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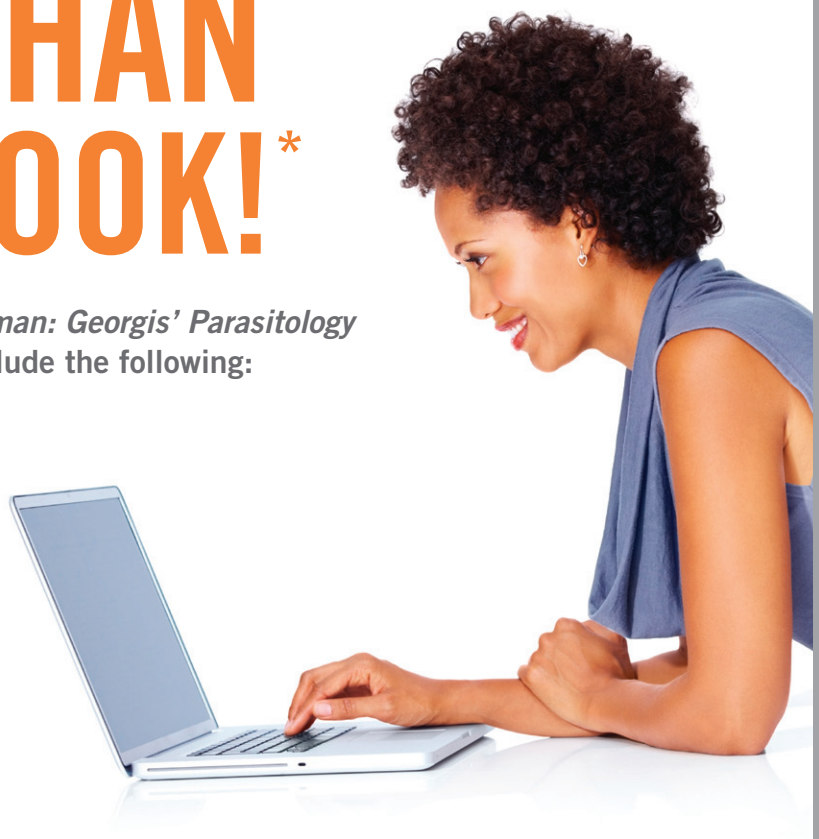

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GEORGIS' PARASITOLOGY FOR VETERINARIANS

10TH EDITION

DWIGHT D. BOWMAN, MS, PhD
Professor of Parasitology
Department of Microbiology
and Immunology
College of Veterinary Medicine
Cornell University
Ithaca, New York

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3251 Riverport Lane
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CONTRIBUTORS

Tad B. Coles, DVM
Medical Writing
Veterinary Consulting
Overland Park, Kansas
Antiparasitic Drugs

Mark L. Eberhard, PhD
Division of Parasitic Diseases and Malaria
Centers for Disease Control and Prevention
Atlanta, Georgia
Histopathologic Diagnosis

Marshall W. Lightowlers, BSc, Hons, PhD
The University of Melbourne Veterinary Hospital
Werribee, Victoria, Australia
Vaccinations

Randy C. Lynn, DVM, MS, DACVCP
Manager, Technical Services
Companion Animal Business Unit
Merck Animal Health
Summit, New Jersey
Antiparasitic Drugs

Susan E. Little, DVM, PhD, DACVM
Regents Professor and Endowed Chair
Department of Veterinary Pathobiology
Center for Veterinary Health Sciences
Oklahoma State University
Stillwater, Oklahoma
Vector-Borne Diseases

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PREFACE

In this, the tenth edition of *Georgis' Parasitology for Veterinarians,* besides the usual updating of details in the text, there have been some significant changes. These changes include altering the order of material presented in the chapter on arthropods, major organizational revisions in the chapter on the protista (protozoa) to match what appears to be the new "systematic synthesis," an attempt to add more images and more reader-friendly text to the chapter on diagnostic parasitology, and finally the expansion of the table on antiparasite vaccines by Dr. Marshall Lightowlers into a full chapter on veterinary vaccines to go along with the chapter on antiparasitic drugs. The book includes many new images, new tables, and updated information, with the hope that it will continue to serve veterinarians and students of veterinary parasitology well.

Again, as stated in the preface of the last edition, for those interested in veterinary parasitology and especially veterinary diagnostics, *Veterinary Clinical Parasitology,* eighth edition, by Dr. Anne M. Zajac of the Virginia-Maryland Regional College of Veterinary Medicine in Blacksburg, Virginia and Dr. Gary A. Conboy is a must-have addition to any library or collection. The book just keeps getting better and better. Again, the proceeds from the sale of *Veterinary Clinical Parasitology* support the continuing efforts of the American Association of Veterinary Parasitologists (AAVP) to provide a centralized vibrant forum for its membership that is also a welcoming presence for new members of the veterinary parasitology community.

Positive things relative to veterinary education have happened since the last edition of *Georgis' Parasitology for Veterinarians*. The field of veterinary parasitology, through the hard work of many members of the AAVP, has become a recognized specialty within the American College of Veterinary Microbiologists, and the first new Diplomates have taken and passed the General and Specialty Parasitology Examinations. The National Center for Veterinary Parasitology (NCVP) at Oklahoma State is going strong as it provides a central nidus for the spread of educated parasitologists nationally. The educators in veterinary parasitology with fiscal assistance from AAVP and the Companion Animal Parasite Council (CAPC) have met every other year for the past 6 years (in Atlanta, Georgia; NCVP in Stillwater, Oklahoma; and the USDA-ARS facility in Beltsville, Maryland) to discuss parasitology education, to share teaching methods and information on curricula, and to define clinical competencies and the means for measuring their completion (Figure 1). Overall, education in veterinary parasitology remains vibrant and strong.

FIGURE 1. Veterinary Educators at the third AAVP/CAPC Parasitology Educators Symposium at the Animal Parasitic Diseases Laboratory of the USDA's Agricultural Research Service (ARS) in Beltsville, Maryland.

An issue of growing concern in veterinary parasitology involves the use of macrocyclic lactones. It is well accepted that there is significant resistance to this class of products in terms of the helminths of sheep and goats and relative to *Parascaris equorum* in horses. In equine parasitology, the recent report by Nielsen et al (2012) on the appearance of the eggs of *Strongylus vulgaris* eggs in the feces of horse herds undergoing selective therapy for cyathostome control is worrisome. Selective therapy was put in place to prevent the potential development of cyathostome resistance, but the concern now is that this practice, which does not target the large strongyles, may be allowing an increase in cases of verminous arteritis from *S. vulgaris*. Thus the practice needs to be reconsidered in light of this finding, and it probably argues strongly for improved methods or the easy diagnosis of *S. vulgaris* infections. Also, the specter of potential resistance of heartworms to macrocyclic lactones as used in heartworm preventives raised its head in a series of publications (Snyder et al, 2011a, 2011b; Blagburn et al, 2011, Bourguinat et al, 2011). The concern over heartworm resistance to macrocyclic lactones is fully warranted because canine heartworm infections can produce dreadful consequences. It is critical that veterinary parasitologists work toward determining whether or not heartworm resistance exists and, if it does exist, how to prevent the spread of any resistant forms. This is an absolutely marvelous class of compounds for both livestock and companion animals, and stewardship by the veterinary parasitology community must be a continued goal.

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ACKNOWLEDGMENTS

For this edition, the person who has been the greatest help in its preparation has been Dr. Araceli Lucio-Forster. While working in the trenches teaching diagnostic parasitology to the students as part of their clinical training, she routinely realizes the deficits in the text, and lets me know what would improve its usefulness. Furthermore, not only does she identify the weaknesses, she presents me with draft remedies, most of which are included in the text. At the same time, Dr. Lucio-Forster also collects images of parasites from all sorts of different hosts to spice up the text and our teaching. Also, in the laboratory, Janice Liotta keeps things running smoothly and has helped repeatedly in collecting specimens for imaging. Other people I need to thank at Cornell University for making this edition possible include the regulars, Drs. Hornbuckle, Barr, Simpson, Smith, Nydam, Ducharme, Miller, Scott, and McDonough, and a series of new folks who have been remarkably helpful and include Drs. Collins, Dykes, Kraus, Rishniw, and Thompson. My colleagues at Cheri-Hill Kennel and Supply have provided many of the specimens that appear as images in this and previous editions of this book, and together we mourn the recent loss of David M. Ulrich, a great friend who was always willing to help with any task at hand and who provided constant insight into the practical side of parasite recovery methodology.

In this edition, I need to make certain that I once again thank my MS and PhD advisor, Dr. M. Dale Little, for spending so many hours in guiding my development as a young and maturing graduate student; I am not certain there were many others who would have put up with me and been such a wonderful mentor at the same time. I need to additionally thank my colleagues at other educational institutions, all of whom make wonderful contributions to our profession. Also, this book would be nothing like it is if it were not for my manifest interactions with members of the different pharmaceutical, biologics, and diagnostic companies and their continued engagement in matters parasitologic and their desire to improve animal health. I need to thank the many nameless veterinarians who listen to me speak on different occasions and do not realize that they teach me more when I am in front of them gauging their responses and listening to their questions than perhaps they are gaining from the words I utter while standing at the front of the room.

Some of the most fun I have been having over the past several years is working with Dr. Alice Lee on the use of alternative methods to count worms in living animals (Lee et al, 2011). Toward this end we have been utilizing endocapsule cameras to gather images as the capsules pass through the intestines of dogs having infections with various helminth parasites and comparing the counts made on images with actual worm counts. The goal remains the development of a means to count worms in living hosts. The dog is the host with which we have worked most closely because it is a monogastric with an intestinal tract similar to human beings for which the camera was developed originally. Things are actually going fairly well with the project. The first dog we ever gave an endocapsule camera, a healthy and very-well-cared-for Great Dane, was discovered in mid-January to have three hookworms by the capsule imaging (Figure 2); the dog had been taken off its parasite control for the winter, which probably allowed the repopulation of the intestine by new adults via larval leak. Worms are definitely visible with the endocapsule camera, and if visible are countable. We have been able to count both *Ancylostoma caninum* and *Toxocara canis* in dogs with an accuracy that is pretty comparable to actual worm counts. We have also seen tapeworms, *Taenia pisiformis*, in some naturally infected dogs (Figure 3).

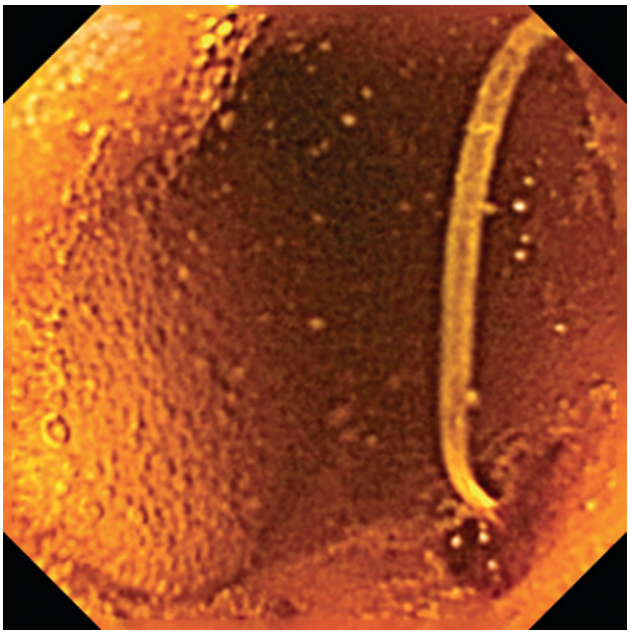

FIGURE 2. Endocapsule image of an adult *Ancylostoma caninum* female in the intestine of a naturally infected Great Dane.

Better images are obtained with endoscopy (Figure 4) but, unfortunately, it is not easy to examine the entire small intestine of a dog in this manner and it does require anesthesia. However, this might be the best means of capturing images of whipworms in living dogs (Figure 5) because the capsules do not enter the cecum as they pass through the canine intestinal tract. These are not the only means of capturing images of living worms in living hosts. Recently in the clinic, images were captured of *Toxocara cati* in the small intestine of a cat with ultrasound (Figure 6). As these imaging modalities are being improved and moved into veterinary medicine, the ability to observe living worms in situ in living hosts is improving to the point that we should not only be able to simply count them, but soon also be able to examine their behavior as never before.

Finally, I need to thank Shelly Stringer, the managing editor for this edition at Elsevier. She has been great. She has put up with a lot of this and that as we have moved toward getting this edition finalized, and still she has always remained remarkably pleasant, upbeat, and helpful. The other folks at Elsevier have also been very helpful and working very hard to keep the book on track; thus again my thanks also to Katie Gutierrez, Brandi Graham, and Courtney Schilling, for all their hard work helping me get this all wrapped

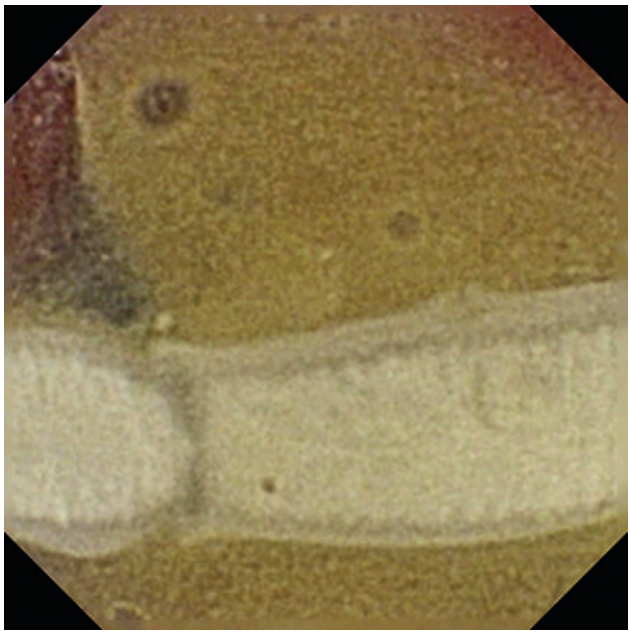

FIGURE 3. Two segments of a *Taenia pisiformis* tapeworm in the intestine of a naturally infected dog.

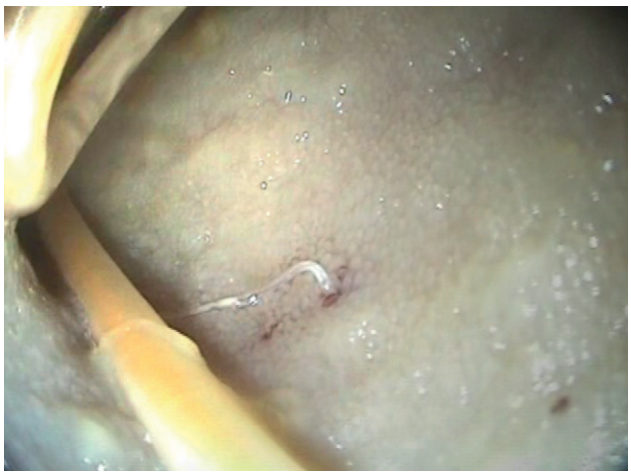

FIGURE 4. Endoscopic view of the intestine of an experimentally infected dog showing portions of two *Toxocara canis* worms and an adult female *Ancylostoma caninum* with her associated feeding site.

up in what I hope is a text that is both useful and pleasing to the eye. Finally, David Stein did a great job as the project manager of the text. He made the whole thing better, from English to layout, from helping with the presentation of italicized headings to understanding the nuances of species, sp., and spp. All the folks at Elsevier have taken a real interest in the text and have made it a much better book that I would have on my own.

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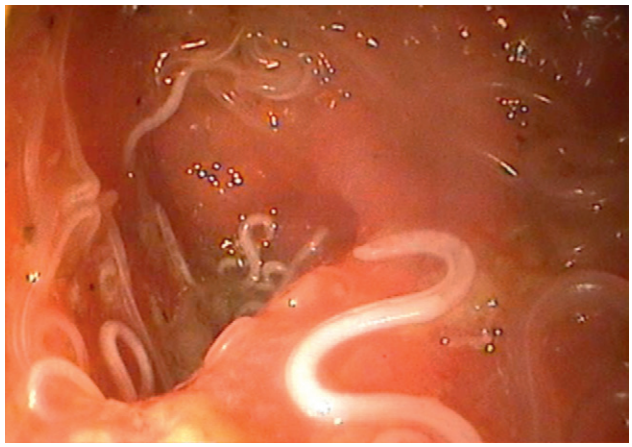

FIGURE 5. Endoscopic view of *Trichuris vulpis* in the cecum of a naturally infected dog.

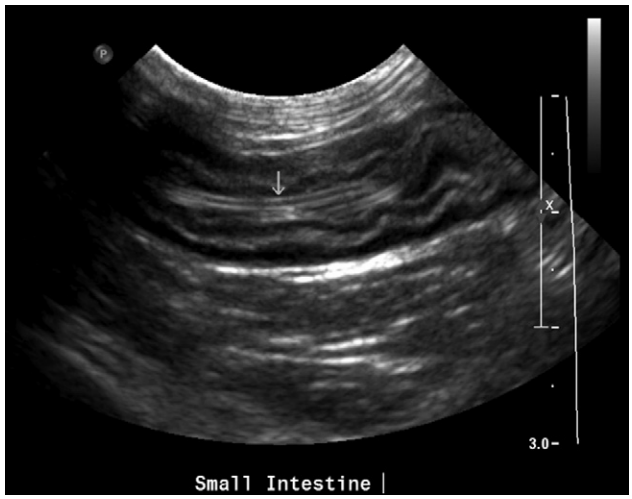

FIGURE 6. Image collected during an ultrasound of a cat at the Cornell University Hospital for Animals showing two *Toxocara cati (arrow)* with their typical appearance as a pair of parallel lines in the small intestine of a naturally infected cat. The movie collected at the time of the procedure is even more impressive.

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CONTENTS

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CHAPTER 1

Introduction

COMMON TERMS IN PARASITOLOGY

A **parasite** is a smaller organism that lives on or in and at the expense of a larger organism called the **host.** A louse is a parasite and so is a virus. The host's expenses in supporting its parasites may be trivial, or they may be substantial or even unbearable. This depends on the number of parasites, the kind and degree of injury they inflict, and the vigor and nourishment of the host. A series of terms (e.g., *mutualism*, *commensalism*, *parasitism*) have been defined to express the degree of unilateral or mutual injury or benefit that is characteristic of particular symbiotic relationships. As a matter of convention, however, if the smaller organism is found in association with humans or with animals or plants that humans esteem, it is called a *parasite*, whether its presence is detrimental, indifferent, or beneficial. This convention is adopted in this book and is harmless enough, provided we remember that parasites vary in pathogenicity.

A **species** of animal is an interbreeding natural population that is reproductively isolated from other such populations. For example, there are two species of rather distantly related ascarid parasites of dogs, *Toxocara canis* and *Toxascaris leonina*. These two species are sufficiently similar in size and appearance to present some difficulty in their differentiation, but although they may share the small intestine of the same dog, they never interbreed. The consequent distinctness of their genetic material is expressed in modest differences in structure and in very substantial differences in life history. *T. canis* and *T. leonina*, however, share enough similarities to make their kinship obvious. We assume that these similarities stem from the evolution of both species from common ancestral stock *(divergent evolution)* because the number and nature of the similarities induce us to reject the alternative explanation—that is, that they represent the adaptations of unrelated forms to the same selection pressures *(convergent evolution)*. We recognize kinship of *T. canis* and *T. leonina* by considering both to be members of the same zoologic order (Ascaridida); each is a leaf, if you will, on the same evolutionary branch.

CONVENTIONS OF TAXONOMIC CLASSIFICATION

Classification is an inductive process. Unfortunately, for those who seek perfection in the correspondence of the classification scheme to the true history of evolution, there is very little objective evidence of the kinship of parasites. The progenitors of the horse *(Equus caballus)* left a clear fossil record of equine evolution, but the ancestors of our parasites merely rotted and withered away, leaving only an occasional trace. The entire hierarchy of taxonomic categories above that of species (genus, subfamily, family, superfamily, suborder, order, class, and phylum) is built of subjective inductions based on degrees of similarity and dissimilarity among the various groups of organisms. Fortunately, the result is nonetheless useful to us in organizing our information about parasites in an orderly and logical way. In short, any particular zoologic classification scheme is no more than an opinion about how the relationships among various groups of organisms may best be expressed.

It is helpful to be acquainted with a few nomenclatural conventions. The full zoologic name of an animal is a **binomen,** consisting of the genus name followed by the species name. The genus name is capitalized and both genus and species names are italicized in print or underlined in manuscript (e.g., *Filaroides milksi*). In taxonomic publications and in other scientific and professional journals, the zoologic name is followed by the name of the person(s) who described the species in question and the date that the description was first published (e.g., *Filaroides milksi* Whitlock, 1956). If, at a later date, another taxonomist decides for one reason or another that this particular species really ought to belong to a different genus, the original describer's name is now placed in parentheses and the name of the taxonomist who moved the species may follow outside the parentheses (e.g., *Andersonstrongylus milksi* [Whitlock, 1956] Webster, 1981). We are not forced to accept Webster's opinion and may continue to call this species by its original name, *Filaroides milksi*, if we believe that we have good enough reason to do so. The species *milksi* is objective in that it is based on real and tangible specimens that Whitlock studied and described in 1956. Assigning *milksi* to any particular genus is, however, largely subjective and based on taxonomic judgment. This is why we frequently come across the same species relegated to two or even more genera.

Certain categories have characteristic suffixes that help to identify them. For example, the genus *Strongylus* belongs to the following hierarchy of higher taxa: subfamily Strongylinae, family Strongylidae, superfamily Strongyloidea, order Strongylida. In this text, the suffixes *-inae*, *-idae*, *-oidea*, and *-ida* are applied to all subfamily, family, superfamily, and order names.

The principal objectives of zoologic nomenclature are to promote stability and universality of zoologic names and to ensure

that each name is unique and distinct. Not every taxonomist is hard at work changing the names to confuse others, as students are prone to suspect.

IDENTIFICATION AND DIAGNOSIS

Identification is determining which taxonomic groups a species belongs to, whereas diagnosis is determining the cause and nature of a case of disease. Both are deductive processes. The diagnosis of parasitism per se requires only that some life stage of the particular species of parasite be identified. Diagnosis of parasitic disease requires much more. In fact, interpretation of the significance of the information regarding the parasite or parasites identified in a particular case of disease frequently taxes our knowledge and interpretive skill to the utmost. In a very few cases, we have a direct cause-and-effect relationship to make it easy. For example, *Haemonchus contortus*, a nematode parasite of sheep, causes disease when the mass of worms present in the abomasum sucks more blood than the sheep can replace, and the disease haemonchosis, manifested as clinical anemia, results. If too few *H. contortus* worms are present to overtax the hematopoietic capacity of the sheep, or if a particular sheep manages to make restitution for blood loss that might lay another low, the case is one of subclinical *H. contortus* infection. Simply put: no anemia, no haemonchosis. One makes the diagnosis of haemonchosis by examining the visible mucous membranes or a sample of blood for evidence of anemia.

Diagnosing haemonchosis is easy. It is very much more difficult to evaluate the clinical significance of most other parasitic infections. For example, when the veterinarian is confronted with a case of chronic diarrhea, finding a few coccidian oocysts in the animal's stool may mislead the veterinarian to neglect other possible causes and jump to the conclusion that the animal has the disease coccidiosis, when in fact the coccidian infection is incidental. The specific identity of the cysts in the feces supplies the diagnostician with a concrete fact that may, in the midst of uncertainty, prove nearly irresistible. A difficult situation indeed—and there are many more like it. In this book, we have tried to present information that is helpful in deciding when parasites are responsible for clinical disease and when they are not. In truth, there is much still to be learned.

Identification of the common parasites of domestic cats, dogs, cattle, sheep, goats, horses, and pigs is a relatively simple matter. Only one semester of study is required to become fairly adept at it. By restricting the scope of the problem to particular host species, it is possible to simplify identification criteria, accommodate reasonably complete sets of illustrations in the available space, and make helpful lists of the kinds of parasites likely to be encountered in particular organs. Chapter 7 is devoted to such criteria, illustrations, and lists. However, when the scope of interest is broadened to include exotic pets and captive and wild mammals and birds, such a detailed approach would inevitably lead to a shelf full of books. Fortunately, many shelves full of books are already to be found in the better academic and municipal libraries, and that is where we must go to get the necessary information. The first step is to determine the scientific name of the host species; if we don't already know it, Google or Wikipedia searches are readily available sources for this information.

Finally, it should be remembered that when we find worms or various diagnostic stages of parasites, our goal is typically to determine the species group to which the individual specimens belong. However, we do not "speciate" parasites, we simply identify them. *Speciation* refers to something done by a creature as it evolves from one species type to another. Therefore, the only thing that can speciate is the creature itself. The term *speciation* should be restricted in its usage to discussions that deal with how species originated, such as discussions about new species and beak shapes on the various finches of the Galapagos.

RELATIONSHIP BETWEEN PARASITES AND HOSTS

Several terms are useful relative to the study of parasitology in general. Animals that live in close association with each other are called **symbionts** living together in the process of **symbiosis.** This has been further characterized for certain specific types of relationships. In the case of **mutualism,** both hosts benefit; this is what occurs with the various ciliates and bacteria that live within the rumen of a ruminant—if they stop functioning, the rumen stops ruminating. When the two organisms just live together and neither "loses" or "wins," the condition is called **commensalism,** and the organisms living in this way are called **commensals.** An example might be the various amoebae that live within the cecum and the colon of cattle and sheep for which no disease has ever been recorded. In the case of **phoresis,** one organism serves to carry the other organism from place to place. This is what takes place in the life history of the fly *Dermatobia hominis*, which uses other flies to carry the larvated eggs to the vertebrate host that becomes infected. Finally, in the case of **parasitism** (quoting Dr. James Law), "one of the two draws its subsistence from the other to the appreciable injury of the latter." The parasite, by definition, has negative effects on its host.

Some terms relative specifically to parasites are used in certain ways by convention. Thus **endoparasites,** parasites within the bodies of hosts, are considered to produce **infections,** whereas **ectoparasites,** those that live on the external surface of a host or in the skin, are said to cause **infestations.** Some parasites are considered to be **obligate parasites:** They always require a host. Other organisms are parasites only if given the opportunity; they are called **facultative parasites** (e.g., *Balamuthia mandrillaris*, *Halicephalobus gingivalis*). Hosts that live only on or in a single host are considered to be **host-specific,** with classic examples being the various lice of birds and mammals. The host in which the adult or sexually reproductive processes of the parasite occur is called a **definitive host.** A host in which there is required development of intermediate or larval stages is called an **intermediate host.** In the case of a **paratenic host,** the host is infected with a parasite that does not undergo any required development, although the parasite sometimes can grow very large in the chain of paratenic hosts that are used (as in the piscine hosts of the larvae of *Diphyllobothrium latum*). Also in the case of paratenesis, the parasites should be transferable from host to host until they ultimately make their way into the final host. Organisms that transmit parasites directly from host to host are termed **vectors. Mechanical vectors** are basically living contaminated syringes, that is, they are not essential in the normal life cycle of the organism being moved from host to host. In the case of a **biologic vector,** the vector is required in the life cycle of the parasite.

Parasites may cycle in animals other than those we consider the host of interest; these hosts are considered **reservoir hosts.** When parasites are present at some stable rate in a population, they are said to be **endemic** (although for animals, the more appropriate term is really **enzootic**). If the disease is present at a very high level in a population, it is said to be **hyperendemic. Endemicity** is often measured in terms of **prevalence**—the percentage of infected

individuals in an area at any given time. **Incidence** refers to the rate at which new infections are occurring within a population (e.g., new cases of heartworm in California in the past 6 months). When a sharp increase in incidence is seen along with a concomitant rise in prevalence, the term that is used is **epidemic**. Similar terms are used specifically for animals—**enzootic, hyperenzootic, epizootic**—but these are unfamiliar to many, so often the terms that pertain to humans are used instead.

The term **zoonosis** means literally "a disease of animals," but the word has come to mean a disease of animals transmitted to people. Hoare (1962) cited four terms to describe transmission of pathogens between humans and animals.

1. **Anthropozoonosis** (etymologically, simply a disease of humans and animals) defines a disease of humans acquired from animals (e.g., rabies, plague, brucellosis, leptospirosis, Rhodesian sleeping sickness, tick-borne encephalitis or relapsing fever, babesiosis, ehrlichiosis, Chagas' disease, trichinosis).
2. **Zooanthroponosis,** considered by some as "reverse zoonosis," defines a disease of animals acquired from people (e.g., transmission of *Entamoeba histolytica* to cats, *Giardia lamblia* to dogs, tuberculosis to cattle, or *Schistosoma mansoni* to baboons).
3. **Amphixenosis** (etymologically, disease of both hosts) defines an infection that is interchangeable between people and other vertebrates (e.g., Chagas' disease, *Schistosoma japonicum*, *Staphylococcus* species).
4. **Anthroponoses** (etymologically, disease of humans) defines infections restricted to humans that evolved from infections of lower animals (e.g., malaria, typhus, relapsing fever).

Other terms presented included *euzoonosis* for infections common to humans and reservoir hosts (probably the same as amphixenosis; e.g., *S. japonicum* in humans and various mammals) and *parazoonosis*, in which humans are infected with a zoonotic agent only rarely (e.g., canine heartworm).

The biology of agents has also been defined relative to zoonosis. **Cyclozoonosis** describes zoonotic agents restricted to vertebrates (e.g., *Taenia solium*). M**etazoonosis** describes agents that cycle between vertebrates and invertebrates (e.g., malaria). **Saprozoonosis** refers to agents cycling between vertebrates and nonanimal hosts (e.g., *Fasciola hepatica*) with metacercariae on vegetation.

To describe the transmission of agents from wild to domestic animals, as well as the transmission of pathogens from domestic animals to domestic or wild animals, I have worked with Dr. Hanna Roisman, the Francis F. Bartlett and Ruth K. Bartlett Professor of Classics, Classics Department of Colby College, Waterville, Maine, to develop terms that aid in defining these conditions. We divided these relationships concerning infections of animals with agents for which they are atypical hosts into three groups (ignoring the infections shared between different wild animals): (1) infection of domestic animals with pathogens of wildlife, (2) infection of domestic animals with pathogens of domestic animals, and (3) infection of wild animals with pathogens of domestic animals.

Zootherionosis* (*zoon*, animal + *therion*, wild animal + *o* + *nosos*, disease) is used to define diseases of domestic animals infected with pathogens of wildlife. The classic example is the infection of imported domestic animals with African wildlife trypanosomes. Other examples include infections with *Leishmania*, plague, Lyme disease, and rickettsiae from rodent reservoirs; the viruses of foot and mouth disease and avian influenza, and Hendra and Nipah viruses; larval infections with *Alaria* species, spargana, tetrathyridia, larval *Baylisascaris procyonis* and *Armillifer armillata*, and bots of *Cuterebra;* and horses and cats, which serve as hosts of the asexual stages of the equine protozoal myeloencephalitis agent, *Sarcocystis neurona*. Cats are lethally infected with *Cytauxzoon felis* of the bobcat. Infections with sexually mature pathogens include the trematodes *Paragonimus kellicotti* in dogs and cats, *Fascioloides magna* in cattle and sheep, *Alaria marcianae* and *Platynosomum fastosum* in cats, and *Heterobilharzia americanum* in dogs; the cestodes *Spirometra mansonoides* in dogs and cats and *Thysanosoma* and *Wyominia* in domestic ruminants; and the nematodes *Parelaphostrongylus tenuis* in ruminants, *B. procyonis*, *Dracunculus insignis*, *Onchocerca*, and *Dioctophyme renale* in dogs, and *Lagochilascaris minor* in cats.

Zootithasonosis* (*zoon*, animal + *tithas*, tamed + *o* + *nosos*, disease) is used for those cases in which a pathogen from one type of domestic animal infects other domestic animals. Feline panleukopenia virus has adapted to dogs, causing a global outbreak of canine morbidity and mortality. Bovine diarrhea virus infects sheep and goats, causing border disease. Cats infect dogs with ringworm, *Microsporum canis*. Cats and ferrets are parasitized with adult canine heartworm, *Dirofilaria immitis*. *Trichostrongylus axei* of ruminants infects the domestic horse. Cats and rabbits develop visceral larval migrans from infection with the dog roundworm, *T. canis*. The cat roundworm, *Toxocara cati*, causes white spot disease in the livers of pigs. Ruminants are infected with taeniid tapeworms of dogs and large cats. The cat can be a host of the coenurus of *Taenia serialis*, which uses dogs as final hosts.

Theriotithasonosis* is used for those cases in which wild animals can be infected with pathogens from domestic animals. Lions in the Serengeti and in captivity have succumbed to a variant of the distemper virus from dogs. Wolves, coyotes, and African wild dogs have been infected with canine parvovirus from domestic dogs. Macropodid marsupials sometimes are infected with ovine Johne's disease bacteria (*Mycobacterium avium* subspecies *paratuberculosis*). Domestic goats infect wild goats with infectious keratoconjunctivitis *(Mycoplasma conjunctivae).* Domestic cattle with contagious bovine pleuropneumonia (*Mycoplasma mycoides* subspecies *mycoides* [small colony type]) have infected African water buffaloes and zebu cattle. *T. canis* routinely infects rodents and birds and can infect tortoises. *Toxoplasma gondii* causes infection in numerous wild animals and has now been reported to cause disease in aquatic mammals. Adult heartworms cause disease in sea lions, and *Dicrocoelium dendriticum* causes infection in deer, rabbits, and woodchucks.

These terms can also be used to represent the sources of human infection. Tables 1-1 and 1-2 were generated for some of the representative parasites of people transmitted from animals: diseases from wild animals or the anthropotherionotic agents and diseases from domestic animals or the anthropotithasonotic agents. Similar tables could be generated for many relationships, such as for disease of dogs and cats from wildlife or disease of otters from dogs and cats as examples of zootherionoses and theriotithasonoses, respectively.

*Because there is no one word in Greek for domesticated animals (unlike *therio* for wild animals), the full words should have been *tithasozootherionosis*, *tithasozootithasozoonosis*, and *theriotithasozoonosis;* however, we opted for simplicity and sonority. The term for infection of wild animals with agents from other wild animals would be *theriotherionosis*, abbreviated to *therionosis*, simply, a disease of wild animals.

REFERENCE

Hoare CA: Reservoir hosts and natural foci of human protozoal infections, *Acta Tropica* 19:281, 1962.

TABLE 1-1 Some Parasites of Zoonotic Importance—Anthropotherionotic Infections (i.e., Diseases of Humans from Wild Animals) †

Parasite	Reservoir Host(s)	Human Disease	Mode of Infection
PROTISTA			
Trypanosoma brucei rhodesiense	Ungulates	East African sleeping sickness	Bite of the tse tse (*Glossina* species)
Trypanosoma cruzi	Mammals	Trypanosomiasis, Chagas' disease	Inoculation of metacyclic trypomastigotes in fresh feces of triatomin bug
Trypanosoma rangeli	Mammals	Trypanosomiasis	Bite of triatomin bug
Leishmania infantum, L. chagasi	Canids	Visceral leishmaniasis	Bite of phlebotomine fly
Leishmania aethiopica	Hyraxes	Cutaneous leishmaniasis	Bite of phlebotomine fly
Leishmania major	Rodents, Gerbillinae	Cutaneous leishmaniasis	Bite of phlebotomine fly
Leishmania tropica	Hyraxes	Cutaneous leishmaniasis	Bite of phlebotomine fly
Leishmania mexicana complex *L. mexicana* *L. amazonensis* *L. venezuelensis*	Rodents, marsupials, primates, other mammals	Cutaneous leishmaniasis	Bite of phlebotomine fly
Leishmania (Viannia) spp. *L.* (V.) *braziliensis* *L.* (V.) *guyanensis* *L.* (V.) *panamensis* *L.* (V.) *peruviana*	Sloths, anteaters, marsupials, canids, rodents	Cutaneous leishmaniasis	Bite of phlebotomine fly
Balantidium coli	Suids	Balantidiosis	Ingestion of cyst passed in feces
Cryptosporidium parvum	Calves and young ungulates	Cryptosporidiosis	Ingestion of oocyst passed in feces
Cryptosporidium ubiquitum	Deer and mammals	Cryptosporidiosis	Ingestion of oocyst passed in feces
Cryptosporidium fayeri	Kangaroos	Cryptosporidiosis	Ingestion of oocyst passed in feces
Cryptosporidium muris	Mice	Cryptosporidiosis	Ingestion of oocyst passed in feces
Cryptosporidium cuniculus	Rabbits	Cryptosporidiosis	Ingestion of oocyst passed in feces
Cryptosporidium tyzzeri, skunk genotype, chipmunk I	Mice, skunks, chipmunks	Cryptosporidiosis	Ingestion of oocyst passed in feces
Toxoplasma gondii	Felids	Toxoplasmosis	Ingestion of oocyst from contaminated environment or bradyzoite in meat
Sarcocystis spp.	Vertebrates	*Sarcocystis* myositis	Ingestion of sporocysts passed in feces
Plasmodium knowlesi	Cercopithic primate	Malaria	Bite of anopheline mosquito
Babesia microti	Rodents	Babesiosis	Bite of *Ixodes scapularis*
Babesia duncani	Rodents (?)	Babesiosis	Bite of ixodid tick
TREMATODA			
Gastrodiscoides spp.	Suids	Paramphistomiasis	Ingestion of metacercaria on vegetation
Fasciola hepatica	Ruminants, suids	Fascioliasis	Ingestion of metacercaria on vegetation
Fasciola gigantica	Ruminants, suids	Fascioliasis	Ingestion of metacercaria on vegetation
Fasciolopsis buski	Ruminants, suids	Fasciolopsiasis	Ingestion of metacercaria on vegetation
Paragonimus westermani, other *Paragonimus* spp.	Felids, canids, mustelids, viverrids	Paragonimiasis	Ingestion of metacercaria in freshwater crab
Paragonimus kellicotti	Mustelids, canids, felids	Paragonimiasis	Ingestion of metacercaria in crayfish
Dicrocoelium dendriticum	Ruminants, woodchucks, rabbits	Dicrocoeliasis	Ingestion of metacercaria in ants
Schistosoma japonicum	Mammals	Schistosomiasis	Penetration of skin by cercaria from freshwater snail
Schistosoma bovis, S. mattheei, S. margrebowiei, S. leiperi	Ruminants, equids, suids	Patent zoonotic schistosomiasis	Penetration of skin by cercaria from freshwater snail

TABLE 1-1 Some Parasites of Zoonotic Importance—Anthropotherionotic Infections (i.e., Diseases of Humans from Wild Animals)—cont'd

Parasite	Reservoir Host(s)	Human Disease	Mode of Infection
Schistosoma rodhaini	Canids, rodents	Patent zoonotic schistosomiasis	Penetration of skin by cercaria from freshwater snail
Avian schistosomes—*Trichobilharzia, Ornithobilharzia, Gigantobilharzia, Austrobilharzia*	Water fowl	Cercarial dermatitis (swimmer's itch)	Penetration of skin by cercaria from freshwater or saltwater snail
Clonorchis sinensis	Canids, felids, other piscivorous hosts	Clonorchiasis	Ingestion of metacercaria in freshwater fish (carp and other cyprinids)
Nanophyetus salmincola	Raccoons, viverrids, canids, felids	Nanophyetiasis	Ingestion of metacercaria in salmon and trout (cyprinids and others)
Opisthorchis felineus	Foxes, suids, rats, mustelids, seals	Opisthorchiasis	Ingestion of metacercaria in freshwater fish (carp and other cyprinids)
Heterophyes heterophyes	Piscivorous mammals, birds	Heterophyiasis	Ingestion of metacercaria in fresh and brackish water fish
Metagonimus yokogawai	Piscivorous mammals, birds	Metagonimiasis	Ingestion of metacercaria in fresh and brackish water fish
Echinostoma ilocanum	Rats, canids	Echinostomiasis	Ingestion of metacercaria in freshwater snails
CESTODA			
Spirometra spp.	Felids, canids, other mammals	Sparganosis	Ingestion of procercoid in copepod or plerocercoid in flesh of paratenic vertebrate host
Diphyllobothrium latum	Bears, canids, felids, phocids	Diphyllobothriasis	Ingestion of plerocercoid in freshwater fish
Diplogonoporus spp.	Cetaceans	Diplogonoporiasis	Ingestion of plerocercoid in salt water fish (e.g., anchovies)
Hymenolepis diminuta	Rodents	Hymenolepiasis	Ingestion of cysticercoid in a beetle
Hymenolepis nana	Rodents	Hymenolepiasis	Ingestion of cysticercoid in a beetle or ingestion of egg
Dipylidium caninum	Felids, canids	Dipylidiasis	Ingestion of cysticercoid in adult flea
Echinococcus granulosus	Canids	Unilocular hydatidosis	Ingestion of eggs passed in feces
Echinococcus multilocularis	Foxes, canids	Multilocular hydatidosis	Ingestion of eggs passed in feces
Taenia saginata	People	Taeniasis	Ingestion of cysticercus in bovid flesh
Taenia solium	People	Taeniasis or cysticercosis	Ingestion of cysticercus in suid flesh (taeniasis) or egg passed in human feces (cysticercosis)
Taenia multiceps	Canids	Coenurosis	Ingestion of eggs passed in feces
Taenia serialis	Canids	Coenurosis	Ingestion of eggs passed in feces
NEMATODA‡			
Trichostrongylus species	Ruminants, others	Trichostrongylosis	Ingestion of larva from contaminated environment
Ternidens deminutus	Primates	Strongylosis	Ingestion of larva from contaminated environment
Oesophagostomum species	Primates, ovines, suids	Oesophagostomiasis	Ingestion of larva from contaminated environment
Mammomonogamus laryngeus	Ungulates	Mammomongamonosis	Unknown
Angiostrongylus cantonensis	Rodents	Meningoencephalitis	Ingestion of larva in mollusk
Angiostrongylus costaricensis	Rodents	Abdominal angiostrongylosis	Ingestion of larva in mollusk
Metastrongylus apri	Suids	Metastrongylosis	Ingestion of larva in earthworm
Ancylostoma braziliense	Felids, canids?	Cutaneous larva migrans (CLM)	Penetration of skin by larva
Ancylostoma ceylanicum	Felids, canids?	Hookworm infection	Penetration of skin by larva

Continued

TABLE 1-1 Some Parasites of Zoonotic Importance—Anthropotherionotic Infections (i.e., Diseases of Humans from Wild Animals)—cont'd

Parasite	Reservoir Host(s)	Human Disease	Mode of Infection
Ancylostoma caninum	Canids	Eosinophilic colitis	Penetration of skin by larva; perhaps ingestion of infective larvae
Strongyloides stercoralis	Canids, primates	Strongyloidiasis	Penetration of skin by larva
Strongyloides fuelleborni	Primates	Strongyloidiasis	Penetration of skin by larva
Strongyloides myopatami	Nutria	Cutaneous larva migrans	Penetration of skin by larva
Strongyloides procyonis	Raccoons	Cutaneous larva migrans	Penetration of skin by larva
Ascaris suum	Suids	Swine ascariasis	Ingestion of egg from contaminated environment
Toxocara spp.	Canids, felids, ungulates, mustelids	Larval toxocariasis (visceral larva migrans)	Ingestion of egg from contaminated environment or larvae in tissue of paratenic host
Baylisascaris spp.	Procyonids, mustelids, ursids	Larval baylisascariasis (visceral larva migrans)	Ingestion of egg from contaminated environment
Lagochilascaris minor	Unknown	Lagochilascariasis	Ingestion of egg from contaminated environment or infective larvae in rodent tissue
Toxascaris leonina	Felids, canids	Visceral larva migrans	Ingestion of egg from contaminated environment or tissue of paratenic host
Anisakis spp.	Cetaceans	Anisakiasis	Ingestion of larva in fish—salmon, cod, herring, mackerel, etc.
Pseudoterranova spp.	Pinnipeds	Anisakiasis	Ingestion of larva in fish—cod, haddock, pollock, halibut, etc.
Gnathostoma spp.	Felids, canids, carnivorous mammals	Larval gnathostomiasis (visceral larval migrans)	Ingestion of larva in freshwater copepod or vertebrate
Physaloptera spp.	Primates, felids, canids, other insectivorous or carnivorous mammals	Physalopteriasis	Ingestion of larva in cockroach, beetles, paratenic vertebrate hosts
Gongylonema spp.	Ruminants, pigs, bears, primates	Gongylonemiasis	Ingestion of larva in cockroach or beetle
Thelazia callipaeda	Felids, canids	Thelaziosis	Deposition of larva from mouthparts of feeding drosophilid fruit fly
Mansonella rodhaini	Chimpanzees	None	Probably bite of *Culicoides*
Dirofilaria immitis	Felids, canids	Pulmonary dirofilariasis	Bite of mosquito
Dirofilaria repens	Felids, canids, other carnivores	Subcutaneous dirofilariasis	Bite of mosquito
Dirofilaria tenuis	Raccoons	Subcutaneous dirofilariasis	Bite of mosquito
Dirofilaria ursi	Bears	Subcutaneous dirofilariasis	Bite of black fly
Dirofilaria subdermata	Porcupines	Subcutaneous dirofilariasis	Bite of mosquito
Dirofilaria striata	Bobcats	Subcutaneous dirofilariasis	Bite of mosquito
Pelecitus scapiceps	Rabbits	Ocular filariasis	Bite of mosquito
Meningonema peruzzii	Cercopithecid monkeys	Cerebral filariasis	Not known
Brugia malayi	Leaf monkeys	Malayan filariasis	Bite of mosquito
Brugia pahangi	Felids, canids	Zoonotic pahangi filariasis	Bite of mosquito
Brugia spp., including *B. lepori* and *B. beaveri*	Many mammals, including rabbits and raccoons	Zoonotic *Brugia*	Bite of mosquito
Molinema arbuta, Molinema sprenti	Porcupines, beavers	Ocular filariasis	Bite of mosquito
Onchocerca gutturosa	Bovids	Zoonotic onchocerciasis	Bite of a black fly
Onchocerca lupi	Canids	Ocular onchocerciasis	Unknown
Trichinella spiralis	Suids, rodents	Trichinosis	Ingestion of larva in meat
Trichinella nativa	Arctic bears	Trichinosis	Ingestion of larva in meat
Trichinella murrelli	Rodents, wild carnivores	Trichinosis	Ingestion of larva in meat
Trichinella britovi	Rodents, wild carnivores	Trichinosis	Ingestion of larva in meat

TABLE 1-1 Some Parasites of Zoonotic Importance—Anthropotherionotic Infections (i.e., Diseases of Humans from Wild Animals)—cont'd

Parasite	Reservoir Host(s)	Human Disease	Mode of Infection
Eustrongylides spp.	Piscivorous birds	Eustrongyloidosis	Ingestion of larva in fish
Dioctophyme renale	Viverrids, piscivorous mammals	Dioctophymatosis	Ingestion of larva in freshwater fish
ACANTHOCEPHALA			
Macracanthorhynchus hirudinaceus	Suids	Macracanthorhychiosis	Ingestion of cystacanth larva in beetle
Moniliformis moniliformis	Rodents	Acanthocephaliasis	Ingestion of cystacanth larva in beetle or cockroach
ARTHROPODA			
Armillifer armillatus	Snakes	Pentastomiasis	Ingestion of egg passed in feces
Porocephalus spp.	Snakes	Pentastomiasis	Ingestion of egg passed in feces
Linguatula serrata	Canids, mammals	Nasopharyngeal pentastomiasis	Ingestion of larval pentastome in herbivore meat
Cheyletiella spp.	Felids, canids, rabbits	Acariasis	Direct contact
Sarcoptes scabiei	Canids, suids, other mammals	Acariasis	Direct contact
Notoedres cati	Felids	Acariasis	Direct contact
Mesostigmatid mites	Birds, rodents	Acariasis	Direct contact with host or its environment/nest
Ctenocephalides felis	Canids, felids	Flea bites	Direct contact
Oestrus ovis	Ovids	Myiasis	Larviposition by adult flies
Gasterophilus spp.	Equids	Myiasis	Oviposition by adult flies

*It is difficult to call some of these infections *zoonotic*. For example, *Taenia solium*, *Taenia saginata*, and *T. asiatica* could not exist without human beings. Similarly, human infections with intestinal sarcocystosis can occur only in humans, so, in a sense, humans are infecting the other hosts. Several of the agents on the list result from human beings' sharing parasites with other species (i.e., amphixenosis) because the parasite is not that finicky about its final host. *Schistosoma japonicum* is a fairly straightforward case of amphixenosis; the schistosome would be just as happy in a cow or in a cat; so is *Trypanosoma cruzi*. It will develop in just about any mammal that is inoculated with metacyclic trypanosomes. Actually, most of the trematode infections in people are cases of amphixenosis, the trematodes can develop in many different hosts that consume the metacercarial stage. This is also the case with *Diphyllobothrium latum* and *Diplogonoporus* spp. If humans choose to display the gustatory habits of a bear or a cetacean, they can also support the maturation of the tapeworm species of those hosts.

†All parasite stages on this list, including eggs, cysts, oocysts, organisms in meat, etc., will die immediately if heated to 65°C.

‡In the case of nematodes, the larval stage infective to the final host is typically the third stage; the exception to this rule is the Trichinelloidea, with species in which the first-stage larva is infective to the final host.

TABLE 1-2 Some Parasites of Zoonotic Importance—Anthropotithasonotic Infections (i.e., Diseases of Humans from Domestic Animals) †

Parasite	Reservoir Host(s)	Human Disease	Mode of Infection
PROTISTA			
Trypanosoma brucei rhodesiense	Native, trypanotolerant cattle	East African sleeping sickness	Bite of the tse tse (*Glossina* species)
Trypanosoma cruzi	Dog, cats, mammals	Trypanosomiasis, Chagas' disease	Inoculation of metacyclic trypomastigotes in fresh feces of triatomin bug
Trypanosoma rangeli	Dog, cats, mammals	Trypanosomiasis	Bite of triatomin bug
Leishmania infantum, L. chagasi	Dogs	Visceral leishmaniasis	Bite of phlebotomine fly
Leishmania (Viannia) species *L.* (V.) *braziliensis* *L.* (V.) *guyanensis* *L.* (V.) *panamensis* *L.* (V.) *peruviana*	Dogs	Cutaneous leishmaniasis	Bite of phlebotomine fly
Balantidium coli	Pigs	Balantidiosis	Ingestion of cyst passed in feces
Cryptosporidium parvum	Calves, lambs, kids	Cryptosporidiosis	Ingestion of oocyst passed in feces
Cryptosporidium meleagridis	Turkeys, chickens	Cryptosporidiosis	Ingestion of oocyst passed in feces
Cryptosporidium canis	Dogs	Cryptosporidiosis	Ingestion of oocyst passed in feces‡
Cryptosporidium felis	Cats	Cryptosporidiosis	Ingestion of oocyst passed in feces‡

Continued

TABLE 1-2 Some Parasites of Zoonotic Importance—Anthropotithasonotic Infections (i.e., Diseases of Humans from Domestic Animals)—cont'd

Parasite	Reservoir Host(s)	Human Disease	Mode of Infection
Cryptosporidium suis, C. andersoni, C. ubiquitum, the horse genotype	Pigs, cattle, sheep and goats, horses	Cryptosporidiosis	Ingestion of oocyst passed in feces
Toxoplasma gondii	Cats	Toxoplasmosis	Ingestion of oocyst from contaminated environment or bradyzoite in meat
Sarcocystis spp.	Cattle, pigs, others	Intestinal sarcocystosis	Ingestion of sarcocyst in meat
Babesia divergens	Cattle	Babesiosis	Bite of *Ixodes ricinus*
TREMATODA			
Gastrodiscoides spp.	Pigs	Paramphistomiasis	Ingestion of metacercaria on vegetation
Fasciola hepatica	Ruminants, pigs	Fascioliasis	Ingestion of metacercaria on vegetation
Fasciola gigantica	Ruminants, pigs	Fascioliasis	Ingestion of metacercaria on vegetation
Fasciolopsis buski	Cattle, pigs	Fasciolopsiasis	Ingestion of metacercaria on vegetation
Paragonimus westermani, other *Paragonimus* spp.	Dogs, cats	Paragonimiasis	Ingestion of metacercaria in freshwater crab
Paragonimus kellicotti	Dogs, cats	Paragonimiasis	Ingestion of metacercaria in crayfish
Dicrocoelium dendriticum	Sheep, goats, cattle	Dicrocoeliasis	Ingestion of metacercaria in ant
Schistosoma japonicum	Mammals	Schistosomiasis	Penetration of skin by cercaria from freshwater snail
Schistosoma bovis, S. mattheei, S. margrebowiei, S. leiperi	Ruminants, horses, pigs	Patent zoonotic schistosomiasis	Penetration of skin by cercaria from freshwater snail
Schistosoma rodhaini	Dogs	Patent zoonotic schistosomiasis	Penetration of skin by cercaria from freshwater snail
Clonorchis sinensis	Dogs, cats	Clonorchiasis	Ingestion of metacercaria in freshwater fish (carp and other cyprinids)
Nanophyetus salmincola	Dogs, cats	Nanophyetiasis	Ingestion of metacercaria in salmon or trout (cyprinids and others)
Opisthorchis felineus	Pigs	Opisthorchiasis	Ingestion of metacercaria in freshwater fish (carp and other cyprinids)
Heterophyes heterophyes	Dogs, cats	Heterophyiasis	Ingestion of metacercaria in fresh and brackish water fish
Metagonimus yokogawai	Dogs, cats	Metagonimiasis	Ingestion of metacercaria in fresh and brackish water fish
Echinostoma ilocanum	Dogs	Echinostomiasis	Ingestion of metacercaria in freshwater snails
CESTODA			
Spirometra spp.	Cats, dogs	Sparganosis	Ingestion of procercoid in copepod or plerocercoid in flesh of paratenic vertebrate host
Diphyllobothrium latum	Cats, dogs	Diphyllobothriasis	Ingestion of plerocercoid in freshwater fish
Dipylidium caninum	Cats, dogs	Dipylidiasis	Ingestion of cysticercoid in adult flea
Echinococcus granulosus	Dogs	Unilocular hydatidosis	Ingestion of egg passed in feces
Echinococcus multilocularis	Dogs	Multilocular hydatidosis	Ingestion of egg passed in feces

TABLE 1-2 Some Parasites of Zoonotic Importance—Anthropotithasonotic Infections (i.e., Diseases of Humans from Domestic Animals)—cont'd

Parasite	Reservoir Host(s)	Human Disease	Mode of Infection
Taenia saginata	People	Taeniasis	Ingestion of cysticercus in beef
Taenia solium	People	Taeniasis or cysticercosis	Ingestion of cysticercus in pork (taeniasis) or egg passed in human feces (cysticercosis)
Taenia asiatica	People	Taeniasis	Ingestion of cysticercus in pork liver
Taenia multiceps	Dogs	Coenurosis	Ingestion of egg passed in feces
Taenia serialis	Dogs	Coenurosis	Ingestion of egg passed in feces
NEMATODAS			
Trichostrongylus spp.	Sheep, goats, cattle, others	Trichostrongylosis	Ingestion of larva from contaminated environment
Oesophagostomum spp.	Sheep, pigs	Oesophagostomiasis	Ingestion of larva from contaminated environment
Mammomonogamus laryngeus	Cattle, other ruminants (?)	Mammomongamonosis	Unknown
Metastrongylus apri	Pigs	Metastrongylosis	Ingestion of larva in earthworm
Ancylostoma braziliense	Cats and dogs	Cutaneous larva migrans (CLM)	Penetration of skin by larva
Ancylostoma ceylanicum	Cats and dogs	Hookworm infection	Penetration of skin by larva
Ancylostoma caninum	Dogs	Eosinophilic colitis	Penetration of skin by larva; perhaps ingestion of infective larvae
Strongyloides stercoralis	Dogs	Strongyloidiasis	Penetration of skin by larva
Ascaris suum	Pigs	Swine ascariasis	Ingestion of egg from contaminated environment
Toxocara spp.	Dogs, cats	Larval toxocariasis (visceral larva migrans)	Ingestion of egg from contaminated environment or larva in tissue of paratenic host
Baylisascaris procyonis	Dogs	Larval baylisascariasis (visceral larva migrans)	Ingestion of egg from contaminated environment
Toxascaris leonina	Cats, dogs	Larval toxascariasis (visceral larva migrans)	Ingestion of egg from contaminated environment
Gnathostoma spp.	Cats, dogs	Larval gnathostomiasis (visceral larval migrans)	Ingestion of larva in freshwater copepod or vertebrate
Physaloptera spp.	Dogs, cats	Physalopteriasis	Ingestion of larva in cockroach, beetles, or paratenic vertebrate host
Gongylonema spp.	Ruminants, pigs	Gongylonemiasis	Ingestion of larva in cockroach or beetle
Thelazia callipaeda	Dogs, cats	Thelaziosis	Deposition of larva from mouthparts of feeding drosophilid fruit fly
Dirofilaria immitis	Dogs	Pulmonary dirofilariasis	Bite of mosquito
Dirofilaria repens	Dogs, cats	Subcutaneous dirofilariasis	Bite of mosquito
Brugia pahangi	Dogs, cats	Zoonotic pahangi filariasis	Bite of mosquito
Onchocerca gutturosa	Cattle	Zoonotic onchocerciasis	Bite of black fly
Onchocerca lupi	Dogs	Ocular onchocerciasis	Unknown
Trichinella spiralis	Pigs, other mammals	Trichinosis	Ingestion of larva in pork or other meat
Dioctophyme renale	Cats, dogs	Dioctophymatosis	Ingestion of larva in freshwater fish
ACANTHOCEPHALA			
Macracanthorhynchus hirudinaceus	Pigs	Macracanthorhychiosis	Ingestion of cystacanth larva in beetle

Continued

TABLE 1-2 Some Parasites of Zoonotic Importance—Anthropotithasonotic Infections (i.e., Diseases of Humans from Domestic Animals)—cont'd

Parasite	Reservoir Host(s)	Human Disease	Mode of Infection
ARTHROPODA			
Linguatula serrata	Dogs, mammals	Nasopharyngeal pentastomiasis	Ingestion of larval pentastome in herbivore meat
Cheyletiella spp.	Cats, dogs, rabbits	Acariasis	Direct contact
Sarcoptes scabiei	Dogs, pigs, others	Acariasis	Direct contact
Notoedres cati	Cats	Acariasis	Direct contact
Mesostigmatid mites	Chickens	Acariasis	Direct contact with host or its environment/nest
Ctenocephalides felis	Cats, dogs	Flea bites	Direct contact
Oestrus ovis	Sheep	Myiasis	Larviposition by adult flies
Gasterophilus spp.	Horses	Myiasis	Oviposition by adult flies
Hypoderma spp.	Cattle	Myiasis	Oviposition by adult flies

*It is difficult to call some of these infections *zoonotic*. For example, *Taenia solium*, *T. saginata*, and *T. asiatica* could not exist without human beings. Similarly, human infection with intestinal sarcocystosis can occur only in humans, so, in a sense, humans are infecting the other hosts. Several of the agents on the list are the result of human beings' sharing parasites with other species (i.e., amphixenosis) because the parasite is not that finicky about its final host. *Schistosoma japonicum* is a fairly straightforward case of amphixenosis; the schistosome would be just as happy in a cow or in a cat; so is *Trypanosoma cruzi*. It will develop in just about any mammal that is inoculated with metacyclic trypanosomes. Actually, most of the trematode infections in people are cases of amphixenosis; the trematodes can develop in many different hosts that consume the metacercarial stage. This is also the case with *Diphyllobothrium latum* and *Diplogonoporus* species. If humans choose to display the gustatory habits of a bear or a cetacean, they can also support the maturation of the tapeworm species of these hosts.
†All parasite stages on this list, including eggs, cysts, oocysts, organisms in meat, etc., will die immediately if heated to 65°C.
‡As of this time, reported only from immunocompromised individuals in the United States.
§In the case of nematodes, the larval stage infective to the final host is typically the third stage; the exception to this rule is the Trichinelloidea with species in which the first-stage larva is infective to the final host.

CHAPTER 2

Arthropods

Arthropods are a group of organisms composed of the familiar insects, spiders, crustaceans (e.g., shrimp), and a few other types of organisms. The body of a typical arthropod is composed of a series of segments, some of which bear jointed legs. Not all arthropods display these characteristics. Body segmentation has all but disappeared with the evolution of mites and ticks, and many insect larvae have no legs. Adaptation to parasitism has led to extreme deviation in body form in certain cases. For example, mites of the genus *Demodex* have evolved into tiny cigar-shaped organisms that fit comfortably into the hair follicles and sebaceous glands of the skin. An even more extreme example is provided by *Sacculina*, a relative of barnacles that grows like a plant's root system in the body of its crab host. Most parasitic arthropods resemble their free-living relatives morphologically but differ from them in quite remarkable physiologic and behavioral adaptations to the parasitic mode of life. For example, the bloodsucking stable fly, horn fly, and tsetse strongly resemble their scavenging cousin, the common housefly, and there is no obvious morphologic difference between the many species of maggots that thrive in decaying plant and animal matter and the "screwworm" that completes its larval development in living flesh. The resemblance of certain parasites to their free-living relatives creates a diagnostic pitfall. Even their presence at the scene of the crime is not sufficient proof of guilt. Fly maggots and coprophilic beetles are frequently found in fecal specimens. In almost every such case, these insects have invaded the fecal mass after defecation and never were parasites at all.

Unfortunately, even when we restrict our consideration to unambiguously parasitic arthropods, we still have too big a chore on our hands. Medical entomology is a formidable subject, and the selection of appropriate information is not always an easy task because certain topics that at first appear to bear directly on current problems of veterinary practice actually lie within the responsibilities of very few veterinarians. For example, information on mosquitoes may occupy half of a textbook of medical entomology, and mosquitoes serve as vectors of such important diseases as equine encephalomyelitis and canine heartworm infection. However, few veterinarians invest the time and effort necessary to acquire a detailed knowledge of mosquitoes because control of these pests is usually the responsibility of the medical entomologist. Of more direct interest to veterinarians are the kinds of parasitic arthropods that live in more prolonged and intimate association with domestic animals. In this book, considerably more attention is therefore devoted to lice, fleas, ticks, and mites than to mosquitoes.

The arthropods of veterinary importance belong to the subphylum Crustacea and the Arthropod Classes Insecta, Arachnida, and Diplopoda. Insects and arachnids compose the bulk of this chapter. The Subphylum Crustacea contains many taxa that serve as intermediate hosts of helminth parasites (copepods, crabs, crayfish, and sow bugs), but only the copepods are discussed herein because they tend to be less familiar to the average person than are crabs, crayfish, and sow bugs. One group of crustaceans, the Pentastomida or tongue worms, are parasites in their own right of the respiratory system of terrestrial vertebrates, reptiles, birds, and mammals and are considered briefly in their own section. The class Diplopoda (millipedes), which contains at least one genus, *Narceus*, which serves as the intermediate host of *Macracanthorhynchus ingens*, a very large acanthocephalan parasite of the raccoon and domestic dog, is mentioned only in passing in this book.

This chapter begins with some of the more common forms—the flies that are well known to most people—and then progresses through the parasitic diptera of importance in veterinary medicine. After the section on flies is a discussion of the fleas. The fleas are then followed by the lice, bugs, and beetles. The next section is devoted to the ticks and mites. The final section of this chapter discusses a few of the crustaceans of veterinary importance.

CLASS INSECTA

STRUCTURE

The body of adult insects consists of the head, thorax, and abdomen. The head consists of a variable number of fused segments and bears two eyes, two antennae, and a complex set of mouthparts. The thorax consists of three segments—the prothorax, the mesothorax, and the metathorax—and bears six jointed legs and four, two, or no wings, depending on the zoologic order to which the insect in question belongs. Thus roaches (Blattodea), caddisflies (Trichoptera), beetles (Coleoptera), and certain bugs (Hemiptera) have four wings, most flies (Diptera) have two, and lice (Mallophaga and Anoplura) and fleas (Siphonaptera) are wingless. When four wings are present, one pair arises from the mesothorax and the second pair from the metathorax. The functional wings of Diptera arise from the mesothorax. The abdomen consists of 11 or fewer segments, of which the terminal ones are modified for copulation or egg laying. As typical arthropods, insects have a chitinous cuticle secreted by the hypodermis, a single layer of columnar epithelial

cells of ectodermal origin that is cast off or molted at intervals to permit growth and metamorphosis. The chitinous cuticle serves as an exoskeleton, thus as both a body covering and a place for attachment of muscles. Heavily chitinized areas or plates of cuticle are connected by thinner, lightly chitinized areas, thus permitting movement and some degree of expansion, as, for example, when the abdomen of a feeding female mosquito fills with blood. Insect muscles are striated and often are capable of extraordinarily rapid contraction. The cuticle is overlain by a thin lipoidal surface layer, the epicuticle, which is impermeable to water but is freely permeable to lipids and lipid-soluble substances.

When a developing insect has grown too large for its cuticle, the hypodermis lays down a new, thin, elastic cuticle under the old one. The old cuticle then splits, and the insect emerges from it. This process is termed **molting;** the shedding of the old external cuticle after the molting process is complete is termed **ecdysis.** Molting with ecdysis divides the life of the individual insect into a series of **stages,** or **instars.** All instars of cockroaches, bugs, and lice resemble their parents except that they are smaller, whereas a newly hatched fly, beetle, or flea looks more like a worm than an insect. The former situation is called **simple metamorphosis (hemimetabolous metamorphosis)** and the series of juvenile instars are called **nymphs,** whereas the latter situation is called **complex metamorphosis (holometabolous metamorphosis),** and the juvenile wormlike stages are called **larvae.** In complex metamorphosis, the complete restructuring necessary for the transformation of the wormlike larva into the adult insect takes place during the **pupal** stage, and all related events are referred to as **pupation.** The exiting of an adult insect from its pupal case is termed **eclosion,** for the purpose of distinguishing between adult emergence from the pupal case and the hatching of a larva from an egg.

ORDER TRICHOPTERA, CADDISFLIES

Trichoptera is a very large group of flies (some 7000+ species) that are better known to fly fishermen than to medical entomologists. These flies have four wings and short mouthparts that are used for consuming water and nectar (Figure 2-1). In species that occur in temperate climates, the adult population is often limited to one generation per year, and they may occur in large blooms. The larvae are aquatic in fresh water and feed on microorganisms or as predators on other insects. The larvae will often construct a portable case in which they live, with only their legs and head protruding. Ultimately, the larva will form a cocoon from which the adult emerges. Males swarm over bodies of water, and females fly into the swarms to be fertilized. The females lay their eggs near water, so the larvae that hatch can make their way into this environment. A good guide to the species of caddisflies has been produced for the fly fishing enthusiast (Pobst and Richards, 1999).

Caddisflies became important in veterinary medicine through work by Madigan and others at the University of California–Davis showing that they serve as vectors of Potomac horse fever's causative agent, *Neorickettsia risticii*. It seems that the caddisflies are intermediate hosts of the metacercarial stage of a trematode parasite of bats: *Acanthatrium oregonense* (family Lecithodendriidae) (Gibson et al, 2005). Unfortunately, these trematodes are often, as in the case of the rickettsial disease of salmon poisoning in dogs, infected with a rickettsia, *N. risticii*. Horses fed mature caddisflies *(Dicosmoecus gilvipes)* developed the clinical and hematologic disease of Potomac horse fever (Madigan et al, 2000). Thus when the horse digests the caddisfly containing the trematode metacercaria, this action releases the *N. risticii* that causes the disease in the horse. The finding is important because it was shown that ticks were not the vector of this rickettsial agent, and because control can be as simple as providing horses with waterers that are covered in some fashion to prevent the bodies of these flies from contaminating the horse's drinking water.

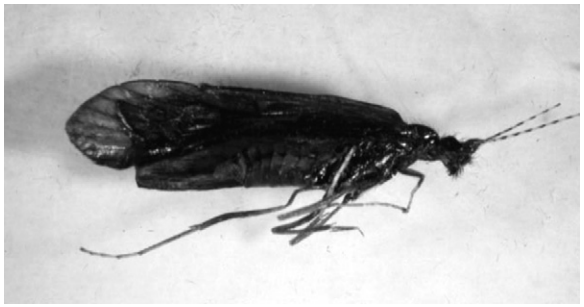

FIGURE 2-1. Caddisfly adult. The larvae of these flies become infected with the metacercariae of trematodes harboring the causative agent of Potomac horse fever. (Courtesy Dr. John E. Madigan, School of Veterinary Medicine, University of California, Davis, California.)

ORDER DIPTERA, FLIES

Adult dipteran flies, except for certain specialized groups such as the parasites of the family Hippoboscidae, have one pair of functional mesothoracic wings. The metathoracic pair are represented by club-shaped balancing organs called **halteres** (Figure 2-2), which are present even in the wingless hippoboscids. Metamorphosis is complex. Although most flies produce eggs or are **oviparous,** a few deposit larvae that have already hatched, and females producing larvae in this manner are said to be **ovoviviparous.** Hippoboscids and tsetses retain their larvae within their abdomens through the third larval instar, and these larvae pupate almost immediately on being born.

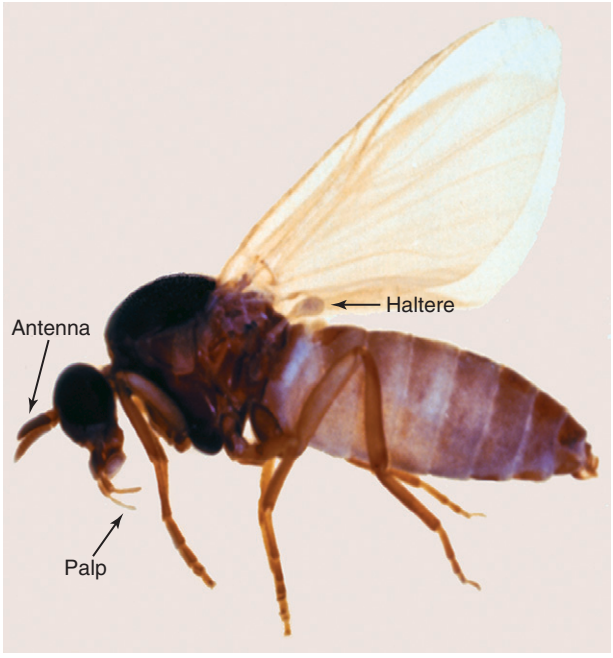

FIGURE 2-2. *Simulium* (Nematocera: Simuliidae), a blackfly. The halteres (*singular*, haltere) are balancing organs that have evolved in Diptera in place of the metathoracic wings. The maxillary palpi are sensory structures associated with the mouthparts. The antennae of blackflies consist of 11 similar segments.

There are three main groups of flies: the gnats and mosquitoes of the Nematocera, the horseflies and deerflies of the Brachycera, and the houseflies, flesh flies and blowflies, botflies, tsetse flies, and keds of the Cyclorrhapha (Box 2-1). All three major groups contain bloodsucking species, many of which serve as disease vectors. In the Nematocera and Brachycera, only the females take blood meals, and, usually, larval development occurs in aquatic environments. Larvae of muscid, sarcophagid, calliphorid, and oestrid cyclorrhaphans can invade living tissues to produce a pathologic condition called **myiasis.** The developmental times of various flies, along with those of some fleas and lice, are presented in Table 2-1.

BOX 2-1 Classification of the Diptera

NEMATOCERA	BRACHYCERA	CYCLORRHAPHA
Culicidae, mosquitoes	Horseflies and deerflies	Muscidae, houseflies
Simuliidae, blackflies		Hippoboscidae, keds
Ceratopogonidae, midges		Sarcophagidae, flesh flies
Psychodidae, sandflies		Calliphoridae, blowflies
		Oestridae and other botflies

Nematocera

Nematocerans are typically small and relatively delicate. The antennae are long and many-segmented, and the individual segments

TABLE 2-1 Some Details on the Times Required for the Life Cycle Stages of Various Diptera, Fleas, and Lice

Group	Egg (Persistence and Time to Hatching)	Larva	Pupa	Male	Female
FLIES: DIPTERA					
Nematocera					
Mosquito	Days to years	7 days	2-3 days	1 wk	4-5 mo; can hibernate
Blackfly	3-7 days diapause	7-12 days	2-6 days	2-10 wk	Weeks to months
Brachycera					
Tabanid	5-7 days 1 generation/yr in temperate climates	1 yr 6 mo-3 yr 1 generation/yr in temperate climates	1-3 wk 1 generation/yr in temperate climates	Few days 1 generation/yr in temperate climates	Months 1 generation/yr in temperate climates
Cyclorrhapha					
Musca	8-12 hr 10-12 generations/ summer	5 days 10-12 generations/ summer	4-5 days 10-12 generations/ summer	<Females 10-12 generations/ summer	2-10 wk; can hibernate 10-12 generations/ summer
Stomoxys	1-3 days	9-60 days	4-9 days	Weeks	Weeks
Haematobia	1 day	4-8 days	6-8 days (overwintering stage)	Weeks	Weeks
Calliphorid	6-48 hr	3-9 days	5-10 days	35 days	35 days
Cochliomyia	11-21 hr	3.5-4.5 days	7 days	Weeks	Weeks
Sarcophagid	Often skipped	14 days		Weeks	Weeks
Melophagus	Skipped	Hours (10-12/female)	3 wk	4 mo (1 mo to mature)	4 mo (1 mo to mature)
Gasterophilus	5 days	9-11 mo	3-5 wk	Weeks (early spring)	Weeks (early spring)
Hypoderma	5-7 days	8-11 mo	4-5 wk	Weeks	Weeks
Oestrus	Skipped	25-35 days or 8-10 mo	Hibernation or 3-6 wk	4 wk	4 wk
FLEAS: SIPHONAPTERA					
Ctenocephalides	2-21 days	9-15 days	7 days-1 yr	Weeks (can be kept alive a long time in laboratory)	
LICE: PHTHIRAPTERA (SIMPLE METAMORPHOSIS)					
Group	**Egg**	**Nymph (No Larvae or Pupae)**		**Adult**	
Pediculus	7-9 days	9-11 days		30 days	
Haematopinus	11 days	11-22 days		14 days	
Felicola	10-20 days	14-21 days		14-21 days	
Trichodectes	7-14 days	14 days		20 days	

*These represent generalities.

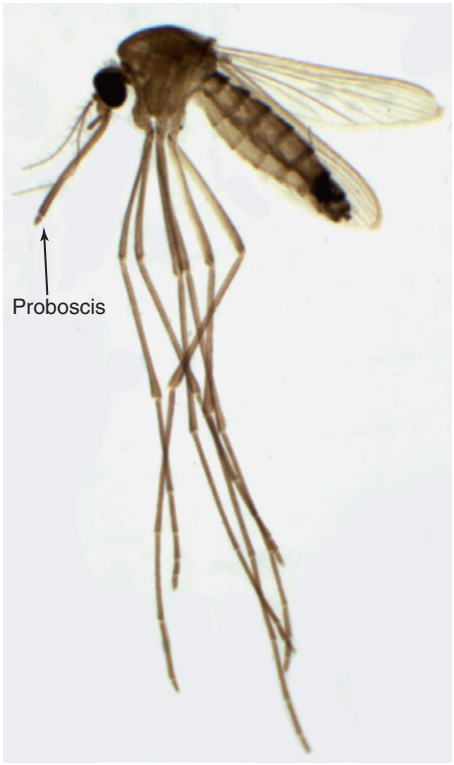

FIGURE 2-3. A mosquito (Nematocera: Culicidae). Note the long antennae and the long mouthparts (proboscis).

resemble one another like beads on a string. Nematocerans generally breed in aquatic or semiaquatic habitats, and their larvae are suitably endowed with appendages for swimming, breathing, and gathering food in water. Only female nematocerans suck blood; the males never do and subsist instead on nectar.

Family Culicidae, Mosquitoes

IDENTIFICATION. Mosquitoes have long, 14- or 15-segmented antennae, an elongated proboscis consisting of a bundle of stylets loosely encased in a sheath formed by the labium, and fringes of scales on the wings (Figure 2-3). These anatomic details are sufficient taxonomic characteristics to reliably distinguish the taxon that we recognize as mosquitoes from other insects with which they might be confused.

LIFE HISTORY. Mosquitoes lay their eggs on water or in dry places that tend to flood seasonally. Eggs laid on water hatch in less than a week. Larvae (Figure 2-4) are air breathers and die within hours if their air supply is shut off by an oil film on the water's surface. The larvae molt four times, usually within the space of 2 weeks, and then pupate. As is characteristic of all nematocerans and brachycerans, the adult emerges through a T-shaped hole in the back of the last larval skin. Culicid pupae are elaborate, free-swimming organisms with a large cephalothorax. As development proceeds, the structures of the adult mosquito become apparent (Figure 2-5). The pupal stage ordinarily lasts from 2 days to a week, but a few hours suffice for certain dry climate species. The adult mosquito emerges through a T-shaped hole in the back of the pupal case as it floats at the water's surface. After about 24 hours, the wings have expanded and hardened, and the mosquito is able to fly. Only female mosquitoes suck blood, the protein of which is necessary for maturation of the ovaries. The female mosquito will very typically feed every few days, with each blood meal being used to nourish the next batch

FIGURE 2-4. Mosquito larva.

FIGURE 2-5. Mosquito pupa. The "trumpets" on the cephalothorax are pupal respiratory structures. The eyes, legs, thorax, and abdomen of the developing adult mosquito can be seen through the pupal cuticle.

of eggs to be produced and laid; after the eggs are laid, the female will then seek out another host. It is the repeated feeding of female mosquitoes on different hosts that makes them such efficient vectors of disease. Males and nonreproductive females get by on nectar and plant juices. The females of some species that

normally feed on blood are sometimes capable of ovarian maturation without a blood meal (i.e., the females are **autogenous**). Other species of mosquitoes feed only on plants, and therefore these species are of little interest as pests or disease vectors. Mammals and birds are preferred hosts (or victims), both of blood-feeding mosquitoes and of the various disease organisms that they transmit.

INJURY. Under ordinary circumstances, the amount of blood lost to a mosquito attack is entirely trivial. Sometimes, however, circumstances favor the simultaneous emergence of enormous swarms of mosquitoes that by their concerted attacks can actually bleed cattle to death. For example, 7 days after Hurricane Allen (August 10, 1980) brought a prolonged drought to an abrupt end and flooded 5000 acres of a Texas ranch, cattle were observed to be visibly distressed by swarms of *Aedes sollicitans* mosquitoes. The next morning, 15 cattle were found dead of exsanguination manifested by extreme pallor of the mucous membranes and postmortal evidence of severe anemia. The interval of 7 days between flooding of the pastures and the sudden death of the cattle corresponded exactly to the time required for *A. sollicitans* to develop from egg to biting adult once its dormancy had been ended by high water. The flood led to the synchronous development of vast numbers of eggs that had accumulated during the prolonged drought, thus producing the enormous swarms of mosquitoes capable of exsanguinating mature cattle overnight. Abbitt and Abbitt, who obtained and thoughtfully analyzed the evidence in this outbreak, estimated that 3.8 million mosquito bites (5300 bites per minute for 12 hours) would be required to remove half of the total blood volume from a 366-kg cow, assuming that a mosquito removes 0.0039 mL per blood meal (Abbitt and Abbitt, 1981). Cats will sometimes develop allergies to mosquito bites that will manifest as large pruritic and erythemic lesions on the nose or other parts of the face (Clare and Medleau, 1997).

DISEASE TRANSMISSION. A **vector** is an animal—often an arthropod—that transmits an infective organism from one host to another. (An inanimate object that serves to transmit infection, such as a doorknob or a dirty tissue, is called a **fomite.**) A vector that transmits infective organisms directly (and, necessarily, promptly) to a recipient host without development or multiplication of the organisms having occurred is called a **mechanical vector.** A **biologic vector,** by contrast, is one in which the infective organisms undergo development, or multiply, or do both, before they are transmitted to the recipient host. Thus a biologic vector is a true host of the disease organism. In the case of sexually reproducing disease organisms such as protozoans and helminths, vectors that host developing or asexually reproducing stages of the organism are termed **intermediate** hosts, whereas vectors that host sexually mature stages are termed **definitive** hosts. Mosquitoes are vectors of many pathogens (Table 2-2). *Culex*, *Aedes*, *Anopheles*, and other genera of mosquitos serve as biologic vectors (intermediate hosts) of filariid worms such as *Dirofilaria immitis*, the canine heartworm, and *Wuchereria bancrofti*, the cause of human lymphatic filariasis. Mosquitoes of the genus *Anopheles* serve as biologic vectors (definitive hosts) of the blood-inhabiting protozoon genus *Plasmodium*, which causes malaria in birds, rodents, and primates. Mosquitoes also serve as biologic vectors of viral encephalitides (e.g., equine encephalomyelitis), West Nile virus, and the viruses of rabbit myxomatosis, fowl pox, and yellow, dengue, and Rift Valley fevers. In the case of viruses, bacteria, and the like, the terms intermediate and definitive are redundant inasmuch as sexual reproduction does not occur in these groups.

TABLE 2-2 Some Pathogens Vectored by Nematoceran Flies

Vector	Some Transmitted Pathogens
Culicidae (mosquitos)	Filariids *Setaria*: horses, cattle, deer Heartworm: dogs and cats *Wuchereria* and *Brugia*: humans and cats Protozoa Malaria *(Plasmodium)*: birds and primates Viruses Equine encephalitis West Nile virus Rift Valley fever
Simuliidae (blackflies)	Filariids *Onchocerca*: horses, cattle, sheep, humans Protozoa Malaria *(Leucocytozoon)*: birds
Ceratopogonidae (biting midges)	Filariids *Onchocerca*: horses *Dipetalonema*: primates Protozoa Malaria *(Leucocytozoon)*: birds Viruses Blue tongue African horse sickness
Psychodidae (sandflies)	Protozoa *Leishmania* species Rickettsia *Bartonella* Viruses 3-Day fever virus

Family Simuliidae, Blackflies

IDENTIFICATION. Blackflies (see Figure 2-2) are small, stout-bodied, black, gray, or yellowish-brown flies with relatively short antennae consisting of nine to 12 (usually 11) similar segments, and short mouthparts with prominent maxillary palps (Figure 2-6).

LIFE HISTORY. Blackflies breed only in running water. Although mountain torrents and temporary upland streams are favored breeding sites of many species, some particularly important species breed in large rivers. Eggs are deposited on the water's surface or on partly submerged stones, twigs, or vegetation. In species that produce several broods per year (**multivoltine** species), larvae hatch from these eggs a few days later, but in species that produce only one brood per year (**univoltine** species), the eggs remain in a protracted state of metabolic quiescence, or **diapause,** and do not hatch until the following year. Blackfly larvae manage to cling to the surfaces of stones in rapidly moving, turbulent streams, in part by means of little hooks on their posteriors and on a short **proleg** near the anterior end of their bodies (Figure 2-7). By flexing their bodies, the larvae are able to move from place to place like inchworms. Blackfly larvae also spin silken strands to help anchor themselves and later to form cocoons, by means of which the pupae continue to cling to the

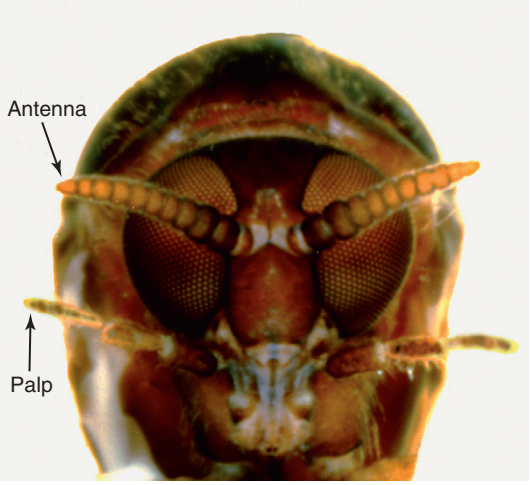

FIGURE 2-6. Head of a blackfly (Nematocera: Simuliidae).

rocks. Adults emerge from these pupae and are carried to the surface in a bubble of air.

INJURY. The female blackfly is a vicious biter. Her mouthparts consist of a bundle of flattened, serrated, bladelike stylets loosely ensheathed by the labium, which itself terminates in a pair of labella. Instead of piercing a blood vessel and feeding from the lumen as a mosquito, bedbug, or sucking louse does, the female blackfly lacerates tissues until a pool of blood forms, and then she imbibes the blood from the pool.

Susceptibility to and severity of host reaction to the bites of many arthropods vary remarkably among individuals. With continued exposure to bites, initially susceptible individuals may become relatively immune, so that they are less frequently bitten or suffer less reaction to the bites. Or, less fortunately, they may become hypersensitive, so that continued attack excites a more severe and sometimes even fatal reaction. Sensitivity to the bites of blackflies is a common phenomenon, and the reactive wheal may continue to itch for many days and tends to be aggravated by scratching. In a hypersensitive person, a single bite may evoke sufficient edematous reaction to force the eyelids shut. Burghardt, Whitlock, and McEnerney (1951) described dermatitis in cattle due to *Simulium*. The lesions consisted of blisters, welts, and scabs affecting the head, thorax, and ears, as well as acute exudative lesions along the midabdominal line. Heavy swarms of blackflies have been known to kill grazing livestock by the thousands. However, the exact cause of death, whether it be anemia, hypersensitivity reactions, or toxin absorbed from fly saliva injected into the bite, remains problematic. During blackfly season, dogs and cats can manifest small pruritic bloody spots on the ears, face, or body. Prevention of such bites is best accomplished using repellents.

DISEASE TRANSMISSION. Blackflies transmit a number of pathogens (see Table 2-2). Blackflies (e.g., *Simulium aureum*, *Simulium jenningsi*, *Simulium vittatum*, *Simulium pictipes*) transmit leukocytozoonosis, a disease of poultry and wild birds caused by several species of the haemosporidian protozoan genus *Leucocytozoon*. Blackflies also serve as obligate intermediate hosts of the filariid nematode *Onchocerca gutturosa*, an apparently innocuous parasite of cattle. In the blackfly, the worm develops from the skin-dwelling microfilarial stage ingested by the fly to the third-stage larval nematode that is infective to the next host. Blackflies (e.g., *Simulium damnosum*, *Simulium ochraceum*) also serve as vectors of the related nematode parasite *Onchocerca volvulus*, which causes human onchocerciasis, manifested by the formation of dermal nodules, and which leads, predominantly in the African form of the disease, to blindness. Because some of these vectors are riverine breeders, the disease tends to be concentrated along river valleys; the ensuing blindness is therefore called "river blindness."

CONTROL. Blackflies attack in swarms during daylight hours and when the air is relatively still. Smoke repels them, and, although chemical repellents afford a degree of protection, campers, gardeners, and livestock usually find their surest relief in the lee of a smudge pot. Livestock should be stabled until sundown during seasons of particularly heavy blackfly attack. Blackflies can be discouraged from attacking horses by the application of several different pyrethroid-containing insecticides/repellents and, to prevent attack of the ears, by the application of petroleum jelly to the inner surface of the pinna; alternatively, fly masks with built-in ear guards may be used.

Family Ceratopogonidae, Biting Midges, "No-See-Ums"

IDENTIFICATION. Ceratopogonids are tiny (measuring less than 2 mm), relatively glabrous flies. Their antennae are long and slender, and their mouthparts are relatively short (Figure 2-8).

LIFE HISTORY. Life histories of various species differ in detail; some species require freshwater and others saltwater habitats. Some breed in water-filled holes in trees, and others in decaying vegetation, sandy and silt soils, and the like. Adults are crepuscular and nocturnal. Only females suck blood, and, although they are fairly strong flyers, they tend to remain close to their breeding grounds. A few, however, may venture forth as far as a half mile when the air is still. Most important species belong to the genera *Culicoides* and *Leptoconops*.

INJURY. The bites of *Culicoides* inflict pain far out of proportion to the size of the fly. In fact, people victimized by these tiny terrors frequently do not realize that they are being tormented by insects, sometimes mistaking them for a bit of cigarette ash because of their small size. *Culicoides* easily pass through standard window screening and make themselves obnoxious to sleepers. In sensitized individuals, the reaction to the bites lasts longer and is more painful than that to mosquito bites.

"Queensland itch," demonstrated by Riek (1953a) as representing allergic dermatitis caused by the development of hypersensitivity to the bites of *Culicoides robertsi*, affects only certain horses. Other horses pastured with the affected ones never show any signs of disease. Initial lesions are discrete papules confined to the dorsal surfaces. Later, hair mats and crusts form and eventually fall off, leaving hairless areas that in severe cases become confluent. Pruritus is intense, and horses may injure themselves by scratching and rolling to relieve the itching. Antihistamine therapy accelerates regression of the lesions (Riek, 1953b).

DISEASE TRANSMISSION. *Culicoides* species transmit the viruses of bluetongue and African horse sickness (see Table 2-2); apparent spread of bluetongue across Europe is a result of the recent spread of its usual vector *Culicoides imicola* and now also other *Culicoides* species that are found in cooler, more northern areas (Sperlova and Zendulkova, 2011). *Onchocerca cervicalis* of horses, *Onchocerca gibsoni* of cattle, and three relatively innocuous filariid parasites of humans *(Dipetalonema perstans*, *Dipetalonema streptocerca*, and *Mansonella ozzardi)* all develop from microfilariae to infective third-stage larvae in the bodies of *Culicoides* organisms. Protozoans transmitted by *Culicoides* include *Hepatocystis* of Old World monkeys and *Haemoproteus* and *Leucocytozoon* of wild and domestic birds.

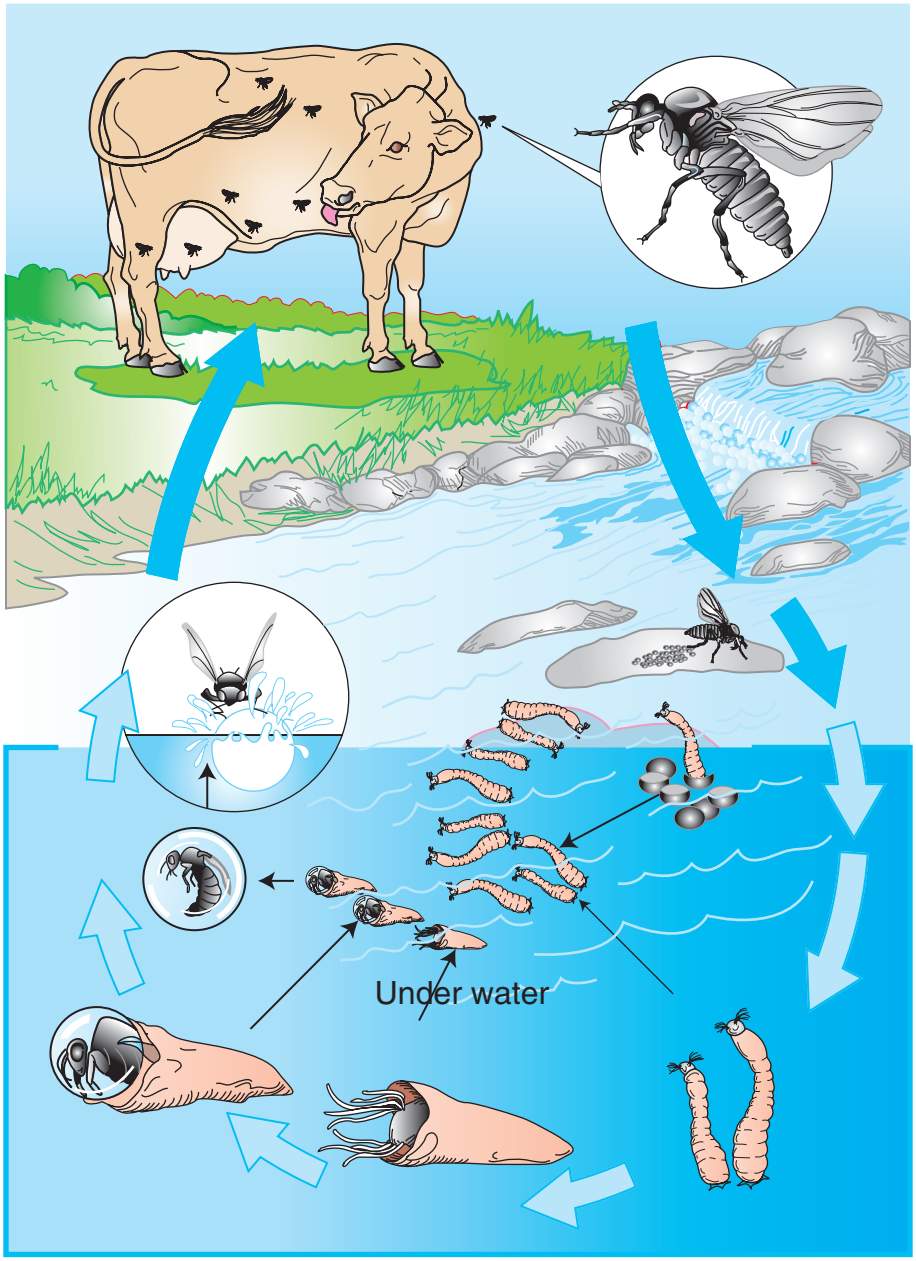

FIGURE 2-7. Life history of a blackfly (Family Simuliidae). The female blackfly deposits her eggs on partly submerged objects in rapidly flowing streams. The larvae that hatch from these eggs cling to the stones and feed on organic matter carried by the current. When ready to pupate, the larvae spin silken cocoons that secure them to the substrate. Adults that emerge from these pupal cases are carried to the surface in a bubble of air and fly off in search of a blood meal.

Family Psychodidae, Subfamily Phlebotomine, Sandflies

IDENTIFICATION. Psychodids are small, dull-colored, slender flies with long antennae; most blood-feeding members are found within the subfamily Phlebotominae. Within the psychodids, the wing veins radiate in nearly straight lines from the base to the tip of the wing (Figure 2-9).

LIFE HISTORY. Psychodids lay their eggs in cracks, crevices, or burrows in which moderate temperatures, darkness, and nearly 100% humidity prevail. They spend at least 2 months as egg, larvae, and pupae but are short-lived as adults. Adult phlebotomine flies are weak flyers and nocturnal in habit. Important species belong to the genera *Phlebotomus* and *Lutzomyia*. *Phlebotomus* occurs in the Old World and *Lutzomyia* in the New World; all species are tropical or relatively subtropical in distribution. Species of *Lutzomyia* are found in the United States, including *Lutzomyia vexator*, *Lutzomyia apache*, *Lutzomyia shannoni*, and others, but it is not clear how many of these species serve as successful vectors of *Leishmania* species in the wild.

DISEASE TRANSMISSION. Phlebotomines transmit *Leishmania* species, which are hemoflagellate parasites of dogs, rodents, and primates, including humans (see Table 2-2). It appears that molecules in the salivary gland secretions of the phlebotomine vector modulate to some extent the course of *Leishmania* development in the host that is bitten (Warburg et al, 1994). Also transmitted by phlebotomines are the Pappataci fever virus (Phlebovirus) and *Bartonella bacilliformis* infection of humans.

CONTROL. Sandflies can be prevented from biting dogs by the use of deltamethrin-impregnated collars and a combination of permethrin and imidacloprid in a spot-on formulation. The collar can provide up to 6 months of protection. Deltamethrin-impregnated collars for the control of canine leishmaniasis (Foglia Manzillo et al, 2006) and the spot-on formulation provide excellent protection between monthly applications (Mencke et al, 2003).

Brachycera

Family Tabanidae, Horseflies and Deerflies

IDENTIFICATION. Tabanids are stout-bodied flies varying from about the size of a housefly to as large as a hummingbird. The short, stout, anteriorly projecting antennae consist of three markedly different segments (Figures 2-10 and 2-11). The first segment is small, the second may be expanded, and the third is marked by annulations that make tabanid antennae appear to have many more than three units. Important genera in the United State are the deerflies *Chrysops* with also *Silvius* in the western states, and the horsefly genera *Tabanus* and *Hybomitra*.

LIFE HISTORY. Female tabanids require a blood meal for maturation of their eggs and obtain it from mammals, reptiles, and,

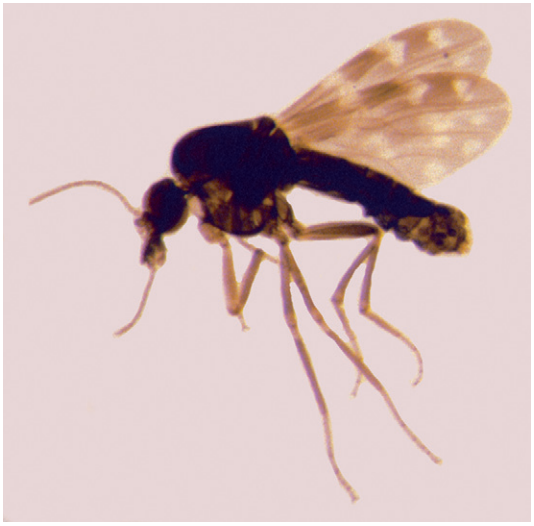

FIGURE 2-8. *Culicoides* (Nematocera: Heleidae), a "no-see-um." *Culicoides* differ from Culicidae mosquitoes in that they are smaller and have a shorter proboscis.

FIGURE 2-9. *Phlebotomus* (Nematocera: Psychodidae). The wing veins radiate in nearly straight lines from the base to the tip of the wing.

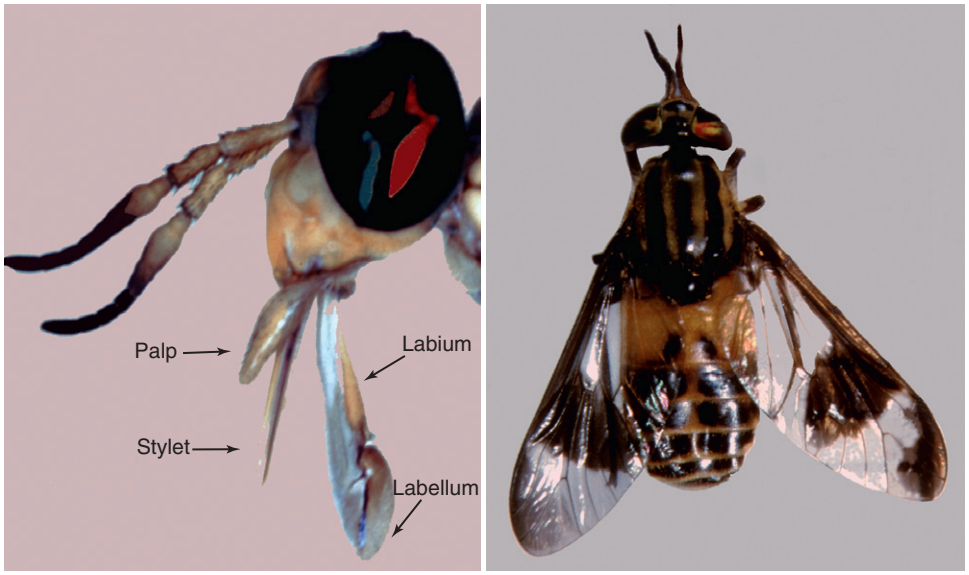

FIGURE 2-10. *Chrysops* (Brachycera: Tabanidae), a deerfly. Because the distal segment of the tabanid antenna is annulated, it gives the impression that the antenna consists of many segments; however, there are only three. The wings often have dark markings.

FIGURE 2-11. *Tabanus* (Brachycera: Tabanidae), a horsefly. The wings are typically without markings.

occasionally, birds. Male tabanids are not blood feeders but subsist on nectar, sap, and aphid feces; the females require these sources of carbohydrate, in addition to blood (Mally and Kutzer, 1984). Except for a few xerophilic species, tabanids tend to be concentrated along watercourses. Eggs are neatly glued in masses of 400 to 1000 to foliage overhanging water. Larvae hatch in a week or so, depending on temperature and relative humidity, and drop into the water. First- and second-stage larvae do not feed, but the third and later stages are aggressively carnivorous or saprophagic and feed on insect larvae, crustaceans, snails, earthworms, young frogs, plant tissues, and dead organic matter, depending on the species of tabanid and the availability of food (Mally and Kutzer, 1984). In temperate regions, larvae overwinter by burying themselves in soil or dead vegetation and pupate the following spring. Thus usually only one generation is produced each year. Adult tabanids are strong flyers and are very difficult to discourage. In Michigan, *Hybomitra* species were found to reach maximum abundance in early summer (May to June), whereas *Chrysops* and *Tabanus* species were most abundant in late summer (early to late July; Strickler and Walker, 1993). In the salt marshes of Cape Cod, Massachusetts, the greenhead flies *Tabanus nigrovittatus* and *Tabanus conterminus* were found to be most active in the afternoon (Hayes et al, 1993). In Florida, peak *Chrysops* activity occurred morning and evening, with a correlation to relative humidity rather than to temperature and light intensity (Cilek and Schreiber, 1996). Konstantinov (1993) showed that masking the visibility of a cow by placing it in a wooded area did not reduce the number of *Hybomitra* flies that found the cow when they were released 150 meters from the host animal.

INJURY. All arthropod attacks cause some annoyance to the host and exact some expenditure of energy in efforts to avoid or relieve their effects. When flies are particularly numerous, pastured livestock may be driven frantic by incessant attacks and can spend so much time and energy combating the onslaught that they cannot rest or graze adequately. The resultant exhaustion always interferes with production and sometimes proves fatal. Certain insects are particularly feared by livestock. Some horseflies are as large as hummingbirds and inflict an excruciating bite. When one of these monsters attacks, horses are likely to bolt, and it behooves the rider or driver to come promptly to their aid. In biting, the mandibles and maxillae of tabanids lacerate the blood vessels and the labella lap up the blood that flows freely from the wound. Repeated attacks in the skinfolds about a cow's udder and in the groove between the udder halves lead to extensive weeping eczematous lesions that may become secondarily infected with bacteria. After a tabanid has finished feeding, the bite wound tends to bleed for many minutes, thus attracting opportunists such as *Musca*. In fact, *Musca* and other flies can often be seen clustered about a feeding tabanid, exploiting the bounty afforded by its sloppy manner of taking a meal. Vicious daylight bloodsuckers, tabanids do not usually attack indoors, but if already feeding when the host enters a building, they will continue to feed until replete. The most efficient solution to tabanid attack is to stable the animals during the hours of peak fly activity.

DISEASE TRANSMISSION. The pain that a tabanid inflicts when it bites tends to increase its efficiency as a mechanical vector of disease organisms. The fly, driven away by its victim's defenses before it has had time to feed to repletion, soon alights on a second host to finish its meal and perhaps to contaminate the wound with fresh, mechanically borne bacteria (e.g., anthrax), viruses (e.g., equine infectious anemia), and the like. The large volume of blood imbibed by each tabanid (up to four times the weight of the fly; Krinsky, 1976) also contributes to its efficiency as a mechanical vector by helping to compensate for the low concentration of microorganisms usually found in blood, and for their failure to multiply in the body of the intermediate host.

Tabanids have been incriminated in the mechanical transmission of anaplasmosis (*Anaplasma* species), anthrax *(Bacillus anthracis)*, tularemia ("deerfly fever," *Francisella tularensis*), and equine infectious anemia virus. Mechanical transmission of equine infectious anemia virus from acutely infected ponies to susceptible ponies occurred after as few as 10 bites by contaminated *Tabanus fuscicostatus*, but all attempts at transmission from a chronically infected pony failed (Hawkins et al, 1973). Mammalian trypanosomes (hemoflagellate protozoans) may be transmitted mechanically or biologically by tabanids, depending on the species involved. Surra *(Trypanosoma evansi)*, a fatal disease of horses, camels, elephants, and dogs in Asia, is transmitted mechanically, and the flies

TABLE 2-3 Some Pathogens Vectored by Brachyceran Flies

Mechanically Vectored	Biologically Vectored
Anthrax Tularemia	Filariids: *Elaeophora*—elk, sheep
Protozoa: *Trypanosoma evansi*—horses, camels, elephants, dogs	Protozoa: *Trypanosoma theileri*—cattle

lose their ability to transmit the infection a few hours after feeding on an animal infected with surra (Table 2-3). *Trypanosoma theileri*, on the other hand, must multiply in the body of the tabanid because it is so scarce in the blood of cattle that one must usually resort to culture techniques to demonstrate its presence there. Otherwise, *T. theileri* would not be distributed throughout the world as a parasite of cattle and their near relatives. A vector in which such parasitic organisms multiply is sometimes referred to as a **cyclopropagative host** to distinguish it from a **cyclodevelopmental host,** in which the parasite actually undergoes ontogenetic development. An example of the latter is *Elaeophora schneideri*, the arterial worm of deer, elk, and domestic sheep in the southwestern United States, which develops from the microfilarial stage in the blood to the infective third stage within the body of the tabanid (Hibler and Adcock, 1971). Additional details concerning disease transmission by tabanids are to be found in the review by Krinsky (1976).

CONTROL. Horseflies and deerflies are very difficult to kill or repel; often the best solution is to stable livestock during the hours of peak fly activity. These flies can use blood from wild animals as food and have larval habitats independent of domestic livestock. Thus unlike flies more directly dependent on their hosts, such as *Stomoxys* and *Haematobia*, these flies can be controlled chemically by repellents alone (Foil and Hogsette, 1994). Konstantinov (1992) showed that only 3% of flies attacking a cow are killed by the cows during their attacks. McMahon and Gaugler (1993) suggested that draining of salt marsh areas to decrease mosquito populations may inadvertently have actually increased habitats preferred by larval tabanids and hence increased the numbers of these biting flies.

Recent work has suggested that zebras might bear black and white stripes for the purpose of preventing tabanid attacks (Egri et al, 2012). This seems to result from their use of polarized light for visual signals. Thus it is suggested that zebras have evolved with just the right quantity and thickness of stripes that when compared with black, brown, or white horses, they have significantly fewer fly attacks.

Cyclorrhapha

The Cyclorrhapha group represents the apex of dipteran evolution, and the common housefly, *Musca domestica*, is a typical example. Instead of the aquatic habitats favored by nematocerans and brachycerans, cyclorrhaphans tend to breed in decaying plant and animal tissues, manure, carrion, and the like. The three larval instars are more or less conical animals with a mouth, usually armed with hooks, at the apex and a pair of prominent respiratory openings called **spiracles** or **stigmata** at the base. Slender larvae of the families Muscidae, Sarcophagidae, and Calliphoridae are usually referred to as **maggots** (Figure 2-12), whereas the rather stout larvae of the family Oestridae and its relatives are called **bots** or **grubs** (compare with Figure 2-25). When the third instar larva enters the pupal stage, its integument hardens to form a puparium, or pupal case. Pupae of most cyclorrhaphan flies are found in decaying organic material or soil. A few species have specialized pupation sites. For example, pupae of the sheep ked *Melophagus ovinus* are found attached to the wool of their host. The adult fly emerges (ecloses) through a circular hole in the anterior end of the puparium. Cyclorrhaphan antennae consist of three dissimilar segments, the third and largest of which bears a frondlike structure called an arista near its proximal end. The antennae are directed ventrally, but the aristae project anteriorly (Figure 2-13). Parasitic specialization has proceeded in two directions in the Cyclorrhapha. In the families Muscidae and Hippoboscidae, specialization has changed from a type adapted to lapping up liquids (e.g., *Musca*) (see Figure 2-13) toward a bayonet-like proboscis for piercing the skin and sucking blood (e.g., *Stomoxys*), and thus toward parasitism in the adult stage. In the families Calliphoridae and Sarcophagidae and in certain members of the family Muscidae, the adult flies have retained their lapping mouthparts and remained scavengers; instead, it is in the larval stages that parasitism has evolved. The botflies (e.g., *Hypoderma*, *Gasterophilus*) have proceeded even further in this direction. Their larvae have become highly specialized host- and site-specific parasites, whereas the mouthparts of the adult flies have become vestigial and nonfunctional. Parasitism by fly larvae is termed myiasis and is of worldwide economic importance.

Family Muscidae

MUSCA

IDENTIFICATION. The genus *Musca* contains 26 species, of which three—*Musca domestica*, the common housefly, *Musca autumnalis*, the face fly, and *Musca vetustissima*, the Australian bush fly—may serve as examples. These three species resemble one another closely enough to require an expert to distinguish specimens on morphologic grounds but differ sufficiently in behavior to make their identities obvious to anyone familiar with their habits. The mouthparts of these all too familiar flies consist of a fleshy, retractable proboscis terminating in a pair of corrugated spongy organs—the labella (see Figure 2-13).

LIFE HISTORY AND DISEASE TRANSMISSION. *M. domestica* lays its eggs on animal manure or on almost any kind of decaying organic material. A female *M. domestica* may deposit 2000 eggs in an average lifetime of 6 to 8 weeks. A tiny, white, first-stage larva (maggot) hatches from the egg in a day or less at summer temperatures. This larva grows, molts twice, and in a few days becomes a fully developed third-stage larva. When ready to pupate, the third-stage larva migrates into a drier medium and shortens, thickens, and becomes darker in color as a result of hardening and tanning of the third-stage cuticle in forming the puparium. The adult fly emerges in 2 or 3 weeks by forcing off the end of the puparium with its **ptilinum,** a bladder-like structure inflated with hemolymph. The ptilinum projects from the **frontal suture** and is withdrawn into the head after the fly has emerged from the puparium. Like the umbilicus of mammals, it is of no further service to the animal. The adult fly then makes its way to the surface of the medium in which the pupa lies buried, expands its wings by pumping hemolymph into the wing veins, and flies away in search of food. The housefly feeds on feces, syrup, milk, decaying fruit, and other dissolved and soluble materials. Houseflies will feed on secretions around the eyes, nostrils, and mouth and on blood that continues to ooze from wounds made by tabanids. *Musca* species annoy horses and cattle to distraction on warm, sunny days. Bacteria, protozoan cysts, helminth eggs, and other disease organisms may be transported from filth to food, body openings, and wounds by way of feces, vomit spots, sticky feet, and body hairs of houseflies. The housefly also serves as a biologic vector of *Draschia megastoma*

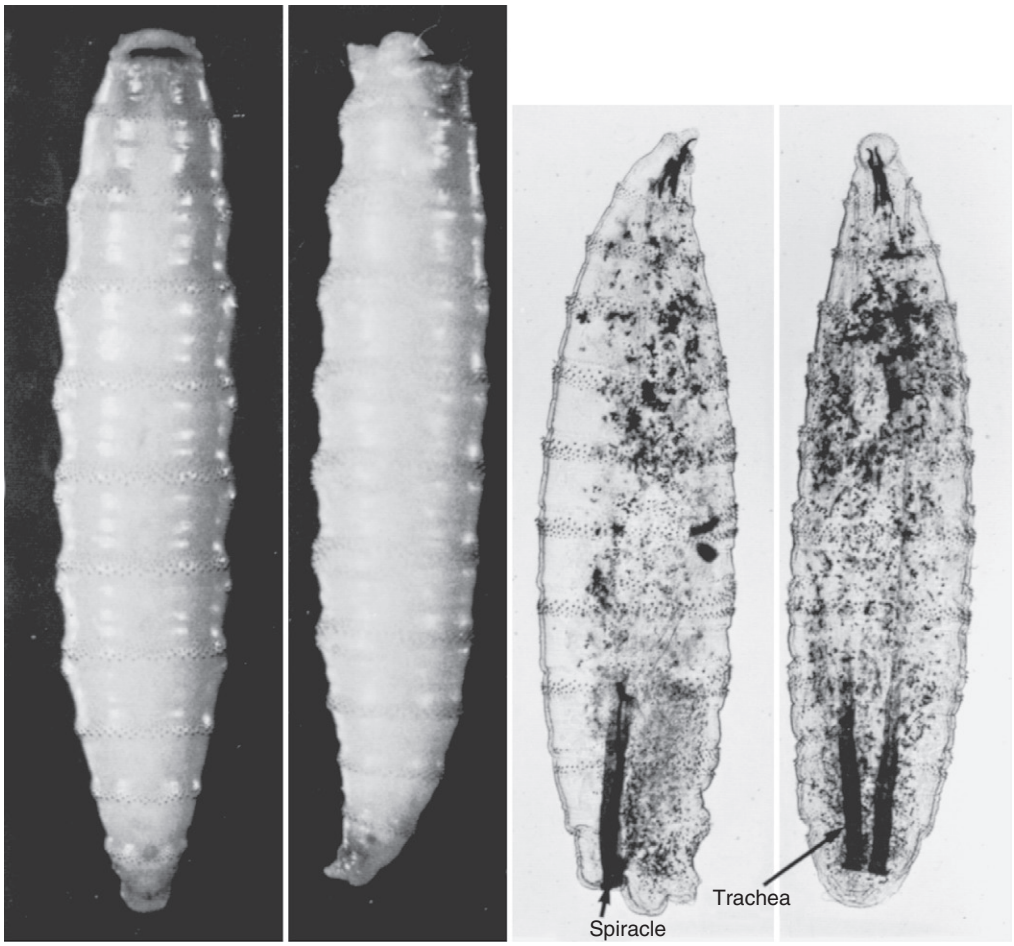

FIGURE 2-12. Muscoid third-stage larva or maggot of the family Calliphoridae. Note the pigmented tracheal trunks leading from the posterior spiracles. Pigmented tracheae are a specific character of *Cochliomyia hominivorax*, the American screwworm. (Specimens courtesy R.J. Gagné.)

and *Habronema muscae*, which are nematode parasites of the stomach of the horse (Table 2-4).

M. autumnalis (the face fly) was introduced into North America from Europe, Asia, or Africa in the early 1950s. These flies crawl about the faces of horses and cattle, feeding on the ocular and nasal discharges induced by their presence, and are extremely annoying to pastured animals. Eggs are deposited in fresh cattle dung, and the larvae pupate in the dried dung or on nearby soil. The adult flies overwinter in buildings. These hibernating adults, like those of the cluster fly, *Pollenia rudis* (a calliphorid fly that as a maggot parasitizes earthworms, and whose adults cluster together inside dwellings in winter to hibernate), cause considerable annoyance to human occupants when, aroused by a spell of warmish weather, they go buzzing and blundering about the house, falling into drinks and making themselves generally disagreeable. It is curious to note that the active adult face fly of summer appears loath to enter buildings and may be observed to swarm off dairy cows as they enter the stable to be milked. They wait outside during milking and swarm back on the cows as they emerge from the stable. This, of course, contrasts with the behavior of *M. domestica*, so appropriately called the housefly. Grazing cattle afflicted with face fly infestations have been shown to increase their herbage dry matter intake as they graze deeper in the sward by taking heavier bites as they try to dislodge the flies from their muzzles (Dougherty et al, 1993). Face flies serve as biologic vectors of *Thelazia* species (eyeworm), a genus of nematode worms

TABLE 2-4 Some Pathogens Vectored by Flies of the Family Muscidae

Fly	Mechanically Vectored	Biologically Vectored
Musca domestica	Suspected of transmitting many agents but seldom shown conclusively	Spirurid nematodes *Draschia megastoma*: horses *Habronema muscae*: horses
Musca autumnalis	Keratoconjunctivitis	Spirurid nematodes *Thelazia* species: cattle
Fannia	Unknown	Spirurid nematodes *Thelazia* species: dogs
Stomoxys	Suspected	Spirurid nematodes *Habronema microstoma*: horses
Haematobia	Suspected	Filariid nematodes *Stephanofilaria*: cattle

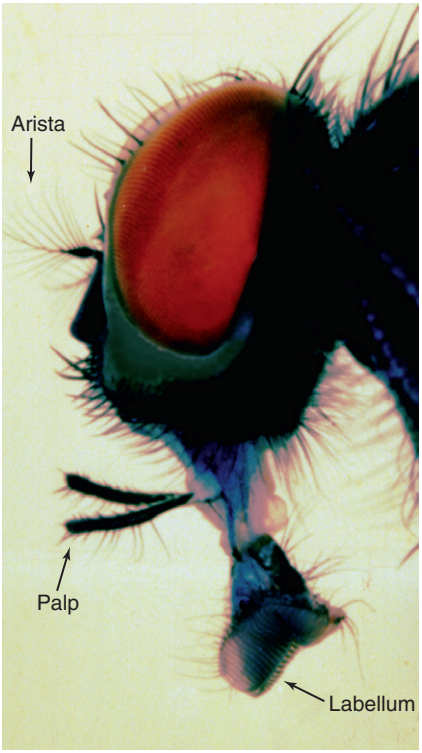

FIGURE 2-13. Head of *Musca domestica* (Cyclorrhapha: Muscidae), the common housefly. The proboscis is retractable into the head.

that infect the conjunctival sacs of horses and cattle (Chitwood and Stoffolano, 1971). *M. autumnalis* also serves as a mechanical vector of infectious bovine keratoconjunctivitis organisms *(Moraxella bovis)*, which can survive for up to 3 days on the legs of the fly (Steve and Lilly, 1965). Cattle protected from face flies had less keratoconjunctivitis and yielded fewer isolates of hemolytic *M. bovis* than did unprotected cattle. "Infection first began to spread from herd to herd after face fly populations exceeded 10/animal for 1 month" (Gerhardt et al, 1982).

M. vetustissima, the Australian bush fly, resembles *M. autumnalis* in preferring to remain outdoors, by breeding in livestock manures, and in crawling about on the faces of livestock. However, *M. vetustissima* differs in displaying an exasperating affinity for the faces of human beings as well as livestock, by involvement of its larvae in wound myiasis, and by an inability to hibernate. Instead of hibernating, *M. vetustissima* reinvades southeastern Australia each spring from the more tropical regions to the north.

In South Africa, *Musca lusoria*, *Musca fasciata*, and *Musca nevilli* have been identified as vectors of a filariid worm, *Parafilaria bovicola*, which lives in the subcutis, bores a hole to the surface, and discharges its eggs in the bloody fluid that weeps from the lesion (Nevill, 1975, 1985; Kleynhans, 1987).

Fannia canicularis (the lesser or little housefly) breeds in ground contaminated with septage drainage and is commonly found associated with large concentrations of chicken manure. These flies can reach impressive numbers as pests and can require pest management. Species of *Fannia* in California are capable of transmission of the canine eyeworm, *Thelazia californiensis*.

CONTROL OF FILTH FLIES. Selection and manner of applying insecticide must conform to regulations that are subject to change. Read the label carefully before applying any insecticide to premises or to domestic animals. Regular spraying of animal sheds, stables, and kennels with residual insecticides should provide good control of flies and other flying insects if reasonable effort is expended to minimize the extent of breeding sites available to these insects. Space sprays, insecticidal baits, and insecticidal resin strips offer additional control. Diazinon, tetrachlorvinphos, and dichlorvos have excellent residual activity against houseflies, face flies, horn flies, stable flies, and mosquitoes for 1 to 4 weeks after application. Spraying resting and breeding areas is often effective. Dichlorvos, pyrethrins, and pyrethroids are used as space sprays for feedlots and sheds. These insecticides may be misted over the backs of animals every 3 to 7 days. Fly baits containing dichlorvos may be sprayed or sprinkled on fly-roosting areas. The sugar fly bait New Improved Golden Malrin contains methomyl and muscalure, a fly-attracting pheromone. The bait is sprinkled around barns. Muscalure attracts and keeps the flies around the bait, thus achieving an increased kill by the insecticide.

Fly control in dairy barns and milk rooms may be achieved with dichlorvos baits, foggers, and sprays. Tetrachlorvinphos and coumaphos are used as sprays or in dust bags and may be applied after milking.

The application of insecticides to lactating cows producing milk for human consumption demands extreme caution because there must be no pesticide in the milk. *Read the label before using any pesticide*. It is against the law to use a pesticide in any manner not specified on the label, and violations with respect to dairy cows are particularly serious. Synergized pyrethroids and some organophosphates are formulated for treating cattle by dust, spray, or backrubber application.

The control of face flies and houseflies on beef cattle and dry dairy cattle may be achieved by regular application of insecticides to animals and fly-breeding sites. Dichlorvos in mineral oil may be smeared daily on the faces of cattle for face fly control. Coumaphos or tetrachlorvinphos may be applied to cattle as a free-flowing dust two or three times a week or self-applied by means of self-treatment dust bags. Pyrethrin or pyrethroid sprays may also be used. Pyrethroid-containing ear tags and similar devices that can be attached to animals allow a continuous, controlled release of insecticides to aid in the control of flies attacking cattle. Coumaphos and diazinon combination ear tags will control face flies and horn flies. Tetrachlorvinphos, a larvicidal organophosphate, prevents the growth of larvae of coprophilic flies in the manure of cattle fed this compound and may be given to lactating dairy cows.

Face fly control on horses may be attempted by application of coumaphos, pyrethrins, or pyrethroids to the entire horse, and by elimination or insecticidal treatment of breeding sites (i.e., cow manure) when feasible. Face flies do not pursue their victims indoors, so stabling horses during hours of peak fly activity often proves to be the best solution.

Biologic control methods using parasitoid wasps have been developed and commercialized for the control of *Musca* species. The larvae of the wasps develop in the maggots of these flies, causing their death. It is possible to purchase parasitized fly pupae from which the adult wasps will emerge, and these can be used to release the wasps on farms. The use of these wasps has proved to be of some benefit when incorporated into integrated fly management programs (Geden et al, 1992).

STOMOXYS

IDENTIFICATION. The stable fly, *Stomoxys calcitrans*, resembles *Musca* species but has a long, pointed proboscis with which it inflicts painful bites instead of the vacuum cleaner affair with which *Musca* sucks up liquids from little puddles. The palpi of *Stomoxys* are shorter than the proboscis (Figure 2-14; compare with *Haematobia* [Figure 2-15]). The third-stage larvae resemble those of *Musca* and have posterior spiracles with sinuous slits, but the spiracles are set farther apart than those of *Musca* (see Figure 2-19).

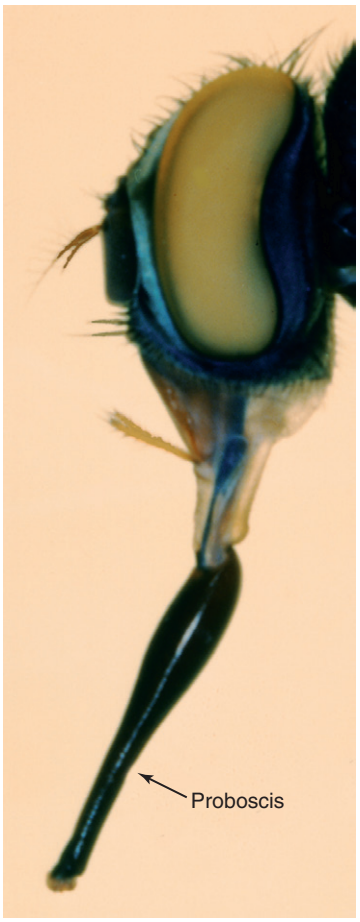

FIGURE 2-14. Head of *Stomoxys calcitrans* (Cyclorrhapha: Muscidae), the stable fly. In feeding, the entire proboscis is thrust into the skin of the host.

FIGURE 2-15. Head of *Haematobia irritans* (Cyclorrhapha: Muscidae), the horn fly. *Haematobia* somewhat resembles *Stomoxys* but is only half as large and has palps almost as long as its proboscis (compare with Figure 2-14).

LIFE HISTORY. Stable flies have a life history similar to that of face flies but differ in preferring decaying organic materials, such as winter hay feeding sites, piles of lawn clippings, damp hay, grain, or animal manure, for egg laying. Stable flies of both sexes feed on blood once or twice a day, depending on the ambient temperature, and suspend operations entirely during cold spells.

INJURY AND DISEASE TRANSMISSION. The presence of *Stomoxys* on grazing cattle will cause increased head and ear movement, skin twitches, and tail swishes. It is interesting to note that the annoyed cattle will increase their herbage dry matter intake and bite masses (Dougherty et al, 1994). The bite of the stable fly is painful and results in the interrupted feeding patterns observed with tabanids. The stable fly serves as a biologic vector of *Habronema microstoma*, a nematode parasite of the stomach of the horse (see Table 2-4).

CONTROL OF STABLE FLIES. Stable flies attack cattle, horses, most other domestic animals, and humans on warm days throughout the summer. Regular application of pyrethrins, synergized pyrethrins, pyrethroids, coumaphos, or dichlorvos is indicated. Efforts to control *S. calcitrans* should include elimination of breeding sites (e.g., lawn clippings, green chop, damp bedding) and application of insecticides to areas where they habitually rest. Repellents in the form of sprays or smears may afford relief for several hours. These flies, with their piercing mouthparts, can theoretically be controlled with systemic and topical insecticides. Chlorpyrifos, coumaphos, phosmet, or tetrachlorvinphos is applied by spray or with self-treatment dust bags or backrubbers. Cyromazine is an insect growth regulator that has been formulated for spreading or spraying on fly breeding sites or as a feed-through product to prevent the development of *S. calcitrans* in horse manure, which will prevent flies from developing in contaminated hay. Winter hay feeding sites, especially around round bale hay feeding sites for cattle, appear to be a site of early emergence of *S. calcitrans* in the spring (Taylor and Berkebile, 2011). The biologic control of *S. calcitrans* by the release of parasitoid wasps seems to require further refinement before routine success occurs in the field (Andress and Campbell, 1994).

HAEMATOBIA

IDENTIFICATION. The horn fly, *Haematobia irritans*, found on the backs of cattle and to a lesser extent on horses, is about half the size of *Stomoxys* and has a relatively shorter proboscis. The palps are nearly long enough to reach the tip of the proboscis, in contrast to those of *Stomoxys* (compare Figures 2-14 and 2-15). Horn flies were first reported in the United States in the fall of 1887, when they were found in Camden, New Jersey. They spread rapidly throughout the United States, appeared in Hawaii in 1897, and have spread through Mexico, Central America, and northern South America (e.g., Guyana; Craig, 1976). The horn fly was also discovered in Argentina, and it has rapidly spread throughout that country (Anziani et al, 1993).

LIFE HISTORY. Horn flies remain on cattle during the warmer seasons of the year, periodically biting their hosts and sucking blood. They are most obvious on the backs of their hosts but take refuge on the ventral abdomen during rain or on particularly hot, sunny days. When a cow defecates, a number of her horn flies swarm to the dropping to lay their eggs and then return to the cow. Larvae hatch in less than a day and crawl into the dropping to feed. Pupation occurs in 4 days, and emergence of the adult follows in 6 more days. In ideal warm, humid weather, the entire cycle from egg to egg requires 2 weeks or less, but in drier, cooler weather, it may require a month or longer. In temperate climates, the horn fly overwinters in the pupal stage, with diapause occurring principally during September (Thomas, Hall, and Berry, 1987).

INJURY AND DISEASE TRANSMISSION. When sufficiently numerous, horn flies can impair milk production and weight gain. Cattle protected from horn fly attack by ear tags impregnated with fenvalerate achieved 18% greater live weight gains than did untreated controls (Foil, DeRoven, and Morrison, 1996; Haufe, 1982). *H. irritans* serves as a biologic vector of *Stephanofilaria stilesi*, a filarioid nematode parasite of North American cattle and

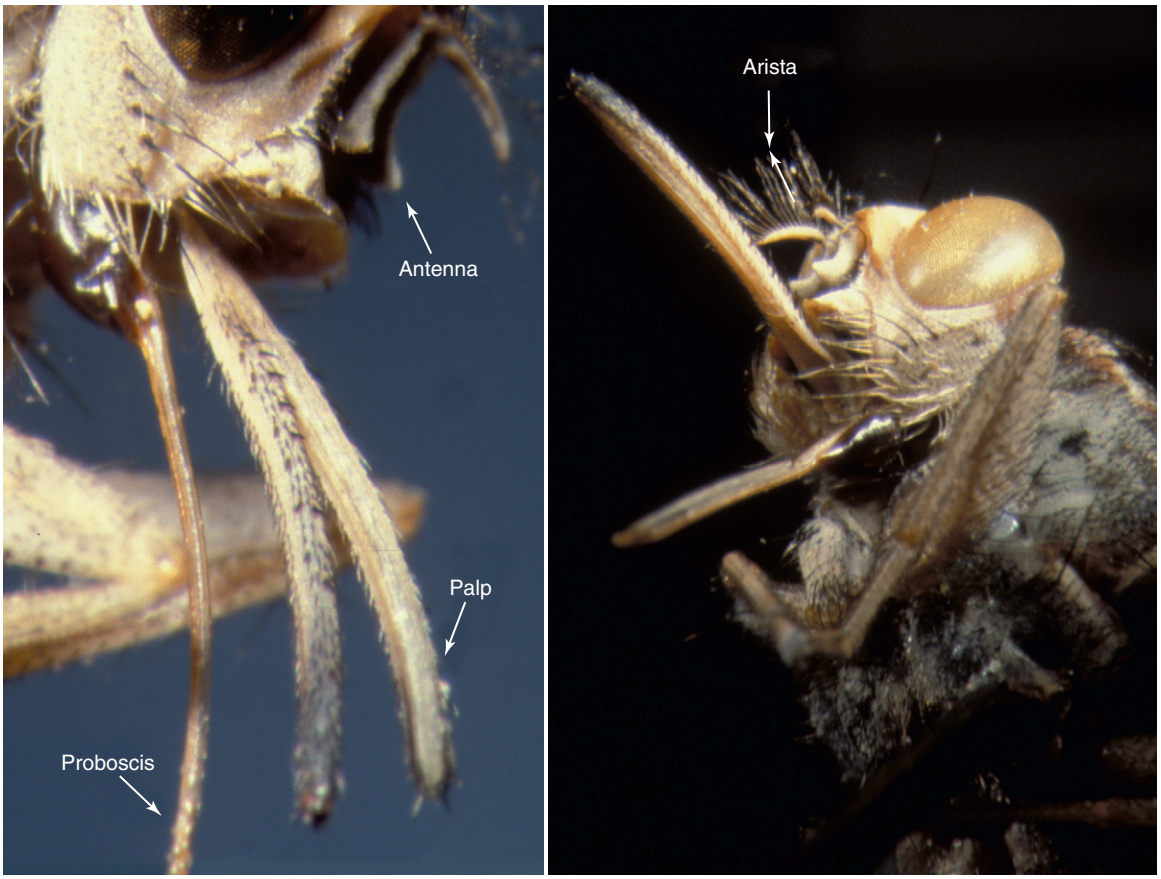

FIGURE 2-16. Head of *Glossina* (Cyclorrhapha: Muscidae), the tsetse that transmits many important species of African trypanosomes.

etiologic agent of stephanofilariasis, a dermatitis usually confined to the midventral region of the abdomen.

CONTROL OF HORN FLIES. Because they remain on the host most of their lives, adult horn flies are vulnerable to effective insecticides applied to cattle by means of sprays, dusts, backrubbers, stock oilers, and insecticide-impregnated plastic ear tags. In fact, horn fly control has depended almost exclusively on insecticides, with the unfortunate development of resistance on the part of the fly to many of them (e.g., DDT, methoxychlor, toxaphene, fenchlorphos, stirofos, permethrin, fenvalerate; Marchionado, 1987). The insecticide tetrachlorvinphos or the synthetic juvenile hormone methoprene may be fed to cattle to render the manure unfit for development and pupation, thus interrupting the life history of *H. irritans*. Treatment of cattle with eprinomectin had very good efficacy against horn flies for at least 2 weeks with good efficacy for longer periods, whereas ivermectin in a pour-on form is effective for at least 4 weeks (Arrioja-Dechert, 1997; Shoop et al, 1996).

The Bruce walk-through horn fly trap affords 50% reduction in horn fly numbers mechanically. Cattle walking through the 10-foot trap contact strips of canvas or carpeting, which dislodge the horn flies on their backs and sides. The host leaves some of its flies behind in the trap, and, provided the process is repeated often enough, the population of horn flies in the herd is significantly reduced (Hall, Doisy, and Teasley, 1987).

GLOSSINA

IDENTIFICATION. Tsetses (*Glossina* species) are localized to Africa and are of significant importance to human and animal health, to the preservation of African wildlife, and to the economy of Africa and the world at large. Each antenna of *Glossina* has a long arista that is "feathered" along one edge. The palps and the long slender proboscis are equal in length, the palps forming a sheath for the proboscis at rest (Figure 2-16).

LIFE HISTORY. The female tsetse bears only one larva at a time. Larval development is completed in the abdomen of the mother, with all three stages feeding on fluids from special uterine glands. It is interesting that milk secretion has evolved independently among both the highest vertebrates and the highest invertebrates. Several blood meals at regular intervals are required to support the larva during its developmental period of roughly 1 to 4 weeks. When extruded by the female tsetse, the fully developed third-stage larva almost immediately burrows into the soil and prepares to enter the pupal stage. A fourth larval stage occurs within the puparium, before metamorphosis to the adult stage at last takes place.

DISEASE TRANSMISSION. The great importance of the tsetse is its role as biologic vector of various trypanosomiases of humans and their domestic animals. African sleeping sickness of humans and "nagana" and related diseases of domestic animals are considered in Chapter 3.

ERADICATION. The tsetse has been eradicated from Zanzibar, the island next to the African continent (Vreysen et al, 2000). Thought has now been given to a major sterile release campaign program, as was used in the control of *Cochliomyia hominivorax* (discussed later) throughout the rest of the African continent (Kabayo, 2002). Some think the entire plan too mammoth and unworkable (Rogers and Randolph, 2002). The International Atomic Energy Agency continues to support the plan, the first releases of sterile males occurred in Ethiopia in June of 2007, and

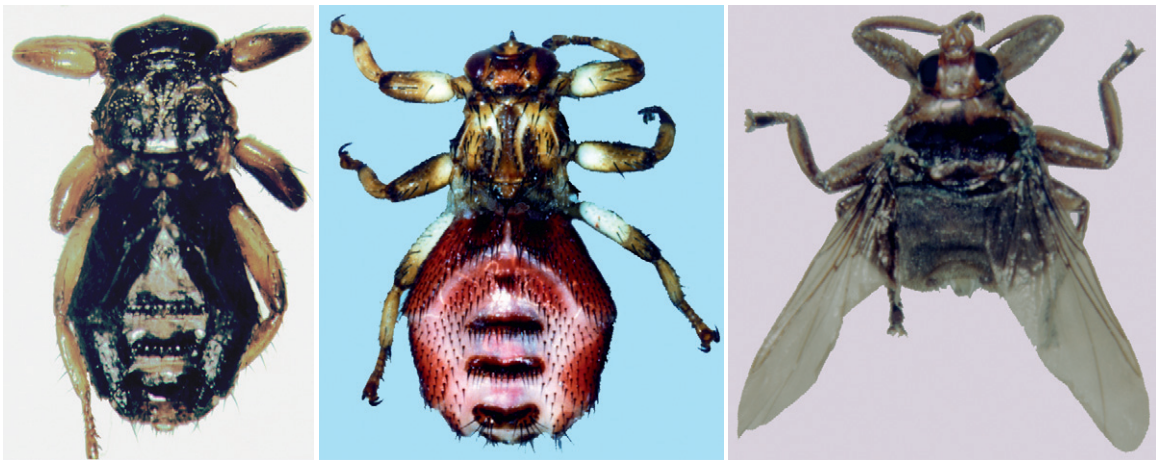

FIGURE 2-17. Examples of family Hippoboscidae. *Left*, *Melophagus ovinus*, the sheep ked; *center*, *Lipoptena cervi*, the deer ked recovered from a horse; *right*, *Pseudolynchia* from a bird.

the Pan African Tsetse and Trypanosomiasis Eradication Campaign (Pattec) held its 10th annual meeting in Accra, Ghana, in June of 2012. There is concern that the complete removal of these great protectors of African wildlife (trypanosomes and their tsetse vectors have protected the game of Africa through the prevention of successful colonization by nonindigenous species) may have a colossal long-term impact on the environment of the African continent if imported species can be allowed to compete for the same geographic regions as the long-protected indigenous wildlife. This potential eradication of the tsetse can be viewed as a major ethical dilemma for veterinarians weighing the advantages of disease eradication, wildlife protection, improved nutrition for the African continent, and ecologic protection.

Family Hippoboscidae, Keds

IDENTIFICATION. Hippoboscids are dorsoventrally flattened, sometimes wingless flies with piercing mouthparts. The antennae are embedded in pits in the sides of the head. *M. ovinus*, the sheep ked; *Hippobosca equina*, the horse louse fly; and *Lipoptena cervi*, the deer ked, are examples (Figure 2-17). *Melophagus* is wingless; the wings of *Hippobosca* remain well developed and functional throughout life; and *Lipoptena* have wings when they emerge from the pupal case. However, the wings of *Lipoptena* break off near the base (see Figure 2-17) once the fly has alighted on a host. *Lipoptena* may attack horses and other domestic animals in addition to deer, and casual observation suggests that their attacks are particularly obnoxious to horses.

LIFE HISTORY. Like tsetses, hippoboscids retain their larvae in their abdomens until they are ready to pupate, nourishing them during development with uterine gland secretions. In the case of *M. ovinus*, larval development requires about a week, and the extruded larva pupates within a few hours. The chestnut-brown pupal cases remain glued to the wool of the host sheep throughout metamorphosis of the adult fly, which emerges in 3 to 6 weeks, depending on the ambient temperature. The entire life of the sheep ked is thus spent on the host. Shearing and organophosphorous insecticides make life very uncertain for these parasites.

Dealate *L. cervi* males and females remain on their normal North American hosts—white-tailed deer *(Odocoileus virginianus)* and wapiti *(Cervus canadensis)*—through most of the year. In spring, larvae are deposited in the haircoat, where they pupate and fall to the ground. Adult *L. cervi* flies emerge from the puparia from September to early December and fly off in search of a host. As soon as the ked alights on a deer, its wings break off and the ked begins to feed. The bite of *L. cervi* is relatively painless to humans but may be followed in several days by a pruriginous welt that may remain intensely pruritic for 2 to 3 weeks (Bequaert, 1942).

DISEASE TRANSMISSION. *M. ovinus* is host to *Trypanosoma melophagium*, which it transmits to sheep. If all keds are removed, the trypanosomes rapidly disappear from the sheep's blood, so it is the ked and not the sheep that represents the true reservoir of infection. Like *T. theileri* of cattle, *T. melophagium* appears to be totally nonpathogenic to its vertebrate host. Recent work in northern India has suggested that the dog fly—*Hippobosca longipennis*—that is found in Africa and Asia and is an intermediate host for the filarioid nematode parasite of the dog—*Acanthocheilonema dracunculoides*—may also be the intermediate host of another distinct canine *Acanthocheilonema* species in dogs in India (Rani et al, 2011).

CONTROL OF HIPPOBOSCIDS. Coumaphos and diazinon as dips or sprays provide excellent control of *M. ovinus* when applied after shearing. In small flocks, diazinon can be applied most conveniently with a garden sprinkling can. Groups of about 20 sheep should be crowded in a small pen so there is just room enough left for one person to move among them. Waterproof overalls and boots should be worn while the insecticide is sprinkled over the backs of the sheep. Ivermectin at 0.2 mg/kg given subcutaneously in sheep controls *M. ovinus* (Molina and Euzeby, 1982). *L. cervi* has been controlled also in red deer and roe deer by the administration of ivermectin (Kutzer, 1988).

What to do about attacks of *L. cervi* on horses, short of keeping them indoors until the alate hippoboscids find their proper hosts, is problematic.

Family Sarcophagidae, Flesh Flies

An adult sarcophagid is about twice as large as a housefly. The thorax is gray with dark, longitudinal stripes, and the abdomen is checkered gray and black (Figure 2-18). Third-stage sarcophagid larvae resemble housefly maggots but are larger. The posterior spiracles are deeply sunken in a rounded concavity; the inner slit of each spiracle is directed down and away from the median line (Figure 2-19). Differentiation of *Sarcophaga* and *Wohlfahrtia* larvae requires that adult flies be reared from them. Place the larvae in question and a piece of liver on 3 to 5 cm of sand or loamy soil in a canning jar. When, after a day or so, the larvae have entered the substrate to pupate, remove the liver to avoid obnoxious odors, and

cover the mouth of the jar with a layer of cheesecloth secured with a rubber band to provide air yet prevent the escape of flies after they have emerged from the pupal cases. The arista of *Wohlfahrtia* bears only very short hairs, whereas the arista of *Sarcophaga* is covered nearly to its tip with long hairs. These rearing instructions serve equally well for calliphorids, but best results are obtained with larvae that are almost ready to pupate, especially when obligate parasitic species are involved. **Facultatively parasitic sarcophorids** will attack wounds and skin under urine- and feces-soaked hair of many mammals. Besides two species—*Wohlfahrtia vigil* and *Cistudinomyia cistudinis*—that are obligate parasites, many species of sarcophagid flies can become facultative parasites, but many of the cases of facultative myiasis are caused by Calliphorid maggots.

FIGURE 2-18. *Sarcophaga* (Cyclorrhapha: Sarcophagidae), a flesh fly. About twice as large as a housefly, *Sarcophaga* is gray, with longitudinal dark stripes on the thorax and a checkered gray and black abdomen.

Family Calliphoridae, Blowflies

IDENTIFICATION. Adult calliphorids are usually intermediate in size between *Musca* and *Sarcophaga* and typically display a brilliant metallic blue, green, copper, or black hue (Figure 2-20). The common names "bluebottle" and "greenbottle" fly refer to the coloration of these flies, which are also called "blowflies" because they "blow" (i.e., deposit) their eggs or larvae in meat. Particular species differ in their preferences regarding the freshness of the meat, from living flesh to carrion in an advanced state of decomposition. Most calliphorids are scavengers or facultative parasites, but a few (e.g., *C. hominivorax*, the American screwworm) are obligate parasites. Third-stage larvae of Calliphoridae are muscoid maggots that differ from those of Sarcophagidae in having posterior spiracles that lie flush with the posterior face of the larva (or, less commonly, are sunken in a shallow, slitlike concavity); the inner slits of the spiracles are directed obliquely downward and toward the median line (see Figure 2-19). Larvae of the very important species *C. hominivorax* may be identified by the dark pigmentation of their tracheal trunks through the last three or four segments (see Figure 2-12).

LIFE HISTORY AND INJURY (MYIASIS). **Myiasis** can be defined and described in different ways. Relative to the biology of the flies, **primary myiasis** typically refers to myiasis in cases in which the insect requires a living host for the larvae to feed on. **Secondary myiasis** is then said to represent cases due to flies that usually feed on dead and decaying flesh sometimes developing in weak, debilitated, wounded, soiled, or immobilized animals. Myiasis can also be described by the site of the lesion (e.g., aural myiasis, nasal myiasis, and so on).

Facultatively parasitic calliphorids are drawn to such attractions as suppurating wounds; skin soiled with urine, vomitus, or feces; and bacterial decomposition products that tend to accumulate in the fleece of a wet sheep. Once established in exudate or necrotic tissue, some kinds of these facultative parasites may later invade living tissue, whereas others do not. For example, the "surgical maggots" of *Phaenicia sericata* and *Phormia regina* are still used occasionally in the treatment of osteomyelitis and other refractory suppurative lesions to clear away necrotic debris and promote

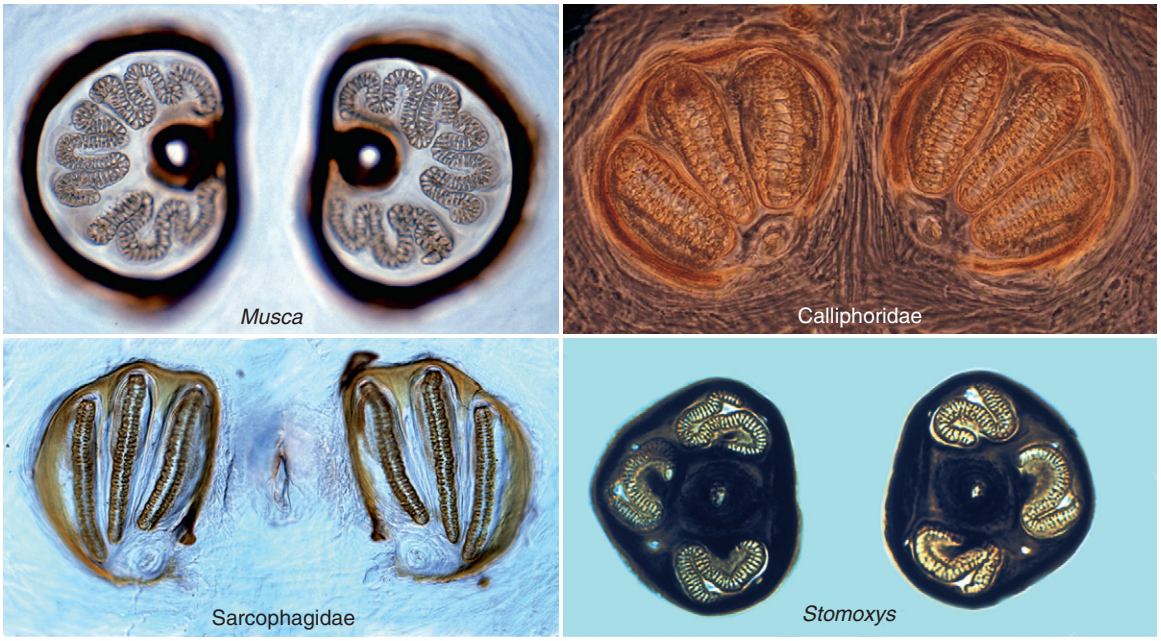

FIGURE 2-19. Muscoid spiracles.

FIGURE 2-20. Calliphorid flies. *Top left*, Mouthparts and head, similar to *Musca. Top right*, Large shiny calliphorid, *Lucilia cuprina*, with another fly that is about the size of the housefly. *Bottom*, Maggots of *Lucilia cuprina* in the fleece of a sheep with wool strike.

healing. Ideally, the surgical maggots do not invade healthy tissue, but strains vary and some of them do not know where to stop. A brave and resourceful gentleman of Dr. Georgi's acquaintance applied this technique in treating his own wounds when a prisoner of war in Vietnam. Once the maggots had done their work to his satisfaction, he flushed them away with his urine.

Wool strike is a common and serious problem in many sheep-raising regions of the world (Figure 2-21). Adult calliphorids are attracted to areas of fleece that have become soiled by feces or urine or were kept damp long enough for bacterial growth to occur and generate odors that lure flies to feed and lay their eggs. The areas involved in wool strike thus include the perineum, the prepuce, and, during periods of considerable rainfall, the water-soaked wool of the flanks, withers, and ventral neck region. Fleece rot, caused by *Pseudomonas aeruginosa*, and dermatophilosis (lumpy wool), caused by *Dermatophilus congolensis*, predispose sheep in Australia to wool strike by *Lucilia cuprina*, and a significantly greater incidence of body strikes was observed in lambs infected with both of these bacteria than with either bacterium alone (Gherardi et al, 1983). Around the world, several genera of calliphorid flies are commonly involved, and each geographic region has its particular scourge among the general assemblage of facultative parasites and scavengers. In Australia, *L. cuprina* stands out as a specialist in wool strike. This fly, although still a facultative parasite, in that it is able to develop in carrion, has become so adept at locating suitable sheep on which to deposit its eggs that it has become the culprit responsible for initiating most cases of wool strike in Australia. The maggots feed on scales and exudate at the surface of the skin, occasionally penetrating underlying tissues. When ready to pupate, the larvae of *L. cuprina* wait until night to leave the carcass (Smith et al, 1981). In this way, the pupae and emerging adults of this highly specialized parasite tend to become concentrated around the preferred resting sites, or camps, of their host species. Once *L. cuprina* has initiated a strike, other species of flies are attracted to feed and lay their eggs in the developing lesions. As the morbid process advances, these less-specialized newcomers tend to replace *L. cuprina*. Toxins absorbed from the myiasis lesion rapidly incapacitate the sheep and lead to its death in a matter of days. Eventually, scavenger species take over the carcass and reduce it to hair and bone. Financial loss caused by wool strike is reckoned in terms of outright death losses, loss of wool, decreased quality of wool, loss of weight, and costs of treatment and preventive measures.

Old, weakened, or paretic dogs with urine-soaked haircoats sometimes develop a form of myiasis analogous to wool strike. As such, an unfortunate animal lies in the "healing rays of the sun," the blowflies are busy laying eggs in its haircoat, and in a few days maggots will be skinning it alive. Frequently, owners who present long-haired dogs with advanced cases of cutaneous myiasis are totally unaware of the mayhem taking place underneath the haircoat. The condition of the patient can be evaluated accurately and effective treatment undertaken only after the hair has been clipped away and all affected areas bathed.

Weakened or defective calves born at pasture are also fair game for members of the family Calliphoridae. It is amazing how quickly the shiny flies appear seemingly out of nowhere and how rapidly their egg masses accumulate about the umbilicus of a newborn calf with cerebellar hypoplasia or muscle contracture. The possibility of myiasis must always be considered in the case of animals incapacitated during warm weather, especially if they are forced to remain outdoors.

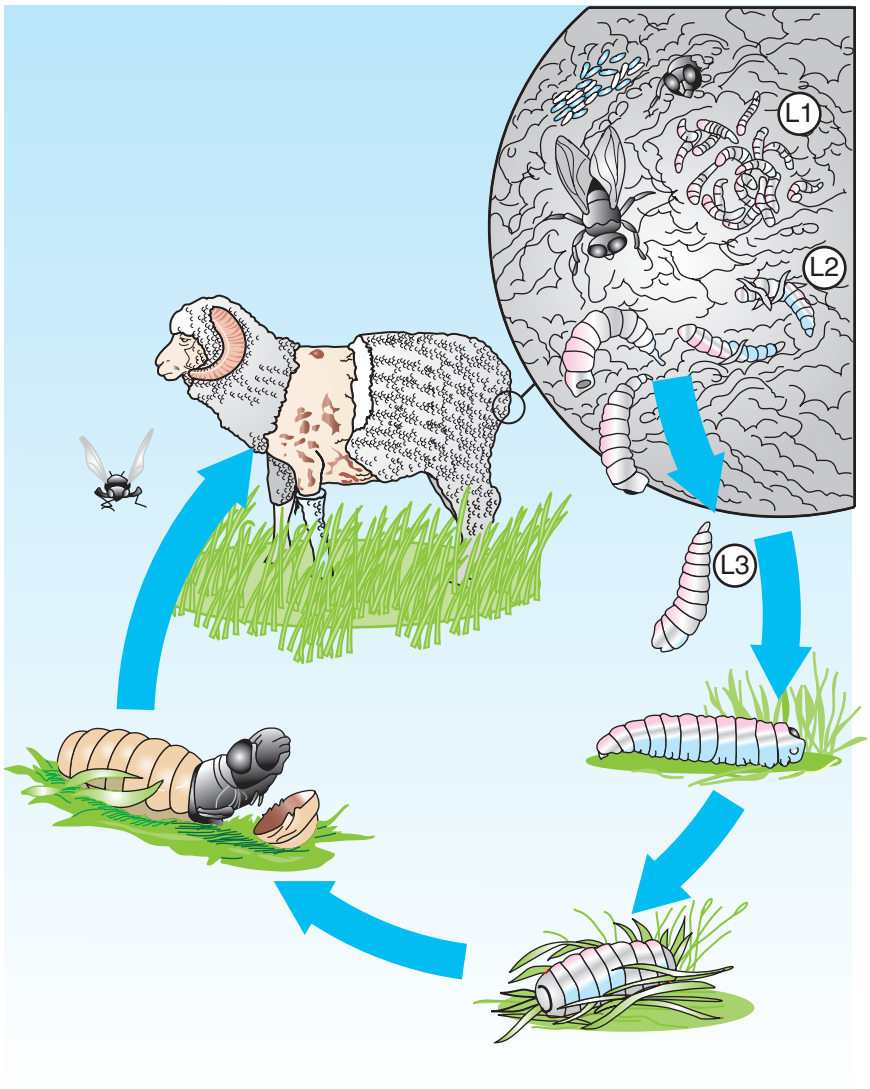

FIGURE 2-21. Life history of the wool strike fly, *Lucilia cuprina.* Adult female *L. cuprina* flies deposit their eggs in moist and soiled wool. Larvae hatch from these eggs, feed on scales and exudate at the surface of the skin, and undergo two molts before falling to the ground to pupate. The adult fly pushes off the end of the puparium by inflating its baglike ptilinum with hemolymph. Having emerged from the puparium, the fly inflates its wings by pumping hemolymph into the wing veins, retracts its ptilinum into the head once and for all, and flies off in search of suitably smelly sheep. The Australian Merino wether in the picture has suffered fly-strike in three stages. The flies first attacked a spot between his shoulders that had collected rainwater and supported bacterial growth. The exudate from this lesion flowed over the wether's shoulders and brisket and greatly extended the area susceptible to fly attack. The shepherd clipped all of the wool from both shoulders and brisket and treated the lesions with an insecticide, but now the flies are attracted to the breech area, which is soiled with feces, so this area too must be clipped and medicated, or the wether will likely die of "crutch strike."

Rabbits, wild animals, and birds may suffer serious losses to myiasis. Domestic rabbits are often victims of myiasis. Flies attracted to lay their eggs on rabbits can cause horrible lesions in rabbits that are housed outdoors even in relatively good conditions for short periods. The lesions are horrible and can be fatal to the rabbit, along with causing severe distress to the owners. This is unfortunately not an uncommon event, and rabbits need to be protected from such infestations (Anderson and Huitson, 2004). Larvae of the sarcophagid fly *Neobellieria citellivora* cause lethal myiasis in ground squirrels *(Spermophilus columbianus)* in Canada (Michener, 1993). Arendt (1985) estimated that infection with larvae of *Philornis deceptivus* (family Muscidae) was responsible for 97% of the mortality observed among pearly-eyed thrasher nestlings *(Margarops fuscatus)* in Puerto Rico. In North America, major causes of avian myiasis are bloodsucking maggots of the genus *Protocalliphora* (Sabrosky, Bennett, and Whitworth, 1989). Larvae of sarcophagid flies, genus *Cistudinomyia*, are capable of causing lethal myiasis in geckos (DeMarmels, 1994).

The American screwworm fly, *C. hominivorax*, is an example of a primary myiasis–producing fly. Females lay their eggs on fresh, uninfected wounds of all kinds. About 200 eggs are deposited in tidy rows. The eggs hatch within a day, and the obligate parasitic maggots commence feeding on living flesh and in so doing produce a foul-smelling, brownish-red discharge. The larvae leave the host in 5 to 7 days and enter the soil to pupate. Adult flies emerge from the pupal cases one to several weeks later. Wherever it occurs, *C.*

hominivorax is a serious menace to man and beast alike. Unconscious victims of accidents or alcohol intoxication lying helplessly exposed have been fatally infected or have had their facial bones completely eaten away by screwworm maggots. Docking and castrating wounds, wire cuts, the navels of newborn animals, tick-bite wounds, shear cuts, needle grass wounds, and even fresh brands may attract the attentions of *C. hominivorax*. A nationwide control program based on treating wounds of all infected animals with insecticidal smears and releasing billions of sterilized flies has succeeded in eliminating screwworm myiasis from the United States and ultimately all of the Americas north of Panama. The adult flies are sterilized by gamma radiation, which induces dominant lethal mutations in the sperm. Because the female screwworm mates only once, and because the wild population of the fly is relatively small, adding hordes of sexually competent but sterile males reduces the probability of successful fertilization to nil. By the use of sterile males produced in Mexico, the American screwworm was eradicated from Libya, where it had been accidentally introduced, probably on imported livestock, in 1988 (Linquist, Abusowa, and Hall, 1992).

TREATMENT OF MYIASIS. Coumaphos is widely employed in the treatment of cutaneous myiasis. This agent may be applied to cattle by dipping, but most commonly it is sprayed or smeared directly on maggot-infested lesions. Ivermectin and doramectin administered subcutaneously to cattle can serve as a prophylactic against infestation by larvae of *C. hominivorax* and appear to be useful aids in the prevention of umbilical myiasis and fly-strike associated with castration (Anziani and Loreficce, 1993; Muniz et al, 1995).

For treatment of wool strike in sheep, coumaphos is recommended in the form of sprays, dips, or local applications to affected areas. All wool soiled or underrun by maggots should be clipped away. Ivermectin when applied as a jetting fluid appears to aid against blowfly-strike of sheep in Australia (Eagleson et al, 1993). Subcutaneous injections of infested sheep in Hungary with ivermectin or moxidectin failed to cause rapid-acting treatment of the infested sheep, and 7 days after treatment, most of the treated sheep were still severely infested (Farkas et al, 1996). Also used in Australia with good success is a jetting fluid with cyromazine, an insect growth regulator that can be mixed with diazinon (Levot and Sales, 1998).

The extent of measures taken to prevent fly-strike in sheep should be proportionate with the degree of risk. Clipping the wool of the breech and the area around the prepuce greatly reduces the amount of moisture and filth that can be retained in those regions of the fleece. Amputating the tails of lambs represents about the minimum of effort that ought to be expended on fly-strike control, but in some parts of the world, lambs manage to grow up with their tails intact. In **Mules' operation,** widely practiced in Australia, with perhaps up to 30 million lambs treated each year, redundant folds of skin from the posterior aspects of the thighs and the tail head are removed with a pair of sharp dagging shears. When the resultant wounds heal, the skin of the breech is drawn taut, thus extending the relatively hairless area immediately surrounding the anus and vulva, thereby reducing the moisture- and filth-carrying capacity of the breech. This operation, carried out in a minute or so without surgical preparation, anesthesia, or aftercare, seems brutal until one has had an opportunity to compare its effects on the patient with those inflicted by *L. cuprina*.

In the case of the backyard dog that has developed a case of fly-strike, as the hair is clipped to determine the extent of the lesion, most of the maggots will be removed in the process. Maggots that remain may be routed by judicious local application of an insecticidal solution. It has been shown that nitenpyram applied at the dose usually given to dogs for flea control can have marked effects on the maggots causing myiasis in dogs, even with maggots of *Cochliomyia hominivorax* (Correia et al, 2010).

Subfamilies of the Oestridae: Oestrinae, Hypodermatinae, Gasterophilinae, and Cuterebrinae; the Botflies

The botflies are highly host-specific (Table 2-5) and site-specific parasites in the larval (i.e., bot) stage and are total slaves to reproduction in the adult stage. The adults have vestigial mouthparts and must carry on their courtship rituals and egg laying using energy stored away when they were larvae. Fully developed bots are larger and stouter than are muscid, sarcophagid, and calliphorid maggots, from which they can readily be distinguished by their posterior spiracles (Figure 2-22; see also Figure 2-19). In fact, in the United States, when found in their accustomed locations in their normal hosts, bots present very little in the way of a diagnostic challenge: a bot in a sheep's nasal passages is an *Oestrus;* a bot in a cow's dorsal subcutis is a *Hypoderma;* a bot in a horse's stomach is a *Gasterophilus;* and there is hardly any sense in making more an exercise of it than that. However, the earlier stages of bots are more difficult to distinguish from maggots, and if a bot is found migrating in other than its normal host, the services of an expert entomologist will be required for identification. First-stage *Hypoderma* larvae have been found migrating aberrantly through the brain of horses, and *Cuterebra* larvae, normally parasites of rodents and lagomorphs, have been found in the brain of cats and dogs and much more commonly in their subcutaneous tissues. *Hypoderma* and *Cuterebra* also occasionally invade humans and migrate subcutaneously. *Oestrus ovis* may larviposit in the eyes of shepherds, thus causing a temporary but painful ocular myiasis.

OESTRUS OVIS. *O. ovis*, the sheep nasal botfly, somewhat resembles a honeybee (Figure 2-23). It is a stout, grayish-brown fly, about 1 cm long and covered with short hairs; the mouthparts are vestigial. These flies are most active during the warmer hours of the day, especially during intervals of bright sunshine. In early morning and late afternoon, they are more likely to be found resting on buildings, tree trunks, water tanks, and the like. It is interesting to watch a mob of Australian Merino sheep on a warm, sunny day with a few scattered clouds. While in the shadow of a cloud, the sheep tend to distribute themselves more or less at random over the paddock, but as the sun emerges from behind the cloud, the sheep immediately huddle together and continue to graze with their heads toward the center of the huddle, only to disperse again with the arrival of the next cloud. This behavior may represent a defensive adaptation to the attack of the larvipositing female *O. ovis*; it seems plausible, at least. While *O. ovis* females are actively depositing their larvae in sheep's nostrils, the sheep hold their noses close to the ground or in each other's fleeces, stamp their feet as if annoyed, and occasionally bolt away. The tiny first-stage larvae may be demonstrated postmortem by sawing the skull in half longitudinally, rinsing the nasoturbinates and nasal sinuses with water, and examining the collected rinsings with a hand lens or a stereoscopic microscope. The fully developed third-stage bots can hardly escape notice in the frontal sinuses.

LIFE HISTORY. On being deposited in the nostril of a sheep, the larva crawls onto the mucous membrane of the nasal passage, where it will remain for at least 2 weeks, anchored to the mucous membrane by its mouth hooks. Larvae arriving late in the season remain arrested in the first stage throughout the winter, and development proceeds only with the return of warm weather. After a sojourn in the nasal cavity, the larvae proceed to the frontal sinuses,

TABLE 2-5 Subfamilies of the Oestridae, Genera, Host Affiliation, and Geographic Range

Subfamily	Genus	Host Group	Range
Oestrinae	*Cephenemyia*	Cervidae	Holarctic
	Cephalopina	Camels	Eurasia, Africa
	Gedoelstia	Antelopes	Africa, south of Sahara
	Kirkioestrus	Antelopes	Africa, south of Sahara
	Oestrus	Ovine, caprine	Worldwide
	Phryngobolus	Elephant	Africa
	Pharyngomyia	Cervidae, zebra, pig, giraffe, hippopotamus, springbuck, sheep	Palearctic
	Rhinoestrus	Equine	Europe, Asia, Africa
	Tracheomyia	Kangaroos	Australia
Hypodermatinae	*Hypoderma*	Bovine	Worldwide
	Oestroderma	Pikas	Asia
	Oestromyia	Mice, marmots, pikas	Eurasia
	Pallasiomyia	Saiga antelope	Eurasia
	Pavlovskiata	Goitered gazelle	Eurasia
	Portschinskia	Mice and pikas	Eurasia
	Przhevalskiana	Caprine and gazelles	Eurasia
	Strobiloestrus	Bovidae, *Kobus* species	Africa
Gastrophilinae	*Cobboldia*	Elephants	Eurasia
	Gasterophilus	Equine	Worldwide
	Gyrostigma	Rhinoceros	Africa
Cuterebrinae	*Cuterebra*	Rodents, lagomorphs, howler monkey	America
	Dermatobia	Nonspecific, larger mammals and birds	America
Gasterophilinae (perhaps)	*Neocuterebra*	African elephant	Africa
	Ruttenia	African elephant	Africa

Adapted from Colwell DD, Hall MJR, Scholl PJ: *Introduction.* In Colwell DD, Hall MJR, Scholl PJ (eds): *The Oestrid Flies: Biology, Host-Parasite Relationships, Impact and Management,* Oxforshire, UK, 2006, CAB International; Pape T: *Phylogeny of the Oestridae (Insecta: Diptera). Syst Entomol* 26:133, 2001; Angulo-Valadez CE, et al: *Nasal bots … a fascinating world! Vet Parasitol* 174:19, 2010.

where development to the third stage is completed (see Figure 2-23). On reaching full development, the third-stage larvae crawl down into the nasal passages, are expelled by the sheep's sneezing, and enter the soil to pupate. Adults may emerge in about 4 weeks in summer but require considerably longer in cool weather. When pupation occurs in autumn, adult flies do not emerge until the following spring. Thus *O. ovis* overwinters both as arrested first-stage larvae in the nasal cavities of sheep and as pupae in soil.

PATHOLOGIC SIGNIFICANCE. Although moderate numbers of *O. ovis* larvae in the nasal and paranasal sinuses do no apparent harm, heavy infections cause sneezing, nasal discharge, and partial blockage of the nasal passages.

TREATMENT OF NASAL BOTS. The larva of *O. ovis* is very susceptible to ivermectin at the standard dosage rate of 0.2 mg/kg (Roncalli, 1984). Nasal bots in sheep have been treated with eprinomectin at both 0.5 mg/kg and 1 mg/kg body weight with efficacies ranging from 83.5% to 100% (Habela et al, 2006; Hoste et al, 2004).

OTHER NASAL BOTS. *Rhinoestrus purpureus* infects horses in parts of Europe, Asia, and Africa; *Cephalopina titillator* infects camels and dromedaries in Africa; and *Cephenomyia* species infect deer, elk, caribou, and other cervids in the Northern Hemisphere. Their life histories generally resemble those of *O. ovis*. However, the third-stage larvae of *R. purpureus* and *C. titillator* are found in the nasal and paranasal sinuses, pharynx, and even larynx, and those of *Cephenomyia* species are found in the pharyngeal pouches (Figure 2-24).

HYPODERMA

IDENTIFICATION. *Hypoderma bovis* and *Hypoderma lineatum*, the heel flies or gadflies, occur in cattle-raising areas of the Northern Hemisphere between 25° and 60° North latitude. The adult fly is about 15 mm long and looks rather like a bumblebee (Figure 2-25). Although these flies have no functional mouthparts for biting, and the process of oviposition on the hairs is presumably painless, cattle tend to become apprehensive and excited at their approach and gallop off aimlessly with their tails held high over their backs. Such behavior, termed "gadding about," tends to involve the whole herd simultaneously in needless, hysterical exertion and distracts it from the more profitable business of grazing. (Agricultural research administrators, practiced in the art of extracting financial support for their institutions from legislative bodies, can tell you exactly how much this form of bovine entomophobia costs the American stockman each year.) The fully developed third-stage *Hypoderma* larva or "cattle grub" is found in walnut-sized lumps, or warbles, on the backs of cattle in spring. Each warble has a small hole at its summit to which the posterior spiracles of the larva are pressed to obtain air. When it emerges or is extracted from the warble, the larva (sometimes also called a warble) is about 25 mm long and is whitish to light brown.

LIFE HISTORY AND PATHOGENESIS. *H. lineatum* and *H. bovis* females glue their eggs to the hairs on the legs of cattle. *H. lineatum* appears with the advent of warm weather and remains active for about 2 months. Then *H. bovis* takes over and persists into summer. The eggs hatch spontaneously in less than a week, and the larvae burrow through the skin and set off on prolonged migrations through the connective tissues of their host. Larvae of *H. lineatum* accumulate in the tissues of the esophagus 5 months later and remain there for about 3 months. Finally, they migrate to the subcutaneous tissues of the back, cut breathing holes in the skin to which they appose their spiracles, and, after molting twice, grow larger. The larvae spend about 2 months in warbles in the backs of infested cows (Pruett and Kunz, 1996). When fully developed (see Figure 2-25), the larvae enlarge their breathing holes, emerge through them, and fall to the ground to pupate. Adult flies emerge from pupal cases about 1 month later and immediately set about their reproductive duties. *H. bovis* larvae tend to accumulate in the spinal canal instead of in the esophagus and appear in the subcutaneous tissues of the back about 2 months later than larvae of *H. lineatum*.

Hypoderma larvae occasionally invade horses and render them useless for equitation by warble formation in the saddle area or even

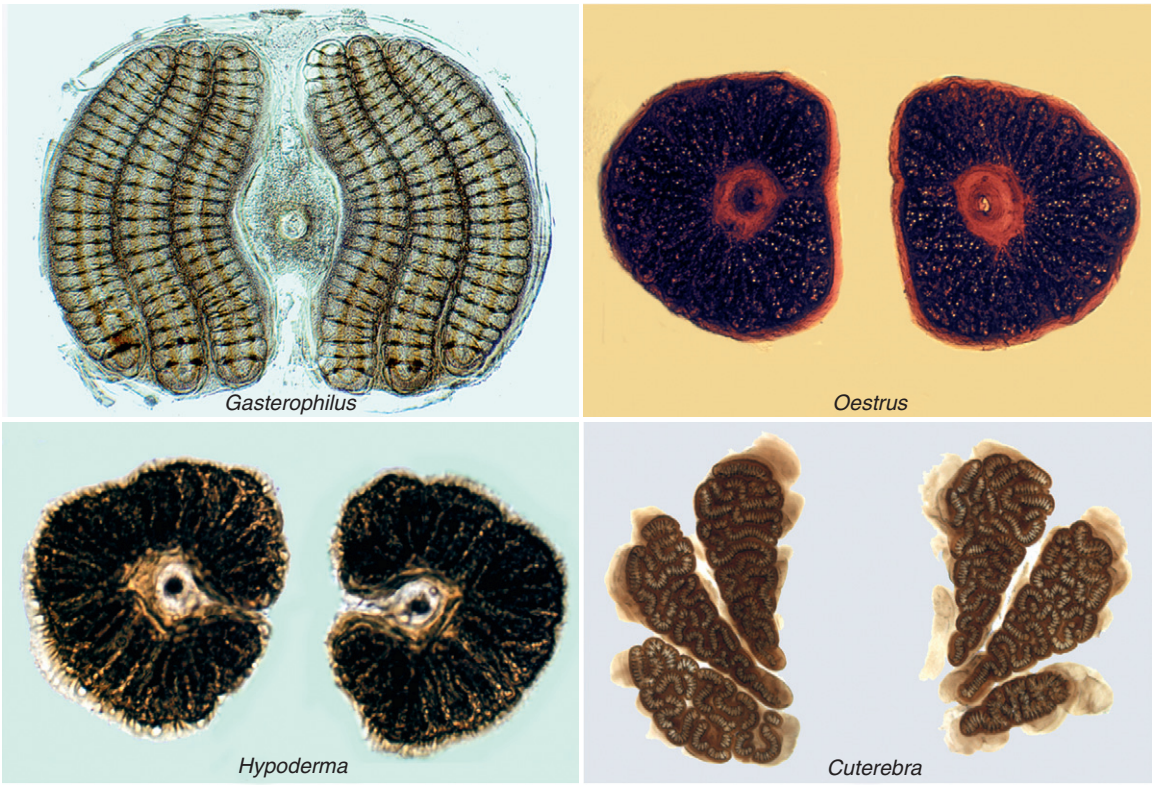

FIGURE 2-22. Bot spiracles (*Gasterophilus* and *Oestrus* ×27; *Hypoderma* ×55; *Cuterebra* ×65).

FIGURE 2-23. *Oestrus ovis. Top*, The adult female fly. *Bottom*, Bots in the nasal sinuses of sheep at necropsy.

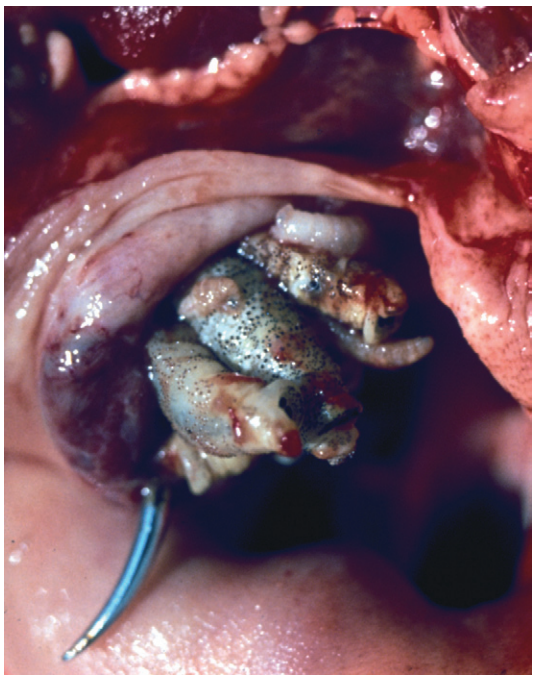

FIGURE 2-24. *Cephenemyia* bots in the retropharyngeal pouch of a deer.

cause fatal neurologic disease by migrating into the brain (Olander, 1967). In humans, *Hypoderma* larvae tend to produce bouts of creeping subcutaneous myiasis ("migrating lumps") as the confused larvae try to find the top of the cow in which they "think" they are migrating. Local paralysis may result from invasion of the spinal cord, and blindness may result from invasion of the eye. These fortunately are rare incidents.

TREATMENT AND CONTROL OF *HYPODERMA*. *Hypoderma* infection is treated most commonly these days with the systemic macrocyclic lactone ivermectin, doramectin, eprinomectin, or moxidectin. Eprinomectin and moxidectin pour-on can be used for treating both beef and dairy cattle. The "safe periods" for applying these insecticides vary for different localities because of differences in fly activity. The insecticides must be applied immediately

FIGURE 2-25. *Hypoderma bovis. Left*, Adult fly. *Right*, Mature bot removed from a warble.

after adult *Hypoderma* activity ceases for the season. Host-parasite reactions manifested clinically by bloat, salivation, ataxia, and posterior paralysis may occur when cattle are treated with larvicidal insecticides while *H. lineatum* larvae are in the esophagus, or while *H. bovis* larvae are in the spinal canal. The host-parasite reaction was once thought to be an anaphylactoid reaction caused by antibodies produced by cattle in response to *Hypoderma* larval antigens. However, experimental evidence indicates that this reaction is caused by a toxin liberated from the dead *Hypoderma* larvae. Injection of phenylbutazone at a dosage rate of 20 mg/kg body weight 20 minutes before injection of larval toxin protected calves against both systemic shock and local inflammatory reactions (Eyre, Boulard, and Deline, 1981). The host-parasite reaction is best treated with sympathomimetic drugs (e.g., adrenaline) and steroids to alleviate local inflammatory reactions. Atropine, the antidote for cholinesterase-inhibiting agents, is contraindicated; host-parasite reaction is not a manifestation of organophosphate toxicity even though it may be precipitated by organophosphate medication.

In cases in which preventive treatment has been neglected, late second-stage and third-stage *Hypoderma* larvae can be safely and quickly removed from the backs of cattle by slowly injecting 1 mL of 3% hydrogen peroxide solution into the breathing hole using a blunt cannula or needle shank of the syringe and taking care not to pierce the grub. Most grubs will emerge within 15 seconds after the foaming action of the hydrogen peroxide begins and leaves behind a cleansed cavity (Scholl and Barrett, 1986).

National eradication efforts directed against *Hypoderma* species have met with success in Denmark, the Federal Republic of Germany, the Netherlands, and the Republic of Ireland, and the incidence in Great Britain was reduced from 38% in 1978 to 0.01% in 1985 (Wilson, 1986). Surveillance against reintroduction of *Hypoderma* species in imported cattle is critical, as evidenced by 19% of tested cattle entering Great Britain in 1993 being seropositive for *Hypoderma* (Sinclair, 1995). In parts of Great Britain where the ox warble has persisted or reappeared, all cattle older than 12 weeks are required to undergo treatment within specific dates, and cattle are routinely inspected at livestock sales and on farms.

RELATED SPECIES. *Hypoderma diana* occurs in deer and occasionally in man in Europe. Other species of *Hypoderma* and genera of warble flies parasitize sheep, goats, and deer in Mediterranean countries and India. *Hypoderma (=Oedemagena) tarandi* is a serious enough pest of reindeer, musk oxen, and caribou in subarctic regions to require prophylactic medication of these wild or semi-wild hosts. In one study, 70% of untreated reindeer harbored more than 100 *H. tarandi* larvae (Washburn et al, 1980). Both ivermectin and doramectin have proved highly effective in the treatment of infection with this parasite.

FIGURE 2-26. Adult female *Gasterophilus intestinalis;* the ovipositor is curved around and under the body.

GASTEROPHILUS

IDENTIFICATION. The adult fly superficially resembles a honeybee, with a long, curved ovipositor carried beneath the abdomen (Figure 2-26). Females may be observed on warm, sunny days hovering near horses and darting very rapidly to attach an egg to a hair.

Eggs are deposited by *Gasterophilus nasalis* females on the hairs of the intermandibular space, by *Gasterophilus hemorrhoidalis* on the short hairs that adjoin the lips, and by *Gasterophilus intestinalis* on the hairs of the forelegs and shoulders (Figure 2-27). An illustrated key for identifying the eggs of the eight species of *Gasterophilus* that occur around the world has been prepared by Cogley (1991).

First-stage larvae of *G. intestinalis* can be found in tunnels in the epithelium covering the dorsal surface of the rostral two thirds of the tongue and in pockets between the molar teeth. Second-stage larvae are found in interdental pockets, attached to the root of the tongue, and attached to the wall of the stomach (Cogley, Anderson, and Cogley, 1982). Less is known regarding the initial migrations of other species of *Gasterophilus*. First- and second-stage larvae of *G. nasalis* are usually completely hidden well below the gum line in interdental pus pockets extending into the root sockets of molar teeth (Schroeder, 1940).

The third-stage larva of *G. nasalis* is yellowish and has one row of spines on each segment (see Figure 2-23); it is usually found in the first ampulla of the duodenum. The following three species of *Gasterophilus* have two rows of spines per segment. The *G. intestinalis* third-stage larva is red, has coarse spines that are blunted at their tips, and attaches in clusters in the nonglandular part of the stomach either near the margo plicatus or in the saccus cecus. The following species have small spines that taper to a fine point: *G. hemorrhoidalis*, which is reddish and is found in the duodenum and rectum of horses in north-central United

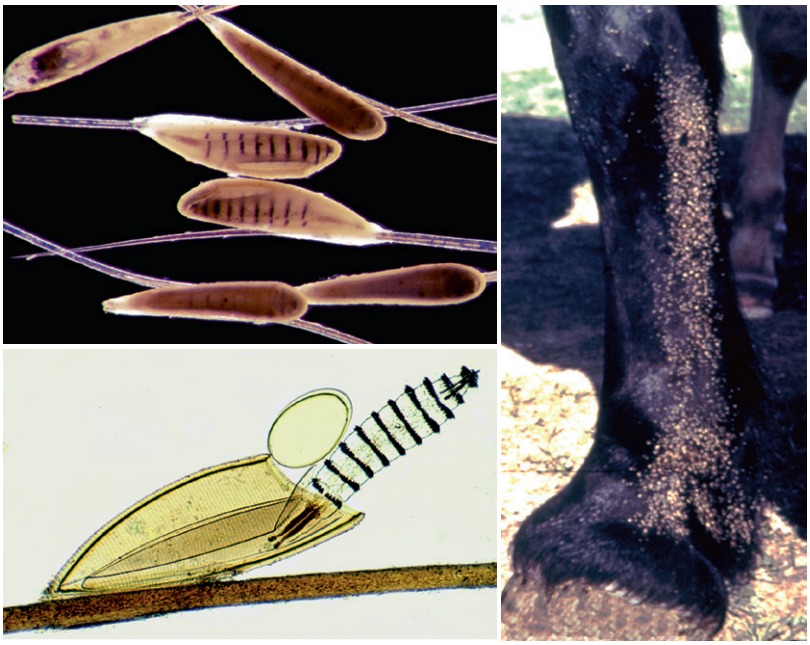

FIGURE 2-27. *Gasterophilus intestinalis. Left*, Eggs of *G. intestinalis* (Cyclorrhapha: Gasterophilidae) on horse hairs; in the bottom image, the operculum has become dislodged and the maggot is partially out of the shell. *Right*, Eggs attached to the hairs on the leg of a horse.

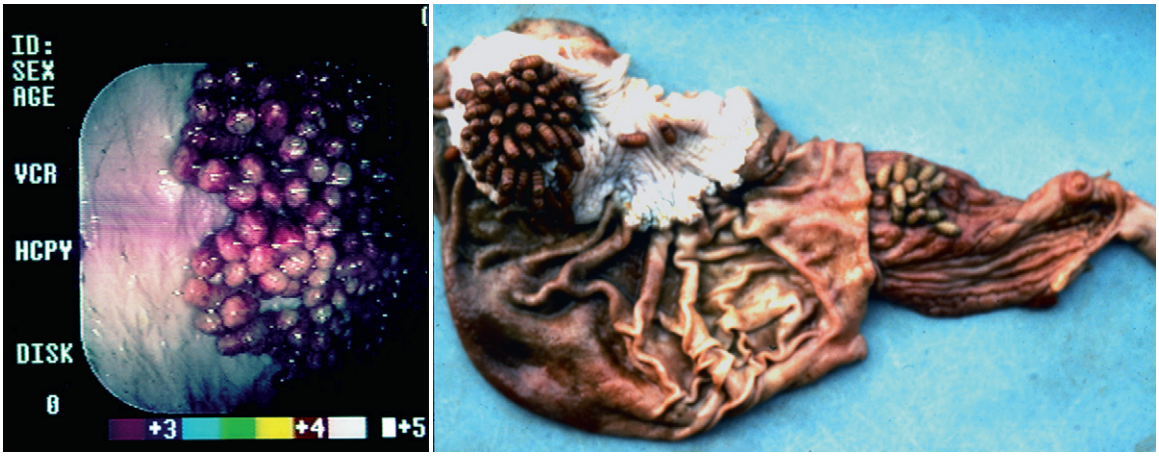

FIGURE 2-28. *Gasterophilus* bots. *Left*, Endoscopy of the bots of *Gasterophilus intestinalis; right,* stomach of a horse with the bots of *G. intestinalis* in its typical predilection site near the margo plicatus and with the bots of *Gasterophilus nasalis* in the first ampulla of the duodenum.

States and Canada; and *Gasterophilus inermis*, which is light yellow and is found in the rectum of European horses. Individual larvae of all species may occasionally be found in atypical locations in the alimentary tract.

LIFE HISTORY. *G. nasalis* females deposit their eggs on the hairs of the intermandibular space. These eggs hatch spontaneously in 5 or 6 days, and the larvae crawl downward toward the chin until they arrive at a point opposite the commissures of the lips, whereupon they proceed directly toward the mouth and pass between the lips. The black eggs of *G. hemorrhoidalis* on the hairs adjoining the lips hatch after 2 to 4 days on contact with moisture, penetrate the epidermis of the lips, and burrow toward the mucous membrane of the mouth (Wells and Knipling, 1938).

The eggs of *G. intestinalis* on the hairs of the front legs are far removed from their destination and depend on direct assistance from the horse to find their way into the mouth (Figures 2-28 and 2-29). Five days after being laid, these eggs contain first-stage larvae that are prepared to hatch rapidly in response to the sudden rise in ambient temperature that occurs when the horse brings its warm muzzle and breath in contact with them; they do not respond to gradual warming (Knipling and Wells, 1935). The larvae then enter the horse's mouth and burrow into the stratified squamous epithelium on the dorsal surface of the tongue. The first- and second-stage larvae of *G. intestinalis* spend about 1 month in the oral cavity. The white first-stage larvae drill burrows up to 13 cm long in the mucosa of the tongue, with "air holes" at an average interval of 4.2 mm to which they apply their caudal spiracles to breathe (Cogley, Anderson, and Cogley, 1982). The burrows typically extend in a rostral to caudal direction, but all terminate several centimeters rostral to the vallate papillae. Having approximately doubled in size during their sojourn in the tongue, the first-stage larvae now enter pockets in the interdental spaces predominantly

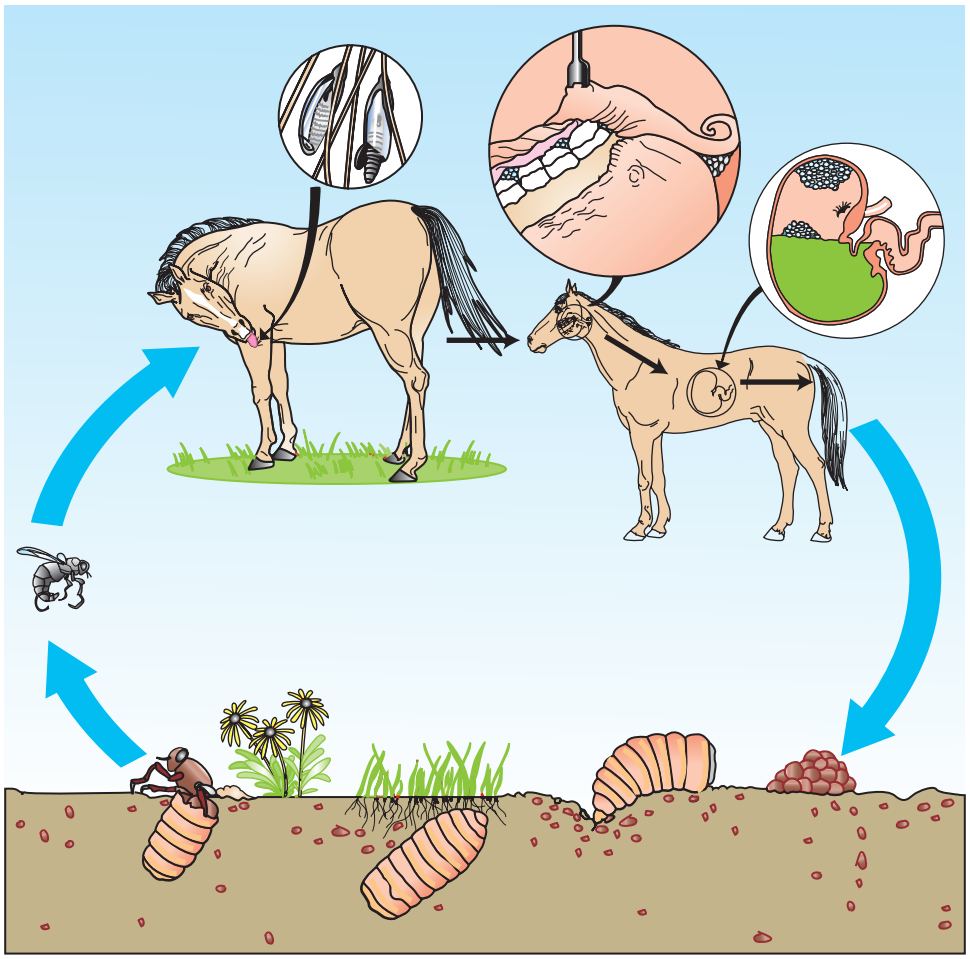

FIGURE 2-29. Life history of the equine stomach bot *Gasterophilus intestinalis.* The female botfly attaches fertilized eggs to the hair shafts of the forelegs and shoulders of horses. First-stage larvae develop in 5 days and stand ready to literally pop out of their shells in response to the warm breath of the horse. Having landed on the horse's face and entered its mouth, the larvae first tunnel quite extensively in the mucous epithelium of the dorsum and sides of the tongue, then enter pockets between the upper molar teeth, where they molt to the second stage. One month after infection, the larvae emerge from the interdental spaces, attach temporarily to the wall of the pharynx, then pass to the stomach, where they molt to the third stage. The third-stage larvae remain attached in the saccus cecus or along the margo plicatus for almost a year. From late spring onward, they let go, pass out in the feces, and pupate in the soil. Adult *G. intestinalis* flies emerge from their pupal cases 3 to 9 weeks later and fly off in search of a horse.

of the upper molar teeth, where they molt from first to second stage. The second-stage larvae develop a red color as a result of synthesis of the insect's own hemoglobin—an adaptation to the low–oxygen tension environment they will presently encounter in the stomach. At last, the second-stage larvae leave the interdental spaces, attach briefly to the root of the tongue, and then proceed to the stomach, where they molt to the third larval stage, or full-grown bot (Cogley, Anderson, and Cogley, 1982). The oral migrations of other species of *Gasterophilus* have not yet been elucidated in such detail as they have been for *G. intestinalis*. However, migration within tissues affords protection from the host's teeth and a source of nourishment and is probably a key feature of the oral migrations of other *Gasterophilus* species as well.

The third-stage larvae remain attached by their mouth hooks to the wall of the stomach *(G. intestinalis)* or duodenum *(G. nasalis)* for up to 12 months (farthest from the intestine—*intestinalis;* farthest from the nose—*nasalis*) (see Figure 7-80). The predilection sites of both species are located above the fluid level in the alimentary tract. In these locations, the bots are surrounded by gas pockets that apparently supply these air-breathing animals with sufficient oxygen (Figure 2-30; Price and Stromberg, 1987). From late spring onward, the larvae release their grip on the mucosa and pass out with the feces to pupate in the soil. Adult botflies emerge from the pupal cases in 3 to 9 weeks, depending on the ambient temperature. Botfly activity continues through summer and fall but ceases completely when cold weather sets in.

IMPORTANCE. Despite the rather impressive oral lesions produced by first- and second-stage larvae and the chronic lesions in gastric and intestinal mucosae caused by attachment of the second and third stages, remarkably little pathologic or experimental evidence associates *Gasterophilus* infection with clinical illness. In fact, many horses support substantial populations of these parasites without apparent ill effect. However, disease is not a simple subject, and *Gasterophilus* infection has been held to etiologic account in cases of stomach rupture, subserosal abscess, splenic abscess, ulceration, and peritonitis (Rainey, 1948; Rooney, 1964; Underwood and Dikmans, 1943; Waddell, 1972). Principato (1988) described, classified, and superbly illustrated the main macroscopic lesions produced by larvae of *G. intestinalis*, *G. nasalis*, *G. hemorrhoidalis*, *G. inermis*, and *Gasterophilus pecorum* in freely ranging horses in Italy.

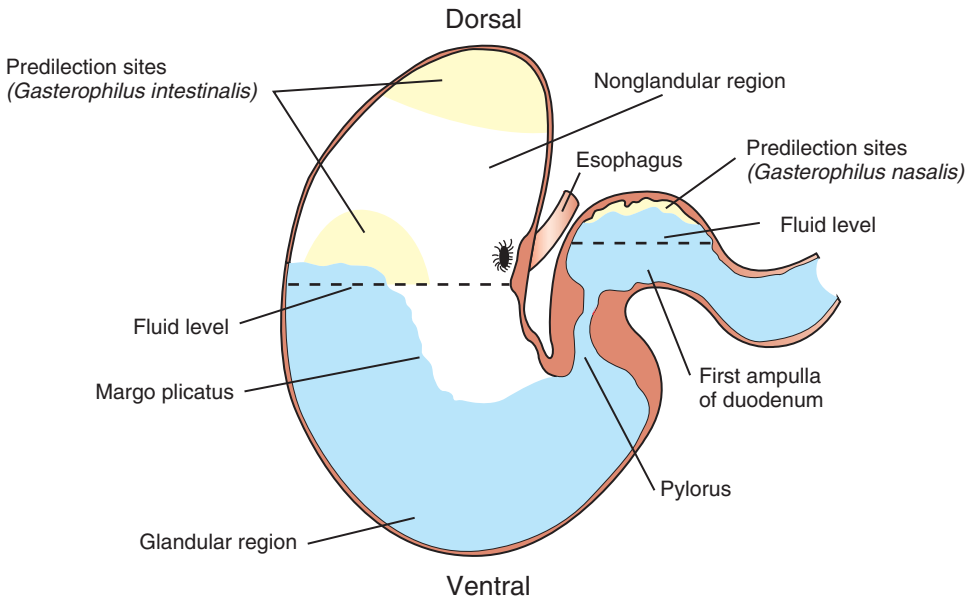

FIGURE 2-30. Predilection sites of *Gasterophilus intestinalis* and *Gasterophilus nasalis* in the stomach and duodenum of the horse. (From Price RE, Stromberg PC: Seasonal occurrence and distribution of *Gasterophilus intestinalis* and *Gasterophilus nasalis* in the stomachs of equids in Texas. *Am J Vet Res* 48:1225, 1987, American Veterinary Medical Association.)

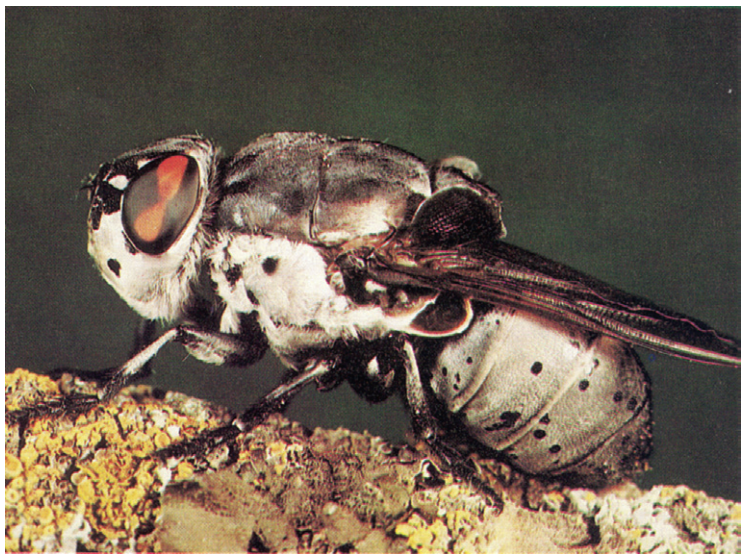

FIGURE 2-31. *Cuterebra jellisoni* (Cyclorrhapha: Cuterebridae), a botfly. The mouthparts of botflies are vestigial. (From Baird CR: Development of Cuterebra jellisoni [Diptera: Cuterebridae] in six species of rabbits and rodents, *J Med Entomol* 8:615, 1971.)

TREATMENT OF GASTEROPHILUS INFECTION. A macrocyclic lactone is now used commonly as treatment. In the southern United States, botflies are active most of the year, and in the case of *G. intestinalis*, the eggs glued to the hairs of the forelegs remain infective long after adult fly activity has ceased. The eggs may be removed from the haircoat with a special fine-tooth comb available from saddlery shops, but the process is rather slow and laborious. If more than a very few horses are involved, the larvae can be lured out of their egg cases by copious sponging with water at 40°C to 48°C (104°F to 118°F) (Knipling and Wells, 1935); the addition of 0.06% coumaphos ensures rapid destruction of these larvae as they emerge. The eggs of *G. nasalis* and *G. hemorrhoidalis* hatch spontaneously when development of the larva is complete.

CUTEREBRA

IDENTIFICATION. The rarely seen (or noticed) adult fly somewhat resembles a bumblebee and has vestigial mouthparts (Figure 2-31). The fully developed third-stage larva is large (up to 45 mm) and dark-brown to black, with the color determined by the stout black spines that cover the body (Figure 2-32). The posterior spiracles consist of groups of elegantly curved openings (see Figure 2-22). Earlier stages are much paler or even white and the posterior spiracles are quite different from those of the third stage, but the dark spines covering the body furnish evidence of the larva's identity as *Cuterebra*. At the present state of knowledge, it is impossible to differentiate species of even fully developed third-stage larvae of *Cuterebra*, except in the few cases in which their life histories have been worked out in detail.

LIFE HISTORY AND PATHOGENESIS. *Cuterebra* species infect rabbits, squirrels, chipmunks, mice, cats, dogs, and occasionally humans (Baird, Podgore, and Sabrosky, 1982). Female *Cuterebra* flies lay their eggs along rabbit runs and near rodent burrows. As the host brushes past, the first-stage larvae hatch

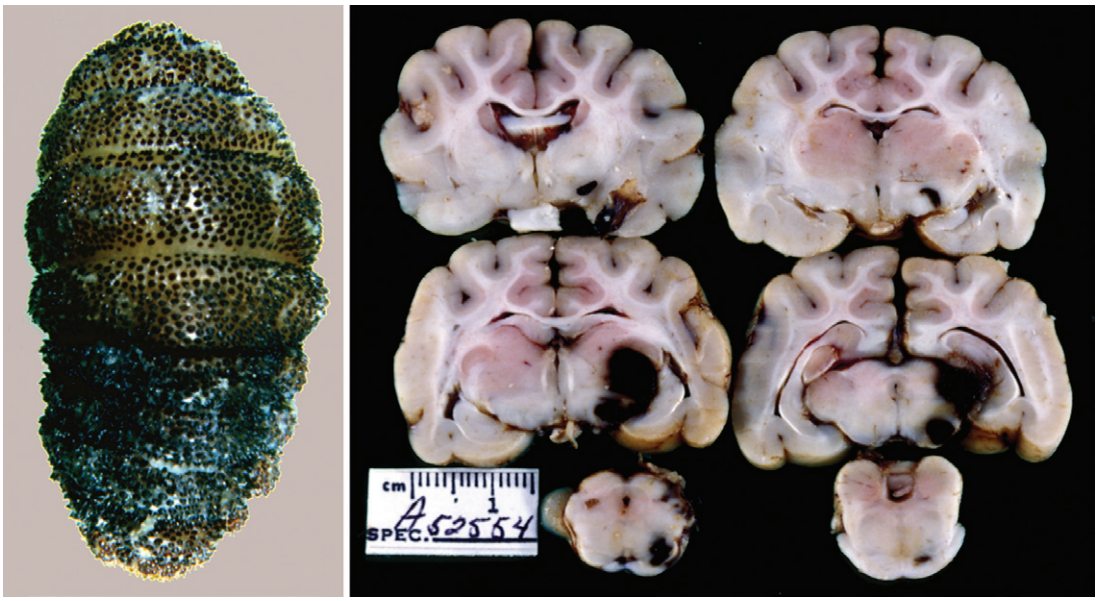

FIGURE 2-32. Cuterebridae. *Left*, A mature bot as it would appear when it is ready to leave the warble and drop to the ground to pupate. *Right*, The brain of a cat that died from the migration of a misguided *Cuterebra* larva.

instantaneously and crawl immediately into the host's fur. These larvae enter the host through its natural body openings and migrate through the body of their host before reaching their final subcutaneous location (see Figure 8-1) (Baird, 1971, 1972; Timm and Lee, 1981). *Cuterebra* larvae are usually found in the cervical subcutaneous connective tissue of cats and dogs during August, September, and October. *Cuterebra* larvae may also be noted in the nasal and oral regions and sometimes migrate through the brain of cats and dogs, with fatal results (see Figure 8-2). Migration of the larva in the brain of cats is believed to cause infarction and to be responsible for feline ischemic encephalopathy (Williams, Summers, and de Lahunta, 1998).

TREATMENT OF CUTEREBRIASIS. A *Cuterebra* larva that has made its way into a warble can be removed by enlarging its breathing hole in the skin sufficiently to allow it to be extracted with forceps, with care taken not to crush the larva in the process. Tranquilization or sedation facilitates restraint but is rarely necessary. The wound heals rather slowly and sometimes suppurates or even sloughs; this may be a result of secondary bacterial infection or leakage of *Cuterebra* antigens into surrounding tissues during extraction. When the worms are in ectopic sites, they may be removed.

Topically applied flea and tick products, such as imidacloprid and fipronil, may kill the young maggots on the haircoats of cats. Also, although no data are available, cats given avermectin heartworm preventives, such as ivermectin, milbemycin, or selamectin, might be protected from *Cuterebra* infection through the killing of larvae during initial phases of their migration in the cat. However, none of these products has been approved for preventing cuterebriasis.

DERMATOBIA

IDENTIFICATION. The adult of *Dermatobia hominis*, another member of the family Cuterebridae, somewhat resembles a brilliant blue calliphorid fly but, like all botflies, has vestigial mouthparts (Figure 2-33). The fully developed third-stage larva is pear-shaped and has posterior spiracles with straight slits deeply sunken in a concavity (see Figure 2-33).

LIFE HISTORY AND PATHOGENESIS. The *D. hominis* female uses a slave to carry her eggs to a prospective host. She captures another fly, usually a bloodsucker such as a mosquito or a stable fly, and glues her eggs to its abdomen. The eggs develop in a week or two, and the larvae inside them stand ready to disembark when the slave fly alights on the skin of a warm-blooded animal to feed. Each *D. hominis* larva that succeeds in penetrating the skin develops at or near the site of penetration in a separate warble. The larva emerges through the breathing hole to pupate about 6 weeks later. The *D. hominis* larva is a serious pest of humans, cattle, sheep, dogs, and other mammals in Central and South America. The adult flies tend to be concentrated at the edges of large forests.

FIGURE 2-33. *Dermatobia hominis. Top*, The adult female fly. *Bottom*, The maggot that crawled out of the arm of an infected human.

Expert Identification of Myiasis Larvae

The major taxa of fully developed myiasis larvae can be identified by means of the criteria set forth earlier. More detailed information can be found in James (1948). However, identification of all three larval stages of even the more common species is a chore for a taxonomic specialist. If preliminary findings are inconclusive, intriguing, or of great practical importance, larvae can be cleaned by shaking them vigorously in water, fixing them in 70% ethyl

FIGURE 2-34. Adult male *Pulex irritans* (Siphonaptera), lateral view, showing the six long legs, the head, three thoracic segments, and the abdomen.

alcohol or 10% formalin, and submitting these specimens for expert identification. Precise identification in certain cases requires rearing the adult fly; instructions are provided under Sarcophagidae in an earlier section. Living larvae may be submitted for expert identification in addition to but not in lieu of fixed specimens; include these in a separate jar loosely packed in moist cotton.

ORDER SIPHONAPTERA, FLEAS

Adult fleas are wingless, laterally flattened insects that have long legs for jumping and a large abdomen (Figure 2-34). Fleas feed on the blood of such animals as dogs, cats, pigs, humans, rodents, and birds. Metamorphosis is complex, with three caterpillar-like larval stages and an enduring pupal stage enclosed in a silken cocoon. Certain hosts develop hypersensitive reactions to flea bites characterized by intense pruritus. A hypersensitive dog or human suffers intolerably from the bites of a small number of fleas that a normal individual would scarcely notice. Various species of fleas transmit plague *(Yersinia pestis)*, murine typhus *(Rickettsia typhi)*, rabbit myxomatosis virus, and feline parvovirus (Torres, 1941) and serve as intermediate hosts of the tapeworm *D. caninum* and the filariid nematode *Acanthocheilonema reconditum*.

Ctenocephalides

Identification

The ubiquitous *Ctenocephalides felis* and the relatively rare *Ctenocephalides canis* are parasites of a very wide range of domestic and wild mammals, including cats, dogs, cattle, and humans. *Ctenocephalides* have both genal and pronotal combs (Figure 2-35); this easily distinguishes them from *Echidnophaga* (Figure 2-36), *Xenopsylla* (Figure 2-37), and *Pulex* (Figure 2-38; see also Figure 2-34), which have neither genal nor pronotal combs, and from certain rodent fleas that have only pronotal combs. *Cediopsylla* (Figure 2-39), a rabbit flea, resembles *Ctenocephalides* in having both genal and pronotal combs but can be distinguished as follows. If a line drawn along the bases of the genal teeth runs parallel to the long axis of the head, the specimen is *Ctenocephalides*, whereas if it runs at an appreciable angle, it is *Cediopsylla*. Do not fail to recognize the eggs and larvae of fleas as such (Figures 2-40 and 2-41).

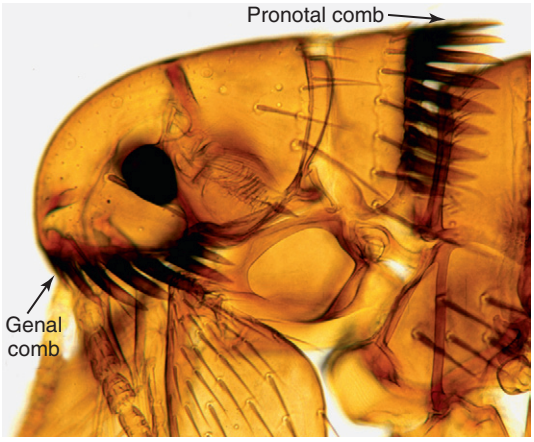

FIGURE 2-35. *Ctenocephalides* (Siphonaptera) of the cat and dog. The bases of the genal teeth of *Ctenocephalides* lie on a line running parallel to the long axis of the head, thus serving to distinguish this genus from certain rodent and leporid fleas that have both genal and pronotal combs.

FIGURE 2-36. *Echidnophaga* (Siphonaptera). *Echidnophaga gallinacea*, the poultry sticktight flea, may be found firmly attached in clusters on chickens' heads and on the eyelids or in the ear canals of dogs, cats, and other animals.

Ctenocephalides lay their eggs on the host. Especially in the case of dogs with thick and soiled haircoats, many of these 0.5-mm-long, glistening, white eggs may remain on the host long enough to hatch; so sometimes, not only adults but eggs and larvae of *Ctenocephalides* are found in the haircoat of infested dogs and cats.

Diagnosis of dog and cat flea infestation is sometimes difficult because only a few fleas are required to cause great misery, especially in a sensitized individual. Flea feces are essentially tiny particles of dried blood. The larval fleas eat their parents' feces, as well as other organic debris. Flea feces may be detected in the haircoat of a dog or cat by a sort of paper chromatography. Suspect detritus may be placed on filter paper or on other absorbent material that has been dampened with dilute soap or detergent solution. Hemoglobin will diffuse out of flea feces in a few minutes and will form a red halo around the speck of debris, or a similarly dampened pledget of absorbent cotton may be rubbed over the haircoat and skin to pick up particles of flea feces; little red spots appear on the cotton.

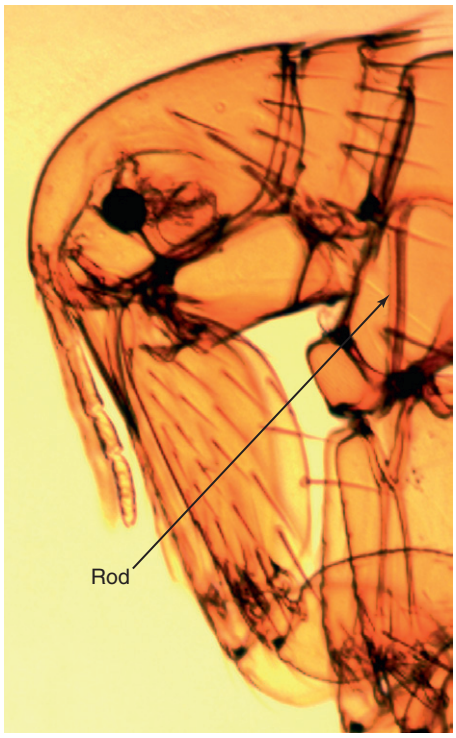

FIGURE 2-37. *Xenopsylla* (Siphonaptera), a rat flea and biologic vector of plague *(Yersinia pestis)* and endemic typhus *(Rickettsia typhi).* The vertical rod on the mesothorax distinguishes this genus from *Pulex*.

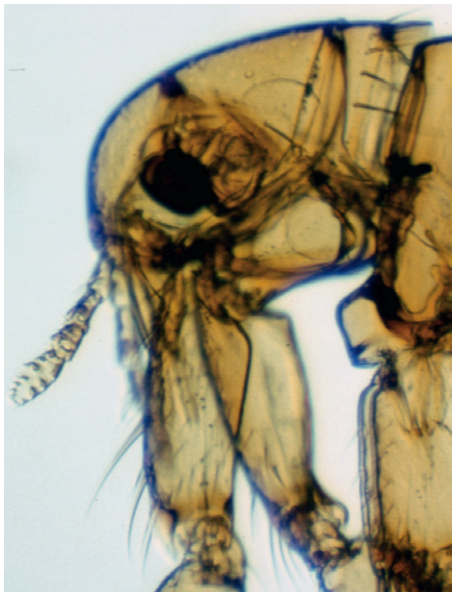

FIGURE 2-38. *Pulex* (Siphonaptera). *Pulex irritans*, the human flea, attacks a wide range of hosts.

Life History

Metamorphosis of fleas is complex, with life stages consisting of egg, larval stages one, two, and three, pupa, and adult (Figure 2-42). The adult *Ctenocephalides* displays little tendency to leave its dog or cat host unless the population approaches about 200. Then, a few fleas may get off occasionally, especially when their host comes in contact with another, possibly less parasitized individual. A common misconception is that *Ctenocephalides* fleas constantly jump on and off their hosts and find new hosts in this manner. In fact, most of the fleas a dog or cat acquires are brand new ones straight out of their pupal cases (Figure 2-43), and it is most important to remember this fact in connection with control efforts. For every flea on the host, there are many eggs, larvae, pupae, phorate pupae (pupal cases containing phorate, from the Greek for "cloaked," adults), and newly emerged adults in the environment, and these tend to be concentrated wherever the host habitually rests. The longer the host

FIGURE 2-39. *Cediopsylla* (Siphonaptera) of the rabbit. The bases of the genal teeth lie on a line running at an angle to the long axis of the head, thus serving to distinguish this genus from *Ctenocephalides*.

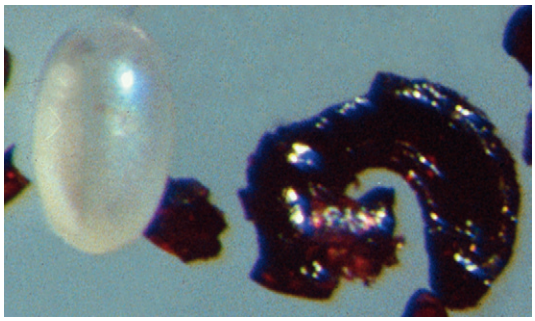

FIGURE 2-40. Egg of *Ctenocephalides* and two masses of flea feces. Flea feces consist essentially of dried host's blood and serve as food for the flea larvae, which have chewing mouthparts.

FIGURE 2-41. Larva of *Ctenocephalides*. Flea larvae are frequently overlooked or misidentified.

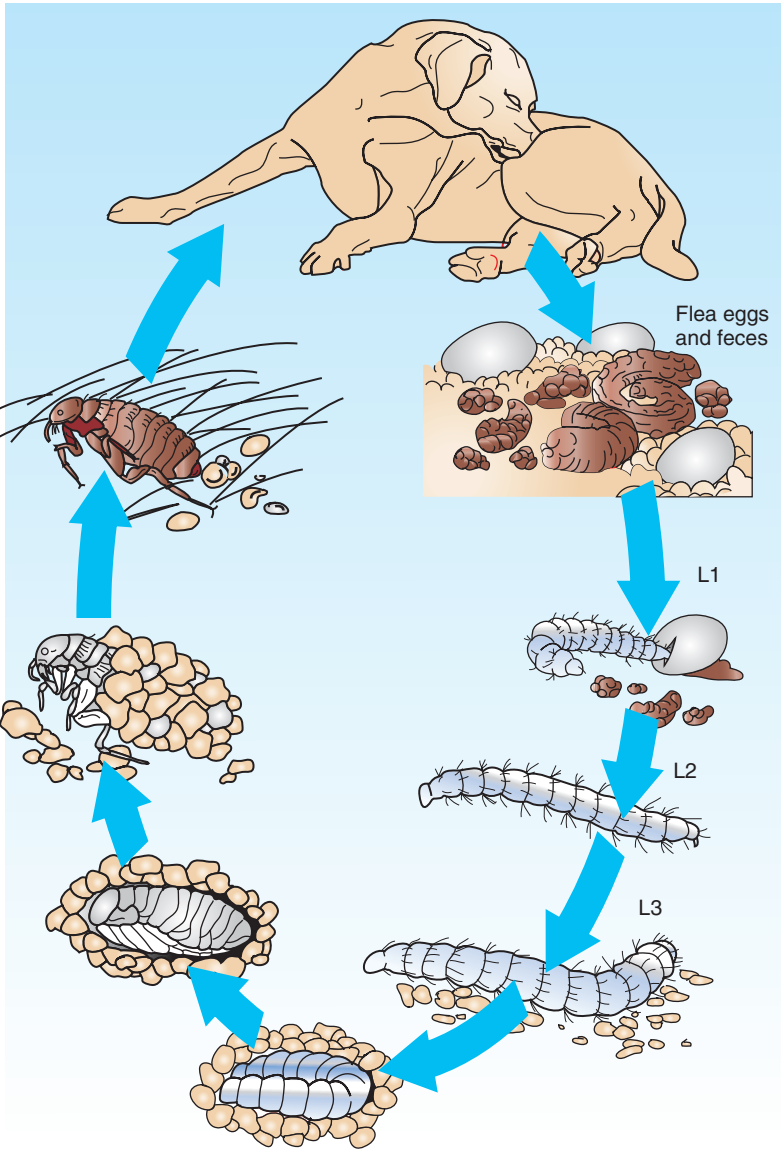

FIGURE 2-42. Life history of *Ctenocephalides felis*. Eggs appear 2 days after male and female fleas arrive on a cat or dog. Most eggs fall out of the pelage and tend to accumulate where the host habitually rests; first-stage larvae (L1) begin to hatch out of them on day 4. Larvae feed on adult flea feces, which, like the eggs, continuously rain down from the coat of an infested dog or cat, and pass through two molts. After about 2 weeks of warm, moist conditions, the third-stage larvae begin to spin cocoons and metamorphose into adult fleas (i.e., pupate). The cocoons are sticky so that fine debris, such as the sand grains in the picture, tends to accumulate on their surfaces. Adults begin to emerge from cocoons at 3 to 4 weeks, females preceding males by several days. Having found a dog or cat, the adult *C. felis* remains aboard, feeding repeatedly and reproducing until it is exhausted and dies or is nipped and swallowed by the host. *C. felis* rarely leaves a suitable host of its own accord.

stays in one place, the more eggs and adult flea feces will be deposited there. Flea feces serve as the principal food at the three larval stages. Development of *C. felis* from egg to adult occurs within the ranges of 13°C to 32°C and 50% to 92% relative humidity and requires from 14 to 140 days at the extremes of temperature. Temperatures above 35°C are lethal to larvae and pupae. Unfed adults may survive for many weeks under cool, humid conditions but probably cannot long withstand the low relative humidities associated with subfreezing conditions (Silverman, Rust, and Reierson, 1981). Unfed *Ctenocephalides* adults can survive for about 2 months while waiting for a host to happen by. People returning home after an absence of several weeks may be greeted by hordes of bloodthirsty fleas that, although preferring to feed on dogs, are quite willing to make do with humans when no dog is available. One of Dr. Georgi's mentors used to deal with this situation as follows. On arriving back in town, he would go directly to the kennel where his dog had been housed during his absence and would take the dog home to collect the hungry fleas that were sure to be lying in wait there. After a brief tour of the house, the dog was immediately taken back to the kennel for a flea bath while the rest of the family retook possession of the house.

Disease and Disease Transmission

C. felis can be pathogenic in its own right simply as a result of the amount of blood that the fleas extract from their hosts. Gravid female *C. felis* consume an average of 13.6 µL of blood per day

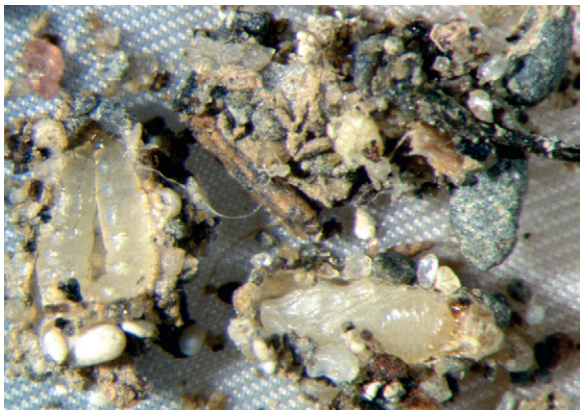

FIGURE 2-43. Cocoons of *Ctenocephalides felis*. *Left*, The cocoon has been opened to reveal the larva within. *Bottom right*, The cocoon shows a flea that has almost completed metamorphosis.

(Dryden and Gaafar, 1991), and 75 females would remove 1 mL of blood from an animal each day. *C. felis* has been reported as the cause of slow death by exsanguination of lambs, sheep, goats, calves, and a jenny (Dryden, Broce, and Moore, 1993; Yaphe, Giovengo, and Moise, 1993; Yeruham and Koren, 2003). It is highly likely that many kittens have succumbed to these infestations if they did not get adequate care (Yaphe, Giovengo, and Moise, 1993), and it is also very likely that many young puppies die yearly of these infestations. If neonatal hookworms are added into the mix, the hematopoietic system of a puppy would be hard-pressed to keep up with several hundred fleas.

C. canis and *C. felis* are true intermediate hosts (biologic vectors) of the tapeworm *D. caninum* and the filariid nematode *A. reconditum*. Fleas acquire *D. caninum* infection as larvae, because these are the stages that have chewing mouthparts suitable for ingesting solid material such as the eggs of this tapeworm. The cysticercoid that develops from the egg is passed along through metamorphosis to the adult flea and infects the dog or cat that chances to ingest that particular flea. Microfilariae of *A. reconditum* are ingested by the blood-feeding adult flea and develop into third-stage larvae capable of infecting a dog. Feline parvovirus, the causative agent of feline panleukopenia, can be transmitted from infected to susceptible cats by *C. felis* (Torres, 1941).

The cat flea has more recently been shown to play an important role in the transmission of other agents of disease in animals and people. It appears that *C. felis* has a role in the transmission of *Rickettsia typhi*, *Rickettsia felis*, and *Bartonella henselae* (Eisen and Gage, 2012). In suburban areas, it has been shown that the opossum, *Didelphis virginiana*, can serve as a reservoir of *R. typhi*, the murine typhus agent, which can be transmitted to people by *C. felis* (Green et al, 2011). *R. felis*, a relatively recently identified species of *Rickettsia* that sometimes causes infection with symptoms in people, is common in cats (also in opossums and dogs) and is commonly found in *C. felis* and other fleas (Eisen and Gage, 2012). It is suspected to make its way into cats and people via flea feces or, perhaps, by flea bite (Eisen and Gage, 2012). The cat flea is now known to be a source of flea feces containing the cat-scratch fever agent, *B. henselae*, and it is believed that disease in people is often introduced through the inoculation by cat scratch of flea feces containing these organisms into wounds caused by a cat having fleas and associated flea dirt (Eisen and Gage, 2012). Investigators (Lappin, 2011), using three groups of specific-pathogen–free cats housed such that contact could occur between pairs of groups, recently showed that if cats with *C. felis* were infected with *B. henselae* and were housed in a central cage, all cats housed on one side without flea prevention became infected with *B. henselae*, while cats housed on the other side and treated monthly with an imidacloprid-moxidectin combination product remained free of *B. henselae*. Thus, this work more firmly incriminates the flea as a vector and shows that flea prevention can aid in the prevention of transmission of *B. henselae*.

Treatment of Infestations With Ctenocephalides

Since the approval in 1994 of lufenuron as a direct-to-pet flea product with remarkable efficacy, which was rapidly followed by the similarly remarkably safe and efficacious fipronil and imidacloprid, a steady stream of new products and novel formulations provided by the pharmaceutical industry to veterinarians have altered the face of flea control in the United States. What had once been to a great extent the provenance of pest control operators became a commonly employed pet-directed control program that for small animals has proved amazingly successful. Dogs and cats never before had the capability of living the flea-free existence that they have enjoyed now for almost 20 years. The current pharmacopeia for flea control contains a few molecules that have been developed as Food and Drug Administration (FDA)-approved products that are internalized by the pet or as Environmental Protection Agency (EPA)-approved products that are topically distributed on the surface of the pet (see Chapter 6). Veterinarians and the public have come to expect these products to work very well, so the bar has been set very high, and the products do deliver.

Flea products approved by the FDA include lufenuron, nitenpyram, spinosad, and selamectin; the first three products are administered orally, and the last, selamectin, is applied topically but enters the bloodstream transdermally. All four products have been approved for cats. Lufenuron works by inhibiting chitin synthesis and is given to dogs and cats; when a flea feeds, the drug makes its way into the flea and then into the larvae, which then will fail to develop; it has no adulticidal action. This product persists in the body of the animal and is used as a monthly flea preventive or as an injectable 6-month formulation for cats. In treating dogs, lufenuron has been combined with milbemycin oxime to provide flea control along with heartworm prevention and treatment and control of internal nematode infections. Nitenpyram is a neonicotinoid insecticide that undergoes rapid oral absorption and rapidly spreads throughout the body of the dog and cat after ingestion; it is known for its very rapid knock-down. Nitenpyram is designed to be given daily as needed to kill adult fleas, and it is approved to be used in conjunction with lufenuron for flea control within a given environment. Spinosad is a product that kills adult fleas through activation of the nicotinic acetylcholine receptors of the flea at a distinct binding site. This product has been formulated to kill adult fleas for a full month after oral treatment, and for dogs, it has been combined with milbemycin oxime, when it functions as a month-long flea adulticide along with the heartworm prevention and internal parasite treatment and control provided by milbemycin oxime. Selamectin is a specially formulated macrocyclic lactone that is administered to animals for heartworm prevention and flea control. This formulation of a macrocyclic lactone is absorbed and then is redeposited in the dermal layer; it persists in the skin of the animal for an extended period to provide flea control.

Products that have been approved by the EPA for use on dogs and cats for flea control are fipronil, imidacloprid, dinotefuran, flumethrin, indoxacarb, pyriproxyphen, and methoprene; the products cyphenothrin and deltamethrin are for use on dogs only. The nature of the EPA approval means that these products are not internalized by the animal. Fipronil is active at the

gamma-aminobutyric acid (GABA)-gated chloride channel and kills adult fleas through contact and ingestion. Fipronil has been formulated into a topical product that provides flea control for a full month. Imidacloprid is another topical product designed for monthly administration that works by binding to nicotinic acetylcholine receptor sites. A third such monthly compound that has been formulated to be applied topically is dinotefuran, another neonicotinoid insecticide. These compounds are sometimes combined with an insect growth regulator—methoprene or pyriproxyphen. Fipronil has typically been combined with methoprene, and imidacloprid and dinotefuran have been combined with pyriproxyphen. These combination products then kill adult fleas and have deleterious effects on the larvae developing in the eggs and on the developing larvae themselves if they do succeed in hatching. Fipronil also has activity against ticks, although the other compounds do not; hence imidacloprid and dinotefuran have been mixed with permethrin products to provide tick control, but these products cannot be applied to cats. Fipronil has also been mixed with amitraz or cyphenothrin to increase its activity against ticks. Two long-acting collars are now available in the United States: one lasts 8 months and contains a mixture of imidacloprid for flea prevention and flumethrin, a second-generation pyrethroid, for tick control on dogs and cats; the other collar for dogs only contains the fourth-generation pyrethroid, deltamethrin, and provides 6 months of protection against both fleas and ticks. Imidacloprid has also been combined with topically applied and absorbed moxidectin in a product that is designed for monthly application to dogs and cats for flea prevention, heartworm prevention, and treatment and control of various intestinal parasites. Indoxacarb is a pro-insecticide that is rapidly metabolized to an active moiety in the flea; it is available as a stand-alone product for flea control on cats and is combined with permethrin to control fleas and ticks on dogs.

Environmental manipulation remains a major aid in controlling fleas, and it does not necessarily require chemicals. A vacuum cleaner will markedly reduce the numbers of eggs, larvae, pupae, and unfed adult fleas in an environment. (Make certain to close and dispose of the bag after vacuuming to prevent the fleas from escaping.) Control efforts should concentrate on places where the dog or cat habitually rests, because this is where eggs and flea feces, the provender of larvae, are most likely to be deposited, and the development of adult fleas is likely to follow. If cage-confined animals are kept in wire-bottom cages elevated at least 13 inches (33 cm) above the ground or floor, any fleas that develop underneath the cage will not be able to jump high enough to get back onto a host (Rothschild et al, 1973). This method has been used successfully in a commercial beagle breeding establishment housing several thousand dogs. Application of this latter environmental control method is clearly limited to strictly confined animals.

Although the excellent products listed above often can bring heavy flea infestations under control, it will sometimes be necessary to treat the environment with insecticides or other mechanical methods. Steam cleaning may be necessary, and it can sometimes help in larger areas. It may be necessary to clean an area and remove all rugs, blankets, sand, or sawdust where animals may have been resting. When in the outdoors, treatment should concentrate in areas that are shaded and out of direct sunlight. Yards and other buildings may be sprayed with cyfluthrin or fenvalerate pyrethroids, but treatments need to be applied as per label instructions.

Other available methods for the control of fleas differ widely in their apparent effects on flea populations under various conditions. Several traps are commercially available for the capture of adult fleas. Some of these traps collect more than 85% of released fleas, whereas others collect only slightly more than 10% (Dryden and Bruce, 1993). Brewer's yeast failed as a repellent to fleas on dogs when fed as a dietary supplement at the rate of 14 g/day (Baker and Farver, 1983). Ultrasonic flea collars also do not repel fleas from dogs, at least under certain laboratory conditions (Dryden, Long, and Gaafar, 1989).

FIGURE 2-44. *Echidnophaga gallinarum* on a chicken in Africa. (Photo courtesy Dr. Jennifer Harrison, DVM, Cornell University, Ithaca, New York, 2010.)

It is important to remember that all insecticides are toxicants, and they are safety tested only for certain animals. Thus, fipronil, which is very, very safe for dogs and cats, has been found to cause severe toxic reactions in rabbits treated for flea control. It is actually against the law to apply EPA-approved products except as stated on the label.

Echidnophaga

Echidnophaga gallinacea, the sticktight flea of poultry, attacks all kinds of domestic birds, as well as dogs, cats, rabbits, horses, and humans, in subtropical America. Dr. Georgi once found several embedded in the eyelids of a cat recently arrived in New York from Alabama. On birds, *E. gallinacea* embeds itself in the skin around the eyes and cloaca and on the combs, wattles, and other glabrous areas (Figure 2-44). These are small fleas with angular heads devoid of genal and pronotal combs; the thoracic tergites (dorsal sclerites of the thorax) are very narrow (see Figure 2-36).

Tunga

Tunga penetrans, the "jigger" or "chigoe," is a small (1-mm) flea of tropical America and Africa that somewhat resembles *Echidnophaga* in having an angular head and narrow thoracic segments and in lacking combs. The impregnated *Tunga* female embeds in the skin of the ankles, in the instep, and between the toes, with only the last few abdominal segments protruding (Figure 2-45). Eggs are retained in the abdomen, and the flea swells to the size of a pea. Lesions caused by this flea are painful and subject to secondary infection and are supposedly the inspiration for the sailor's oath, "I'll be jiggered" (Chandler and Read, 1961).

Xenopsylla

Xenopsylla is a widely distributed genus of rat fleas that also attack humans and are an important vector of plague *(Yersinia pestis)* and murine (endemic) typhus *(Rickettsia typhi)*. Combs are absent, and the head is smoothly rounded, thus distinguishing *Xenopsylla* from the foregoing genera; it differs from *Pulex* in having a vertical rod on the mesothorax (see Figure 2-37).

Disease Transmission

Plague is normally a disease of rodents caused by the bacterium *Yersinia pestis* and transmitted by various fleas, of which *Xenopsylla*

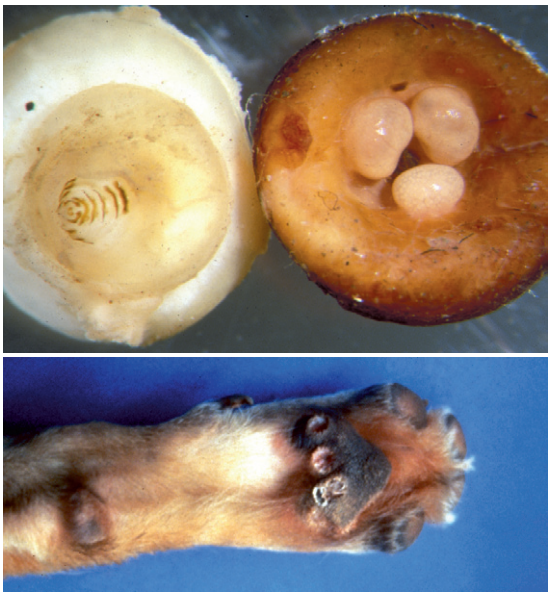

FIGURE 2-45. *Tunga penetrans*. *Top, Tunga* specimens from a goat and a pig in Ecuador. *Left*, The posterior end of the goat specimen shows normal terminal segments. *Right*, The anterior of the pig specimen has three large sacculations, with the head within enclosed in the space between them. *Bottom*, The paw of a dog with several of these fleas embedded in the skin.

cheopis stands out, especially in relation to human infection. The great plague pandemics that decimated civilization during the Middle Ages may have been precipitated by large-scale plague mortality among rodent cohabitants of humans, resulting in the vector turning to humans for its blood meals and communicating *Y. pestis* to them in the process.

Plague was introduced into North America, probably in the San Francisco, California, area, around 1900. It has spread throughout North America in the wild rodent and flea population and is now found in a large portion of the United States, basically throughout the west up until it stops parallel with the eastern boundary of the Texas panhandle. In the west, especially in the four-corners area of New Mexico, Arizona, Utah, and Colorado, plague is seen in people, dogs, and cats and typically is introduced by hitch-hiker fleas of rodents. It has been noted that some cases in people have been due to flea bites acquired while sleeping in bed with a dog that has brought fleas home on its haircoat (Gould et al, 2008). In areas such as these, it is probably prudent to maintain one's pet dog (and cat) on a flea product that provides activity against adult fleas.

Pulex

Pulex irritans, the human flea, is widely distributed and attacks a wide range of hosts, including humans, swine, and dogs. *Pulex* resembles *Xenopsylla* but lacks the mesothoracic rod (see Figures 2-34 and 2-38).

ORDER PHTHIRAPTERA, LICE

Two main kinds of lice are represented by the suborder Anoplura, or bloodsucking lice, and by three suborders—Ischnocera, Amblycera, and Rhychophthirina—that are for simplicity grouped here under the heading Mallophaga, or chewing lice (Table 2-6). Anoplurans have piercing mouthparts consisting of three stylets that, in fixed specimens, are usually concealed within the relatively narrow head (Figure 2-46). Anoplurans are parasites of placental animals only. Mallophagans have stout mandibles on the ventral side of their relatively broad heads (Figure 2-47), and these lice feed on epidermal scales, feathers, and sebaceous secretions of birds and mammals. Both anoplurans and mallophagans spend

TABLE 2-6 Lice Found on Domestic Animals and Humans

Host	Anoplura	Mallophaga
Dog	*Linognathus setosus* *Heterodoxus spiniger*	*Trichodectes canis*
Cat	None	*Felicola subrostratus*
Cow	*Haematopinus eurysternus* *Haematopinus quadripertusus* *Haematopinus tuberculatus* *Linognathus vituli* *Solenopotes capillatus*	*Damalinia bovis*
Horse	*Haematopinus asini*	*Damalinia equi*
Pig	*Haematopinus suis*	None
Sheep	*Linognathus ovillus* *Linognathus pedalis* *Linognathus africanus*	*Damalinia ovis*
Goat	*Linognathus africanus* *Linognathus stenopsis*	*Damalinia caprae* *Damalinia crassipes* *Damalinia limbata*
Rat	*Polyplax spinulosa*	None
Mouse	*Polyplax serrata*	None
Guinea pig	None	*Gliricola porcelli* *Gyropus ovalis* *Trimenopon hispidum*
Human	*Pediculus humanus capitus* *Pediculus humanus humanus* *Pthirus pubis*	None

FIGURE 2-46. Head and thorax of an anopluran louse. The bloodsucking stylets occupy the median plane of the head; the mouth is at the arrow.

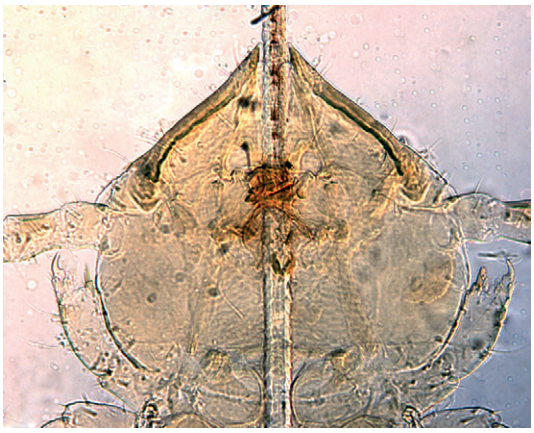

FIGURE 2-47. Mandibles of a mallophagan louse, *Felicola subrostratus*, grasping a cat hair.

FIGURE 2-48. *Haematopinus eurysternus* (Anoplura) of cattle. All tarsal claws are of equal size.

their entire lives among the hairs or feathers of their hosts and display a high order of host specificity. Even the eggs are securely attached to the hairs or feathers of the host (see Figure 2-49). The lice that hatch from these eggs are tiny replicas of the adults; they molt several times but undergo only minor changes in appearance (i.e., **simple metamorphosis**). The cycle from egg to egg requires several weeks, and only one or two eggs may be found developing within the abdomen of a female louse at any one time, but enormous populations may develop notwithstanding. The hatching process itself is of passing interest. The young louse swallows air and ejects it through its anus to form a cushion of compressed air that forces the animal against the operculum (i.e., lid) of the eggshell until it pops open. Thus it may be said (with due application of etymology and low humor) that "every louse is hoisted by its own petard."

Because of the sedentary habits of lice, one searches for them by carefully examining the haircoat or plumage of the host. The only exception to this generalization, the human body louse *Pediculus humanus humanus*, clings to the fibers of the clothing instead of body hairs while it feeds on its host. With a little practice, bloodsucking lice and chewing lice can be distinguished by inspection. This plus high host specificity simplifies identification, especially for hosts that have only one species of louse (e.g., *Haematopinus suis* on *Sus scrofa*, *Felicola subrostratus* on *Felis catus*). The next simplest case involves one anopluran and one mallophagan species per host species (e.g., *Haematopinus asini* plus *Damalinia equi* on *Equus caballus,* and *Linognathus setosus* plus *Trichodectes canis* on *Canis familiaris*). Cattle *(Bos taurus)* present a more complex case; they are infested by three anoplurans and one mallophagan, and attention to generic morphologic characteristics is required for their differentiation. Occasionally, a few lice are collected from sources other than their normal host. For example, *Pthirus pubis*, the human crab louse, has been reported now and again on dogs. In such cases, it is necessary to note the obvious morphologic differences displayed by *L. setosus*, the anopluran normally found on dogs, and *P. pubis*, denizen of human pubic hairs, to avoid misdiagnosis.

Lice are well-adapted parasites and are usually more of a nuisance than a threat to their hosts. The role of the human body louse in the spread of *Rickettsia prowazekii*, the causative agent of epidemic typhus, is an outstanding exception, and a few other examples of lice serving in the role of biologic vectors and intermediate hosts can be cited. Lice can also be mechanical vectors; the hog louse, *Haematopinus suis*, and other blood-feeding arthropods serve as mechanical vectors of swinepox virus, which can persist in *H. suis* for up to a year (Schang, Pave, and Larroux, 1952). It takes a large population of lice to directly drain the vitality of their host, and usually contributory conditions such as stress of inclement weather, crowding, poor nutrition, and individual diathesis can be demonstrated in cases of clinical illness related to louse infestation. If a very large number of lice are found on cattle, on a puppy, or on a stock of laboratory rats, there is something wrong in the way the animals are kept, and merely spraying insecticide to kill the lice falls far short of full clinical management of the case.

Suborder Anoplura

Anoplurans—about 400 some species—have pincer-like tarsal claws for clinging to the hairs of their hosts. The size of these claws is related to the diameter of the hair shaft and is probably an important factor in establishing host specificity and site specificity. Without hair, these lice are helpless; they pass from host to host most efficiently when a "bridge" of hair exists between host individuals. This is why *P. pubis* is frequently transmitted during sexual intercourse. According to Chandler and Read (1961), the French call this parasite "papillon d'amour."

Haematopinus

All tarsal claws are of equal size, and the lateral margins of the abdomen are heavily sclerotized (Figure 2-48). The two other anopluran genera found on cattle, *Linognathus* and *Solenopotes*, differ in having smaller claws on their first pair of legs. Species of *Haematopinus* that infest domestic animals include *H. asini* of horses, *H. suis* of swine (Figure 2-49 and see Figure 7-103), and *Haematopinus eurysternus*, *Haematopinus quadripertusus*, and *Haematopinus tuberculatus* of cattle. *H. eurysternus* is a common parasite of domestic cattle *(B. taurus)* in North America and tends to concentrate on the neck, poll, brisket, and tail, but in heavy infestation it may be generally distributed over the body. *H. quadripertusus*, normally a tropical and subtropical parasite of *Bos indicus* and *B. indicus–B. taurus* hybrids, lays its eggs in the tail switch but may be found

FIGURE 2-49. *Left*, *Haematopinus suis* (Anoplura) of swine. *Right*, Two *Haematopinus asini* clinging to horse hairs, and several operculate eggs glued to the hair of the equine host.

around the eyes and long hairs of the ears (Roberts, 1952). *H. tuberculatus* is an Old World parasite of water buffalo *(Bubalus bubalus)* and of domestic cattle associated with them (Meleney and Kim, 1974).

Heavy infestations of *H. eurysternus* are capable of causing severe anemia in adult range cattle (Peterson et al, 1953). Certain individuals are predisposed to the growth of large populations of lice, whereas other members of the same herd support only light infestations. These "louse breeders," as they are called, are likely to perish during winter storms, weakened as they are by their louse burdens. Such animals may be saved by insecticide applications. The rate of increase in hematocrit is, however, considerably slower than one would expect in a simple blood loss anemia.

Linognathus

Unlike *Haematopinus*, the first pair of tarsal claws of *Linognathus* is smaller than the second and third pairs, and the lateral margins of the abdomen are not heavily sclerotized (Figure 2-50). *Linognathus* differs from *Solenopotes* in having more than one row of setae per abdominal segment and in lacking a sternal plate and protuberant abdominal spiracles. Species of *Linognathus* infesting domestic animals include *Linognathus vituli* of cattle; *Linognathus ovillus*, *Linognathus pedalis*, and *Linognathus africanus* of sheep; *Linognathus stenopsis* and *L. africanus* of goats; and *L. setosus* of dogs and foxes (Figure 2-51).

Solenopotes

Solenopotes capillatus, the "little blue louse" of cattle, is distinguished from *Linognathus* in having only one row of setae per abdominal segment, a sternal plate at least half as wide as it is long, and protuberant abdominal spiracles (Figure 2-52).

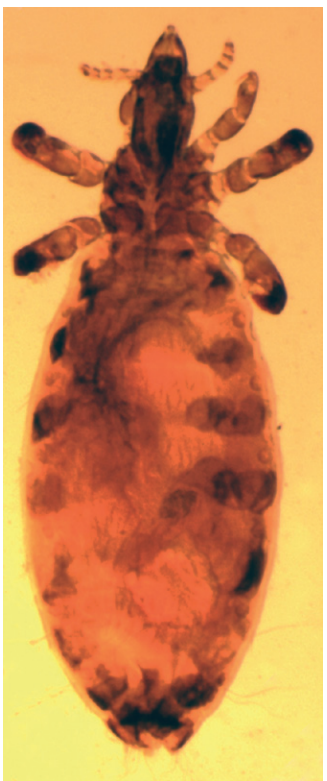

FIGURE 2-50. *Linognathus vituli* (Anoplura) of cattle. The first pair of tarsal claws is smaller than the second and third pairs. Spiracles are flush with the surface of the abdomen, and more than one row of setae is present per abdominal segment.

Polyplax

Polyplax spinulosa is a parasite of the rat, and *Polyplax serrata* is a parasite of the mouse (Figure 2-53 and see Figure 7-113). Both of these anoplurans may develop into serious nuisances in laboratory animal colonies and, when sufficiently abundant, may even bleed animals to death (Figure 2-54). Treatment of infested rats has been performed with the topical spray application of fipronil (Diaz, 2005).

FIGURE 2-51. *Linognathus setosus* (Anoplura) of dogs and foxes.

Pthirus

The large tarsal claws of *P. pubis* (Figure 2-55) are adapted to the coarse hairs of the pubic and perianal regions, armpits, mustache, beard, and, particularly in young children, the eyebrows and eyelashes; these latter two furnish the nearest approximation to a pubic

FIGURE 2-52. *Solenopotes capillatus* (Anoplura) of cattle. The first pair of tarsal claws is smaller than the second and third pairs. Spiracles protrude above the surface of the abdomen, and only one row of setae is present per abdominal segment.

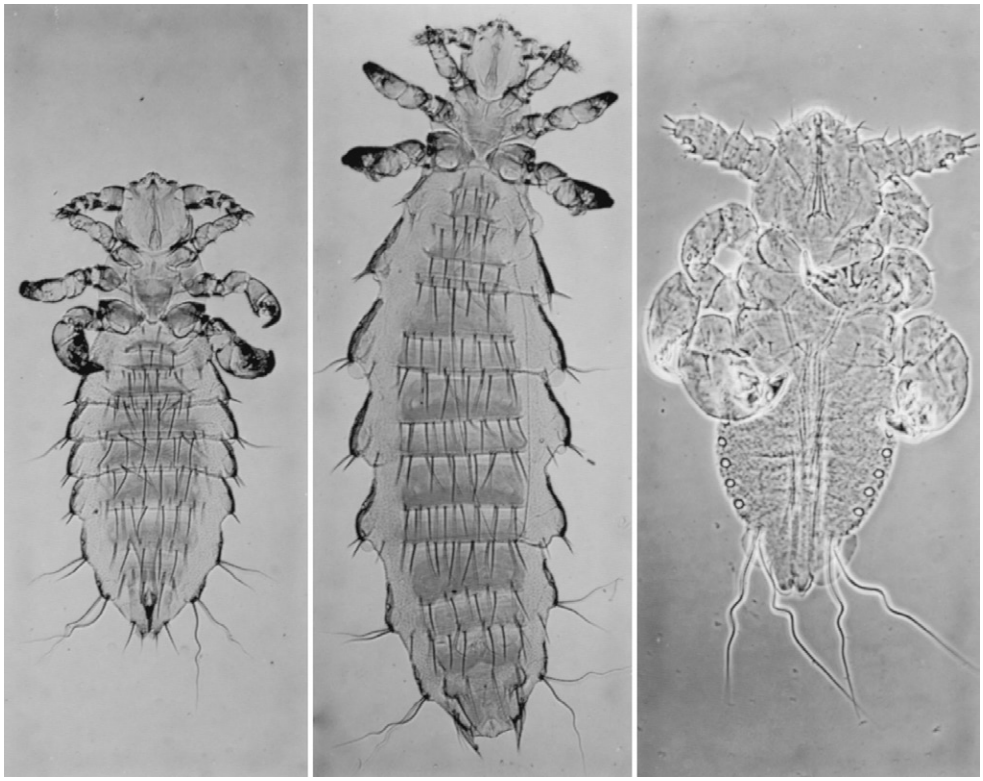

FIGURE 2-53. *Polyplax serrata* (Anoplura) of the mouse. *Left*, Male. *Center*, Female. *Right*, Nymph.

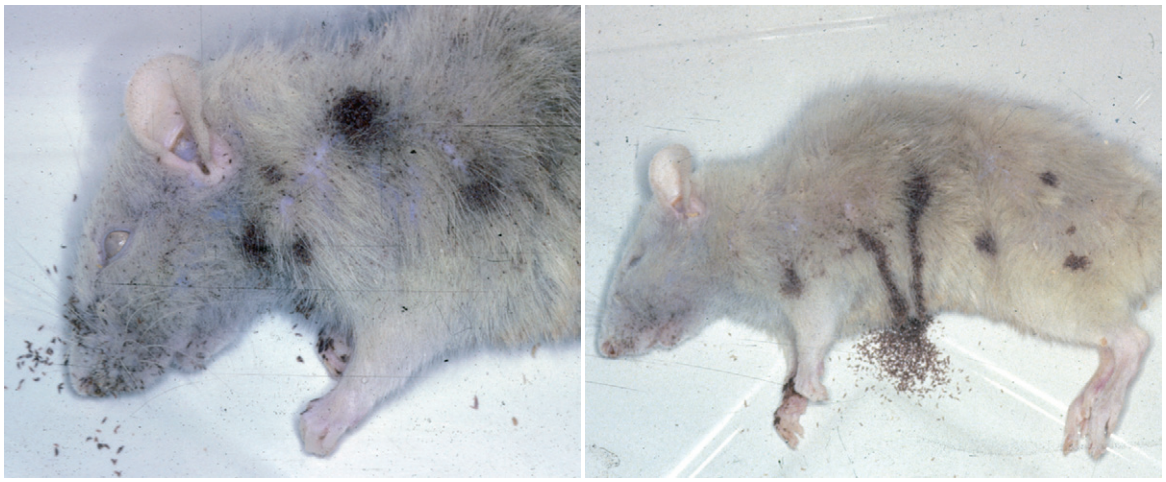

FIGURE 2-54. *Polyplax spinulosa* leaving a rat that died of the effects of its louse population. Urged on by the heat of an incandescent bulb, these lice are emulating their host's legendary tendency to flee from unpromising situations. This is a general phenomenon among the more mobile ectoparasites and can be exploited to advantage in diagnosis. However, if it is necessary to euthanize the host, do not use chloroform, ether, or other agents that will surely kill the parasites as well as their hosts.

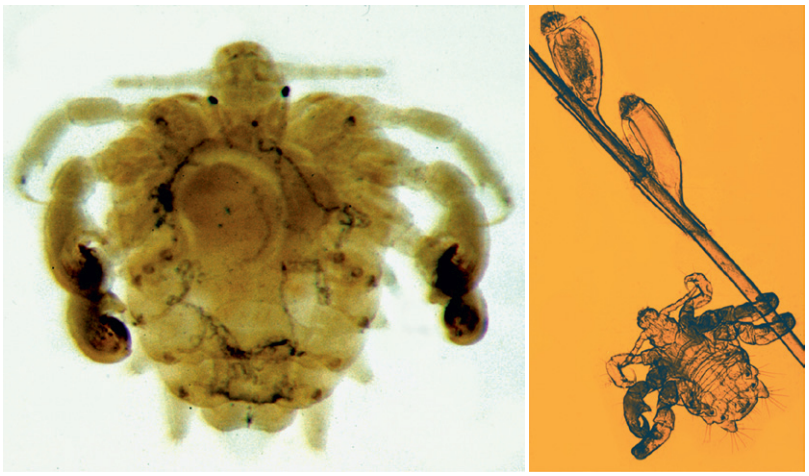

FIGURE 2-55. *Pthirus pubis* (Anoplura). *Left*, An adult female human crab louse. *Right*, A louse and two eggs on a pubic hair. Dogs occasionally acquire *Pthirus pubis* by contact with infested humans or their clothing.

hair that a child has to offer. Pruritus is intense, and a papular dermatitis with discoloration of the skin develops. Once feeding, these lice display a marked disinclination to move and tend to remain fixed at one point for days while their feces accumulate about them. The life cycle requires about 1 month from egg to egg, so that considerable time may elapse between acquisition and awareness of infestation. Sexual contact is the principal means of transmission between individuals, but often towels, clothing, and bedding used by an infested person are blamed for a bit of social dalliance. On some occasions, fomites and close contact are probably responsible for other family members or the family dog developing infestations with this parasite. During crises of this sort, the dog may be presented to the veterinarian for euthanasia in the mistaken belief that the dog is the culprit and reservoir of pestilence. Dealing with a family outbreak of crab lice and a falsely incriminated dog requires considerable tact.

Pediculus

The human head louse, *Pediculus humanus capitis*, stays mainly on the human head, especially around the ears and the nape of the neck (Figure 2-56). Dogs are rarely infested. Eggs are attached firmly to the hairs and hatch within a week. Infestation spreads rapidly because of the ease with which hairs are shed and wafted about. Outbreaks of head lice may occur under the best conditions of sanitation and personal deportment. The human body louse, *Pediculus humanus humanus*, does not cling to hair. Instead, this louse clings to the fibers and deposits its eggs in the seams of clothing. Except in very heavy infestations, all people need do to be rid of body lice is to remove their clothing. When people are unable to bathe and change clothing for extended periods, as, for example, during wars and natural disasters, body louse populations are likely to expand rapidly. Under such circumstances, epidemic typhus *(R. prowazekii)*, which is transmitted by the body louse, is likely to break out, and it is not for mere comfort's sake that vigorous delousing measures must be adopted.

Humans and gorillas share species of pubic lice (*P. pubis* on people and *Pthirus gorillae* on gorillas), but none are found on chimpanzees (Reed et al, 2007). At the same time, chimpanzees are host to *Pediculus schaeffi*, humans are host to *P. humanus*, and gorillas are not parasitized by species in this genus.

FIGURE 2-56. *Pediculus humanus capitis* (Anoplura), the human head louse, collected from a child attending public school in Ithaca, New York.

FIGURE 2-57. *Damalinia (Holokartikos) crassipes* (Mallophaga: Ischnocera) of the goat. Typical of ischnocerans parasitizing mammals, *D. crassipes* has three-segmented antennae.

The Mallophaga

Some 4000 species of mallophagans, or chewing lice, are parasites of birds and mammals. All bird lice are biting lice, and many species of them are known. Mallophagans ingest a variety of epidermal materials. Some readily ingest feather keratin and can be cultured on this substance in vitro. A few, such as *Heterodoxus spiniger* of the dog and related amblyceran parasites of birds, are blood feeders (Agarwal, Chandra, and Saxena, 1982).

Because their hosts are insectivorous and very fastidious, bird lice are in constant danger of being eaten by their host instead of vice versa. However, they tend to be far less sluggish than their relatives that parasitize mammals; many have long legs to help them keep "one step ahead," and they frequently develop enormous populations. Mallophagans may cause their hosts considerable irritation when present in large numbers, especially in situations in which it is difficult for the animals to groom themselves, as in the case of stanchioned cattle. The three suborders of chewing lice are Ischnocera, Amblycera, and Rhynchophthirina.

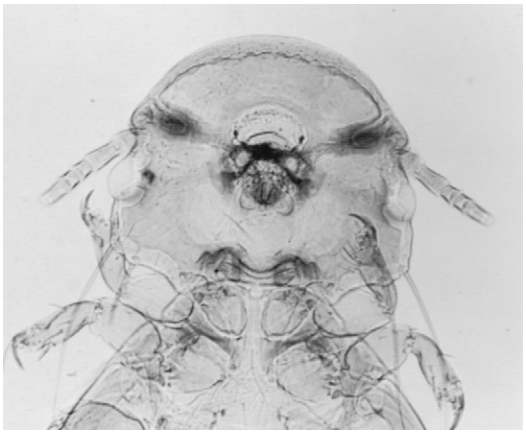

FIGURE 2-58. *Goniocotes* sp. (Mallophaga: Ischnocera) of the chicken. Typical of ischnocerans parasitizing birds, *Goniocotes* has five-segmented antennae.

Suborder Ischnocera

Ischnocerans have salient antennae that are three-jointed in species infesting mammals (Figure 2-57) and five-jointed in species infesting birds; all lack maxillary palps (Figure 2-58).

DAMALINIA (***BOVICOLA***). Species infesting domestic mammals include *Damalinia bovis* on cattle, *Damalinia equi* (also goes by the name *Werneckiella equi*) on horses (see Figure 2-45), *Damalinia ovis* on sheep, and *Damalinia caprae*, *Damalinia limbata*, and *Damalinia (Holokartikos) crassipes* on goats.

TRICHODECTES. *T. canis*, the canine chewing louse (Figure 2-59 and see Figure 7-49), may serve as intermediate host (cyclodevelopmental vector) of the tapeworm *Dipylidium caninum*, although fleas of the genus *Ctenocephalides* are far more important in this respect. *T. canis* must be differentiated from the anopluran *L. setosus* and from the warm climate amblyceran, *H. spiniger*.

FELICOLA. *F. subrostratus* is the only louse found on cats (Figure 2-60). This louse is characterized by the triangular shape of the anterior portion of the head.

Suborder Amblycera

Amblycerans have club-shaped antennae that lie in grooves in the head and four-segmented maxillary palpi (Figure 2-61). Many amblycerans are parasites of birds, but one species, *H. spiniger*, is a parasite of dogs in warm climates, and three species—*Gliricola porcelli*, *Gyropus ovalis*, and *Trimenopon hispidum*—are parasites of the guinea pig (Figure 2-62 and see Figure 7-117).

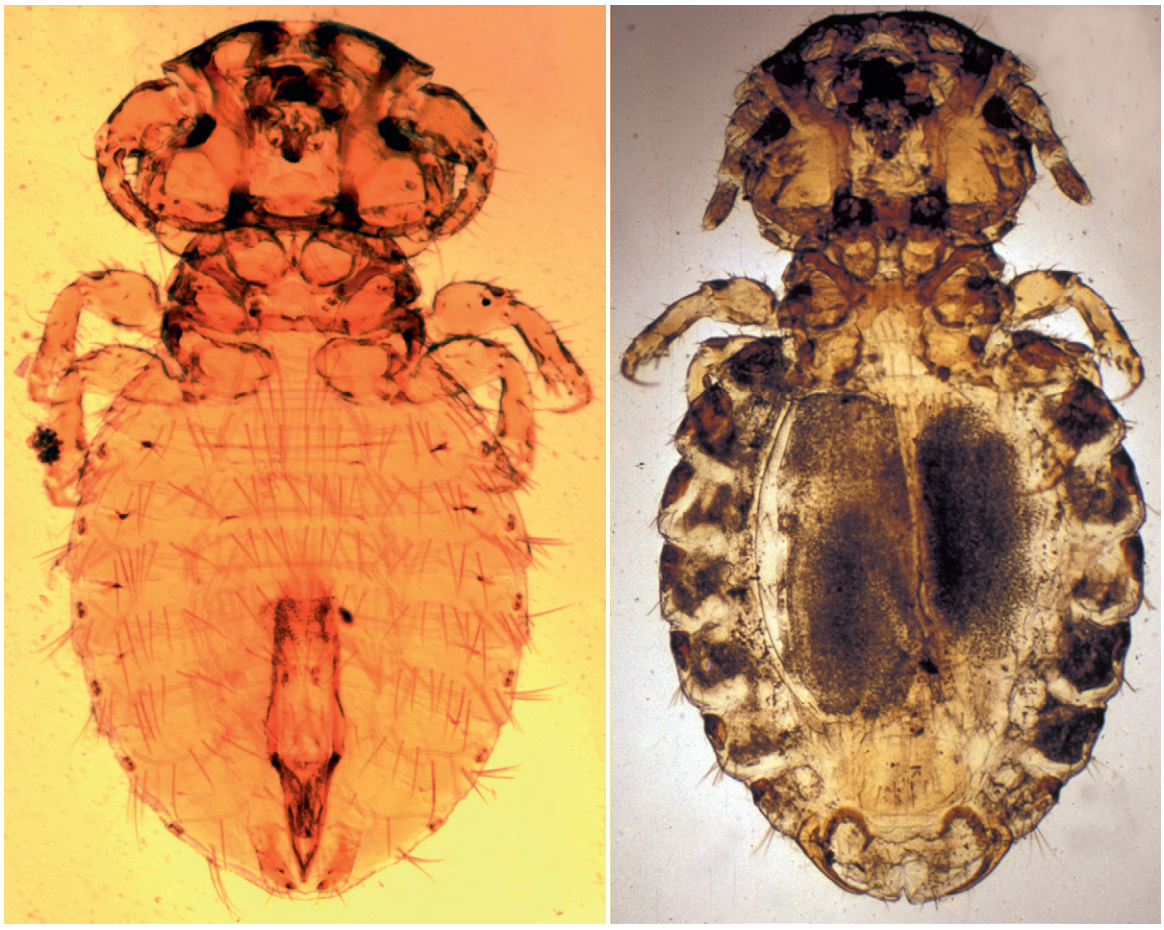

FIGURE 2-59. *Trichodectes canis* (Mallophaga: Ischnocera) of the dog. Male on left, female on right.

FIGURE 2-60. *Felicola subrostratus* (Mallophaga: Ischnocera) of the cat.

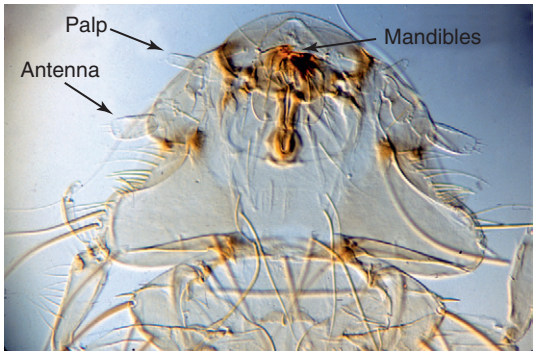

FIGURE 2-61. *Menopon* sp. (Mallophaga: Amblycera) of the chicken.

Suborder Rhynchophthirina

Haematomyzus species are parasites both of Asian and African elephants and of wart hogs (Figure 2-63). The preferred location on elephants is the posterior aspect of the ears and adjacent areas of the head and neck.

TREATMENT OF LOUSE INFESTATIONS

Dogs and Cats

Topical monthly application products have been found to be very efficacious in treating mallophagan infestations in dogs and cats. Selamectin has been shown to have high efficacy in the treatment of lice on dogs and cats (Shanks et al, 2003). *Trichodectes canis* has been shown to be treated with both fipronil and imidacloprid

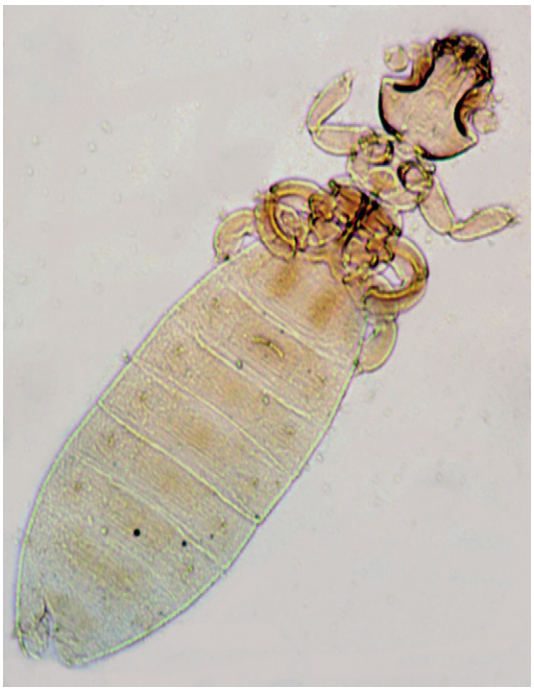

FIGURE 2-62. *Gliricola porcelli* (Mallophaga: Amblycera) of the guinea pig.

FIGURE 2-63. *Haematomyzus elephantis* (Mallophaga: Rhynchophthirina) of the elephant.

(Hanssen et al, 1999; Pollmeier et al, 2002). Fipronil has also been shown to be highly efficacious against *F. subrostratus* (Pollmeier et al, 2004). Lice are readily controlled with carbaryl-containing shampoo, spray, or dip. Usually two treatments are adequate when applied at an interval of 1 week.

In the case of dogs, the anopluran *Linognathus setosus* has been very successfully treated with both imidacloprid and selamectin (Gunnarsson, Christensson, and Palmer, 2005; Hanssen et al, 1999).

Beef and Nonlactating Dairy Cattle

Most cases of louse infestation in cattle are mild and are manifested only by occasional scratching and restlessness on the part of the animals. However, as populations increase through the winter and early spring, the degree of irritation to the animals (and to any sympathetic observer) verges on unbearable, and treatment must be carried out. Coumaphos, chlorpyrifos, and tetrachlorvinphos as sprays, dips, or pour-ons provide excellent control of lice. The macrocyclic lactones administered subcutaneously are highly effective against anopluran infestations in cattle. The pour-on formulations of the macrocyclic lactones also provide good efficacy against *D. bovis*. It has been shown in New York State that calves housed in outdoor hutches have markedly lower louse infestation rates than calves held in collective stalls or pens in barns (Geden, Rutz, and Bishop, 1990).

Dairy Cattle

Tetrachlorvinphos, synergized pyrethrins, permethrin, and coumaphos are applied to lactating dairy cows as sprays, in dust bags, in backrubbers, and as sprinkle-on dusts. Two applications should provide good control. Eprinomectin is efficacious against louse infestations of lactating cows.

Swine

Coumaphos and tetrachlorvinphos provide good control of lice when applied as sprays or poured on the topline from shoulders to hips. It is good practice when treating swine to apply insecticide also to the bedding of the holding pens. Usually two applications are adequate. Ivermectin, doramectin, and moxidectin all have excellent efficacy against *H. suis*.

Horses

Lice are found on horses principally during winter and spring. Two spray applications of coumaphos 2 weeks apart should provide adequate control. In cold weather, dusting horses with synergized pyrethrins is a less stressful procedure.

Elephants

Treatment of *Haematomyzus elephantis* infestations with oral administration of ivermectin at doses in the range of 0.059 to 0.087 mg/kg body weight was found to be highly effective (Karesh and Robinson, 1985).

Guinea Pigs

Guinea pigs have been successfully treated for infection with *Gliricola porcelli* with a single topical application of a solution containing 10% (w/v) imidacloprid and 1% (w/v) moxidectin (Kim et al, 2008).

Humans

Treatment for lousy people is to be done under the supervision of a human physician. However, the veterinarian has a role in protecting pets from the all too common implication that they are the source of the human infestation. People get their lice from other people. Treatments for people containing various insecticides are typically available in the form of creams, lotions, or shampoos; in the United States, these products can usually be procured as over-the-counter products. Usually one application suffices, but treatment may need to be repeated with heavy infestations. Lice and their eggs are killed by exposure to a temperature of 50°C for 30 minutes, so sufficiently rigorous laundering can be an effective adjunct in control (Kraus and Glassman, 1976). If a home has an infestation of some of the family members, toys, brushes, combs,

and so on can be placed in the clothes dryer in a pillowcase and dried to destroy any lice or eggs that might be present on these items.

ORDER HEMIPTERA, BUGS

Hemipterans have two pairs of wings (which may be vestigial), a triangular shield between the wing bases, four-segmented antennae, and a three-segmented beak that is directed caudally beneath the head when not in use (Figures 2-64 and 2-65).

Development occurs by means of **simple metamorphosis.** Some hemipterans feed on plants; some kill insects and suck their juices; and some are bloodsuckers and pests of rodents and humans, occasionally attacking other animals. Predacious reduviids (assassin bugs) inflict painful bites, and many such species have been reported to attack humans, but the bites of the more specialized parasitic reduviids (cone-nose bugs) and cimicids (bedbugs) are painless.

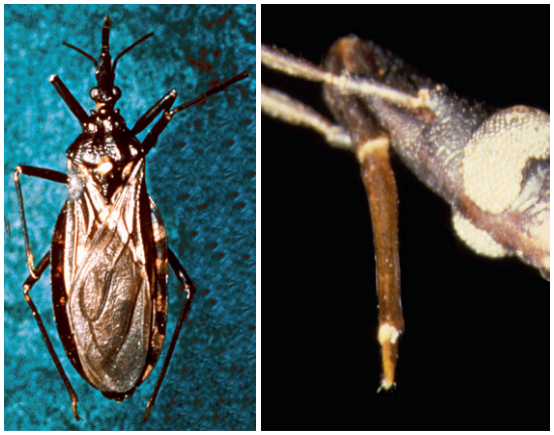

FIGURE 2-64. Triatomin bug (Hemiptera: Reduviidae), an assassin bug. Vector of *Trypanosoma cruzi* in North America. *Right*, The proboscis of the bug that is partially inserted into the host when the bug feeds.

FIGURE 2-65. *Cimex lectularius* (Hemiptera: Cimicidae), the bedbug.

Family Reduviidae, Assassin Bugs, and Kissing or Cone-Nose Bugs

The reduviids (see Figure 2-64) have wings and a characteristic three-segmented beak. The parasitic species of the subfamily Triatominae, which feed exclusively on the blood of vertebrates, have a more slender beak than the predatory species and are able to feed painlessly enough so as not to awaken a sleeping host. They hide in crevices by day and attack their sleeping hosts by night in the manner of bedbugs, argasid ticks, and some species of mesostigmatid mites. Triatominae of the genera *Triatoma*, *Rhodnius*, and *Panstrongylus* transmit American trypanosomiasis or Chagas' disease *(Trypanosoma cruzi)*. *T. cruzi* is transmitted in the feces of the bug; therefore this is defined as transmission via the **posterior station.** This definition is provided for the purpose of distinguishing this type of transmission from the **anterior station** transmission (via mouthparts and bite) of trypanosomes by tsetses and a few trypanosomes, such as *Trypanosoma rangeli*, which are transmitted by the bites of triatomin bugs.

Examination of triatomin bugs in Texas revealed bugs in 97 of 254 Texas counties and bugs infected with *T. cruzi* in 48 counties (Kjos, Snowden, and Olson, 2009). *Triatoma gerstaeckeri* was the most common bug, followed by *Triatoma sanguisuga*. *T. gerstaeckeri*, *T. sanguisuga*, and *Triatoma lecticularia* were associated with human dwellings, and when collected in or near those dwellings, half of the bugs were found infected with *T. cruzi*. The bug *T. sanguisuga* may also play a minor role in the transmission of equine encephalomyelitis.

Family Cimicidae, Bedbugs

Bedbugs (see Figure 2-65) have oval, dorsoventrally flattened bodies, vestigial wings, three-segmented beaks, and a disagreeable odor. They are nocturnal and secretive bloodsucking parasites of humans, chickens, bats, and nesting birds. Like triatomids, bedbugs hide in crevices by day and attack their sleeping host at night. They lay their eggs in their hiding places and molt five times at approximately weekly intervals, taking one blood meal between molts and another before egg laying. Bedbugs can endure starvation for several months. Although such a blood-feeding pattern as this would seem ideally suited to the transmission of disease organisms, bedbugs, although frequently indicted, have yet to be convicted on any such counts.

There is every reason to believe that bedbugs would feed on dogs and cats in a household if they were available. Bedbugs typically do not stay upon the host on which they feed, but leave after completing their bloodmeal. However, in Scotland, bedbugs were found in larger numbers by an owner on a long-haired cat; in fact, the way the owners found they had a bedbug infestation was by holding and petting their cat (Clark, Gilleard, and McGoldrick, 2002).

ORDER BLATTODEA, COCKROACHES

Cockroaches are important as intermediate hosts of certain parasitic worms such as the spirurid nematodes *Spirura*, *Oxyspirura*, and *Gongylonema;* the acanthocephalans *Moniliformis*, *Prosthenorchis*, and *Homorhynchus;* and the pentastomid *Raillietiella*. They also serve as mechanical vectors of filth-borne diseases of humans. Inspection of premises where food is prepared is often a veterinary function. Presence or absence of cockroaches is an important criterion of the adequacy of food sanitation (Figure 2-66).

ORDER COLEOPTERA, BEETLES

Beetles have hard, shell-like outer wing covers called **elytra** that lack venation (Figure 2-67). Development occurs via **complete metamorphosis;** the larvae are grubs.

FIGURE 2-66. A cockroach, *Periplaneta americana* (Blattaria).

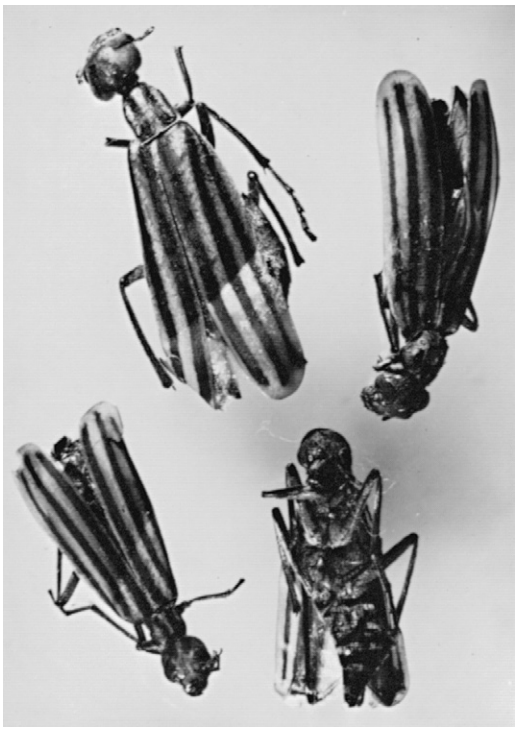

FIGURE 2-68. *Epicauta* sp. striped blister beetles. Consumption of alfalfa hay containing dead striped blister beetles causes acute cantharidin toxicosis in horses. (Courtesy Dr. R.J. Panciera.)

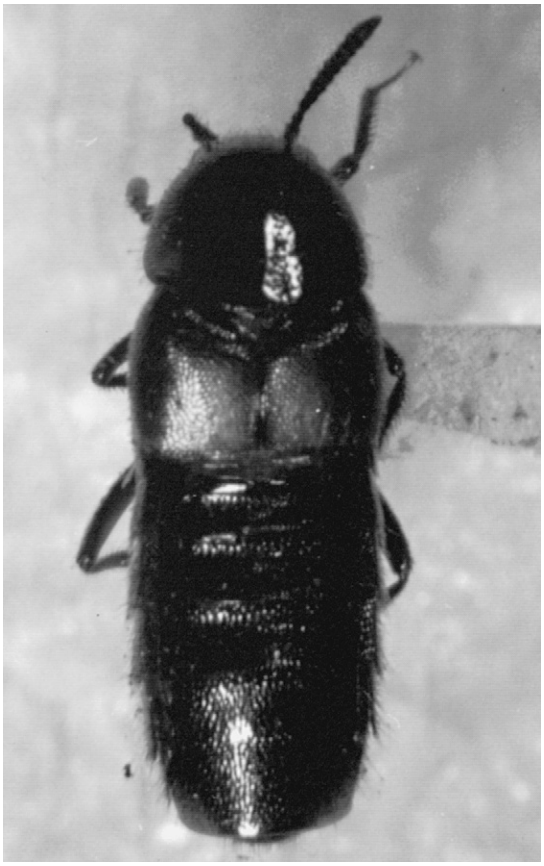

FIGURE 2-67. A beetle, *Aleochara bimaculata* (Coleoptera; Staphylinidae). This beetle is an ectoparasite on horn fly and face fly pupae as a larva and feeds on fly eggs as an adult. The elytra of this beetle cover only the anterior portion of the abdomen.

Beetles, like cockroaches, are important as intermediate hosts of parasitic worms that infect domestic animals and humans. The spirurid nematodes *Gongylonema* and *Physocephalus*, the acanthocephalans *Macracanthorhynchus* and *Moniliformis*, and the cestodes *Hymenolepis* and *Raillietina* (not to be confused with the pentastomid *Raillietiella* or, for that matter, with the mesostigmatid *Raillietia)* all develop in beetles to the stage infective for the vertebrate host.

Some species of beetles are extremely toxic. For example, **blister beetles** (*Epicauta* species) (Figure 2-68) release an irritant and vesicant chemical **(cantharidin)** when crushed during single-operation mowing and crimping of alfalfa hay. Hay containing these crushed beetles is lethal for horses and may remain so even after years of storage. Clinical signs of cantharidin toxicosis include abdominal pain, fever, depression, frequent urination, shock, and, occasionally, synchronous diaphragmatic flutter; mortality may exceed 70% of affected individuals. Hematologic findings included hemoconcentration, neutrophilic leukocytosis, and hypocalcemia. As in all clinical poisonings, locating the source of the toxic agent is essential both for reaching a definitive diagnosis and for preventing further losses; the beetles should be sought in hay fed to affected horses (Schoeb and Panciera, 1978, 1979). The lethal dose of cantharidin for the horse is probably less than 1 mg/kg body weight (Beasley et al, 1983).

Dung beetles (some 14,000 species of the family Scarabaeidae) are very important in the grazing ecology because they break up, remove, and bury manure (Figure 2-69). Without their services, ruminant and horse dung tends to accumulate on the pasture, where it breeds flies, physically interferes with the growth of grass, and discourages grazing in the immediate vicinity. Besides simply clearing the surface of pastures, dung beetles enhance fertility and filth by burrowing in the soil and carrying their little balls of dung down into the burrows, where it is attacked by bacteria and fungi

FIGURE 2-69. Dung beetle from Canton, Ohio, rolling a ball of dung to burial. (Photo courtesy Lawrence L. Bowman.)

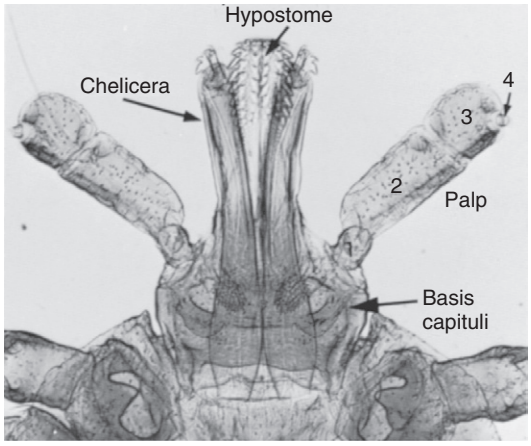

FIGURE 2-70. Capitulum of *Amblyomma*.

and the nutrients therein are made available to plants. Australia has gone so far as to import dung beetles from Africa in a successful effort to reduce accumulations of cattle dung on pasture and the fly populations that breed therein. Administration of ivermectin to grazing cattle suppressed not only target organisms but dung beetle populations as well. This unforeseen effect of anthelmintic medication may have potentially disastrous effects on dung removal and soil nutrient cycling, at least under some environmental conditions and dosage regimens (Coe, 1987; Wall and Strong, 1987).

The small **hive beetle,** *Aethina tumida*, was introduced into the United States sometime around 1998 (Elzen et al, 1999). The beetle is now known to be in 30 states, most of which are east of the Mississippi River. The beetles enter the hives of the European honeybee *(Apis mellifera)*; the beetle larvae feed on honey in the combs and cause the bees to flee the hive. This is one of several recently introduced arthropod pathogens of honeybees that have caused severe damage to these important pollinators throughout the United States.

CLASS ARACHNIDA

Although the class Arachnida includes spiders, scorpions, whip scorpions, and other forms that are of occasional interest to veterinarians, the following exposition is restricted to ticks and mites. Larval stages of both ticks and mites normally have three pairs of legs, and nymphs and adults have four pairs. The head, thorax, and abdomen are fused; antennae and mandibles are absent. The mouthparts (palps, chelicerae, and hypostome), together with the basis capituli, form a capitulum, or gnathosome (Figure 2-70).

SUBORDER METASTIGMATA, TICKS

All ticks are bloodsucking parasites. The hypostome is armed with backward-projecting teeth, and the chelicerae are armed with movable denticles (see Figure 2-70). The lateral stigmata are caudodorsal to the fourth coxae (Figure 2-71) and lack the sinuous peritremes characteristic of the somewhat similar suborder Mesostigmata.

The greatest importance of ticks involves the large number and variety of microbial diseases that they transmit among domestic animals (Table 2-7). These diseases are also listed later in the discussion on the particular genera involved as vectors. Other injuries inflicted by ticks include toxicosis, the bite wound, worry, and blood loss. Two major families of ticks have been identified: the Argasidae, or **soft ticks,** and the Ixodidae, or **hard ticks.** (A third family

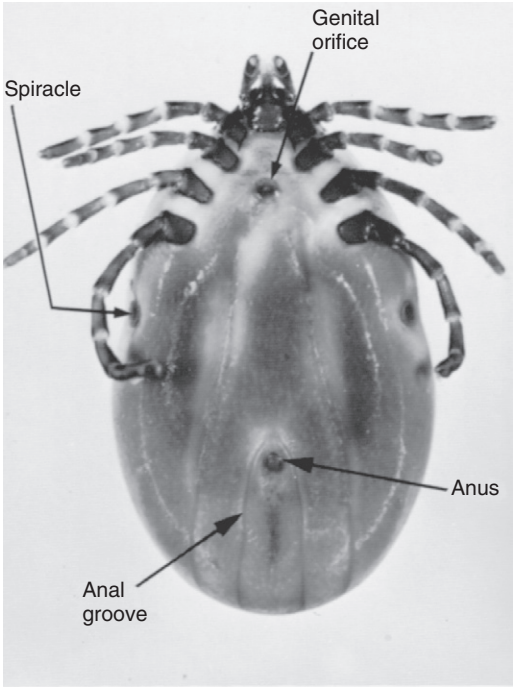

FIGURE 2-71. Ventral aspect of *Ixodes*. The anal groove of *Ixodes* curves anteriorly around the anus.

of ticks, the Nuttalliellidae, represented by a single species in the genus *Nuttalliella*, are of real importance only to acarologists.) Besides having markedly different morphology, soft and hard ticks vary greatly in their behavior. The Argasidae family tends to be composed of species that live in nests or burrows from where they surreptitiously feed quickly on unsuspecting hosts. Ixodid ticks tend to spend most of their lives in fields or scrub areas, where they await passing hosts. These ixodid ticks then attach and remain attached to their hosts for up to several days before they release and drop to the ground.

Family Argasidae

The family Argasidae, or soft ticks, is small, consisting of 140 species belonging to four genera—*Argas*, *Ornithodoros*, *Otobius*, and *Carios*. *Carios* species are limited to bats and will not be considered further here. Argasids live in nests, burrows, buildings, and sleeping places of their host animals and are distributed mostly in arid

TABLE 2-7 Some of the Common Agents Vectored by North American Ticks, the Disease, and Hosts of Concern

Genus	Species	Organism	Disease	Primarily Affected Host(s)
Argas	*A. persicus*, others	*Borrelia anserina*	Avian spirochetosis	Turkeys, chickens, other birds
Ornithodoros	Several	*Borrelia* spp.	Tick-borne relapsing fever	Various mammals, humans
		Unknown	Epizootic bovine abortion	Cattle, deer
		Possibly *Borrelia coriaceae*		
Otobius	*O. megnini*	*Francisella tularensis*	Tularemia	Wild cervids, cattle, horses, goats, sheep, dogs, humans
Ixodes	*I. scapularis* *I. pacificus*	*Borrelia burgdorferi*	Lyme disease, borrelioses	Dogs, cats, cattle, horses, humans
		Anaplasma phagocytophilum	Human granulocytic anaplasmosis	Rodents, humans, dogs, ruminants
		Powassan virus (Flaviviridae)	Tick-borne encephalitis	Humans, various mammals
Haemaphysalis	*H. leporispalustris*	*Francisella tularensis*	Tularemia	Lagomorphs, rodents, carnivores
Rhipicephalus	*R. annulatus*, *R. microplus*	*Borrelia theileri*	Bovine borreliosis	Cattle
		Babesia bigemina	Bovine babesiosis	Cattle
		Anaplasma marginale, *A. centrale*, *A. ovis*	Anaplasmosis	Cattle, sheep, other ruminants
	R. sanguineus	*Anaplasma platys* and other species	Canine anaplasmosis	Dogs
		Babesia canis, *B. gibsoni*	Canine babesiosis	Dogs
		Ehrlichia canis	Canine monocytic ehrlichiosis	Dogs and humans
		Mycoplasma haemocanis	Canine hemotropic mycoplasmosis	Dogs
		Hepatozoon canis	Hepatozoonosis	Dogs
		Rickettsia rickettsii	Rocky Mountain spotted fever	Dogs and humans
Dermacentor	*D. andersoni* *D. variabilis*	*Rickettsia rickettsii*	Rocky Mountain spotted fever	Dogs and humans
		Francisella tularensis	Tularemia	Dogs, cats, humans
		Coxiella burnetii	Q fever	Large domestic livestock, humans, various mammals
	D. variabilis	Reoviridae (Coltivirus)	Colorado tick fever	Rodents, carnivores, humans, domestic animals
		Cytauxzoon felis	Cytauxzoonosis	Cats
		Ehrlichia chaffeensis	Human monocytic ehrlichiosis	Humans, deer, dogs
	D. andersoni	*Anaplasma marginale*, *A. centrale*, *A. ovis.*	Anaplasmosis	Cattle, sheep, other ruminants
		Francisella tularensis	Tularemia	Sheep, horses, rabbits, game birds
		Colorado tick fever virus (Reoviridae)	Colorado tick fever	Rodents, carnivores, humans, domestic animals
	D. occidentalis	*Anaplasma marginale*, *A. centrale*, *A. ovis.*	Anaplasmosis	Cattle, sheep, other ruminants
Amblyomma	*A. americanum* *A. maculatum*	*Coxiella burnetii*	Q fever	Large domestic livestock, humans, various mammals
	A. americanum	*Ehrlichia ewingii*	Canine and human granulocytic ehrlichiosis	Dogs, humans, white-tailed deer
		Cytauxzoon felis	Cytauxzoonosis	Cats
		Francisella tularensis	Tularemia	Lagomorphs, rodents, carnivores
	A. maculatum	*Hepatozoon americanum*	American hepatozoonosis	Coyotes, dogs

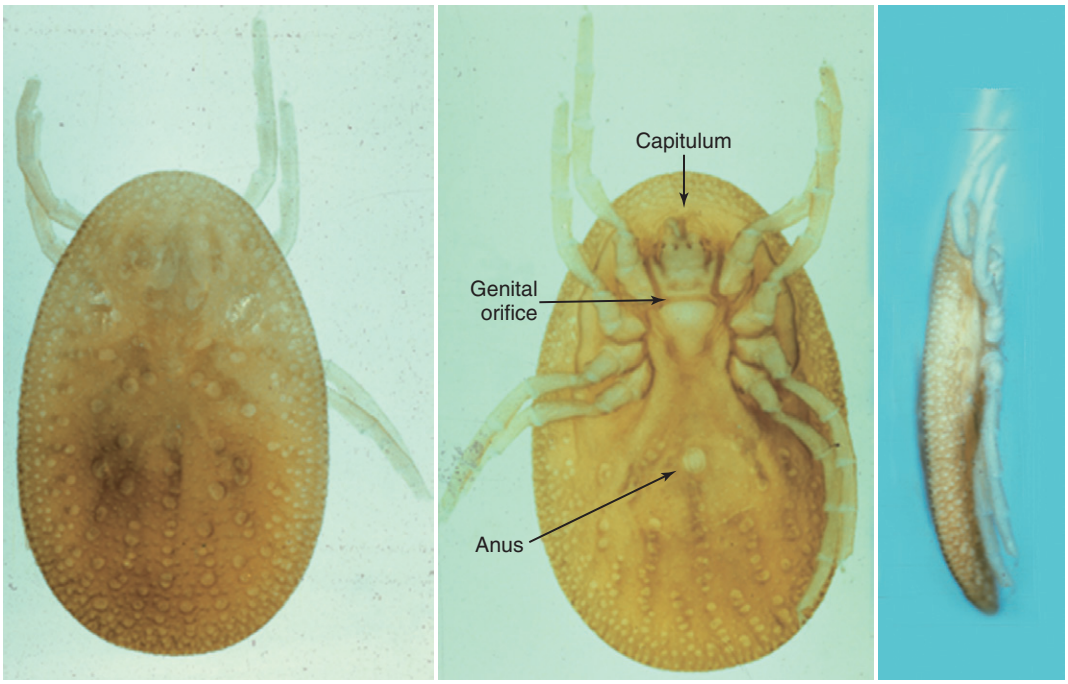

FIGURE 2-72. *Argas. Left*, Dorsal aspect. *Center*, Ventral aspect. *Right*, Lateral aspect.

regions or in drier habitats in moist regions. Life stages consist of the egg (laid in several batches of hundreds), larva, two or more nymphal stages, and adult male and female. Unlike ixodid nymphs and adults, which require several days to complete engorgement and feed only once during each stage, argasid nymphs and adults feed to repletion on their sleeping hosts in minutes or hours and feed repeatedly. Female argasids lay a clutch of eggs after each blood meal. Argasid larvae, on the other hand, feed for several days, and *Otobius* nymphs may remain in the external ear canal of cattle for several weeks.

Argas

IDENTIFICATION. *Argas* species are 5- to 10-mm, flattened, ovoid, and yellow to reddish-brown ticks with leathery, mammillated, and wrinkled dorsal and ventral surfaces meeting at a sharp lateral margin. The mouthparts are on the ventral surface and thus are hidden when the tick is viewed from above (Figure 2-72). *Argas* is rarely found on the host; to find these ticks, search cracks and crannies in the hen house. In the United States, *Argas* is restricted to areas along the Gulf of Mexico and the Mexican border.

LIFE HISTORY. Female *Argas* ticks deposit their eggs in clutches of 25 to 100 in the crevices that serve as hiding places during the day. Several clutches are laid, each preceded by a blood meal lasting 45 minutes or less. The six-legged larva hatches in 1 to 4 weeks, attaches to a host, and feeds for about 5 days; the larva is thus active day and night. When replete, the larva leaves the host and finds a hiding place in which to spend a week or so molting into a nymph. The eight-legged nymph feeds at night and undergoes a second molt to a second nymphal stage, which again feeds and undergoes a third molt into an adult male or female. Although development from egg to adult may be completed in as few as 30 days, lack of suitable hosts may prolong the process. Larvae and nymphs may survive for months and adults for longer than 2 years without a blood meal. Trying to starve them out does not pay.

DISEASE TRANSMISSION. In South America, *Argas* species transmit fowl or avian spirochetosis *(Borrelia anserina)*, via tick fecal contamination, to domestic poultry, grouse, canaries, guinea fowl, and pigeons (see Table 2-7). Ticks may remain infective for 6 months or longer and may transmit the spirochetes to their offspring via the ovaries (transovarial transmission). *Argas* species also transmit a rickettsial agent, *Aegyptianella pullorum*, to chickens and geese in the tropics and subtropics of the Old World.

TICK PARALYSIS. Infestation with larvae of *Argas persicus* can result in fatal flaccid paralysis of young chickens (Rosenstein, 1976).

Ornithodoros

IDENTIFICATION. *Ornithodoros* differs from *Argas* in being more globular, in lacking a sharp lateral margin, and in not appearing distinctly ovoid when viewed from above. The body is flattened in unfed specimens but is strongly convex dorsally when distended with blood. These ticks (Figure 2-73) are found in cracks and crannies of avian roosts and nests, in rodent burrows, and in the resting places of large mammals.

LIFE HISTORY. Species of *Ornithodoros* differ with respect to whether the larvae feed, the number of nymphal instars present (three to five), and host and lair preferences. *Ornithodoros hermsi* is a rodent parasite in the Rocky Mountain and Pacific Coast states, breeding in rodent burrows and rodent-infested buildings, whereas *Ornithodoros coriaceus* of California and Oregon attacks deer and cattle from the soil of their bedding areas. As typical argasids, *Ornithodoros* can survive unfed for months or even years.

DISEASE TRANSMISSION. *Ornithodoros* are most important as vectors and reservoirs of relapsing fever spirochetes (*Borrelia* spp.) of humans (see Table 2-7). Infection may be maintained in tick populations for many years by transovarial transmission of the spirochetes from female ticks to their offspring and tends to remain endemic in wild rodent populations. Tick-borne relapsing fever typically involves an individual or a small group of campers who have slept in a tick-infested cabin out in the wilderness (Cutler, 2009). Because the *Ornithodoros* ticks involved in transmission are nocturnal and surreptitious, relapsing fever victims are frequently unaware of recent tick exposure.

Otobius

IDENTIFICATION. Larvae and two nymphal stages of *Otobius megnini*, the spinose ear tick, parasitize the ear canals of cattle, remaining in a particular host for as long as 4 months. Other domestic animals and humans also sometimes serve as hosts. One of Dr. Georgi's former students reported that he had suffered several painful attacks by *Otobius*. As implied by the common name, the cuticle of *Otobius* is covered by spines. The second nymphal stage is particularly distinctive (Figure 2-74).

LIFE HISTORY. Larvae feed in the ear canal and molt into the first nymphal stage, which in turn feeds in the same host's ear canal and molts into the second nymphal stage, which again feeds but leaves the ear canal and drops to the ground to molt to the adult stage. Adult *Otobius* have vestigial hypostomes and do not feed; they copulate within a day or two after emergence, and the females oviposit in the soil. Larvae survive unfed for as long as 2 months.

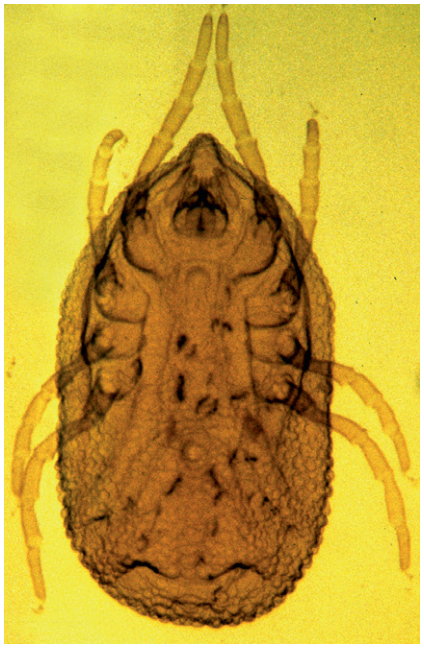

FIGURE 2-73. *Ornithodoros.*

Thus, *Otobius* differs from *Argas* and *Ornithodorus* in being a one-host tick and in laying only one clutch of eggs.

Family Ixodidae

Members of the family Ixodidae, or **hard ticks,** have a shield, or **scutum,** that covers the entire dorsal surface of the male but only part of the dorsal surface of the female (Figure 2-75). The size of the scutum remains constant during engorgement of a female, and consequently it covers a progressively smaller proportion of her dorsum. A tick's eye, if present, is a mere roundish lucent area at the margin of the scutum about opposite the second coxa. The scutum and the posterior edge of the body may bear a series of indentations or folds along the margin; these are called **festoons.** Also, the scutum may have colored patterns on the surface (an **ornate scutum**) or it may be without coloration (an **inornate scutum**). The large **stigmata** (respiratory openings attached to the tracheal system) are behind the last pair of legs on the sides of the body. The anterior end of the tick houses the feeding apparatus, which consists of a **basis capituli** that is adjacent to the body. On the anterior portion of the basis capituli are the **palps,** one on each side of the paired **chelicerae** and the central **hypostome.** The palps consist of four segments, with the distal fourth pretty well buried in the third. Each chelicera has large cutting blades on the distal end, and the hypostome has numerous small teeth or denticles.

Eggs are laid in a single clutch of thousands. Ixodid larvae, nymphs, and adults feed only once, and several days are usually required for complete engorgement. Ixodids usually live outdoors and attach to passing host animals. Two molts occur: the first from larva to nymph, and the second from nymph to adult. Species that complete both molts without leaving the host are called **one-host ticks;** species whose engorged nymphs drop off to molt are called **two-host ticks;** and those whose nymphs and larvae drop off to molt are called **three-host ticks.** *Dermacentor variabilis* is a three-host tick whose larvae and nymphs engorge on small mammals and whose adults engorge on dogs. *Rhipicephalus sanguineus* is a three-host tick whose larvae, nymphs, and adults all engorge on dogs. The individual or species identity of the host has no bearing on the use of these terms. What is important relative to these terms is that a one-host tick or a three-host tick that feeds on only one host is often easier to control through management of the single host than is a three-host tick that has different hosts throughout the environment. For example, if cattle are hosts to a

FIGURE 2-74. *Otobius megnini. Left*, First nymph. *Right*, Second nymph.

FIGURE 2-75. *Amblyomma maculatum.* The male *(left)* has an ornamented scutum that covers the entire body. In the case of the female *(right)*, the scutum is also ornate but covers only a portion of the dorsal surface of the tick. As the female engorges, the scutum remains constant in size and, at last, covers only a small proportion of the fully engorged female.

one-host tick, dipping and other applications of chemotherapeutic agents or vaccination of the cattle will have effects on all life stages of the tick. If three hosts were involved, the first host might be a rodent, the second a rabbit or a bird, and the third cattle. Thus it would be more difficult to manage these two- or three-host systems because it would be difficult to manage or treat all three hosts involved. That three-host ticks may feed on several different hosts during their lives, from small rodents to large mammals, makes them perfect vectors for the transmission of zoonotic agents to humans (e.g., the larva feeds on a rodent, the nymph or adult will feed on humans); thus transmission from rodents to humans becomes a real possibility. This is exactly what occurs in the case of Lyme borreliosis.

Two- and three-host ticks can transmit disease organisms via **interstadial transmission;** that is, infection acquired by a larval tick is carried through the molt to the nymphal stage and then is conveyed to the host on which the nymph feeds, or infection acquired by a nymph is carried through the molt and is conveyed to the host on which the adult tick feeds. Thus three-host ticks can transmit disease organisms interstadially through larva-to-nymph and nymph-to-adult transitions, whereas two-host ticks are limited to the latter. In **transovarial transmission,** the disease organisms are passed from the adult female tick to her larvae through infection of her ovaries. *Babesia bigemina* is transmitted from the adult female *Rhipicephalus* (formerly *Boophilus*, this genus has now been subsumed within the genus *Rhipicephalus*) tick to her progeny by way of her ovaries. Transovarial transmission of disease organisms is the only mechanism that allows one-host ticks, such as *Rhipicephalus annulatus*, to serve as vectors.

Ixodid ticks found attached to domestic animals may be removed individually by cautious traction with thumb forceps. The long hypostomes of *Ixodes*, *Amblyomma*, and *Hyalomma* are effective anchors. *Dermacentor*, *Rhipicephalus*, and *Haemaphysalis* compensate for their shorter hypostomes by secreting cement in which the mouthparts are embedded that attaches them securely to the skin (Moorhouse, 1973; Moorhouse and Tatchell, 1966). Therefore, unless reasonable care is exercised, the capitulum may be torn away and remain embedded as a foreign body in the skin of the host. Outdoor areas suspected as sources of ixodid tick infestation may be surveyed with a drag made by attaching one edge of a square yard of flannel to a stick and drawing it slowly over the vegetation. Hungry ticks will climb aboard the passing drag and can then be removed at intervals and placed in specimen bottles.

Veterinarians should carefully examine the ticks they encounter in practice. If a specimen is found that looks different from normal ticks, it should be sent to a diagnostic laboratory for expert identification. However, many practical problems can be solved by generic identification of adult ixodid ticks, and criteria for accomplishing this goal are presented here. No attempt is made here to identify larvae and nymphs beyond the family level; larvae have six legs (Figure 2-76) and nymphs have eight legs and a scutum of the female type, but the genital aperture is absent (Figure 2-77). A key to the nymphs of ixodid ticks that may be helpful to veterinarians has been presented elsewhere (Bowman and Giovengo, 1991).

In the following outline of genera of ixodid ticks, the **character in bold type** is sufficient or is nearly sufficient to represent the genus alone, provided that the corresponding morphologic feature of the specimen is seen and correctly interpreted. Any ixodid tick must have one or another of these characters, and they serve as convenient starting points for identifying specimens; however, to be on the safe side, check each subsidiary character as well. Further details may be found in *Ticks of Veterinary Importance*, Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), Agriculture Handbook No. 485.

Approximately 700 species of hard ticks are included within a total of 12 genera. Currently recognized genera are *Amblyomma*, *Anomalohimalaya*, *Bothriocroton*, *Cosmiomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Ixodes*, *Margaropus*, *Nosomma*, *Rhipicentor*, and

FIGURE 2-76. Six-legged *Ixodes* larva.

FIGURE 2-77. Eight-legged *Ixodes* nymph. Although difficult to discern in this figure, an anterior anal groove can be found in the nymphal and larval stages of ticks of the genus *Ixodes*.

Rhipicephalus, with the genus *Boophilus* becoming a subgenus of the genus *Rhipicephalus* (Horak, Camicas, and Keirans, 2002). The five genera that are found in North America include *Ixodes*, *Haemaphysalis*, *Rhipicephalus*, *Dermacentor*, and *Amblyomma*. The other genera from outside North America are sometimes found here on imported animals.

Genera Found in North America

Ixodes

IDENTIFICATION. **The anal groove forms an arch anterior to the anus;** this can be seen with oblique illumination of uncleared specimens (Figure 2-78). Other genera have a groove posterior to the anus or no groove at all. *Ixodes* species have no eyes, festoons, or scutal ornamentation; their palpi are broadest at the junction of segments two and three (Figure 2-79).

LIFE HISTORY AND DISEASE TRANSMISSION. *Ixodes holocyclus* of Australia is the most virulent tick paralysis producer

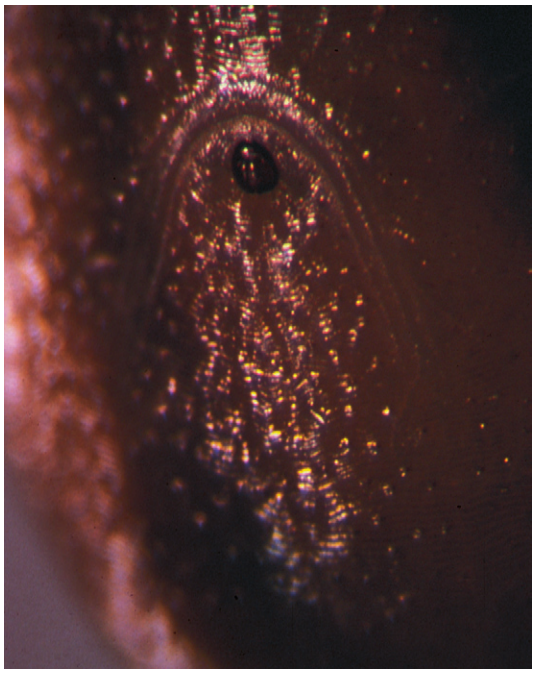

FIGURE 2-78. The anus and the anterior anal groove present on the posterior ventral surface of all stages of ticks in the genus *Ixodes*.

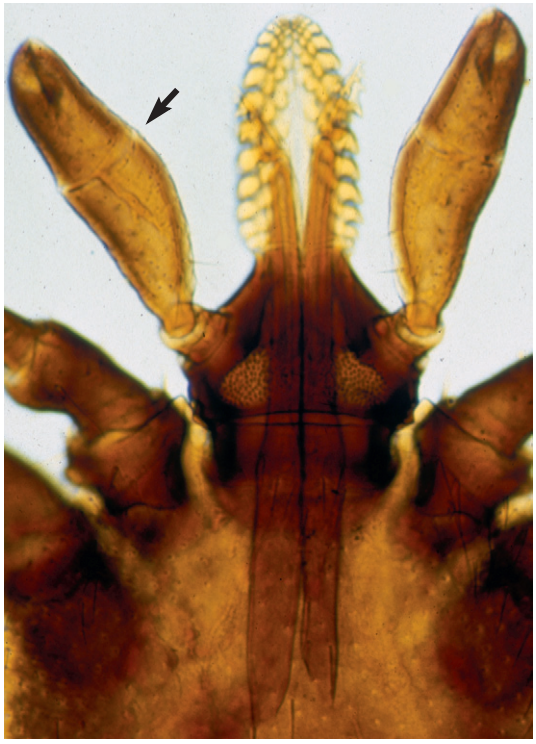

FIGURE 2-79. Capitulum of *Ixodes*. The palps of *Ixodes* are broadest at the junction of the second and third segments *(arrow)*.

known. *Ixodes pacificus* is known to cause tick paralysis in North America.

Species of *Ixodes* are the major vectors of Lyme disease in North America and in Europe. Nymphs and adults of *Ixodes scapularis* in the eastern and north-central United States, a three-host tick that normally feeds on mice and voles as larvae and nymphs and on deer as adults, transmit microtine piroplasmosis *(Babesia microti)*, Lyme

disease *(Borrelia burgdorferi)*, and human granulocytic anaplasmosis *(Anaplasma phagocytophilum)* to dogs (Hinrichsen et al, 2001; Lissman et al, 1984), humans (Burgdorfer et al, 1982; Spielman, 1976; Weil et al, 2012), and other animals (see Table 2-7). In the northeastern United States, the white-footed mouse, *Peromyscus leucopus*, is the principal reservoir host for *B. burgdorferi* and serves as host for larvae and nymphs of *I. scapularis*; the white-tailed deer, *O. virginianus*, serves as host to the adult tick (Lane and Burgdorfer, 1987). *I. pacificus* is a major vector of Lyme disease and human granulocytic anaplasmosis in western United States (Piesman, 1991). The incidence of human Lyme disease in May and June coincides with the activity of nymphs that were infected as larvae the previous summer. Thus nymphs feed in each transmission season before larvae do. The white-tailed deer plays a dominant role as principal host of the adult *I. scapularis* tick, which feeds on this host from late fall through winter (Matushka and Spielman, 1986). *I. scapularis* has been shown to be a vector of Powassan virus in the United States, which causes tick-transmitted viral encephalitis (Anderson and Armstrong, 2012). These zoonotic infections occur mainly in New England and in the north-central United States. A fatal case of deer tick virus encephalitis has occurred in Maine (Tavakoli et al, 2009).

In Europe, species of *Ixodes* are vectors of bovine piroplasmosis and various viral diseases, including louping ill (ovine encephalomyelitis) and tick-borne encephalitis virus, which cause disease in people. It appears that tick-borne encephalitis virus can also cause disease in dogs (Pfeffer and Dobler, 2011).

Haemaphysalis

IDENTIFICATION. The palpi have laterally flared second segments (Figure 2-80)**.** Avoid confusing these structures with the hexagonal basis capituli of *Rhipicephalus*. Like *Ixodes*, these ticks have neither eyes nor scutal ornamentation, but they differ in having festoons and a posterior anal groove.

LIFE HISTORY. Larvae and nymphs of *Haemaphysalis leporispalustris*, the rabbit tick, feed on ground-nesting birds and small mammals, and the adults attach to rabbits, especially to the ears and around the eyes. Occasional specimens are collected from cats.

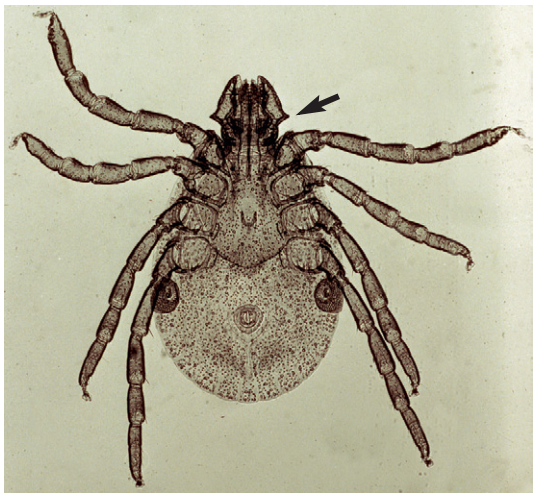

FIGURE 2-80. *Haemaphysalis.* The second palpal segment *(arrow)* is flared laterally.

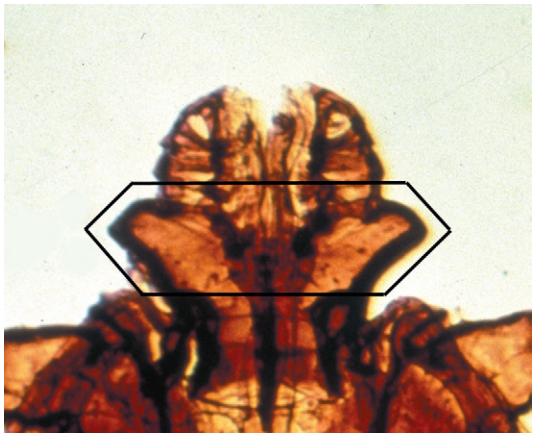

FIGURE 2-81. Capitulum of *Rhipicephalus.* The basis capituli is hexagonal.

Rhipicephalus

IDENTIFICATION. The basis capituli is hexagonal (Figure 2-81)**; eyes and festoons are present, but the scutum is unornamented** (Figure 2-82)**; males have salient adanal and accessory shields** (Figure 2-83)**.**

LIFE HISTORY AND DISEASE TRANSMISSION. Larvae, nymphs, and adults of *R. sanguineus*, the brown dog tick, all feed on dogs and sometimes on humans (Figure 2-84). Originally a tropical species, *R. sanguineus* has taken advantage of central heating to spread into the temperate zones, where it often generates enormous populations in homes, kennels, and veterinary hospitals; it cannot survive the winter outdoors in the North. Dogs living in temperate regions frequently acquire their *R. sanguineus* ticks in such infested premises, but during summer, infestation may occur outdoors. Therefore if enduring results are to be achieved, elimination of these ticks must include acaricidal treatment of both the dog and the home or kennel. The latter procedure is a job for a professional exterminator. Development from egg to egg may be completed over slightly longer than 2 months under favorable conditions; unfed adults may survive for well over a year. A household, including two dogs and the client's wife and mother-in-law who never left England, apparently acquired infestations with *R. sanguineus* through the introduction of ticks into the client's car when he gave rides to a neighbor's dogs while at his summer home in France (Jagger, Banks, and Walker, 1996). He brought the ticks home to England from France in his car, indicating just how mobile are the ticks of this species.

R. sanguineus transmits canine piroplasmosis *(Babesia canis)* transovarially and canine monocytic ehrlichiosis *(Ehrlichia canis)* interstadially (see Table 2-7). *R. sanguineus* has recently been implicated in an outbreak of Rocky Mountain spotted fever (RMSF) in Native American lands in Arizona; before this, *R. sanguineus* was considered a vector of RMSF only south of the U.S. border (Demma et al, 2005; Folkema et al, 2012).

***Rhipicephalus* (formerly *Boophilus*) *annulatus*,** the transovarial vector of bovine piroplasmosis, was brought to the Americas on cattle from Africa and the Mediterranean Coast of Europe. This species and a few others were placed until recently in their own genus, *Boophilus*, but this is now considered a synonym of *Rhipicephalus* (Barker and Murrell, 2004). Other African species of *Rhipicephalus* serve as vectors of the devastating East Coast fever *(Theileria parva)* and other forms of bovine theileriosis, bovine piroplasmosis *(B. bigemina)*, and the virus of Nairobi sheep disease. *R. annulatus* (Figure 2-85) is similar to *R. sanguineus* in that the adults have a hexagonal basis capituli, eyes, and an unornamented scutum, and the males have adanal and accessory shields. However, ***R. annulatus* differs from *R. sanguineus* in that it has palpi that**

FIGURE 2-82. *Rhipicephalus sanguineus* male *(left)* and female *(right)*. The posterior of the scutum on the male bears indentations and festoons along the posterior margin *(arrow)*. The eyes are the relatively lighter areas on the sides of the scutum of the male and the female that are at about the level where the second pair of legs extend from the ventral surface.

FIGURE 2-83. Ventral aspects of a male *Rhipicephalus (left)* and a male *Dermacentor (right)*. Coxae of male *Dermacentor* progress in size from the first to fourth coxa *(1 to 4)*. On the posterior of the male *Rhipicephalus* can be seen the large and pronounced adanal shields on each side of the anus.

are ridged dorsally and laterally, and the adults of *R. annulatus* lack festoons.

R. annulatus was eradicated from the United States over 40 long years of dipping cattle that began in 1906. Losses from piroplasmosis were estimated then at $40 million to $100 million per year, at a time when cattle were selling at 2 to 4 cents a pound. Eradication was favored by the affinity of this tick species for cattle and by its one-host life history, which made it possible to destroy a substantial proportion of the tick population each time the cattle were dipped (Figure 2-86). However, it remains apparent that constant vigilance at the U.S.-Mexico border is critical in keeping the United States free of this dangerous tick vector (Lohmeyer et al, 2011). Comparable efforts to eradicate any species with broader host preferences, especially those feeding on wildlife, would have been much more difficult. *Rhipicephalus microplus*, also a piroplasmosis vector, has a broader host range that includes horses, goats, sheep, and deer. *Rhipicephalus* specimens encountered in the field in North America should be immediately reported to state or federal authorities because of the one-host (cattle) nature of *R. annulatus* and its great vector potential for transmitting bovine piroplasmosis.

Dermacentor

IDENTIFICATION. The basis capituli is rectangular as viewed from above (Figure 2-87). Coxae of males progress in size from the first to the fourth (see Figure 2-83). *Dermacentor* resembles

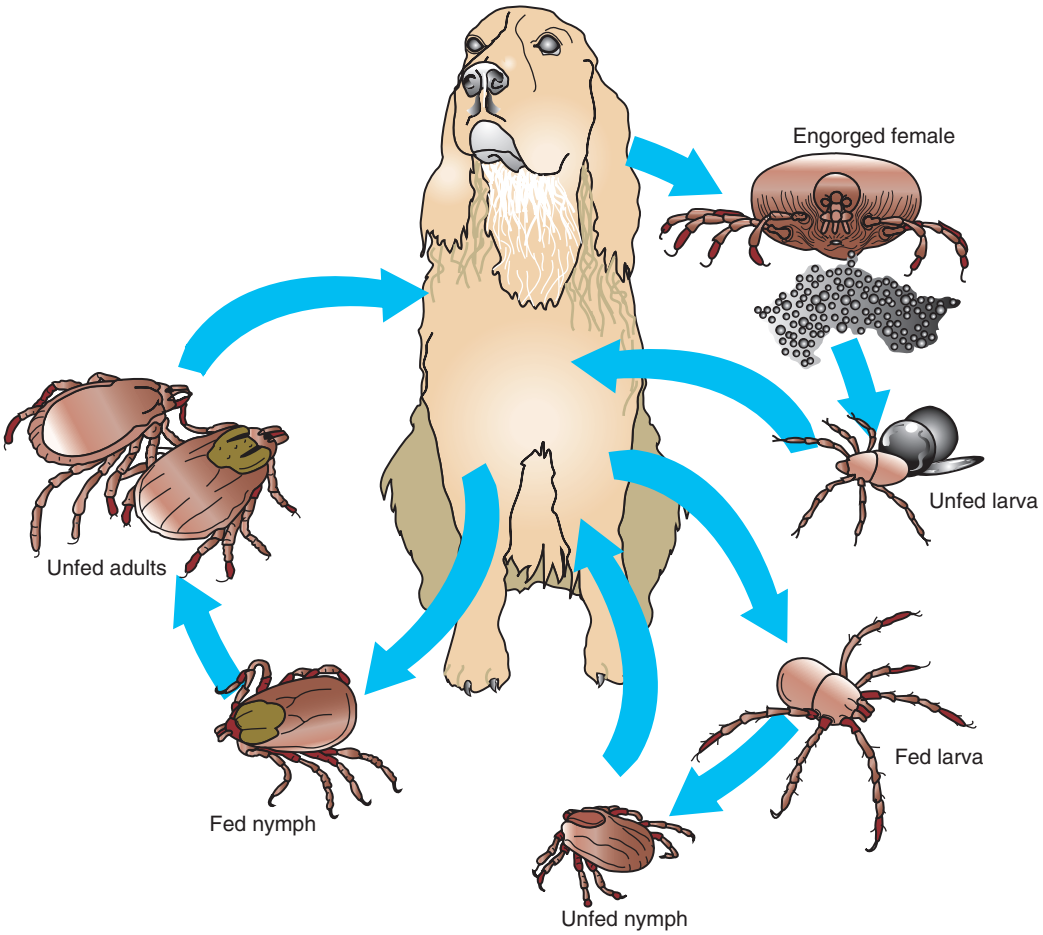

FIGURE 2-84. Life history of the brown dog tick, *Rhipicephalus sanguineus*. Six-legged larvae feed on the dog for a few days, drop off, and molt to the eight-legged nymphal stage. Nymphs feed on the dog for about a week, drop off, and molt into male and female adults. Females are fertilized on the dog, feed for 1 to 3 weeks, and become greatly engorged with blood before dropping to the floor to lay their clutches of 2000 to 4000 eggs several weeks later. Eggs emerge from the genital opening one at a time and accumulate in front of the female tick over a period of several more weeks. The complete cycle requires 2 to 3 months, which is fast compared with that of most tick species.

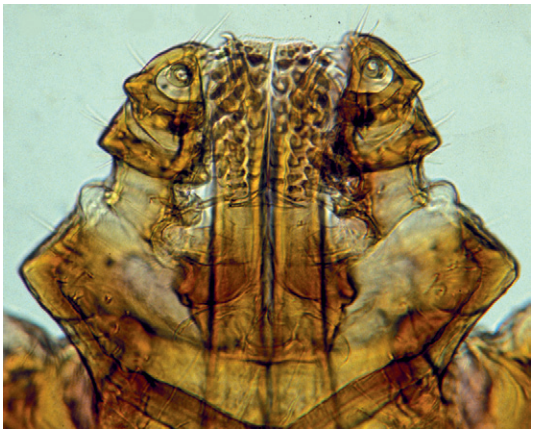

FIGURE 2-85. Capitulum of *Rhipicephalus (Boophilus) annulatus.* The basis capituli is hexagonal, and the palpi are ridged dorsally and laterally *(arrows)*.

FIGURE 2-86. Cow with a fairly large number of attached *Rhipicephalus (Boophilus) annulatus* ticks.

Rhipicephalus in having eyes and 11 festoons, but the basis capituli is rectangular, **the scutum is ornamented** (Figure 2-88)**, and the males lack adanal shields.** *Dermacentor* (*Anocentor*) *nitens*, the tropical horse tick, has no ornamentation on its scutum and only seven festoons.

LIFE HISTORY AND DISEASE TRANSMISSION. *D. variabilis*, the American dog tick, is widely but discontinuously distributed over the eastern half and West Coast of the United States and over parts of Canada and Mexico. Larvae and nymphs engorge on small rodents; adults engorge on humans, dogs, horses, cattle, and

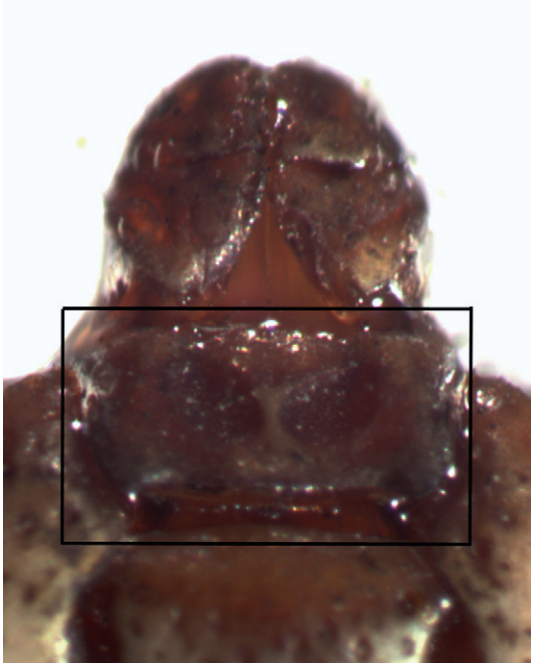

FIGURE 2-87. Capitulum of *Dermacentor*. The basis capituli is rectangular.

FIGURE 2-88. Dermacentor male. Notice the ornamented scutum.

wildlife. *D. variabilis* transmits RMSF *(Rickettsia rickettsii)* and tularemia *(F. tularensis)* and causes tick paralysis (see Table 2-7). Adult females feed to repletion over several days, becoming larger each day (Figure 2-89).

Dermacentor andersoni, the Rocky Mountain wood tick, requires 1 to 3 years to complete its life history, depending on the latitude, altitude, and abundance of small mammals on which it feeds as larva and as nymph. *D. andersoni* transmits RMSF, tularemia, Colorado tick fever, and Q fever and causes tick paralysis (see Table 2-7).

D. nitens, the tropical horse tick, is limited, in the United States, to the southern portions of Florida and Texas. Preferring the external ear canals of horses but also found on other sites and other

FIGURE 2-89. *Dermacentor* female ticks that have engorged for different numbers of days from 1 to 5.

hosts such as cattle, sheep, goats, and deer, *D. nitens* is the vector of equine piroplasmosis *(Babesia caballi)* (see Table 2-7).

Other North American species of *Dermacentor* include *Dermacentor albipictus*, the winter tick that causes heavy losses among deer, elk, and moose; *Dermacentor nigrolineatus*, the brown winter tick; and *Dermacentor occidentalis*, the Pacific Coast tick. In moose *Alces alces*, infestation with *D. albipictus* causes hair loss, which progresses rapidly from February to April and may amount to as much as 44% of the haircoat. McLaughlin and Addison (1986) estimated that loss of 30% of its hair in a winter environment of –20°C would double the daily energy requirements of an otherwise normal 230-kg yearling moose. The increased catabolic rate imposed by hair loss then leads to reduction in body fat stores and to lowered resistance to disease and predation.

Amblyomma

IDENTIFICATION. **The mouthparts are much longer than the basis capituli; the second palpal segment is at least twice as long as the third** (see Figure 2-68)**.** Eyes and festoons are present, scutum is ornamented, and adanal shields are absent. *Aponomma elaphensis* resembles *Amblyomma* but is smaller and lacks eyes; it is a parasite of a rat snake in Texas.

DISEASE TRANSMISSION. In the United States, species of *Amblyomma* that attack humans, livestock, dogs, and cats (e.g., *A. americanum* [Figure 2-90], *Amblyomma maculatum* [see Figure 2-75], *Amblyomma cajennense*, *Amblyomma imitator*) are distributed mainly in the southeastern coastal states of Missouri, Oklahoma, and Texas, but specimens may occasionally be found as far north as Ithaca, New York. These species have been incriminated in the transmission of RMSF, *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, and tularemia, and in the causation of tick paralysis (see Table 2-7). *A. americanum* has been incriminated now as being the vector of *Cytauxzoon felis*, a fatal protozoal disease of cats that is probably maintained in the wild in the bobcat, *Lynx rufus* (Reichard et al, 2009; Birkenheuer et al, 2008). *A. maculatum*, the Gulf Coast tick that has a more southerly range than *A. americanum*, has now been incriminated as the vector (through tick ingestion) of American hepatozoonosis, caused by *Hepatozoon americanum*, a horrible debilitating disease of dogs in the southeastern and south central United States (Mathew et al, 1998), and it appears that a major reservoir for this infection may be the coyote *(Canis latrans)* (Garrett, Kocan, and Reichard, 2005). It has recently been learned that *Amblyomma cajennense* is present in fairly good numbers in south Texas, and it is suspected that it vectored *Theileria equi* between horses in this area (although transmission to date has been verified only in the laboratory) (Scoles et al, 2011).

African species of *Amblyomma* transmit heartwater *(Ehrlichia ruminantium)* of cattle, sheep, and goats, as well as the virus of Nairobi sheep disease. *Amblyomma dissimile*, the iguana tick, and *Amblyomma tuberculatum*, the gopher tortoise tick, are parasites of reptiles and amphibians; the latter is the largest ixodid tick found in North America, with engorged females reaching a length of

FIGURE 2-90. *Amblyomma americanum.* The male has an ornamented scutum with festoons. The scutum of the female bears a single, large, light-colored dot, hence the name *lone-star tick*. The mouthparts of *Amblyomma* are relatively proportionately longer than those of other ticks commonly found in the United States.

FIGURE 2-91. An engorged *Amblyomma* female next to a U.S. quarter for size comparison purposes.

25 mm (Figure 2-91). The largest tick, *Amblyomma varium*, is a parasite of sloths in South America.

Genera Not Found in North America

Hyalomma

Hyalomma resembles *Amblyomma* in having mouthparts much larger than the basis capituli but differs in that the second and third palpal segments are approximately the same length (Figure 2-92). Eyes are present, and festoons are irregularly coalesced; the male has adanal and accessory shields.

Margaropus

Margaropus resembles *Rhipicephalus*, but the palps are not ridged and the legs of the male progress in size from the first to the fourth.

Rhipicentor

Rhipicentor resembles *Rhipicephalus* dorsally and *Dermacentor* ventrally; eyes and festoons are present, adanal and accessory shields are absent, and fourth coxae are greatly enlarged.

Direct Effects of Ixodid Ticks on the Host

Tick Toxicosis

In North America, the species most frequently involved in tick paralysis are *D. andersoni*, *D. variabilis*, *A. americanum*, and *A. maculatum*. Tick paralysis is an ascending paralysis caused by absorption of toxins from the saliva of engorging female ticks. The tick injects a considerable volume of saliva into the wound in part as an aid to digestion, and in part as a means of disposing of surplus water extracted from the blood meal. A single female tick can produce paralysis in humans, dogs, or cats, especially if the site of attachment is near or on the head, but paralysis does not invariably occur even if many ticks of a suitable species are present. Usually, heavy infestations are required to produce tick paralysis in cattle. The first

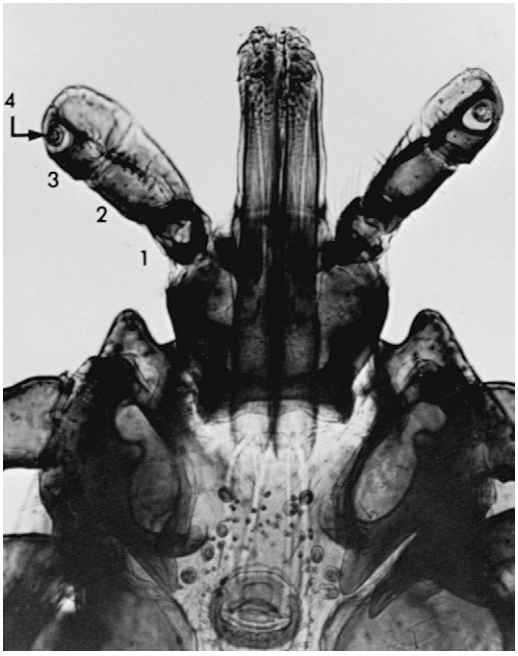

FIGURE 2-92. Capitulum of *Hyalomma*. Palpal segments two and three of *Hyalomma* are approximately the same length, whereas the second palpal segment of *Amblyomma* is about twice as long as the third.

clinical sign is incoordination of the hindquarters that rapidly proceeds to complete paralysis and spreads to the forequarters, the neck, and finally the respiratory muscles, with fatal consequences. Removal of engorging ticks usually leads to gratifyingly rapid recovery. In Australia, *I. holocyclus*, a parasite of the bandicoot and other marsupials, causes a particularly severe form of tick paralysis in domestic animals. Of 577 Australian dogs affected and seen by veterinarians in 1998, 5% of the dogs died from the disease (Atwell, Campbell, and Evans, 2001). Effective treatment of paralysis caused by *I. holocyclus* requires administration of specific antitoxin and general supportive treatment, as well as removal of all ticks from the victim. Even larvae and nymphs of *I. holocyclus* are potentially capable of inducing paralysis when present in sufficient numbers. However, as is the case with other tick paralysis species, the engorging female of *I. holocyclus* is usually responsible. The surest prevention of tick paralysis lies in careful daily examination of exposed animals and removal of ticks. Because clinical signs of paralysis do not begin to appear until the ticks have been feeding

for at least 4 days, they should be large enough to be found relatively easily before clinical signs develop. In areas of heavy exposure, weekly acaricidal dipping is necessary. It is sometimes difficult to know if a dog has an attached tick; a case of tick toxicosis due to an *I. holocyclus* occurred in the United Kingdom in a dog very recently brought from Australia that appeared to have been infected while with the transport agency. In this case, the owner noticed the signs of ataxia and found the tick attached to the pinna of the ear, and the dog made a full recovery (Adamantos, Boag, and Church, 2005).

The Bite Wound

Ixodes, *Amblyomma*, and other genera with long mouthparts produce deep, painful bite wounds that tend to become inflamed, secondarily infected with bacteria, and flyblown. In Great Britain, secondary infection of *Ixodes ricinus* bites with *Staphylococcus* results in both local and metastatic abscessation (tick pyemia) in lambs. In the Gulf Coast states, *A. maculatum*, which prefers to attach to the ears of larger mammals, causes such pain and swelling that cattle are unable or at least reluctant to flick their ears and thus ward off flies. Before screwworm control, such ears were prone to invasion by larvae of *C. hominivorax*, frequently with loss of the external ear or death.

Blood Loss and Worry

Sir Arnold Theiler once collected half of the *Rhipicephalus decoloratus* ticks from a horse that had died of acute anemia. His collection weighed 14 lb (Theiler, 1911). That horse's tick burden must have contained about 13 L of blood. This example may appear extreme to those of us who dwell in temperate zones and experience only an occasional mosquito or blackfly bite, but there are places in the tropics where light-colored cattle are so totally covered by the dark bodies of engorging ticks that they appear from a distance to be black. Also, dogs and other hosts lose significant quantities of blood to the ticks that feed on them; volumes consumed by a replete adult female are for each species: 1.45 mL for *D. variabilis*, 0.81 mL for *A. americanum*, 0.55 mL for *R. sanguineus*, and 0.51 mL for *I. scapularis* (Koch and Sauer, 1984). Loss of blood, pain from and swelling of bite wounds, secondary infection, myiasis, and absorption of toxins, in moderate and varying proportions, result in a form of ill thrift referred to as "tick worry." Because tick worry for many hosts is the most common practical consequence of tick infestation, it may be even more important than the more dramatic ones.

Treatment and Control of Tick Infestations

Dogs and Cats

Ticks on dogs and cats are most easily treated by prevention with topical application of fipronil and other tick products. Fipronil has been found to be an excellent means of preventing tick infestations of dogs and cats. It has been combined as with amitraz or cyphenothrin to increase its acaricidal properties, but these products can be used only on dogs. Other topical products include pyrethrin and permethrin, and permethrin is another product that cannot be used on cats. Permethrin has been combined with imidacloprid for use on dogs to control both ticks and fleas. Another approach is to use collars containing amitraz, tetrachlorvinphos, deltamethrin, or flumethrin; some of these chemicals kill fleas as well, but others have additional compounds added to kill adult fleas or insect growth regulators that have detrimental effects on flea development. Flumethrin-containing collars are safe for dogs and cats. Control of *R. sanguineus* in buildings may be achieved by spraying with permethrin or cyfluthrin and could require the use of professional exterminators.

Lactating Dairy Cattle

For lactating dairy cattle, coumaphos and dichlorvos are applied as sprays or in backrubbers for the control of ticks. Permethrin-containing ear tags can be used in lactating dairy cattle. No restrictions apply when these products are used as recommended.

Beef and Nonlactating Dairy Cattle

For beef cattle and nonlactating dairy cattle, coumaphos and dichlorvos may be used as dips and sprays in the control of ticks. Several ear tags for cattle have activity against ticks, with different products being labeled for different species, including *D. variabilis*, *A. americanum*, *A. maculatum*, and *Boophilus (Rhipicephalus)* species, and the spinous ear tick, *Otobius megnini*. *O. megnini* ear ticks can be treated on individual animals with insecticidal dusts or emulsion concentrates instilled into the ear canal from squeeze bottles or an oil can. Ivermectin, doramectin, and moxidectin all provide some level of protection against ticks, but none of these products is currently labeled for tick control. There are vaccines that have been used for the control of *Rhipicephalus annulatus* and *Rhipicephalus microplus* in countries outside of the United States.

Horses

In horses particularly, tick attachment sites may become markedly irritated, leading to an itch-scratch cycle marked by serious self-mutilation. Permethrin and cypermethrin with or without synergists are used in spray or dust formulations.

Environment

Mainly driven by the fear people have of becoming infected with Lyme disease by tick bite, attempts have been made to develop means of controlling ticks within the environment. One means used is removal of an essential host. This has been tried for *I. scapularis* by eliminating all deer in an area (Wilson et al, 1988). Such a drastic method may produce significant tick reductions, although alternative hosts may be found that allow the ticks to persist in the environment in lower numbers. Also, methods have been examined for reducing the numbers of ticks on deer by the use of ivermectin-treated feed bait (Pound et al, 1996); this method shows some potential for control. Another approach has been to attack the larvae of ticks by using acaricidal-impregnated baits or nesting material containing ectoparasiticides, which mice and rats carry to their nesting areas (Mather, Ribiero, and Spielman, 1987); again, this method can be quite successful in controlling ticks in isolated areas. In addition, work is under way to produce vaccines against ticks that cause the host to produce antibodies that the tick ingests while feeding that damage the gut of the feeding tick (Willadsen et al, 1995); such vaccines are likely to be used more and more widely as they become available for use in cattle, dogs, and cats.

Sometimes, premises will need to be sprayed for control, especially in the case *of R. sanguineus*. In the past, ranchers in the West periodically would burn pastures to reduce tick numbers. In cases with severe tick pressure, it may not be sufficient to simply treat the pets in a household; it may also be necessary to also treat certain areas with acaricides, most likely along areas where lawns and wooded areas come in contact. Conversely, when a backyard pet is treated with an excellent anti-tick product and comes into contact with ticks in its environment, the number of ticks within an area can be markedly reduced.

SUBORDER MESOSTIGMATA, MESOSTIGMATID MITES

Mesostigmatids, as the name implies, have their **stigmata** (respiratory pores) in the middle of their bodies. A stigma lies between the

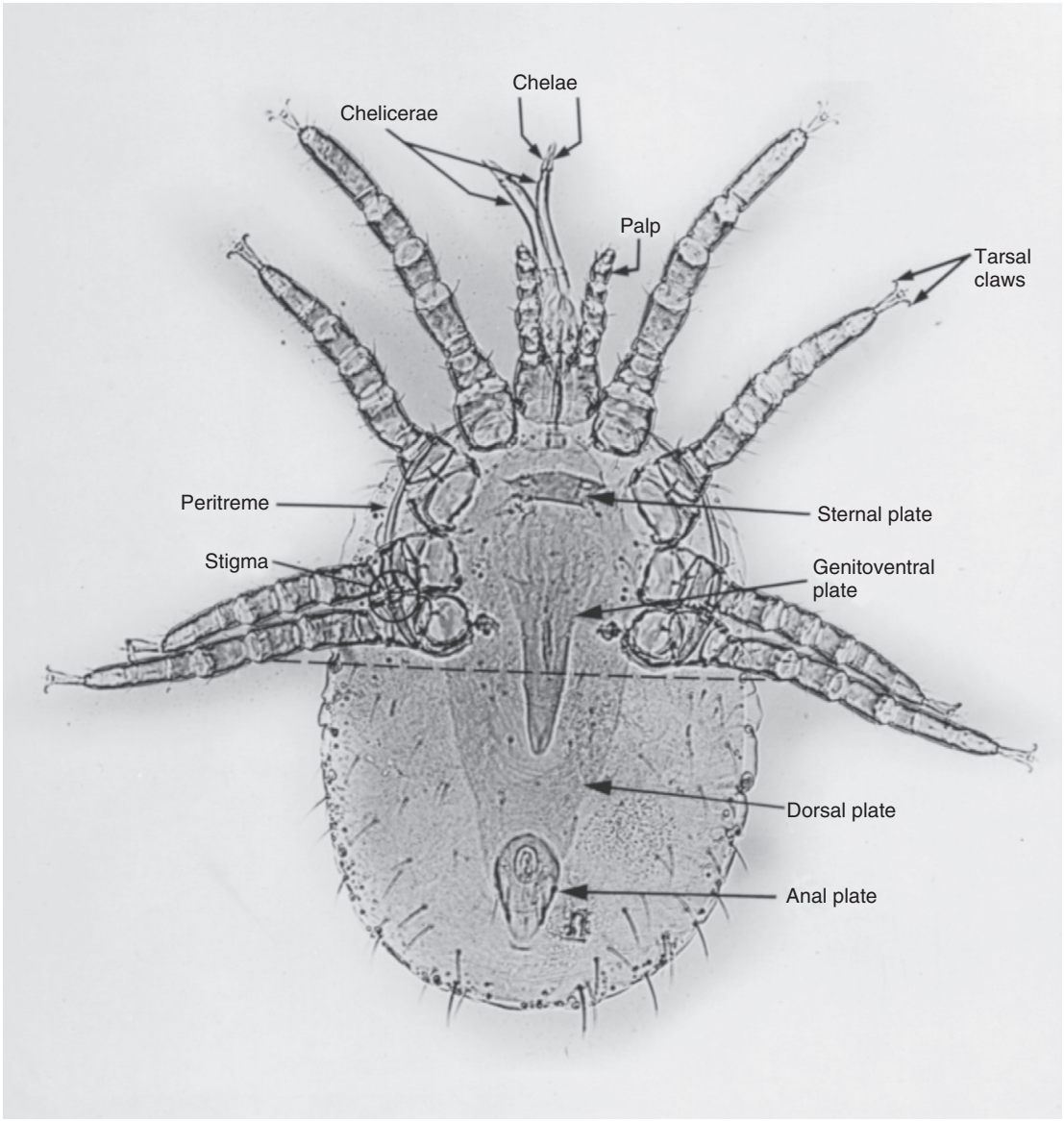

FIGURE 2-93. *Ornithonyssus sylviarum*, a bloodsucking mesostigmatid mite. The legs are confined to the anterior half of the body of mesostigmatid mites; the stigma is located between the third and fourth coxae and has a peritreme. The chelae of *Ornithonyssus* are much larger than those of *Dermanyssus*.

third and fourth coxae on each side of the body and is connected to a sinuous **peritreme.** The coxae are evenly spaced and crowded into the anterior half of the body; the tarsi are generally armed with claws; and the ventrum is armored with sclerotized plates (Figure 2-93).

Families Dermanyssidae and Macronyssidae

Bloodsucking mesostigmatid mites that parasitize birds (e.g., *Dermanyssus gallinae*, *Ornithonyssus sylviarum*) and rodents (e.g., *Ornithonyssus bacoti*, *Liponyssoides sanguineus*) frequently turn on the human inhabitants of a building when deprived of their normal hosts, as may occur when fledglings leave their nests or after rodents have been exterminated. Generic or even familial identification of these mites is sufficient to establish the general nature of the epidemiologic situation, but specific identification sometimes provides a very helpful lead in the search for nests. For example, a hospital administrator submitted a specimen of a mite that was causing great consternation by its abundance in the hospital linens. Dr. Georgi identified the specimen as a dermanyssid mite and advised the gentleman to hunt for bird or rodent nests. A few days later, he reported no success in finding nests of either kind. However, by that time, the specimen had been shown to an expert acarologist who identified it as *Dermanyssus hirundinis*, a relatively host-specific parasite of swallows. Thus advised, the hospital administrator knew just where to look, and the problem was quickly solved.

Dermanyssids and macronyssids all look very much alike on casual inspection, but because they vary significantly in habits and host preferences, accurate identification is a prerequisite to effective control. The **chelicerae** (piercing mouthparts), **chelae** (scissor-like structures on the end of the chelicerae), and form and septation of various sclerotized plates serve as the main taxonomic characters used in differentiating these mites.

Dermanyssus *(Dermanyssidae)*

The chelicerae are long and slender and the chelae minute (Figure 2-94). A single dorsal plate is present; the sternal plate has two pairs of setae; and the anus is in the posterior half of the anal plate.

FIGURE 2-94. Gnathosome of *Dermanyssus gallinae.* The chelicerae of *Dermanyssus* are slender and whiplike, and the chelae are very small.

FIGURE 2-95. Living *Ornithonyssus sylviarum* crawling on a chicken feather collected from litter. Note the dark X-shaped gut.

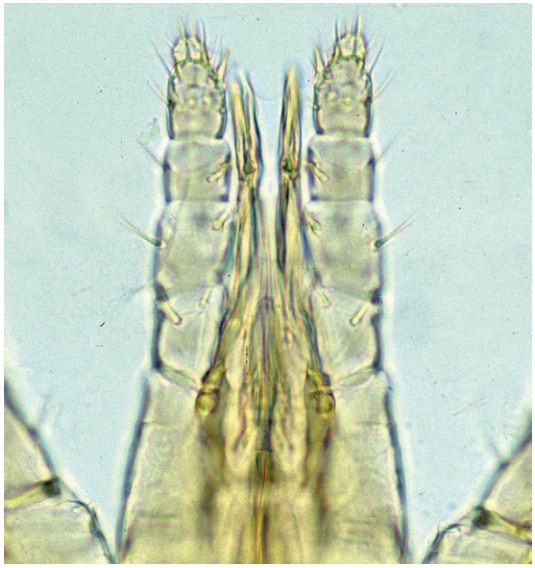

FIGURE 2-96. Gnathosome of *Ophionyssus*.

Dermanyssus mites are infrequently found on the bird because these mites hide in nests, roosts, and the like during the day and attack the sleeping bird at night. Life stages include the egg, which is deposited in the diurnal hiding places of the mites; the six-legged, nonfeeding larva; and the blood-feeding protonymph, deutonymph, and adult male or female. A generation can be completed in as little as a week, and large populations may build up in chicken houses or birds' nests. The adults can survive starvation for months. *Dermanyssus* mites remove enough blood to kill nestlings and reduce egg production. Ramsay and Mason (1975) reported a case in a dog that was so severe that mites crawling through the hair resembled the "walking dandruff" usually associated with *Cheyletiella* infestations. Their importance as disease vectors is unclear.

Liponyssoides (Dermanyssidae)

The chelicerae are long and slender and the chelae minute. Two dorsal plates are present; the anterior plate is 10 times as large as the posterior; and the sternal plate has three pairs of setae. *Liponyssoides (Allodermanyssus) sanguineus*, a parasite of the house mouse, *Mus musculus*, and other small rodents, is the vector of rickettsial pox *(Rickettsia akari)* of humans. People are occasionally infected in the United States, and most are described as being from New York City (Madison et al, 2008); dogs can also become ill if infected with *R. akari* (Zavala-Castro et al, 2009).

Ornithonyssus (Macronyssidae)

The chelicerae are much stouter than those of *Dermanyssus*, and the chelae are easily visible under ordinary magnification. A single dorsal plate is present, and the anus is in the anterior half of the anal plate (see Figure 2-93). When the mite is alive, the gut often appears black or dark red (Figure 2-95). Common species include *O. sylviarum*, the northern fowl mite; *Ornithonyssus bursa*, the tropical fowl mite; and *O. bacoti*, the tropical rat mite. *Ornithonyssus* species remain on the host much of the time and cause considerable loss of blood. Persons handling eggs from laying flocks heavily infested with *O. sylviarum* may experience annoyance and serious discomfort from the bites of these mites. *O. bacoti* is an important pest in laboratory rodent stocks and serves as an intermediate host for *Litomosoides carinii*, a filariid parasite of the cotton rat, *Sigmodon hispidus*. *L. carinii* is a favorite laboratory model for testing antifilarial drugs.

Ophionyssus (Macronyssidae)

Ophionyssus natricis, the snake mite, is a formidable bloodsucking pest that tends to thrive on captive snakes (Figure 2-96).

FIGURE 2-97. *Raillietia auris*, a mesostigmatid parasite of the ear canal of cattle. In this reflected light photomacrograph, the specimen appears as it would under a stereoscopic microscope or a powerful hand lens.

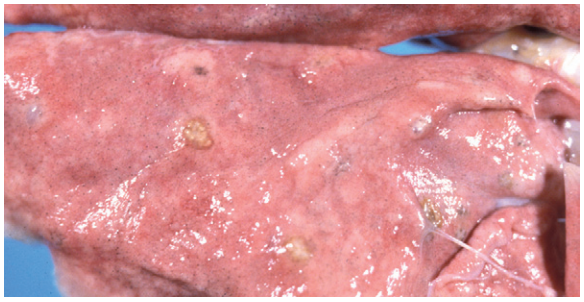

FIGURE 2-98. *Pneumonyssus simicola* lesions in the lungs of a macaque.

Snakes have been treated with injectable ivermectin (Stanchi and Grisolia, 1986).

Family Raillietidae

Raillietia

Raillietia auris (Figure 2-97), long considered a harmless parasite of the ears of cattle, has been shown to cause ulceration and blockage of the auditory canals by pus with resultant loss of hearing (Heffner and Heffner, 1983). Jubb, Vasallo, and Wroth (1993) reported that infestations with this mite were associated with calves circling, ataxia, and unilateral facial paralysis. In their work, calves were cleared of their infestations with the application of flumethrin to the ear canal, whereas the topical application of flumethrin or subcutaneous ivermectin was unsuccessful.

Family Halarachnidae

Pneumonyssus

Groups of *Pneumonyssus simicola* mites may be found in the lung parenchyma of most if not all *Macaca mulatta* monkeys (see Figure 8-8). The lesions are pinhead or larger, whitish or yellow foci (Figure 2-98) that have soft or empty centers and contain mites and a black pigment. These lesions are scattered throughout the lungs and may be mistaken for those of tuberculosis. It is difficult to correlate clinical signs of pulmonary acariasis with the degree of pathologic change in the lungs, and antemortem diagnosis is difficult. Monkeys can be reared free of *Pneumonyssus* infection if they are separated from their mothers at birth and are reared in isolation from adult monkeys. The histopathologic diagnosis of *P. simicola* infection is discussed in Chapter 8.

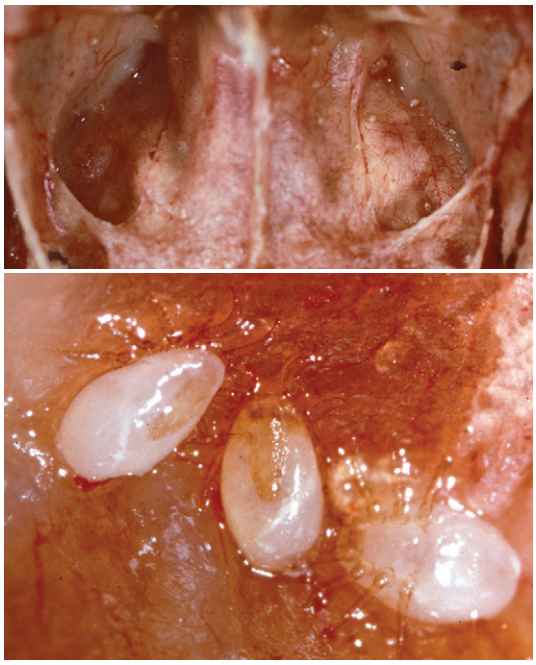

FIGURE 2-99. *Pneumonyssoides caninum. Top*, View into the nasal sinuses of a dog at necropsy showing the mites in situ. *Bottom*, Closer view of three adult mites.

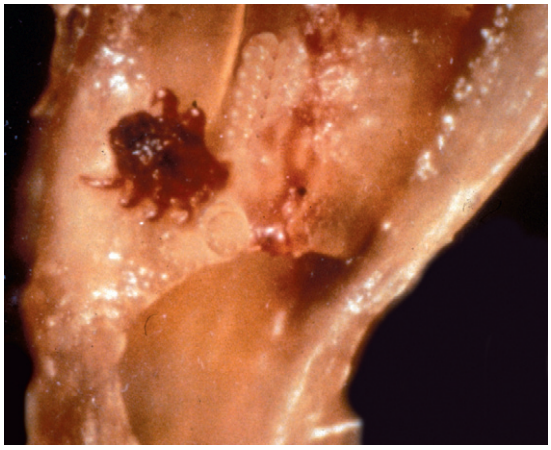

FIGURE 2-100. *Sternostoma* mite in the trachea of a bird.

Pneumonyssoides

A parasite of the nasal and paranasal sinuses of dogs (Figure 2-99 and see Figure 7-45), *Pneumonyssoides caninum* sometimes causes chronic sneezing and epistaxis. Occasionally, nasal discharge has been reported in dogs with this infestation (King, 1988). Rhinoscopy and nasal swabbing are aids to diagnosis. Treatment of *P. caninum* is easily induced by the subcutaneous administration of ivermectin (Mundell and Ihrke, 1990); it can also be successfully treated with two topical applications of a combination of imidacloprid and moxidectin (Ritter, 2008).

Family Rhinonyssidae

Sternostoma

Sternostoma tracheacolum is a bloodsucking mite of the respiratory passages, including the abdominal air sacs, of canaries, finches, and a wide range of other wild and domestic birds (Figure 2-100). *S. tracheacolum* infection may not be apparent clinically or may

cause chronic respiratory illness manifested by loss of voice, shaking of the head, and sneezing. Diagnosis in the living bird is facilitated by moistening and parting the feathers in the neck region and transilluminating the trachea with a strong light; the mites appear as shadowy spots in the trachea. On necropsy examination, these mites appear to the unaided eye as black spots in the posterior nares, trachea, air sacs, lung tissues, and abdominal cavity (Kummerfeld and Hinz, 1982). In an outbreak in an aviary housing Lady Gould's Amadines *(Chloebia gouldiae)*, the birds were treated with topical application of selamectin (Zeissler, 2009).

Family Varroidae

Varroa

Varroa destructor (formerly known as *Varroa jacobsoni*) is a parasite of honeybees that was introduced into the United States sometime in the 1980s. Mites and other parasites of bees are a serious threat to U.S. agriculture. All one has to do is visit a local lawn with clover and notice that no honeybees or very few honeybees are present; some have estimated that more than 95% of the wild honeybees in the United States have been eliminated by these parasites. Wild honeybees are no longer considered effective as providers of pollination for farmers. Within commercial hives, in the winter of 1995, losses ranged from 40% in Delaware to 80% in Maine. Although bees produce honey valued at some $125 million, it is even more important to note that they are responsible for pollinating nearly $15 billion worth of crops in the United States each year (Doebler, 2000). *V. destructor*, an external parasite of honeybees, is very large; females are 1 to 1.5 mm in diameter, reddish to dark brown, and easy to observe on bees with the naked eye. The mites suck hemolymph from both adult bees and the brood, preferring the blood of drones. A female mite enters a brood cell about 1 day before capping and becomes sealed in the brood capsule with the larval bee. The female then lays eggs, and the developing larval mites feed off the developing bee. When the adult bee emerges from the brood cell, the mites in the cell will have developed to adulthood and mated, and the females will be ready to enter a new cell. The disease is spread between hives by mites attached to worker bees. Untreated infestations of hives destroy colonies. Treatment of infested colonies is performed using formulations that contain the miticide flumethrin, fluvalinate, oxalic acid, formic acid, or thymol. It should not be forgotten that bees are food animals, and that strips should not be used during honey flow, or when honey that may be removed for human consumption is present. Veterinarians need to be aware that pesticides may be used in ways that can lead to honey contamination (Harman, 1998). The development of resistance to these agents in mites is also problematic; the fact that bees are also arthropods makes it difficult to increase treatment levels because bees are usually killed through the same pharmacologic pathway.

SUBORDER ASTIGMATA, ASTIGMATID MITES

In contrast to mesostigmatids, astigmatid mites lack stigmata, and respiration is integumental; the first and second coxae are widely separated from the third and fourth, the ventrum is devoid of conspicuous plates, and some **tarsi** are equipped with **sarcoptiform pretarsi,** a sucker **caruncle** that is supported on a thin terminal stalk, the **pedicel.** Astigmatids include the mange mites, certain hair-clasping mites, two internal parasites of chickens, and the grain mites.

Mange mites (families Sarcoptidae, Knemidocoptidae, and Psoroptidae) cause mange or scabies, a dermatitis characterized by pruritus, alopecia, and epidermal hyperplasia with desquamation. Rubbing and scratching by the host frequently result in deeper wounds that ooze serum and blood. These coagulate, gluing hair, epidermal debris, and foreign matter together to form crusts and scabs. Secondary bacterial infection may complicate the situation.

The typical distribution and manner of spread of mange lesions vary with the host and parasite species and are often characteristic enough to permit accurate diagnosis by an experienced observer. However, recovery and identification of mites are necessary for positive diagnosis. Negative scrapings are inconclusive. Therefore, typical mange lesions should be subjected to persistent examination until mites are found, or until further scraping would do excessive injury to the patient. For lesions with minimal epidermal hyperplasia and lesions caused by deeply burrowing mites (e.g., *Sarcoptes*, *Demodex*), dip a scalpel blade into glycerin or mineral oil, pinch a fold of skin firmly between thumb and forefinger, and, while holding the blade at right angles to the skin, scrape until blood begins to seep from the abrasion. Much of the detritus will adhere to the layer of mineral oil on the scalpel blade and may be transferred to a microscope slide and searched for mites. For lesions with marked epidermal hyperplasia and exfoliation and lesions caused by superficially dwelling mites (e.g., *Chorioptes*) and lice, scrape the detritus into an ointment tin using the cover as a scraper. Examine the scrapings under a stereomicroscope or hand lens to find the mites crawling about. If no mites are observed directly, recourse may be digestion of the skin scrapings in potassium hydroxide, as described in Chapter 7.

Generic differentiation of mange mites likely to be encountered in routine veterinary practice requires little more than examination of their pretarsi (Figures 2-101 and 2-102). If the pretarsus has a long, unsegmented pedicel (stalk), the specimen is most likely *Sarcoptes* or *Notoedres*. If the pretarsus has a long, three-segmented pedicel, the mite is bound to be *Psoroptes*. Pretarsi with short pedicels are found on *Chorioptes* from ungulates and *Otodectes* from dogs; species identity of the host is a sufficiently reliable differential criterion in this case. *Knemidokoptes* females lack pretarsi, but the males have pretarsi resembling those of *Sarcoptes*. Certain particularly destructive manges, such as psoroptic mange in sheep and

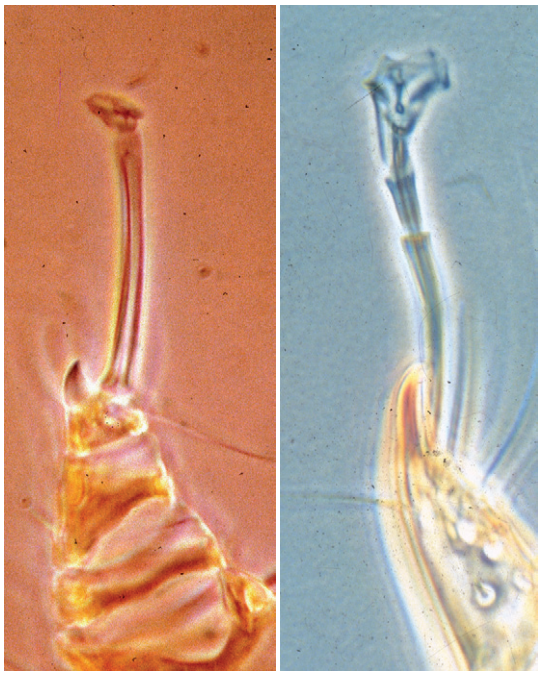

FIGURE 2-101. Pretarsi of *Sarcoptes (left)* and *Psoroptes (right).* Both have long pedicels; the pretarsus of *Psoroptes* is jointed.

cattle and sarcoptic mange in cattle, should be reported to state animal disease control authorities.

Family Sarcoptidae

Sarcoptes

The pretarsi have long, unsegmented pedicels, and the anus is at the posterior edge of the body (Figure 2-103; see also Figure 2-101). *Sarcoptes scabiei* causes sarcoptic mange or scabies of humans, dogs, foxes, horses, cattle, and others. Sarcoptic mange of cattle is reportable. Although *S. scabiei* infests a wide range of hosts, a considerable degree of host specificity has arisen among populations of this parasite, so that scabies of pigs tends to spread more readily among pigs, scabies of humans tends to spread more readily among humans, and, when interspecific transmission does occur, the resulting dermatitis tends to be atypical and transient. In fair-skinned human subjects with relatively mild infestations, it is possible to see the tiny serpentine tunnels that trace the wanderings of the egg-laying female mite as she burrows through the epidermis. Along the course of the burrow, dark areas representing eggs and accumulations of feces may be observed, and, at the end of the tunnel, the mite may be found and lifted out with the point of a needle. Hair obscures such lesions on domestic animals, and it may be that many relatively mild cases of sarcoptic mange are overlooked. As few as 10 to 15 mites constitute a case of ordinary (but nonetheless unendurable) human scabies, but thousands to millions may be found on a mangy pig or fox. It is curious, however, that *Sarcoptes* mites are frequently difficult to find on dogs, even those exhibiting advanced lesions.

Sarcoptic mange of domestic animals usually starts on relatively hairless areas of skin and may later generalize. In dogs, the lateral aspect of the elbow and the pinna of the ear are favorite starting places; the lesions consist of follicular papules, areas of erythema, crusts of dried serum and blood, and excoriations from scratching to relieve the intense pruritus (see Figure 8-3). Secondary bacterial infection is a frequent complication. In swine, sarcoptic mange usually starts around the eyes and on the nose, back, sides, and inner surface of the thighs; lesions may progress to hyperkeratosis and exfoliation of epidermal debris (see Figure 8-4). The red fox, *Vulpes vulpes*, is affected by a lethal form of sarcoptic mange in which the epidermis may undergo a 10-fold increase in thickness and may contain countless hordes of mites. Sarcoptic mange in cattle is fortunately very rare in the United States. Infestation in cattle can often become a horrible generalized disease that requires treatment and quarantine; cattle can have numerous highly pruritic lesions that can cause severe self-trauma. In an outbreak of sarcoptic mange in a herd of cattle in New York State, the prevalence of udder cleft dermatitis was determined both before and after treatment of the mite infestations with eprinomectin (Warnick et al, 2002). Control of the mange in the cattle had only a moderate effect on the prevalence of udder cleft dermatitis and did not eliminate the condition from the herd.

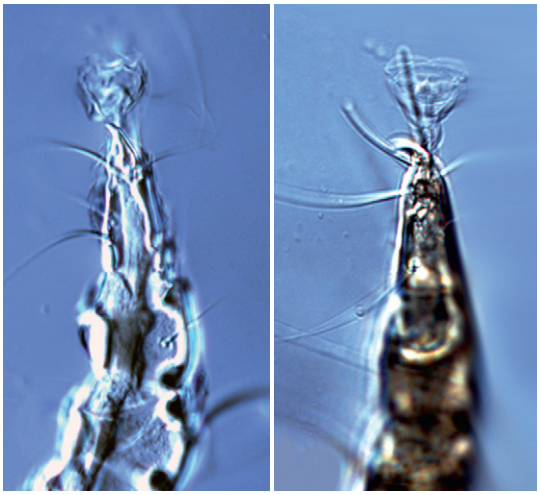

FIGURE 2-102. Pretarsi of *Otodectes (left)* and *Chorioptes (right)*. Both have short pedicels. *Otodectes* is a parasite of the ear canal of carnivorans; *Chorioptes* is a parasite of the epidermis of ungulates.

Notoedres

A parasite of cats, rats, rabbits, and occasionally and temporarily of humans, *Notoedres* much resembles *Sarcoptes* in that the pretarsi have long, unsegmented pedicels, but it is smaller and its anus is

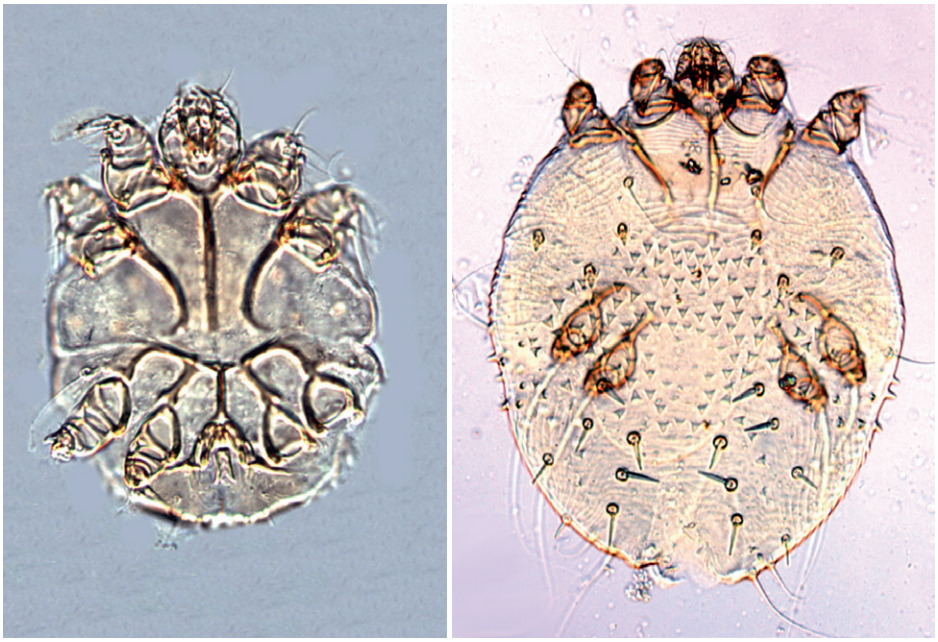

FIGURE 2-103. *Sarcoptes* male *(left)* and female *(right)*.

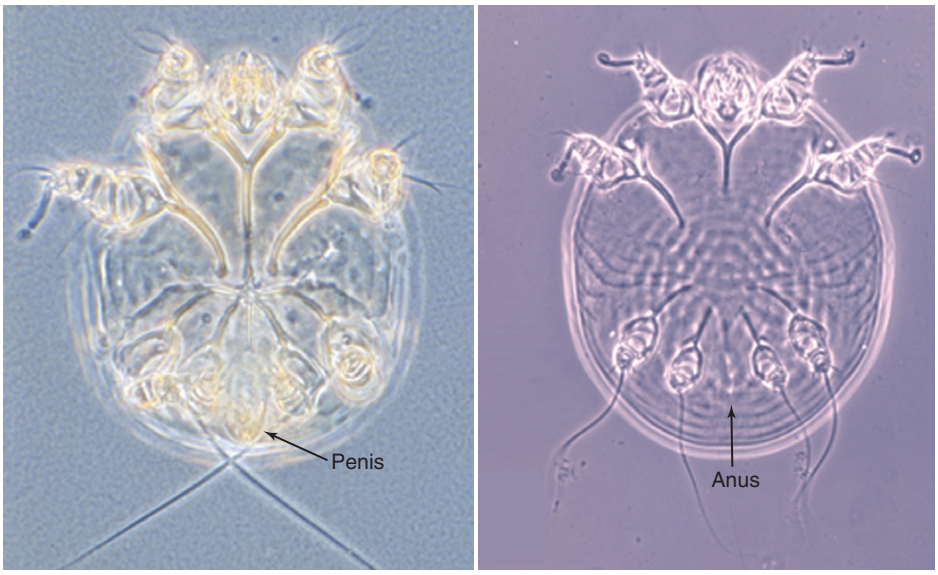

FIGURE 2-104. Notoedres male *(left)* and female *(right)*.

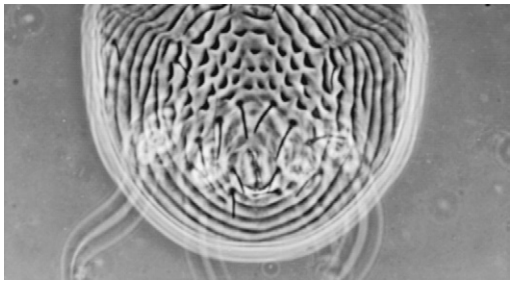

FIGURE 2-105. *Notoedres cati.* Same as Figure 2-101, *right*, but with dorsal anus of female in focus.

FIGURE 2-106. A group of kittens with typical notoedric mange lesions on the ear margins.

on the dorsal surface instead of on the posterior margin of the body (Figures 2-104 and 2-105). Face mange of cats caused by *Notoedres cati* starts on the medial edge of the pinna of the ears and then spreads over the ears, face, paws, and hindquarters by contiguity and contact. The lesions of notoedric mange consist principally of alopecia and marked hyperkeratosis with abundant epidermal flakes; mites are easily demonstrated (Figure 2-106 and see Figure 8-5). An epizootic of notoedric mange has been reported in the Florida Keys, where more than 500 cats were examined (Foley, 1991a). Major signs included pruritus, self-mutilation dermatitis, gray crusts on the skin, secondary pyoderma, and hypertrophied skin. This is the typical cause of mange in cats.

Not all cases of feline mange are caused by *Notoedres*. For example, in the case of an exotic cat, a half dollar–sized area of dermatitis on the top of a pet ocelot's head was tentatively diagnosed as notoedric mange. However, a scraping revealed that the villain was *Sarcoptes* and raised the possibility of an infested human contact. In fact, the owner had been suffering from a severe itch below her breasts but had not connected her discomfort with her ocelot's skin lesion. In this particular case, it was not at all clear who had harbored the mites first, but that, after all, is an academic question. What is important is that correct generic identification of the parasite led to effective control through appropriate medication of both infested individuals. Cases of sarcoptic mange have also been reported in domestic cats. Most recently, four cases of crusted scabies were described; two of the cats were from areas frequented by foxes and two from homes where a dog had, or had been treated for, sarcoptic mange (Malik et al, 2006).

Cosarcoptes, Prosarcoptes, Pithesarcoptes, and *Kutzerocoptes*

The first three genera are parasites of Old World monkeys (Cercopithecidae), and the last one is a parasite of New World monkeys (Cebidae). All resemble *Sarcoptes* morphologically, biologically, and pathogenetically. Mange of monkeys, at least that caused by *Cosarcoptes scanloni*, may be transmissible to humans (Smiley and O'Connor, 1980).

Trixacarus caviae

A parasite of the guinea pig, *T. caviae* closely resembles *Sarcoptes scabiei* but is only half as large; the anus is on the dorsal surface of the female and on the posterior margin of the body of the male. *Trixacarus* causes pruritus so intense that affected guinea pigs are subject to fits and seizures brought on by vigorous scratching or manipulation of the skin (Kummel et al, 1980). Mange in guinea pigs has been successfully treated with ivermectin administered subcutaneously.

Family Knemidocoptidae

Knemidokoptes

Knemidokoptes mutans causes scaly leg in chickens, turkeys, pheasants, and other gallinaceous birds. The mites burrow in the epidermis of the legs, causing the scales to lift and become loosened

and the legs to become thickened and deformed (Figure 2-107). To demonstrate mites, simply remove a loose leg scale and examine the underside of it with a hand lens. The female *K. mutans* is about 0.5 mm in diameter; the legs are very short and lack pretarsi (see Figure 2-107). Males are much smaller and have longer legs equipped with pretarsi resembling those of *Sarcoptes*.

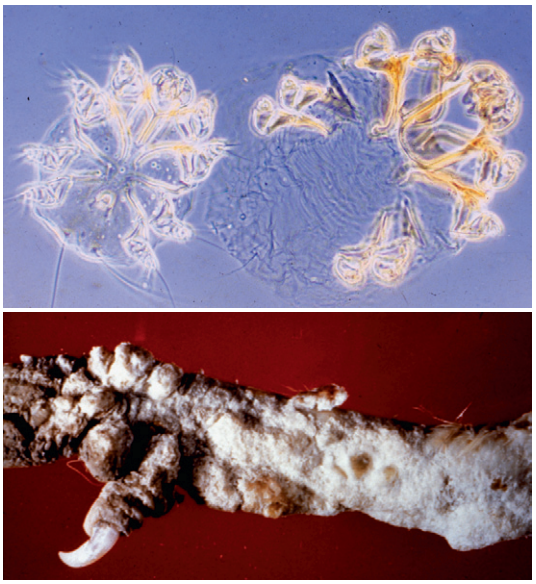

FIGURE 2-107. *Knemidokoptes* male *(left)* and female *(right)* and lesions caused to the leg of an infested chicken *(bottom)*.

Knemidokoptes pilae and *Knemidokoptes jamaicensis* cause mange of the legs, base of the beak, vent area, and back of parakeets and canaries, respectively. Lesions respond well to daily applications of mineral oil to all areas where mites are likely to be found, including the vent area. The oil tends to loosen crusts, which should be carefully removed. Rotenone-orthophenylphenol (Goodwinol ointment) or ivermectin mixed with a few drops of dimethyl sulfoxide (DMSO) and applied to lesions with a cotton swab is a suitable topical treatment. Ivermectin administered orally or intramuscularly at 0.2 mg/kg presents several advantages over topical acaricides: Only one or in particularly serious cases two treatments are necessary. It does not mat down feathers or get in the birds' eyes and is apparently well tolerated (Ryan, 1986).

Knemidokoptes gallinae, the depluming mite of chickens, pigeons, pheasants, and geese, is found at the base of the feathers on the back, on top of the wing, and on the vent, breast, and thighs. It causes intense pruritus, leading in turn to feather pulling.

Family Psoroptidae

Psoroptes

The legs are long, and the pretarsi have long, three-segmented pedicels (Figures 2-108 and 2-109; see also Figure 2-101). *Psoroptes ovis* causes a very serious and reportable form of mange (scabies or "scab") in cattle, sheep, and horses. Psoroptic mange is prevalent among cattle herds in the southwestern United States but is relatively rare elsewhere in North America. *Psoroptes cuniculi* is very common and causes ear canker in rabbits and a less severe form of otic acariasis in goats and horses.

Psoroptes ovis does not burrow in the epidermis but remains at the base of the hairs and pierces the skin with its stylet-like

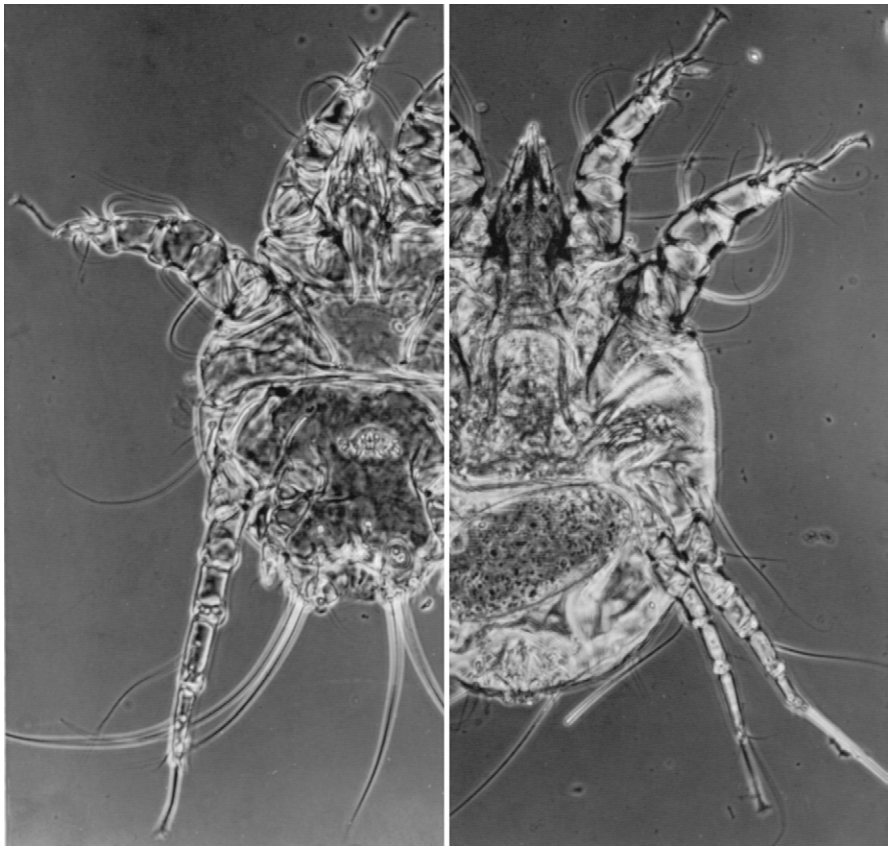

FIGURE 2-108. *Psoroptes* male *(left)* and female *(right)*.

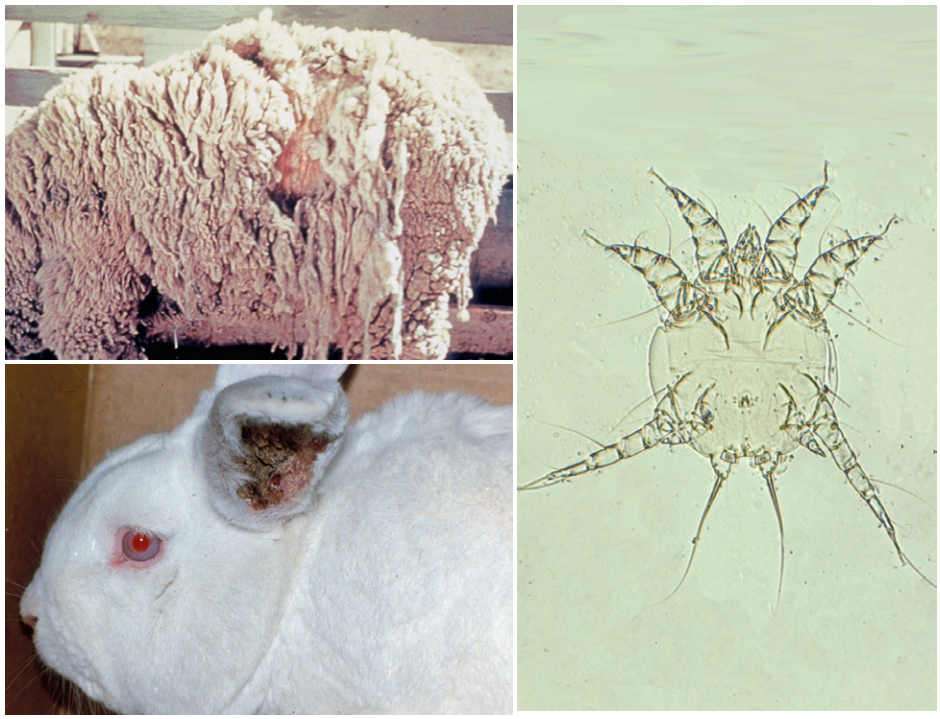

FIGURE 2-109. Psoroptic mange on a sheep *(top left)* and ear canker in a rabbit *(bottom left)*. *Right*, An adult male *Psoroptes*.

chelicerae. This manner of feeding results in exudation of serum, which hardens to form a scab. Mites are best demonstrated under the edges of these scabs, so it is inefficient to submit great wads of wool to the laboratory, especially if the scabs are not included in the shipment. Psoroptic scab is particularly devastating in sheep, especially those maintained principally for the production of high-quality wool. Pruritus is usually intense. At first, tags of wool are observed projecting from the fleece and clinging to fence posts, door jambs, trees, and other convenient objects against which an itchy sheep might obtain some measure of relief (see Figure 2-109). Progressively more and more wool is shed or rubbed away by the frantic sheep, and pustules appear on the denuded, hardened, thickened, and excoriated skin. As the pustules become confluent and overlain by a scab of coagulated serum and foreign material, the area ceases to be suitable for the mites, and they move on to fresh territory. In this way, the lesions tend to spread over the surface of the body. The sheep become greatly debilitated by psoroptic scab and may even die of it. *Psoroptes ovis* may survive off the host for several days or weeks. Therefore, effective control requires both acaricidal treatment of all infested livestock and disinfection or 2- to 4-week vacating of contaminated enclosures and vehicles (Wilson, Blachut, and Roberts, 1977).

P. cuniculi is a ubiquitous parasite of the external ear canal that can frequently be demonstrated in apparently normal rabbits. When infested rabbits are placed under stress, as for example, when a doe kindles, the population of mites tends to explode and the ear canal is laid waste as a result (see Figure 2-109 and see Figures 7-108 and 7-109). A full-blown case of ear canker without secondary bacterial infection will respond amazingly well and will heal in a dramatic fashion after the subcutaneous administration of ivermectin. Prevention is possible by weekly instillation of a few drops of mineral oil into the ear canal of each rabbit in the colony. *P. cuniculi* produces a less severe form of otic acariasis in goats and horses.

Chorioptes bovis

Pretarsi of *Chorioptes bovis* have short, unsegmented pedicels on the first, second, and fourth pairs of legs of the female and on all legs of the male; the male has two turret-like lobes on the posterior margin of the body (Figure 2-110). *C. bovis* is a cosmopolitan, superficially dwelling parasite that displays a distinct preference for the tail, escutcheon, and legs of cattle, where it feeds on epithelial debris. Although cattle are the principal hosts, *C. bovis* also may be found on the tail and legs of horses, sheep, and goats and in the ear canal of rabbits. Asymptomatic infestation is far more common than obvious dermatitis.

Chorioptic mange in cattle usually appears during late winter as a superficial, mildly pruritic, flaky dermatitis involving the tail, escutcheon, and hind legs (Figure 2-111). Whereas stanchioned animals are made miserable because they are unable to take appropriate action to relieve the itching, for unconfined cattle, chorioptic mange probably is not much more serious a burden than a crop of chewing lice and, like a suit of woolen underwear, may help keep them warm by encouraging physical activity. Chorioptic mange tends to disappear soon after the cattle are turned out to pasture in spring. *C. bovis*, like the pinworm *Oxyuris equi*, is an identifiable cause of tail rubbing in horses.

C. bovis causes exudative dermatitis on the lower legs and scrota of rams. In extreme cases, the crusts may be 5 cm thick. Deterioration of semen quality was associated with chorioptic mange lesions covering more than one third of the scrotum and was apparently related to elevation of testicular temperature (Rhodes, 1975).

Otodectes cynotis

Pretarsi of *Otodectes cynotis* have short, unsegmented pedicels on the first and second pairs of legs of the female and on all legs of the male; the body of the male is only weakly bilobed posteriorly (Figure 2-112; see also Figure 2-102). *O. cynotis* infests the external ear canal and adjacent skin of dogs, cats, foxes, and ferrets,

FIGURE 2-110. *Chorioptes* male *(left)* and female *(center)*. The female has pretarsi on the first, second, and fourth pairs of legs, and the male has pretarsi on all four pairs. *Right*, *Chorioptes* male and deutonymph; the deutonymph has pretarsi on the first and second pairs of legs.

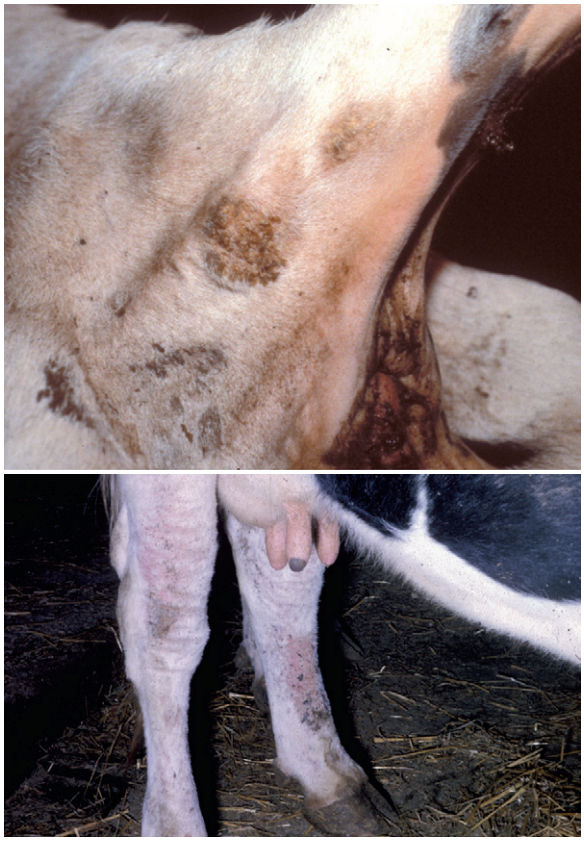

FIGURE 2-111. Chorioptic mange on cows.

causing intense irritation. Occasional reports have described people, and a recent report from Japan discussed five cases in which people were suffering intermittent tinnitus and had mites removed from their eardrum that were identified as *O. cynotis*; four of the five patients routinely slept in the same bed as their pet cat, and the fifth patient lived with five cats (Kato et al, 2011). Copious production of dark cerumen is always considered characteristic of otodectic otitis; however, cats with massive numbers of mites—thousands—tend to have ears that contain light tan to gray, sandy, or flakelike material, rather than dark-brown to black waxy material. Perhaps the dark cerumen represents a later stage in the infestation, or perhaps it occurs in animals better able to control the infestation. In any event, the aural pruritus sometimes causes the animal to rub and scratch its ears and shake its head violently enough to produce hematoma of the aural pinna. The mites may be demonstrated by swabbing the ear canal with a cotton applicator and then placing the applicator on a dark background under a lamp or on a sunny windowsill. The heat will drive the mites out of the debris, and they will be seen as tiny white specks moving against the dark background. The number of mites present in the cat's ear can be quite remarkable. Preisler (1985) reported more than 8500 mites in the ear canal of a cat. When large numbers of mites are present in the canal, the cat's ear tends to contain a dry, waxy, light-colored, parchment-like material in sheets, with large numbers of mites present in each layer.

Other Astigmatid Mites

Hair-clasping mites of the superfamily Listrophoroidea have one or more pairs of legs variously flattened, bowed, or otherwise modified for clasping a hair (see Figure 7-110). Examples include *Chirodiscoides caviae*, a parasite of guinea pigs (Figure 2-113), and *Myocoptes musculinus*, a parasite of rodents (Figure 2-114). *Lynxacarus radovskyi* is a hair-clasping mite of domestic cats in Florida, Puerto Rico, Hawaii, Australia, and Fiji (Figure 2-115); hordes of these tiny mites clinging to the hairs impart a scruffy appearance (Greve and Gerrish, 1981). Not all hair-clasping mites belong to the superfamily Listrophoroidea or even to the suborder Astigmata. For examples of exceptions, see *Myobia* and *Radfordia* later.

Feather mites occur in variety and in abundance. Most are members of several superfamilies of Astigmata. Feather mites are usually external, but some live within the quills. Others, such as members of the family Epidermoptidae, burrow in the skin and may cause a mangelike condition. Astigmatid feather mites may be

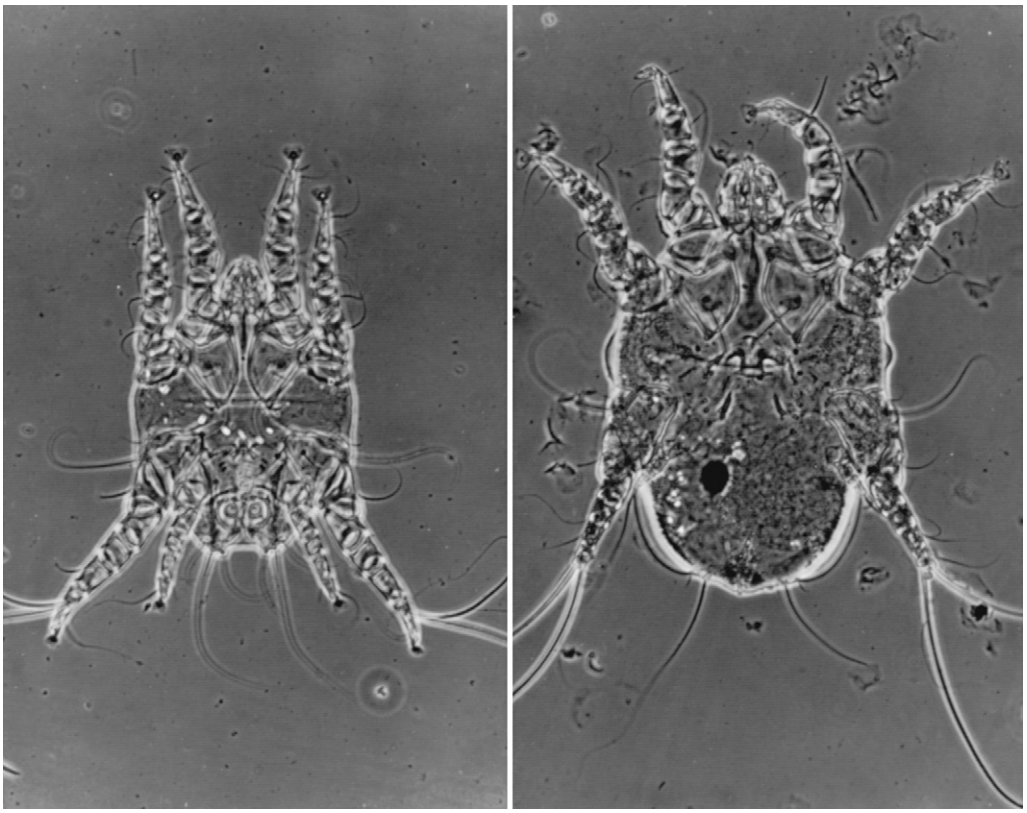

FIGURE 2-112. *Otodectes* male *(left)* and female *(right)*. The female has pretarsi on the first and second pairs of legs; the male has pretarsi on all four pairs.

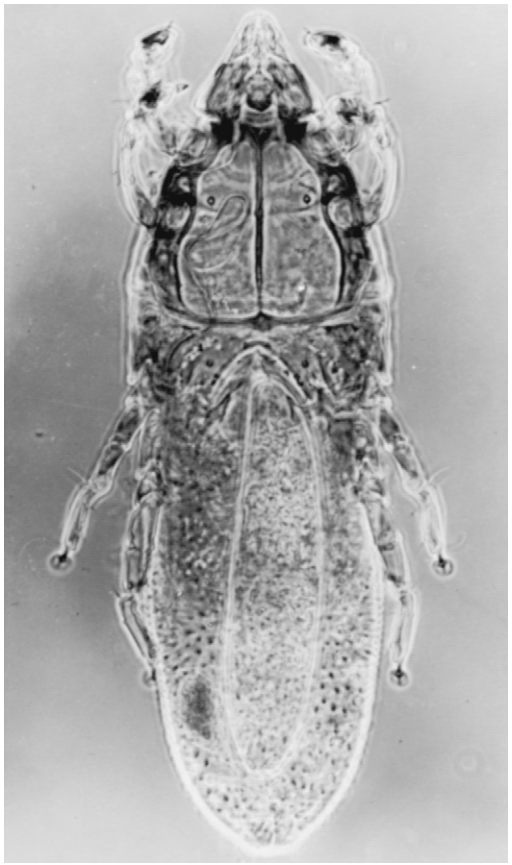

FIGURE 2-113. *Chirodiscoides caviae* female.

distinguished from prostigmatid feather mites such as *Syringophilus* by their sarcoptiform pretarsi.

Two families of Astigmata have evolved as internal parasites of birds: *Laminosioptes* (Laminosioptidae) occur in subcutaneous nodules in chickens, and several genera of the family Cytoditidae are parasites of the air sacs and respiratory passages of chickens, canaries, and other birds.

Members of the families Acaridae and Glyciphagidae are free-living mites that feed on organic matter. They may be found in grain, cheeses, dried fruit, and other stored food products. Contact with these mites and their detritus may cause urticaria and dermatitis in human beings. "Grain mites" are frequently found as pseudoparasites in fecal smears. They may be distinguished from parasitic astigmatids by the shape of the female genital opening, which is a transverse or U-shaped slit in parasites, but a more or less longitudinal slit in grain mites.

SUBORDER CRYPTOSTIGMATA, ORIBATID MITES

The Cryptostigmata, or oribatid mites, are free-living inhabitants of humus, some of which serve as intermediate hosts to tapeworms of the family Anoplocephalidae. When ingested by an oribatid mite, the larva in the egg of the tapeworm *Moniezia* develops into a cysticercoid, the larval stage of which is infective for the ruminant definitive host.

SUBORDER PROSTIGMATA, PROSTIGMATID MITES

The Prostigmata (with the stigmata if apparent located anterior to the first pair of legs) is a polyphyletic amalgamation that includes both free-living species and such diverse obligate parasites as pilosebaceous mites *(Demodex)*, hair-clasping mites *(Myobia)*, and "chiggers" (Trombiculidae).

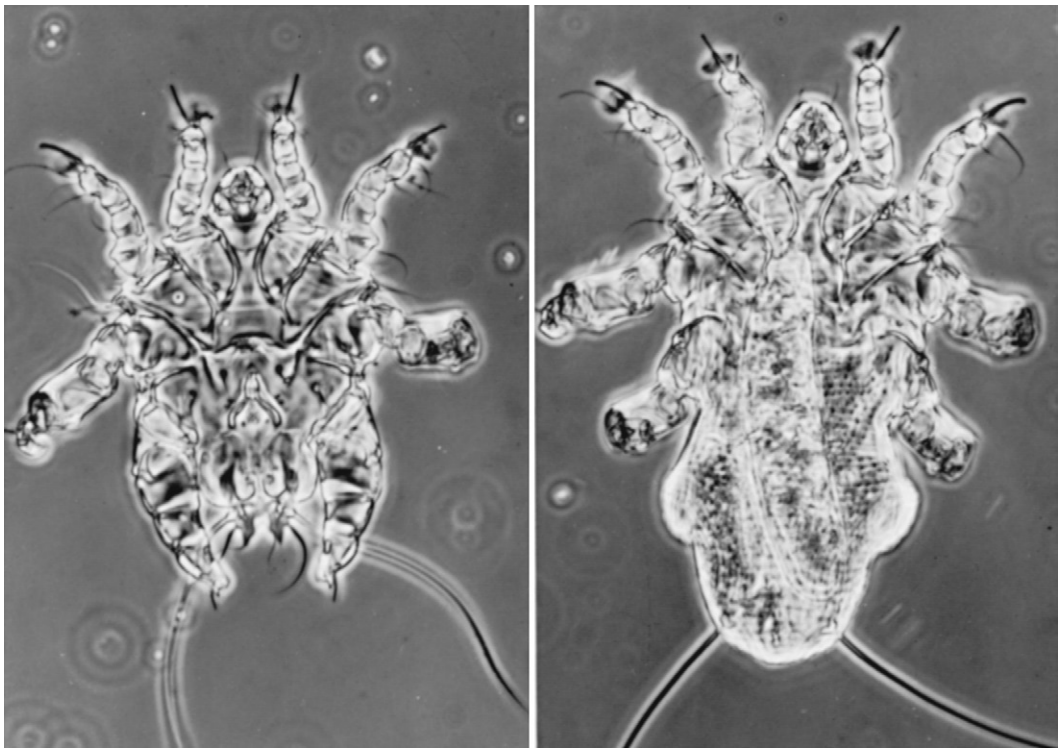

FIGURE 2-114. *Myocoptes musculinus* male *(left)* and female *(right)*, an astigmatid hair-clasping parasite of laboratory rodents. Notice how the third pair of legs of the male and the third and fourth pairs of legs of the female are modified for hair clasping. The first two pairs of legs have sarcoptiform pretarsi.

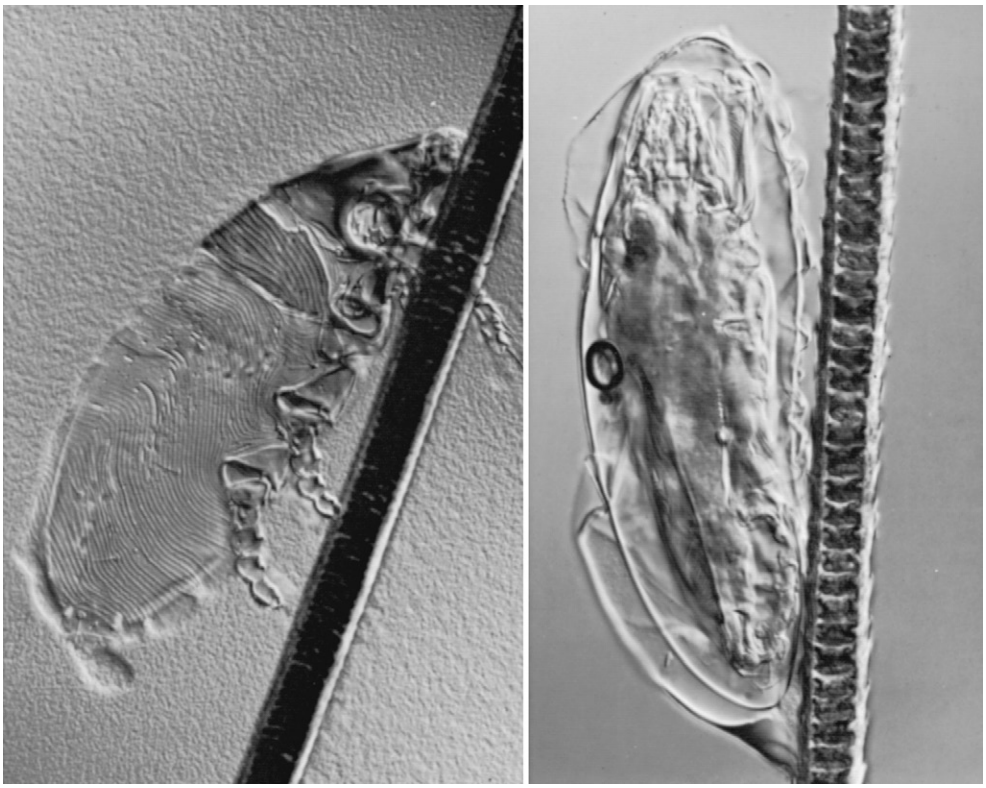

FIGURE 2-115. *Lynxacarus radovskyi* (Listrophoroidea), a hair-clasping mite of the cat. *Left*, Adult mite. *Right*, Egg with larva. (Specimens courtesy Dr. Robert Foley.)

FIGURE 2-116. *Demodex canis (left)* and *Demodex cati (right).*

Family Demodicidae

Demodex

These tiny, wormlike mites with short, stubby legs (Figure 2-116) live in the hair follicles and sebaceous glands of mammals. Several distinct species of *Demodex* often parasitize the same host animal, but each species tends to be restricted to a particular habitat. For example, two species, *Demodex folliculorum* and *Demodex brevis*, live in the skin of almost every human face—*D. folliculorum* in the hair follicles and *D. brevis* in the sebaceous glands (Desch and Nutting, 1972), where they eat the epithelial cells. Some important pest species are as follows.

Demodex canis is present in small numbers in the skin of most normal dogs (see Figure 2-116 and Figure 8-6). Pups acquire *D. canis* infection from their dams during the nursing period, and most cases of demodectic mange occur between 3 and 6 months of age. Affected dogs harbor much larger than normal populations of *D. canis*, apparently as a result of immunodeficiency, and display circumscribed areas of erythema and alopecia around the eyes and mouth and over bony projections on the extremities. There is no evidence of pruritus. If the lesions remain thus localized, the prognosis for clinical recovery is excellent; most such cases are mild, and the animals recover spontaneously with attainment of sexual maturity. However, a few cases persist; these tend to become generalized and intractable and may prove fatal. In generalized demodicosis, the hair becomes sparse over wider expanses and the skin becomes coarse, dry, and erythematous ("red mange"). Concomitant staphylococcal pyoderma is the rule in generalized cases; pustules develop, break open, and ooze. Severe cases are associated with a disagreeable odor. Generalized canine demodicosis is difficult to ameliorate and probably impossible to cure. Two further mites of the genus *Demodex* have been described from the dog (Desch and Hillier, 2003). *D. injai* is about twice the length of *D. canis*, appears to be found mainly in the sebaceous glands, and appears most often associated with dorsal seborrheic dermatitis. The third *Demodex* species, *Demodex cornei*, is shorter and stouter than *D. canis* and lives in association with stratum corneum rather than hair follicles. The three species from the dog have now been carefully measured and figured (Izdebska and Fryderyk, 2011). The signs caused by *D. cornei* appear similar to those of *D. canis*, and the sign associated with *D. injai* is an oily coat on the dorsum of the neck and trunk (Tater and Patterson, 2008). All three forms will respond to acaricidal therapies.

Demodex cati is rarely noticed (see Figure 2-95). Dermatitis associated with *D. cati* is usually localized on the head and in the ear canals. A more superficially dwelling and much shorter *Demodex*, *Demodex gatoi*, like the small form from the dog, appears to be associated with the stratum corneum of cats (Desch and Stewart, 1999). It appears that cats also may harbor a third species that has been seen but not yet described in detail; this is another short form, and its habitat within the skin is unknown (Desch and Stewart, 1999).

Demodex bovis mites are part of the normal fauna of bovine skin, but sometimes pinhead- to egg-sized nodules appear, usually on the neck and forequarters (see Figure 8-7). Occasionally only the eyelids, vulva, or scrotum is involved. If a fresh nodule is nicked with a sharp scalpel, thick, toothpaste-like pus that contains masses of *D. bovis* mites can sometimes be expressed, but older lesions consist only of scar tissue and are devoid of mites. Bovine demodectic mange is practically incurable, even though individual lesions typically regress, because new nodules form to take their place. However, an unusual case of bilateral lower palpebral demodicosis in a dairy cow, characterized by chronic eosinophilic granulomatous cellulitis but without appreciable pus formation, resolved spontaneously within 3 months (Gearhart, Crissman, and Georgi, 1981).

Demodex ovis is rarely noticed but is probably rather common; mites infest the meibomian glands and the hair follicles and sebaceous glands of primary hairs of the general body skin but are most numerous on the neck, flanks, and shoulders. A second species parasitizing sheep, *Demodex aries*, appear to be confined to areas with very large sebaceous glands such as the vulva, prepuce, and nostrils (Desch, 1986).

Demodex caprae causes a nodular dermatitis in milk goats.

Demodex caballi is a harmless parasite of the meibomian glands of horses. The horse is also host to a second species, *Demodex equi*, which is about half as long (190 to 232 m) as *D. caballi* (Desch and Nutting, 1978).

Demodex cuniculi is a relatively rare parasite of the rabbit.

Demodex phylloides is found in nodules around the eyes and on the snouts of pigs. These lesions later spread over the underside of the body.

Family Cheyletiellidae

Cheyletiella species are easily recognized by their big palpal claws, M-shaped gnathosomal peritremes, and comblike tarsal appendages (Figure 2-117). *Cheyletiella yasguri* occurs on dogs, *Cheyletiella blakei* on cats, and *Cheyletiella parasitivorax* on rabbits. Humans may serve as an accidental or transitory host. Pups infested with *C. yasguri* develop "walking dandruff" on their backs—a dermatitis with branlike exfoliative debris that stirs with the movements of these rather large mites. The Georgis observed a caged cat that passed *C. blakei* in its feces for several weeks. Presumably this cat was ingesting these mites while grooming itself, but no skin lesion was macroscopically visible, and no mites could be found in the fur.

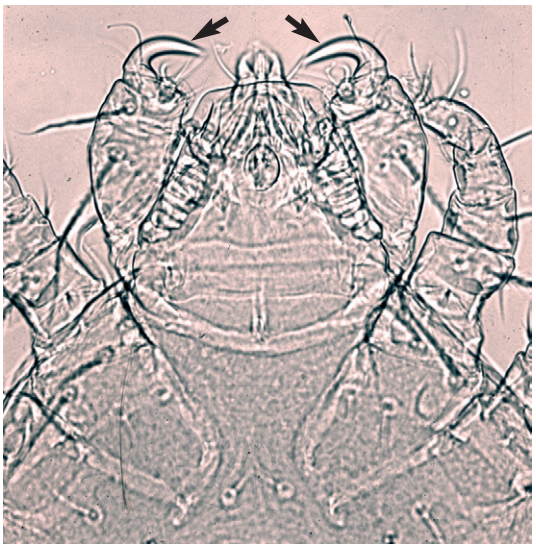

FIGURE 2-117. The anterior end of *Cheyletiella yasguri;* note the formidable palpal claws *(arrows)*.

Other genera of the family Cheyletiellidae are parasites of birds. *Cheyletiella* species survive longer off the host than other mange mites, and the premises may remain a source of reinfestation after treatment of affected animals.

Family Psorergatidae

Psorobia ovis, the sheep itch mite, sporadically causes pruritus and fleece derangement in sheep by rubbing and through chewing by the infested host. The course is very chronic. Lambs younger than 6 months appear unaffected, and generalization may require 3 or 4 years. The mite is minute and almost discoidal, and has radially arranged legs. *Psorobia bos* is a nonpathogenic mite of cattle. *Psorergates simplex*, the subcutaneous mite of mice, may cause a mangelike condition. To examine mites, skin an infested mouse and look for pockets of mites on the underside of the dermis.

Family Myobiidae

Myobiid mites cause dermatitis in stocks of laboratory rodents. In myobiids, the first pair of legs is modified for clasping hair (Figure 2-118), whereas in *Myocoptes* species, the third pair of legs of the male and the third and fourth pairs of the female are so modified (see Figure 2-114). *Myobia musculi* attacks laboratory mice, and *Radfordia ensifera* attacks laboratory rats. Alopecia and erythema of the dorsal neck region are typical; severe cases are characterized by self-inflicted excoriations. Stress of overcrowding is frequently responsible for converting an asymptomatic infestation of hair-clasping mites into an outbreak of serious skin disease.

Family Harpyrhynchidae

Harpyrhynchids are rounded mites resembling psorergatids that cause mangelike conditions in birds. Several genera include species that burrow in feather follicles or form large crusted cysts in the skin.

Family Syringophilidae

Syringophilids are nonpathogenic inhabitants of the lumen of feather quills.

Family Trombiculidae

Larvae (chiggers) of the family Trombiculidae are parasitic, but the nymphs and adults are free-living. These bright red or orange,

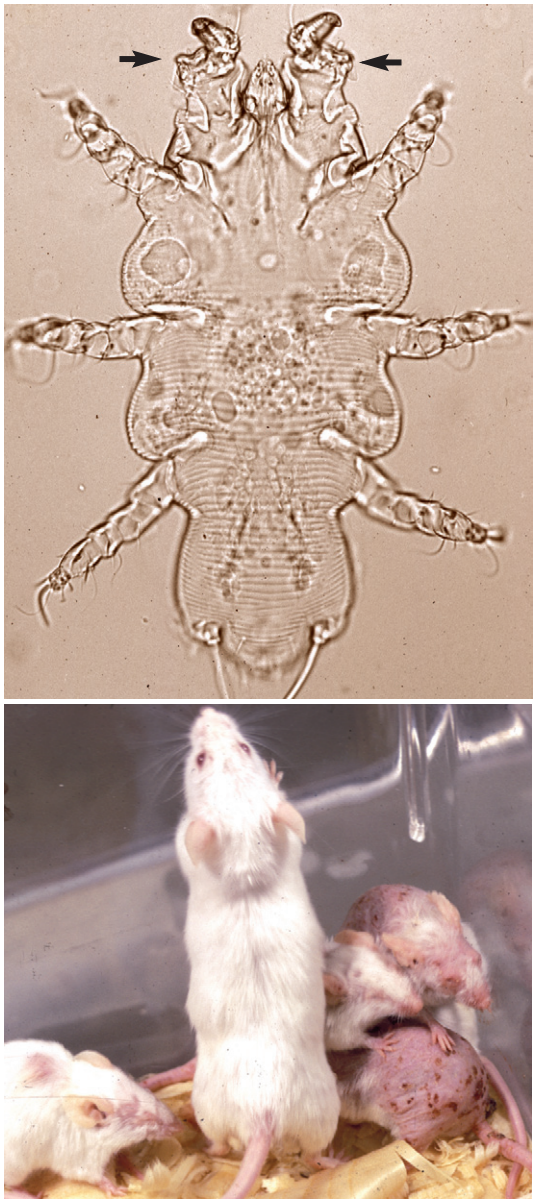

FIGURE 2-118. *Myobia musculi (top)*, a myobiid hair-clasping parasite of laboratory rodents. The first pair of legs *(arrows)* is modified for hair clasping. The mice *(bottom)* are suffering from an infestation with this mite.

six-legged larvae are likely to be found on the skin or in the ears of cats or dogs, on the faces or pasterns of sheep and other ungulates, and under the wings or around the vents of chickens and other birds (Figure 2-119). Infestation is usually acquired in wild or semiwild landscapes; the distribution of these nuisances is remarkably spotty, but wherever they are found, chiggers are infamous. Microscopically, the scutum is useful for recognizing a chigger as such and for identifying genera and species with the help of keys. Focus on the dorsal surface (the surface opposite the one with the coxae) to see the scutum (Figures 2-120 and 2-121). Chiggers remain on the skin for several days unless dislodged by the scratching host; their saliva, when injected into the skin, disintegrates host cells, and the resulting material is taken into the mite as food. The surrounding skin hardens, and a tube called a *stylostome* is formed, in which the mouthparts remain until the chigger is replete or dislodged. The fully developed stylostome extends from the surface of the epidermis into

FIGURE 2-119. Living chigger from the ear of a cat in Maryland. (Courtesy Dr. Craig Greene, VCA Newark Animal Hospital, Newark, Delaware.)

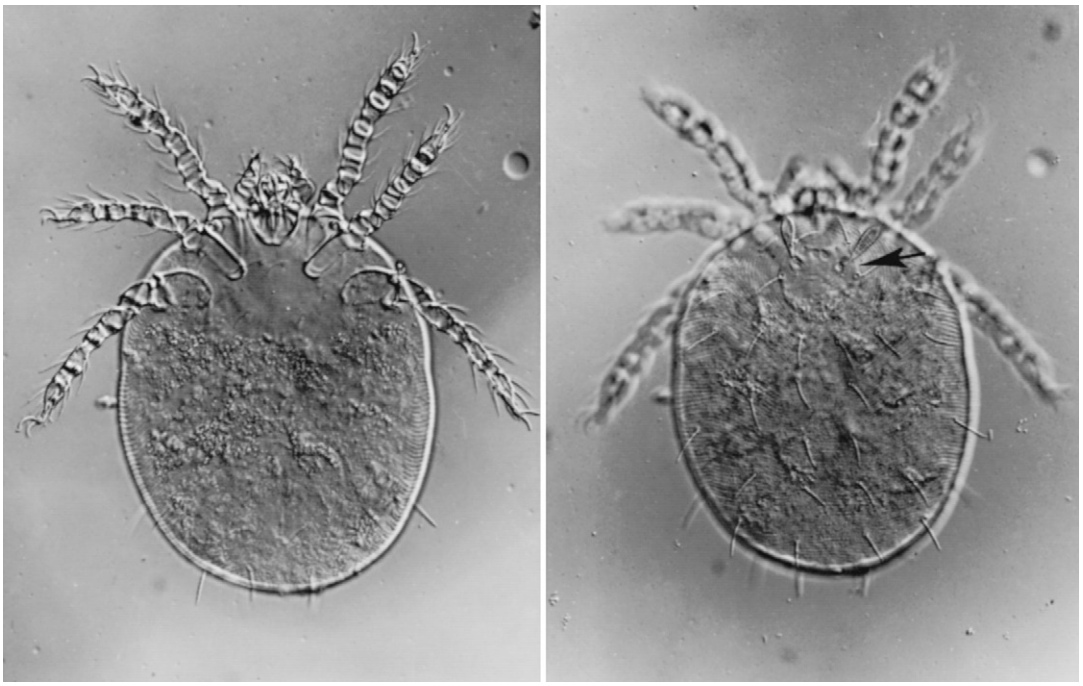

FIGURE 2-120. *Walbachia americana*, a trombiculid mite (chigger). *Left*, The ventral surface is in focus. *Right*, The dorsal surface. The scutum *(arrow)* with its two sensillae (large plumose setae) and four or five setae is helpful in identifying chiggers; it is on the dorsal surface near the anterior end of the body.

the dermis and is lined by necrotic cells of the stratum germinativum (see Figure 8-9). Pruritus is intense and may be protracted for many days after the chigger has been removed. Twenty-four hours after infestation with more than 2000 larvae of *Neotrombicula autumnalis*, two male Yorkshire Terriers developed paresis involving first the hind legs, then the forelegs. The nervous signs disappeared within 3 days after repeated acaricidal (propoxur) and symptomatic therapy (Prosl, Rabitsch, and Brabenetz, 1985).

Recently, a new syndrome, called *straelensiosis*, was reported to be affecting dogs in Europe (Bourdeau et al, 2001). This condition is caused by a trombiculid mite, *Straelensia cynotis*, which was described by Fain and Le Net (2001). Some 22 dogs over a period of 5 years in the south of France had been found to have chronic, painful, extensive-to-generalized dermatitis that was associated with papular crusts and suppurations. Most of these cases occurred in hunting dogs. This larval trombiculid enters the hair follicle, where it stays for an extensive period with its stylostome directed into the dermis (Figure 2-122). Besides other cases from France, cases have been reported in Spain, Portugal, and Italy (Seixas et al, 2006; Degorce-Rubiales et al, 2008; Ramirez et al, 2009; Vercelli and Cornegliani, 2011).

Family Pyemotidae

Pyemotes

"Hay itch mites" of the genus *Pyemotes* are parasites of various insect larvae that are grain-destroying pests. *Pyemotes tritici* is a tiny elongate mite that becomes enormously distended when gravid; males and females are sexually mature at birth. People and domestic animals that come into contact with infested grains, straw, hay, and the like may be attacked by these mites and may develop an erythematous and intensely pruritic papular and vesicular rash. An outbreak of dermatitis in 12 horses and in many persons in Florida was attributed to *P. tritici* received in a shipment of alfalfa hay (Kunkle and Greiner, 1982).

Family Tarsonemidae

Acarapis woodi is the tracheal mite of honeybees. These mites entered the United States through Texas and Florida in 1984, coming from Mexico and Europe. The mites, along with *Varroa* and the beetle *Aethina*, have been responsible in part—along with the currently unexplained "colony collapse disorder"—for the remarkable reduction of honeybee populations in the United States.

FIGURE 2-121. Scutum of *Neotrombicula* sp. (Trombiculidae: Prostigmata).

These small mites live within the trachea of the honeybee. Female mites move from bee to bee, entering the adult bee through the first thoracic spiracle. Large numbers can build up in the tracheal tubes. Mites within the tubes cause problems with thermoregulation of hives in winter and cause bees to die outside of hives because they cannot elevate their metabolic rate high enough to stay warm when they fly on cool days. Treatment has been provided by adding menthol chips or oil of wintergreen to hives (Williams, 2000). Although this seems to afford some protection, it may be that most susceptible hives have already disappeared.

Treatment of Astigmatid and Prostigmatid Mite Infestations

Dogs and Cats

SARCOPTES. Selamectin is probably the treatment of choice for sarcoptic mange in dogs and is labeled for this application (Shanks et al, 2000). Topical imidacloprid (10% w/v)/moxidectin (2.5% w/v) has also been found to be highly efficacious against sarcoptic mange in dogs (Fourie, Heine, and Horak, 2006). In addition, subcutaneous ivermectin is routinely used to treat sarcoptic mange. Other effective acaricides include amitraz, benzyl benzoate, lime sulfur, phosmet, and rotenone. In most cases, treatment with these compounds must be repeated several times over a period of weeks.

S. scabiei and other sarcoptiform parasites may temporarily infest people who come into intimate contact with mangy dogs and cats. In this case, acaricidal treatment of the pet is the key to lasting success in curing the people. On the other hand, proper scabies contracted from another human being causes very persistent dermatitis and misery unless effectively treated and, of course, has little or nothing to do with dogs and cats.

NOTOEDRES. Selamectin topically applied to cats will treat notoedric mange (Itoh et al, 2004). Ivermectin (0.3 mg/kg) has been successfully used to treat numerous cats with notoedric mange (Foley, 1991a). The standard previous treatment for *N. cati* infestations in cats was lime sulfur. With lime sulfur, the cat first is bathed and then is dipped or washed with a 1:40 solution of lime sulfur in warm water. This treatment is applied weekly for at least 6 weeks.

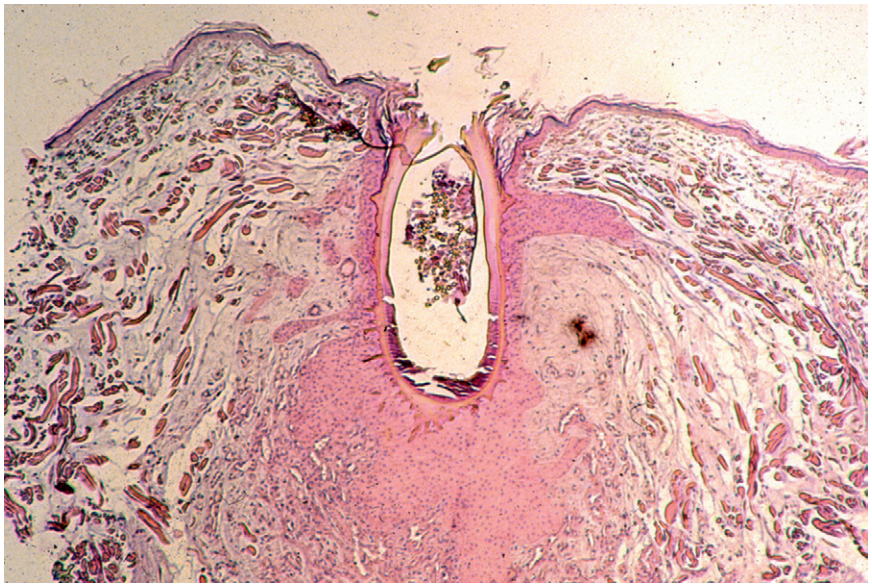

FIGURE 2-122. *Straelensia cynotis* in a hair follicle. (Courtesy Dr. Maja Suter, Institute of Animal Pathology, University of Berne, Berne, Switzerland.)

OTODECTES. Selamectin is approved for the treatment of ear mites in cats. Ivermectin and milbemycin oxime have both been formulated as otic suspensions that are approved for treating ear mites in cats. *Otodectes cynotis* has also been successfully treated with topical application of moxidectin/imidacloprid (Fourie, Kok, and Heine, 2003). Subcutaneous ivermectin (0.2 to 0.225 mg/kg) injected on one or two occasions with a 3-week interval between injections has proved highly successful in treating ear mites in cats (Foley, 1991b). *Otodectes* ear infestations also respond to pyrethrin- and rotenone-containing compounds. With these products, the ear canal should be thoroughly cleaned before instillation of the acaricidal solution. Application of 1 to 2 mL of mineral oil to the ear canal followed by 30 seconds of massage repeated every 2 or 3 days will often cure dogs and cats of their ear mite infestations.

DEMODEX. Fortunately, most dogs with lesions of demodectic mange have a localized *D. canis* infestation that will respond successfully to topical treatment. The localized form of demodectic mange may be controlled by applying rotenone ointment or benzyl benzoate lotion. These drugs have very little residual activity and therefore must be applied daily.

Treatment of generalized demodectic mange is a challenge and frequently proves to be a frustrating experience for the veterinary dermatologist and the client alike. Currently recommended therapies include amitraz rinses every 7 to 14 days and oral daily ivermectin, milbemycin, or moxidectin that may continue for a month or longer (Mueller, 2004; Tater and Patterson, 2008). Amitraz, approved for the control of generalized demodicosis, is applied topically as an aqueous suspension (10.6 mL of concentrate per 2 gal or 7.6 L of water) at 2-week intervals for a total of three to six applications (Folz et al, 1983). It is recommended that treatment be continued until no viable mites can be found in skin scrapings at two successive treatments. A brood bitch with asymptomatic *D. canis* infection may be bred, but a bitch with demodectic mange or a history of demodectic mange should be spayed.

Numerous cases of *Demodex gatoi* have been reported, and a recent report from Finland described 11 cats in six households with a *D. gatoi* infestation that developed quite severe signs (Saari et al, 2009). For these cats, treatment was difficult, lime sulfur dips were unsuccessful, imidacloprid with moxidectin was unsuccessful, and selamectin several times was unsuccessful, as were ivermectin injections, although oral ivermectin every other day for 10 weeks seemed to be effective. The authors reported that they successfully treated cats in three of the households using amitraz baths.

CHEYLETIELLA. Infection with *C. yasguri* in dogs has been successfully treated with topical application of imidacloprid/moxidectin (Loft and Willesen, 2007) or selamectin (Mueller and Bettenay, 2002). Infestations also responded well to the application of topical 65% permethrin, and all mites were gone within 1 week of treatment (Endris et al, 2000). Milbemycin oxime has been used to treat dogs with naturally occurring cheyletiellosis (White, Rosychuk, and Fieseler, 2001). Canine cheyletiellosis has also been successfully treated with fipronil (Chadwick, 1997). *Cheyletiella* is susceptible to amitraz as well. In cats, infestations with *C. blakei* have been successfully treated with topical application of fipronil (Scarampella et al, 2005) or selamectin (Chailleux and Paradis, 2002). The premises should be sprayed with a residual insecticide to destroy these rather hardy mites.

C. yasguri of dogs and *C. blakei* of cats also attack people, especially those who share their beds with pets. Curiously, *C. blakei* rarely produces obvious lesions on cats, but the owner may be aware of frequent bites. If *C. blakei* infestation is suspected, one can attempt to collect mites from the fur with a bit of Scotch tape. However, the diagnosis is more often reached fortuitously when the mites or their eggs are found in a routine fecal flotation. Because cats so meticulously groom themselves, a fecal flotation often affords a better sample of what is on the cat's exterior than is obtained by direct examination.

Ruminants

CHORIOPTES. Eprinomectin can be applied topically to lactating dairy cattle without withholding of milk and is approved for the treatment of chorioptic mange. Alpacas and llamas have also been successfully treated with eprinomectin applied topically (Plant, Kutzler, and Cebra, 2007). In a severe case of mange in a herd of alpacas involving infestations with *Psoroptes*, *Sarcoptes*, and *Chorioptes*, treatment with injectable ivermectin rapidly cleared the animals of their *Psoroptes* and *Sarcoptes* mites, but *Chorioptes* mites required further treatment with topically applied ivermectin (Geurden, Deprez, and Vercruysse, 2003). Chorioptic mange usually responds to standard louse treatments. Coumaphos or lime sulfur suspension as spray or dip controls chorioptic mange mites on lactating dairy cows.

SARCOPTES. Sarcoptic mange should be reported to state disease control authorities and treatment carried out under their supervision. Lactating dairy cows can now be treated with eprinomectin, which is labeled for *Sarcoptes* infestations of cattle. Sarcoptic mange of beef cattle and nonlactating dairy cattle is treated with avermectins, ivermectin, moxidectin, doramectin, or eprinomectin. It also can be treated with sprays or dips containing lime sulfur, phosmet, and tetrachlorvinphos.

PSOROPTES. Psoroptic scabies in cattle or sheep should be reported to state disease control authorities and treatment carried out under their supervision. Coumaphos, phosmet, and hot lime sulfur are approved by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) as official dips for psoroptic scabies in cattle, and injectable ivermectin is approved as a systemic acaricide (Wright, 1986). Most of the macrocyclic lactones are labeled for treating psoroptic mange in cattle. Cattle treated with ivermectin must be isolated from untreated cattle for 2 weeks after treatment and withheld from slaughter for the required period. Ivermectin has not been approved for the treatment of psoroptic mange in sheep in the United States. Holding facilities vacated by sheep or cattle with psoroptic mange should be left vacant for at least 2 weeks to give the mites time to die off before new stock is housed (Wilson, Blachut, and Roberts, 1977).

DEMODEX. Two goats with nodular demodicosis were treated successfully with no scarring or skin depigmentation (Strabel et al, 2003). One of the goats was treated with weekly oral ivermectin; the other was treated with selamectin. It seems highly probable that these treatments will also work in sheep and in cattle under most circumstances.

Horses

Severe irritation caused by mange mites may lead to serious self-mutilation by affected horses. Treatment with macrocyclic lactones has proved efficacious (Osman, Hanafy, and Amer, 2006). Mange is contagious and sometimes communicable. Isolate mangy horses and sterilize all water buckets, brushes, curry combs, and the like. Stalls should be thoroughly disinfected or left vacant for 2 to 3 weeks.

Swine

Ivermectin is highly effective in treating sarcoptic mange in swine.

Ferrets, Lagomorphs, and Pocket Pets

Notoedric mange *(Notoedres douglasi)* affecting two fox squirrels *(Sciurus niger)* responded dramatically to a single subcutaneous injection of ivermectin at 0.5 mg/kg body weight (Evans, 1984). A case of notoedric mange that developed in an African pygmy hedgehog *(Atelerix albiventris)* was treated with injectable moxidectin (Pantchev and Hofmann, 2006).

O. cynotis has been effectively treated in ferrets with topical selamectin (Miller et al, 2006), and it has been shown that topical application of imidacloprid and moxidectin is efficacious in ferrets (Sueur et al, 2011).

M. musculinus and *M. musculi* infestations were eliminated from laboratory mice by subcutaneous injection of ivermectin 0.2 mg/kg body weight (Wing, Courtney, and Young, 1985). More recently, a group of mutant mice with sickle-cell anemia were cleared of their infection by being cross-fostered on outbred mothers that were treated with topical ivermectin (Huerkamp et al, 2005). Topical selamectin has been shown to eliminate infestations of mice with *M. musculinus*, *M. musculi*, and *R. ensifera;* this treatment also controlled the pinworms *Syphacia obvelata* and *Aspiculuris tetraptera* (Gönenç et al, 2006). In addition, a single treatment with topical moxidectin has been used successfully in the treatment of *M. musculinus* infestations (Pullium et al, 2005). Application of 0.5 mg active permethrin per mouse as 0.25% dust mixed with the bedding was convenient and eliminated *M. musculi* infestations in experimental mice (Bean-Knudsen, Wagner, and Hall, 1986).

Guinea pigs infected with *T. caviae* have been shown to respond well to treatment with ivermectin (Mandigers, van der Hage, and Dorrstein, 1993; McKellar et al, 1992). Treatment has been administered orally, subcutaneously, or percutaneously. It seems highly likely that other avermectins would also be efficacious in the guinea pig.

Ear canker in rabbits *(P. cuniculi)* responds to two subcutaneous injections of ivermectin at 0.2 mg/kg administered 14 days apart (Bowman, Fogelson, and Carbone, 1992). Rabbits can also be treated very successfully with topical selamectin (McTier et al, 2003), topical imidacloprid/moxidectin (Hansen et al, 2005), or topical eprinomectin (Ulutas et al, 2005). The mites *C. parasitovorax* and *Listrophorus gibbus*, along with the flea *C. felis*, have been successfully treated in rabbits with a topical formulation of imidacloprid and permethrin (Hansen et al, 2006). When pesticides and anthelmintics are applied to rabbits, it must be remembered that rabbits remain in the unusual regulatory position of still being considered a minor species food animal in the United States, and the reality is that they are often still consumed as food.

CLASS CRUSTACEA

COPEPODS

Copepods are crustaceans of importance to veterinary medicine because they serve as intermediate hosts of both cestodes and nematodes. Three major groups of copepods are known: calanoids, cyclopoids, and harpacticoids; cyclopoids make up the group that typically has been found to be important intermediate hosts of the parasites of domestic animals. Copepods have shrimp-shaped bodies and five pairs of swimming legs (Figure 2-123). The antenna on each side of the head usually branches into two stalks. There may or may not be a single simple eye. Copepods reproduce sexually, and males often have a modified antenna that is used in copulation. Females typically carry egg sacks that contain developing eggs. Most copepods are grazers of phytoplankton, but some can be carnivorous, and a few are parasites in their own right. Eleven molts occur, separating 12 larval stages. The first five molts produce six larval forms of the naupliar type, the next five molts produce the developmental stages called copepodites (typically, a new body segment is added with each molt), and the final molt produces the adult male or female. While grazing, the copepods will ingest the coracidia of tapeworms or the hatched larvae of nematodes. They will then serve as transport hosts or as required intermediate hosts. Important parasites that use copepods include *Spirometra*, *Diphyllobothrium*, *Gnathostoma*, and *Dracunculus* species.

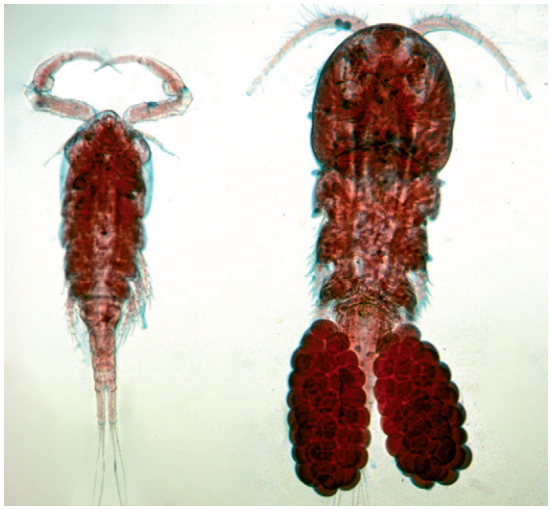

FIGURE 2-123. Copepods, male and female, stained. The female bears the two large egg sacks that are typical of many of these free-living crustaceans.

PENTASTOMIDA

Pentastomids, or tongueworms, are highly specialized crustaceans, as unlikely as that may seem. The adult parasites live in the respiratory passages of predacious reptiles, birds, and mammals. The body is annulated, and the anterior, subterminal stoma is flanked by two pairs of retractable hollow fangs or hooks (Figure 2-124 and see Figure 8-10). Eggs containing four- or six-legged larvae are discharged with the nasal secretions or are swallowed and passed in the feces (Figure 2-125). If ingested by an appropriate intermediate host, usually a member of some species likely to fall prey to the predator in question, these larvae invade the tissues, develop, and encyst in the viscera as nymphs that resemble the adults in all particulars except for mature reproductive organs (see Figures 8-11 to 8-13).

Linguatula serrata occurs in the nasal and paranasal sinuses of dogs and cats, where it causes bleeding, catarrhal inflammation, and some impediment to respiration. Cattle, sheep, rabbits, and other animals serve as intermediate hosts; fully developed nymphs, the form infective for carnivorans, are found encysted in the lymph nodes and serous membranes.

Kazacos et al (2000) reported on a Basenji-cross dog that had been born and spent time in Cameroon, Africa. It seems that the dog must have ingested some quantity of python feces containing the eggs of pentastomes of the genus *Armillifer*. The dog had been ill for several years, and when it became acutely ill 2 years after first admission, it was unresponsive to treatment and was euthanized. It was found to have a massive visceral infection with the nymphs of this pentastomid (Figures 2-126 and 2-127).

FIGURE 2-124. Stoma and hooks of a pentastomid nymph from a South American otter.

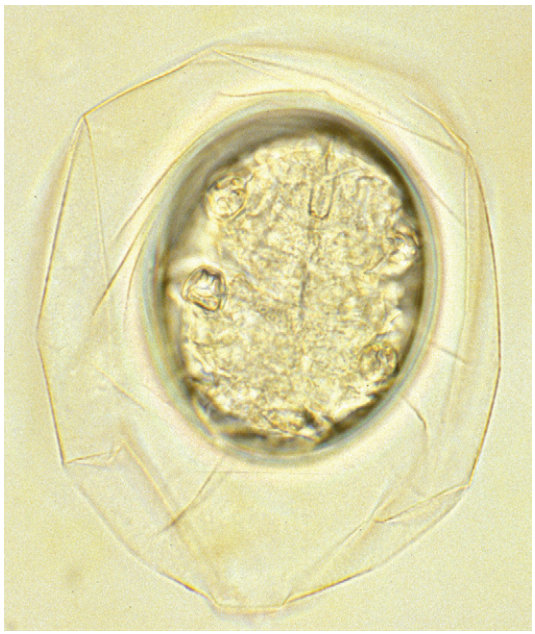

FIGURE 2-125. Egg of the pentastomid *Rheighardia sternae* from the feces of a gull.

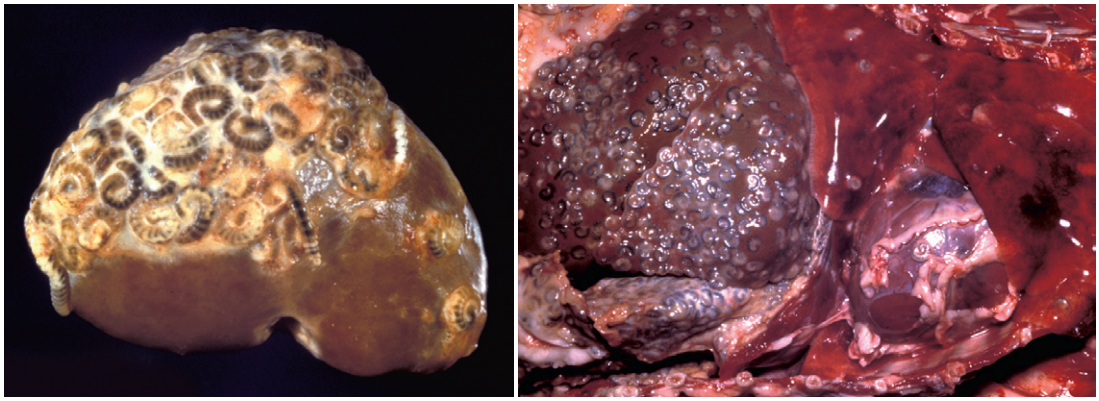

FIGURE 2-126. *Armillifer armillatus. Left*, A kidney removed from an infected dog in which the other viscera *(right)* have the liver, lungs, and heart containing large numbers of the very large coiled nymphs of this pentastomid parasite, whose adults are found in pythonid snakes. (Courtesy Dr. Kevin R. Kazacos, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana.)

FIGURE 2-127. *Armillifer armillatus.* Nymphs of this pentastomid teased from the tissues of the dog in Figure 1-125. Several of the nymphs have been damaged during the teasing process. (Courtesy Dr. Kevin R. Kazacos, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana.)

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CHAPTER 3

Protista

The eukaryotic organisms have undergone a systematic reorganization over the last several decades, and the process is still in a state of flux, so stability remains tenuous at different levels within the systematic schemata that are proposed. In general, the eukaryotes have been divided now into four large groups: the Archaeoplastida (the plants), the Unikonts (animals, fungi, and amoebae), the Excavata (many of the better known flagellate parasites), and the SAR group (Stramenopiles; Alveolates, including Ciliata and Apicomplexa; and Rhizaria) (Hampl et al, 2009). This chapter will discuss members of the Excavata, the Alveolates, a Stramenopile, and a few Unikonts (true amoebae). This classification scheme makes it difficult to talk in terms of the classical "protozoa" because the protozoa actually were representatives of many unrelated groups, often related to various multicellular forms. However, after one gets past the larger groupings and their new names, information about individual parasites remains the same.

A vast majority of the eukaryotes are unicellular organisms that are related to other multicellular forms (e.g., malaria being related to the brown algae that compose the giant kelp forests of northern oceans). However, in veterinary medicine, interest is focused on agents that cause disease, and these tend to be unicellular forms. The popular term *protist* is applied to these eukaryotes with a unicellular level of organization, and it is understood that when cell differentiation occurs in these groups, it is restricted to the purposes of sexual reproduction, motility, alternate vegetative morphology for different habitats (e.g., intestinal lumen vs. liver tissue), and quiescent and resistant transmission stages, such as cysts (Adl et al, 2005). Knowledge of these relationships is important for at least two good reasons: If you know that two things are related biologically and you learn about one, you will also know something about the other; and often if one agent is killed by chemicals of a certain class, then these same drugs often will work on its relatives.

Most protists are free-living organisms, and of those that live as parasites in the bodies of mammals, only a small proportion is associated with disease. Their etiologic significance is sometimes unclear. For example, certain intestinal flagellates multiply when the host has diarrhea. In such cases, the presence of large numbers of flagellates in the fecal smear is the result rather than the cause of the diarrhea. On the other hand, there are protists that indeed behave as primary pathogens, and they are responsible for some of the most important diseases of domestic animals and humans. These diseases include the malarias, piroplasmoses, coccidioses caused by apicomplexans, and trypanosomiases caused by kinetoplastid hemoflagellates.

EXCAVATA

The Excavata is a group that contains several groups of importance in human and veterinary medicine. These groups include Trypanosomatida (with *Trypanosoma* and *Leishmania*), Trichomonadida (*Tritrichomonas* and relatives), Diplomonadida (*Giardia* and relatives), and Heterolobosea (*Naegleria* and relatives). These organisms all tend to multiply asexually by binary fission, and certain species form resistant cysts. Most of these have a eukaryote flagellum, undulipodium, for locomotion during many of their life stages, and it is for this reason that they used to be grouped under the name flagellates. The parasitic Excavata of interest to veterinarians can be divided into two main groups according to their location in the host's body and their type of life history. The Trypanosomatida live in the blood, lymph, and tissue spaces and are typically transmitted from host to host by bloodsucking insects. The Diplomonadida and the Trichomonadida are associated with the alimentary or genital tract, usually in intimate association with the mucous membranes. They are transmitted from host to host in the feces or in genital effluvia, and some are transmitted as trophozoites (e.g., *Trichomonas*), others as cysts (e.g., *Giardia*). The Heterolobosea are dimorphic facultative parasites that are free-living as flagellate or amoeboid forms; when parasitic in animal tissues, they are seen as an amoeboid form that is found in different host tissues (e.g., brain, skin, liver).

KINETOPLASTEA

Trypanosomatida

Trypanosoma

A trypanosome is an elongated, spindle-shaped cell with a single nucleus lying near the middle of its length and a single flagellum that arises near a large mitochondrion with copious DNA called a **kinetoplast** and passes out of the anterior end of the cell (Figure 3-1). During development in both mammalian and arthropod hosts, trypanosomes can undergo considerable morphologic change. Four morphologic forms are distinguished in the case of *Trypanosoma cruzi*. The **amastigote** lacks a flagellum, whereas the other three forms all have a flagellum but differ with respect to the

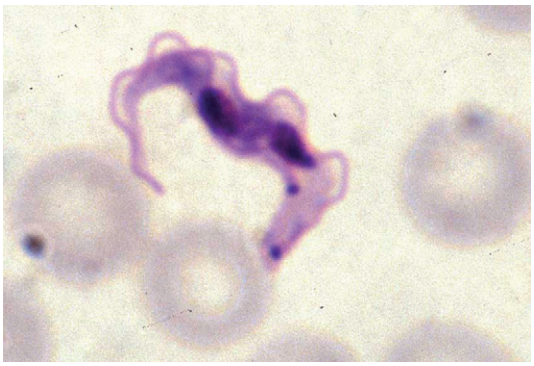

FIGURE 3-1. Giemsa-stained trypomastigote of *Trypanosoma brucei*. With tsetse-transmitted trypanosomes, dividing forms, like the one in this image, can be observed in blood smears.

location of the kinetoplast. The kinetoplast lies posterior to the nucleus in the **trypomastigote,** immediately anterior to the nucleus in the **epimastigote,** and near the anterior end of the cell in the **promastigote.** The flagellum lies at the edge of an undulating membrane as it courses from the kinetoplast to the anterior end of the cell body of the trypomastigote. Infection of the arthropod host occurs when it ingests the blood of an infected mammal. Infection of the mammalian host occurs by one of two mechanisms, depending on the species of trypanosome involved: through the bite of the infected arthropod or by contamination of the host's mucous membranes or abraded skin by its feces. The former are called **salivarian,** and the latter **stercorarian trypanosomes** (*stercus* is Latin for feces). Most salivarians are pathogenic and most stercorarians are nonpathogenic, but the pathogenic stercorarian *T. cruzi* is an important exception to this generalization.

TSETSE-TRANSMITTED SUB-SAHARAN AFRICAN TRYPANOSOMES. Tsetse-transmitted trypanosomes are of major significance in sub-Saharan Africa (see Figure 3-1). *Trypanosoma brucei* and *Trypanosoma congolense* cause fatal nagana disease in domestic ruminants but are only mildly pathogenic among the indigenous wild ruminants. Wild ruminants thus serve as reservoirs of *T. brucei* and *T. congolense*, which are conveyed through the bites of tsetses (*Glossina* species) to domestic livestock. These trypanosomes and tsetses defend vast areas of African grazing lands against invasion by domestic livestock. Humans have been striving to introduce their domestic animals into these areas for a long time without remarkable success, and where humans have succeeded, overgrazing of the grasslands has had a part to play in local desertification.

T. brucei multiplies by longitudinal binary fission in the blood, lymph, and cerebrospinal fluid of the mammalian host. The trypomastigotes, the only stage in the mammalian host, that are ingested by the tsetse when it feeds on the blood of an infected mammal multiply in the insect's midgut, undergo metamorphosis, and migrate to the salivary glands, where they reach the infective metacyclic trypomastigote stage and are then ready to be injected into the mammalian host at the next feeding. *Trypanosoma gambiense* and *Trypanosoma rhodesiense*, the etiologic agents of African sleeping sickness in humans, are closely related to *T. brucei*.

Some sub-Saharan African tsetse-transmitted trypanosomes have been taken out of Africa to other areas where they are mechanically transmitted by other dipteran biting flies. *Trypanosoma vivax* is a tsetse-transmitted trypanosome of considerable importance to livestock in West Africa, where the reservoir hosts are wild ungulates. The living trypanosome is active in fresh blood films, hence the name *vivax*. In cattle, the infection may occur without signs, or acute or chronic disease may be present. In peracute disease, high parasitemia may be associated with extensive hemorrhages throughout the mucosal and serosal surfaces of the body. In chronic disease, cattle become anemic and emaciated, with signs of severe wasting. Similar disease has been reported in goats and sheep. *T. vivax* has been exported from Africa to South America, where it causes disease in horses, cattle, buffalo, sheep, and goats (Da Silva et al, 2011); is transmitted by tabanid flies (Desquesnes, 2004); and uses deer as a reservoir (Fiasson et al, 1948).

NON-TSETSE DIPTERAN-VECTORED TRYPANOSOMES. *Trypanosoma evansi* occurs in Africa north of the Sahara, Asia, and tropical America, and causes surra of all species of domestic animals. Flies of the family Tabanidae and vampire bats serve as vectors. In South American horses, *Trypanosoma equinum* causes a disease called *mal de caderas*, which is similar to surra in its biology and disease manifestations.

SEXUALLY TRANSMITTED TRYPANOSOMES. *Trypanosoma equiperdum* is unique among trypanosomes in not requiring an intermediate host. Transmission among hosts occurs through direct sexual contact and results in the equine venereal disease called *dourine*. The acute stage is characterized by swelling of the genitalia and a mucoid discharge in which *T. equiperdum* can usually be demonstrated. As acute signs subside, circular, flattened, "silver dollar" plaques appear in the skin and then disappear within several hours or days to be replaced by others. The chronic stage of dourine is marked by emaciation, paresis, intermittent fever, and finally death. Dourine was eradicated from the United States in 1920 and again in 1949 but has since reappeared at least once. The eradication of *T. equiperdum* from North America was made possible to a great extent by the work of a Canadian veterinarian, Edward Watson, who worked on the disease for some 15 years, was the first to identify the trypanosome in horses in North America, and developed a complement fixation test that could be used to identify infected horses in the field. Identified horses were then destroyed. Thus, within 16 years, the disease had been identified and eradicated from the Canadian provinces (Derbyshire and Nielsen, 1997).

NONPATHOGENIC TRYPANOSOMES. Not all trypanosomes transmitted by the bites of arthropod vectors are exotic and tropical, but most of those that are found in North America are considered to be nonpathogenic. *Trypanosoma cervi* was identified in 29 of 45 Alaskan reindeer *(Rangifer tarandus)* examined over a 2-year period and in 98% of white-tailed deer *(Odocoileus virginianus)* in southern Florida examined over a 5-year period (Telford et al, 1991). *Trypanosoma cervi* also infects elk and mule deer in the United States and is present apparently without pathogenic effect (Kingston, Morton, and Dietrich, 1982). *Trypanosoma theileri* (pronounced "tyler-eye") is a harmless parasite of cattle transmitted by tabanid flies, and *Trypanosoma melophagium* is an equally harmless parasite of sheep transmitted by the sheep ked, *Melophagus ovinus;* both are distributed worldwide. Occasionally, *T. theileri* contaminates culture media that have been enriched with "sterile" bovine serum, much to the surprise and confusion of the microbiologist. It is interesting that *M. ovinus*, which is first cousin to a tsetse, is almost universally infected with a trypanosome, albeit fortunately a harmless one.

AMERICAN TRIATOMIN-TRANSMITTED TRYPANOSOMES. *T. cruzi* (Figure 3-2), the etiologic agent of American trypanosomiasis (Chagas' disease) of human and dog, is transmitted by triatomin bugs of the genera *Triatoma*, *Rhodnius*, and *Panstrongylus* in South and Central America and in Texas, Arizona, New Mexico, California, and Oklahoma (Fox et al,

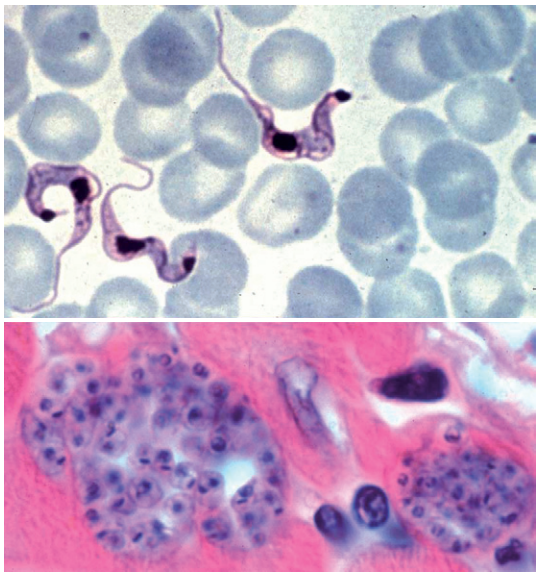

FIGURE 3-2. *Trypanosoma cruzi.* Top image is a trypomastigote in a Wright's-stained buffy coat preparation from a naturally infected dog. Bottom image shows the amastigote stages in heart muscle. (Top specimen courtesy Dr. Stephen C. Barr.)

1986; Kjos et al, 2009). Opossums, armadillos, rats, guinea pigs, cats, raccoons, and monkeys serve as reservoirs of infection in the wild. Five of 400 raccoons *(Procyon lotor)* examined in Maryland were infected (Walton et al, 1958); 104 of 221 raccoons (47%) were found to be seropositive in South Carolina and Georgia (Yabsley and Noblet, 2002). *T. cruzi* has been observed in hunting dogs in central Virginia that had lymphadenopathy but did not yet have clinical signs of cardiomyopathy (Barr et al, 1995). Autochthonous cases of *T. cruzi* continue to occur in the United States from time to time in dogs (Bern et al, 2011; Nabity et al, 2006; Patel et al, 2012).

In the vertebrate host, *T. cruzi* amastigotes (cells that contain a nucleus and a kinetoplast, but with no or only a very rudimentary undulipodium) multiply by binary fission in cells of the mononuclear phagocyte system, neural cells, glial cells, and most importantly cardiac and smooth muscle cells (see Figure 8-15). Amastigotes released by rupture of the host cell change into trypomastigotes, which then appear in the circulating blood to invade other cells or to be ingested by the bug as it feeds. Trypomastigotes of *T. cruzi* are rarely, if ever, seen dividing in blood smears prepared from circulating blood. The trypanosomes multiply and undergo metamorphosis in the bug's hindgut and are eventually passed in the feces of the bug, which almost invariably defecates while feeding on its sleeping victim. Trypanosomes enter the body by going through the oral, nasal, and conjunctival mucosae or by rubbing of infectious bug feces into abrasions or the bug's bite wound in the skin. Infection can also occur through the placenta or by blood transfusion, and accidental self-injection presents a potential hazard of infection to persons handling blood samples from infected animals, even those specimens in which trypomastigotes cannot be demonstrated in blood films. Trypomastigotes are difficult to demonstrate in the blood of long-term carriers, and one must turn to serology, culture, polymerase chain reaction (PCR), or xenodiagnosis for recourse. In xenodiagnosis, uninfected bugs are allowed to feed on the suspected individual, and their hindguts are later examined for trypanosomes—a cumbersome and inefficient procedure at best. In dogs, acute disease is characterized by lymphadenopathy and clinical signs associated with

FIGURE 3-3. *Leishmania infantum.* Top image is a macrophage from bone marrow of an infected dog containing large numbers of amastigotes. Bottom image is a dog from Brazil infected with *L. infantum (L. chagasi)* showing typical cutaneous manifestation of a long-standing infection.

acute myocarditis: pale mucous membranes, lethargy, ascites, hepatomegaly, splenomegaly, and tachyrhythmia (Barr, 1991). Signs during the chronic stage of the disease are related to congestive myocardial failure (da Pascon et al, 2010). Megaesophagus and other megasyndromes described in humans with chronic Chagas' disease have not been reported in dogs.

Leishmania

Leishmania donovani and *Leishmania infantum* are the major causes of visceral leishmaniasis (kala-azar); *L. infantum* is often called *Leishmania chagasi* by many workers when discussed in hosts in the Americas. *Leishmania tropica* and related species cause several clinical forms of cutaneous leishmaniasis in humans, dogs, rodents, and wild mammals in Eurasia and Africa. *Leishmania mexicana* is a complex of species in the Americas causing cutaneous lesions that use various animal reservoir hosts. *Leishmania braziliensis* and related species cause mucocutaneous leishmaniasis in the Americas.

VISCERAL LEISHMANIASIS. Leishmanial organisms live as amastigotes within macrophages throughout the body of the vertebrate host (Figure 3-3). The disease is spread by the bite of a phlebotomine sandfly, with the important genera being *Phlebotomus* in the Old World (Africa and Eurasia) and *Lutzomyia* in the New World (the Americas). Europeans introduced visceral leishmaniases in the form of *L. infantum* into the Americas during the period of colonization. The disease tends to be concentrated in areas around the Caribbean, in parts of sub-Saharan Africa, and in Brazil.

Small pockets of the disease can be found in other parts of the world also.

The stage in the macrophage of the vertebrate host is the amastigote (see Figure 8-16). When an amastigote in a macrophage is ingested by the sandfly, which feeds on superficial tissues and juices of a host, the amastigotes differentiate into promastigotes. Within the fly, the promastigotes multiply and produce very large numbers. Promastigotes migrate to the pharynx of the fly, and a few days later they reach the hypostome of the fly, where the promastigotes are often sufficient in number to block the feeding ability of the fly. The process of development in the fly from infection to being infective takes about a week. The next time the fly bites, it will prick the skin and inject a number of promastigotes, and because the flies have difficulty feeding, they often remain hungry and feed more than when uninfected. The promastigotes will then be ingested by macrophages and carried throughout the body of the host.

Within the human host, the macrophages serve to provide a means by which the parasite is disseminated throughout the body. The tissues most commonly found to harbor large numbers of parasites include the spleen, liver, bone marrow, intestinal mucosa, and mesenteric lymph nodes. The large numbers of organisms that can develop in the bone marrow can cause decreased red blood cell and platelet numbers. Dogs often also develop cutaneous lesions.

In the 1980s, autochthonous visceral leishmaniasis was reported in a colony of American foxhounds in Oklahoma (Anderson et al, 1982) and in a colony of English foxhounds in Ohio (Swenson et al, 1988). In 1999 additional infections were reported in a group of working foxhounds kenneled at a hunt club in New York State. Further testing of foxhounds from around the United States revealed that of 11,000 foxhounds in U.S. and Canadian hunt packs, some 12% had antibodies to *Leishmania* organisms, although most were without signs (Enserink, 2000). Infected dogs were found in 21 states in the United States and in southern Canada, with most cases in the eastern portion of North America. It remains unclear how the infection was spread between these dogs. It was suggested that transmission was conducted perhaps by sandflies when the dogs were taken to southern states for hunts; however, increasing evidence recently suggests that transplacental transmission of canine leishmaniasis can occur (Boggiatto et al, 2011; Rosypal et al, 2005). A few cases of visceral leishmaniasis have also been reported to occur in dogs other than foxhounds that have never left the United States or Canada (Schantz et al, 2005).

Visceral leishmaniasis in dogs (see Figure 3-3) often is seen with cutaneous manifestations. Dogs are considered major reservoirs for human infection with this parasite and have been the targets of eradication programs similar to rabies control programs (Oliveira-dos-Santos et al, 1993). The need to develop a means of preventing canine infections on a large scale has resulted in attempts to develop vaccines that will do this (Mayrink et al, 1996). Work has also shown that the routine monthly or biweekly spot-on application of imidacloprid and permethrin could prevent transmission, as reported in kenneled dogs in an area with a high prevalence in southern Italy (Otranto et al, 2007).

CUTANEOUS LEISHMANIASIS. Autochthonous cases of cutaneous leishmaniasis are reported on occasion in animals from the United States, and it is believed that these are regularly being transmitted to animals and people from phlebotomine sandfly bites. Most cases in domestic animals have been reported in cats, and a number of cases typically presenting as nodular lesions on the face and identified molecularly as *L. mexicana* have been reported from Texas, as have autochthonous infections with lesions in people (Trainor et al, 2010; Wright et al, 2008). Horses sometimes have cutaneous leishmaniasis, and lesions on two horses in Puerto Rico were demonstrated to contain organisms (Ramos-Vara et al, 1996). A case of *Leishmania siamensis* was reported from a lesional mass on the ear of a horse in Florida (Reuss et al, 2012). Both canine cutaneous leishmaniasis and canine visceral leishmaniasis are sometimes imported with dogs that have vacationed in areas where these diseases are enzootic.

Trichomonadida

Trichomonads are characteristically pear-shaped, with a single nucleus and a rodlike axostyle that protrudes from the more pointed posterior end. Three to five anterior flagella are present, and an undulating membrane with a trailing flagellum runs along its free edge. Trichomonads do not have a cyst stage involved in their transmission between hosts. Pseudocysts have now been described in the case of *Tritrichomonas foetus* from the prepuce of bulls (Pereira-Neves et al, 2011); however, at this time, it remains unclear whether or not they are an important part of the transmission biology of this parasite. Special techniques are required for the differentiation of trichomonad genera on purely morphologic grounds, and the identification of species is currently aided by molecular comparisons. Therefore, practical diagnosis is based on host and site specificity, the number of anterior and trailing flagella, and more and more on the utilization of molecular methods.

Tritrichomonas foetus

Tritrichomonas foetus (Figure 3-4) is found in the vagina, uterus, macerated fetus, prepuce, penis, epididymis, and vas deferens. The organism displays considerable pleomorphism, varies from 10 to

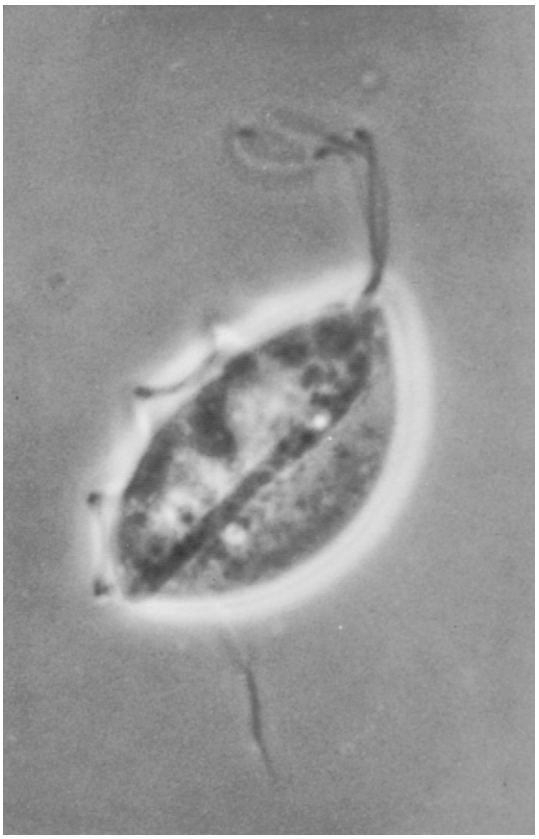

FIGURE 3-4. *Tritrichomonas foetus.* Electronic flash, phase contrast micrograph of living organism from a culture provided by Dr. S. J. Shin. The three anterior flagella, the undulating membrane, the trailing flagellum, and the axostyle are clearly visible.

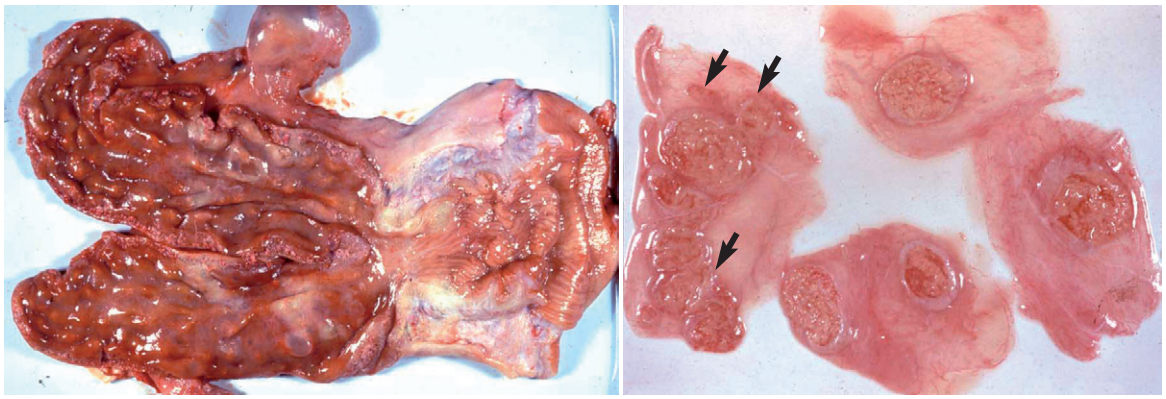

FIGURE 3-5. *Tritrichomonas foetus. Left*, Opened bovine uterus showing mild diffuse endometritis and inflammatory exudate in the form of a cloudy exudate on the endometrial surface. *Right*, Bovine chorioallantois. Cotyledons with placental edema; arrows indicate areas of adventitial placentation.

25 µm in length, and has three anterior flagella and a long, trailing flagellum that extends beyond the undulating membrane. In collecting samples to isolate *T. foetus*, it is important to avoid fecal contamination and consequent potential confusion with intestinal flagellates.

Bovine genital trichomoniasis is a venereal disease manifested in cows and heifers by infertility, abortion up to 5 months after breeding, pyometra, and occasional fetal mummification (Figure 3-5). Infection in beef cattle remains relatively common in some parts of the United States; 16% of 57 herds sampled in California had at least one infected bull (BonDurant et al, 1990). Although infection is inapparent in bulls, *T. foetus* trophozoites can be demonstrated by direct microscopic examination or by culture of preputial swabs or washings. The infected bull is usually responsible for spreading trichomoniasis in the herd, and artificial insemination is recommended as a control measure when feasible. *T. foetus* trophozoites are transferred from the penis to the vagina during copulation. However, semen usually is not infectious unless contaminated with preputial fluid during artificial collection. Semen contaminated in this way remains infectious despite the addition of diluents, antibiotics, and freezing (Fitzgerald, 1986). Infected bulls should be culled, and in those situations in which artificial insemination is impractical, they should be replaced with younger, uninfected bulls. However, failure on the part of the artificial insemination technician to observe effective hygienic precautions in conducting vaginal examinations for the detection of estrus may totally negate the benefits of artificial insemination as a control measure (Goodger and Skirrow, 1986).

T. foetus can usually be demonstrated in the vaginal secretions or washings of virgin heifers 14 to 20 days after service by an infected bull. Infected cows should be given at least 4 months of sexual rest, during which time *T. foetus* trophozoites usually disappear from the reproductive tract. Whether inseminated naturally or artificially, cows and heifers must first be given sexual rest so their reproductive tracts will be cleared of *T. foetus* before gestation begins; otherwise the infection will be perpetuated in the developing embryo (Fitzgerald, 1986). Diagnosis has been aided by the use of the InPouch TF transport and culture kit, available from Biomed Diagnostics, San Jose, California (Parker, Campbell, and Gajadhar, 2003). For diagnosis, PCR has been shown to be more sensitive than culture methods, and in some cases, one negative PCR result may be sufficient for interstate shipment. The preferred sample from a bull is a collection of vigorous back-and-forth scrapings of the glans penis and prepuce into a sterile infuse pipette with negative pressure from a 12-cc syringe. For cows, cervical mucus or uterine fluid collected through an insemination or infusion pipette with negative pressure from a syringe is the preferred sample. Currently there is no treatment for bovine trichomoniasis; there are vaccines available to prevent this infection (see Chapter 9).

Surveys have found varying levels of infection of *T. foetus* among cattle in the United States. In Alabama between 2002 and 2005, only 1 of 374 samples was confirmed positive (Rodning et al, 2008). Testing of 1984 beef bulls in 59 herds in Florida revealed an overall prevalence of 6% with a herd prevalence of 30.4%, with more bulls being found in herds with more than 500 cows (Rae et al, 2004). In Nebraska, of 121 bulls, 24 were found infected by real-time PCR, with a positive correlation between the number of nonpregnant cows in a herd and the presence of infected bulls (Ondrak et al, 2010).

TRITRICHOMONAS BLAGBURNI. Cats are host to a large bowel trichomonad that is considered by many to be the same as *T. foetus* of cattle (Levy et al, 2003), although others consider it to be slightly different genetically, and believe that the cat form has never been recovered from naturally infected cattle (Slapeta et al, 2010). A similar problem has been noted relative to the cattle parasite and *Tritrichomonas suis* of the nasal cavity and large bowel of pigs; some researchers consider them identical and others disagree with that opinion (Lun et al, 2005; Reinmann et al, 2012). Potential sharing by cats and pigs of a species that is of significant economic importance in cattle is of course of great importance for those interested in the epizootiology of the cattle disease and is of significant concern in countries where the disease in cattle has been eradicated (Reinmann et al, 2012). Recently the species that is thought to be the causative agent of disease in cats has been assigned the species name *Tritichomonas blagburni* based on molecular gene sequencing differences, differences in pathogenicity of the feline and bovine isolates, and the results of cross-infecton studies using isolates from cats and cattle (Stockdale Walden et al, 2013).

It is assumed that cats are infected by the oral ingestion of trophozoites, very likely by direct fecal-oral contact. It has been shown that the trophozoites of *T. blagburni* from cat feces can survive transit through the alimentary tract of slugs (van der Saag, McDonell, and Slapeta, 2011). Infections with *T. blagburni* are felt to be most common in young purebred domestic show cats that are group housed and in other cats housed in high-density populations (Gookin et al, 2004; Tolbert and Gookin, 2009). This infection is associated with intermittent large bowel diarrhea. Diagnosis is often made using the InPouch TF transport and culture kit (Gookin et al, 2003), which typically is combined with PCR for species

confirmation (Tolbert and Gookin, 2009). The current treatment of choice to clear these cats of their infection consists of oral administration of ronidazole at 30 to 50 mg/kg every 12 hours for 14 days, with post-treatment testing to verify clearance (Gookin et al, 2006); in some cats, a reversible neurotoxicity similar to that seen with metronidazole has been reported (Rosado, Specht, and Marks, 2007). It appears that dogs with diarrhea may also be infected with *T. foetus* on some occasions (Gookin et al, 2005; Tolbert et al, 2012), but now the question arises as to whether this is due to *T. foetus* or *T. blagburni*.

Other *Trichomonas* Species

Trichomonas vaginalis causes vaginitis in women; it is transmitted by sexual intercourse, with men playing the role of asymptomatic carrier. This is one of the more common sexually transmitted diseases of people around the world. *Trichomonas gallinae* causes necrotic ulcerations in the esophagus, crop, and proventriculus of pigeons, turkeys, and chickens and occasionally in hawks that are fed infected birds. *Trichomonas* species occur as oral parasites on various hosts and tend to multiply in the presence of pyorrhea, much as their intestinal counterparts multiply in the presence of diarrhea.

Pentatrichomonas Species

Cats, dogs, people, and other animals can also be infected with *Pentatrichomonas hominis*, a parasite that is sometimes considered to be associated with diarrhea (Gookin, Stauffer, and Levy, 2007; Kim et al, 2010; Tolbert et al, 2012). Diagnosis of this infection is also facilitated by the use of the InPouch TF transport and culture kit (Gookin et al, 2003). Treatment, when given, typically takes the form of metronidazole.

Histomonas meleagridis

Histomonas meleagridis is a cosmopolitan parasite of the cecum and liver of turkeys, chickens, pheasants, guinea fowl, and the like. The cecal nematode *Heterakis gallinarum* serves as transport host for *H. meleagridis*. When a bird ingests an infective *H. gallinarum* egg, it acquires a nonpathogenic nematode and a pathogenic protozoan parasite at one stroke. The protozoan, released from the nematode larva, spends about a week as a flagellate resident of the cecal lumen before it loses its flagella and invades the subepithelial tissues of the wall as an amoeboid organism. Inflammation and necrosis of the cecal wall and liver are particularly severe and cause a high rate of mortality in turkeys. *H. meleagridis* trophozoites discharged in bird droppings perish within hours, but they remain infective for years within the larvated eggs of *H. gallinarum* in soil. Earthworms serve as paratenic hosts for *H. gallinarum* larvae, and because birds like to eat them, they actually facilitate infection with both this nematode and its protozoan guest. The disease is typically of little consequence in chickens, but it can cause high mortality in turkeys. The only drug currently approved for use in turkeys with this conditions is nitarsone, an arsenical, which is presented in a combination with bacitracin as a feed additive as an aid in the prevention of histomoniasis; it must be withdrawn 5 or more days before slaughter.

Nonpathogenic Intestinal Trichomonadida

Nonpathogenic species of trichomonads occur in the cecum and colon of various domestic and wild animals. These organisms tend to multiply in fluid feces, and many cases of diarrhea are mistakenly attributed to them for this reason. Their abundance in fluid feces is often the effect and not the cause of the diarrhea. Many different genera and species of these intestinal flagellates have been described over the years but are now overlooked because most have been found not to be pathogenic and because little time has been spent in careful examination of saline wet mounts of fresh feces or fixed and stained fecal preparations.

Diplomonadida (*Giardia* and Relatives)

Giardia

The number of species of *Giardia* that exist is open to question, and the names of species in current usage are in a state of flux (Thompson et al, 2000; Bowman, 2005). Species in people have been called *Giardia lamblia*, *Giardia duodenalis*, *Giardia intestinalis*, or *Giardia enterica*. Some species are recognized as distinct (e.g., *Giardia muris* in mice, *Giardia agilis* in amphibians, *Giardia psittaci* in birds). On the basis of molecular biology, the groups of *Giardia* are currently discussed in terms of Assemblages. In most cases, when *Giardia* is isolated from a host and is examined via molecular methods, the association is such that Assemblages A and B are considered to be mainly those found in human beings, Assemblages C and D make up the majority of organisms found in dogs, Assemblage E makes up the group most typically found in hoofed stock (cattle, sheep, goats, pigs, horses, and so on), Assemblage F consists of the forms from cats, and Assemblage G represents the form from rats. The newest group is Assemblage H, which has been found in seals. Assemblages C and D have never been recovered from a human in the United States, and most interest in the zoonotic transmission of *Giardia* has focused on Assemblage A, which is the most common non–host-specific assemblage that appears in animals. Subtyping data do not support a widespread occurrence of zoonotic transmission, even with this Assemblage in which it has been shown that humans are most commonly infected with subtype AII while animals are most commonly infected with subtype AI (Feng and Xiao, 2011).

Unfortunately, this is not always as simple as the ABCs, especially since much of the work to date has not included subtyping of the different Assemblages recovered. DNA of *Giardia* from 131 positive samples from dairy cattle in New York State that underwent genotyping included 102 that were Assemblage E and 29 that were Assemblage A, and all animals that were positive for Assemblage A were younger than 84 days and were found on only 5 of 22 farms (Mark-Carew et al, 2012). Sometimes Assemblage A may be found in a cat (Vasilopulos et al, 2007) or a dog (Hopkins et al, 1997). In Ontario, Canada, among canine fecal samples submitted by veterinary clinics, 74 of 75 *Giardia*-positive canine samples contained Assemblage C or D, and 1 of the 75 samples contained Assemblage B (McDowall et al, 2011). In this same study, 12 of 13 feline samples contained Assemblage A, the other contained Assemblage B, and none contained the feline Assemblage F. One study used four different molecular loci to examine *Giardia* from samples of feces of dogs in Croatia that lived in households versus samples from dogs that lived in animal shelters (Beck et al, 2012). The predominant Assemblages in both sets of dogs were C and D, and the authors believed that this showed that dogs were at only minimal risk of being reservoirs of zoonotic isolates. However, it was difficult to obtain good resolution and congruence with the different diagnostic loci relative to the shelter dogs; this was thought to be a result of their having mixed infections with the human-infecting Assemblages A and B. Another study is much different from all the others because the vast majority of dogs had neither C nor D: Of 148 DNA samples from *Giardia*-positive canine feces from the western United States, 41% were identified as the human-infecting Assemblage B, 28% as the potentially human-infecting Assemblage A, and only 31% as the canine-infecting Assemblage C or D (Covacin et al, 2011).

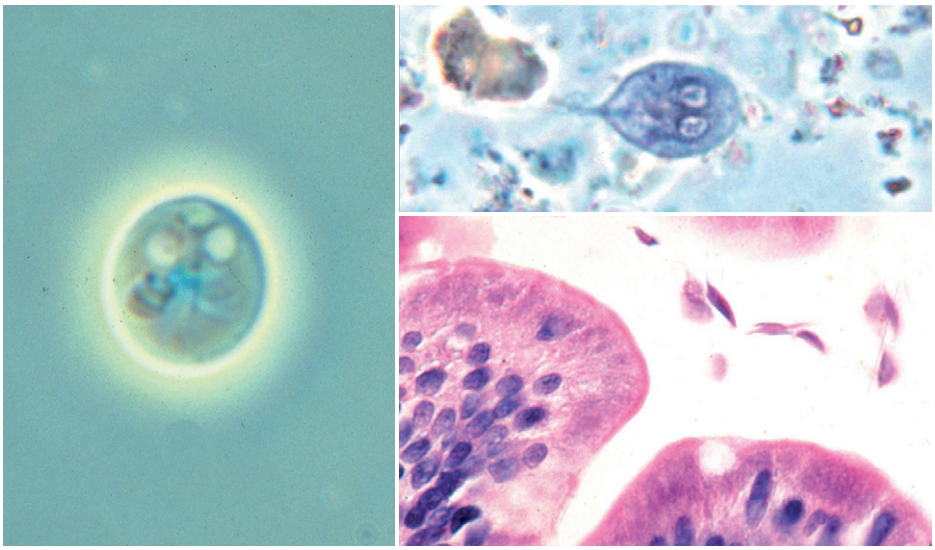

FIGURE 3-6. *Giardia. Left*, Cyst passed in feces. Phase contrast micrograph showing two of the four nuclei near the top of the image. *Top right*, *Giardia* trophozoite in trichrome-stained fecal smear. *Bottom right*, Section through intestinal mucosa of an infected animal with detached trophozoites present within the lumen.

Giardia trophozoites are adapted for attachment to the mucous epithelial cells of the small intestine (Figure 3-6). The *Giardia* trophozoite is shaped like a teardrop (see Figure 3-6), with one side pushed in to form a sucking disc. Within the cell are two nuclei, each with a large endosome (Feulgen-negative nucleolus) that makes the organism look like a tennis racket with eyes when viewed bottom side up under the compound microscope. Other subcellular structures include two slender axonemes, four pairs of flagella, and a pair of median bodies. All of the other intestinal flagellates are found in the cecum and colon, but *Giardia* parasitizes the small intestine, in which the trophozoites attach to the mucosal cells by their sucking discs. Trophozoites usually form infective cysts before passing out with the feces. The mature cyst containing two potential trophozoites is the form usually found in the feces of infected hosts (see Figure 3-6). Although trophozoites may also be passed, especially with diarrheal stools, they are incapable of causing infection and soon die; if they go into fresh water, they will lyse owing to their inability to osmoregulate.

In dogs, diarrhea may begin as early as 5 days after exposure to infection (Abbitt et al, 1986); cysts first appear in the feces after about a week or two. In cats, *Giardia* trophozoites are found in the jejunum and ileum instead of the duodenum. The principal clinical sign is persistent diarrhea resulting from intestinal malabsorption; the feces of infected cats are often mucoid, pale, soft, and more than usually malodorous (Kirkpatrick, 1986). In calves, *Giardia* was associated with chronic diarrhea marked by high morbidity, negligible mortality, absence of response to electrolytes and antibiotics, and clinical improvement within 48 hours of treatment (St Jean et al, 1987). In lambs, careful examination of production parameters in bottle-reared and experimentally infected animals showed that neonatal giardiasis caused extended times for lambs to reach slaughter weight and decreased carcass weight (Olson et al, 1995). The enumeration of cysts in the feces of ewes around lambing time revealed a periparturient increase in cyst production by ewes that peaked between the time of lambing and 4 weeks afterward (Xiao, Herd, and McClure, 1994). In an outbreak in central Italy, lambs with naturally acquired giardiasis developed a malabsorption syndrome, decreased weight gain, and impaired feed efficiency that responded to treatment with fenbendazole at 10 mg/kg for 3 days (Aloisio et al, 2006). *Giardia* infection in humans may be inapparent or may cause severe enteritis.

DIAGNOSIS. Trophozoites may be demonstrated in direct smears of diarrheal feces (see Figure 3-6); trophozoites often cannot be demonstrated in formed stools. Cysts (see Figure 3-6 and Figure 7-104) may be concentrated by fecal flotation in zinc sulfate of specific gravity 1.18 but tend to shrink and become distorted beyond recognition in sucrose and other flotation media. Phase contrast microscopy is helpful in identifying *Giardia* trophozoites and cysts. If phase contrast microscopy is unavailable, a drop of Lugol's solution of iodine at the edge of the coverslip will stain the trophozoites and cysts, making them easier to identify by increasing the contrast of nuclei within the organisms. *Giardia* cysts are frequently found in the normal stools of asymptomatic hosts, but in occasional cases of clinical giardiasis, neither cysts nor trophozoites can be found in the feces. Several antigen detection kits are designed for use with the feces of animals and people (Garcia and Shimizu, 1997), and the IDEXX SNAP test is now routinely used in-house by veterinarians (Carlin et al, 2006).

TREATMENT. Dogs may be treated for giardiasis with fenbendazole at the same dosage used for helminths (Barr, Bowman, and Heller, 1994; Zajac et al, 1998). Dogs have also been treated with a combination febantel-pyrantel-praziquantel (37.8 mg/kg, 7.56 mg/kg, 7.56 mg/kg, respectively) product for 3 days with successful clearance of cysts from most dogs (Payne et al, 2002). Treatment with albendazole (25 mg/kg every 12 hours for a total of four doses) has been shown to stop the shedding of *Giardia* cysts by infected dogs (Barr et al, 1993). Albendazole therapy has the potential of inducing bone marrow toxicosis in dogs and cats; therefore veterinarians should observe caution in using this drug for treating giardiasis (Stokol et al, 1997). Other treatments that have been used for canine giardiasis include quinacrine (6.6 mg/kg twice a day for 5 days), metronidazole (22 mg/kg orally twice a day for 5 days), and tinidazole (44 mg/kg once daily for 3 to 6 days) (Zimmer and Burrington, 1986). Dogs in a kennel situation were cleared of their infections using ronidazole at 30 to 50 mg/kg twice daily for 7 days in conjunction with chlorhexidine shampoos and disinfection of the pens with 4-chlorine-M-cresol (Fiechter,

Deplazes, and Schnyder, 2012). However, within 1 to 2 months after treatment, in spite of the maintenance of a high level of hygiene, all dogs again tested positive by antigen detection, cyst detection, or both.

Giardia infection in cats may be treated safely and effectively with metronidazole, 22 to 25 mg, orally, twice a day for 5 to 7 days (Scorza and Lappin, 2004; Zimmer, 1987). Cats have also been successfully treated with a combination of febantel (37.8 mg/kg), pyrantel (7.56 mg/kg), and praziquantel (7.56 mg/kg) for 5 days (Scorza, Radecki, and Lappin, 2006).

The question now is this: When does one treat a dog or a cat that is positive for *Giardia*? A few years ago, when all *Giardia* were considered zoonotic and the diagnosis was made only in dogs with clinical signs, it was easy to recommend that infected dogs be treated. Now, with improved diagnostics that can detect fecal antigen and with increased awareness that *Giardia* can cause disease, the question very commonly involves whether to treat dogs and cats with no clinical signs. Do you sell a puppy if it is shedding *Giardia* cysts in its feces? Can a shelter in good conscience place an infected kitten in a foster home? In a survey from around the United States using the IDEXX SNAP test, some 15% of dogs and 10% of cats were shedding antigen in their feces (Carlin et al, 2006). Also, about 7% of the world's human population harbors *Giardia* in the small intestine. It seems that animals can sometimes be shedding A and B and thus may be a source of zoonotic infection to humans; however, it also appears that people usually become infected with *Giardia* from other people, and people often are asymptomatic carriers. However, members of the public would prefer to blame animals rather than their human associates. A survey of human fecal samples from the Rocky Mountains of the United States found that 1.4% contained *Giardia lamblia*, 1.5% contained *Entamoeba coli*, 1.1% contained *Endolimax nana*, and 0.1% contained *Iodamoeba butschlii* (Church, Neill, and Schotthoefer, 2010); the sources of the commensals *E. coli*, *E. nana*, and *I. butschlii* are humans, not dogs or cats.

If one is deciding whether to provide regular treatment of dogs and cats for giardiasis when no clinical signs are present, one should give some thought to the potential of selecting for resistant isolates. Some documented cases of human giardiasis appear totally refractory to treatment with albendazole, tinidazole, furazolidone, paromomycin, and metronidazole (Brasseur and Favennec, 1995; Lopez-Velez et al, 2010; Nash et al, 2001). Similarly, many veterinary practitioners have reported cases of *Giardia* in dogs that are refractory to routine treatment. Although in vitro, it is fairly easy through drug selection to develop *Giardia* strains that are resistant to the routine chemotherapeutic agents, especially the nitroimidazoles, in only a few instances have resistant strains been isolated from refractory human cases (Lemée et al, 2000; Tejman-Yarden et al, 2011). Recent work has shown that metronidazole-resistant strains have some difficulty attaching to surfaces compared with strains that are not resistant, and that resistant strains are less able to successfully colonize the intestine in suckling mice and gerbil models; it is believed that this is a fitness trade-off relative to being able to survive in the presence of high drug concentrations (Tejman Yarden et al, 2011). However, because some resistant isolates could infect these hosts, albeit at lower levels, the authors caution that "viewed from the perspective of drug resistance rather than biological fitness, our data provide strong new support for the notion that clinically relevant Mz [Metronidazole] resistance can and does exist in *Giardia*." Thus, and especially if zoonotic assemblages of *Giardia* are occurring in dogs and cats, trying to clear every pet that has no clinical signs of *Giardia* infection should be a matter of concern; it is possible that such treatments might be hastening the development of resistant forms, and that some of these forms could colonize humans, as well as dogs and cats.

Fenbendazole and albendazole administered to calves at varying doses for different periods have proved efficacious against *Giardia* (O'Handley et al, 1997; Xiao, Saeed, and Herd, 1996). All treatments with fenbendazole with a single dose of 10 mg/kg, with 10 or 20 mg/kg administered daily for 3 days, or with 0.833 mg/kg administered daily for 6 days were effective. For albendazole, a dose of 20 mg/kg administered daily for 3 days was effective.

For treatment of giardiasis in parakeets, three doses of dimetridazole at 1.5 mg/30 g of body weight given at 12-hour intervals by stomach tube were more effective than supplying drinking water containing 200 ppm of this chemical for 5 days. Metronidazole therapy was not effective (Scholtens, New, and Johnson, 1982).

Control of *Giardia* infection involves prevention of fecal contamination of feed and water supplies and sanitation and disinfection of the environment with Lysol (2% to 5%), Sterinol (1%), or chlorine bleach (sodium hypochlorite, 1%) (Kirkpatrick, 1986).

Heterolobosea (*Naegleria* and Relatives)

The amoeba-like creatures in the Excavata of interest in veterinary parasitology are the facultative amoebae in the genus *Naegleria*. Facultative amoebae are free-living most of the time but can cause serious disease if they enter vertebrate hosts (Visvesvara and Schuster, 2008a and 2008b). Members of the genus *Naegleria* are protists that have an amoeboid feeding form that lives and moves about on surfaces in warmer waters, a biflagellate dispersal form, and a cyst form. They become pathogenic typically when they find their way into the nostrils; then the amoeboid form causes fatal fulminant primary amoebic meningoencephalitis as they make their way into the brain, where they feed and reproduce extremely rapidly. Infections are most commonly described in human cases where death has typically occurred within a week or so after the event in which the amoebae were apparently acquired (Schuster and Visvesvara, 2004). A case of fatal primary amoebic encephalitis due to *Naegleria fowleri* was reported in a tapir in an Arizona zoo (Lozano-Alarcon et al, 1997). A case was reported in a sheep as well (Fuentealba et al, 1992). Also, cases of fatal *N. fowleri* have been reported in cattle, including 9 cases in 10- to 20-month-old heifers in California in the summers of 1998 and 1999, when summer temperatures sometimes exceeded 42°C (Daft et al, 2005; Morales et al, 2006; Pimentel et al, 2012).

SAR

The SAR is the joining of three large groups of plastid-containing organisms: the Stramenopiles, the Alveolata, and the Rhizaria. Within the SAR, members of the Alveolata are best known to parasitologists because they include the ciliates and the Apicomplexan parasites causing cryptosporidiosis, coccidiosis, toxoplasmosis, and malaria. The Stramenopiles contain the brown algae, the diatoms, water molds, and the terrestrial downy mildews of plants; of importance to animal parasitology is the fact that currently placed within the Stramenopiles are the opalines of amphibia and *Blastocystis hominis*, a potentially zoonotic agent that is considered by some a cause of large bowel disease in people and other animals. The Rhizaria contains the diatoms and the foraminifera, and it currently contains the haplosporidian parasites of great importance

in shellfish culture; however, this group is not discussed further in this book.

ALVEOLATA

The grouping of the Alveolata can perhaps be most readily appreciated if one considers them to be covered with the classical "pellicle," the trilaminar membrane obvious in the electron micrographs of different Apicomplexan parasites. The external surface is covered with alveoli, which can be crudely considered as flattened membranous bubbles on the external surface of the cell. It appears that the movement of these alveoli is what provides gliding motility in the case of the Apicomplexa.

Ciliophora (Ciliates)

Ciliates are fairly well known to everyone from the images they have seen of a swimming *Paramecium* at some point in their lives. They are characterized as being covered with cilia and as possessing dimorphic nuclei; they include one macronucleus and one to several micronuclei. Only one ciliate is of any marked significance in veterinary, and human, medicine—*Balantidium coli*.

Balantidium coli

B. coli, a normal element of the intestinal fauna of the pig and rat, is very large as single cells go, measuring up to 150 µm in length (Figure 3-7). *B. coli* is reported with some regularity from primates and ostriches. The cell surface is covered with cilia (*singular*, cilium) arranged in rows, with a tuft of longer ones surrounding the peristome, or "cell mouth." Prominent organelles include a large macronucleus, a smaller micronucleus, two contractile vacuoles, and a number of food vacuoles in the cytoplasm. *B. coli* reproduces by transverse fission and forms cysts up to 60 µm in diameter. Comparison of all internal transcribed spacer genes of *B. coli* from a human and from two pigs, four ostriches, and four gorillas did not seem to support separate species designations for the forms found in pigs and ostriches (i.e., they should still be considered part of the species *B. coli*) (Ponce-Gordo, Fonseca-Salamanca, and Martínez-Díaz, 2011). A number of articles from Asia have described disease due to *B. coli* that occurred in cattle over the past few years, but at this time, no molecular analyses have been performed on the agent involved nor have attempts been made to distinguish the observed *Balantidium* from the similar-looking commensal ciliate *Buxtonella* that might be present in these animals. It seems that it may be prudent to wait until further work is performed before discussing cattle as another reservoir host of *B. coli*, or as an animal that may readily develop disease from this protozoal parasite of pigs.

Although harmless to the pig and usually harmless to humans, *B. coli* occasionally causes ulceration of the human large intestine, manifested clinically as diarrhea and occasionally as dysentery (diarrhea with abdominal pain, straining, and blood and mucus in the stools) (see Figure 8-17). Diagnosis of *B. coli* infection is based on the demonstration of motile trophozoites in direct smears of diarrheal feces or cysts in flotation preparations of formed feces. Acute enteritis characterized by watery diarrhea and lethargy involving four gorillas in the Los Angeles Zoo was attributed to *B. coli* infection (Teare and Loomis, 1982). Lowland gorillas affected with balantidiasis did not accept metronidazole well and were treated with intramuscular injections of dehydroemetine dihydrochloride (Gual-Sill and Pulido-Reyes, 1994). Another lowland gorilla was reported to have recently died of acute balantidiasis that may have been caused by exacerbation of an existing *B. coli* infection by an acute bacterial infection (Lankester et al, 2008). Besides in pigs and primates, *B. coli* has caused large bowel disease with ulceration in dogs and horses (Headley, Kummala, and Sukura, 2008).

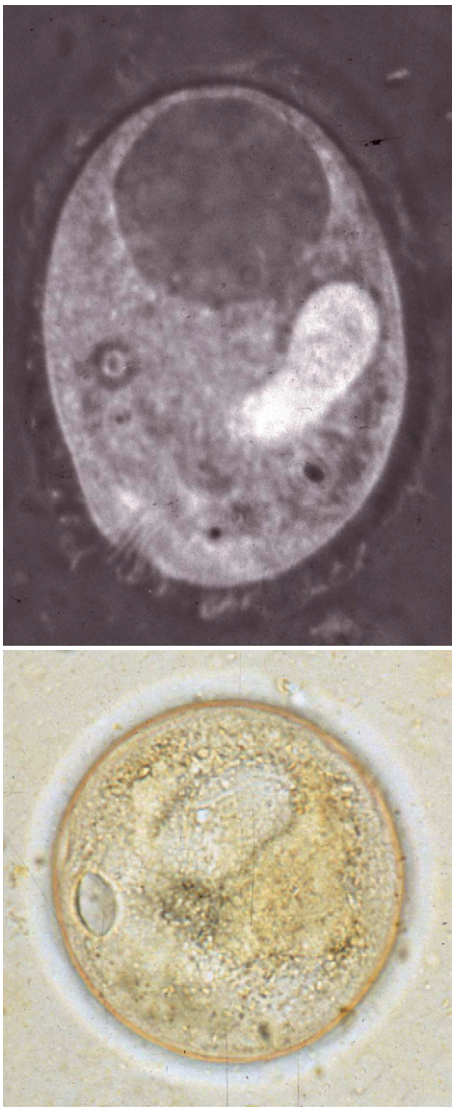

FIGURE 3-7. *Balantidium coli. Top*, Trophozoite (electronic flash photograph) of motile ciliate. *Bottom*, Cyst. Trophozoites abound in the large intestine of normal swine, and cysts are passed in their feces. *B. coli* has been incriminated in human colonic disease ranging from mild colitis to an ailment resembling amoebic dysentery.

Symbiotic Ciliates

The forestomachs of ruminants and the ceca and colons of horses abound with large, somewhat bizarre ciliates that are neither pathogenic nor indispensable to their hosts (Figure 3-8). Sometimes they are found in the lungs of ruminants at necropsy as the result of agonal inspiration of ruminal contents and nothing more (see Figures 8-18 and 8-19).

Also commonly seen in the feces of cattle are the cysts of *Buxtonella sulcata*, an apparent commensal that looks very similar with the untrained eye to the cysts of *Balantidium coli*.

Apicomplexa

The Apicomplexa is a group characterized as being parasites of cells, most typically intracellularly; having stages capable of gliding motility; having an apical complex within at least some of the life stages; and possibly undergoing sexual conjugation. Four groups

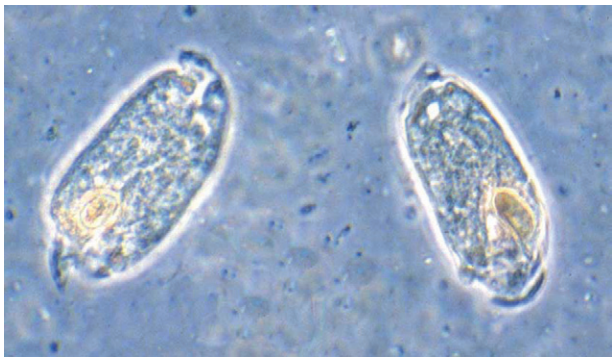

FIGURE 3-8. Ciliates from the large intestine of a horse.

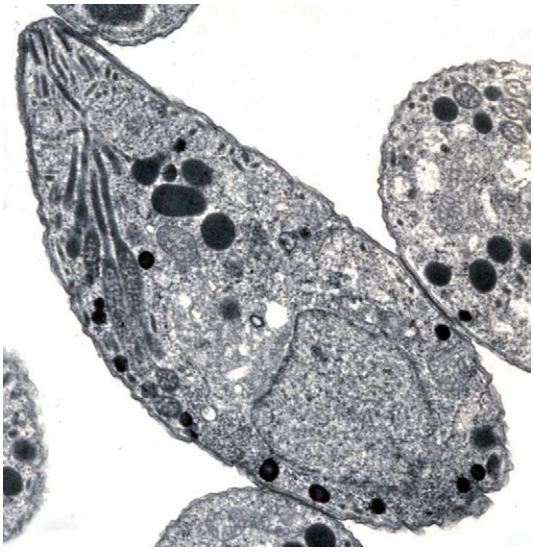

FIGURE 3-9. Tachyzoite of *Toxoplasma gondii* from a mouse. (TEM courtesy Dr. John F. Cummings.)

will be discussed herein relative to veterinary parasitology: the Gregarinasina, a group mainly parasitizing invertebrates that now includes *Cryptosporidium*; the Coccidiasina, which includes the well-known agents of coccidiosis, toxoplasmosis, neosporosis, and sarcocystosis; the Piroplasmorida, including the agents of babesiosis, theileriosis, and cytauxzoonosis; and the Haemosporida, which includes the malaria parasites. Two of these groups—Gregarinasina and the Coccidiasina—are placed within the larger group, the Conoidasida, because of their very obvious possession of a conoid apparatus; the other two groups—Piroplasmorida and Haemosporida—are placed within the Aconoidasida because their conoid is absent or inapparent in most life stages. The Aconoidasida are typically parasites of blood cells that are transmitted by blood-feeding arthropods. The Conoidasida tend to be parasites of the intestinal tract with various cystic stages passed in the feces of the host, although some have life cycles that involve blood-feeding arthropods in atypical fashion, so that they must be ingested by the next host to continue the life cycle.

The functional unit of apicomplexan ontogeny is the **zoite**—a motile, banana- or cigar-shaped cell, rounded at one end and pointed at the other (apical) end (Figure 3-9). It is the haploid zoite that migrates within the host and invades cells, and it is the zoite that represents the beginning and the end point of every coccidian life process. Relationship to a particular portion of the life history is denoted by a prefix. Thus, **sporozoites** are infective forms found in sporulated **oocysts** (pronounced "oh′-oh-sists"); sporozoites are the result of the reduction divisions that occur in the oocyst as a result of fusion of the gametes. Sporozoites invade host cells, in which they form many **merozoites** or **schizozoites** through a kind of multiple internal fission called **schizogony** (pronounced "ski-zog-o-ne′"; synonym, **merogony**); **tachyzoites** divide rapidly, **bradyzoites** divide slowly, and so on.

Gregarinasina of the Conoidasida

The gregarines are mainly known as parasites of marine, freshwater, and terrestrial invertebrates. Within these animal hosts, they parasitize the intestinal tract, body cavity, or reproductive system. Gregarines move by gliding and can be much larger than the cells they parasitize. They have an obvious conoid, which they insert within the membrane of the cells upon which they feed through a process known as **myzocytosis.** They undergo sexual conjugation through a joining of isomorphic gametocytes in a process known as **syzygy.** They are passed from host to host typically via oocysts formed after sexual conjugation. These oocysts contain sporocysts and are known to veterinary parasitologists mainly through observation of the oocysts of a gregarine parasite of the earthworm, *Monocystis lumbrici*, which are passed in the feces of animals that ingest earthworms (or sometimes, in the case of carnivores, after they have ingested animals, such as shrews, that typically feed on earthworms).

CRYPTOSPORIDIUM. The genus *Cryptosporidium* is currently considered by many parasitologists to be more closely related to the gregarines than to the coccidia and malarias (Carreno, Martin, and Barta, 1999). Again, this seems a purely academic exercise, but it helps explain the superficial relationship between these organisms and the mucosal cells that they parasitize, and why almost all anticoccidials and antimalarials have proved inadequate in the control of infection with this parasite. Of course, in 10 years we might change our minds again.

The genus *Cryptosporidium* has undergone a proliferation of species over the past few years at a fairly frantic clip (Kvac et al, 2013). Important species in veterinary medicine that are now recognized include the following as parasites of the small intestine: *Cryptosporidium parvum* in calves younger than 30 days of age; *Cryptosporidium ryanae* in slightly older calves, up to about 1 year of age; and *Cryptosporidium bovis*, which is more common in adult cows; also recognized are *Cryptosporidium xiaoi* in sheep, *Cryptosporidium suis* in swine, *Cryptosporidium ubiquitum* in deer, *Cryptosporidium canis* in dogs, *Cryptosporidium felis* in cats, *Cryptosporidium meleagridis* and *Cryptosporidium baileyi* in birds, *Cryptosporidium wrairi* in guinea pigs, and *Cryptosporidium tyzzeri* in mice. The *Cryptosporidium* of horses appears to be a distinct genotype, and it has been shown to be zoonotic (Burton et al, 2010); it does appear, however, that horses might sometimes be infected with *C. parvum* (Perrucci et al, 2011). Parasites of the stomach include *Cryptosporidium muris* in the mouse, *Cryptosporidium serpentis* in the snake, and *Cryptosporidium andersoni* in the abomasum of the cow. The important species in people is *Cryptosporidium hominis*. The zoonotic species that commonly infects people, and often veterinary students, is *C. parvum*. Other species that show up as rare zoonoses in people, often the immunocompromised, are *Cryptosporidium canis*, *Cryptosporidium felis*, *C. meleagridis*, *C. muris*, and *Cryptosporidium suis*. Sheep, goats, horses, and related animals seem to share *C. parvum* with calves, at least right now. Several species from cervids will probably be described sometime soon.

LIFE HISTORY. The transmission stage is the infective oocyst (5 to 8 μm in diameter, depending on the species) (Figures 3-10

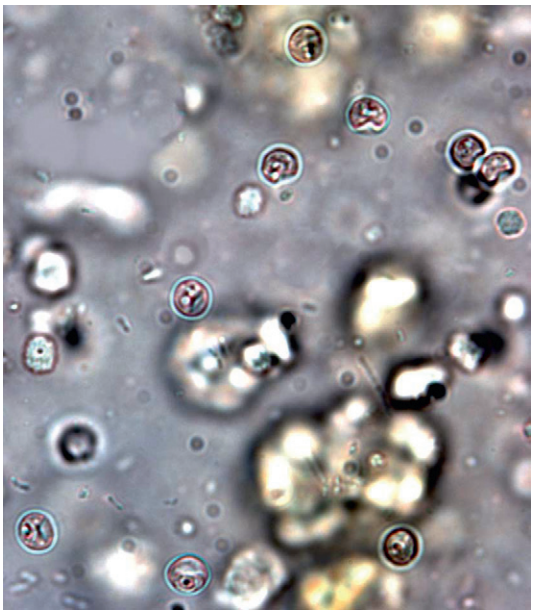

FIGURE 3-10. Oocysts of *Cryptosporidium parvum* in a sugar flotation preparation from calf feces.

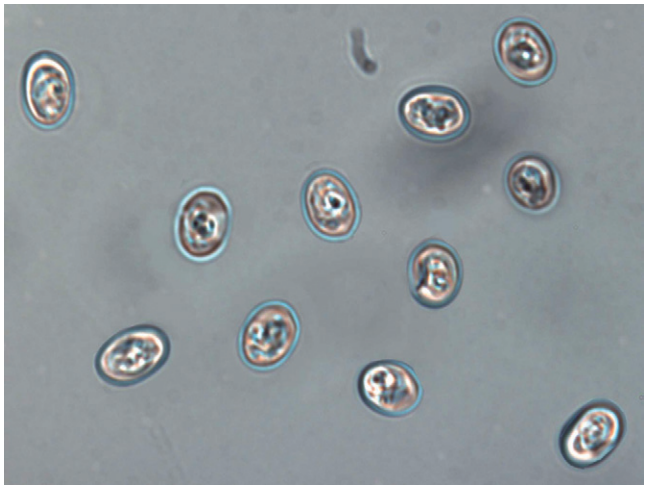

FIGURE 3-11. *Cryptosporidium andersoni* oocysts in a sugar preparation from infected cow feces.

and 3-11) containing four sporozoites that is discharged in the feces and serves to disseminate the infection. The oocysts remain viable for months unless exposed to extremes of temperature (below 0°C, above 65°C), desiccation, or impracticably concentrated disinfectants (5% ammonia, 10% formalin). When ingested by a suitable host, the oocyst opens along a preexisting suture line to release the four sporozoites that invade the microvillous border of the gastric glands (*C. muris;* Tyzzer, 1907, 1910) or the lower half of the small intestine (*C. parvum;* Tyzzer, 1912) (see Figure 8-29). In parasitophorous vacuoles in the microvillous border just under the surface of the cells they infect, the very small cryptosporidia undergo **schizogony** (asexual multiplication through synchronous division), **gametogony** (development of male and female gametes), fertilization (joining of a male with a female gamete), and **sporogony** (production of haploid sporozoites, the stage that infects the cells of the next host). Some oocysts go through excystation internally, providing the mechanism for autoinfection that accounts for the chronicity of certain cases in immune-sufficient hosts and lethal hyperinfection in immune-deficient hosts.

CLINICAL SIGNS. Inapparent infection is common in many mammalian, avian, reptilian, and piscine hosts. For example, *Cryptosporidium* was found in the microvillous borders of enterocytes of 5% (184 of 3491) of 1- to 30-week-old pigs submitted for diagnostic necropsy, but according to Sanford, "Only 26 per cent of the cryptosporidia-infected pigs had diarrhea and most of those had other primary diarrheagenic agents or lesions capable of causing their diarrhea" (Sanford, 1987). On the other hand, debilitating diarrhea may be associated with infection (e.g., in calves within the first 3 weeks of life). Although *C. parvum* is usually the culprit in clinical cryptosporidiosis in mammals, *C. andersoni* may cause mild diarrhea in cattle of all ages, especially young adults. Immunocompromised hosts may develop a life-threatening hyperinfective form of cryptosporidiosis, as is the case with many human patients with acquired immunodeficiency syndrome (AIDS) (Ma and Soave, 1983). Severe cryptosporidiosis has been reported to be associated with immunocompromise induced by feline leukemia virus (FeLV) in a cat (Monticello et al, 1987) and in Arabian foals with inherited combined immunodeficiency. In the latter case, however, it was not possible to separate the effects of *Cryptosporidium* and those of concurrent adenoviral infection (Snyder, England, and McChesney, 1987).

DIAGNOSIS. *Cryptosporidium* oocysts are difficult to see on fecal slides because they are colorless, transparent, and small; *C. parvum* measures 5.0 µm by 4.5 µm (see Figure 3-10), and *C. andersoni* measures 7.4 µm by 5.6 µm (see Figure 3-11) (Upton and Current, 1985). Concentrated sucrose solution (specific gravity 1.33) is the flotation medium of choice for concentrating oocysts of *Cryptosporidium*. The coverslip variant of the flotation concentration technique, which is described in Chapter 7 in the section on qualitative fecal examination, can be used. The oocysts appear as tiny subspherical objects that may be dented by osmotic extraction of water by the hypertonic medium. They tend to lie immediately below the coverslip, so one should focus on the top of an air bubble to find the best focal plane for *Cryptosporidium* oocysts. The oocyst walls may have a pinkish hue that helps in finding them; the pinkish hue is caused by chromatic aberration and is best developed by objective lenses of modest quality. The cyst walls are clear and colorless under a highly corrected objective lens. Questionable objects may be examined under the highest magnification to demonstrate the sporozoites. Phase contrast microscopy is helpful, and several staining procedures (e.g., methylene blue, Giemsa stain, iodine wet mount, modified Kinyoun acid-fast smear) have been recommended to increase the optical contrast and to stain confusing yeasts differentially. However, the most serious obstacle to correct microscopic diagnosis of cryptosporidiosis is inexperience and insecurity on the part of the microscopist. The best procedure is to keep examining feces from 1- to 3-week-old calves with the 40× objective and suitably stopped brightfield illumination until the *Cryptosporidium* oocysts are seen. If in doubt, one should check for sporozoites under higher power. Once the oocysts are seen, the most essential ingredient of accurate diagnosis will have been found. Exacting microscopic technique pays dividends, especially as one nears the resolution limits of the light microscope. Köhler illumination, which is described in all microscope manuals, is indispensable. Also useful for laboratories are various fluorescently labeled antibodies that bind to the oocyst, but these methods require the availability of a microscope equipped with an ultraviolet light source and appropriate filter sets. Several assays designed for in-office use have been approved for detection of the *C. parvum*

antigen in human feces, and the test, although expensive, appears to work well on bovine samples as well.

TREATMENT. No effective specific treatment for *Cryptosporidium* infection in animals is known. For people, the U.S. Food and Drug Administration (FDA) has approved the use of nitazoxanide in oral suspension for the treatment of diarrhea caused by *Cryptosporidium* (and *Giardia*). Nitazoxanide administered to experimentally infected calves at a dose of 1.5 g twice a day for 5 days significantly reduced the duration of oocyst shedding and improved fecal consistency (Ollivett et al, 2009). Paromomycin at 150 mg/kg once a day for 5 days has been used in the treatment of dogs and cats. For cats, drugs used have included tylosin at 10 to 15 mg/kg three times a day for 14 to 21 days, and azithromycin at 5 to 10 mg/kg twice a day for 5 to 7 days.

Coccidiasina of the Conoidasida

The Apicomplexa of interest within the Conoidasida are all obligate intracellular parasites that cause disease by destroying their host cells. Coccidians are transmitted mainly by fecal contamination and reproduce through rigid sequences of asexual and sexual phases of multiplication and development that, in an important minority of cases, require an alternation of hosts. In some instances (e.g., *Hepatozoon*, *Schellackia*), the cycle utilizes blood-feeding arthropods as hosts, wherein the oocysts develop and the next host is infected when it eats the infected arthropod.

***EIMERIA*.** This genus of coccidian is characterized by the presence of oocysts that when sporulated have four sporocysts, each with two sporozoites. The wall of the oocyst is often brownish in color, and many species have a polar cap. Members of this genus all have direct life cycles. Thus, the general form of the direct coccidian life cycle is represented by the genus *Eimeria*, species of which include gastrointestinal parasites of a wide range of vertebrate hosts.

GENERAL *EIMERIA* LIFE HISTORY. This life history includes both asexual multiplication and sexual multiplication. Sexual multiplication culminates in the formation of **oocysts,** which are discharged with the feces, and in the development, within each of these oocysts, of eight infective organisms, known as **sporozoites.** The life history of *Eimeria* should be learned by heart because it serves as a basis for that of all of the other coccidians. Figure 3-12 may prove helpful in mastering the following details.

Schizogony (Merogony). If the infective, **sporulated oocyst** is ingested by a suitable host, the **sporozoites** emerge, and each may enter an epithelial or lamina propria cell, round up as a **trophozoite** (see Figure 8-20), grow larger, and become a first-generation **schizont** (pronounced "skiz-ont" or "shyz-ont," a bit of the tomāto or tomăto dilemma; for our purposes, schizont = **meront**). The trophozoite, schizont, and all other intracellular stages of *Eimeria* are surrounded by a membrane-lined parasitophorous vacuole in the host cell cytoplasm or, in some cases, nucleoplasm. This schizont produces first-generation merozoites that burst the cell and invade fresh cells to become second-generation schizonts (see Figures 8-21 and 8-22). Several more schizogonic generations may occur, but two or three represents the limit for many of the important species of *Eimeria*. The number of asexual generations, the type and location of the host cells parasitized, and the number of merozoites formed at each generation depend on the species of coccidium in question. The salient attributes of schizogony include the following: (1) an exponential increase in the number of zoites arising from a single sporozoite; (2) destruction of host cells in proportion to the degree of infection; and (3) automatic suspension of the asexual process after a fixed number of repetitions.

Gametogony. A merozoite produced by the final schizogony (i.e., a **telomerozoite**) enters a fresh host cell and develops into either a male or a female (**microgamont** or **macrogamont,** respectively) or a developing sex cell (see Figure 8-23). The macrogamont (female) enlarges, stores food materials, and induces hypertrophy of both cytoplasm and nucleus of its host cell. When mature, it is called a **macrogamete** or **female sex cell.** The microgamont (male) undergoes repeated nuclear division and becomes multinucleate. Each nucleus is finally incorporated into a biflagellate **microgamete** or **male sex cell.** Of the many microgametes formed by the microgametocyte, only a small fraction will find and fertilize macrogametes to form **zygotes.** A wall is formed around the zygote by the coalescence of hyaline granules at its periphery to form an **oocyst** (see Figure 8-24).

Sporogony. The oocyst is released by rupture of the host cell and passes out with the feces to undergo **sporulation.** Within a day or two, if provided with adequate moisture, moderate temperatures, and sufficient oxygen, the single cell **(sporont)** in the oocyst divides into four **sporoblasts.** Each sporoblast develops into a **sporocyst,** which contains two haploid **sporozoites,** thus becoming an infective, sporulated oocyst and completing the cycle (Figure 3-13). The life history of *Eimeria* is presented again in schematic form in Figure 3-14.

***EIMERIA*-INDUCED COCCIDIOSIS.** A particular species of *Eimeria* tends to be restricted to a narrow range of hosts, but each host species may be parasitized by a number of different species of coccidians simultaneously. Antemortem diagnosis of coccidian infection (i.e., coccidiosis) is based on identification of oocysts in the host's feces. Host specificity and the form of the oocyst usually suffice for identification to the species level, but micrometry and sporulation of the oocysts must occasionally be resorted to in order to distinguish among certain species (see Chapter 8). Postmortem diagnosis is based on gross and microscopic lesions, which vary considerably with the host and parasite species involved, and on demonstration of the sexual or asexual stages of the parasites. Schizonts, gamonts, oocysts, and intermediate stages lie surrounded by their parasitophorous vacuoles in the cytoplasm (or nucleus, in a few cases) of enterocytes, lamina propria cells, or endothelial cells of the central lacteal of the villus. Although these are most elegantly displayed by histologic techniques, direct smears or squash preparations are just as dependable as hematoxylin and eosin (H&E) slides and are quicker and less expensive. Oocysts and merozoites can often be demonstrated in smears or concentrates of intestinal contents. Contrast microscopy or staining with Wright's or Giemsa stain is helpful in demonstrating sporozoites.

It is important to understand that the mere identification of coccidian oocysts in the feces of a host does not justify a diagnosis of the disease coccidiosis unless the history and the clinical signs are in accord. Large numbers of oocysts may be counted in the feces of perfectly healthy hosts, and surveys reveal prevalence rates on the order of 30% to 50% in a wide range of host species. On the other hand, severe and even fatal coccidiosis sometimes occurs during the early asexual stages of infection, before oocysts have had time to develop. In such cases, disease is manifest, but oocysts have not yet appeared in the feces. Chronic diarrhea, the cardinal sign of coccidiosis, results from the destruction of intestinal epithelium by hordes of multiplying organisms. Diarrhea has many causes, only one of which is coccidian infection, so the diagnosis of coccidiosis is always uncertain in individual cases. In other words, diarrhea plus oocyst shedding does not infallibly equal coccidiosis. However, as regularly recurring episodes of illness characterized by diarrhea in successive populations of calves, lambs, kids, piglets, chicks, ducklings, poults, or other domestic or wild animals,

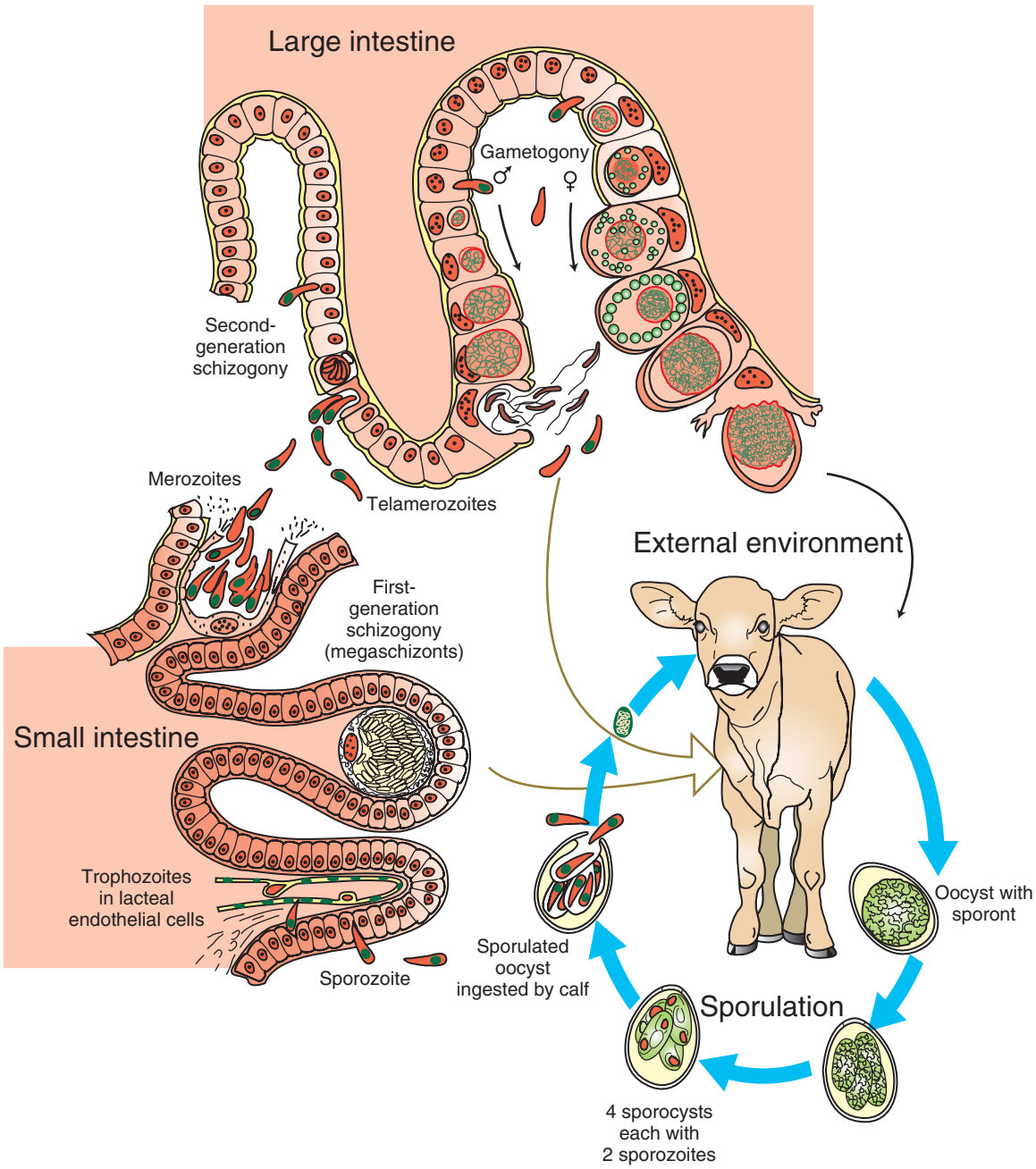

FIGURE 3-12. Life history of *Eimeria bovis*. The details of eimerian ontogeny are set forth in the text. *E. bovis* first-generation schizonts are megaschizonts that develop in central lacteal cells of the small intestine. Second-generation schizogony and gametogony occur in epithelial cells of the large intestine. Clinical signs are associated with the large intestinal phase of the infection.

outbreaks of coccidiosis become predictable events and leave the diagnostician in little doubt as to the cause. Given a closed breeding colony with reasonably steady environmental conditions, clinical coccidiosis will regularly appear in each new wave of young mammals or birds unless effective prophylactic measures have been exercised.

It is often repeated that coccidian infection is self-limiting, implying that the population of infecting organisms grows to a maximum and then more or less abruptly fades away to extinction or to a low level as the host develops immunity. Small numbers of oocysts may be shed in the feces for several weeks or even months, but infection remains otherwise inapparent. Should the now relatively immune host be exposed to a different species of coccidian, the same pattern will be repeated. Thus immunity to coccidian infection tends to be highly specific and reasonably protective but incomplete. Some animals shed oocysts while remaining healthy for months or years. Such animals have sufficient protective immunity to limit but not exclude infection in the presence of continued exposure.

The disease coccidiosis results from overwhelming infection or from the interaction of moderate levels of infection and stress. The level of environmental contamination with oocysts is best affected by removing all manure and getting all surfaces as clean as feasible. No reliable and practical disinfectant is available. Drying and direct sunlight are most effective in destroying oocysts when these agents happen to be available. Administration of anticoccidial drugs

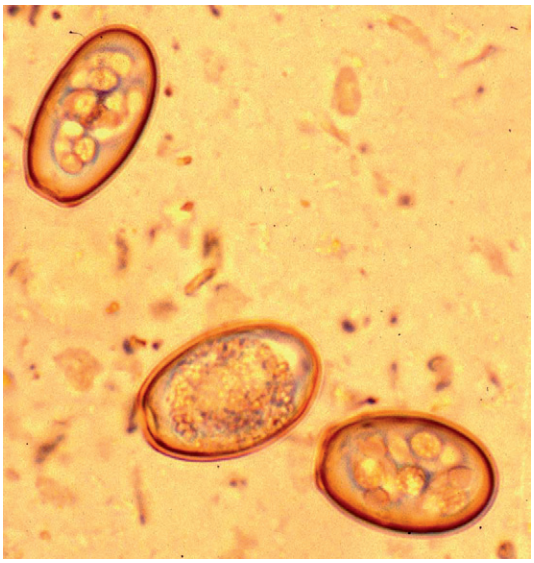

FIGURE 3-13. *Eimeria magna* oocysts, sporulated, from the feces of a domestic rabbit.

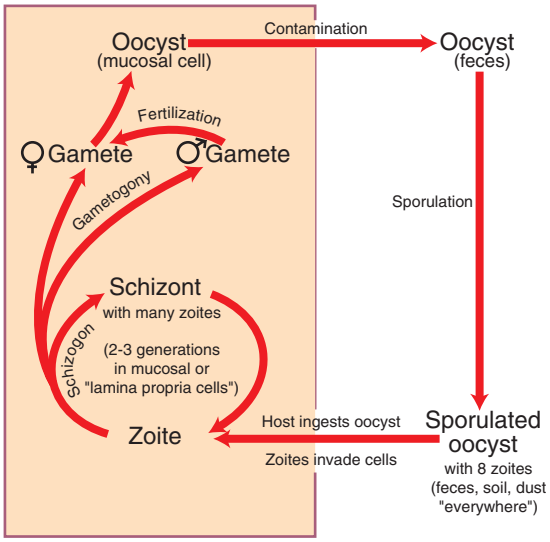

FIGURE 3-14. Life history of a typical *Eimeria* species.

(coccidiostats) during exposure of young susceptible animals permits infection to occur and immunity to develop but limits infection sufficiently to abort disease. Such drugs are virtually indispensable to intensive systems of poultry, goat, cattle, sheep, dog, and cat production.

***EIMERIA* IN CATTLE.** All calves experience infection with one or more species of *Eimeria* during their first year of life (see Figure 7-68), so finding a few oocysts in the diarrheal feces of a sick calf does not itself justify a diagnosis of coccidiosis. However, authentic outbreaks of coccidiosis do occur, especially in cattle up to 2 years of age, and these outbreaks are most often attributed to *Eimeria zuernii* or *Eimeria bovis*. Both of these species undergo two asexual cycles; the first culminates in the formation of schizonts in the lamina propria cells *(E. zuernii)* or the endothelial cells of the lacteals *(Eimeria bovis)* of the villi of the lower ileum. The megaschizonts of *E. bovis* are macroscopically visible (about 250 µm) and contain more than 100,000 merozoites. The schizonts of *E. zuernii* are unobtrusive because of their small size and deeper location. The second-generation schizonts are microscopic and occur in the epithelial cells of the cecum and colon, which are also the site of gametogony. Onset of clinical signs coincides with the beginning of gametogony and results from the mechanical disruption of mucosal cells by the sexual stages. In severe cases, so few epithelial cells remain that serum and blood are lost from the capillaries of the denuded lamina propria. The prepatent period (i.e., the time from infection until diagnostic stages appear in the feces) for *E. bovis* is 16 to 21 days; the prepatent period for *E. zuernii* is 12 to 14 days. *Eimeria alabamensis* and *Eimeria auburnensis* are occasionally incriminated in outbreaks of clinical coccidiosis (Radostits and Stockdale, 1980).

Winter coccidiosis in calves characterized by bloody diarrhea and tenesmus is a distinctive clinical entity. Severe cold weather and other stresses may precipitate clinical disease at infection levels that otherwise might not produce symptoms.

Nervous coccidiosis may affect as many as one third of the cattle in some herd outbreaks of coccidiosis, especially in beef cattle in northwestern United States and western Canada. In addition to acute diarrhea, affected animals display muscular tremors, convulsions, opisthotonus, nystagmus, blindness, and a mortality rate of about 50%. The pathogenesis of nervous coccidiosis is unknown, but more than 90% of cases occur during the coldest months of the year—from January to March. Canadian workers have reported the presence of a heat-labile toxin in the serum of calves with nervous coccidiosis that can transfer neurologic signs to inoculated mice (Isler, Bellamy, and Wobeser, 1987); of interest, no follow-up to this report has taken place in the past 25 years.

Treatment—Cattle. Clinical coccidiosis in calves caused by *E. bovis* and *E. zuernii* may be treated with amprolium (thiamine antagonist), monensin (ionophore), or sulfa drugs (e.g., sulfamethazine, sulfadimethoxine, sulfaquinoxaline). Actually, once oocysts have appeared in the feces, it is too late in the course of infection for specific chemotherapy to benefit the animal appreciably. Chemotherapy is certainly outweighed in importance by supportive therapy, especially that directed toward maintenance of fluid balance. Amprolium may be administered for 5 days in the drinking water at a concentration intended to deliver a dose of 10 mg/kg body weight per day. Usually it is better to administer medication individually to clinically ill animals because the sickest and neediest animals are the least likely to receive their share with mass treatment methods. Sulfamethazine is administered orally at a dosage rate of 140 mg/kg body weight daily for 3 days (Radostits and Stockdale, 1980). Amprolium (10 mg/kg/day for 5 days) and sulfaquinoxaline are included. Sulfaquinoxaline (6 mg/lb/day for 3 to 5 days) may also prove useful in treating calves with clinical signs of coccidiosis.

For prophylaxis, amprolium is administered to calves in the feed or drinking water for 21 days during natural exposure to oocysts at a concentration intended to deliver 5 mg/kg per day. Decoquinate is recommended as an aid in the prevention of coccidiosis caused by *E. bovis* and *E. zuernii* in ruminating calves and older cattle. Decoquinate is fed at a dosage level of 0.5 mg/kg for at least 28 days during periods when there is risk of exposure to oocysts; it is ineffective in the treatment of already established infection. Lasalocid is sold as a feed additive and is administered at 1 mg/kg daily. Horses must not be allowed to ingest feed that contains lasalocid. Monensin is sold as a feed additive for improved feed efficiency and the control of coccidiosis and is fed at the rate of 100 to 360 mg per head per day. Horses must never be allowed access to feed containing monensin because the toxic dose for this species is only about one tenth of that for cattle (Langston et al, 1985). Also, several sulfa-based products are available for coccidiosis control.

***EIMERIA* IN SHEEP AND GOATS.** At one time, sheep and goats were thought to share the same species of *Eimeria;* however, two complete sets of species names are gradually emerging to reflect the predominant opinion that sheep and goats harbor similar but distinct sets of coccidian species (see Figure 7-83 and Table 7-3). Specific diagnosis is based on morphologic identification of oocysts in sugar flotation concentrates of feces. Micrometry and sporulation of oocysts in 1% of potassium dichromate solution may be resorted to if necessary for differentiating similar species.

In sheep, clinical coccidiosis is especially likely to occur after shipping and is probably precipitated by the associated stress. In lambs experimentally infected with *Eimeria ovinoidalis*, oocysts appear in the feces about 14 days after infection, and if the infections are severe, deaths will occur, beginning about 3 weeks after infection. Goats appear to be much more susceptible to their *Eimeria* infections, and coccidiosis is a serious problem in raising kids in many goat herds. Clinical signs typically follow weaning by 2 to 3 weeks, but coccidiosis should be suspected whenever diarrhea is observed in kids older than 2 weeks. Weaker, more heavily infected kids are likely to die; the stronger and less heavily infected survive but fail to grow normally. Pasty to watery diarrhea and dehydration are typical. Bloody stools and tenesmus, frequently observed in calves with coccidiosis, are not typical of the disease in sheep and goats. Diarrhea may precede oocyst shedding by several days. In such cases of suspected prepatent coccidiosis, direct fecal smears should be examined for merozoites. Necropsy examination reveals many 3- to 6-mm irregular, whitish, raised lesions. Smears or squash preparations made from these lesions reveal *Eimeria* at various stages of development.

Treatment—Sheep. The animals most at risk are lambs at weaning in grazing pens or when placed in feedlots; it is often important to begin treatment before or immediately after animals are moved into one of these environments. Decoquinate, lasalocid, and sulfaquinoxaline are approved for control of coccidiosis in sheep. Sulfaquinoxaline is to be administered in the water for 3 to 5 days. Decoquinate is administered as for cattle at 0.5 mg/kg for at least 28 days. Lasalocid is administered to sheep in feed so that they get between 15 and 70 mg per head per day. Again, do not let horses get at feed containing lasalocid.

Treatment—Goats. Decoquinate and monensin are approved for prevention of coccidiosis in nonlactating goats. For prophylaxis, herd conditions may require that kids be medicated continuously from 2 weeks until they are several months of age. Decoquinate may be mixed with feed to supply 0.5 mg/kg per day, or it may be mixed with salt (4 lb of 6% decoquinate premix with 100 lb of salt). Monensin is fed at the rate of 20 g of monensin sodium per ton on a 90% dry matter basis. This is offered as the sole ration. Do not let horses get into feed containing monensin. Amprolium is not approved for administration to goat kids in the United States. Experimentally, amprolium may be administered to goat kids with coccidiosis at a considerably higher dose rate than is recommended for calves (25 to 55 mg/kg of body weight). Overdose with amprolium may lead to fatal polioencephalomalacia from thiamine deficiency. Sulfa drugs may be used for treating coccidiosis only in sufficiently hydrated kids because these drugs damage the kidneys if insufficient water is available to keep them in solution.

Older goats, while remaining free of clinical signs, may shed oocysts for extended periods and serve as the ultimate source of infection for kids. In problem herds, it may prove necessary to isolate kids at birth from their dams, feed them artificially, and include a coccidiostat in the starter ration for several months. In more favorable situations, it may suffice to provide a clean, disinfected stall and wash the doe's udders carefully before kids are allowed to nurse. Stress or exposure to a previously unencountered species of *Eimeria* may lead to temporary bouts of diarrhea in adult goats. Much more information and perspectives on goat coccidiosis other than those presented here can be found in the text by Smith and Sherman (2009).

***EIMERIA* IN LLAMAS.** Llamas and alpacas are host to a number of species of *Eimeria*, and they can develop coccidiosis, especially as crias. Species include *Eimeria alpacae*, *Eimeria ivitaensis*, *Eimeria lamae*, *Eimeria macusaniensis*, *Eimeria punoensis*, and *Eimeria peruviana*. The species that is considered of significance is *E. macusaniensis* (see Figure 7-22), which can cause severe disease in animals on its own and, perhaps through severe necrotizing or hemorrhagic enteritis, may predispose animals to overgrowth of *Clostridium perfringens* with associated enterotoxemia (Cebra et al, 2007; Johnson, Stewart, and Perkins et al, 2009; Rosadio et al, 2010).

Treatment—Llamas. Treatment in llamas has included amprolium hydrochloride (10 mg/kg every day for up to 15 days) or sulfadimethoxine (110 mg/kg every 24 hours for up to 10 days); on occasion, treatment has included anti-inflammatory drugs. For oocysts of *E. macusaniensis* that were 3.5 to 7 years old, originally from a guanaco, success in infecting both llamas and an alpaca is achieved with prepatent and patent periods of 30 to 40 days in alpacas and 14 to 55 days in llamas; in the alpaca, the prepatent period was longer, and the alpaca was patent for only 1 day (Jarvinen, 2008). Based on the timing of disease appearance, it would appear that moving the animals onto new pasture that had been contaminated with oocysts and left vacant for some time puts them at great risk of disease development during the prepatent portion of the life cycle.

GENERAL TREATMENT AND CONTROL OF EIMERIOSIS IN RUMINANTS. Treatment of isolated cases of fully developed coccidiosis consists of supportive therapy because by the time oocysts are detected in the feces, no available drug will have much effect on the population of coccidians infecting that particular host. Controlling coccidiosis in populations of susceptible animals is a challenging proposition, and heavy reliance is placed on chemicals administered prophylactically. The objective of anticoccidian prophylaxis is to afford sufficient protection to the exposed animal to allow it to develop immunity without getting sick in the process. The chemicals reduce the magnitude of the challenge and thereby prevent coccidiosis; they do not prevent the infection. However, the chemicals cannot be expected to perform miracles. Too much contamination of the environment with oocysts and, even more important, too much stress placed on the hosts are conditions that cannot be overcome by the best of chemicals.

Whatever chemical agent is chosen, efficient control of coccidiosis requires that exposure of ruminants to oocysts and stressful conditions be minimized. Adequate stall space, clean mangers, plenty of clean air, and dry footing are as essential as they are rare. Never mix calves, sheep, or goats of different ages or sizes in the same pen. As a matter of regular routine, all animals should be observed attentively for several minutes a day at a time when neither the stockman nor his stock has other urgent business. If any sick animals are observed, they should be removed to a separate pen for supportive treatment. This measure is doubly beneficial: it reduces exposure of the sick to unnecessary stress and of the healthy to extra oocysts. As soon as coccidiosis has been diagnosed in one or a few of the animals, all of the other young ruminants on the premises should be treated prophylactically with an anticoccidial agent. Coccidial infection (coccidiasis) is inevitable. Coccidial disease (coccidiosis) can be prevented or at least ameliorated by sound husbandry and appropriate medication.

***EIMERIA* IN HORSES.** *Eimeria leuckarti* is the only species of enteric coccidian reported from North American horses (see Figure 8-25). A survey of naturally acquired *E. leuckarti* infection conducted on 13 Kentucky breeding farms revealed *E. leuckarti* in 67 (41%) of the foals on 11 of those farms. Oocysts, demonstrated by flotation in concentrated sucrose (SG 1.275), were first shed in the feces when the foals were 15 to 123 days old and continued to be shed sporadically for as long as 4 months (Lyons, Drudge, and Tolliver, 1988); a similar survey of three farms in Kentucky in 2004 revealed oocysts in 36%, 41%, and 85% of the 79 foals examined on the farms (Lyons et al, 2007).

Oral administration of 50,000 to 2 million oocysts to yearling ponies led to patent infections in 33 to 37 days, and oocyst shedding continued for as long as 12 days. Schizonts were not observed; gametocytes developed in lamina propria cells of the villi in the small intestine. No clinical signs of disease were observed in these artificially infected ponies (Barker and Remmler, 1972). *E. leuckarti* infection therefore appears to be prevalent, at least in foals in Kentucky, but relatively harmless.

Treatment—Horses. *E. leuckarti* appears to be nonpathogenic, which is fine because no treatment is available for this infection.

***EIMERIA* IN PIGS.** Pigs are host to eight or more *Eimeria* species (see Figure 7-97). The *Eimeria* of pigs are considered to be fairly inconsequential as parasites in most instances; however, there have been cases both in natural infections and following experimental inoculation in which pigs have developed diarrhea as the result of *Eimeria* infections. The parasite of pigs that is considered of consequence is *Cystoisospora suis* (discussed later), which causes disease in neonatal pigs for which treatment is often provided.

***EIMERIA* IN RABBITS.** Rabbits are host to a number of *Eimeria* species (see Figure 104), and one, *Eimeria stiedae*, can be highly pathogenic. This is a relatively unusual *Eimeria* species in that it often will be found in the epithelium of the biliary system, it can cause marked hypertrophy of the epithelium and significant pathogenic changes on the surface of the liver as large white foci that may be visible on necropsy, and it often is fatal.

Treatment—Rabbits. In laboratory settings, rabbits are being treated with toltrazuril or ponazuril. Similarly, some pet rabbits are being treated with ponazuril. It must be remembered that the rabbit still is considered a food animal by many people in the United States; therefore, when treatment is administered, there *must* be concern as to the ultimate fate of the animal being treated.

***EIMERIA* IN POULTRY.** The subject of coccidiosis in domestic poultry forms too large and complex a body of information to be accommodated on these pages. The reader is referred to standard texts on avian diseases.

***KLOSSIELLA*.** *Klossiella* is a genus of the apicomplexan parasites that appears to have a direct life cycle similar to *Eimeria* but that occurs in renal epithelia. *Klossiella equi* is a parasite of the renal epithelium of the horse, and *Klossiella muris* is a parasite of the renal epithelium of the mouse (see Figure 8-30). The life histories of these parasites have yet to be worked out in detail; neither species appears to be pathogenic under ordinary circumstances. However, tubular necrosis and nonsuppurative interstitial nephritis in an older, immunocompromised pony have been reported (Anderson et al, 1988). Reinemeyer, Jacobs, and Spurlock (1983) were the first to demonstrate the sporocysts of *K. equi*; these were observed in the urine of a 2-year-old standardbred gelding with immunodeficiency. These transmission stages, like the pathologic changes reported by Anderson et al (1988), are rarely observed.

***CYSTOISOSPORA*.** The common canine and feline coccidia were once in the genus *Isospora*, but after a taxonomic revision, the coccidial species affecting dogs and cats are in *Cystoisospora* (Barta et al, 2005). Members of *Cystoisospora* are more like other genera found in carnivores (e.g., *Hammondia*, *Toxoplasma*, *Besnoitia*, *Sarcocystis*) in that the oocysts where sporulated contain two sporocysts, each with four sporozoites, and they have a similar ability to utilize prey hosts to aid in making their way back into a carnivore. Dogs, cats, and pigs can be infected by and can develop coccidiosis from ingestion of the oocysts of their respective species of *Cystoisospora*, but the sporozoites coming out of these oocysts can encyst (singly without any multiplication) in the tissues of rodents and birds. Thus, dogs, cats, and pigs can become infected with *Cystoisospora* through ingestion of paratenic hosts.

Fecal samples of dogs and cats often contain various pseudoparasites that arise from the various gustatory habits of their hosts. A dog with a history of recurrent diarrhea and oocyst shedding seems like an "open-and-shut case" until the oocysts turn out to belong to an *Eimeria* parasite of a squirrel. In this case, it may be that dietary indiscretion, not protozoan infection, is to blame for the diarrhea. In fact, almost all puppies and kittens experience infection with *Cystoisospora* at some time during the early months of their lives. Puppies and kittens raised in elaborately controlled pathogen-free colonies are always at risk of getting infected, even when very stringent levels of sanitation are practiced.

***CYSTOISOSPORA* IN DOGS.** In dogs, the common species causing coccidiosis are *Cystoisospora canis*, *Cystoisospora ohioensis*, and *Cystoisospora burrowsi;* the species with the largest oocyst is *C. canis* (see Figure 7-24). The prepatent period of *C. ohioensis* is 6 to 7 days, and for *C. canis*, it is 10 to 12 days; the patent period for *C. ohioensis* is only 2 to 7 days, while for *C. canis* it is 10 to 11 days (Buehl et al, 2006; Mitchell et al, 2007). When dogs are fed the spleen or liver of chickens given oocysts of *C. ohioensis*, the prepatent period occurs slightly later at 10 days post ingestion (Massad et al, 2009). In a large study of fecal samples submitted for diagnostic work in Vienna, Austria, it was found that dogs shedding oocysts had a greater chance of having diarrhea, both hemorrhagic and nonhemorrhagic, than dogs without parasites or dogs with parasites other than *Cystoisospora* (Buehl et al, 2006). Clinical signs may precede oocyst shedding in particularly acute infections with *C. canis* (Mitchell et al, 2007). Diarrhea is copious and watery and may persist for several weeks. Response to treatment is seldom dramatic.

***CYSTOISOSPORA* IN CATS.** In cats, the two species that occur are *Cystoisospora felis* and *Cystoisospora rivolta;* the species with the larger oocyst is that of *C. felis* (Figure 3-15), which tends to be more ovoid than spheroid like other *Cystoisospora* oocysts. The life cycle of feline *Cystoisospora* can be direct or indirect (Figure 3-16). When oocysts are ingested, the prepatent periods are 4 to 7 days for *C. rivolta* and 7 to 11 days for *C. felis* (Petry et al, 2011).

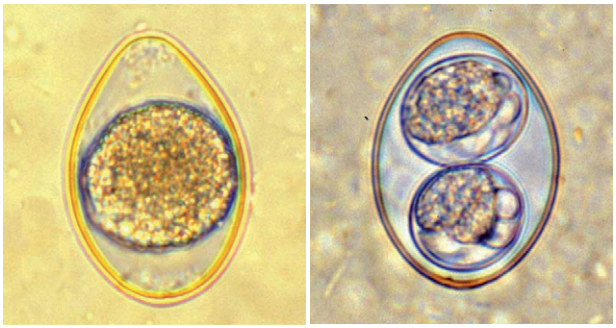

FIGURE 3-15. *Cystoisospora felis* unsporulated oocyst *(left)* and sporulated oocyst *(right)*.

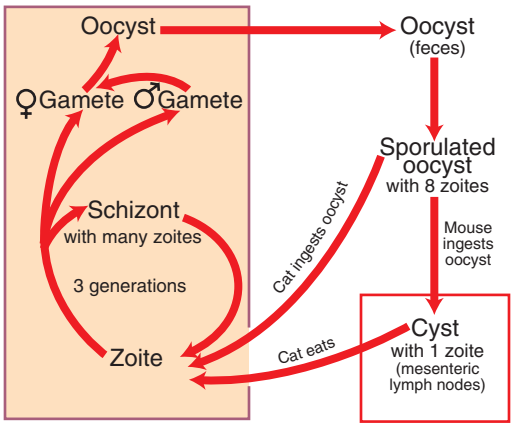

FIGURE 3-16. Life history of *Cystoisospora felis*.

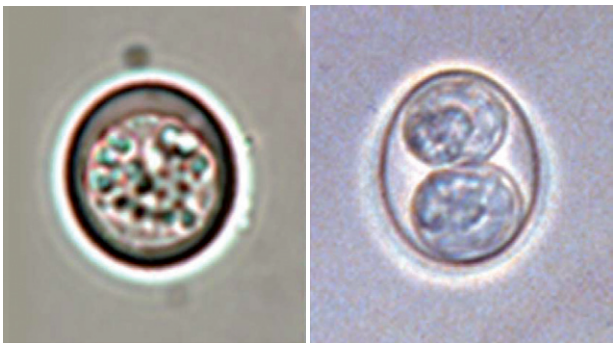

FIGURE 3-17. Oocysts of *Toxoplasma gondii*, unsporulated *(left)* and sporulated *(right)*.

The patent periods are longer than 2 weeks for *C. rivolta* and 10 to 11 days for *C. felis*. When cystozoites from infected mice are fed, the prepatent period is quicker in *C. felis* but similar in the case of *C. rivolta* (Frenkel and Dubey, 1972a). *C. rivolta* is pathogenic for newborn but not weaned kittens (Dubey, 1979); *C. felis* is pathogenic for both young and older kittens (Andrews, 1926; Shaw, 1971). Clinical signs range from neutrophilic infiltration and hypersecretion to enteritis, emaciation, and death depending on the dose of oocysts consumed.

Treatment of Cystoisosporosis in Dogs and Cats. Coccidiosis outbreaks in dogs and cats involving *Cystoisospora* species can be controlled with sulfonamide drugs. Sulfadimethoxine is administered to dogs for the treatment of coccidian enteritis according to the following schedule: 55 mg/kg for the first day and 27.5 mg/kg for the next 4 days or until the dog is symptom free for at least 2 days. In Europe, approval has been given for a suspension of 18 mg/mL of toltrazuril for coccidiosis treatment and 0.9 mg/mL of emodepside for treatment of roundworms in puppies. The dosage is 0.5 mL per kg body weight orally. This product has been found efficacious in reducing oocyst shedding in both puppies and kittens (Altreuther et al, 2011; Petry et al, 2011). Ponazuril has been used in the United States at 20 mg per kilogram daily for 1 to 3 days; it must be remembered that in the United States, this is an off-label use of this product.

***CYSTOISOSPORA* AND *EIMERIA* IN PIGS.** Pigs are host to eight species of *Eimeria* and one of *Cystoisospora*, of which only the latter appears to be of significant clinical importance (Vetterling, 1965). *Cystoisospora suis* causes neonatal coccidiosis in 1- to 2-week-old pigs (Lindsay, Current, and Taylor, 1985). Clinical signs include diarrhea, dehydration, and weight loss; morbidity tends to be high, and mortality low or moderate. Susceptibility to infection falls rapidly with age. Although 400,000 oocysts of *C. suis* may kill a 1-day-old pig, only mild and transient diarrhea ensues if infection is postponed until the pig reaches 2 weeks of age. The prepatent period is 5 days, and oocyst shedding lasts 1 to 3 weeks. Pigs surviving infection with *Cystoisospora suis* are solidly immune to reinfection with this species. Porcine neonatal coccidiosis is to be differentiated from enteritides associated with *Strongyloides ransomi*, toxigenic *Escherichia coli*, transmissible gastroenteritis virus, rotavirus, and *Clostridium perfringens* type C. *Cystoisospora suis* infection is rarely observed in adult swine (Lindsay, Blagburn, and Powe, 1992; Stuart et al, 1980). It has been shown that infection with *C. suis* is common among pig farms in Ontario, Canada, with oocysts being detected on 70% of farms examined; that litters of pigs in which *C. suis* was detected were more likely to have diarrhea than uninfected pigs; and that pigs from infected litters were on the average 1.4 kilograms lighter than pigs from uninfected litters (Aliaga-Leyton et al, 2011a and 2011b).

Treatment. Medication of piglets with neonatal coccidiosis appears to be futile. Rigorous sanitation probably represents the most effective investment. "The following sanitation program has been recommended: steam clean farrowing crates; wet down the crates with an ammonia-orphenol-containing disinfectant and let them stand overnight; and steam clean the following day" (Stuart and Lindsay, 1986). Work in Canada showed that farms that used a detergent when cleaning farrowing crates were 10 times less likely to be positive for *C. suis* than farms that did not (Aliaga-Leyton, 2011a). In Europe today and until 2005 in Canada, Baycox (toltrazuril) from Bayer HealthCare was approved for the treatment of coccidiosis in piglets. Use in Canada was discontinued at the request of Health Canada based on the concern that Canadian scientists could not rule out the possibility that it might have health effects on consumers. In Europe, Baycox is also approved for the treatment of chickens and cattle with coccidiosis.

TOXOPLASMA

LIFE HISTORY. *Toxoplasma gondii* is an enteric coccidian of the domestic cat *(Felis catus)* and other members of the family Felidae. Domestic cats and other members of the carnivore family Felidae are the only known definitive hosts of *T. gondii* (hosts in which microgametes and macrogametes are formed); therefore, only felids shed oocysts of this parasite in their feces (see Figures 7-54 and 8-34). The domestic cat is considered the common final host in most of the world. The oocyst is small (11 to 13 µm; Figure 3-17), contains a single sporont, and is noninfective when passed in the feces. Sporulation is completed in 1 to 5 days and results in formation of two sporocysts, each of which contains four sporozoites. Fully sporulated oocysts are infective on ingestion to essentially all warm-blooded animals, including cats (Figure 3-18). Therefore almost any warm-blooded animal may serve as a paratenic host of *T. gondii* (Dubey, 2010). A paratenic host is a host in which a parasite may grow or multiply, but growth or development is not required for the parasite to complete its life cycle.

On ingestion, sporulated oocysts rupture in the intestine and release the sporozoites. These enter and multiply in cells of the intestine and associated lymph nodes to form rapidly multiplying stages, **tachyzoites** (see Figure 3-9), which spread to all other tissues of the body; there they invade cells and continue to multiply (Figure 3-19). Eventually, tissue cysts containing slowly dividing forms, **bradyzoites,** are formed in the brain, striated muscles, and liver and remain viable for the life of the host (Figure 3-20; and see Figure 8-35). Bradyzoites are infective on ingestion to essentially all warm-blooded animals and behave in a manner similar to

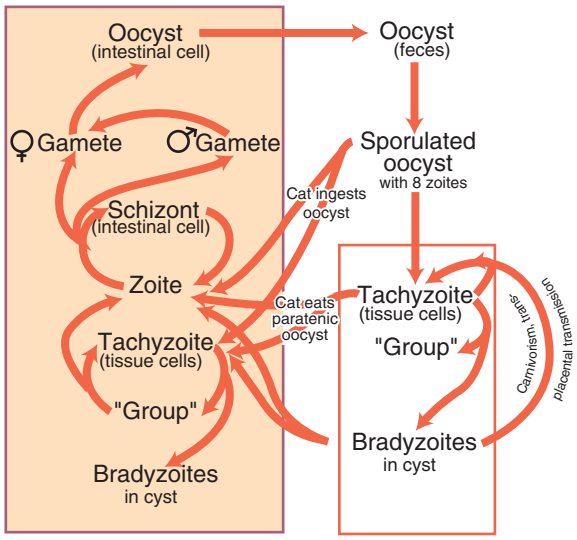

FIGURE 3-18. Life history of *Toxoplasma gondii*.

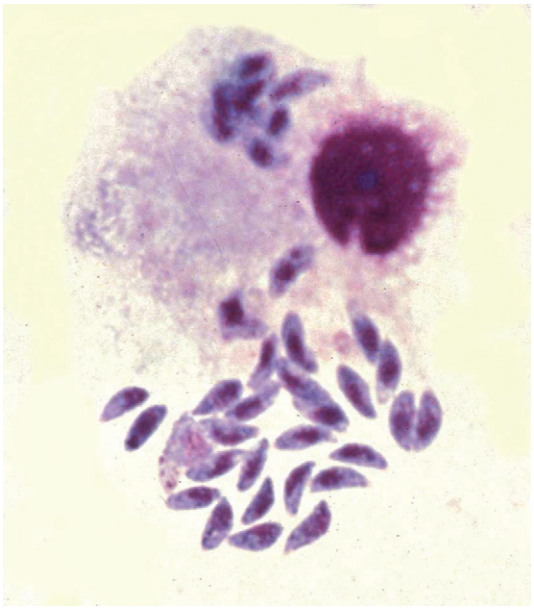

FIGURE 3-19. Tachyzoites of *Toxoplasma gondii* and the pulmonary macrophage of a naturally infected cat (Giemsa stain).

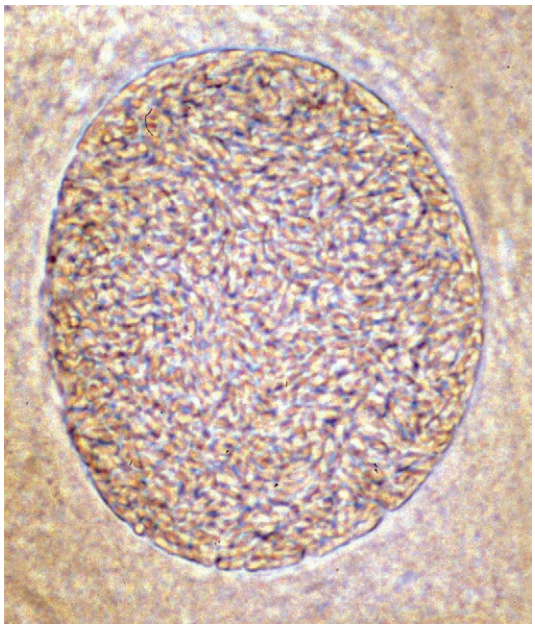

FIGURE 3-20. *Toxoplasma gondii* cyst in a mouse's brain. This fresh temporary mount is prepared by simply squashing the brain tissue between the coverslip and the slide.

that just described for sporozoites. Historically, bradyzoites were differentiated from tachyzoites by the fact that they stained with the periodic acid–Schiff reagent, indicating that they contain glycogen, and were able to withstand pepsin digestion fluids. Thus, paratenic hosts become infected with *T. gondii* by ingesting sporulated oocysts from cat feces or by ingesting bradyzoites in the tissues of other paratenic hosts. Transplacental transmission of tachyzoites from dam to fetus in utero also occurs but varies in importance depending on the species of host involved (Dubey, 2010). Tachyzoites and bradyzoites divide by a process called **endodyogeny** that is distinct from schizogony in that each tachyzoite or bradyzoite produces only two daughter cells.

When a member of the cat family ingests tissue cysts of *T. gondii* (see Figures 3-18 and 3-20), the bradyzoites penetrate the epithelial cells of the small intestine, undergo a series of asexual cycles, including both schizogonous and endodyogonous cycles, and finally undergo the sexual cycle, which culminates in the shedding of oocysts. Cats shed *Toxoplasma* oocysts in their feces 3 to 10 days after eating mice infected with encysted bradyzoites but not until 19 to 48 days after ingesting sporulated oocysts (Dubey and Frenkel, 1976). Apparently, the asexual reproduction preceding formation of bradyzoites in the paratenic host satisfies a major portion of the developmental requirements preceding sexual reproduction. Cats may also serve as paratenic hosts inasmuch as multiplication of tachyzoites and cyst formation occur in their extraintestinal tissues; cats are also capable of developing systemic disease (Dubey and Prowell, 2013).

IMPORTANCE. It is the explosive multiplication of tachyzoites of *T. gondii* and their destruction of host cells that causes the majority of disease associated with this parasite. Cats, like other animals, can develop severe toxoplasmosis (Meier, Holzworth, and Griffths, 1957). Reports of fatal cases describe cats that have presented initially with fever, anorexia, vomiting, and diarrhea (Spycher et al, 2011). Cats that present with toxoplasmosis may survive if administered appropriate treatment (Dubey and Prowell, 2013).

In any host, if the tachyzoites make their way through the placenta and into the fetus, the effects can be devastating. Abortion in sheep and goats due to congenital toxoplasmosis is well known and of considerable significance to farmers and veterinarians around the world (Dubey, 2010). In the case of sheep, abortions are still considered by most to be due to the acquisition of an infection during midpregnancy at 60 to 90 days after fertilization. Infection of ewes in the last month of pregnancy is liable to produce only minimal signs in infected lambs. There is a vaccine approved for sheep in Europe and New Zealand (see Chapter 9). It is also believed that most ewes will not abort during a second pregnancy. Goat does that are pregnant are very likely to abort if infected during pregnancy, and it is believed that goats may abort as the result of toxoplasmosis more than once. Goats vaccinated with the commercial Toxovac S48 live vaccine that was developed for the prevention of abortion in sheep have been shown to be protected against abortion (Chartier and Mallereau, 2001). A mouse bioassay of heart tissue from 112 meat goats purchased from a retail store near Beltsville, Maryland, revealed that 29 contained viable *T. gondii* (Dubey, Rajendran, Ferreira, et al, 2011).

In humans, upon first exposure to *T. gondii*, an adult with an intact immune system will typically suffer a brief if unpleasant illness marked by variable combinations of fever, myalgia, lymphadenopathy, anorexia, and sore throat that is probably rarely diagnosed as to exact cause. The situation is far graver for those with deficient immune responses, such as fetuses, neonates, older adults, and those with congenital or acquired immunodeficiency disease. The greatest concern attaches to exposure of human fetuses to the hazard of death, congenital malformation, or mental retardation that may result from exposure of the nonimmune mother to *T. gondii* infection during pregnancy. Although women with circulating antibody to *T. gondii* need not worry about exposing their unborn babies to congenital toxoplasmosis, such women account for only about 10% to 15% of the population at risk. The other 85% to 90% must be careful to avoid cat feces and uncooked meat during pregnancy (Jones et al, 2001).

Adult cattle appear to be resistant to toxoplasmosis (Dubey, 2010), and a survey by the U.S. Department of Agriculture (USDA) of meat from supermarkets in the United States (2094 samples each of commercially raised beef, chicken, and pork from 698 retail meat stores) revealed no toxoplasmosis in the beef when pools (6 meat samples per pool) were fed to cats (Dubey et al, 2005a). No reports have described clinical toxoplasmosis in adult cattle, and *T. gondii* organisms have been isolated from aborted fetuses on only two occasions (Dubey, 2010).

T. gondii infection is highly prevalent in pigs, and uncooked pork may be an important source of human infection (Dubey, 2010). However, commercial pork in the United States, probably as a result of pigs being raised indoors and flash-frozen as part of processing, contains very little in the way of toxoplasmosis. Of 2094 samples of purchased commercial meat from the United States, only seven pooled samples (six samples per pool) produced oocysts when fed to cats (Dubey, 1986a). *T. gondii* was isolated from the hearts of 17 of 33 pigs from two organic farms in the state of Michigan (Dubey et al, 2012).

Reports in France have described people contracting toxoplasmosis from ingestion of raw horsemeat that probably originated in Canada or Brazil (Pomares et al, 2011).

The USDA survey of chicken meat in 2094 samples from supermarkets in the United States revealed no toxoplasmosis in the commercial chicken samples (Dubey et al, 2005a). However, backyard and free-range chickens are very commonly infected with *T. gondii* (Dubey, 2010).

Humans may contract toxoplasmosis by ingesting sporulated oocysts from the feces of an infected cat or by eating uncooked meat of animals containing *T. gondii* cysts. "Pregnant women should eat only adequately cooked meat and either leave the cleaning of cat litter pans to someone else or wear disposable gloves" (Frenkel and Dubey, 1972b). They are also well advised to wash lettuce and other fresh vegetables carefully; avoid contact with newborn lambs, kids, and fetal membranes; and shun unpasteurized goat's milk. Meats that probably pose the greatest risk of infection are undercooked mutton, goat, range-fed pork, and range-fed chicken.

TREATMENT. A cat shedding oocysts of *T. gondii* should be hospitalized to prevent exposure of its owner until it stops shedding oocysts, usually within 2 weeks. Reinfection, if it occurs, results in low-grade shedding of oocysts of short duration. Intercurrent *Cystoisospora* infection may also trigger a brief output of *T. gondii* oocysts. However, in general, having once passed through a patent *T. gondii* infection, the particular cat remains a relatively minor source of infection. Thus the cat that has a history of shedding *T. gondii* oocysts and/or is serologically positive is probably a safer pet than the cat that has never been exposed to this organism (Dubey, 1986b).

Cats clinically ill with toxoplasmosis can be treated with clindamycin hydrochloride. The drug should be given orally with food. Start at 25 mg/kg twice daily and work up to 50 mg/kg twice daily. If the cat goes off its feed, withhold the drug for 24 hours and then start the clindamycin again at the level of 25 mg/kg. Cats should be treated for a minimum of 2 weeks. Cats can also be treated with clindamycin phosphate intramuscularly at 12.5 to 25 mg/kg twice daily, pyrimethamine orally at 0.25 to 0.5 mg/kg with 30 mg/kg of sulfonamide per kilogram given twice daily, or trimethoprim and sulfadiazine orally at 15 mg/kg twice daily, all for 4 weeks (Lindsay et al, 1997). Pyrimethamine causes megaloblastic anemia or leukopenia, and therapy should be discontinued if no response is seen in 30 days. Based on the efficacy of treatment of toxoplasmosis in mice with ponazuril (Mitchell et al, 2004), this product may offer a means of reducing the shedding of oocysts in the feces of cats.

NEOSPORA. *Neospora caninum* is a coccidian with a life cycle that is similar to that of *T. gondii*, but instead of cats, *N. caninum* uses dogs and other canids as its final host and cattle as an intermediate host (Dubey and Schares, 2011). It was originally described as a parasite of the neurologic tissues of the domestic dog (Dubey et al, 1988), and then it was found to also be a cause of abortion in cattle (Anderson et al, 1991). In 1998, it was demonstrated the *N. caninum* of cattle uses dogs as definitive hosts (McAllister et al, 1998), producing oocysts that are shed in the feces after ingestion of tissue cysts from beef. Oocyst shedding by dogs was confirmed (Lindsay, Dubey, and Duncan, 1999), but it has been hard to get dogs to produce large numbers of oocysts on a regular basis. Coyotes, *Canis latrans*, have been shown capable of producing oocysts of *N. caninum* when fed meat of the white-tailed deer, *Odocoileus virginiana*, suggesting a potential sylvatic cycle in the United States (Rosypal and Lindsay, 2005). Similarly, pigeons have been experimentally infected with *N. caninum* and are considered as possible intermediate hosts in the wild (Mineo et al, 2009). The gray wolf, *Canis lupus*, has also been found to be shedding oocysts of *N. caninum* in the wild (Dubey et al, 2011a).

No cases of neosporosis have been reported from humans; therefore *N. caninum* is not considered to be a zoonotic agent.

NEOSPOROSIS IN DOGS. In the dog, the disease has often presented as littermate pups dying of signs related to polyradiculitis (Bjerkås, Mohn, and Presthus, 1984; Core, Hoff, and Milton, 1983). Puppies usually are born without signs, and they typically begin to show signs progressing toward paralysis of the rear limbs beginning 3 weeks after birth (Dubey and Schares, 2011). The cysts in neural tissues (Figure 3-21) are characterized by the possession of a cyst wall that is thicker than that of *T. gondii*. In these transplacentally infected puppies, the typical presentation is of a flaccid hindlimb paresis, sometimes with contracture. In cases of adult onset of the disease, presentation includes neurologic signs, nodular dermatitis, pneumonia, urine and fecal incontinence, hepatitis, myocarditis, and myositis.

Diagnosis and Treatment of Neosporosis in Dogs. Diagnosis is often made quite readily from the classical presentation of flaccid posterior limb paralysis. This can be combined with serology, molecular methods, and demonstration of the organisms in biopsies or at the necropsy of littermates that have succumbed to the infection (see Figure 8-36). Sometimes, these puppies will be infected with both *T. gondii* and *N. caninum*. No drugs that will kill the *N. caninum* tissue cysts have been identified (Dubey and Schares, 2011).

NEOSPOROSIS IN CATTLE. *N. caninum* is a major cause of bovine abortion among dairy cows around the world (Anderson et al, 1991; Barr et al, 1997; Dubey and Schares, 2011). Abortions

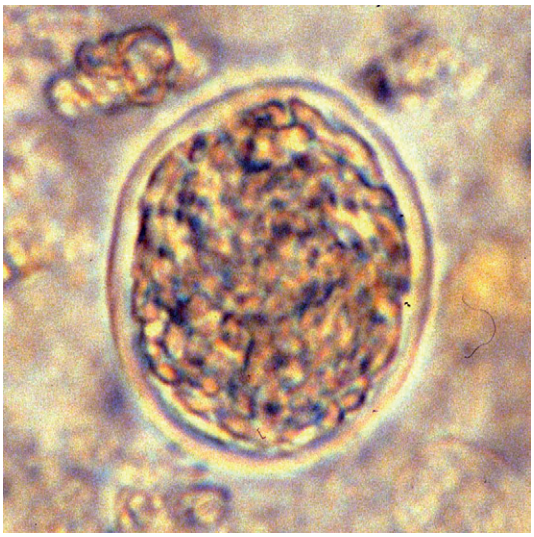

FIGURE 3-21. *Neospora caninum* cyst from a homogenate of the brain of a naturally infected dog. Note the thickness of the cyst wall compared with that of *Toxoplasma gondii*.

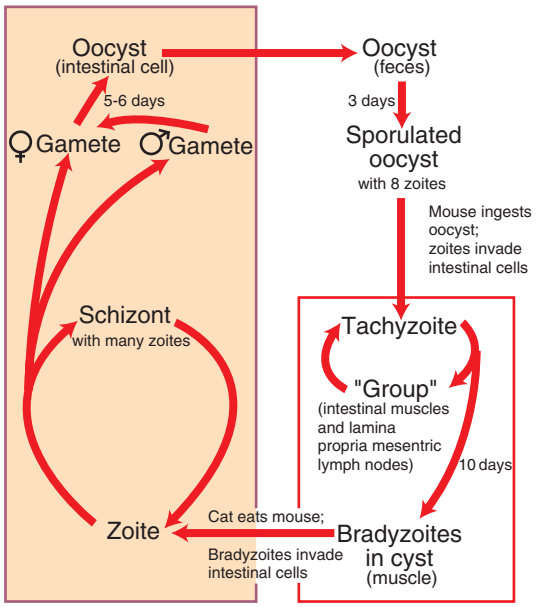

FIGURE 3-22. Life history of *Hammondia hammondi*.

due to this parasite are common, and between 10% and 20% of abortions in dairy cows probably are caused by *N. caninum*. *Neospora* abortions tend to peak at midgestation, and calves infected in utero after this time tend to survive. Abortions may occur in subsequent pregnancies, but more typically, future births produce calves that are congenitally infected. It seems that serologically positive calves will ultimately give birth to calves that are infected and seropositive. It has been suggested that seropositive cows produce less milk than seronegative cows and are more likely to be culled earlier (Thurmond and Hietala, 1996 and 1997; Tiwari et al, 2007); however, the effects on milk production remain unresolved (Dubey and Schares, 2011).

Diagnosis and Treatment of Neosporosis in Cattle. Several diagnostic assays, immunologic and molecular, are now available for the detection of *N. caninum* infection. Also available for cattle are tests for antibodies in bulk milk. However, diagnosis of the presence of antibodies to *N. caninum*, the presence of DNA, or even the presence of organisms in aborted tissues does not necessarily mean that the infection was the cause of the abortion. In California and the Netherlands, it appears that in cases where all other causes of abortion were ruled out, only about 20% of all abortions were *N. caninum* associated (Dubey and Schares, 2011). Treatment of lactating dairy cows is especially problematic, and no drug therapy is currently available.

NEOSPOROSIS IN HORSES. It seems that horses are infected with a different species than *N. caninum*, but it is difficult to know with certainty how many horses might have acquired infection with *N. caninum* because of cross-reacting serology (Dubey and Schares, 2011). *Neospora hughesi* was described in 1998 on the basis of material collected from a horse (Marsh et al, 1998). The differentiation was based on molecular differences among equine, bovine, and canine isolates. Differences between *N. hughesi* and the bovine and canine isolates were later confirmed with material collected from a horse in Oregon (Dubey et al, 2001a). In cases where viable organisms have been isolated from horses, all have been identified as *N. hughesi* (Dubey and Schares, 2011).

HAMMONDIA. The oocysts of species of *Hammondia* are morphologically virtually indistinguishable from one another and from those of *Toxoplasma* and *Neospora*. *Hammondia hammondi* is a parasite of the cat that, unlike *Cystoisospora felis*, multiplies in the tissues of an intermediate host such as pigs, rats, mice, goats, hamsters, and dogs. *Hammondia heydorni* is a similar parasite that uses dogs and coyotes as the final host, and cattle, sheep, goats, camels, water buffalo, guinea pigs, and dogs as intermediate hosts. The form that produces oocysts in the feces of foxes has been recognized as a new species, *Hammondia triffittae*, which seems to use similar intermediate hosts as are used by *H. heydorni* (Gjerde and Dahlgren, 2011). In the intermediate hosts, the zoites first multiply rapidly (tachyzoites); they then form cysts in which they multiply slowly (bradyzoites). The net result is the multiplication and storage of zoites in cysts in the tissues of an animal that is likely to fall prey to a cat or dog final host. As indicated in Figure 3-22 for *H. hammondi*, only sporulated oocysts from cat feces are infectious for mice, and only bradyzoites from mouse tissues are infectious for cats. Thus *H. hammondi* has an obligatory two-host life history. Tachyzoites are neither infectious to cats nor transmissible to the progeny of pregnant female mice via the placenta, as is true of *T. gondii*. This obligatory heteroxenous life cycle appears to be typical of all members of the genus *Hammondia*.

SARCOCYSTIS. Species of *Sarcocystis*, like *H. hammondi*, have an obligatory two-host life history but differ in that only sexual reproduction occurs in the definitive host, and that sporogony is completed there. Fully sporulated oocysts and sporocysts are discharged in the host's feces, and no development occurs in the external environment. Asexual reproduction, including schizogony and sarcocyst formation, occurs only in the intermediate host. The bradyzoites in sarcocysts differ from those in *Hammondia* cysts in that they develop into gametocytes instead of schizonts when ingested by the definitive host. Bradyzoites represent a state of arrested development, or hypobiosis. Like sporozoites in a sporulated oocyst, bradyzoites in a sarcocyst must enter a definitive host to develop further. The life history of *Sarcocystis* is portrayed diagrammatically in Figure 3-23.

The host relationships of several species of *Sarcocystis* are summarized in Table 3-1. Normally the carnivorous host becomes infected by eating the infected flesh of the herbivorous host, and the herbivorous host becomes infected by ingesting sporocysts from the feces of the carnivorous host (see Figure 7-54). Schizogony and encystment occur exclusively in the herbivorous host, and gametogony, fertilization, and sporulation occur exclusively in

TABLE 3-1 Host Relationships of Some Species of *Sarcocystis*

	DEFINITIVE HOSTS		
Intermediate Hosts	**Dog**	**Cat**	**Human**
Cattle	*Sarcocystis cruzi*	*Sarcocystis hirsuta*	*Sarcocystis hominis*
Sheep	*Sarcocystis tenella* *Sarcocystis arieticanis*	*Sarcocystis gigantea*	*Sarcocystis medusiformis*
Goat	*Sarcocystis capracanis* *Sarcocysts hircicanis*	*Sarcocystis moulei*	—
Swine	*Sarcocystis miescheriana*	*Sarcocystis porcifelis*	*Sarcocystis suihominis*
Horse	*Sarcocystis bertrami* *Sarcocystis equicanis*	—	—
Cottontail rabbit	*Sarcocystis leporum*	—	—
Mouse	—	*Sarcocystis muris*	—
Mule deer	*Sarcocystis hemionilatrantis*	—	—
White-tailed deer	*Sarcocystis ococoileocanis*	*Sarcocystis odoi*	—

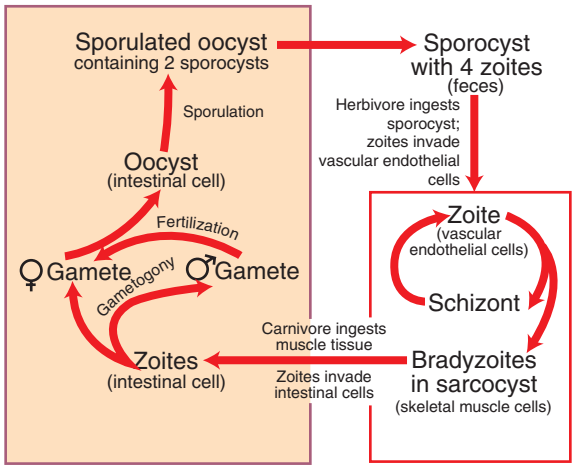

FIGURE 3-23. Life history of *Sarcocystis* species.

the carnivorous host. *Sarcocystis* usually causes no illness in the carnivore, but schizogony in the endothelium of the herbivore may result in serious or fatal disease.

Cattle become infected with *Sarcocystis cruzi* when they ingest its sporocysts discharged in dog feces. Two schizogonic generations occur in the vascular endothelium, the first generation principally in the endothelium of the mesenteric arteries and the second in the endothelium of capillaries throughout the body (see Figure 8-31). At least one more schizogonic generation occurs in circulating mononuclear cells. Merozoites released from second- or later-generation schizonts enter striated muscle cells and, in certain cases, nerve cells to form sarcocysts (see Figures 8-32 and 8-33). Sarcocyst formation is a slow process requiring several months. The dog becomes infected when it consumes uncooked beef containing sarcocysts of *S. cruzi*. Thus the cycle of infection can be interrupted by cooking beef scraps to be fed to dogs or by preventing canine fecal contamination of cattle feedstuffs. The economic importance of subclinical bovine sarcocystosis remains to be assessed, but clinical disease and death losses have occurred in cases in which 10,000 or more sporocysts were ingested over a short time (Dubey and Fayer, 1983; Frelier, 1977). Clinical signs in cattle are associated with release of the second wave of merozoites about 4 to 6 weeks after infection and consist of protracted fever, anemia, lymphadenopathy, anorexia, diarrhea, hypersalivation, weakness, and hair loss about the eyes, the neck, and, perhaps most noticeably, the tail switch.

Infection of sheep with 10,000 to 50 million *Sarcocystis tenella* sporocysts was studied experimentally. A total of 25 to 50 million sporocysts led to death in 16 to 19 days from occlusion of the mesenteric arteries by first-generation schizonts. Sheep infected with 10 million and fewer sporocysts had anemia, hepatitis, and myocarditis related to the second schizogonic generation. Neurologic signs and lesions of encephalomyelitis were also observed in these artificial *S. tenella* infections in sheep (Dubey, 1988).

SARCOCYSTIS NEURONA. *S. neurona* causes severe neurologic disease in horses of both sexes and all ages. Clinical signs include stumbling, paresis, lameness, ataxia, recumbency, constipation, urinary incontinence, diaphoresis, muscle atrophy, and other manifestations of neural degeneration depending on the location of the lesions (MacKay, 1997; Mayhew and Greiner, 1986). The equine protozoal myeloencephalitis (EPM) organism has been identified as *S. neurona* (described by Dubey et al, 1991). Horses can be infected and can develop signs of acute infection by intravenous inoculation of merozoites grown within autologous peripheral blood mononuclear cells (Witonsky et al, 2008). Opossums are the hosts that shed sporocysts into the environment (Figure 3-24). Opossums shed sporocysts of *S. neurona* and at least four other distinct sporocysts in their feces as determined morphologically and molecularly (Cheadle et al, 2001a). One characteristic of this infection is the formation of schizonts by dividing cells in the equine tissues (Figures 3-25 and 3-26). It has also been shown that if ponies are given sporocysts of *S. neurona*, the parasite can be found in the mesenteric lymph nodes within a day, within the liver 2 days after infection, and within the lungs by 7 days post infection (Elitsur et al, 2007). Microscopic lesions consistent with equine protozoal myeloencephalitis appeared in the brain and spinal cord of the ponies 7 to 9 days after they were given the sporocysts. It is also now known that cats, striped skunks, and nine-banded armadillos can be infected with muscle stages of *S. neurona* (Cheadle et al, 2001b and 2001c; Tanhauser et al, 2001). It has also been discovered that raccoons are capable of having myocarditis and encephalitis due to *S. neurona* infection (Hamir and Dubey, 2001). It seems that sarcocysts may develop in some infected horses (Mullaney et al, 2005), but this remains to be verified.

Diagnosis. An antemortem diagnosis is based solely on clinical signs of neurologic disease, and none of these is

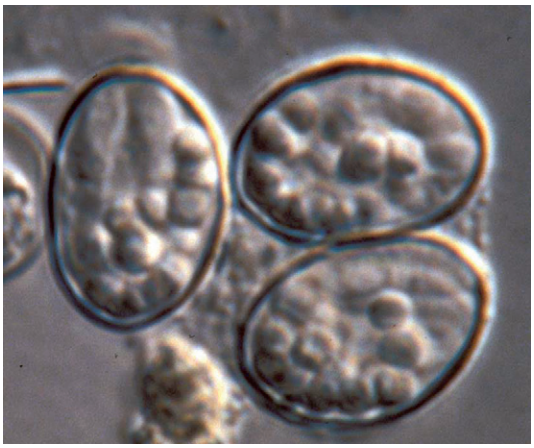

FIGURE 3-24. Sporulated sporocysts of *Sarcocystis neurona* passed in the feces of an opossum fed infected muscle from an experimentally infected cat. (Courtesy Dr. J. P. Dubey, USDA, Beltsville, Maryland.)

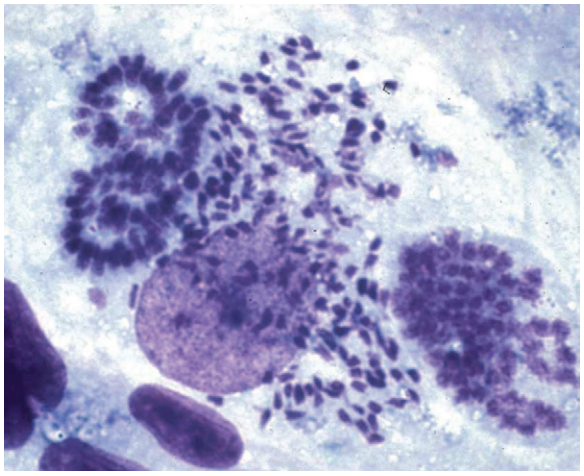

FIGURE 3-25. Schizonts of *Sarcocystis neurona* in a culture of bovine turbinate cells (Giemsa stained). Culture was initiated with merozoites from the nervous tissue of a naturally infected horse.

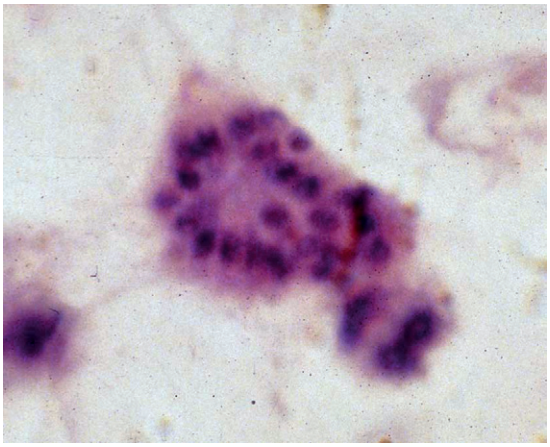

FIGURE 3-26. Section of nervous tissue from a horse showing the characteristic rosette of organisms that is not uncommonly seen in infections with *Sarcocystis neurona*, the causative agent of equine protozoal myeloencephalitis (EPM).

pathognomonic. Several laboratories provide diagnostic tests consisting of Western blot analysis of serum or cerebrospinal fluid or molecular methods using PCR to assist with diagnosis. A positive diagnosis is based on histopathologic demonstration of the EPM organisms in association with lesions in the central nervous system (see Figure 3-26).

Treatment. FDA-approved treatments for EPM currently include ponazuril at 5 mg/kg for 28 days (Marquis with 15% W/W ponazuril) and diclazuril at 1 mg/kg for 28 days (Protazil antiprotozoal pellets, 1.56% diclazuril). Also routinely used still are sulfonamide (sometimes with trimethoprim) plus pyrimethamine. Treatment is not always consistent between horses; it seems that not all respond to therapy (Bello and Allen, 2008). No vaccine is currently available.

CLINICAL SARCOCYSTOSIS IN DOGS AND CATS. Infections with *Sarcocystis* in dogs and cats are usually incidental findings in histologic sections. However, these hosts do occasionally develop disease associated with these infections. Two dogs from the United States (Colorado and Montana) and one from Canada (British Columbia) developed massive sarcocystosis (Chapman, Mense, and Dubey, 2005; Sykes et al, 2011). The Canadian dog presented with fever, dehydration, abdominal pain, and ataxia, with occasional petit mal seizure activity, and clinical signs had progressed 5 weeks later to an inability to walk and dysphagia with muscle atrophy, weight loss, and generalized pain on palpation. The dog's condition improved after clindamycin treatment, and biopsy revealed massive numbers of sarcocysts within muscle cells. The dogs from the United States also initially presented with lethargy and anorexia that progressed to neurologic deficits and muscle wasting (Sykes et al, 2011), and again, muscle biopsy showed that massive numbers of muscle cells were infected. Both of these dogs were initially treated unsuccessfully with clindamycin, and one dog died. The other dog subsequently responded to treatment with decoquinate.

In a cat with sarcocystosis, the initial presentation was for spinal pain, ataxia, and anisocoria (Bisby et al, 2010). Intracellular protozoa were noted on cerebrospinal fluid analysis. Serology was positive for *Sarcocystis* and negative for *Toxoplasma*. The cat was treated with several compounds, including clindamycin, ponazuril, and pyrimethamine with trimethoprim sulfonamide, and ultimately improved. The organism was identified as either *S. neurona* or *S. dasypi*, which uses opossums as the final host.

CLINICAL ENCEPHALITIC SARCOCYSTOSIS IN SHEEP. Apicomplexan encephalomyelitis of adult sheep may be caused by *S. tenella* and other *Sarcocystis* species (Dubey, 1988).

CLINICAL ENCEPHALITIC SARCOCYSTOSIS IN CATTLE. Encephalitis in an 18-month-old steer was apparently caused by a *Sarcocystis*-like organism (Dubey, Perry, and Kennedy, 1987). Similar acute disease due to *Sarcocystis* has been described in a naturally infected calf and a heifer in South Africa (van der Lugt et al, 1994).

***TOXOPLASMA, NEOSPORA,* AND *SARCOCYSTIS* IN SEA OTTERS AND OTHER AQUATIC MAMMALS.** Several reports have described *Toxoplasma*, *Neospora*, and *Sarcocystis* inducing antibody responses or causing serious disease in seals, walruses, otters, dolphins, and sea lions (Conrad et al, 2005; Dubey et al, 2001b, 2003, and 2005b; Dubey, Chapman, and Rosenthal, 2006; Dubey and Thomas, 2012; Honnold et al, 2005). Infection is caused by oocysts washing into the aquatic environment and perhaps being concentrated by filter-feeding shellfish, on which some of these animals feed (Shapiro, Miller, and Mazet, 2012). The challenge involves how to minimize the exposure of these aquatic animals, some of which are on the endangered species list, to the oocysts

shed in the feces of terrestrial carnivores that are then washed into various tidal basins and estuaries.

BESNOITIA. Large cysts (up to 3 mm in diameter) containing bradyzoites may be found in the skin, where they cause lesions (see Figure 8-37). Important species with cysts in domestic animals include *Besnoitia besnoiti* of cattle, *Besnoitia bennetti* of donkeys, and *Besnoitia caprae* of goats. Species with cysts in various wildlife hosts include *Besnoitia tarandi* and *Besnoitia darlingi* in opossums, *Besnoitia wallacei* in rats, *Besnoitia neotomofelis* in woodrats, *Besnoitia oryctofelisi* in rabbits, and *Besnoitia jellisoni* in mice (Dubey and Yabsley, 2010; Mehlhorn et al, 2009). Where the life cycles have been fully elucidated (i.e., for *B. darlingi*, *B. wallacei*, *B. oryctofelisi*, and *B. neotomofelis*), the final host has been a cat. When cats are fed tissue containing cysts, they shed oocysts in the feces that morphologically resemble those of *T. gondii*.

BESNOITIA BESNOITI. This infection with its noticeable cysts has been known to occur in cattle in Africa, Asia, Israel, and Venezuela, and from some southern European countries such as France, Spain, and Portugal. In 2008, the infection appeared in a herd in Germany, where 120 cattle were diagnosed clinically; it has been classified by the European Food Safety Authority as an emerging disease in Europe (Gollnick et al, 2009, 2010). A long-standing belief suggests that biting flies play a role in the transmission of *B. besnoiti*; however, although work is ongoing in Europe to test this hypothesis (Liénard et al, 2013), it has not yet been verified experimentally. Older work showed that the cat is the definitive host of this species of *Besnoitia*, as has been documented for other species (Peteshev, Galuzo, and Polomoshnov, 1974; Peteshev and Polomoshnov 1976). Of course, it is possible that both biological transmission via cat feces and mechanical transmission by biting flies may occur.

BESNOITIA BENNETTI. *Besnoitia bennetti* seems to be a species of *Besnoitia* restricted to donkeys. Besnoitiosis has appeared in miniature donkeys in the United States and has been identified as *Besnoitia bennetti* by molecular methods (Elsheikha et al, 2005; Ness et al, 2012). Tissues mainly affected by the cysts in these donkeys include the sclera of the eyes, the oropharyngeal region, and the testes. Donkeys were the only equid infected in the areas where it has occurred, and the mode of transmission remains unknown, as it is for *B. besnoiti*. Treatments with ponazuril, trimethoprim-sulfamethoxazole, and nitazoxinide have not been successful in clearing the infection in almost all cases where they have been used.

HEPATOZOON. The species of *Hepatozoon* commonly causing disease in dogs in the United States is now recognized as *Hepatozoon americanum* (Macintire et al, 1997; Panciera et al, 1997; Vincent-Johnson et al, 1998). Throughout the rest of the world, the disease seems to be caused by a different species, *Hepatozoon canis* (Smith, 1996). The vector of *H. americanum* is now known to be *Amblyomma maculatum* (Mathew et al, 1998, 1999), and reservoirs are known to include coyotes (Garrett et al, 2005). In the case of *H. canis*, dogs acquire their infection by ingesting an infected tick, *Rhipicephalus sanguineus*; in Brazil, it has been shown that *H. canis* can also be transmitted by *Amblyomma ovale* (Baneth, 2011).

In the life cycle of this parasite, ticks become infected by ingesting a blood meal that contains neutrophils and monocytes that harbor gamonts of the parasite. Sexual replication in the gut of the tick results in the production of oocysts containing infective sporozoites. After dogs become infected by ingesting the tick, schizonts are found in various tissues, and finally, the gamonts occur in white blood cells.

HEPATOZOON CANIS. *H. canis* typically seems to cause subclinical infection, and the diagnosis is typically based on the finding of gamonts in the peripheral blood. Also, the meronts typically seen in multiple organs of the body with the occurrence of *H. canis* infection in dogs from other parts of the world are not seen with any regularity in dogs infected in the United States. On the other hand, cases of *H. canis* have been identified as occurring in several dogs in the United States (Little and Baneth, 2011).

In infected nymphal *R. sanguineus*, gamonts were in syzygy 24 hours after infection (Baneth, Samish, and Shkap, 2007). Early oocysts were then detected 4 days after the ticks had fed. Mature oocysts in adult ticks were infective to dogs 35 days post molt or 53 days after nymph attachment.

In dogs fed infective oocysts, the bone marrow was found to contain a first round of merogony that was completed around 3 weeks post infection with the production of some 20 to 30 merozoites per meront (Baneth, Samish, and Shkap, 2007). Also typical meronts in the spleen are apparently formed by schizogony around a central residual body that appeared mature at 24 days. Beginning about a month after onset of the infection, mature gamonts began circulating in leukocytes in the peripheral blood. The dogs had fevers from day 16 to day 27, and two dogs developed skeletal pain and recumbency, which again are not typically seen in naturally infected dogs with *H. canis*.

Treatment. *H. canis* is treated with imidocarb dipropionate at 5 to 6 mg/kg every 14 days until gamonts are no longer present in blood smears, but treatment frequently does not clear the animals of all organisms (Baneth, 2011). Macintire et al (1997) suggested that primaquine phosphate has proven efficacious in treating dogs infected with *H. canis* in Africa. If few circulating gamonts are found in a dog, the prognosis is good; however, the prognosis must remain guarded in dogs that present with high numbers of circulating gamonts in their blood.

HEPATOZOON AMERICANUM. In the case of infection with *H. americanum*, severe disease typically occurs, with dogs having marked neutrophilic leukocytosis. Dogs with *H. americanum* often have significant joint pain associated with myositis and periosteal bone proliferation, which can be revealed in radiographs. Lesions occur primarily on the diaphysis of the more proximal long bones of the limbs; however, flat and irregular bones are frequently involved (Panciera et al, 2000). Lesions involving metacarpals, metatarsals, and digits are infrequent. The earliest observed periosteal lesions in experimentally infected dogs were noted 32 days after exposure to sporulated oocysts of *H. americanum*, with hypertrophy and hyperplasia of osteoprogenitor cells, and osteoblasts appearing in the cellular zone of the periosteum. The osseous lesions are similar to those of hypertrophic osteopathy in domestic dogs and other mammalian species.

The life cycle of *H. americanum* has been shown to include the use of mammalian paratenic hosts containing cystozoites. It has been shown that rabbits (including European and North American swamp rabbits and cottontail rabbits), cotton rats, and mice can have persistent cystozoites of *H. americanum* within their tissues, including the spleen and myocardium (Allen et al, 2011; Johnson et al, 2008, 2009). Dogs that ingest infected rabbits will have gamonts appear in their blood within 5 weeks of eating the infected rabbits. Thus, dogs that hunt can become infected by eating a prey animal without ever ingesting a tick.

Diagnosis of *H. americanum* infection typically requires the examination of muscle tissue collected at biopsy or during necropsy to reveal the schizonts. With *H. americanum* infection, a large cystic form of the organism occurs in skeletal muscles that has not been observed in other parts of the world. Several laboratories now perform molecular analysis of blood or biopsy material to make specific diagnoses.

Treatment. In a report of two cases of infection in the United States, treatment with toltrazuril failed to prevent relapse in most of the 11 treated dogs; treatment of three dogs with a combination of trimethoprim sulfate, pyrimethamine, and clindamycin also failed to prevent relapse (Macintire et al, 1997). Of the 22 dogs reported in the study by Macintire et al (1997), seven were humanely killed because of chronic wasting, six died of the disease, three were lost to follow-up, and six were alive at the time of the report. Three of the living dogs were free of clinical signs, whereas the other three dogs had chronic wasting disease with intermittent periods of remission and relapse. Treatment to prevent remission after clinical improvement is provided with the administration of decoquinate twice a day for up to 2 years. It is often necessary to provide supportive therapy with nonsteroidal anti-inflammatory agents to relieve pain and fever.

***HEPATOZOON* IN CATS.** *Hepatozoon* has been found on occasion in cats and other felids around the world (Baneth, 2011). At this time, it is not known whether the species in cats is the same as that in dogs. In several districts of Bangkok, Thailand, where both canine and feline hepatozoonoses are present, 32.3% of 300 cats were positive by PCR, and more cats were infected in areas where higher percentages of dogs were infected with *H. canis* (Jittapalapong et al, 2006). On the other hand, the species of *Hepatozoon* found in a cat from Spain appeared to be based on genetic relationships among 18S rDNA hypervariable region sequences to be more closely aligned with that of bobcats in Georgia than with that of *H. americanum* (Allen et al, 2011). Thus, it seems possible and perhaps likely that cats may be found to be infected with their own species or collections of species of *Hepatozoon*. At this time, the vectors for feline hepatozoonosis are not known.

In feline hepatozoonosis, gamonts circulate usually in less than 1% of neutrophils. Meronts have been identified in the myocardium and skeletal muscle of domestic cats and wild felids along with associated elevated activities of creatine kinase (Baneth, 2011).

Piroplasmorida of the Aconoidasida

The Piroplasmorida are apicomplexan parasites that are transmitted by feeding ixodid ticks. In this case, sexual conjugation takes place within the stomach of the tick, and sporozoites are generated within the salivary glands. Stages in the vertebrate host include meronts, merozoites, and gamonts. A recent examination of the molecular phylogeny of the Aconoidasida has divided the group into eight Clades (Lack, Reichard, and van den Bussche, 2012). In general, *Babesia* is placed in Clade I, *Theileria* in Clade III, and *Cytauxzoon* in Clade V. Then, for the remaining Clades, Clade II contains the equine parasite *Theileria equi*; Clade IV contains a species from a giraffe and a seagull; Clade VI, "the Duncani" group, contains *Babesia conradae* and zoonotic *B. duncani*; Clade VII contains two species from birds; and Clade VIII contains the zoonotic *Babesia microti* of rodents and most of the *Babesia* species that have been described from felids.

***BABESIA*.** *Babesia* species are apicomplexan parasites of the erythrocytes of their vertebrate hosts (Figure 3-27); the erythrocyte is the only vertebrate host cell infected. Typically, *Babesia* stages that are seen in red blood cells are large compared with the *Theileria* species, which occur in the same host, but unfortunately, there are exceptions. For members of the genus *Babesia*, sexual conjugation occurs within the intestinal lumen of the tick, and sporogony occurs within the epithelium of the tick's intestinal wall. The products of sporogony enter the tick's hemocoel, where they further multiply within various cells of the tick, including those of the ovary, in such a way that the female tick passes the infection to her eggs and to the larvae that hatch from these eggs. Sporozoites also are found in the salivary glands and undergo further multiplication when the *tick* is feeding; they are passed in the saliva as the tick continues to feed.

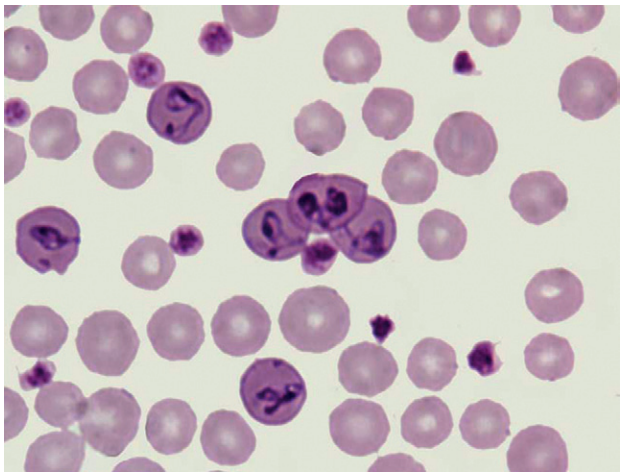

FIGURE 3-27. *Babesia bigemina* in Giemsa-stained blood film from a cow.

BABESIA IN CATTLE

Texas Fever. *Babesia bigemina* (see Figure 3-27) causes bovine piroplasmosis (Texas fever), a disease characterized in the acute phase by pyrexia (up to 42° C), hemoglobinuria, anemia, icterus, and splenomegaly. The apple-seed–like merozoites (also called *piroplasms*) are found in pairs in the erythrocytes, which they destroy, releasing hemoglobin in the process and giving rise to the characteristic clinical manifestations. Some of the merozoites appear to differentiate into gamonts, but unlike the Haemosporidia, the gamonts are difficult to differentiate morphologically from the merozoite stages. Transmission of infection among cattle occurs through the bite of the one-host ticks *Rhipicephalus (Boophilus) annulatus* and *Rhipicephalus (Boophilus) microplus*; and again, since the piroplasms multiply in the ovary of the female tick, transovarial transmission occurs, and the larvae that hatch from her eggs are infected.

Calves are much less susceptible than older cattle. The greater susceptibility of older hosts holds for all species of *Babesia* and is greatly increased by splenectomy.

Texas fever was once endemic south of the thirty-fifth parallel in the United States (southern border of Tennessee), but a cattle-dipping campaign launched in 1906 to eradicate *R. annulatus* virtually eliminated the disease by 1940. This prodigious effort was successful mainly because of the high degree of specificity displayed by *R. annulatus* for its bovine host. Other mammals can serve as hosts, but most *R. annulatus* ticks are found on cattle. Therefore, when the cattle were rounded up for dipping, most of the feeding tick population was rounded up with them. *R. microplus*, on the other hand, infests a broad range of hosts. Thus when *R. microplus* is present in high numbers as a major vector, eradication of bovine piroplasmosis is virtually impossible with contemporary methods.

Because the disease has been eradicated from the United States of America but not from the United Mexican States, *R. microplus* and *R. annulatus* border incursions are controlled by the USDA APHIS Fever Tick Eradication Program (http://www.cfsph.iastate.edu). Personnel, including mounted inspectors called "tick riders," enforce a tick-free quarantine zone. Before cattle and horses are moved out of the zone, they must be inspected and treated with acaricides. If ticks are found on ranches, they are placed under quarantine for 6 to 9 months. Because of the involvement of

R. microplus, there is concern that this tick can become re-entrenched by using deer to support its presence. There are vaccines available for bovine babesiosis in Australia (see Chapter 9).

OTHER *BABESIA* SPECIES OF LIVESTOCK. *Babesia bovis, Babesia divergens*, and *Babesia argentina* cause bovine piroplasmosis in various parts of the world. In the United Kingdom, piroplasmosis is transmitted between cattle by *Ixodes ricinus*. Each species of *Babesia* tends to use one or more different species of tick vectors. Other species of *Babesia* infect goats *(Babesia ovis)*, horses *(Babesia caballi)*, and swine *(Babesia trautmanni)*. The United States is considered to be free of *B. caballi*, and its one-host tick vector, *Dermacentor nitens*, has not been reported in Florida since 1990 (Short et al, 2012). The tick *D. nitens* can transmit the organism transovarially to its eggs, and although the cycle is maintained from one generation to the next, it does not continue unless the second generation has an opportunity to feed on an infected horse (Schwint et al, 2008). This means that if it were introduced, re-eradication would be easier than it would be if the tick were here.

CANINE BABESIOSIS. This disease is cosmopolitan in distribution (Lobetti, 1998). Dogs are infected by two species of these parasites, *Babesia canis* and *Babesia gibsoni*. In a survey of 673 canine blood samples tested for *Babesia* DNA by PCR, the 144 positive samples came from 29 states and one Canadian province (Birkenheuer et al, 2005). Of these samples, 91% (131) were recognized as the small form of *Babesia*, *B. gibsoni*, and 10 were recognized as the larger *Babesia* form, *B. canis vogeli* (three samples did not match current recognized species). Almost all samples representing *B. gibsoni* (122 of 131) were from American pit bull terriers. Six of the 10 *B. canis vogeli* cases were seen in greyhounds. Babesiosis is diagnosed by identification of the stages in stained blood films; typically, this is now followed by a molecular assay for species determination.

Babesia canis. This is the larger form occurring in dog red blood cells, with pear-shaped trophozoites, 4 to 5 µm long, typically found in pairs in the erythrocytes. The species *B. canis* has been divided into three subspecies: *B. canis canis* of Europe, transmitted by *Dermacentor reticulatus*; *B. canis vogeli* of northern Africa and North America, transmitted by *R. sanguineus*; and *B. canis rossi* of southern Africa, transmitted by *Haemaphysalis leachi*. Fortunately, the subspecies in North America and Europe do not produce the fulminant form of disease seen in southern Africa (Jacobson, 2006). A survey of greyhounds in Florida has shown that large numbers (46% of 383 greyhounds) have antibodies to *B. canis* (Taboada et al, 1992), but most do not have clinical signs. The disease when present in dogs in the United States typically manifests with depression, anemia, anorexia, lethargy, and splenomegaly. The strain of *B. canis* in Florida appears mainly to cause disease in puppies, for which the major diagnostic feature is anemia. Typical treatment of *B. canis* consists of imidocarb dipropionate (5 to 7 mg/kg subcutaneously or intramuscularly, repeated in 14 days), which is approved for this use in the United States (Irwin, 2010). There are vaccines available for the prevention and control of *B. canis canis* and *B. canis rossi* (see Chapter 9), the species not routinely found in the United States.

Babesia gibsoni. *B. gibsoni* is a smaller *Babesia* that occurs in the dog; it is 3 µm long and usually is round to oval in shape. In other parts of the world, *B. gibsoni* is transmitted by *R. sanguineus*, *Haemaphysalis bispinosa*, and *Haemaphysalis longicornis.* However, it is now fairly well accepted that most transmission of *B. gibsoni* in the United States occurs directly between dogs through wounds received when fighting (Yeagley et al, 2009). The disease can range from subclinical to fatal, with acute disease including hemolytic anemia, thrombocytopenia, splenomegaly, lymphadenomegaly, anorexia, lethargy, pyrexia, and vomiting. Dogs often recover with treatment but remain subclinically infected. Treatment with atovaquone (13.5 mg/kg orally three times daily) and azithromycin (10 mg/kg orally every 24 hours) will reduce or eliminate *B. gibsoni* parasitemias.

Babesia conradae. This second small form of *Babesia* is found in dogs and occurs in southern California. This form seems to be related to forms found in people and wildlife in the same area (Kjemtrup et al, 2006). *B. conradae* is more pathogenic in dogs than *B. gibsoni* (Kjemtrup and Conrad, 2006). Treatment with a 10-day course of atovaquone (13.3 mg/kg orally every 8 hours) and azithromycin (10 to 12.5 mg/kg orally each day) removes clinical signs and clears the agent from the blood of infected dogs (di Cicco et al, 2012).

Babesia microti. This species of *Babesia* routinely infects rodents and is transmitted by the tick *Ixodes scapularis*; this is the species that many clients living in these areas will be familiar with because of their concerns over human health, because cases occur in people in New England and the upper Midwest (centered in Minnesota and Wisconsin). This species has not been found in dogs in these same geographic areas.

THEILERIA. The genus *Theileria* differs from *Babesia* in that schizonts occur in lymphocytes and induce the infected lymphocytes to undergo division and proliferation. Also, typically no transovarial transmission occurs in the case of ticks infected with *Theileria* species. *Theileria parva*, the causative agent of East Coast fever of African cattle, occurs in the erythrocytes, lymphocytes, and endothelial cells and is transmitted interstadially by *Rhipicephalus* and *Hyalomma* species. East Coast fever is characterized by dyspnea, emaciation, weakness, tarry feces, and exceptionally heavy mortality. There are vaccines available for preventing disease due to *T. parva* and to *Theileria annulata* in Africa (see Chapter 9). Some species of *Theileria* are seen in deer in the United States, such as *Theileria cervi*, which is transmitted by *Amblyomma americanum* (Reichard and Kocan, 2006).

THEILERIA EQUI. *Theileria equi* of the horse has been demonstrated to have schizont stages in lymphocytes and was carefully redescribed as a *Theileria* species (Mehlhorn and Schein, 1998). The stages in the lymphocytes are considered a pre-erythrocytic form (Mehlhorn and Schein, 1998), and if they behave as do the exoerythrocytic stages of malaria, transfer of circulating merozoites by blood transfusion from one horse to another will institute an intraerythrocytic cycle of parasitemia with no chance of schizonts in lymphocytes. The lymphocytic schizogonous form of the disease should only follow the bite of an infected tick.

The United States was considered free of *T. equi* until 2008, when an outbreak occurred in Florida, where 20 horses on seven different properties were found to be infected (Short et al, 2012). The index case presented with a slightly elevated rectal temperature, tachycardia, pale and yellow-tinged mucous membranes, icteric sclerae, and hematuria during the first 24 hours of hospitalization. Organisms were seen by the veterinarian on a stained slide. Ultimately, the source of this outbreak was believed to be two infected horses from Mexico, and the agent was spread mechanically between horses via shared needles and the practice of blood transfusions to artificially increase red blood cell (RBC) numbers before races. A second *T. equi* outbreak occurred in 2009 in Texas, where 292 of 360 horses on a single ranch were found to be positive (Scoles et al, 2011). In this outbreak, the index case was a mare that presented with signs of theileriosis, and the infection was diagnosed with a serum antibody test. Ultimately, when 2500 horses in the outbreak area were examined in Texas, 413 were found positive. A national testing program put in at that time examined blood

samples from 200,000 horses; this examination disclosed that 179 horses were positive for *T. equi* and 10 for *B. caballi*, which was unrelated to the Texas outbreak. These positive horses were all members of two groups: horses that were imported when a less sensitive antibody test was employed; and horses, similar to those in the Florida outbreak, that were participating in unsanctioned horse racing.

Treatment. As part of the research surrounding the outbreaks, it was found that treatment of horses with imidocarb dipropionate at 4 mg/kg intramuscularly every 3 days for a total of four injections would clear most horses (verified by DNA clearance as detected by PCR and sub-inoculation into splenectomized horses), and that a second treatment would remove all organisms from horses not cleared with one treatment (Ueti et al, 2012). With this method, all horses on the ranch where the outbreak occurred were treated and were verified to be *T. equi* negative.

CYTAUXZOON. The genus *Cytauxzoon* is defined as distinct from *Theileria* in that the schizonts that occur in the vertebrate occur in macrophages rather than lymphocytes. Some would prefer to combine the genus under *Theileria*. However, the name *Cytauxzoon* does such a marvelous job of describing the disease and its effects on the feline host cell with the resulting pathogenesis that it would be sad to lose the name to synonymy, and the recent molecular phylogeny argues for its preservation as well (Lack, Reichard, and van den Bussche, 2012).

CYTAUXZOON FELIS. Cytauxzoonosis of cats, caused by *Cytauxzoon felis*, is a sporadic but rapid and often fatal disease of domestic cats that occurs predominantly in the south central and southeastern United States (Blouin et al, 1984; Bondy et al, 2005; Jackson and Fisher, 2006). Clinical signs consist of pyrexia, anemia, icterus, and dehydration; death occurs within a few days, and the most common signs of the infection are pancytopenia and icterus. Blood smears stained with Wright's or Giemsa reveal 1- to 2-µm organisms with light blue cytoplasm and a dark red nucleus in the erythrocytes (Figure 3-28). Late in the course of cytauxzoonosis, enormous reticuloendothelial cells packed with schizonts appear in the peripheral blood. Histologically, parasitized reticuloendothelial cells nearly occlude the lumens of small and medium-sized veins in the lungs, spleen, and lymph nodes (Haber and Birkenheuer, 2005; Wightman, Kier, and Wagner, 1977). In a report on 34 cases from the mid-Atlantic states, 32 infected cats succumbed to the infection (Birkenheuer et al, 2006).

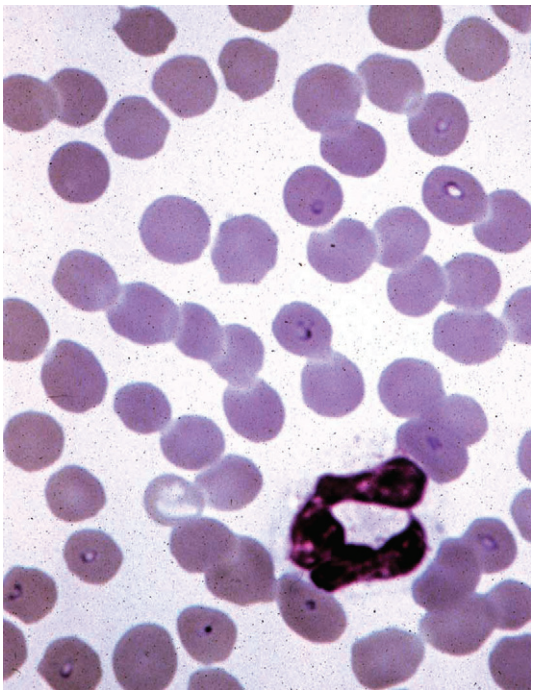

FIGURE 3-28. Giemsa-stained blood film from a cat showing the appearance of *Cytauxzoon felis* organisms within the red blood cells. (Courtesy Dr. Tracy W. French.)

The bobcat *Lynx rufus* is considered the natural reservoir of this infection and typically has a parasitemia without clinical signs (Shock et al, 2011), although fatal experimental infections in bobcats have been reported (Kier, Wightman, and Wagner, 1982). *Dermacentor variabilis* nymphs that fed on a splenectomized parasitemic bobcat as adults transmitted fatal cytauxzoonosis to two splenectomized domestic cats (Blouin et al, 1984). *Amblyomma americanum* has been shown to be a highly competent vector of *C. felis* (Reichard et al, 2009, 2010), and in several concurrent trials, *C. felis* was not transmitted to domestic cats by *D. variabilis* (Reichard et al, 2010). Also, when ticks were collected from the field in Oklahoma, C. *felis* was found in *A. americanum* but not *D. variabilis*. The range of feline cytauxzoonosis cases corresponds very well to the distribution of *A. americanum* in the United States; it also matches well the distribution of *C. felis* in bobcats, with the exception of North Dakota, where 2% of bobcats are infected and *A. americanum* is not present (Shock et al, 2011).

Cats can survive natural infections with *Cytauxzoon felis* (Brown et al, 2010; Walker and Cowell, 1995). Four of 18 cats without clinical signs from northwestern Arkansas and northeastern Oklahoma were identified by finding the organisms in red blood cells, and the parasitemia persisted for up to 154 days (Meinkoth et al, 2000). It has been shown that cats that have survived clinical cytauxzoonosis and become carriers can serve as reservoirs for tick-transmitted transfer of the infection and fatal disease to other cats (Reichard et al, 2009).

Iatrogenic cytauxzoonosis was induced in a specific pathogen-free cat by the inoculation of mononuclear cells from a Florida panther *(Felis concolor coryi)*, in an attempt to determine whether the panther was infected with feline immunodeficiency virus (Butt et al, 1991). The cat died 12 days after inoculation with typical schizonts of *C. felis* occluding the pulmonary veins (see Figure 8-39). Again, bobcats may die of the infection under natural conditions (Nietfeld and Pollock, 2002). A tiger in Florida housed in a private breeding facility died of clinical cytauxzoonosis: "Following chemical immobilization on day 3 of illness, two female lone star ticks *(Amblyomma americanum)* were removed from the inguinal skin" (Garner et al, 1996).

Prevention and Treatment. Cats, if they go outside in areas where cytauxzoonosis is known to occur, need to be protected against tick bites. The current treatment of choice for clinical cytauxzoonosis appears to be a 10-day course of atovaquone (15 mg/kg every 8 hours) and azithromycin (10 mg/kg orally every 24 hours), which provides a better chance of survival to discharge (60%) compared with treatment with imidocarb (3.5 mg/kg intramuscularly, followed a week later by a second injection), which had a survival rate to discharge of 26% (Cohn et al, 2011). Cats with lower parasitemia, lower total bilirubin, and higher white blood cell counts were more likely to survive.

Haemosporida of the Aconoidasida

These organisms tend to be transmitted by biting flies. This mode of transmission, along with the presence of flagella on the microgametes that appear in the gut of the insect, along with the development after gamete fusion of a motile ookinete with a conoid, is what sets these parasites apart from the Piroplasmorida.

PLASMODIUM. *Plasmodium* species are the causative agents of malarias of humans, nonhuman primates, rodents, birds, and reptiles (mainly lizards). Mammalian malarias are transmitted by anopheline mosquitoes, and avian malarias by culicine mosquitoes; the vectors of reptilian malarias are largely unknown.

LIFE HISTORY. Sporozoites injected into the host by the infected mosquito during feeding enter cells such as hepatocytes, become trophozoites, and undergo schizogony. This first multiplication of plasmodia in hepatocytes is termed **pre-erythrocytic schizogony.** Merozoites released when the hepatocyte ruptures invade erythrocytes or reticulocytes of the circulating blood, pass through a trophozoite phase, and then undergo **erythrocytic schizogony.** In certain species of *Plasmodium*, particularly *Plasmodium vivax*, some schizonts enter a quiescent stage and are called **hypnozoites;** these will later reactivate and are responsible for relapses after therapeutic elimination of erythrocytic infection. Merozoites released when infected erythrocytes rupture reinvade other erythrocytes and again undergo schizogony. Each generation of erythrocytic merozoites occupies approximately 24, 48, or 72 hours, depending on the species of *Plasmodium* involved. Synchronization of schizogony and consequent erythrocyte destruction leading to cyclic bouts of chills and fever are typical of certain malarias, particularly those of humans. Eventually, some merozoites develop into microgametocytes or macrogametocytes, which are the stages infective for the mosquito. When a suitable species of mosquito feeds on a malarious host, microgametocytes and macrogametocytes in the blood meal mature, and the microgametes fertilize the macrogametes to form zygotes. The zygotes then elongate to form motile ookinetes, which migrate to the hemocoel side of the mosquito's midgut, where each develops into an oocyst. Thousands of sporozoites develop within each oocyst by a budding process similar to schizogony and are released into the hemocoel when the oocyst ruptures. Those sporozoites that reach the salivary glands are ready to infect another host the next time the mosquito takes a blood meal and thus complete the rather involved life history of *Plasmodium*. In humans, the symptoms of malaria are extremely variable, and diagnosis depends on the demonstration of plasmodia in fixed, stained blood smears. Fatality can usually be attributed to cerebral involvement, renal failure, or pulmonary hemorrhage.

IDENTIFICATION. Differentiation of species of *Plasmodium* is based on study of Giemsa-stained thin blood smears and recognition of rather subtle morphologic features of the early trophozoite ("ring form") (Figure 3-29), amoeboid late trophozoite, schizont, and male and female gametocytes. The color and distribution of hematin in the cytoplasm of the parasite, as well as cytoplasmic stippling and other morphologic alterations of the infected erythrocyte, are also taken into account. The diagnosis of malaria is clearly a job for an expert. Antigen detection methods similar to those used in animal medicine for heartworm and viruses are now available for human malarias.

SIMIAN MALARIA. About 20 species of *Plasmodium* have been described from nonhuman primates, some of which (e.g., *Plasmodium knowlesi*) can be transmitted to humans through the bites of infected anopheline mosquitoes. The diagnosis of simian malaria is of particular interest to laboratories where imported primates are experimental animals (Coatney et al, 1971). Old World monkeys may also be infected with *Hepatocystis*.

AVIAN MALARIA. Avian malaria is a complex of diseases caused by many species of *Plasmodium* (Figure 3-30). *Plasmodium* species are important to zoo and conservation veterinarians dealing with penguins because they often succumb to infection from species that they do not typically encounter (Bueno et al, 2010; Levin et al, 2009). Avian *Plasmodium* species often differ from those of mammals in that there is a prehepatic schizogonous stage in vascular endothelial cells. *Haemoproteus* and *Leucocytozoon*, considered later, also cause malaria-like infections in birds.

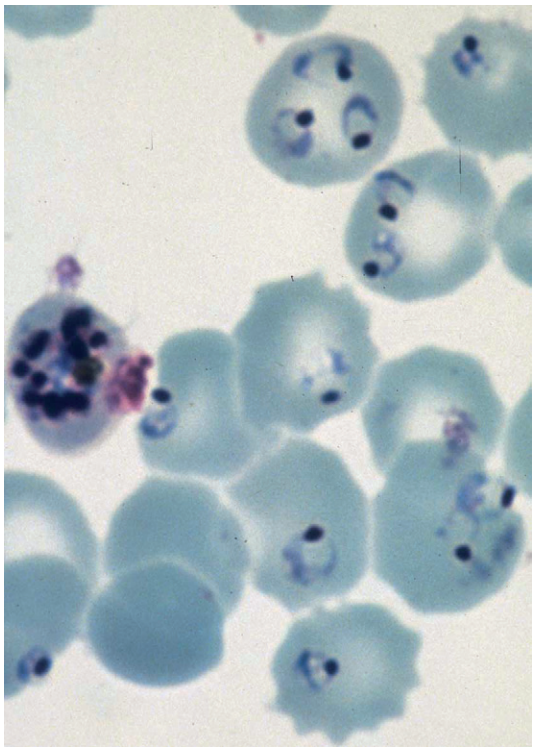

FIGURE 3-29. *Plasmodium falciparum*, human malaria, ring-stage trophozoites in red blood cells.

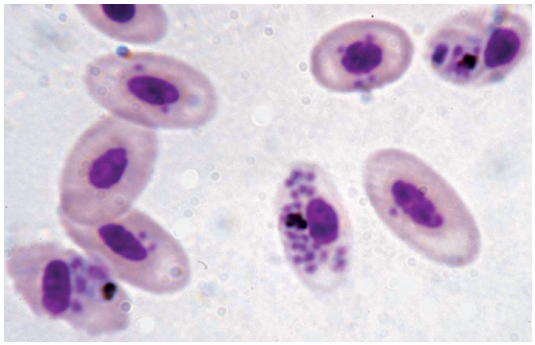

FIGURE 3-30. Schizonts of *Plasmodium gallinarum* in chicken red blood cells. (Specimen courtesy Priscilla Maldonado, New York University.)

HAEMOPROTEUS. *Haemoproteus* species are parasites of birds, turtles, and lizards. Schizogony occurs in vascular endothelial cells of various organs, and only gametocytes appear in circulating erythrocytes. In blood films stained with Giemsa, the gametocytes appear as elongated, sometimes horseshoe-shaped cells embracing the erythrocyte nucleus; the cytoplasm of the gametocyte contains pigment granules accumulating as a result of the incomplete digestion of hemoglobin (Figure 3-31). Various species of *Haemoproteus* are transmitted by *Culicoides*, Hippoboscidae, and *Chrysops*, which become infected when they ingest erythrocytes containing gametocytes. Fertilization, development of oocysts, and salivarian transmission of sporozoites to the vertebrate host resemble the corresponding events in the life history of *Plasmodium*. *Haemoproteus* is essentially nonpathogenic.

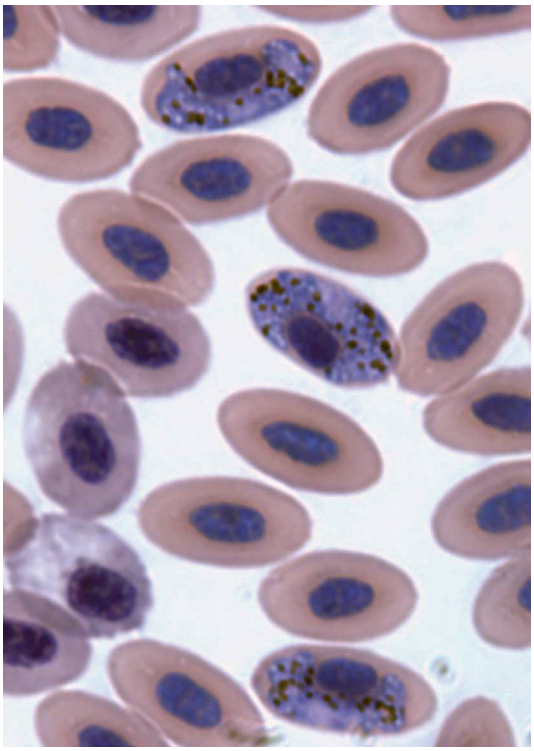

FIGURE 3-31. *Haemoproteus* sp. in avian red blood cells (Giemsa stain).

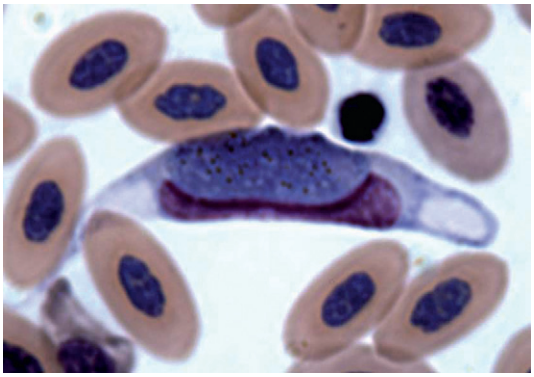

FIGURE 3-32. *Leucocytozoon* sp. in a blood smear from a red-tailed hawk (Giemsa stain).

LEUCOCYTOZOON. *Leucocytozoon* species are parasites of domestic and wild birds; *Leucocytozoon simondi* causes acute, fatal disease in ducks and geese, as does *Leucocytozoon caulleryi* in chickens and *Leucocytozoon smithi* in turkeys. Schizogony occurs in hepatocytes and vascular endothelial cells of various tissues (see Figure 8-38), producing merozoites that invade erythroblasts, erythrocytes, lymphocytes, and monocytes, and there develop into gametocytes. *Leucocytozoon* gametocytes differ from those of *Plasmodium* and *Haemoproteus* in not containing pigment granules and in greatly distorting the host cell (Figure 3-32). Some gametocytes are round and push the host cell nucleus to one side so that it forms a cap on the parasite. Others are oval or elliptical in cells that become elongated and bizarre in appearance as the parasite grows. *Simulium* species serve as intermediate hosts.

HEPATOCYSTIS. *Hepatocystis* species are parasites of the lower monkeys, fruit bats, and squirrels of the Old World.

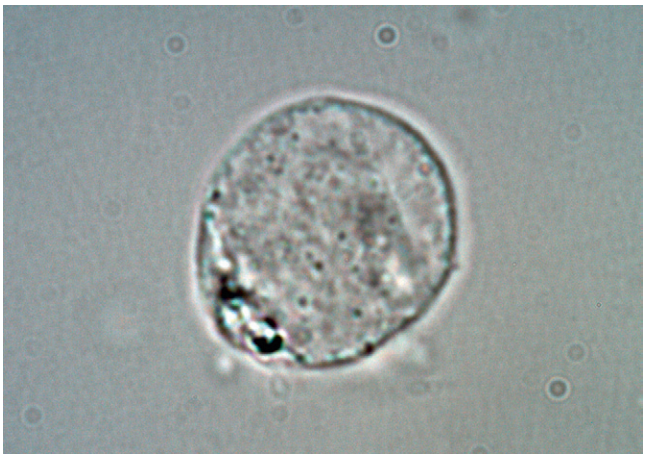

FIGURE 3-33. *Blastocystis hominis* in culture. Vacuolate cyst–like form representative of the stage typically seen in feces.

Schizogony occurs in hepatocytes, requires 2 months, and results in large schizonts called **merocysts.** Merozoites released from meronts invade erythrocytes and develop into gametocytes. *Culicoides* species are the probable vectors.

STRAMENOPILES

The Stramenopiles are one of the three groups that along with the Alveolata and Rhizaria form the phylogenetic grouping known as SAR. Most Stramenopiles, like the ciliates, are not parasitic. The Stramenopiles include diatoms, water molds, downy mildews, golden algae, and giant brown algae. One group known to parasitologists placed within this group are the organisms known as the Opalinids, giant ciliate–like creatures that live in the posterior bowel of frogs. The reason the group is considered here is that this is currently where the organism *Blastocystis* has been placed. *Blastocystis hominis* has long been associated with loose stools in people, but until this systematic change in status, it had typically been considered a yeast, not a protist (Zierdt, 1988). It is now also being considered as a potential water-borne zoonotic agent (Leeli et al, 2012).

BLASTOCYSTIS HOMINIS. This parasite is not uncommon in human fecal samples, sometimes being wall to wall under the coverslip in centrifugal zinc-sulfate flotations on fresh feces. The cyst passed in the feces has a thick wall and can measure anywhere from 6 to 40 µm in diameter, typically 6 to 15 µm. Characteristically, these cysts contain a large central body resembling a vacuole that has a narrow rim of cytoplasm and inclusion bodies (Figure 3-33). When fecal slides are stained with trichrome stain, the central body will typically appear green and the inclusion bodies a dark red.

The biology is not known in detail. It is assumed that the cyst is the transmission stage that is passed from host to host via direct fecal contact in water or contaminated food. Upon cyst ingestion, there is infection of epithelial cells where they take multivacuolar and amoeboid forms. It has been proposed that the multivacuolar forms produce thin-walled cysts that repopulate the intestine via internal autoinfection, and that the amoeboid forms can either divide or produce thick-walled cysts.

Molecular systematics have currently divided morphologically similar organisms into somewhere around 14 subtypes (STs). In general, subtypes 1 to 4 are found in humans and other primates, ST5 is in pigs, ST6 and ST7 are typically in birds, ST8 and ST9 are in primates and birds, ST10 is in cattle, ST11 is in elephants,

ST12 is in giraffes and kangaroos, ST13 is in the Australian macropodid quokka, and ST14 is in calves (Parkar et al, 2010; Santin et al, 2011; Fayer, Santin, and Marcarisin, 2012). It is not all this neat, unfortunately, and this is where it begins to become a bit more complicated relative to the zoonotic issue.

Pigs are infected with ST5, and it seems rather well associated with pigs. In Spain, on large intensive pig farms, the prevalence of *B. hominis* was related to age (Navarro et al, 2008). Piglets had an infection rate of 15%, weaners 75%, 2- to 4-month growers 70%, 4- to 6-month growers 60%, and sows 10%. It has been stated that cysts can remain viable in swine manure slurries (Snell-Castro et al, 2005).

Two studies have directly examined the issue of zoonotic transmission: one in zoo animals and zoo keepers (Parkar et al, 2010), and one in pets and their human family members (Nagel et al, 2012). In the study of zoo keepers, 12 of 19 were found to have *B. hominis*, and only 2 of 22 workers without animal contact had cysts in their feces. Of the 11 sequenced zoo keeper isolates, 6 were ST3, and 4 were ST1. In the study examining various therapies for *B. hominis* patients in Australia, samples were also collected from their pets, and both were sequenced. ST1, ST3, and ST4 were found in the patients, and in all eight cases, similar matching subtypes were found in the pet dog or cat.

UNIKONTS

Some of the amoebae have been removed from the Protista and moved over to the world of animals and fungi. The groups left within the Excavata were *Naegleria* and its allies. Included with the amoebae that were moved to the Unikonts are all the other parasitic amoebae: those of the intestinal tract (*Entamoeba* and its associates) and some of the facultative amoebae important in human and animal medicine, including *Acanthamoeba* and *Balamuthia*.

AMOEBOZOA

Entamoebida

Entamoeba

Entamoeba histolytica is principally a parasite of the large intestine that causes amoebic dysentery in humans, an endemic disease of the tropics that occurs sporadically in temperate regions. Amoebic abscess of the liver is a serious, frequently life-threatening sequela. Humans also host a few nonpathogenic amoebae *(Entamoeba dispar*, *Entamoeba hartmanni*, *Entamoeba coli* [see Figure 7-122], *Iodamoeba buetschlii*, and *Endolimax nana)*. Dogs are believed to serve in some areas as occasional hosts of the pathogenic *Entamoeba histolytica* and the commensal, *Entamoeba dispar.* Other amoebic trophozoites and cysts frequently appear in fresh fecal smears of perfectly healthy cattle, sheep, goats, horses, and swine but are usually overlooked. They have been described in the past as separate species (e.g., *Entamoeba bovis*, *Entamoeba ovis*) but have received almost no attention in recent years.

Special cases exist in which amoebae are of clinical importance, notably in primates. For example, a case of gastric amoebiasis characterized by anorexia, diarrhea, and weight loss was reported in the silvered leaf monkey *(Presbytis cristatus)* (Palmieri, Dalgard, and Connor, 1984). The normal high pH level (5.0 to 6.7) of the stomach of leaf monkeys and the stress of capture, shipment, and confinement were considered to have contributed to the extensive gastric involvement observed. *Entamoeba invadens* causes severe disease and death in captive reptiles. For example, 200 of 500 red-footed tortoises *(Geochelone carbonaria)* imported into southern Florida died over a period of 2 months, showing signs of anorexia, listlessness, and diarrhea. Necropsy examination revealed necrosis of the duodenal mucosa and multifocal hepatic necrosis. Amoebae were found in both duodenal and hepatic lesions histologically (Jacobson, Clubb, and Greiner, 1983).

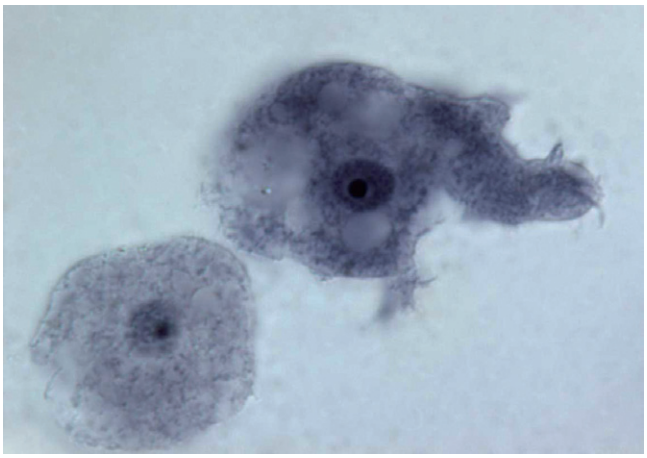

FIGURE 3-34. *Acanthamoeba* trophozoites from culture. Note the filamentous pseudopods and the large central karyosome within the nucleus.

The parasitic amoebae reproduce asexually, usually by binary fission. Actively parasitic forms, called **trophozoites,** display amoeboid motion when recovered from fresh feces and kept at body temperature. Most species form cysts, which in certain cases are multinuclear. Trophozoites are more likely to be found in fluid feces, and cysts in formed feces.

Treatment of *Entamoeba histolytica* Infection

Little is known about the treatment of canine amoebiasis. In humans, metronidazole is the drug of choice in the treatment of intestinal and hepatic amoebiasis and is therefore a logical choice for treating canine amoebiasis. Roberson (1997) suggests oral administration of 50 mg of metronidazole per kilogram body weight daily for 5 days.

Acanthamoebida

Facultative Amoebiasis

ACANTHAMOEBA. The facultative amoebae that are found within the Unikonts do not have a flagellate form. The best known members are those species in the genus *Acanthamoeba* (Figure 3-34; and see Figure 8-14) that cause chronic amoebic encephalitis (Sell et al, 1997; Visvesvara and Schuster, 2008a and 2008b) and acanthamoebic keratitis (Schaumberg et al, 1998). Cases of acanthamoebic encephalitis have been reported in gorillas, monkeys, dogs, sheep, cattle, horses, kangaroos, birds, reptiles, amphibians, and fish (Reed et al, 2010; Visvesvara and Schuster, 2008a and 2008b). A dog has also been reported to have succumbed to acanthamoebiasis mainly of the lungs, liver, and kidneys secondary to long-term immunosuppressive therapy for steroid-responsive meningitis-arteritis (Kent et al, 2011).

BALAMUTHIA. The amoeba, *Balamuthia mandrillaris*, was found first to cause disease in a mandrill from the San Diego Zoo (Visvesvara et al, 1993). This parasite has since killed gorillas, baboons, gibbons, monkeys, horses, sheep, and dogs (Hodge et al, 2011; Visvesvara and Schuster, 2008b; Mätz-Rensing et al, 2011). Human cases have also been reported (Bravo and Seas, 2012).

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CHAPTER 4

Helminths

Of the creatures known as worms or helminths, the group that most commonly comes to mind, the Annelid worms including the earthworms and various terrestrial and aquatic oligochaetes and polychaetes, usually does not consist of parasites, although they may serve as various intermediate and paratenic hosts. The typical parasitic worms of vertebrates belong to the phyla Platyhelminthes (flatworms, flukes, and tapeworms), Nematoda (roundworms), Acanthocephala (thorny-headed worms), and occasionally larvae of the group known as the Nematomorpha (Gordian worms). Tongue worms of the parasitic class Pentastomida are wormlike in appearance but being of the phylum Arthropoda are discussed in Chapter 2. This list of parasitic helminth taxa does not exhaust Nature's bounty of "small, elongate, and slender, creeping or crawling animals, usually soft-bodied, naked, and limbless or nearly so; any animal having a real or fancied resemblance to an angleworm or earthworm" (*Webster's New International Dictionary,* ed 2, Springfield, Massachusetts, 1935, G & C Merriam Co). However, it does include all of the worms in which veterinarians are particularly interested.

PHYLUM PLATYHELMINTHES

The Platyhelminthes differ markedly from the groups of the Ecdyozoa, which includes the Arthropods, Nematodes, Acanthocephala, and Nematomorpha, in that platyhelminths unlike the Ecdysozoa are not covered with an external cuticle and do not molt (i.e., shed their cuticle as they grow or metamorphose). Platyhelminths are covered by a syncytium of cells and have no body cavity (coelom), which is why they are called *acoelomates.* The platyhelminths are bilaterally symmetrical animals that are typically hermaphroditic. The digestive tract if present typically has only one opening, the mouth, with digested food being regurgitated from the oral opening; the Cestoidea do not have a digestive tract in any of the life stages (i.e., in neither larvae or adult stages). The excretory system is a tubular network with clumps of flagellum-bearing cells, the so-called flame cells, which propel waste along the collection system to the excretory pore. Almost invariably, reproduction results in the formation of eggs that leave the body of the adult form via the genital pore.

The parasitic Platyhelminthes, which contains a number of free-living species in several different groups, also contains the parasitic forms placed together in three classes, the Trematoda, Monogenoidea, and Cestoidea, within the Superclass Neodermata. The planarians that sometimes are found by aquarists in fish tanks and then are brought to veterinarians for identification as possible parasites are actually in one of the other groups of mostly free-living, carnivorous platyhelminths. Most of the members of the other groups of the Platyhelminthes are better known to naturalists than they are to veterinarians.

Of the three classes of parasitic forms within the subclass Neodermata, the two with most direct relevance to current veterinary medicine are the Trematoda and the Cestoidea. Within the Trematoda are the digenetic trematodes or flukes of importance to veterinary medicine, with adult stages occurring in the intestine, bile ducts, lungs, blood vessels, or other organs of their vertebrate final hosts. The Cestoidea group includes the tapeworms that are parasites of the intestine of vertebrates with larvae that are parasites of different vertebrates or invertebrates. The Monogenoidea undergo direct development and are mainly ectoparasitic on the gills of aquatic and amphibious animals. *Gyrodactylus* and *Dactylogyrus,* for example, are common and pathogenic monogenean parasites of the skin and gills of aquarium fishes. These parasites are currently of interest to few veterinarians, but as fish farming increases, as the popularity of aquarium fish continues to rise, and as more veterinarians assume roles as experts in this area, they will require greater coverage in textbooks on veterinary parasitology.

CLASS TREMATODA

Subclass Digenea

Life History

The subclass Digenea is so called because its members undergo indirect development with asexual and sexual generations parasitizing alternate hosts. The asexual generations typically occur in mollusks, often snails, and the sexual generations are found in vertebrates. Second-intermediate hosts in the life cycles often serve as a means of getting the trematode from its molluskan host to the vertebrate, where it will mature. All flukes infecting dogs, cats, ruminants, horses, and swine are digeneans. The life history of *Fasciola hepatica,* depicted in Figure 4-1, is typical of the Digenea and is a good place to start, because the life cycle is simple and the only host other than the vertebrate final host is a snail.

Adult *F. hepatica* flukes (Figure 4-2) live in the bile ducts of ruminant and other mammalian hosts. Their eggs are carried first to the bowel lumen with the bile and then to the exterior with the feces. When deposited, each of these eggs consists of a fertilized ovum and a cluster of vitelline cells enclosed in an operculated capsule (Figure 4-3). Only if the egg falls into water will a ciliated larva called a **miracidium** develop inside it (Figure 4-4). The miracidium is completely covered with cilia and has a conical papilla at its anterior end for boring into the snail intermediate host, a pair of eye spots, a brain, a rudimentary excretory system, and a cluster

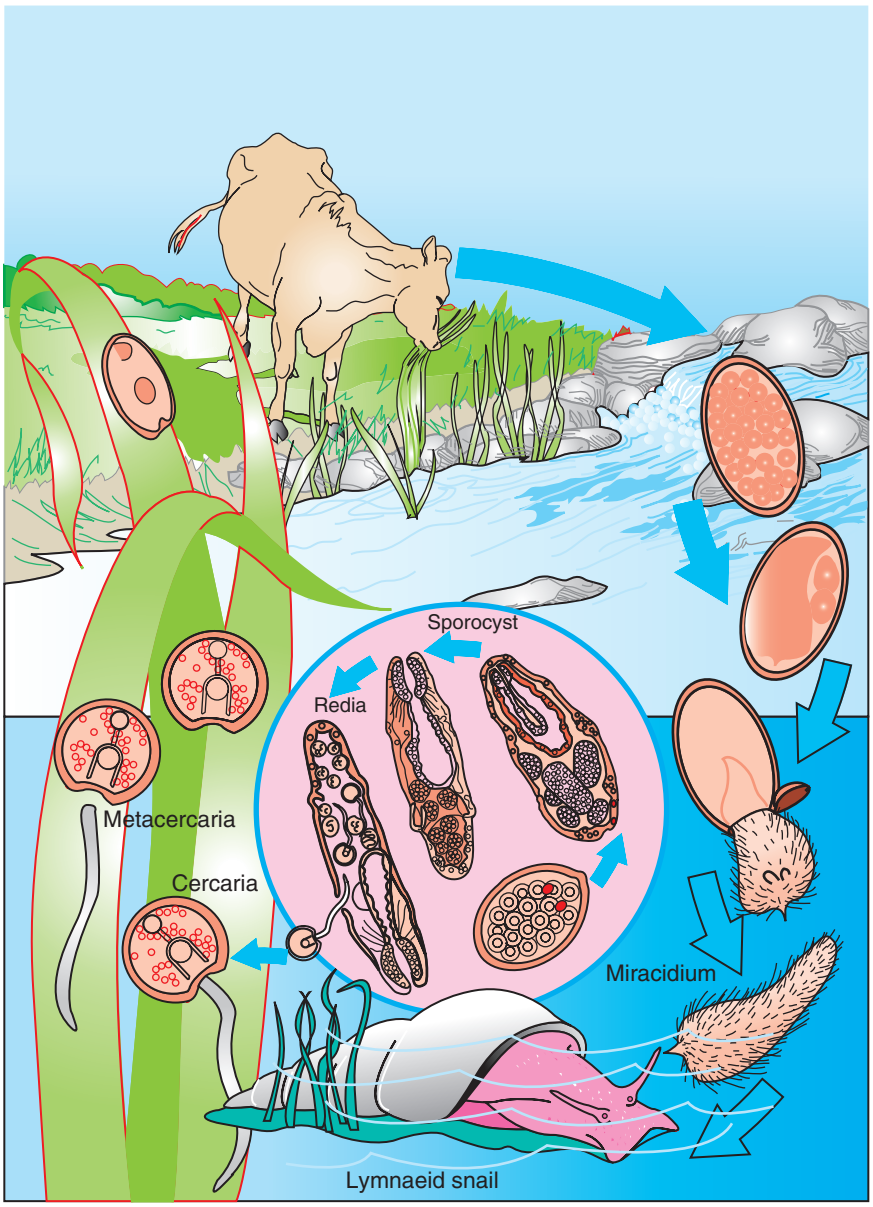


FIGURE 4-1. Life history of *Fasciola hepatica*. Adult liver flukes produce fertile eggs that leave the host by way of the common bile duct and intestinal tract. If these eggs are carried to water, a ciliated miracidium develops within them over a period of several weeks or months, depending on the temperature of the water. On hatching, the miracidia seek certain species of lymnaeid snails, in which they develop and multiply through one generation of sporocysts and two of rediae. Second-generation rediae produce free-swimming cercariae that leave the snail and encyst as metacercariae on various submerged objects, including aquatic vegetation. Ruminants and other animals become infected with *F. hepatica* when they ingest aquatic plants contaminated with metacercariae.

of germinal cells, the progenitors of the next generation of larvae (Figure 4-5). The miracidium, which is fully developed and ready to hatch after 2 to 4 weeks at summer temperatures, escapes from the egg capsule by pushing aside the operculum and swims about in search of a suitable species of snail (e.g., *Lymnaea truncatula*). If it fails to find such a snail within 24 hours, the miracidium exhausts its energy stores and dies.

If the miracidium is more fortunate, it bores into the snail's body, loses its ciliated covering, migrates to the gonad or digestive gland (often referred to as the *liver*), and forms a **sporocyst.** Each germinal cell, by growth and repeated divisions, becomes a germinal ball, and each germinal ball develops into a **redia** (Figure 4-6). The rediae grow until they burst the sporocyst wall and thus are liberated into the tissues of the snail. The redia has a mouth and digestive organs and eats its way through the snail's tissues. Like the sporocyst, the redia is packed with germinal balls, which are the progenitors of a second generation of rediae. Not all families of trematodes have redial stages, and some may have two or three redial generations.

In the case of *F. hepatica*, each germinal ball of second-generation rediae develops into yet a third kind of larva, the **cercaria** (Figure 4-7). The cercaria is a tadpole-like larva with a discoidal body and a long tail for swimming. The cercaria displays certain adult organs (e.g., oral and ventral suckers, mouth, pharynx, forked intestine, excretory canals with flame cells) and primordia of the reproductive organs. Special secretory cells alongside the pharynx are purely larval structures; they secrete a cyst wall within which the final larval stage will lie in wait for a grazing ruminant. When fully developed in a month or two of summer temperatures, the cercaria leaves the redia through a birth pore and makes its way out through

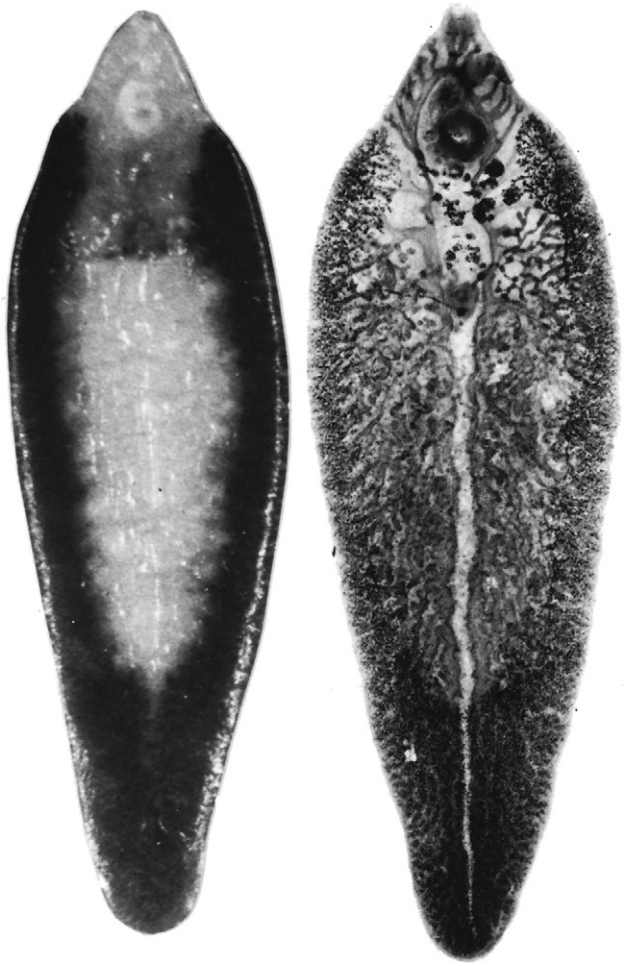


FIGURE 4-2. Adult *Fasciola hepatica* liver fluke. *Left,* An uncleared specimen. *Right,* A cleared, stained specimen.

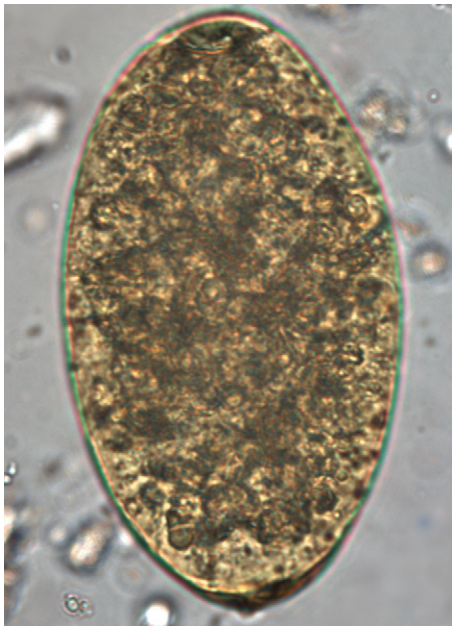


FIGURE 4-3. Egg of *Fasciola hepatica* from feces.

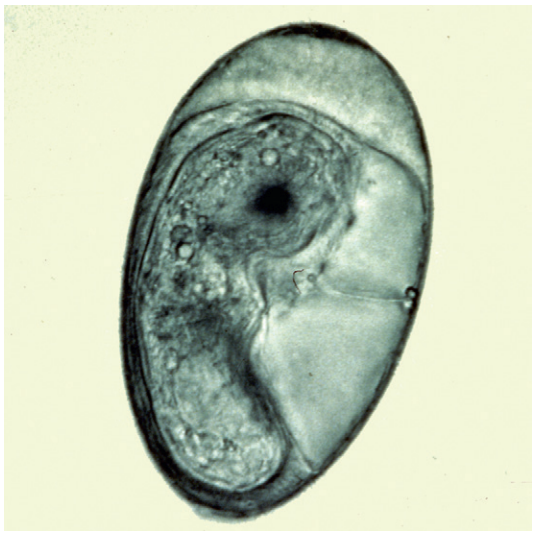


FIGURE 4-4. Egg of *Fasciola hepatica* containing a fully developed miracidium.

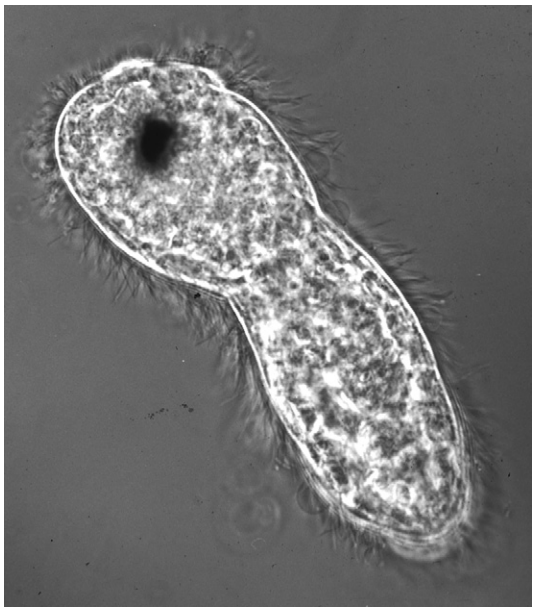


FIGURE 4-5. Miracidium of *Fasciola hepatica* swimming; electronic flash photomicrograph.

the snail's tissues and into the surrounding water. After a brief swim, the cercaria migrates a short distance above the water level on the surface of some plant and encysts, losing its tail in the process to become a **metacercaria,** the stage that is infective to sheep and other grazing mammals (Figure 4-8).

When ingested, the metacercarial cyst wall is digested in the host's small intestine. The young fluke penetrates the wall of the intestine and crosses the peritoneal space to the liver, which it penetrates (see Figure 8-41). After several weeks of boring about in the hepatic parenchyma, the young flukes enter the bile ducts, mature into sexually active adult flukes, and begin laying eggs at about a month and a half after infection. The complete life cycle of *F. hepatica* thus encompasses 3 or 4 months under favorable conditions. Therefore exposure to this parasite and patent infection tend to be rather more widely separated in time than is the case with most ruminant parasitisms.

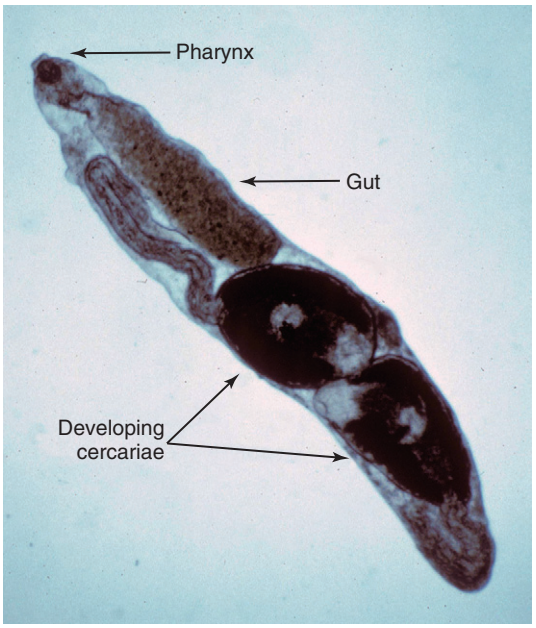


FIGURE 4-6. Redia of *Fascioloides magna* from a snail showing developed cercariae inside. (Courtesy Dr. Gary A. Conboy, Atlantic Veterinary College, University of Prince Edward Island, Canada.)

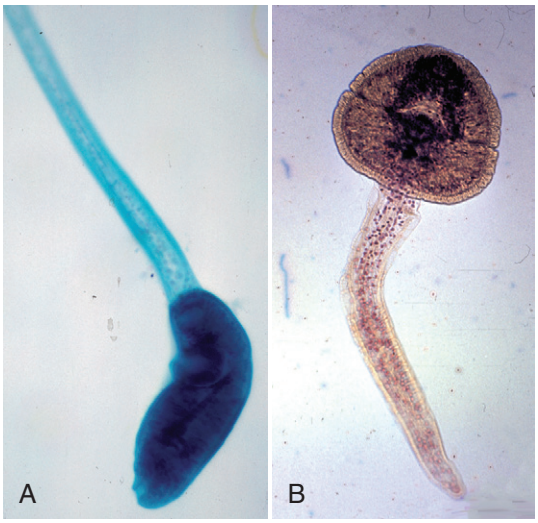


FIGURE 4-7. Cercariae of *Fasciola hepatica*.

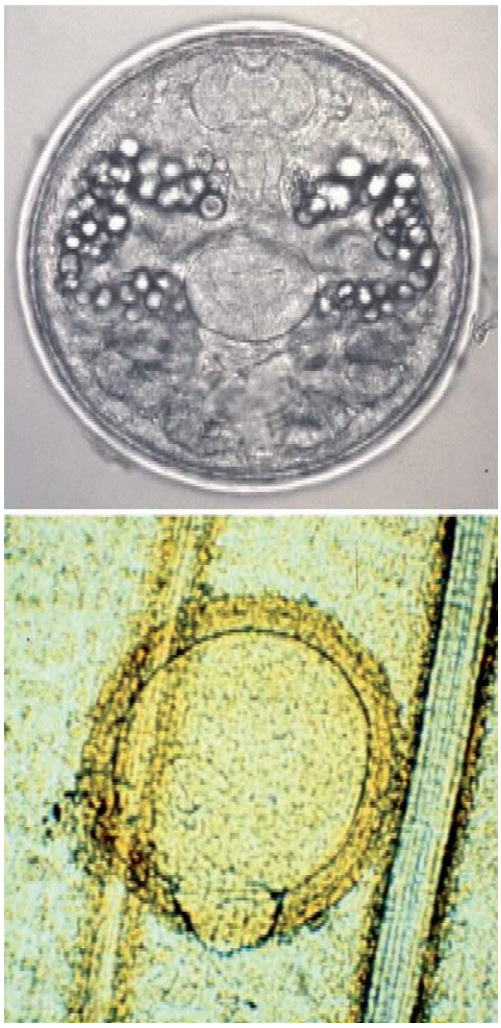


FIGURE 4-8. Metacercariae of *Fasciola hepatica*. *Top,* Free cyst. *Bottom,* Cysts on vegetation.

Digenean trematodes are very discriminating in their choice of snail hosts, and the geographic distribution of trematode species is therefore largely dictated by the geographic distribution of suitable species of snails. Adult trematodes, on the other hand, seem to be able to make do with a rather broad range of definitive host species.

The metacercarial stage determines what food the host must eat to become infected with an adult fluke. The strategies used by different trematodes vary (Figure 4-9). The metacercariae of fasciolids and paramphistomatids encyst on vegetation and have a strategic advantage when it comes to getting into grazing ruminants. For the troglotrematids, heterophyids, and opisthorchiids, the metacercariae encyst in intermediate hosts such as fish, crayfish, and crabs, and fish-eating mammals tend to serve as the final hosts. The diplostomids are found within amphibians or other vertebrate paratenic hosts, whereas the dicrocoeliids encyst in arthropods. The schistosomatids differ from other trematodes in that there is no metacercarial stage; rather, the cercariae penetrate the skin of the final host. Sometimes humans eat foodstuffs that put them in contact with possible trematode infections (e.g., *F. hepatica* has found its way into humans by way of watercress, *Dicrocoelium dendriticum* has entered humans through ingestion of ants containing metacercariae).

Identification

An adult digenean trematode typically is little more than a bag of reproductive organs with both sexes represented. Typically, it consists of two testes and one ovary, the anatomic positions of which provide diagnostic criteria. The genital pore may be identified by the convergence of male and female reproductive ducts. Usually the presence of a cirrus, or intromittent organ, helps to identify the male duct, and a procession of well-tanned eggs the female duct. The oral sucker surrounds the mouth, which is connected by way of the esophagus to a pair of blind ceca. The ceca are simple tubular sacs in most species but are intricately branched in the family Fasciolidae. The ventral sucker or acetabulum is often but not always near the genital pore. In the family Heterophyidae, both the ventral sucker and the genital pore are enclosed in an invagination, the ventrogenital sac, and an extra genital sucker or gonotyl surrounds the genital opening. The anatomic structures most used as taxonomic characters are labeled in Figure 4-10.

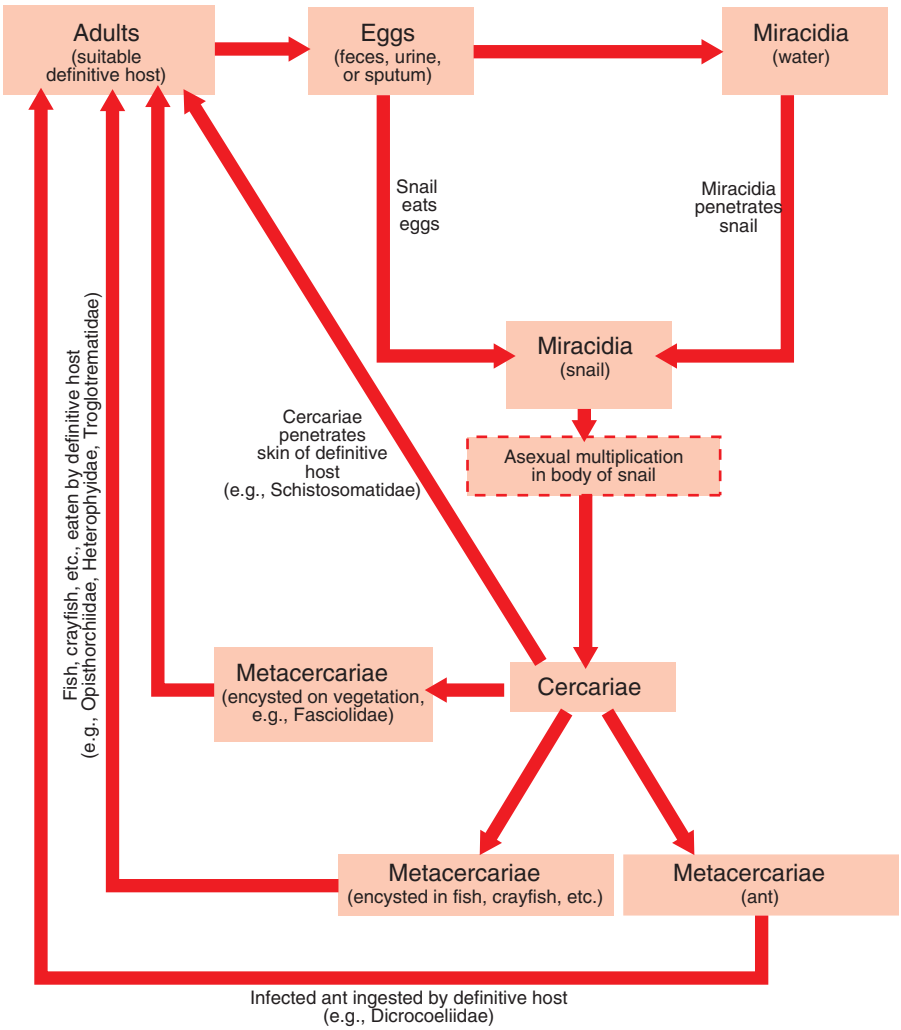


FIGURE 4-9. Some life history variations followed by trematode parasites of domestic animals.

Diagnostic criteria sufficient for identification of these families are presented in the following discussion. In general, identification of trematodes to family level combined with the host and organ listings provided in Chapter 7 will result in a sufficiently precise diagnosis to serve practical needs. An excellent guide to the identification of families and genera of trematodes of North America north of Mexico is S.C. Schell's *Handbook of Trematodes of North America North of Mexico* (Moscow, Idaho, 1985, University Press of Idaho). Because only a limited set of trematode species is likely to be found in domestic animals in any particular locality, knowledge of the endemic species is valuable. Sometimes the only way to acquire this information is to submit collections for expert identification. The specimens should be relaxed by overnight storage at 5°C and fixed in formaldehyde and acetic acid in alcohol (FAA), or shipped fresh and packed in plenty of ice in a well-insulated container (if the intention is to also seek molecular identification, forego the formaldehyde and acetic acid, and fix the flukes in 70% ethanol).

A Few Representative Families of Trematodes

Information on the geographic distribution and biology of some trematodes of veterinary importance can be found in Table 4-1.

Trematodes Acquired by Eating Metacercariae Encysted on Vegetation

FAMILY FASCIOLIDAE

IDENTIFICATION. The body is large and leaflike, with suckers close together at the anterior end; the ceca have numerous diverticula; and the ovary and testes are dendritic (Figure 4-11; see also Figure 4-2). *F. hepatica* and *Fasciola gigantica* are parasites of the liver and bile ducts of herbivorous mammals and man; *F. gigantica* is more restricted to the tropics. *Fascioloides magna* is a parasite of the liver of the white-tailed deer, but it will also infect other ruminants. *Fasciolopsis buski* is a parasite of the small intestine of pigs and humans in Asia; the ceca of this species do not have diverticula. Antemortem diagnosis of chronic fascioliasis occurs by demonstration of the large operculate eggs (see Figure 4-3) in the feces. Saturated sucrose floats but distorts the eggs, which nevertheless remain recognizable. Sedimentation techniques are preferred, however.

LIFE HISTORY. The life history of *F. hepatica* as presented in the preceding section is typical of the family. The geographic distribution of *F. hepatica* is worldwide but discontinuous. In North America, *F. hepatica* is found in the Gulf Coast States, the Pacific Northwest, the Caribbean, and eastern Canada. The lymnaeid snails that serve as intermediate hosts require neutral soils that remain reasonably moist throughout the year and tend to flourish

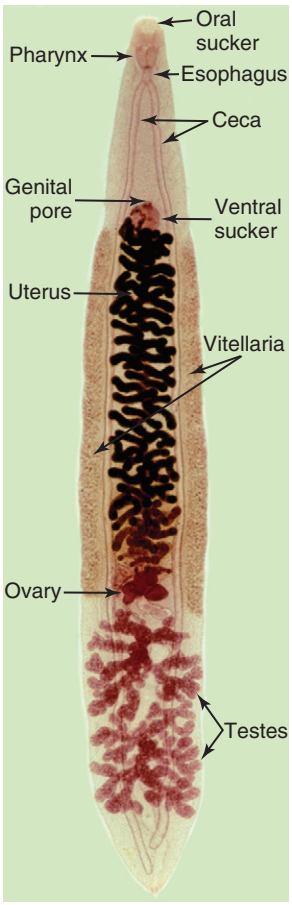


FIGURE 4-10. *Clonorchis sinensis* (Opisthorchiidae).

FIGURE 4-11. Liver flukes of ruminants. *Fasciola hepatica, Fasciola gigantica,* and *Fascioloides magna* belong to the family Fasciolidae. The small flukes scattered about are *Dicrocoelium dendriticum* of the family Dicrocoeliidae.

where winters are not so cold as to destroy the eggs and juvenile stages, thus permitting the parasite population to survive its season of hardship in both definitive host and environment. Because soil characteristics may vary dramatically within very short distances, it is not uncommon for one "fluky pasture" to contain all of the *F. hepatica* snails and metacercariae, whereas the rest of the farm may afford safe grazing. Small streams, ponds, and marshy areas are obvious snail-breeding areas, but any depression (e.g., rut, dead furrow) that can hold a bit of water for a while can serve as a source of infection during periods of adequate rainfall.

Transmission of fascioliasis occurs between February and July in Louisiana (Malone et al, 1984), but in the northwestern states, transmission gradually builds through the pasture season and reaches a peak during November (Hoover et al, 1984). Summer drought tends to interrupt the cycle on the Gulf Coast, whereas winter cold tends to do the same in the Northwest. However, special circumstances may produce unexpected results. For example, outbreaks of fascioliasis during periods of drought form an apparent paradox that can be explained as follows. When drought has laid waste to the rest of the pasture, green vegetation is still to be found at the water hole, and livestock may be forced to graze on aquatic plants, which they ordinarily eschew as unpalatable. Such plants are likely to be heavily contaminated with the resistant metacercariae of *F. hepatica,* and concentrated grazing on them may result in serious levels of infection. Because metacercariae are extremely resistant to drying, infection may follow feeding of hay grown on infested meadows far removed from the scene of an outbreak.

F. magna, one of the largest known trematodes, is widely scattered over North America; this fluke has also been reported in deer in Europe (Czech Republic, Slovakia, Hungary, Croatia, and eastern Austria). Adult *F. magna* organisms are found in cysts that communicate with the bile ducts of its normal definitive host, the white-tailed deer *(Odocoileus virginianus)*. In cattle, these cysts usually do not communicate with the bile ducts, and in sheep and goats, the young *F. magna* fail to mature and the juvenile flukes wander aimlessly and destructively in the liver tissue (see Figure 7-72). Therefore *F. magna* infection is nonpatent in cattle, sheep, and goats and cannot be diagnosed by fecal examination in these hosts. Aimlessly migrating juvenile *F. magna* are likewise very destructive in llamas, *Dama* deer, sika deer, and other cervids at game farms and petting zoos, where white-tailed deer probably serve as the source of infection. It has recently been shown that American bison *(Bison bison),* are not susceptible to infection with *F. magna,* although they can be infected with *F. hepatica* (Foreyt and Drew, 2010).

IMPORTANCE. Several clinical syndromes may be associated with liver fluke infection, depending on the numbers and stage of development of the parasite and on the presence or absence of *Clostridium novyi.* **Acute fluke disease** occurs during invasion of the liver by young flukes from recently ingested metacercariae. In heavy invasions, the trauma inflicted by young flukes tunneling about in the liver and the consequent inflammatory reaction result in highly fatal clinical illness characterized by abdominal pain with a disinclination to move. Postmortem examination reveals an abdominal cavity containing blood-stained exudate and an enlarged, friable liver covered with fibrin tags; large numbers of young flukes can be recovered from the cut surfaces. Heavy invasions of the sort associated with acute fluke disease may occur when lambs are turned onto pastures containing marshy areas that were heavily contaminated the previous season.

In certain cases, all that is needed to precipitate rapidly fatal disease is a minor trauma that provides clostridial organisms with

TABLE 4-1 Information on Some Trematodes of Veterinary Importance

Family	Genera and Species	Geographic Distribution	Hosts	Location in Host	Disease	Length of Adult	Length of Egg	Second Intermediate Host	Prepatent Period
Fasciolidae	*Fasciola hepatica*	Tropics and United States	Herbivorous mammals	Bile ducts	Hepatic fibrosis	3 cm	120 μm	Metacercariae on vegetation	60 days
	Fasciola gigantica	Africa	Humans	Bile ducts	Hepatic fibrosis	5 cm	120 μm	Metacercariae on vegetation	60 days
	Fasciolopsis buskii	Asia	Pigs and humans	Intestine	Intestinal upset	8 cm	120 μm	Metacercariae on vegetation	90 days
	Fascioloides magna	United States and Europe	White-tailed deer	Liver (cysts)	Hepatitis, kills other cervids and small ruminants, nonpatent cysts in cattle	10 cm	120 μm	Metacercariae on vegetation	270 days
Paramphistomidae	*Paramphistomum* and *Cortylophoron*	Worldwide	Ruminants	Rumen	Intestinal damage by immature flukes	10 mm	120 μm	Metacercariae on aquatic vegetation	80 days
Troglotrematidae	*Nanophyetus salmincola*	North Pacific rim	Dogs and cats	Intestine	Transmits *Neorickettsia helminthoeca*	1 mm	80 μm	Fish	7 days
	Paragonimus kellicotti	Eastern United States	Minks, dogs, cats	Lungs	Cysts in lungs	6 mm	90 μm	Crayfish	30 days
Heterophyidae	*Cryptocotyle*	United States: East Coast	Birds	Intestine	Enteritis	2 mm	30 μm	Fish	14 days
	Heterophyes	Middle East	Dogs and cats	Intestine	Enteritis	2 mm	30 μm	Fish	14 days
Opisthorchidae	*Opisthorchis*	Asia and Europe	Dogs and cats	Bile ducts	Very little	6 mm	30 μm	Fish	30 days
	Metorchis	United States	Foxes, pigs	Bile ducts	Very little	6 mm	30 μm	Fish	17 days
	Clonorchis	Asia	Dogs and cats	Bile ducts	Very little	6 mm	30 μm	Fish	60 days
Dicrocoelidae	*Dicrocoelium dendriticum*	New York, Quebec, British Columbia, Europe	Sheep, cattle, pigs, deer, woodchucks	Bile ducts	Fibrosis with chronic disease	10 mm	40 μm	Ants	80 days
	Platynosum fastosum	Caribbean and southern United States	Cats	Bile ducts and gallbladder	Hepatitis, fibrosis, vomiting, jaundice, diarrhea	7 mm	45 μm	Lizards	30 days

Diplostomatidae	*Alaria canis*	Northern United States and Canada	Dogs and foxes	Intestine	Very little	4 mm	100 μm	Frogs, paratenic hosts	35 days
	Alaria marcianae, Fibricola texensis	Southern United States	Raccoons and opossums						
Schistosomatidae	*Schistosoma mansoni*	Worldwide	Humans	Mesenteric veins	Hepatic fibrosis	10-20 mm; sexes separate	55-145 μm; lateral spine	None, penetrate skin	60 days
	Schistosoma haematobium	Africa	Humans	Veins of urinary bladder	Erosion of bladder wall	10 mm; sexes separate	60 × 140 μm; terminal spine	None, penetrate skin	70-84 days
	Schistosoma japonicum	Asia	Humans, cats, mammals	Mesenteric veins	Hepatic fibrosis	10 mm; sexes separate	58 × 85 μm; no spine	None, penetrate skin	35-42 days
	Schistosoma bovis	Africa	Cattle	Mesenteric veins	Hepatic fibrosis	10 mm; sexes separate	62 × 207 μm; terminal spine	None, penetrate skin	42 days
	Schistosoma margrebowiei	Africa	Horses, ruminants	Mesenteric veins	Hepatic fibrosis	10 mm; sexes separate	60 × 80 μm; no spine	None, penetrate skin	38 days
	Bivitellobilhania loxodontae	Africa	Elephants	Mesenteric veins	Hepatic fibrosis	10 mm; sexes separate	71 × 87 μm; no spine	None, penetrate skin	Not known
	Heterobilharzia americana	United States	Raccoons, dogs, opossums	Mesenteric veins	Hepatic fibrosis	10 mm; sexes separate	70 × 87 μm; no spine	None, penetrate skin	60 days
	Bird genera	Worldwide	Birds	Skin	Dermatitis in mammals	10 mm; sexes separate	Varied	None, penetrate skin	

some damaged and poorly oxygenated tissue in which to multiply and secrete their deadly toxins. Even the minor trauma associated with the migrations of a few *F. hepatica* (or *Taenia hydatigena* larvae) is enough to provide an appropriate environment for *C. novyi.* As is typical of clostridial infections, sheep die so fast that they hardly have time to be sick. Necropsy reveals focal liver necrosis and extensive subcutaneous hemorrhage; the latter is possibly responsible for the colloquial name "black disease." *C. novyi* also causes a lethal condition called "big head" in young rams, but here the precipitating trauma results from contests of physical prowess instead of parasite migrations.

Chronic fluke disease is associated with the presence of adult trematodes in the bile ducts and is characterized by the classical clinical signs of liver fluke infection. Gradual loss of condition, progressive weakness, anemia, and hypoproteinemia with development of edematous subcutaneous swellings are noted, especially in the intermandibular space and over the abdomen. Necropsy reveals distended, thickened bile ducts packed with adult trematodes. In cattle, the fibrotic ducts later calcify to produce what looks like a branching system of clay pipes. Isseroff, Sawma, and Reino (1977) demonstrated that the bile duct hyperplasia of fascioliasis is related to the excretion of large amounts of the amino acid proline by *F. hepatica.* Isseroff, Spengler, and Charnock (1979) have adduced evidence to the effect that proline synthesis and excretion by *F. hepatica* may account, at least in part, for the anemia that often accompanies infection with this fluke.

The presence of one fluke leads to condemnation of the liver in slaughtering establishments inspected by the U.S. Department of Agriculture (USDA). Tindall (1985) reported that almost one third of livers from cattle raised in Puerto Rico were condemned during the year ending in October 1984. Following Puerto Rico, in order of percentage of livers condemned, were Florida, Nevada, Oregon, Idaho, Utah, Washington, and California. Probably, liver condemnations far outweigh losses caused by clinical fascioliasis in economic importance. Briskey, Scroggs, and Hurtig (1994) examined livers from seven slaughterhouses in 17 of the western United States and found 368 of 1913 livers positive for liver flukes. *F. magna* causes considerable economic loss by producing wasted cattle livers condemned as unfit for human consumption, and its destructive migrations in the livers of sheep and goats virtually preclude small ruminant production in endemic areas.

TREATMENT AND CONTROL. Clorsulon (Curatrem) is administered to cattle orally as 8.5% suspension at a dosage rate of 7 mg/kg for treatment of immature and adult *F. hepatica* infections (Malone, Ramsey, and Loyacano, 1984; Courtney, Shearer, and Plue, 1985; Yazwinski et al, 1985). The dose of clorsulon (2 mg/kg) administered with ivermectin as Ivomec Plus is fully efficacious only against the adults of *F. hepatica*. Clorsulon is not licensed for use in dairy cattle of breeding age, and cattle must not be treated within 8 days of slaughter.

Albendazole is indicated for the removal of liver fluke from cattle at a dosage rate of 10 mg/kg of body weight and from sheep at 7.5 mg/kg. Albendazole is not licensed for use in dairy cattle of breeding ages, and cattle must not be treated within 27 days of slaughter. Albendazole (15 mg/kg) was effective in eliminating adult *F. hepatica* and in reducing the death rate among naturally infected goats in Montana (Leathers et al, 1982).

Other effective flukicides (diamphenethide, nitroxynil, oxyclozanide, rafoxanide, triclabendazole) are not available in the United States. There does appear to be resistance of *F. hepatica* to triclabendazole (Gordon et al, 2012).

F. magna presents a more difficult problem in domestic ruminants. Both clorsulon (24 mg/kg) and albendazole (26 mg/kg) were reasonably effective against immature and adult *F. magna* in its natural host, the white-tailed deer (Foreyt and Drawe, 1985). However, a drug must kill essentially all immature *F. magna* to benefit infected sheep and goats because survival of only a few young flukes is potentially lethal in these hosts. In sheep, a single treatment with clorsulon (15 mg/kg) 8 weeks after inoculation with metacercariae of *F. magna* was not sufficiently effective to be of practical value (Conboy, Stromberg, and Schlotthauer, 1988), whereas closantel (15 mg/kg orally or 7.5 mg/kg intramuscularly) was considered to "meet the need" (Stromberg et al, 1985). Unfortunately, closantel is unavailable to veterinarians in the United States.

Theoretically, aquatic snails can be controlled by draining swamps or by broadcasting molluscicides on the snail-infested waters. However, the continued existence of flukes where they have always been indicates that snail control measures are impracticable in many cases. Areas connected by streams with other snail-infested regions are generally not amenable to snail control measures. Periodic anthelmintic medication may help to reduce contamination of pastures with fluke eggs. When periods of drought or cold destroy *F. hepatica* eggs and snails weakened by infection with this parasite, control measures based on anthelmintic medication alone may produce satisfactory results. On the other hand, when large populations of eggs and infected snails are able to survive the year around, these must be attacked directly as well.

FAMILY PARAMPHISTOMATIDAE

IDENTIFICATION. Several flukes with similar life cycles have the ventral sucker at the posterior end of the body, whereas the ventral sucker of other trematodes is either on the ventral surface of the body or absent (Figure 4-12). The flukes making up what is discussed here as the Paramphistomidae are now considered to be in a dozen or so distinct families within the larger superfamily, the Paramphistomoidea; many are found in the stomach and rumen

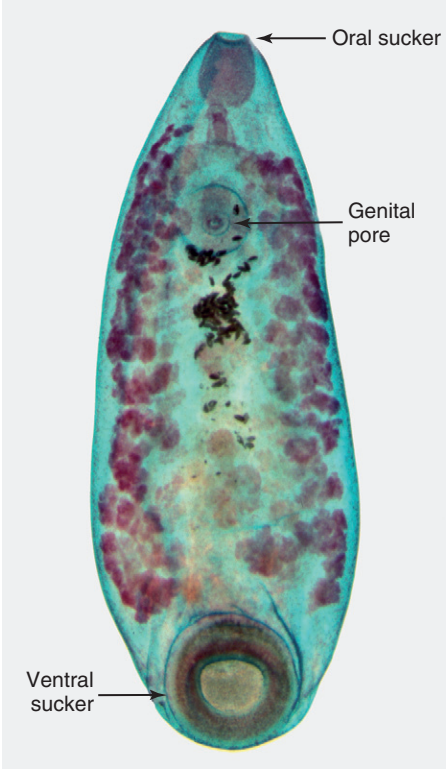


FIGURE 4-12. A rumen fluke of the family Paramphistomatidae.

while others are found in the intestine, often in the cecum and colon. For the sake of simplicity they are grouped here under this one heading familiar to most parasitologists.Genera and species include *Paramphistomum, Calicophoron,* and *Cotylophoron* (rumen flukes) (family Paramphistomidae); *Gastrodiscoides hominis* (a parasite of the intestine of humans, monkeys, and apes) (family Gastrodiscidae); and *Megalodiscus* species (parasites of the colon and cloaca of frogs) (family Cladorchiidae).

LIFE HISTORIES. Eggs of *Paramphistomum cervi* are undeveloped when passed in the feces of cattle, sheep, and goats. Miracidia develop in eggs deposited in water and hatch to invade snails of the genera *Physa, Bulinus, Galba,* and *Pseudosuccinea,* in which cercariae develop through one sporocyst and two redial stages. On emergence from the snail, the cercaria swims away to encyst on aquatic vegetation. Thus the extramammalian portion of the life history of a fluke in the genus *Paramphistomum* is very much like that of one in *Fasciola.* Metacercariae of *Paramphistomum* species excyst in the upper small intestine and migrate through the abomasum back to the rumen. With heavy infection, migration to the rumen tends to be prolonged, and disease of several months' duration may result; in an outbreak in calves in Great Britain, the described signs were diarrhea with depression, dehydration, and anorexia (Millar, Colloff, and Scholes, 2012). Once the flukes have finally arrived in the rumen and reticulum, the adult paramphistomes are relatively harmless (Rolfe and Boray, 1987).

Instead of encysting on aquatic vegetation as do other paramphistomatids, *Megalodiscus* cercariae encyst on the skin of frogs and tadpoles. The frogs become infected when they eat pieces of molted epidermis or tadpoles bearing metacercariae.

TREATMENT. Clorsulon at 2 mg/kg in combination with ivermectin at 0.2 mg/kg was ineffective in treating immature rumen flukes (Rolfe and Boray, 1993). Both hexachlorophene in a single dose of 20 mg/kg and oxyclozanide in two doses of 19 mg/kg 3 days apart were highly efficient against juvenile and adult paramphistome flukes, predominantly *Calicophoron calicophorum,* in cattle (Rolfe and Boray, 1987). Unfortunately, neither of these chemicals is available for use in domestic ruminants in the United States.

Trematodes Acquired by Eating Fish, Crayfish, Crabs, and Other Intermediate Hosts

FAMILY TROGLOTREMATIDAE

IDENTIFICATION. These are small flukes with an oral sucker that is nonterminal. The genital pore is immediately posterior to the ventral sucker, a cirrus sac is present, and the paired testes are large and oval and are located at about the level of the mid hindbody. The species of Troglotrematids of veterinary importance is *Nanophyetus salmincola,* which lives in the small intestine of its piscivorous host (Figure 4-13).

LIFE HISTORY OF *NANOPHYETUS SALMINCOLA*. The adult flukes are found attached to the mucosa of the small intestine of piscivorous carnivorans of the Pacific Northwest. Eggs are undeveloped when passed in the host's feces. Miracidia require about 3 months to develop in eggs laid in water and hatch spontaneously still later. The miracidia penetrate the freshwater snail *Oxytrema silicula,* in which cercariae develop in rediae. After emergence from the snail, these cercariae penetrate the skin of salmonid fishes and encyst in various tissues. Eating salmon or trout infected with metacercariae of this trematode infects the dog, cat, coyote, fox, bear, raccoon, or mink. *N. salmincola* is host in turn to a rickettsial agent, *Neorickettsia helminthoeca,* the causative agent of "salmon poisoning" in dogs. Salmon poisoning, characterized by hemorrhagic enteritis and lymph node enlargement, is diagnosed by the presence of trematode eggs in the patient's feces and is usually fatal unless treated with broad-spectrum antibiotics.

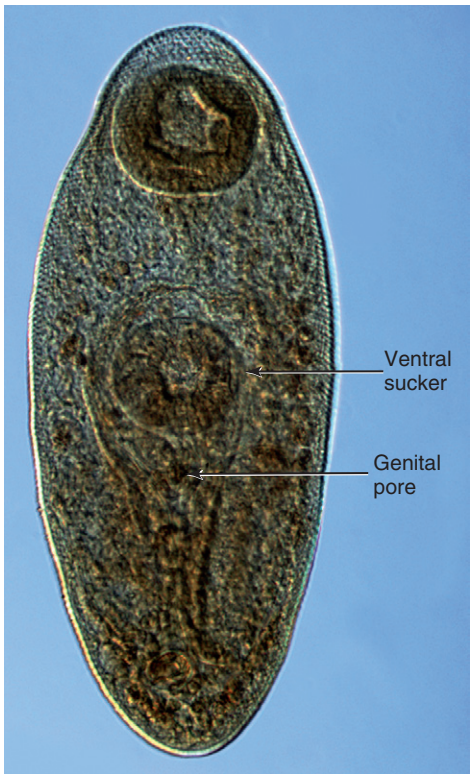


FIGURE 4-13. *Nanophyetus salmincola* (Troglotrematidae).

TREATMENT. Praziquantel, 7 mg to 38 mg administered subcutaneously or intramuscularly, has also been shown to be highly effective in the removal of *N. salmincola* from dogs and coyotes (Foreyt and Gorham, 1988).

FAMILY PARAGONIMIDAE

IDENTIFICATION. The most notable characteristic among the members of the only genus in this family, *Paragonimus,* is the habit of encysting in pairs in the lungs of mammals. They differ from other similarly appearing trematodes in lacking a cirrus sac and in having a highly dendritic vitellarial network. The only genus in this family is *Paragonimus* (Figures 4-14 and 4-15), which uses crabs and crayfish as the second intermediate hosts in its life cycle. The species of importance in North America is *Paragonimus kellicotti;* various other species are found in other parts of the world, with the exceptions being Europe and the continents of Australia and Antarctica. In the United States, cases appear to occur throughout the eastern half of the country.

LIFE HISTORY OF *PARAGONIMUS KELLICOTTI*. *Paragonimus kellicotti* occurs, usually in pairs, in pulmonary cysts (see Figure 8-44). Cats, dogs, and many species of wild mammals in North America may become infected by eating crayfish containing the encysted cercariae or by eating animals that have recently fed on crayfish. The large, vase-shaped eggs (see Figures 7-34, *B,* and 8-45) are swept up the tracheobronchial tree, swallowed, and passed out with the feces. If the eggs arrive in water, miracidia develop and hatch in about 2 weeks and enter an operculate snail, *Pomatiopsis lapidaria,* in which cercariae develop through one sporocyst and two redial stages. The cercariae leave the snail and encyst as metacercariae in freshwater crayfish. Radiographically demonstrable cysts develop in the lungs of cats at 28 days, and eggs are first shed in the feces about a month after infection. Signs of respiratory disease may be associated with *P. kellicotti* infection. The other

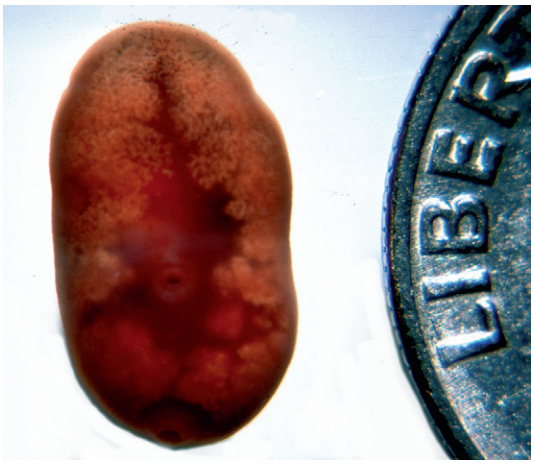


FIGURE 4-14. *Paragonimus kellicotti.* Living adult worm recovered at necropsy from a cyst in the lung of a cat.

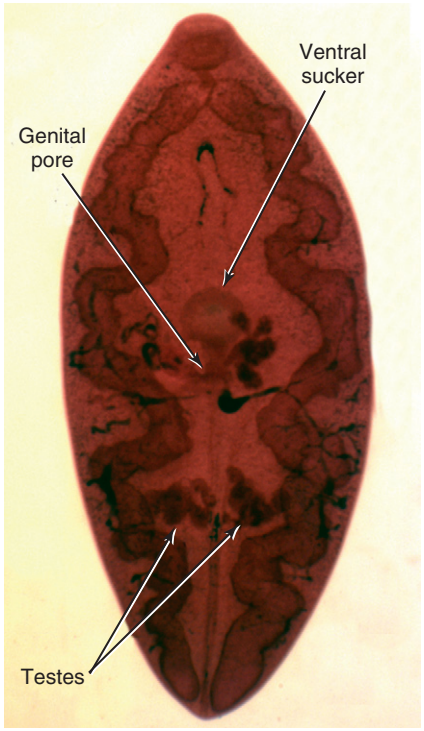


FIGURE 4-15. *Paragonimus kellicotti.*

species of *Paragonimus* around the world also use freshwater crabs and crayfish as their second intermediate hosts.

TREATMENT. Praziquantel, 23 mg/kg three times a day for 3 days, has been shown to be highly efficacious in removing *P. kellicotti* from the lungs of cats and dogs (Bowman et al, 1991). Fenbendazole, 50 mg/kg for 10 to 14 days, is also highly effective against these lung flukes (Dubey, Miller, and Sharma, 1979), as is albendazole, at a dosage rate of 25 mg/kg twice daily for 10 days (Johnson et al, 1981).

FAMILY HETEROPHYIDAE

IDENTIFICATION. The ventral sucker and the genital pore are withdrawn in a ventrogenital sac; one or more gonotyls (muscular suckers surrounding the genital pore) may be present (Figure 4-16).

Metagonimus yokogawai and *Heterophyes heterophyes* are parasites of cats, dogs, pigs, and humans in East Asia; infection is acquired

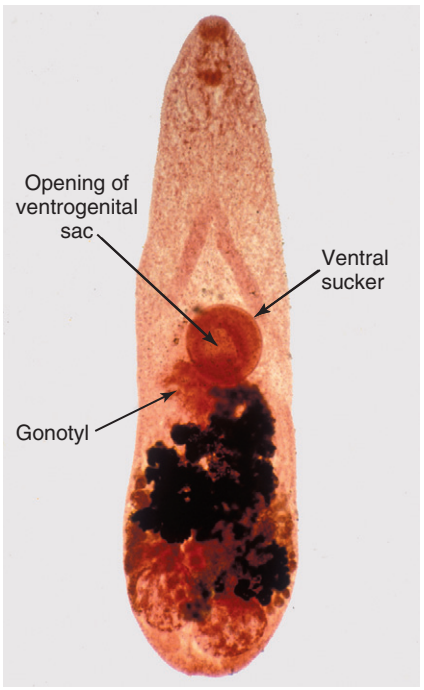


FIGURE 4-16. *Heterophyes* sp. from a dog in Lebanon.

by eating insufficiently cooked fish in which metacercariae have encysted.

Cryptocotyle lingua, a parasite of gulls and terns, produces severe enteritis in dogs, foxes, and minks a few days after they have eaten a small North Atlantic fish, the cunner, in which metacercariae are found in the subcutaneous tissues surrounded by black host capsules. The appearance of infected fish has led to the colloquial name, "black spot disease." A black host capsule is also observed surrounding various other species of trematode metacercariae and is not peculiar to *C. lingua.* Cercariae of *C. lingua* develop in the periwinkle *Littorina littorea,* a marine snail.

FAMILY OPISTHORCHIIDAE

IDENTIFICATION. The uterus and the ovary are anterior to the testes. No cirrus sac is present, and the genital pore is immediately anterior to the ventral sucker of these flat, translucent, fusiform, or oval parasites of the bile and pancreatic ducts of mammals, birds, and reptiles (Figure 4-17; see also Figure 4-10). Opisthorchiids might be confused with dicrocoeliids because they are similar in size, shape, and location in the host, but in dicrocoeliids, the ovary is posterior to the testes. Species include *Opisthorchis tenuicollis, Opisthorchis felineus, Metorchis conjunctus, Metorchis albidus, Parametorchis complexus, Clonorchis sinensis,* and others.

LIFE HISTORY OF *OPISTHORCHIS TENUICOLLIS*. Adult trematodes are parasites of the bile and pancreatic ducts and small intestine of dogs, cats, foxes, pigs, and humans. When deposited in the host's feces, eggs containing miracidia are eaten by a snail *Bithynia tentaculata,* in which cercariae develop in rediae. The cercariae encyst as metacercariae in carp, bream, and roach. The definitive host becomes infected by eating these freshwater fish.

IMPORTANCE. Opisthorchids display a rather low order of host specificity, and each species is capable of infecting many species of fish-eating mammals. Uncomplicated infection with moderate numbers of opisthorchiids is usually asymptomatic, but chronic infection with heavy worm burdens may lead to severe hepatic insufficiency.

TREATMENT. Praziquantel at 30 mg/kg PO once daily should be efficacious (Hong et al, 2003).

FIGURE 4-17. *Parametorchis* sp. (Opisthorchiidae).

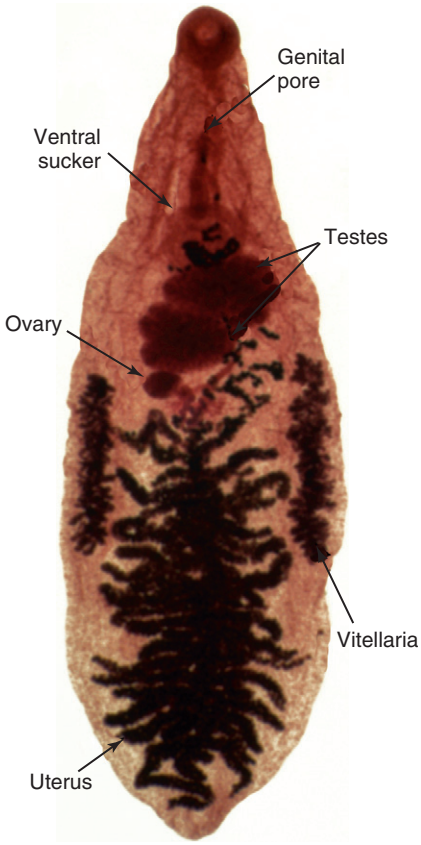


FIGURE 4-18. *Dicrocoelium dendriticum* (Dicrocoeliidae).

Trematodes Acquired by Eating Arthropods or Vertebrate Paratenic Hosts

FAMILY DICROCOELIIDAE

IDENTIFICATION. The body is translucent. The ovary is posterior to the testes of these parasites of the gallbladder and bile and pancreatic ducts of mammals, birds, and reptiles (Figures 4-18 and 4-19; see also Figure 8-47).

DICROCOELIUM DENDRITICUM. Whereas most trematode life histories involve water, this species is adapted to a sequence of hosts that frequent dry habitats. Adult *D. dendriticum* are parasites of the bile ducts of sheep, cattle, pigs, deer, alpacas, woodchucks, and cottontail rabbits. Embryonated eggs deposited in the host's droppings are ingested by the terrestrial snail *Cionella lubrica,* in which long-tailed cercariae develop in daughter sporocysts. As the cercariae leave the sporocysts, the snail secretes mucus around masses of them to form so-called slime balls, in which they are expelled from the snail. These slime balls are apparently esteemed as food by the ant *Formica fusca,* in which the cercariae encyst as metacercariae. The definitive host becomes infected by inadvertently ingesting infected ants while grazing; the metacercariae excyst in the small intestine and migrate up the common bile duct into the finer ramifications of the biliary tree.

Importance. *D. dendriticum* causes no clinical illness in cattle, lambs, or yearling sheep, but these trematodes are long-lived, and pathologic changes in the liver increase in severity and extent with the duration of the infection. Therefore in older sheep, *D. dendriticum* infection causes progressive hepatic cirrhosis manifested clinically as cachexia, lowered wool production, decreased lactation, and premature aging. In short, *D. dendriticum* makes sheep husbandry unprofitable by curtailing the reproductive life of the ewe flock. Llamas and alpacas in Switzerland and southern Germany have been found infected with disease and showing a decline in general condition, recumbence, decreased body temperature, and anemia, with major alterations in the liver at necropsy with cirrhosis,

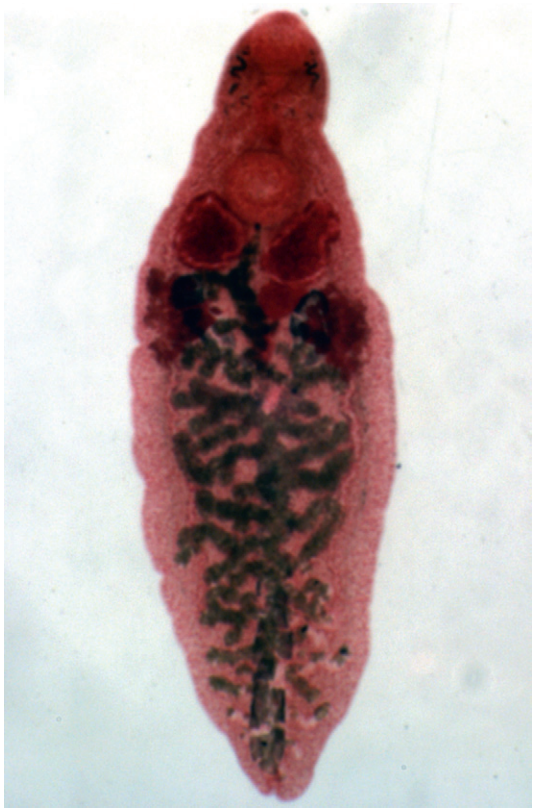


FIGURE 4-19. *Platynosomum fastosum* (Dicrocoeliidae).

abscesses, granulomas, and large numbers of *D. dendriticum* (Wenker et al, 1998).

Treatment. Albendazole administered orally to sheep at 15 to 20 mg/kg is highly effective against adult *D. dentriticum* (Theodorides, Freeman, and Georgi, 1982). Llamas were successfully treated with a single oral dose of praziquantel at 50 mg/kg (Wenker et al, 1998).

PLATYNOSOMUM FASTOSUM. This parasite of the bile and pancreatic ducts of cats occurs in southeastern United States and the Caribbean (see Figure 4-19). The life cycle involves cercariae with very short tails that develop in sporocysts in terrestrial snails that eat the trematode eggs, a second intermediate host; terrestrial isopods (pill bugs) that ingest the sporocysts with cercariae after they leave the snail; and encysted metacercariae in the bile duct and gallbladder of amphibians and reptiles. Infection in cats is acquired by eating lizards, toads, geckos, and skinks containing metacercariae (Chung, Miyahara, and Chung, 1977; Eckerlin and Leigh, 1962). The infection has been taken to Hawaii in introduced anoles (Goldberg and Bursey, 2000). In St. Kitts in the West Indies, a survey of 100 stray cats revealed that 81% were infected with *Platynosomum;* this finding was based on the finding of eggs in the feces (Krecek et al, 2010). It has been reported that infection with *P. fastosum* can be associated with the development of cholangiocarcinomas in infected cats (Andrade et al, 2012).

Treatment. Praziquantel 20 mg/kg markedly reduced the number of *Platynosomum* eggs passed in the feces of cats (Evans and Green, 1978). On the suggestion to a practitioner in Florida that it might be possible to treat platynosomiasis with elevated doses of praziquantel, the reply was that the last infected cat with hepatic dysfunction died when so treated. Thus it was thought that surgical removal of the flukes was the best course of therapy. Albendazole is another logical choice.

EURYTREMA PROCYONIS. This common parasite of the pancreatic duct of the raccoon is thought to use as the second intermediate host arthropods that become infected when they ingest the sporocysts that are extruded by infected snail, as occurs with *D. dendriticum* and *P. fastosum.* A related species, *Eurytrema pancreaticum,* in Southeast Asia and China, uses grasshoppers as the second intermediate host and ruminants as the final host. *E. procyonis* was reported from a New York State domestic cat with a 2-year history of weight loss and vomiting, probably resulting from pancreatic fibrosis and atrophy (Anderson, Georgi, and Car, 1987). In another cat seen in New York, ultrasonography after the discovery of eggs in the feces revealed an enlarged hypoechoic pancreas, a distended and thickened pancreatic duct, and elevated pancreatic lipase immunoreactivity (Vyhnal et al, 2008). After oral treatment of this cat with 34 mg praziquantel, 34 mg pyrantel, and 170 mg febantel, for 5 days, the feces was negative for eggs, most of the ultrasonographic abnormalities had resolved, and pancreatic lipase immunoreactivity levels had normalized. Treatment of the cat with 30 mg/kg of fenbendazole daily for 6 days resulted in detection of no additional eggs in the feces (Roudebush and Schmidt, 1982).

Trematodes Acquired by Eating Amphibia or Vertebrate Paratenic Hosts

FAMILY DIPLOSTOMIDAE.

IDENTIFICATION. The body of these intestinal parasites of birds and mammals is divided into a flattened or spoon-shaped forebody containing oral and ventral suckers and a bulbous tribocytic organ, and a cylindrical hindbody containing the reproductive organs (Figure 4-20). The forebody will wrap around the mucosa of the intestinal tract, forming a firm attachment between the fluke and the host's intestinal epithelium (Figure 4-21; see also Figure 8-49). Diplostomatids are most likely to be confused with members of the families Strigeidae, which have cup-shaped forebodies and leaflike tribocytic organs, and Cyathocotylidae, which have bulbous tribocytic organs but undivided bodies.

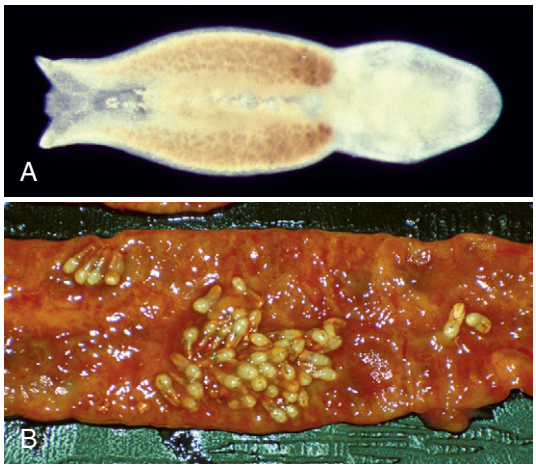


FIGURE 4-20. *Alaria canis* (Diplostomatidae). **A,** Living specimen detached from mucosal epithelium showing the forebody and the hindbody, with the forebody having a ventral groove for wrapping around a bit of host mucosa. **B,** Flukes attached to the intestinal mucosa.

FIGURE 4-21. *Alaria* sp. (Diplostomatidae) attached to the mucosa of a dog's small intestine.

***ALARIA* SPECIES.** The large, unembryonated egg (see Figure 7-34, *A*) is passed in the feces of the infected canid (Figure 4-22). If the egg is deposited in water, a miracidium develops and hatches in about 2 weeks to penetrate a snail of the genus *Helisoma,* in which cercariae develop in daughter sporocysts. Each cercaria that succeeds in penetrating the skin of a tadpole transforms into a special larval stage called a *mesocercaria,* which is limited to *Alaria* species and a few closely related genera. If the tadpole is eaten by a frog, snake, or mouse, the mesocercariae take up residence and

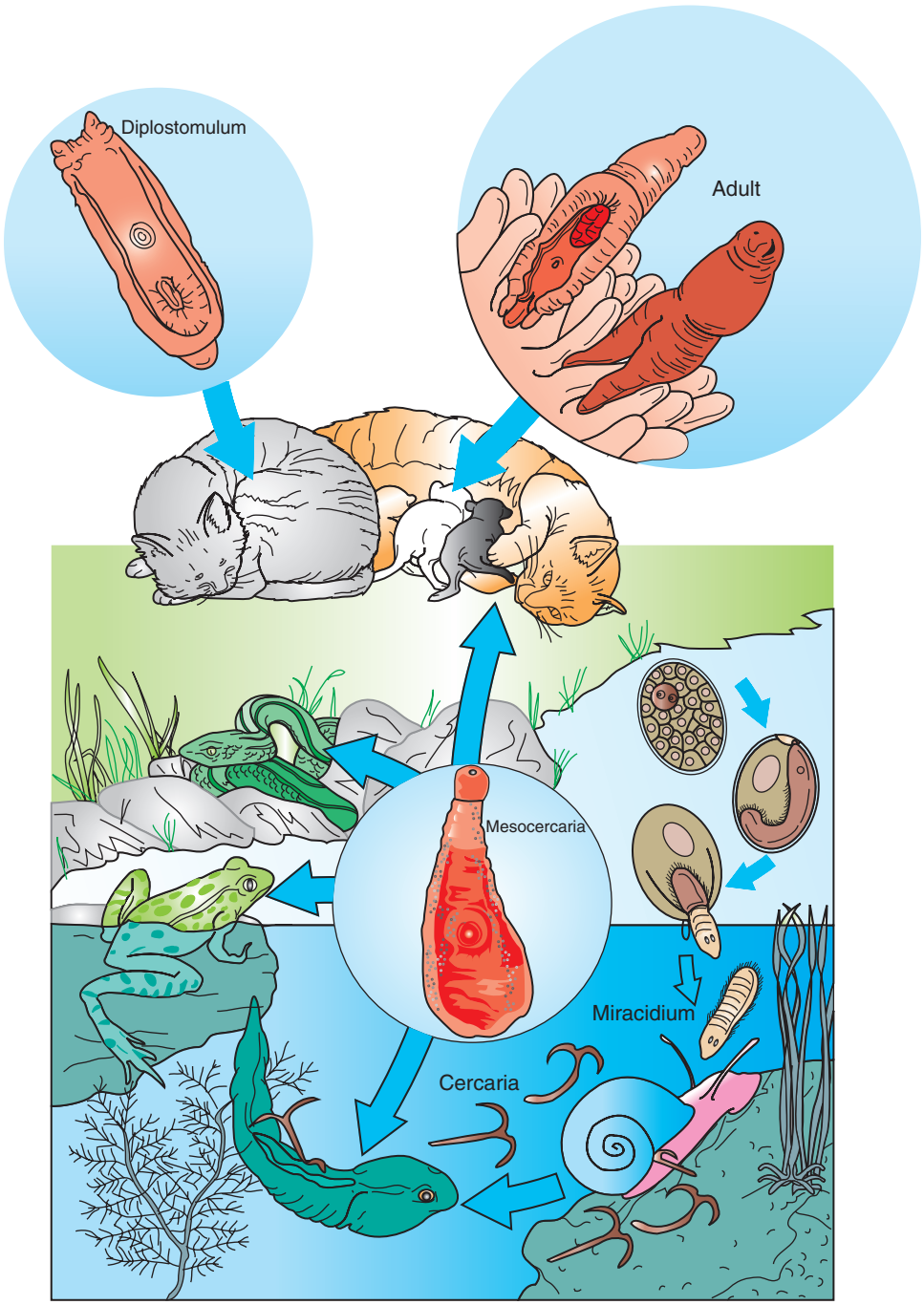


FIGURE 4-22. Life history of *Alaria marcianae* (Diplostomatidae). Miracidia develop in eggs deposited in water, hatch, and enter planorbid snails of the genus *Helisoma,* where they develop into forked-tailed cercariae. Cercariae penetrate the skin and enter the tissues of tadpoles of the leopard frog *Rana pipiens,* where, undergoing only minor changes, they remain as mesocercariae. If the tadpole is eaten by a frog, snake, bird, or mammal, the mesocercariae invade the tissues of these paratenic hosts but again remain mesocercariae. However, when mesocercariae in tadpoles or any of the paratenic hosts are ingested by a male or nonlactacting female cat, they penetrate the diaphragm and develop into metacercariae of the diplostomulum type in the lungs. Finally, diplostomula pass up the trachea and down the esophagus to mature and reproduce in the small intestine. If mesocercariae are ingested by a lactating queen, they migrate to the mammary glands and are shed in the milk to develop into adult worms in the kittens. Some mesocercariae remain in the tissues of the queen to infect future litters. (Diagram and notes modified from Pearson [1956] and Shoop and Corkum [1984].)

wait for their new host to fall prey to a dog or other suitable definitive host. The frog, snake, or mouse that harbors these mesocercariae is called a **paratenic host** or **collector host,** which, by definition, is a host in which immature stages may survive indefinitely but undergo no essential development, and which can be passed by ingestion from paratenic host to paratenic host without further development. The paratenic host helps to distribute the parasite in space and time and often bridges the gap of food preferences or overcomes some other obstacle to the union of parasite and definitive host. When a dog eats a paratenic host, the mesocercaria migrates directly through the diaphragm to the lungs, where it transforms into a metacercaria. In a few weeks, the

metacercaria migrates up the trachea, is swallowed, and matures in the intestine. Eggs appear about 3 to 5 weeks after ingestion of mesocercariae (see Figure 4-22).

Mice infected with mesocercariae transmit *Alaria marcianae* to their sucklings through the milk, and when mature, these offspring can transmit the infection in the same fashion. If a female cat becomes infected with *A. marcianae* during lactation, the mesocercariae will not develop into metacercariae in her lungs but instead will migrate to her mammary glands to infect her kittens. The kittens then behave as definitive hosts and develop patent infections (Shoop and Corkum, 1984).

Importance. Adult *Alaria* organisms are attached to the mucous membrane of the small intestine but apparently do their host little harm. However, because the mesocercariae migrate through the lungs and sometimes wander into other tissues, they may at times cause clinical illness. For example, a case of human infection with mesocercariae of *Alaria americana* terminated fatally as a result of extensive pulmonary hemorrhage. Circumstances suggested that the person had eaten inadequately cooked frogs' legs while hiking (Freeman et al, 1976).

Treatment. Infection with the adult trematode within the intestinal tract of dogs and cats can be treated with praziquantel and probably epsiprantel. The typical cestocidal dosage will be efficacious in most cases.

Trematodes as Disease-Carrying Agents

FAMILIES LECITHODENDRIIDAE, HETEROPHYIDAE, AND TROGLOTREMATIDAE. Potomac horse fever caused by *Neorickettsia risticii* has been shown to be transmitted in a fashion similar to salmon poisoning disease of dogs caused by *Neorickettsia helminthoeca* (Kanter et al, 2000; Madigan et al, 2000). In the case of *N. risticii,* trematode parasites of bats in the genera *Acanthatrium* and *Lecithodendrium* of the family Lecithodendriidae transmit the agent from bat to bat via metacercariae encysted within caddisflies that acquire their infection as aquatic larvae (Figure 4-23). Horses become infected when the adult flies fall into and are consumed with feed or water. A great deal remains to be learned about *Neorickettsia* species and their transmission by trematodes. Elokomin fluke fever causes milder infections in dogs in northwestern United States and appears to be a variant of *N. helminthoeca* that also uses *Nanophyetus salmincola* as its vector. In Japan and Southeast Asia, a *Neorickettsia* transmitted by the Heterophyid fluke *Stellantochasmus falcatus* (the SF agent) has been routinely isolated from metacercariae in fish, but the agent does not cause disease in people or other animals. The SF agent from *S. falcatus* was found when metacercariae from fish were examined to look for *Neorickettsia sennetsu,* the agent of Sennetsu fever in Japan; however, although the SF agent was isolated, *Neorickettsia sennetsu* has not yet been found in any trematode.

Trematodes Acquired by Skin Penetration

FAMILY SCHISTOSOMATIDAE. Schistosomiasis caused by *Schistosoma mansoni, Schistosoma haematobium,* and *Schistosoma japonicum* is second only to malaria as a scourge of mankind, especially in South America, Africa, and Eastern Asia. Domestic animals in various tropical areas may be affected with *Schistosoma bovis* (cattle and sheep), *Schistosoma indicum* (horses, cattle, goats, and, in India, buffalo), *Schistosoma nasale* (cattle in India), *Schistosoma suis* (swine and dogs in India), and *Schistosoma mattheei* (sheep, southern Africa). In the Philippines, Indonesia, and limited areas in China, *S. japonicum* is a serious parasite of humans and animals alike. In North America, schistosomes present only two isolated problems: *Heterobilharzia americana,* a parasite of raccoon, nutria, bobcat, rabbit, and dog in the area extending from Florida along the Gulf Coast into Texas and north at least as far as Kansas; and "swimmer's itch," a dermatitis caused by cercariae of wild waterfowl schistosomes (*Trichobilharzia, Austrobilharzia,* and *Bilharziella* species) penetrating and abortively migrating in human skin. Of course, many cases of human schistosomiasis exist in North America among immigrants from endemic localities, but human schistosomiasis is unlikely to become endemic in the United States because the snail intermediate hosts (*Biomphalaria, Tropicorbis, Oncomelania,* and *Bulinus* species) do not occur here.

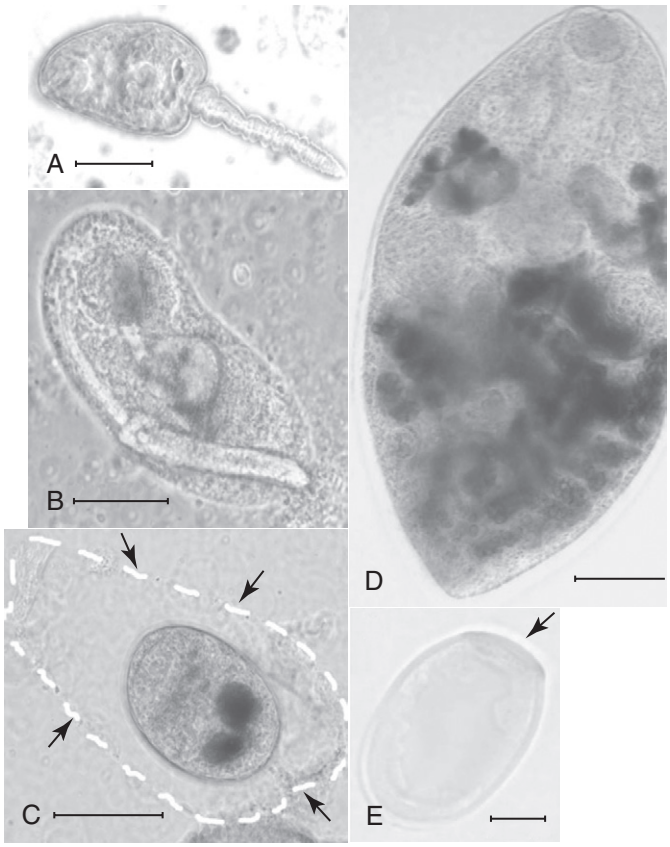


FIGURE 4-23. Phase-contrast photomicrographs showing representatives of the *N. risticii*–positive trematode life stages. **A,** A cercaria released from the *Elimia virginica* snail (first intermediate host of the trematode). Bar, 20 µm. **B,** A sporocyst found within the *E. virginica* snail. Bar, 20 µm. **C,** A metacercaria found within the mayfly second intermediate host. Arrows and white dashed line indicate the outer cyst wall consisting of insect tissue. Bar, 200 µm. **D,** The gravid adult life stage of the trematode found within the definitive host: the big brown bat *(Eptesicus fuscus).* Bar, 200 µm. **E,** A trematode egg obtained from the *E. fuscus* definitive host. Arrow indicates the operculum. Bar, 5 µm. (With permission from Gibson KE, Rikihisa Y: Molecular link of different stages of the trematode host of *Neorickettsia risticii* to *Acanthatrium oregonense, Environ Microbiol* 10:2064, 2008.)

IDENTIFICATION. The sexes are separate, with the slender female lying in the gynecophoric canal of the somewhat stouter male (Figure 4-24). Adult schistosomes are parasites of veins of the digestive and urinary tracts of birds and mammals. Other trematodes are hermaphroditic and parasitize tissues other than blood vessels. Eggs lack an operculum and contain a fully developed miracidium when discharged in the feces (e.g., *Schistosoma mansoni, S. japonicum*) or urine (e.g., *S. haematobium*); eggs of some species are armed with a spine. Other trematode eggs have a polar operculum and lack a spine. Schistosome eggs hatch on exposure to water, so feces must be suspended in 0.85% sodium chloride (NaCl)

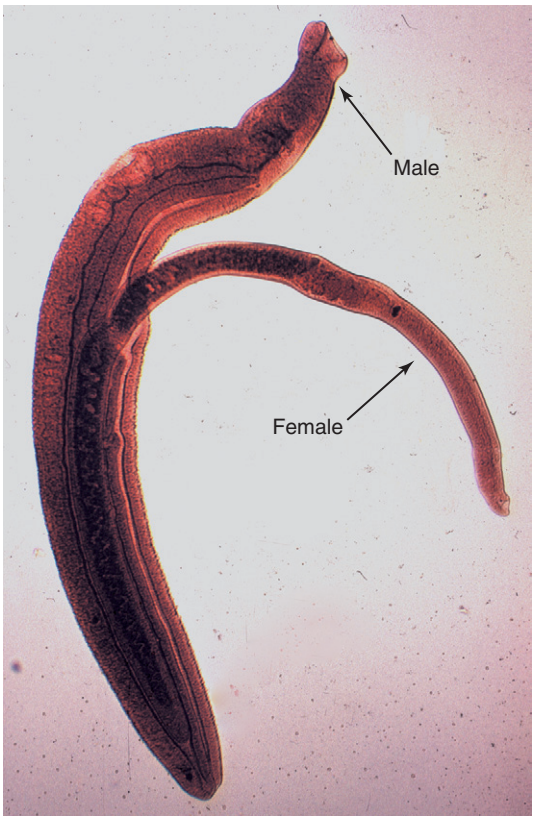


FIGURE 4-24. *Schistosoma mansoni* (Schistosomatidae). The body of the slender female can be seen protruding from the gynecophoric groove of the stouter male.

solution when eggs of these parasites are sedimented. A miracidium hatching technique (Goff and Ronald, 1980) increases the probability of detecting patent *H. americana* infection in dogs. The eggs of *H. americana* are rather spherical and possess only a slight bump on one side rather than a spine, as is seen in *Schistosoma mansoni* and *S. haematobium.*

LIFE HISTORY OF *HETEROBILHARZIA AMERICANA*. The miracidium hatches soon after the egg comes in contact with water and then enters a freshwater snail, *Lymnaea cubensis,* in which cercariae develop in daughter sporocysts. On emergence from the snail, the cercariae penetrate the skin of a raccoon, nutria, bobcat, rabbit, or dog and migrate by way of the lungs to the liver. After a period of development in the liver, mature males and females make their way to the mesenteric veins and mate, with the more or less cylindrical female lying in the gynecophoric groove of the male (see Figure 8-50). The prepatent period is about 60 days, and the eggs, laid in the terminal branches of the mesenteric veins, passively work through the bowel wall to the lumen and escape with the feces (see Figures 8-51 and 8-52). The eggs evoke a granulomatous reaction that eventually prevents their egress and favors their carriage to other organs, with the consequent production of widely disseminated granulomas. The life histories of other schistosomes differ only in detail from those of *H. americana.*

IMPORTANCE. *H. americana* has long been known to cause significant liver and bowel wall pathology in dogs that is associated with lethargy, weight loss, vomiting, diarrhea, hyporexia, anorexia, hypercalcemia, and polyuria/polydipsia in more than 50% of affected animals (Kvitko-White et al, 2011; Fabrick, Bugbee, and Fosgate, 2010). It has also been shown to be associated with hepatic, pulmonary, and intestinal granulomas in the horse (Corapi et al, 2011). In the examination of 12 horses with hepatic disease from the Gulf Coast and from southeastern regions of Texas, it was believed that the clinical disease that had been observed in two of the horses was a result of the large number of granulomas in the liver and of associated granulomas involving the heart (Corapi et al, 2012). The examined horses had focal granulomas that measured from 1 to 4 mm in diameter and numbered from a few dozen to several thousand. Granulomas were found in other tissues, including the serosa of the small intestine and colon, the mediastinum, the lungs, and the heart. Eight of 11 horses from which fresh or frozen liver tissue was derived were verified to be infected with this trematode by amplification of a portion of the *H. americana* SSU rRNA gene.

TREATMENT. For treatment of *H. americana* infection, fenbendazole administered orally at 40 mg/kg for 10 days completely removed *H. americana* from one artificially infected dog, whereas an untreated control dog remained infected (Ronald and Craig, 1983). Praziquantel (25 mg/kg daily for 2 or 3 days; given orally or via subcutaneous injection) appeared curative in most dogs, and dogs that were hypercalcemic after having not responded to other therapies to lower their calcium levels were normocalcemic between 36 and 48 hours after starting praziquantel treatment (Fabrick, Bugbee, Fosgate, 2010).

CLASS CESTOIDEA

Cestoidea is the class of Platyhelminthes that is composed of the various organisms known as *tapeworms.* The Class is divided into 18 orders, but only two are of significance to most veterinary practitioners because most of the diverse forms are found in sharks and fish. The two groups of interest to veterinarians are the Cyclophyllidea, which are found mainly in terrestrial vertebrates, and the Diphyllobothriidea, which have aquatic stages as part of their life cycles. The order Diphyllobothriidea used to be called the Pseudophyllidea, but this name was suppressed and replaced (Kutcha et al, 2008).

Tapeworms resemble trematodes in having acoelomate parenchymatous bodies and in having both sexes represented in the same individual. An adult tapeworm is essentially a chain **(strobila)** of independent, progressively maturing reproductive units, one end of which is capable of attachment to the wall of the host's intestine by a **holdfast** organ or **scolex.** In a fully developed adult tapeworm, all stages of development are displayed in a linear array, starting at the scolex and terminating at the distal end. Although from a reproductive viewpoint, a tapeworm appears to be a colony instead of an individual, all segments are served by common osmoregulatory and nervous systems, and the animal moves in a rhythmic and coordinated manner by means of the concerted activity of two zones of muscle fibers found in each segment. No organs of prehension or digestion are present, and all nutrients are absorbed through the tapeworm's specialized integument. The body of an adult tapeworm is so flattened that for the purposes of argument, it can be said to have two surfaces and two edges. This shape affords maximum surface area per unit volume—a distinct asset for an animal that absorbs all of its nourishment through its skin. Some tapeworms grow to considerable size. The strobila of the adult tapeworm, *Taenia saginata,* for example, may contain as many as 2000 segments and can reach a length of 3.6 meters (30 feet) in the human small intestine (Arundel, 1972).

Tapeworms are hermaphrodites; the zygote produced by fertilization is wrapped with yolk cells and is surrounded by a shell applied by the female reproductive tract. The first larval stage develops within the eggshell and is called an **oncosphere.** The fully formed oncosphere consists of a **hexacanth embryo** (the six

hooklets are used for motility). When the oncosphere is eaten by the appropriate host, it develops in the body cavity or tissues of that host into the second larval stage. In the case of most cyclophyllidean tapeworms, only one intermediate host is found between the larva-containing egg and the intestinal tract of the final host, where the adult tapeworm develops. Tapeworms of the genera *Diphyllobothrium* and *Spirometra* within the Diphyllobothriidea require a second host to house the transformation of the second larval stage into the third larval stage, which is infective to the final host. There is one cyclophyllidean tapeworm genus, *Mesocestoides,* which requires two hosts for larval development, but it is not yet known which host eats the oncosphere to get the life cycle started. Also, there is one cyclophyllidean tapeworm species, *Vampirolepis (Hymenolepis, Rodentolepis) nana,* a parasite of rodents, humans, and other primates that is exceptional among tapeworms in being able to complete its life history from hexacanth embryo to adult tapeworm within a single individual. The second and third larval stages of these various tapeworms have their own names, which are presented later in the discussion of their respective life histories; often the larval tapeworm produces clinical disease that is much worse than that caused by the adult tapeworm.

The Diphyllobothriidea are associated with aquatic food chains and use free-living crustaceans, copepods, as the first intermediate host in which the **oncosphere** develops into a second larval stage called a **procercoid.** The second intermediate host may be a fish, amphibian, or reptile that supports development of the **procercoid** into a third larval stage called a **plerocercoid.** The definitive host is infected when it ingests a plerocercoid in a second intermediate host or any of a series of paratenic hosts. The Cyclophyllidea is a group of tapeworms associated with terrestrial food chains that contains five families of veterinary importance: Taeniidae, Mesocestoididae, Anoplocephalidae, Dipylidiidae, and Hymenolepididae. The intermediate host may be a mammal (Taeniidae) or an arthropod (Anoplocephalidae, Dipylidiidae, Hymenolepididae). The genus *Mesocestoides* remains an enigma; the final host is a mammal, and the second can be a mammal, bird, or reptile, but so far, the first intermediate host has not been identified. In a teleologic sense, the objective of larval development is to form a holdfast in an intermediate host that is likely to be ingested by a suitable definitive host. Because this objective has been reached in such diverse hosts as mites and cattle, considerably greater variation in size and form has been noted among larval cestodes than among adults. It is at this point that uniformity of structure and function gives way to diversity. Therefore details of larval development are discussed in connection with life histories in the following characterization of cestode families.

When an infective tapeworm larva first arrives in the intestine of its definitive host, most of the infective larva's body is digested away, leaving only the scolex and a bit of undifferentiated tissue called the *neck.* The scolex attaches to the intestinal wall, and the neck begins to bud off segments. These segments remain attached to one another to form the chain mentioned previously. At first, the segments remain undifferentiated, but ovaries, testes, vitellaria, and other reproductive organs gradually begin to take shape in the segments some distance removed from the neck. These reproductive organs gradually mature, eggs and sperm are formed, and fertilization occurs. Depending on the kind of tapeworm, the fertilized eggs may be discharged through a uterine pore or may accumulate in the segment. Therefore the terminal segments of a mature tapeworm are found to be empty in the former case and packed full of eggs like ripe seedpods in the latter.

The anatomic details and nomenclature of the genitalia are important in detailed taxonomic work but need not be emphasized here because a reliable identification usually can be made on the basis of host identity and somewhat more accessible morphologic features as outlined later. However, differences do exist between cyclophyllideans and diphyllobothriideans that are important in diagnosis and in understanding their particular life histories. Information on the geographic distribution and biology of some cestodes of veterinary importance can be found in Table 4-2.

Diphyllobothriidean Tapeworms

The holdfast of these adult tapeworms has only two shallow, longitudinally grooved bothria for locomotion and attachment (Figure 4-25). The two most important genera, *Diphyllobothrium* and *Spirometra,* have no hooks to assist the weak grip of the bothria. The considerable area of contact between the long chain of broad segments and the intestinal mucosa apparently affords sufficient traction to maintain the tapeworm in place. The tapeworm segments have a midline uterine pore that permits the escape of eggs (Figure 4-26). Segments over a considerable length of the strobila discharge their eggs until their supply is exhausted, and the terminal segments of these tapeworms become senile rather than gravid and are usually detached in short chains rather than individually. Thus the diagnosis of these tapeworm infections often depends on distinguishing the operculate eggs in fecal sediments from those of trematodes, which sometimes is not an easy matter.

FIGURE 4-25. *Diphyllobothrium latum* (Diphyllobothriidae), scolex of stained, permanent mount.

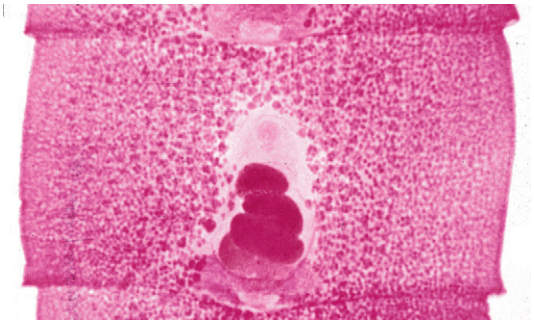


FIGURE 4-26. Mature segment of *Diphyllobothrium latum*.

The Diphyllobothriidean oncosphere and its two membranes are surrounded in turn by an operculate shell (Figure 4-27). The outermost membrane remains behind in the shell when the oncosphere, now surrounded only by its ciliated inner membrane or **embryophore,** pops open the shell's operculum and swims away (Figure 4-28). The ciliated Diphyllobothriidean oncosphere is called a **coracidium.**

Family Diphyllobothriidae

IDENTIFICATION. The scolex of *Diphyllobothrium latum* and *Spirometra mansonoides* has two slitlike grooves (see Figure 4-25). Mature segments are broader than long (Figure 4-29; see also Figure 4-26). The uterus, which consists of a spiral tube with four to eight loops on each side, opens to the outside through a midventral uterine pore behind the genital pore. The reproductive organs are concentrated at the centers of the segments (see Figure 4-29). Operculated eggs are discharged through the uterine pore.

LIFE HISTORY. The two important genera of Diphyllobothriidae in veterinary medicine, *Diphyllobothrium* and *Spirometra,* differ in that one exclusively uses aquatic intermediate hosts and the other uses amphibious and terrestrial intermediate hosts. *Diphyllobothrium* species use copepods and fish. *Spirometra* species use copepods, amphibians, reptiles, birds, and mammals.

D. latum has a life cycle that requires two intermediate hosts, of which the first is a copepod and the second is a vertebrate. When ingested by a copepod, the **coracidium** (oncosphere with ciliated embryophore) develops into a solid, wormlike **procercoid** within the body cavity (Figure 4-30). When the infected copepod is ingested by a second intermediate host, the procercoid enters its musculature or connective tissues and develops into a **plerocercoid** (Figure 4-31). The plerocercoid is notable for its ability to parasitize a series of predatory paratenic hosts until a suitable definitive host is found. Thus when a pike eats a minnow infected with the plerocercoids of *D. latum,* these merely invade the flesh of the pike and remain plerocercoids. However, when a human, a dog, or a cat eats

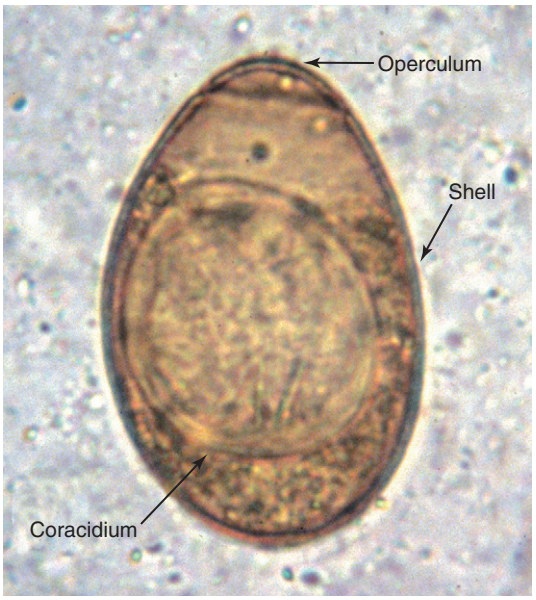


FIGURE 4-27. Egg of *Spirometra mansonoides* (Diphyllobothriidae). The capsule of diphyllobothriid eggs is operculate; this one contains a fully developed coracidium.

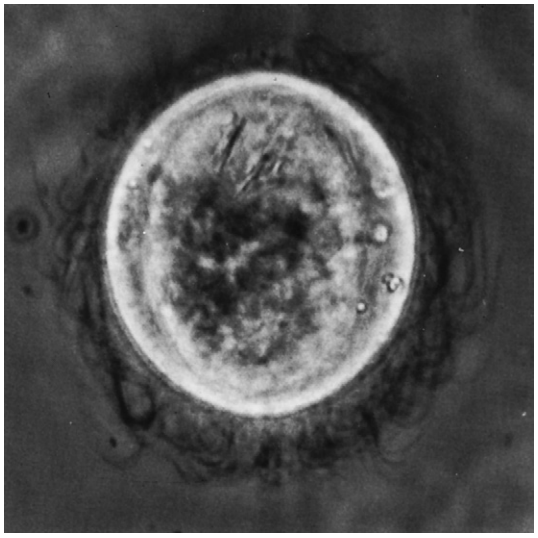


FIGURE 4-28. Coracidium of *Spirometra mansonoides.* Phase contrast electronic flash photomicrograph of the free-swimming organism. (Courtesy Dr. Justus Mueller.)

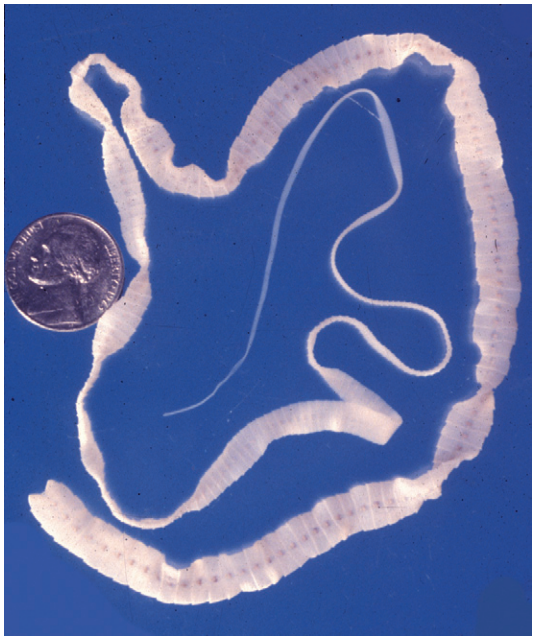


FIGURE 4-29. *Spirometra mansonoides* (Diphyllobothriidae), entire specimen from a cat. Note how small the scolex is relative to the mature segments, and also the central location of the genitalia throughout the length of the tapeworm.

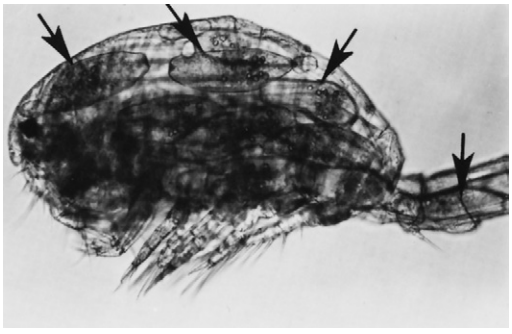


FIGURE 4-30. Copepod *(Cyclops vernalis)* with body cavity filled with three different procercoids *(arrows)* of *Spirometra mansonoides;* electronic flash photomicrograph of living organisms.

TABLE 4-2 Information on Some Tapeworms of Veterinary Importance

Cestode	Final Hosts	Geographic Distribution	Egg	Intermediate Hosts	Larval Stage	Scolex	Segment	Prepatent Period	Comments
DYPTHILODBOTHRIIDEA									
Diphyllobothriidae									
Diphyllobothrium	Dogs, cats, humans, bears, pigs, seals	Cold climate, fresh water	Operculate 66 × 44 μm	Copepod*; fish†	Plerocercoid	Slitlike, no hooks	Square, medial uterine pore	40 days	Humans can be infected with adult form
Spirometra	Dogs, cats, lynx, raccoons	United States, Australia, Asia	Operculate 66 × 44 μm	Copepod*; tadpoles, snakes, rodents†	Plerocercoid "sparganum"	Slitlike, no hooks	Square, medial uterine pore	15-30 days	Causes human sparganosis
CYCLOPHYLLIDEA									
Taeniidae									
Taenia pisiformis	Dogs	Worldwide	Taeniid egg, 30 μm	Cottontail rabbits	Cysticercus	Muscular, 4 suckers, hooks claw hammer shaped	Square segments, single lateral pore	56 days	
Taenia hydatigena	Dogs	Worldwide	Taeniid egg, 30 μm	Mainly sheep	Cysticercus	Muscular, 4 suckers, hooks claw hammer shaped	Square segments, single lateral pore	51 days	
Taenia ovis	Dogs	Worldwide, absent in United States	Taeniid egg, 30 μm	Mainly sheep	Cysticercus	Muscular, 4 suckers, hooks claw hammer shaped	Square segments, single lateral pore	42-63 days	
Taenia saginata	Humans	Worldwide	Taeniid egg, 30 μm	Cattle—muscle	Cysticercus	No hooks on scolex	Square segments, single lateral pore	70-84 days	Scolex without hooks
Taenia solium	Humans	Worldwide	Taeniid egg, 30 μm	Pigs—muscle	Cysticercus	Muscular, 4 suckers, hooks claw hammer shaped	Square segments, single lateral pore	35-84 days	Causes human cysticercosis
Taenia asiatica	Humans	Southeast Asia	Taeniid egg, 30 μm	Pigs and cattle—liver	Cysticercus	No hooks on adult scolex	Square segments, single lateral pore	70-84 days	Adult scolex without hooks
Taenia taeniaeformis	Cats	Worldwide	Taeniid egg, 30 μm	Mice and rats	Strobilocercus	Muscular, 4 suckers, hooks claw hammer shaped	Square segments, single lateral pore	40 days	
Taenia serialis	Dogs	Worldwide	Taeniid egg, 30 μm	Cottontail rabbits	Coenurus	Muscular, 4 suckers, hooks claw hammer shaped	Square segments, single lateral pore	1-2 months?	Cats have had coenurosis

Taenia multiceps	Dogs	Worldwide, absent in United States and New Zealand	Taeniid egg, 30 μm	Mainly sheep	Coenurus	Muscular, 4 suckers, hooks claw hammer shaped	Square segments, single lateral pore	30 days	
Echinococcus granulosus	Dogs, canids	Sheep areas	Taeniid egg, 30 μm	Mainly sheep	Unilocular hydatid cyst	Muscular, 4 suckers, hooks claw hammer shaped	Worms and segments very small, usually not seen	45-60 days	Causes human unilocular hydatidosis
Echinococcus multilocularis	Foxes, dogs	Holarctic	Taeniid egg, 30 μm	Mice and rats	Multilocular hydatid cyst	Muscular, 4 suckers, hooks claw hammer shaped	Worms and segments very small, usually not seen	28 days	Causes human alveolar hydatidosis
Anoplocephalidae									
Moniezia benedeni	Cattle	Worldwide	Square	Oribatid mites	Cysticercoid	Unarmed	Much wider than long	40 days	
Moniezia expansa	Sheep	Worldwide	Square to round	Oribatid mites	Cysticercoid	Unarmed	Much wider than long	25-45 days	
Moniezia caprae	Goats	Worldwide	Square to round	Oribatid mites	Cysticercoid	Unarmed	Much wider than long	???	
Thysanosoma actinoides	Ruminants—not cattle	Mountainous North and South America	Elongate, small	Book lice (Psocidae)	Cysticercoid	Unarmed	Fringed	???	
Anoplocephala magna	Equids	Worldwide	Round	Oribatid mites	Cysticercoid	Unarmed	Much wider than long	4-6 weeks	Eggs hard to find in feces; segments usually not seen
Anoplocephala perfoliata	Equids	Worldwide	Round	Oribatid mites	Cysticercoid	Unarmed with lappets	Usually see whole worm	4-6 weeks	
Paranoplocephala mammilana	Equids	Worldwide	Round	Oribatid mites	Cysticercoid	Unarmed	Much wider than long	4-6 weeks	
Dipylidiidae									
Dipylidium caninum	Dogs, cats, other felids and canids	Worldwide	Eggs passed in egg packets	Fleas	Cysticercoid	4 suckers, retractable rostellum	Pumpkin seed–shaped segments with lateral pores on both sides	21 days	Occasionally, adult worms in children
Mesocestoididae									
Mesocestoides spp.	Raecoons, dogs, cats	Worldwide	Eggs confined to parauterine organ	First host still unknown; reptiles and mammals†	Tetrathyridium	Muscular 4 suckers; no hooks	Small, sesame seed–like, with contained parauterine organ	20-30 days	Dogs can be infected with tetrathyridia. Rare human infections

*First intermediate host.
†Second intermediate and paratenic hosts.

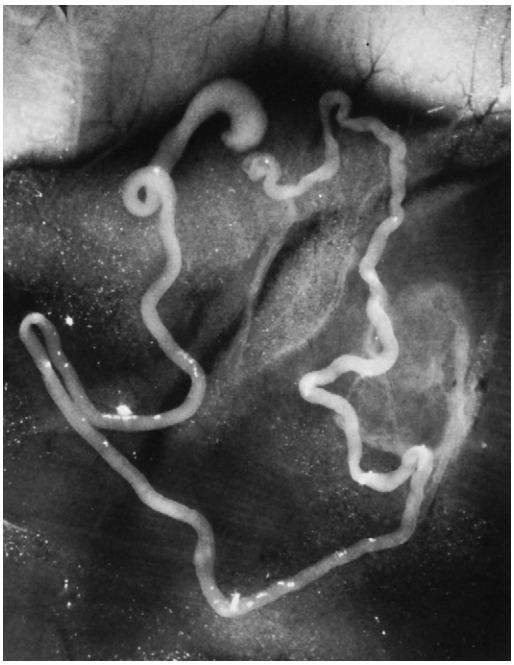


FIGURE 4-31. *Spirometra mansonoides* plerocercoid larva in the subcutaneous tissues of a white mouse. (Photograph [about twice natural size] courtesy Dr. Robert Smith; culture courtesy Dr. Justus Mueller.)

either the minnow or the pike, the plerocercoid matures into an adult tapeworm, with the **prepatent period** (i.e., the time between infection and the appearance of detectable stages, which in this case are eggs found in the feces) being about 5 or 6 weeks. *D. latum* procercoids develop in copepods of the genus *Diaptomus,* and its plerocercoids develop in fish. Definitive hosts of *D. latum* include humans, dogs, mongooses, walruses, seals, sea lions, bears, foxes, and minks (Wardle and McLeod, 1952).

Spirometra mansonoides procercoids develop in copepods of the genus *Cyclops.* Its plerocercoids develop in "any class of vertebrates except fishes"; even kittens fed procercoids support development of plerocercoids, which appear in the flat muscles of the body wall and subcutaneous fascia (Mueller, 1974). The natural intermediate host is probably the water snake *Natrix,* and the natural definitive host is probably the bobcat *Lynx rufus.* Other definitive hosts of *S. mansonoides* include the domestic cat and dog and the raccoon (Mueller, 1974). The life history is illustrated in Figure 4-32; animals can begin shedding eggs in the feces as soon as 10 days after ingestion of the larval plerocercoid stage. A case of proliferative sparganosis (i.e., where the plerocercoid undergoes asexual multiplication in the host) was reported in a 21-month-old border collie in Tampa, Florida (Drake et al, 2008). The dog presented with forelimb lameness, signs of pain, and subcutaneous edema. The dog was treated with praziquantel, fenbendazole, and nitazoxanide, but the infection progressed and the dog was euthanized 133 days after presentation.

An Eastern Asian species, *Spirometra mansoni,* does use frogs, rabbits, and birds for development of the plerocercoid (see Figure 8-68). Not so very long ago, it was the custom in parts of Eastern Asia to apply the incised body of a freshly caught frog as a poultice to wounds, sore eyes, and the like. (This behavior is not so unlike the once common application of a raw steak to "reduce the bruising" associated with a black eye.) The plerocercoids *of Spirometra mansoni,* if present in the tissues of the frog, would then transfer to the human host and migrate about in the subcutaneous connective tissues—a condition dubbed **sparganosis** in the human medical literature. The plerocercoids **(spargana)** of *S. mansonoides* are also capable of causing human sparganosis, as Mueller and Coulston (1941) demonstrated by experiments on themselves in which they inserted spargana into the tissues of their arms.

D. latum and *S. mansonoides* are usually less obtrusive than other tapeworm parasites of dogs and cats because they do not detach segments but release their eggs more or less continuously through the uterine pores of their mature segments. Therefore the client usually is unaware of *Diphyllobothrium* and *Spirometra* infection unless a whole tapeworm or a long chain of senile segments is discharged at once. *Diphyllobothrium* infection is acquired by eating uncooked predatory freshwater fish. *S. mansonoides* can be passed experimentally from the copepod through such diverse second intermediate hosts as frogs and mice. In the type locality (Syracuse, New York), *Natrix,* a water snake, is frequently found infected with *S. mansonoides* plerocercoids.

Families of Cyclophyllidean Cestodes

Compared with the rest of the mature worm, which may be several meters long in the larger species, the **scolex** is minute, frequently measuring less than a millimeter. The cyclophyllidean scolex has four radially disposed muscular suckers that serve for attachment and locomotion (Figure 4-33). These suckers and the tissue immediately surrounding them are quite mobile. Dr. Georgi described watching a severed scolex of *Taenia pisiformis* "walk" with remarkable agility across the bottom of a Petri dish. Each sucker in turn was advanced on a stalk of tissue and fixed to the bottom of the dish. Then the scolex was drawn toward the point of fixation by contraction of the stalk of tissue, another sucker advanced, and so on. At the apex of most cyclophyllidean scolices is a dome-shaped projection, the **rostellum,** which is sometimes retractable into the scolex and may be armed with small hooks. In the family Taeniidae, a nonretractable rostellum is armed with two concentric rows of hooks. Strong muscles operate these hooks in a concerted and rhythmic clawing motion. The points are projected in a manner similar to a cat baring its claws, but in a centrifugal direction. This clawing motion ceases once the scolex has found safe anchorage in the intestinal wall (see Figure 7-42). Cyclophyllidean families that lack rostellar hooks (e.g., Anoplocephalidae, Mesocestoididae) tend to have more strongly developed suckers.

Segments of cyclophyllidean strobila have genital pores for fertilization but no opening to allow the eggs to escape from the uterus. Therefore the eggs accumulate until the segment becomes packed full like a ripe seedpod. As they reach the end of the chain, these gravid segments are detached and pass out with the feces or crawl out the anus onto the perianal skin. Therefore cyclophyllidean infections are usually diagnosed by identifying gravid segments on the host or in its environment.

Cyclophyllidean oncospheres are fully developed when passed in the feces of the definitive host and are immediately infective for the intermediate host. These oncospheres lack a true shell and technically should not be called *eggs*, but most authors call them this and so shall we. The outer membrane of the cyclophyllidean oncosphere serves as a protective capsule in some species. However, the outer membrane of taeniid oncospheres is delicate and usually has been lost by the time they appear in a host's feces. The inner embryonic membrane (embryophore) serves as a protective coat for the taeniid oncosphere. In anoplocephalids the embryophore is a distinctive pear-shaped body (pyriform apparatus), and in taeniids it consists of a rather thick layer of prismatic blocks. The eggs of *Dipylidium caninum* are clustered in packets formed by outpocketings of the uterine wall.

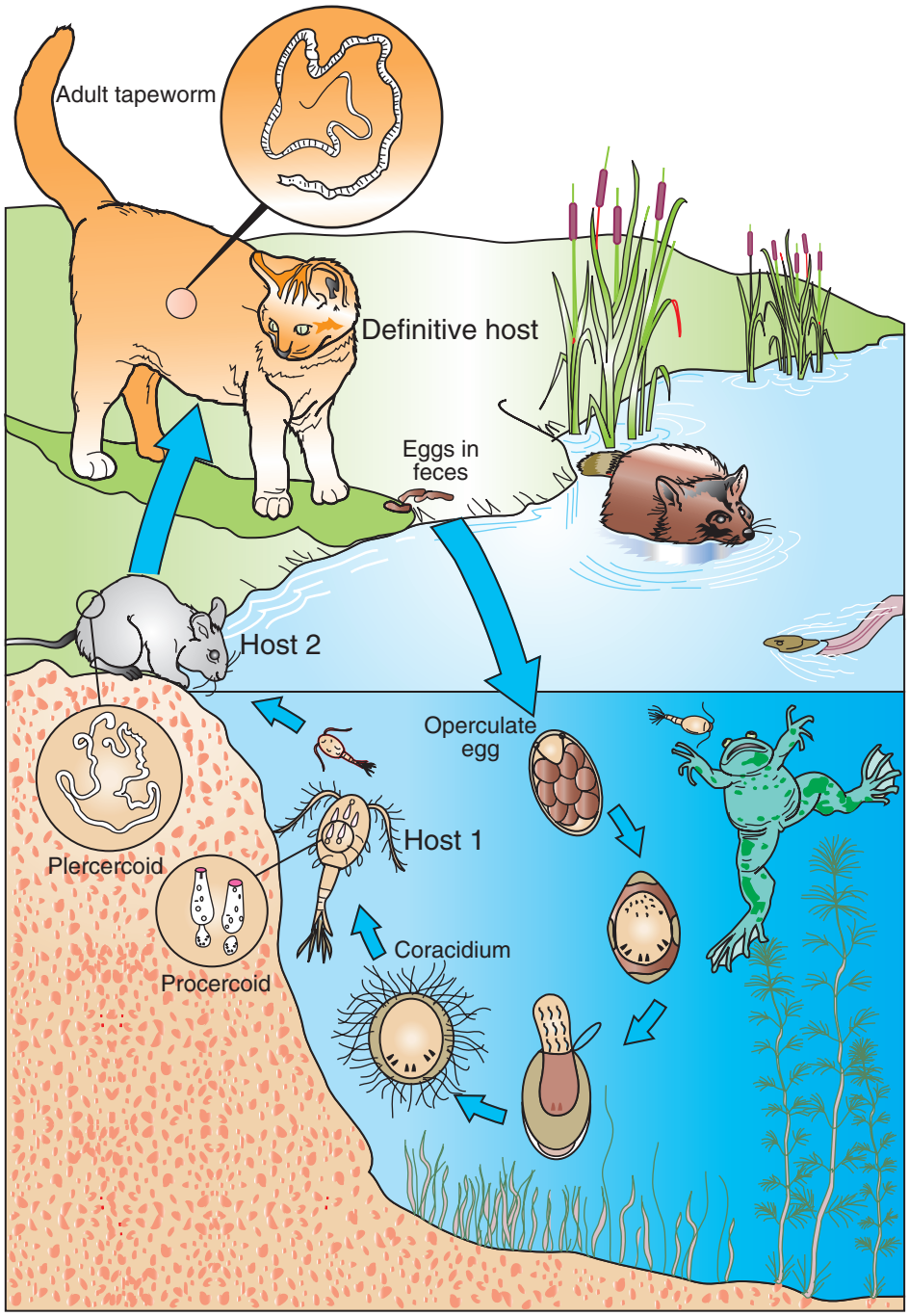


FIGURE 4-32. Life history of *Spirometra mansonoides,* a pseudophyllidean tapeworm. Coracidia develop and hatch from eggs deposited in water and swim about until they are ingested by copepods of the genus *Cyclops.* Shedding its ciliated coat, the hexacanth embryo develops into a procercoid larva in the body cavity of the copepod. If an infected copepod is swallowed by any vertebrate except a fish, the procercoids develop into plerocercoids, which tend to locate in subcutaneous tissues and flat muscles of the body wall. Plerocercoids survive predation of their hosts and remain plerocercoids in their new hosts unless the new host happens to be a cat. Plerocercoids develop into adult *S. mansonoides* tapeworms in the small intestine of the domestic cat and bobcat.

Teratologic development of cestode larvae is not at all uncommon, and occasionally cases are observed in which larval tapeworm tissue behaves much like a malignant neoplasm. For example, Williams, Lindsay, and Engelkirk (1985) reported a fatal case of peritoneal cestodiasis in a dog from which parasites actually passed out through a poorly healing laparotomy incision, and 500 mL of parasite tissue was recovered from the peritoneal cavity at necropsy. The parasites were too abnormal both grossly and histologically to be identified, even by careful comparison with specimens of the most likely candidates, *Mesocestoides corti, Taenia crassiceps,* and *Taenia multiceps*.

Family Taeniidae

TAENIA

IDENTIFICATION. Adult tapeworms of the genus *Taenia* measure from tens to hundreds of centimeters in length, depending on the species in question and the degree of maturity of the specimen. The scolex has four suckers and a nonretractable rostellum

FIGURE 4-33. Holdfast and neck of *Taenia* sp., showing four suckers and nonretractable rostellum with hooks.

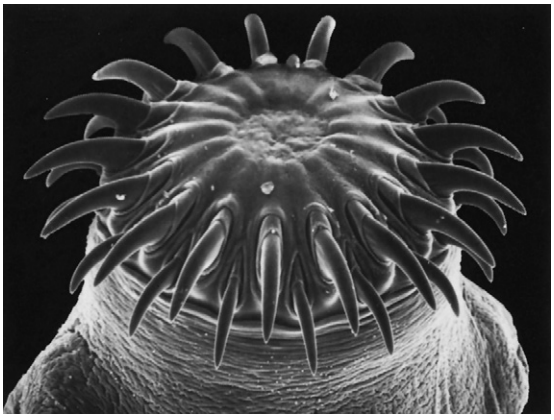


FIGURE 4-34. *Taenia taeniaeformis* (Taeniidae). The rostellum of taeniid tapeworms is nonretractable and is armed with a row of long hooks and a concentric row of short hooks. (Scanning electron micrograph by Dr. Ronald Minor.)

armed with two rows of hooks (Figure 4-34; see also Figure 4-33). The segments are more or less rectangular, with unilateral genital pores alternating irregularly from one side to the other along the strobila (Figure 4-35). The eggs in gravid segments are typical of the family (Figure 4-36). Differentiation of genera and species is based on number and sizes of rostellar hooks and on morphology of mature segments and may require the services of an expert (Verster, 1969). Taeniid tapeworms of the genus *Echinococcus* are recognized by their very small bodies, millimeters in length, composed of only four or five segments, along with hooks and eggs that are morphologically similar to those of other taeniid tapeworms. Species of the genus *Taenia* are fairly restricted in their final host usage, where the adult worm is found in the small intestine. *Taenia* species commonly occurring as adults in dogs include *T. pisiformis, T. hydatigena, Taenia ovis, Taenia serialis,* and *T. multiceps*. *Taenia* species occurring as adults in human beings include *Taenia solium, T. saginata,* and *Taenia asiatica.* The common *Taenia* species occurring in adult form in the domestic cat is *Taenia taeniaeformis.* Common species of *Echinococcus* in canids include *Echinococcus granulosus* and *Echinococcus multilocularis* (also found on occasion in domestic cats). In South America a species of *Echinococcus* in felids is *Echinococcus oligarthus; Echinococcus vogeli* is a species that cycles between the bush dog, *Speothos venaticus,* and the paca, *Cuniculus paca.* In Tibet the species *Echinococcus shiquicus* cycles between the Tibetan fox, *Vulpes ferrilata,* and the plateau pika, *Ochotona curzoniae.*

LIFE HISTORY. Gravid taeniid segments (Figure 4-37) are shed and exit from the carnivorous definitive host through the anus; the segments of *Echinococcus* species are so small that they are never observed, and often only free eggs are passed in the feces. The segments crawl about on the pelage of the host or surface of the fecal mass, emptying themselves of their eggs (oncospheres) in the process. Therefore any segment collected after it has been out for longer than a few minutes may contain few if any eggs. If ingested by a suitable vertebrate intermediate host (usually a species normally taken as prey by the definitive host), the egg hatches and the hexacanth embryo enters the wall of the intestine and migrates to its organ of predilection, usually the liver, and peritoneal membranes or skeletal and cardiac muscles. Here the **hexacanth embryo** grows, cavitates, and differentiates to form the second larval stage, which is infective to the definitive host. The fully developed second larval stage of the family Taeniidae consists of a fluid-filled bladder with one or more scolices (often called a **bladderworm**) and is surrounded by a connective tissue capsule formed by the vertebrate intermediate host.

Until the middle of the nineteenth century, the relationship of bladderworms to tapeworms was not recognized. Therefore different stages of the same species were described and named as distinct species belonging to separate phyla. For example, *Cysticercus cellulosae* was placed in the now defunct phylum Cystica, whereas its parent, *T. solium,* was referred to the defunct phylum Vermes. The older names of the larval stages are still occasionally used to identify the morphologically different larval stages of tapeworms. Such usage is helpful in describing pathologic specimens because it eliminates the need for writing "the cysticercus of *Taenia* such-and-such." However, because the specific names of adult and larval stages often differ, these additional names can add to the confusion that sometimes surrounds events in the development of different species of tapeworm. Therefore their use has been minimized herein as much as possible.

When a second larval stage of a taeniid tapeworm is ingested by a suitable definitive host, the bladder is digested away, the scolex embeds itself in the mucosa of the small intestine, and the neck begins to bud off segments to form the strobila. Eggs of taeniid tapeworms first appear in the feces 6 to 9 weeks after ingestion of the larva. Williams and Shearer (1982) observed a prepatent period of 34 to 80 days for *T. taeniaeformis* in cats, and infections remained patent for 7 to 34 months.

Four basic kinds of taeniid second-stage larvae are known: **cysticercus, strobilocercus, coenurus,** and **hydatid.** Members of the genus *Taenia* typically form cysticerci, strobilocerci, and coenuri, depending on the species in question. A **cysticercus** (Figures 4-38, 4-39, and 4-40; see also Figure 8-60) consists of a single bladder with one scolex. A **strobilocercus** (Figure 4-41; see also Figure 8-61) is a cysticercus that has already begun to elongate and segment while still in the intermediate host, and a **coenurus** (Figure 4-42) consists of a single bladder with many scolices, each with the potential for developing into a mature tapeworm (see Figure 8-62). Hydatids are formed by members of the genus *Echinococcus* and are of two kinds: **unilocular hydatid cysts** (see Figure 8-64) and **alveolar hydatids** (see Figure 8-57), both of which often

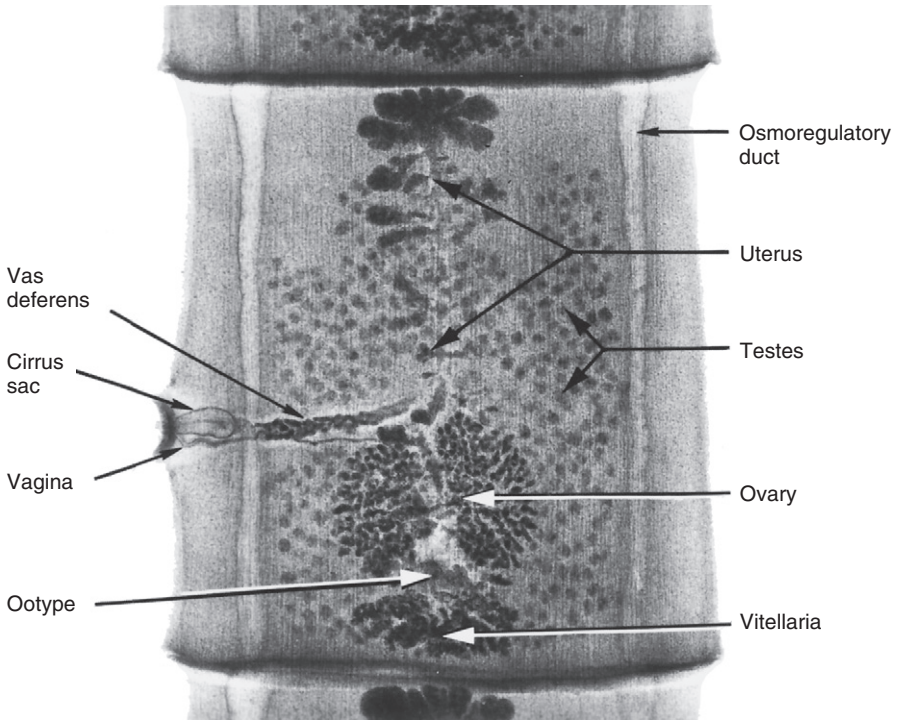


FIGURE 4-35. Mature *Taenia* segment.

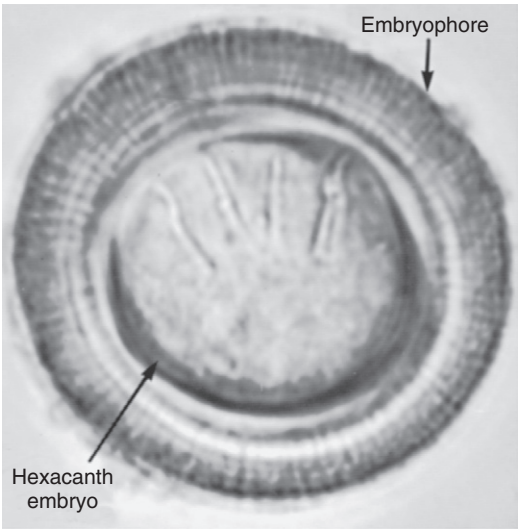


FIGURE 4-36. Egg of *Taenia taeniaeformis* (Taeniidae) of the cat. The capsule of taeniid eggs is fragile; eggs in fecal smears have usually lost their capsules.

contain thousands of scolices. Usually, one *Taenia* oncosphere develops into only one bladderworm. However, in the case of *T. crassiceps*, asexual multiplication (budding) results in many cysticerci surrounded by a single host-tissue capsule (see Figure 8-63). Such a structure may easily be mistaken for a hydatid cyst by the unwary observer. Many coenuri branch and ramify extensively to form very complex structures, and teratologic malformations may result in diverse and complex structures.

CYSTICERCOSIS. *Taenia hydatigena* is a canine taeniid tapeworm with a cysticercus (see Figure 4-38) that migrates through the liver tissue and encysts on the peritoneal membranes of cattle, sheep, swine, and certain wild ungulates. Massive invasions, as occur when entire tapeworm segments are ingested, result in acute traumatic hepatitis, and even small numbers of migrating *T. hydatigena* larvae are capable of precipitating "black disease" in the presence of *C. novyi.* However, frank disease is rarely caused by this larval tapeworm, and the principal economic loss results from condemnation of infected livers by meat inspection authorities. Rare cases of human cysticercosis and coenurosis are also caused by larvae of canine taeniids.

Taenia ovis, a second canine taeniid tapeworm with a cysticercus that infects the cardiac and skeletal muscles of sheep, represents the most important pathologic lesion found by U.S. inspectors in imported Australian mutton. In one instance $1,540,000 worth of boneless mutton (12.5% of the total shipment) had to be sold as pet food or shipped back to Australia (Arundel, 1972). This tapeworm recently was recognized as the cause of serious losses at abattoirs in Canada; from February to May of 2008, 12% of lambs sent to slaughter in Ontario were condemned as the result of infection with the cysticerci of *T. ovis* (Menzies, 2011). A trace-back of 237 carcasses condemned in Ontario between 2009 and 2011 revealed that they originated from 133 farms across Canada (de Wolf, 2011). Vaccines for sheep have been developed with very good efficacy against the development of the cysticercoid stage, but unfortunately they have yet to be commercially employed to any great extent.

Taenia pisiformis is a third canine taeniid tapeworm; the cysticercus is found in the liver and peritoneal cavity of rabbits. This tapeworm is the most common taeniid tapeworm of dogs in the United States. This indicates how well the process works, because every dog that is so infected must have eaten a rabbit or parts of a rabbit, and this means that many rabbits are infected by grazing near where *T. pisiformis* segments have been shed.

Taenia saginata is a taeniid tapeworm of human beings that has an "unarmed" scolex (i.e., the scolex has no hooks). The cysticercus of *T. saginata* encysts in the striated muscles of cattle, especially the heart and muscles of mastication. The cysticercus, similar to the adult form, has an unarmed scolex. Taeniid eggs survive the rigors

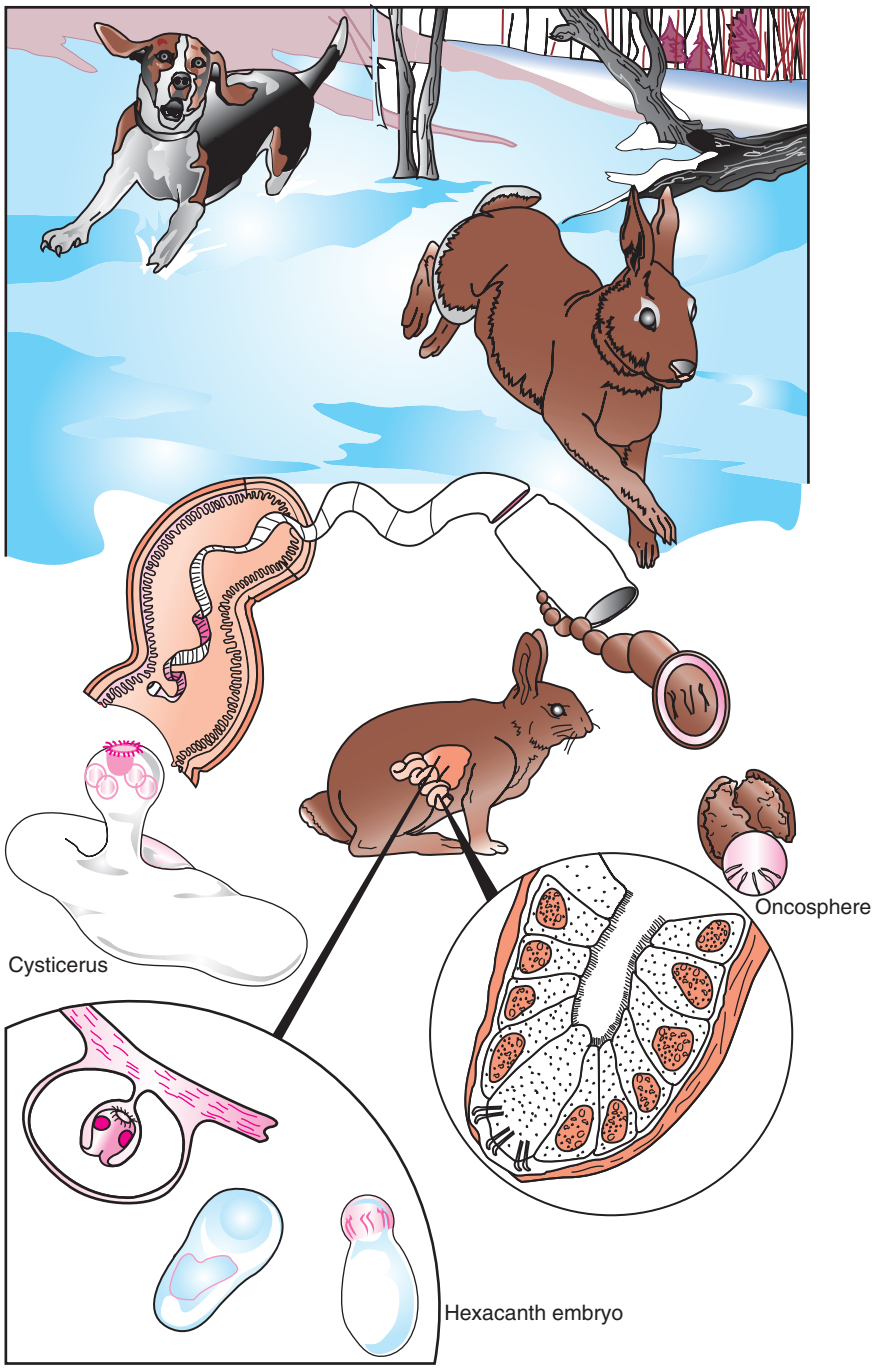


FIGURE 4-37. Life history of *Taenia pisiformis,* a cyclophyllidean tapeworm. Oncospheres (eggs) of *T. pisiformis* are shed in the feces of dogs. If ingested by a cottontail rabbit, *Sylvilagus floridanus,* the hexacanth embryo hatches, invades the mucosa of the small intestine, and makes its way to the liver. Tunneling through the liver, the hexacanth grows, cavitates to form a bladder, and develops a holdfast organ complete with two rows of hooks and four suckers. Fully developed cysticerci may remain in the liver, but they are more often found encapsulated on the peritoneal surfaces of the mesentery. When a dog eats an infected rabbit, the bladder is digested, leaving only the holdfast and adjacent neck. The holdfast attaches to the wall of the small intestine, and segments begin to form at the neck.

of the septic tank, as well as many contemporary municipal sewage treatment processes; because defecating out-of-doors is unavoidable when hunting or camping out (and because the segments can leave the host by crawling out through the anal opening), it is easy to see how cattle pastures can become contaminated with *T. saginata* eggs. The cysticerci that develop when these eggs are ingested by cattle are relatively inconspicuous and are easily overlooked by the lover of rare or raw ("cannibal sandwich") beef. Consequently, *T. saginata* is a common parasite in the United States and would be far more common but for the vigilance of our meat inspectors. Condemnation of carcass meats for the presence of *T. saginata* cysticerci can result in great economic loss. Sometimes this loss is concentrated in a particular lot of cattle and is borne by a single producer. Under such circumstances, the economic loss caused by *T. saginata* ceases to be an abstract number and becomes of immediate concern, not only for the unlucky producer, but for his

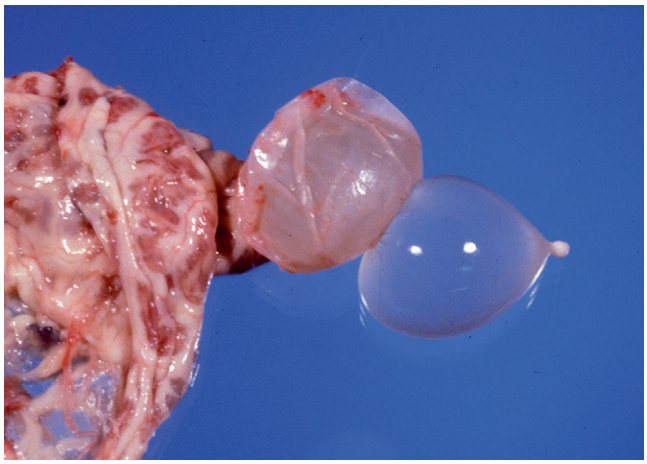


FIGURE 4-38. Cysticercus of *Taenia hydatigena* (Taeniidae) from the mesenteries of a sheep.

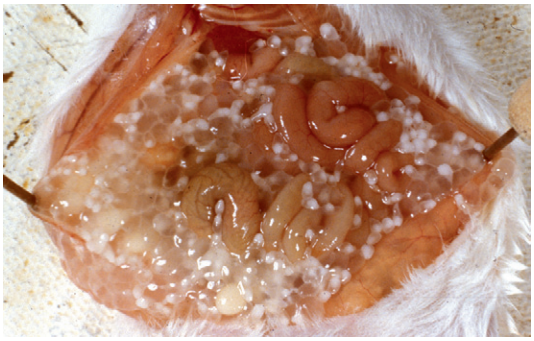


FIGURE 4-39. Cysticerci of *Taenia crassiceps* in the abdominal cavity of an albino mouse that was injected with 10 of these self-replicating cysticerci.

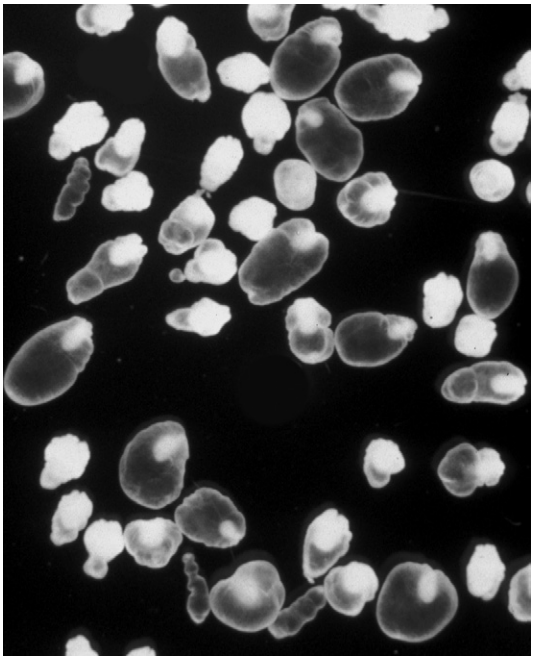


FIGURE 4-40. Cysticerci of *Taenia crassiceps* as they appear in a petri dish.

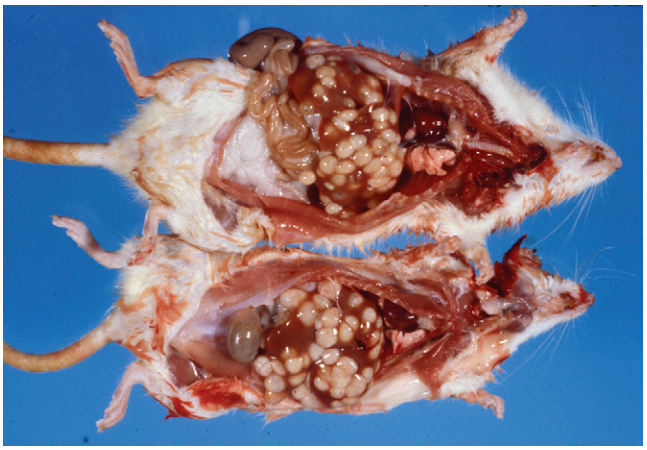


FIGURE 4-41. Strobilocerci *Taenia taeniaeformis* in the liver of two experimentally infected rats.

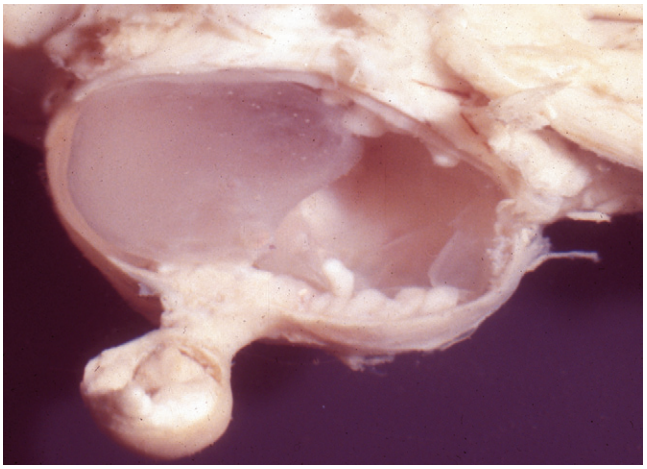


FIGURE 4-42. A coenurus of *Taenia serialis* from the subcutaneous axillary region of a chinchilla.

veterinarian, too. The problem is that some person, most likely a farm or feedlot employee, has a tapeworm and has defecated or shed segments in or near the cattle feed. People are generally uncooperative under such circumstances, and the culprit is rarely identified.

Taenia asiatica is a taeniid tapeworm of human beings found in southeast Asia (e.g., Thailand, Indonesia, Korea, Taiwan, the Philippines). The scolex of the cysticercus has hooks, is found in the liver of pigs (occasionally cattle), and does not occur in muscle. When this form develops to the adult stage in humans, the hooks are lost, and the tapeworm appears morphologically and molecularly very similar to *T. saginata*. This form has caused significant economic losses in pigs in the area where it occurs and is thought to be maintained by the habit of humans eating raw liver. This form may be considered a separate species but has also been designated a separate subspecies with the name *Taenia saginata asiatica* (Fan et al, 1995; Hoberg et al, 2001).

Taenia solium is the "armed" taeniid tapeworm of human beings; the "armed" refers to the fact that the scolex has hooks both as a larval cysticercus and as an adult. The cysticercus of the human tapeworm *T. solium,* unlike those of *T. saginata* and *T. asiatica,* represents a significant hazard to human health. People become infected with the adults of *T. solium* by ingesting the cysticerci in undercooked pork. After the tapeworm matures, the person's feces contain a steady supply of eggs, which may be conveyed to the

mouth at any time by a lapse in personal hygiene. When the eggs reach the stomach, the oncospheres hatch out, enter the gut wall, and wander far and wide in the body, slowly developing into cysticerci. Apparently the milieu intérieur of humans resembles that of swine closely enough to satisfy the developmental requirements of the cysticercus. In humans the signs depend on where the cysticerci localize, and sites may include most typically muscle, but also, eye, brain, or spinal cord; a number of autochthonous human cases have occurred in the past few years (Sorvillo et al, 2011). On rare occasions dogs can also be infected with these cysticerci (see Figure 8-60).

STROBILOCERCUS. *Taenia taeniaeformis,* the common taeniid tapeworm of domestic cats, has a larval stage that is termed a strobilocercus (see Figure 4-41; see also Figures 8-54 to 8-56 and 8-61). This larval stage is not of any significant zoonotic potential. A survey of euthanized cats from shelters in Oklahoma revealed that 30 of 116 cats were positive for *T. taeniaeformis,* and of these 30 cats, only 8 were diagnosed through centrifugal sugar flotation (Adolph et al, 2011).

COENUROSIS. *Taenia multiceps* is a canine taeniid tapeworm, for which the larval stage is a coenurus that invades the cranial cavity of sheep, goats, and sometimes cattle. As the cyst grows over a period of 6 or 8 months, neurologic signs of progressive space occupation slowly develop. Blindness, incoordination, walking in circles, and pressing the head against walls, tree trunks, and the like may be observed. Finally, the animal lies down and dies. The most common diseases that might be confused with cerebral coenurosis are bacterial encephalitis (listeriosis) and parelaphostrongylosis. Intracranial surgery is the only cure for cerebral coenurosis but lies beyond economic reality for sheep unless the shepherd is very skillful with his jackknife. The location of the larva within the skull makes some people wonder how the scolices ever reach a dog's stomach, but they must not realize that a good stout dog can crush a sheep's skull with one bite. As in the case of *Taenia hydatigena* and *Taenia ovis,* control can be attained only by excluding dogs and other canids from sheep pastures. Unfortunately, this is often next to impossible.

Taenia serialis is another taeniid tapeworm of canids with a larval stage that is a coenurus. In this case the larval stage typically develops in the subcutaneous tissues or viscera of rabbits. Cerebral coenurosis in cats appears in isolated cases (Georgi, de Lahunta, and Percy, 1969; Hayes and Creighton, 1978; Kingston et al, 1984; Smith et al, 1988). Marked by severe neurologic disturbances, it is invariably fatal. The responsible species is probably *T. serialis* owing to the disappearance of *T. multiceps* from the United States.

ECHINOCOCCUS

IDENTIFICATION. The genus *Echinococcus* contains two species of special importance to veterinary medicine—*E. granulosus* and *E. multilocularis*; these are very small (2 to 8 mm long) adult tapeworms that have only four or five segments, of which only the terminal segment is gravid (Figure 4-43). In *E. granulosus,* 45 to 65 testes are generally distributed, and the genital pore is located at or posterior to the middle of the segment. In *E. multilocularis,* 17 to 26 testes are found posterior to the genital pore, which is located anterior to the middle of the segment. **Caution:** Human hydatid infection may be acquired by ingesting the eggs of *Echinococcus* species; wear gloves and wash carefully when handling the feces of potentially infected carnivores.

E. granulosus is endemic in North and South America, England, Africa, the Middle East, Australia, and New Zealand. *E. multilocularis* is endemic in north-central Europe, Alaska, Canada, and the central United States as far south as Illinois and Nebraska (Ballard and Vande Vusse, 1983). A hydatid cyst was reported from the lungs of a deer in Sullivan County, New York, in 2005 (Chico, 2005).

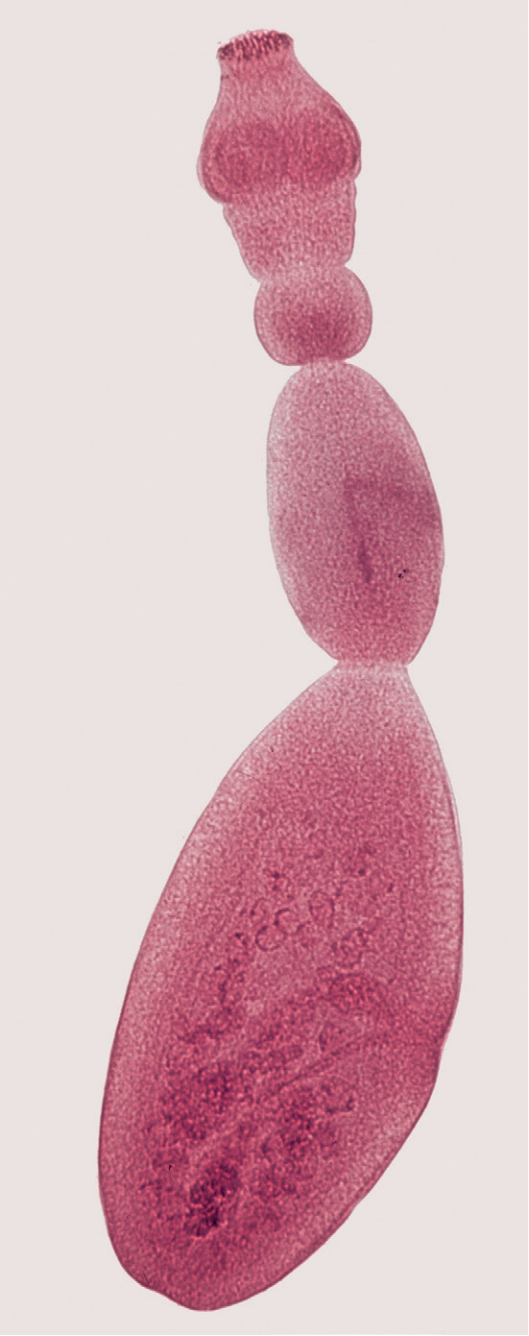


FIGURE 4-43. *Echinococcus granulosus* (Taeniidae), entire worm.

LIFE HISTORY. *E. granulosus* is a parasite in adults of the dog, coyote, wolf, and dingo. Its larva is a **unilocular hydatid cyst** in sheep, swine, cattle, humans, moose, caribou, kangaroos, and others. Species vary in their suitability as intermediate hosts. Hydatid cysts found in sheep are usually fertile, whereas those in cattle tend to be sterile. Subspecies of *E. granulosus* differ in their preferences for intermediate hosts. For example, *E. granulosus granulosus* hydatids belong to the subspecies adapted to sheep and humans, whereas *E. granulosus equinus* is the subspecies found in horses, asses, and mules. The hydatid membrane may bud off daughter cysts either internally or externally. The whole structure occupies progressively more space as it grows, but hydatid cysts do not infiltrate, in

contrast to alveolar hydatids. Pathogenic effects of hydatid cysts include pressure atrophy of surrounding organs and allergic reactions to hydatid fluid leaks. Rupture of a fertile hydatid cyst may scatter bits of germinative membrane, scolices, and brood capsules throughout the pleural or peritoneal cavity, resulting in multiple hydatidosis. Pulmonary hydatid cysts may rupture into a bronchus, the contents may be coughed up, and the lesion may be healed. Hydatid cysts that remain intact eventually die and degenerate, but the course is protracted.

E. multilocularis is a parasite of canids, mainly foxes and wolves in arctic regions. The larval stage, the **alveolar hydatid cyst,** develops in the liver of voles and lemmings (see Figures 8-57 and 8-58). The alveolar hydatid is characterized by exogenous budding that continuously proliferates and infiltrates surrounding tissue. As with unilocular hydatid cysts, the alveolar hydatid contains many small scolices, each of which is termed a **protoscolex** (plural, **protoscolices**). People become infected when they ingest the egg of *E. multilocularis*.

UNILOCULAR HYDATID DISEASE. The unilocular hydatid cyst, the second-stage larva of *E. granulosus,* is infective to dogs and other canids that serve as definitive hosts (Figure 4-44). Starting as an oncosphere measuring less than 30 µm in diameter, the larva grows very slowly and infrequently exceeds more than a few centimeters in diameter in slaughtered sheep and cattle. Because humans live longer, a fertile hydatid infecting man may grow very large and may interfere with the function of neighboring organs by pressing against them. The hydatid membrane is surrounded by, but usually is not attached to, an inflammatory connective tissue capsule (see Figure 8-64). The space between the host and the parasite generally contains a small volume of clear, colorless, or light-yellow liquid. Brood capsules, each containing many scolices, develop from the germinal epithelium lining the laminated hydatid membrane (Figure 4-45). Some of these rupture, releasing scolices to form sediment of so-called "hydatid sand" in the hydatid fluid (Figure 4-46). Endogenous daughter cysts may be found free in the fluid-filled cyst cavity or attached to the germinal epithelium. Exogenous daughter cysts are relatively unusual; they may be found in the pericystic space between the hydatid membrane and the host connective tissue capsule. "Sterile" hydatids, so-called because they lack protoscolices, often form in cattle and swine, making the diagnosis sometimes difficult and presumptive.

ALVEOLAR HYDATID DISEASE. Alveolar hydatid cysts are the second larval stage of *E. multilocularis* (the first is the hexacanth embryo within the egg) and contain protoscolices that are infective to dogs, foxes, and cats, which serve as definitive hosts (Figure 4-47). Alveolar hydatids may develop in voles, lemmings, cattle, horses, swine, and humans. In humans the cysts are typically "sterile" and become a proliferating germinal membrane that continuously proliferates and infiltrates surrounding tissue like a malignant neoplasm. Alveolar hydatid infection proves invariably fatal in a few years. In North America the largest numbers of cases in human beings have occurred in areas where the parasite has entered the peridomestic cycle by infecting dogs and rodents in Native American villages. This occurred in St. Lawrence Island, Alaska, where a large number of villagers were infected with this parasite. Cases continue to be reported from Alaska, and one case was reported from the lower 48 United States—Minnesota. In central Europe, almost 600 cases have been reported in recent years, most from eastern France to western Switzerland. Often in people the entire cyst cannot be removed by surgical resection because of its indiscrete boundaries, making it more difficult to treat than the discrete cysts of unilocular hydatidosis. Patients are often placed on long-term anthelmintic therapy with products such as albendazole.

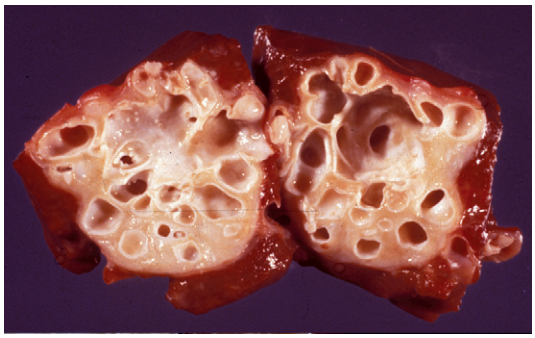


FIGURE 4-44. A hydatid cyst *(Echinococcus granulosus)* in the liver of a horse. This horse displayed no clinical signs of hepatic involvement despite the presence of 20 to 30 cysts like the one illustrated.

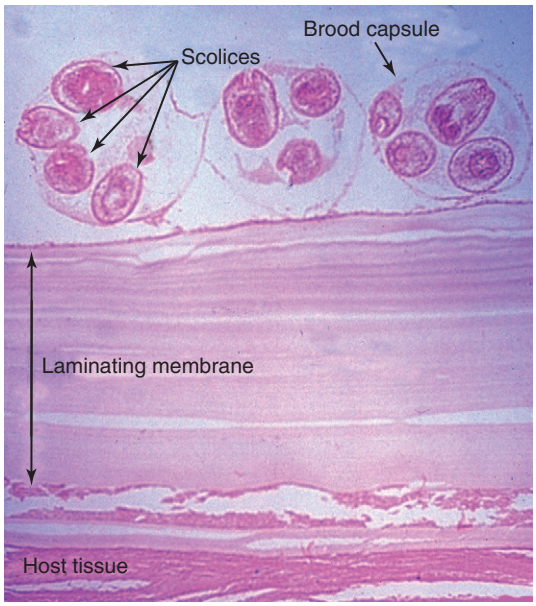


FIGURE 4-45. *Echinococcus granulosus* hydatid cyst; histologic section of cyst wall with three brood capsules, each containing three or more protoscolices.

Of 408 patients alive in 2000 whose cases were reported to the central European hydatid registry, only 4.9% were considered to have been cured of infection.

CONTROL. Both *E. granulosus* and *E. multilocularis* tend to establish sylvatic cycles when suitable predator-prey relationships exist in the wildlife population of a region. Therefore *E. granulosus* cycles are maintained among wild ruminants and wolves in the Canadian north woods and among wallabies and dingoes in Australia. Natural nidi of *E. multilocularis* are maintained in various rodents and foxes. The sylvatic cycle reaches humans through their domesticated animals. Dogs that scavenge the entrails of wild game infected with *Echinococcus* species become direct sources of hydatid infection for humans and their domestic animals. Contamination of pastures with the feces of infected wild carnivorans also results in hydatid infection of domestic ruminants and swine. The establishment of a pastoral cycle may then result from the feeding of uncooked offal from these domestic animals to dogs and, in the case of *E. multilocularis,* to cats (Figure 4-48).

The direct source of human infection is, in most instances, the domestic dog or cat, and scrupulous hygiene is the first line of defense. Periodic anthelmintic medication of dogs or cats, depending on the species of tapeworm involved, carries the threat one step

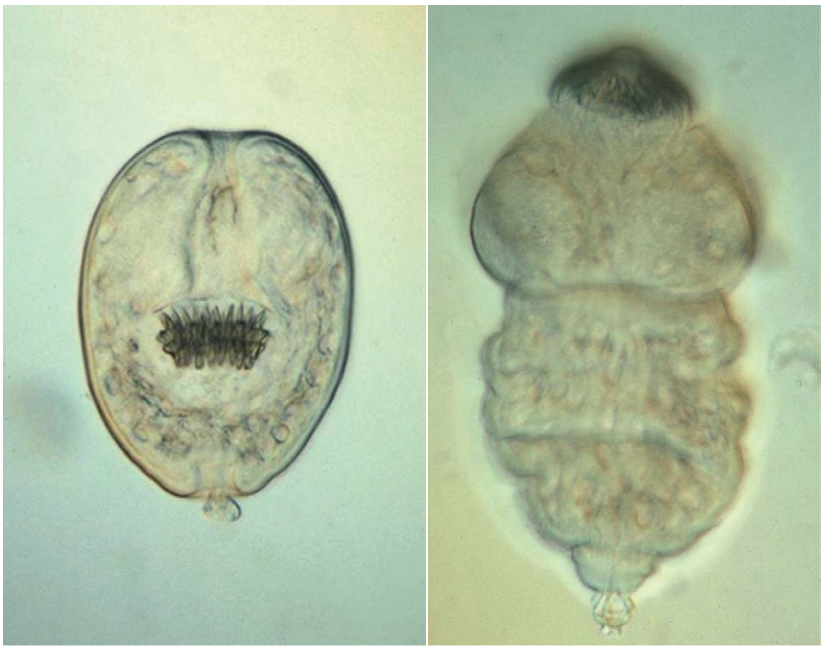


FIGURE 4-46. Protoscolices of *Echinococcus granulosus* from a hydatid cyst. The one on the left is invaginated, whereas the one on the right is evaginated.

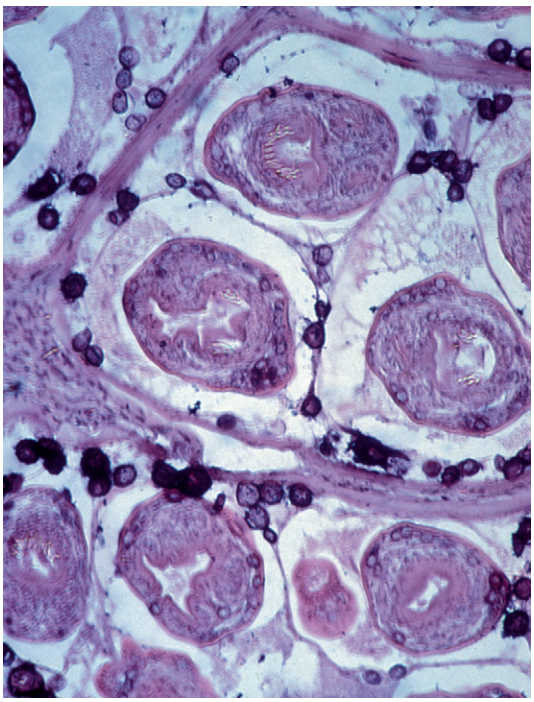


FIGURE 4-47. *Echinococcus multilocularis* alveolar hydatid.

farther away. In the case of a well-established sylvatic cycle, this is about as far as it is practical to go. *Echinococcus* infection may be reduced to an insignificant incidence in cases in which it is limited to a pastoral cycle and thus is accessible to manipulation by humans. Destruction of all stray dogs, regimented anthelmintic medication of the rest, and prohibition against feeding of uncooked offal to dogs and cats are mandatory.

A campaign against hydatid disease was begun in Iceland in 1864. At the outset, about one in six or seven people and virtually all ages of slaughter sheep and cattle harbored hydatid cysts, and about one fourth of dogs were infected with the adult worm. By 1900 the human infection rate had fallen dramatically and had basically reached the point of nonexistence. The campaign, devised by Dr. Harald Krabbe of the Royal Veterinary and Agricultural University of Copenhagen, consisted of alerting the public to the need to observe strict hygiene in dealing with dogs, destroying all cysts and infected offal, and administering mandatory anthelmintic medication to all dogs (Palsson, 1976). Thus salutary results in *Echinococcus* control can be achieved in a century or so, provided there is no sylvatic cycle to complicate the issue. In Australia, for example, a sylvatic cycle involving kangaroos and *Canis dingo* would have to be considered in any eradication attempt. "Obviously the denial of sheep offal to domestic dogs will not eliminate infection if dogs have access to macropods in dingo-infested areas" (Herd and Coman, 1975). In the United States, *E. granulosus* appears to be most prevalent in sheep-raising areas of Utah (Loveless et al, 1978) and California. In California the spread of echinococcosis appears to be related to a quaint trans-human form of husbandry in which bands of sheep migrate from place to place under the control of contract Basque shepherds from Spain and France. These shepherds, for the most part, are ignorant of the epidemiology of hydatid disease and feed their dogs mostly on dead sheep (Araujo et al, 1975). A review of human deaths associated with hydatid disease among U.S. citizens revealed that 41 such deaths were reported between 1990 and 2007 (Bristow et al, 2012). Thirty of the 41 cases were seen in foreign-born citizens, but 11 cases were noted in U.S.-born citizens; it was not possible to ascertain where these people acquired their infection, but these data indicate that transmission to humans may be occurring in the United States.

Vaccines are available that have been successful in preventing the development of hydatid cysts in sheep. These vaccines are currently undergoing field trials in various parts of the world and may go a long way in providing new means of eradication of this parasite in certain locales.

Other Cyclophyllidean Tapeworms

The second larval stage of all of the following cyclophyllidean families are **cysticercoids** of one kind or another. A cysticercoid

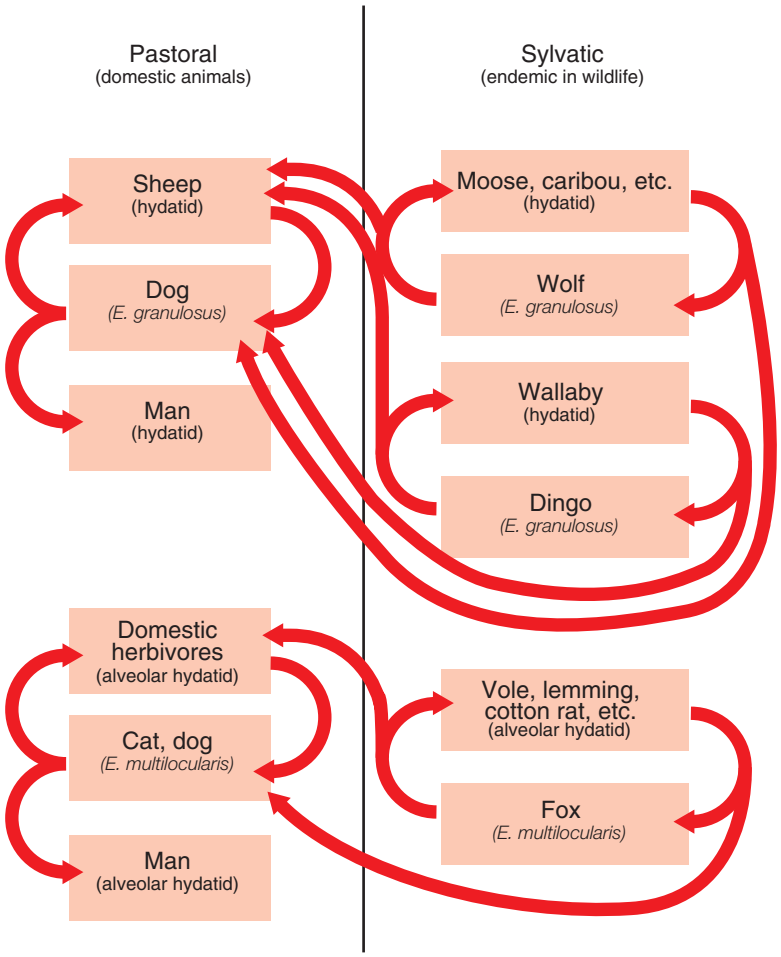


FIGURE 4-48. Pastoral and sylvatic cycles of *Echinococcus granulosus* and *Echinococcus multilocularis.*

may be thought of as a cysticercus small enough to fit into the body of an arthropod. It is small and solid rather than cavitated (the cysticercoid is solid; the cysticercus has a fluid-filled bladder) but has an inverted (or at least introverted) scolex. The cysticercoids of *Mesocestoides* species have yet to be identified, remarkable as that may seem in this enlightened age. The specialists nevertheless seem certain that a cysticercoid stage of *Mesocestoides* must precede the well-known tetrathyridium found in a wide range of mammals, birds, and reptiles.

Family Anoplocephalidae

IDENTIFICATION. *Moniezia* organisms have unarmed scolices with four large suckers and very wide segments with bilateral genitalia. They are found in the small intestine of cattle, sheep, and goats (*Moniezia benedeni, Moniezia expansa,* and *Moniezia caprae*). Interproglottidal glands at the posterior margin of each segment extend the full width of *M. expansa* but occupy only the midzone of the *M. benedeni* segment (Figure 4-49). The egg of *M. benedeni* found in cattle feces is one of the few eggs that appear square, and the pear-shaped (pyriform apparatus) characteristic of anoplocephalid eggs can be seen internally (Figure 4-50).

Thysanosoma actinioides, the fringed tapeworm, is found in the common bile duct and duodenum of virtually all ruminant species except cattle. Ligature of the bile duct within 5 minutes of slaughter has revealed that these worms are probably found almost exclusively in the intestine of the living animal (Boisvenue and Hendrix, 1987). The endemic areas of *T. actinioides* are the western parts of

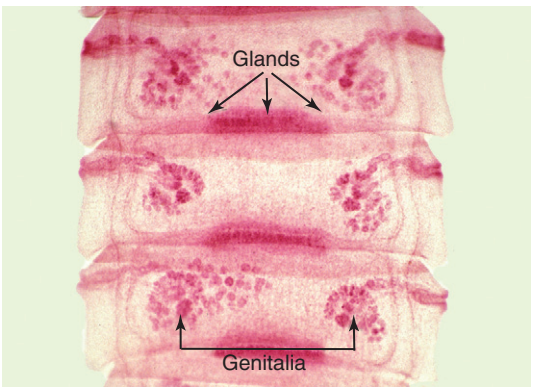


FIGURE 4-49. Mature segments of *Moniezia expansa* (Anoplocephalidae).

North and South America, especially mountainous areas. *Wyominia tetoni* is found in mountain sheep *(Ovis canadensis). T. actinioides* has wide segments with bilateral genitalia and a fringe of outgrowths at the posterior border of each segment. *W. tetoni* resembles *T. actinioides,* but its segments are not fringed. *Thysaniezia, Stilesia,* and *Avitellina* species are exotic anoplocephalids of ruminants.

Anoplocephala magna and *Paranoplocephala mamillana* (Figure 4-51) are relatively harmless parasites in the small intestine of horses. *Anoplocephala perfoliata* (Figure 4-52) is found mainly in the cecum but also tends to cluster in the ileum near the ileocecal valve,

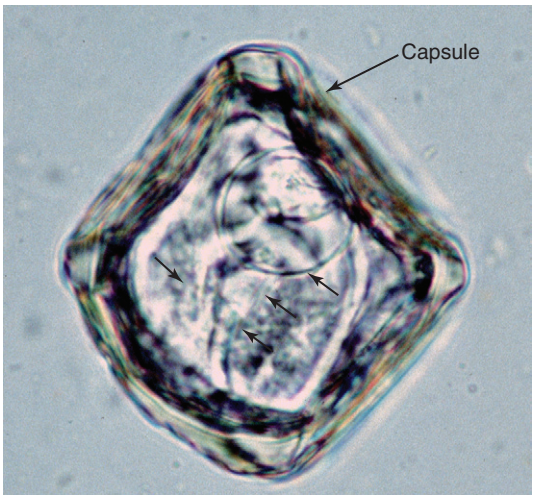


FIGURE 4-50. Egg of *Moniezia* sp. (Anoplocephalidae) of ruminants. The pear-shaped embryophore *(arrows)* is typical of anoplocephalid eggs.

where it is associated with ulceration and reactive inflammation of the ileal wall. This clustering results in ulceration of the mucous membrane and inflammation with thickening and induration of deeper layers of the intestinal wall. These pathologic changes probably account for some cases of persistent diarrhea and may predispose to intussusception of the ileum into the cecum or rupture of the bowel wall in the vicinity of the ileocecal valve (Barclay, Phillips, and Foerner, 1982; Beroza et al, 1983). Proudman and Edwards (1993) published work showing an association between infection with *A. perfoliata* and ileocecal colic in horses. Diagnosis of *A. perfoliata* infection is based on distinguishing the eggs from those of *A. magna* and *P. mamillana*. *A. perfoliata* eggs and segments frequently cannot be demonstrated by flotation or sedimentation techniques in the feces of horses known to be heavily infected with this parasite—a paradox for which we are unable to offer a satisfactory explanation. For this reason, an enzyme-linked immunosorbent assay (ELISA) has been used to examine the immunoglobulin G (IgG) of horses to detect infection with this parasite. In a case-controlled study with this means of detection, horses with tapeworms had a 26 times greater risk of developing spasmodic colic (Proudman, French, and Trees, 1993). However, when a matched case-control study was used with 46 pairs of horses in, Ontario, Canada, along with an ELISA to monitor infection status, no association was found between the presence of *A. perfoliata* and the development of colic in infected horses (Trotz-Williams et al, 2008). However, it does need to be remembered that horses have to be treated occasionally with something other than a macrocyclic lactone to clear them of their tapeworms, which can reach very high numbers without treatment.

LIFE HISTORY. The life histories of only a few anoplocephalids have been documented, but they describe an arthropod intermediate host in which the infective cysticercoid develops. Infection purportedly results from the incidental ingestion of these infected arthropods by the grazing animal. Free-living oribatid mites serve as hosts for cysticercoids of *Moniezia* species of sheep and cattle, *Bertiella* species of primates, and *Cittotaenia* species of the European wild rabbit. *T. actinoides* is apparently transmitted by "booklice" or "barklice" of the family Psocidae, order Psocoptera. Psocopterans resemble mallophagan lice but are entirely free-living and have no other known relationship to parasite life histories.

CONTROL. All tapeworms of cattle, sheep, and goats belong to the family Anoplocephalidae. Pasture renovation is recommended to destroy the surface layer of humus and thus the habitat

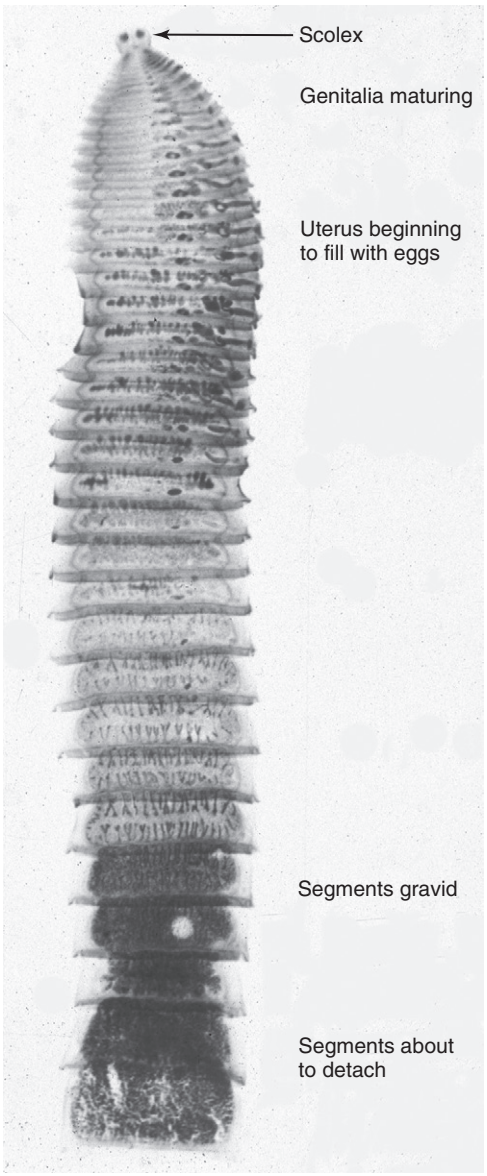


FIGURE 4-51. *Paranoplocephala mamillana* (Anoplocephalidae), entire tapeworm.

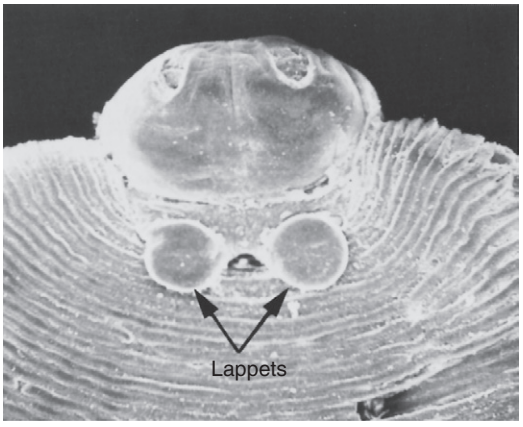


FIGURE 4-52. *Anoplocephala perfoliata* (Anoplocephalidae), scanning electron micrograph. The scolex of *A. perfoliata* is about 2 mm in diameter and has four large suckers and four projections called *lappets*.

of oribatid mites, which are the intermediate host of at least some of these cestodes. However, little experimental evidence is available to support this recommendation. Fortunately, adult tapeworms are relatively nonpathogenic. Species that invade the bile ducts cause condemnation of the liver at slaughter, in this way leading to considerable economic loss.

Family Dipylidiidae

IDENTIFICATION. In *Dipylidium caninum, Diplopylidium* species, and *Joyeuxiella* species, the scolex has four suckers and a retractable rostellum armed with several circles of thornlike hooks (Figure 4-53). Segments are shaped like cucumber seeds and have bilateral genital pores. The genital apertures of *D. caninum* lie slightly behind the middle of the segment (i.e., away from the scolex), and each egg capsule may contain from 5 to 30 eggs (Figure 4-54). The genital apertures of the Middle Eastern, African, and Australasian parasites *Diplopylidium* and *Joyeuxiella* lie before the middle of the segment (i.e., toward the scolex), and each capsule contains a single egg.

A survey of euthanized cats from shelters in Oklahoma revealed that 40 of 116 cats were positive for *D. caninum,* and none were diagnosed using centrifugal sugar flotation (Adolph et al, 2011).

LIFE HISTORY. Cysticercoids of *D. caninum* develop in fleas (*Ctenocephalides* species) and biting lice *(Trichodectes canis),* and the dog acquires this tapeworm while nipping its insects (Figure 4-55). Children also may become infected in this way. Cysticercoids of *Diplopylidium* and *Joyeuxiella* develop in coprophagous beetles; reptiles and small mammals serve as second intermediate hosts.

D. caninum requires only 2 to 3 weeks to develop from a cysticercoid into a segment-shedding tapeworm. Therefore the benefits of anthelmintic therapy are particularly short-lived unless fleas and biting lice also are brought under control. It has been shown that developing cysticercoids require a day or so in a flea that has found a mammalian host to be warm enough to finish their ultimate development to the infective stage (Pugh, 1987).

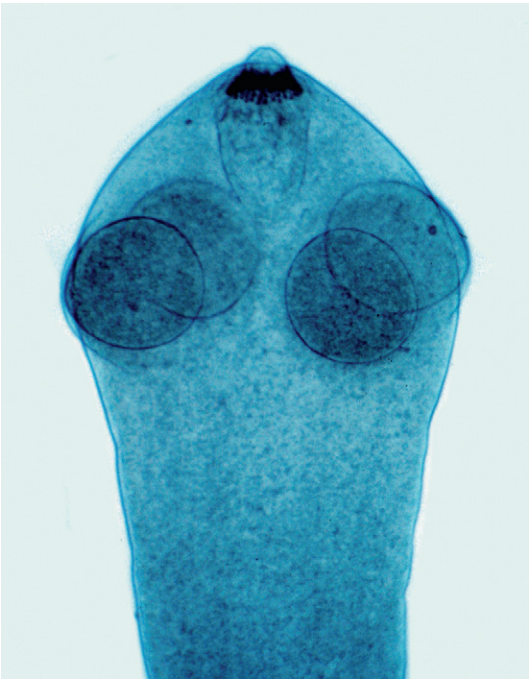


FIGURE 4-53. *Dipylidium caninum* (Dipylidiidae); scolex of fresh stained specimen. The scolex of *D. caninum* is less than 0.5 mm in diameter; the rostellum is retractable and is armed with small, thornlike hooks.

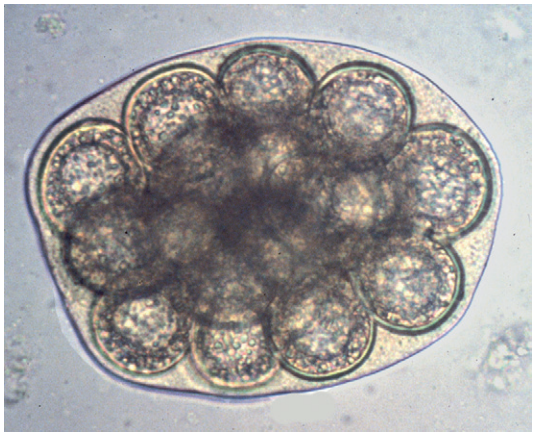


FIGURE 4-54. Egg packet of *Dipylidium caninum.*

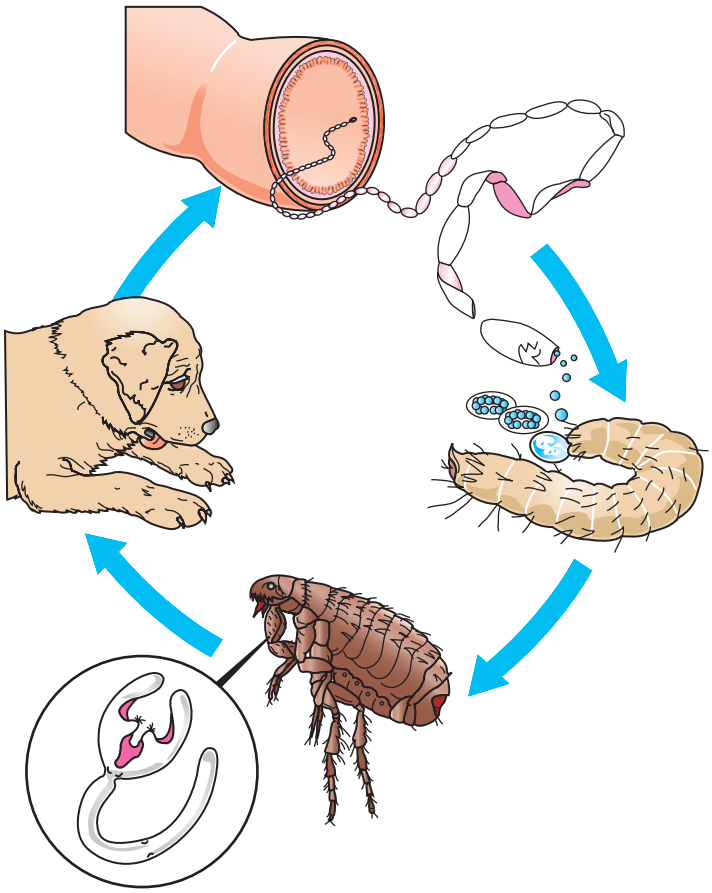


FIGURE 4-55. Life history of *Dipylidium caninum.* Gravid segments discharge their egg packets as they move about. Larvae of *Ctenocephalides* chew their way into egg packets and ingest the oncospheres of the tapeworm. The hexacanth embryo enters the body cavity of the flea larva and remains there through its metamorphosis. After the adult flea emerges from the cocoon, the hexacanth develops into a cysticercoid in 2 or 3 days. If such a flea is ingested by the definitive host, as during self-grooming, the cysticercoids develop into adult tapeworms in the small intestine.

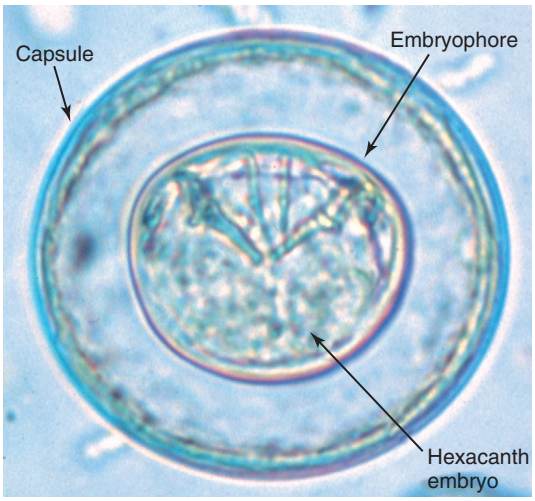


FIGURE 4-56. Egg of *Hymenolepis diminuta* (Hymenolepidae), a common parasite of rodents.

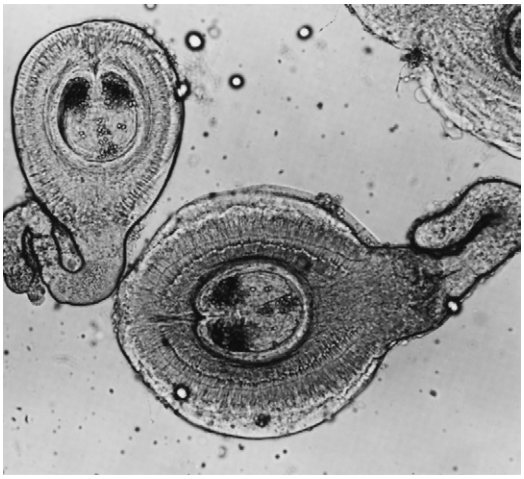


FIGURE 4-57. Cysticercoids of *Hymenolepis diminuta.*

Family Hymenolepididae

The family Hymenolepididae contains many species that occur in birds and two mammalian parasites. *Hymenolepis diminuta* is a parasite of the small intestine principally of rodents but occasionally also of dogs and even humans (Ehrenford, 1977). The eggs of this tapeworm can be found in the feces (Figure 4-56). The cysticercoid of *H. diminuta* develops in fleas, flour beetles, and a rather wide range of other insects (Figure 4-57). *Vampirolepis (Hymenolepis, Rodentolepis) nana* is also a parasite of rodents and humans; its second larval stage is a cysticercoid in fleas and flour beetles or in the intestinal mucosa of its definitive host. *V. nana* can complete its life history within the intestinal tract of a mouse or a human. Some of the eggs hatch within the intestine, and the hexacanth embryos burrow into the mucous membrane to form cysticercoids that later reenter the lumen to complete their development as mature tapeworms. The rest of the eggs pass out with the feces to await ingestion by flour beetles or fleas, in which the cysticercoids develop. Thus *H. diminuta* requires fleas, flour beetles, or other insects as intermediate hosts, whereas *V. nana* may or may not. Because the eggs discharged in feces are infective to humans, *V. nana* infection in laboratory rodent stocks constitutes something of a health hazard for personnel. A survey of mice supplied as pets or as feed for pet reptiles in stores in New York City revealed that half the mice were infected with *V. nana,* and that mice from each of the three stores sampled were positive (Roble, Gillespie, and Lipman, 2012). Because *H. diminuta* infection requires ingestion of an infected insect, human infection with this tapeworm is less probable, but it does occur. Hymenolepids have three testes and a single ovary; *V. nana* has a single circle of hooks on its scolex, whereas *H. diminuta* has no hooks.

Family Mesocestoididae

IDENTIFICATION. The scolex of *Mesocestoides* species has four suckers but no hooks (Figure 4-58). Mature segments have a mediodorsal genital pore, and eggs accumulate in a special, thick-walled parauterine organ as the segments mature (Figure 4-59). Gravid segments detach from the strobila and carry their relatively small burden of oncospheres to the outside world.

LIFE HISTORY. The complete life history of the genus *Mesocestoides* has yet to be worked out. The larval form infective for the definitive host is a third larval stage called a **tetrathyridium** and is found in the peritoneal cavity of mammals and reptiles and in the lungs of birds (see Figures 8-65 to 8-67). A cysticercoid larval stage is hypothesized to precede the tetrathyridium, possibly developing from the oncosphere in a coprophagic insect (Loos-Frank, 1991).

Mesocestoides infection of dogs and cats results from predation on snakes, birds, and small mammals. Some clients find it difficult to accept that their civilized pets are using their long, sharp teeth in an atavistic way, especially sportsmen with expensive bird dogs and vegetarians with cats. However, the carnivoran must be denied prey if *Mesocestoides* infection is to be prevented. Most taeniids have about a 2-month prepatent period, but *Mesocestoides* organisms may start discharging segments hardly more than 2 weeks after infection, thereby imparting the impression that the anthelmintic has not worked at all. To make matters worse, *M. corti* tapeworms multiply asexually in the intestines of dogs. If this species is not totally eliminated by anthelmintic medication, it will repopulate the intestine even without further exposure (Eckert, von Brand, and Voge, 1969). This disease, which is caused by the asexual multiplication of the larvae of this parasite, has been examined as to survivability in 60 dogs undergoing treatment for this infection (Boyce et al, 2011). In this careful analysis, it was found that survival for 1 year was 60.5%, and that dogs treated aggressively with surgery and peritoneal lavage along with high doses of fenbendazole were five times less likely to die than dogs not treated aggressively. The dogs in these cases presented with clinical signs that included ascites (60%), anorexia/weight loss (42%), vomiting (23%), diarrhea (9%), and tachypnea (9%); subclinical infections (22%) were incidentally detected, typically during ovariohysterectomy or neutering. In some cases of peritoneal mesocestodiasis, treatment with praziquantel has proved more efficacious than treatment with fenbendazole (Papini et al, 2010).

Treatment of Adult Tapeworm Infections

Dogs and Cats

Adult tapeworm infections cause little harm or inconvenience to dogs and cats. It is true that infected dogs frequently sit down and drag their bottoms, but so do uninfected dogs. No doubt a tapeworm segment wandering about the perineum tickles. Although this phenomenon must certainly be included in the list of causes for pruritus ani, distended anal sacs are more frequently to blame. The veterinarian who treats pruritus ani by expressing anal sacs will obtain better results than another who prescribes anthelmintics for this condition.

A tapeworm segment crawling about on a pet's tail or freshly passed feces offends most clients, and the civilized world makes

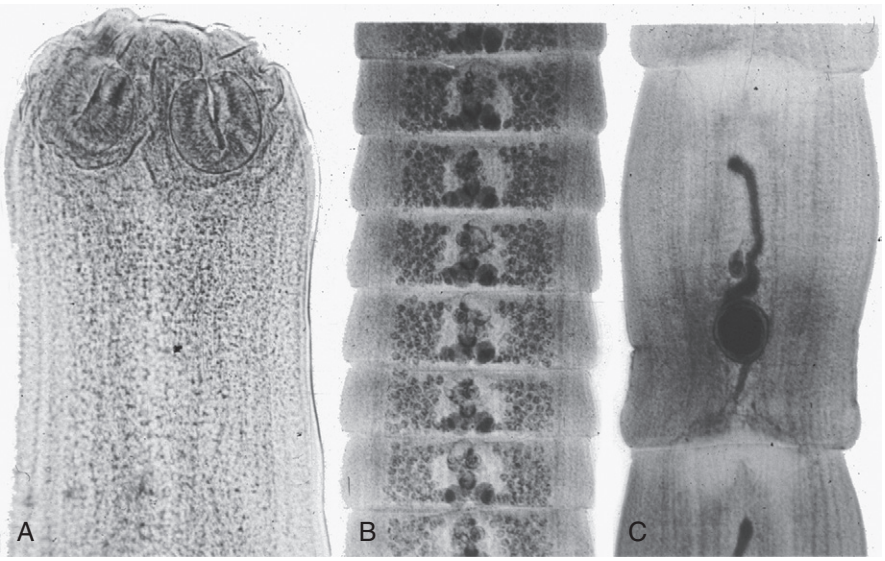


FIGURE 4-58. *Mesocestoides* sp. **A,** Scolex. **B,** Mature segments. **C,** Gravid segments.

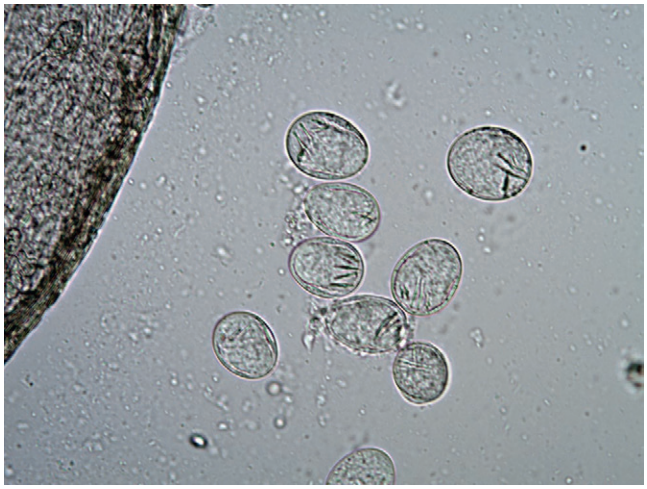


FIGURE 4-59. Eggs of *Mesocestoides* that have been removed from the parauterine organ by pressure applied to the coverslip over a segment placed on a microscope slide.

quite a business of poisoning tapeworms. For lasting results to be obtained, the source of infection must also be dealt with, or the segments will reappear and the client may not.

Many drugs have proven efficacy against the tapeworms affecting dogs and cats. Both praziquantel and epsiprantel show activity against one or more tapeworm genera, but almost all drugs fail against one or another genus.

The cestocidal drug praziquantel, in a single 5-mg/kg oral or subcutaneous dose, eliminates 100% of both immature and adult *T. hydatigena, T. pisiformis, Taenia ovis, T. taeniaeformis, E. granulosus, E. multilocularis, M. corti,* and *D. caninum* from dogs and cats (Anderson, Conder, and Marsland, 1978; Dey-Hazra, 1976; Rommel, Grelck, and Hörchner, 1976; Thomas and Gönnert, 1978). Praziquantel at a dosage of 7.5 mg/kg for 2 consecutive days eliminated 100% of *Diphyllobothrium erinacei,* and a single dose of 35 mg/kg eliminated all *D. latum* from infected cats (Sakamoto, 1977). Praziquantel in combination with pyrantel pamoate and febantel also has been shown efficacious in removing infections with *E. granulosus* and *E. multilocularis.* Epsiprantel at 2.75 mg/kg in cats and 5.5 mg/kg in dogs is efficacious against *D. caninum*, *T. pisiformis,* and *T. taeniaeformis.* Doses of 7.5 mg/kg were required to clear all dogs of infection from adult *E. multilocularis* (Arru, Garippa, and Manger, 1990). Cats in the United States can also be treated with a topical praziquantel (12 mg/kg) and emodepside (3 mg/kg) formulation that has excellent efficacy against both tapeworms, *T. taeniaeformis* and *D. caninum,* as well as the hookworm *Ancylostoma tubaeforme* and the roundworm *Toxocara cati.*

Fenbendazole administered for 3 days at 50 mg/kg is effective against *T. pisiformis*.

Ruminants

For *Moniezia* infection in the United States, fenbendazole has been approved as a cattle anthelmintic at 5 mg/kg. Overseas, fenbendazole is marketed for *Moniezia* control at a higher dose of 7.5 mg/kg. Albendazole also can be used in cattle in the United States for treatment of *Moniezia* infection at the approved dose of 10 mg/kg. Oxfendazole is approved for treating *Moniezia* infection in cattle at a dose of 4.5 mg/kg.

Albendazole is effective against *Thysanosoma* infection in sheep at 7.5 mg/kg. Fenbendazole at 10 mg/kg also appears to be effective (Bergstrom, Taylor, and Presgrove, 1988), as is praziquantel at 40 mg/kg (Martinez, 1984).

Stilesia (exotic) infections are difficult to treat. Praziquantel at a dose of 2.5 mg/kg was extremely effective against *Moniezia* infection in sheep, but doses of 8 to 15 mg/kg were required for the treatment of *Avitellina centripunctata, Stilesia globipunctata,* and *Stilesia hepatica* (Bankov, 1975, 1976; Thomas and Gönnert, 1978).

Horses

Lyons et al (1992) found praziquantel at 1 mg/kg to be highly effective in the removal of *A. perfoliata* from horses. Formulations that deliver ivermectin (0.2 mg/kg) with praziquantel (1 mg/kg or 1.5 mg/kg) and that deliver moxidectin (0.4 mg/kg) with praziquantel (2.5 mg/kg) are available to treat horses in the United States. Although pyrantel is not labeled for the treatment of tapeworms in horses in the United States, Slocombe (1979) found pyrantel at 13.2 to 19.8 mg base per kilogram highly effective; daily feeding of pyrantel tartrate (2.64 mg/kg) to horses significantly reduces tapeworms in both adult horses and yearlings, with most treated animals becoming free of this parasite (Greiner and Lane, 1994; Lyons et al, 1997).

PHYLUM NEMATODA

Body form is remarkably constant among nematodes—a fact that may simplify the anatomy lesson but that somewhat aggravates the difficulties of identification and taxonomic classification. It is helpful in understanding nematode anatomy and physiology to appreciate the significance of the nematodes' unique high-turgor pressure method of maintaining sufficient corporeal rigidity to permit rapid locomotion by sinusoidal undulation. Crofton (1966) brilliantly expounded these relationships in his book *Nematodes,* and the following discussion represents a summary of his exposition.

Nematodes have a relatively large body cavity **(pseudocoelom)** containing fluid under pressure that varies up to one half atmosphere above that of the surrounding medium (see Figures 8-78, 8-85, and 8-96 to 8-98). The body cuticle contains inelastic collagen fibers so arranged that an increase in internal pressure causes an increase in length but minimal change in diameter. This anisometric cuticle and high internal pressure thus maintain a relatively constant body diameter. Nematodes do not have a circular muscle layer. Rather, all of the somatic musculature is oriented longitudinally and is divided into dorsal and ventral fields by lateral expansions of the hypodermis, the **lateral chords.** A muscle cell of either field is connected by a cytoplasmic process to its respective (dorsal or ventral) median nerve. Thus dorsal and ventral flexion of the body is made possible by independent contraction of the corresponding muscle field, and longitudinal waves of contraction result in the sinusoidal pattern characteristic of nematode locomotion.

The high internal pressure also exerts its influence on the structure and organization of the internal organs. For the lumen of the intestine to be filled with food, some sort of pump is essential to overcome the tendency of the pseudocoelomic fluid pressure to collapse it, and most nematodes have a well-developed muscular esophagus for this purpose. Defecation, on the other hand, is accomplished by the contraction of a dilator ani muscle (there is no sphincter) that opens the end of the digestive tube and allows it to empty.

The basic **excretory system** consists of paired unicellular glands with a common midventral excretory pore in the neck region (near the circumesophageal nerve ring) and ducts that, in some forms, run nearly the full length of the body in the substance of the lateral chords. In the Ascaridoidea and related groups, the excretory system is composed of a single very large cell with a very large nucleus, with the pore being located near the nerve ring or anteriorly between the subventral lips.

Male nematodes are smaller than the females of their species. Their caudal ends may terminate in a cuticular expansion supported by muscular rays. This so-called **copulatory bursa** reaches its highest development among the strongylids and is used to grasp the female (Figure 4-60). The Strongylida are therefore considered the "bursate" nematodes, whereas the Oxyurida, Ascaridida, and Spirurida constitute a group of nematodes considered to be the "abursate" nematodes. Within the Dioctophyamatoidea of the order Enoplida, male *Dioctophyme* and *Eustrongyloides* have a round, sucker-like bursa, but these worms are sufficiently distinct that there should be no confusion with the strongylids. The **copulatory spicules** used to dilate the vulva of the female are cuticular structures that develop by sclerotization of folds of the dorsal wall of the cloaca. Spicules are often paired, but some species have only one (e.g., *Trichuris* species) or none (e.g., *Trichinella* species); they vary greatly in size and shape among species and are often used as diagnostic characters. In many species, accessory sclerotizations of the cloacal wall serve as guides for the spicules. A spicule guide in the dorsal wall is called a **gubernaculum,** and one located in the ventral wall is called a **telamon.** The primary male reproductive organs consist of a single convoluted tube with regions structurally and functionally differentiated as testis, seminal vesicle, and vas deferens. The terminal portion of the vas deferens with its strong muscular coat, called the **ejaculatory duct,** empties into the **cloaca.** Some male nematodes have two reproductive ducts, but none of these are animal parasites.

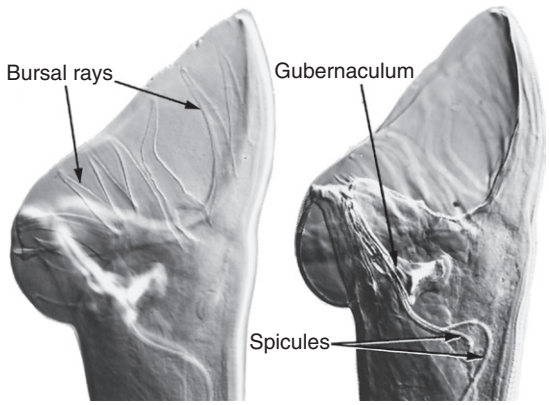


FIGURE 4-60. Surficial *(left)* and sagittal *(right)* aspects of the copulatory bursa of *Cyathostomum labiatum,* a typical member of the order Strongylida, superfamily Strongyloidea.

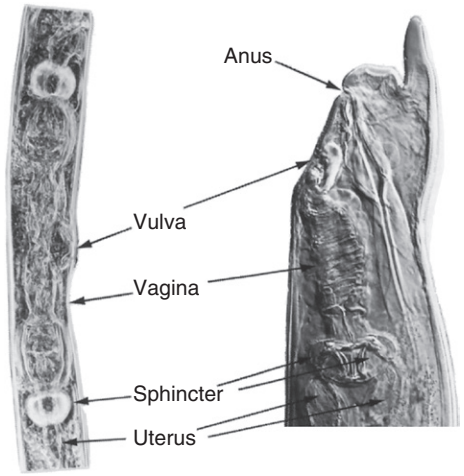


FIGURE 4-61. Ovijectors of a representative of the superfamily Trichostrongyloidea *(left)* and of the superfamily Strongyloidea *(right)* (×64).

The female reproductive system is also tubular and usually has two branches (i.e., **didelphic**) but may be monodelphic or even multidelphic. Regions structurally and functionally differentiated as ovary, oviduct, uterus, and vagina communicate through the vulva with the exterior. The vulva is ventral in position and may be located near the oral end **(opisthodelphic),** the caudal end **(prodelphic),** or the middle of the body **(amphidelphic).** The location and special anatomic features of the vulva are useful in identification (Figure 4-61). In female strongylids, a muscular ovijector regulates the discharge of eggs from the uterus. The eggs contained in the terminal portion of the uterus are valuable aids in identifying nematodes. See Chapter 7 for illustrations of nematode eggs.

All rational control efforts are based on an understanding of the life history and behavior of both host and parasite. A general outline of the ontogenetic development of a nematode is shown in Figure 4-62. What appear to be a rich and confusing diversity of

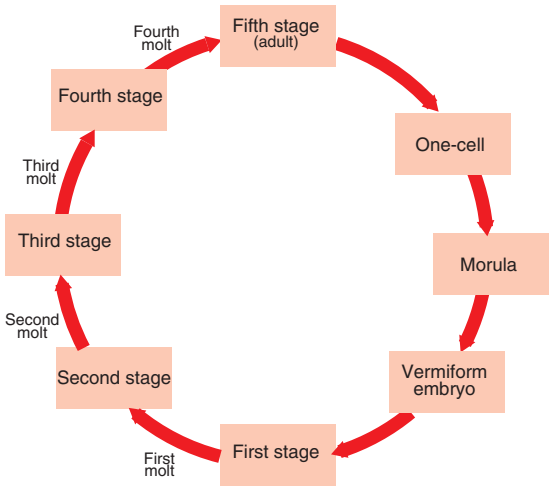


FIGURE 4-62. Stages and transitions in the ontogenetic development of a nematode.

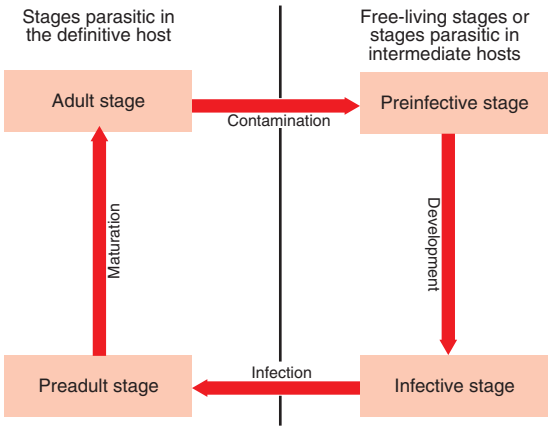


FIGURE 4-63. A generalization of nematode life histories, emphasizing the stages and transitions of greatest importance for diagnosis, treatment, and control. As used here, the term *preadult stage* refers to all stages of parasitic larval development from entry of the parasite into the host to attainment of sexual maturity. Maturation represents the length of time required for this transition. Similarly, *preinfective stage* represents all developmental stages leading up to the infective stage, and *development* represents the time required for that transition.

life histories among various orders of nematodes can all be related and rationalized according to this basic pattern. Embryonic development is, of course, a continuous process, with change accompanying every cell division. The "one-cell," "morula," and "vermiform embryo" stages are arbitrarily chosen from this continuum because they are the stages of egg development most frequently encountered in diagnostic procedures. The difference between a vermiform embryo and a first-stage larva is that the former contains only cell clusters as organ primordia, whereas the latter displays clearly recognizable organs such as esophagus, intestine, and excretory glands. A microfilaria is an example of a vermiform embryo, which develops into a larva only after it has been ingested by a mosquito. Each larval stage is separated from the next by a molt marked by metamorphosis of the larva and ecdysis or casting off of the cuticle from the preceding stage.

The nematode life history also can be generalized from the standpoint of important events related to diagnosis, treatment, and control. Figure 4-63 presents these events as four stages (adult, preinfective, infective, and preadult) separated by four transitions (contamination, development, infection, and maturation). In mastering the details of any particular nematode life history, the process of integrating these two schemes is a profitable intellectual exercise. The prepatent periods of the more important veterinary species of nematodes are presented in Table 4-3.

The systematics used in this text discussion of nematodes follow those of the CIH Keys to the Nematode Parasites of Vertebrates, which appeared as a series between 1974 and 1983 (Anderson, Chabaud, and Willmott). More recently, other classifications have appeared, and one of the more commonly used is that of Dorris, De Ley, and Blaxter (1999; Figure 4-64). Because this classification scheme is used more and more commonly, it seems useful to provide it here for users of this text. The differences between the two systems are minimal. In the system used here, the Nematode Class Adenophorea (*Trichinella*, whipworms, and relatives) is represented in the clade groupings as Clades I and II. The major number and groups of nematodes discussed in the text that follows are placed in the Class Secerntea (horse strongyles, ascarids, hookworms, lungworms, pinworms, and heartworm), which is included in Clades III, IV, and V in the other presentation. Thus, Clade I contains the Enoplida with the Dioctophymatoidea, Trichinelloidea, and Muspiceoidea; Clade III contains the abursate Oxyurida, Ascarida, and Spirurida; and Clade V contains the bursate Strongylida. The only real difference is that *Strongyloides* is placed in

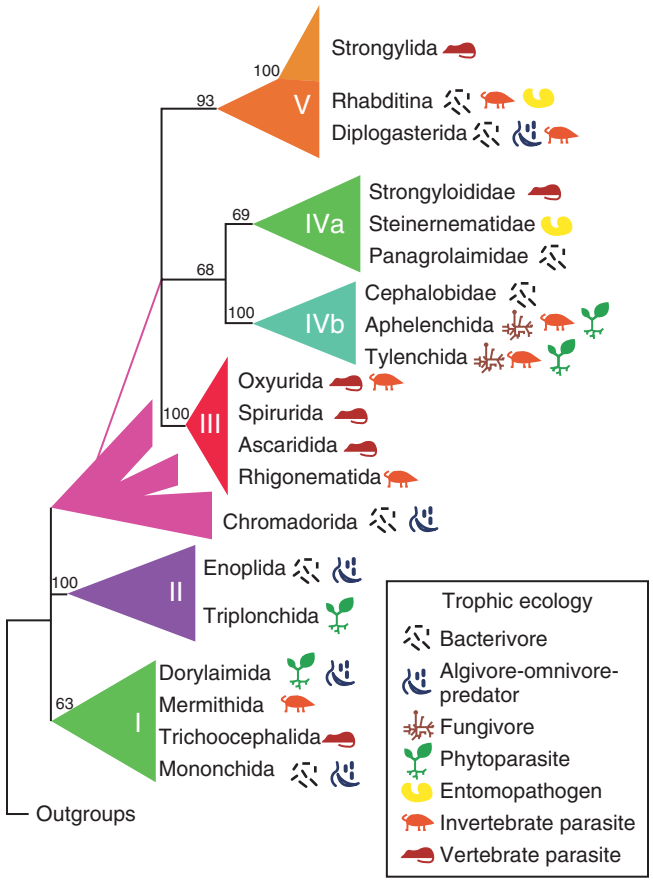


FIGURE 4-64. Phylogenetic structure of the Nematoda revealed by analysis of full-length small-subunit rDNA sequences. (From Dorris M, De Ley P, Blaxter ML: Molecular analysis of nematode diversity and the evolution of parasitism, *Parasitol Today* 15(5):188-193, 1999.)

Clade IV away from some of the other rhabditoid nematodes found in Clade V. One should not get lost in either classification scheme or in the minor quibbles of systematists. For the most part, in the world of veterinary medicine, what practitioners typically want to know is how it relates to things they already know well, so the

TABLE 4-3 Some Nematode Prepatent Periods*

Parasite	Prepatent Period	Comments
SECERNENTEA		
STRONGYLIDA		
Trichostrongyloidea		
Trichostrongylus	¾ month	Arrested larvae
Ostertagia	¾ month	Arrested larvae
Haemonchus placet	1 month	
Haemonchus contortus	¾ month	
Cooperia	½ month	
Nematodirus	¾ month	
Hyostrongylus	¾-1 month	
Dictyocaulus	1-1¼ month	
Strongyloidea		
Cyathostominae	2½-4 months	Arrested larvae
Strongylus vulgaris	6-7 months	
Strongylus equinus	9 months	
Strongylus edentatus	11 months	
Triodontophorus	3-6 months	
Chabertia	1¼ months	Arrested larvae
Oesophagostomum	1½ months	Arrested larvae
Stephanurus dentatus	9-16 months	
Ancylostomatoidea		
Ancylostoma caninum	½ month	Arrested larve/ transmammary infection
Ancylostoma tubaeforme	½ month	Arrested larvae
Uncinaria stenocephala	½ month	Arrested larvae
Uncinaria lucasi	½ month	Arrested larve/ transmammary infection
Bunostomum	1½-2¼ months	
Metastrongyloidea		
Crenosoma	¾ month	
Filaroides hirthi	1¼ months	Potential autoinfection
Filaroides osleri	6 months	Potential autoinfection
Aelurostrongylus abstrusus	1¼-1½ months	
Protostrongylus	1¼-1½ months	
Metastrongylus	¾-1 month	
Muellerius	1½ months	
Parelaphostrongylus tenuis	2¾-3 months	Not patent in most domestic animal hosts
RHABDITIDA		
Strongyloides stercoralis	½ month	Transmammary infection
Strongyloides papillosus	¼-½ month	Transmammary infection
OXYURIDA		
Oxyuris equi	4-5 months	
ASCARIDIDA		
Ascaris suum	2 months	
Parascaris equorum	2½ months	
Toxocara vitulorum	¾ month in calves	Transmammary infection
Toxascaris leonina	2 months	Paratenic hosts
Toxocara canis	1-2 months	Transplacental infection, paratenic hosts
Toxocara cati	2 months	Paratenic hosts
SPIRURIDA		
Gongylonema	2 months	Intermediate host, dung beetle or cockroach
Draschia	2 months	Intermediate host, Musca
Habronema	2 months (?)	Intermediate host, Musca/Stomoxys
Thelazia	¾-1 month	Intermediate host, Musca or fruit flies
Setaria	8-10 months	Vector: mosquitoes
Onchocerca	10+ months	Vector: blackflies or ceratopogonids
Elaeophora	4½ months	Vector: tabanids
Dirofilaria	6½-7 months	Vector: mosquitoes
Acanthocheilonema reconditum	2-3 months	Vector: fleas
Dracuncula insignis	10-12 months	Intermediate host, copepod
Spirocerca lupi	6-8 months	Intermediate host, dung beetle or paratenic host, vertebrate
ADENOPHOREA		
Trichinelloidea		
Trichuris vulpis	2½-3 months	
Trichinella spiralis	¼-½ month	Find adults in diarrheic feces
Dioctophymatoidea		
Dioctophyme renale	4-5 months	Eggs in urine

*All periods are presented as months postinfection in a naive animal.

questions become more like these: "Does it migrate through the lungs?" "How does it compare with heartworm?" "Does a cow get it while grazing?" "Can you kill it with ivermectin?" And yes, it is sometimes hard to see it, but the ordering schemes actually provide a latticework for constructing some sort of mental order.

SECERNETEAN NEMATODES

The Secernentea nematodes include the majority of the parasitic forms of nematodes. Besides the Stryongylida, Rhabditia, Oxyuridam, Ascaridida, and Spirurida, they also include a group, the Tylenchida, that is mainly free-living but that includes some species parasitic in invertebrates, including insects, mites, and leeches. The Secernentean parasites of vertebrates follow the rule of the infective third stage, wherein the vertebrate host is almost invariably infected by a third-stage larva. This is not the same in the Adenophorea, to be discussed later, in which species of the Trichinelloidea are infective to the vertebrate as a first-stage larva and where *Dictophyma renale* is infective to the vertebrate as a third-stage larva.

ORDER STRONGYLIDA

The order Strongylida is composed of four superfamilies: (1) Strongyloidea, the large bowel strongyles of horses and the nodular worms of ruminants, swine, and primates; (2) Trichostrongyloidea, the abomasal and small intestinal hairworms of ruminants; (3) Ancylostomatoidea, the hookworms of diverse mammals; and (4) Metastrongyloidea, the lungworms. One of the most important genera of nematodes *(Dictyocaulus)* that live in the lungs, hence lungworms, falls within the Trichostrongyloidea rather than the Metastrongyloidea superfamily, but there are always exceptions to be resolved.

Morphology

The strongylid mouth, or **stoma,** presents important diagnostic characteristics that are the same for both male and female and usually sufficient for generic identification. Strongyloids have well-developed **buccal capsules** often armed, at the base, with teeth (Figure 4-65). Ancylostomatoids also have well-developed buccal capsules, but these are permanently flexed dorsally and armed on their ventral (leading) edge with formidable pointed **teeth** or rounded **cutting plates** (Figure 4-66). In the Trichostrongyloidea, the buccal capsule usually is reduced in size but may be equipped with a tooth or a lancet in bloodsucking species (Figure 4-67). In the typical metastrongyloid, the buccal capsule is absent.

Male nematodes of the order Strongylida have a caudal **copulatory bursa** that consists of dorsal, lateral, and ventral expansions of the body cuticle **(lobes)** supported by muscular processes called **rays** (Figure 4-68). The dorsal lobe contains one ray that is usually median in position and variously branched. The lateral lobes each contain an externodorsal ray adjacent to the dorsal lobe and three rays arising in a group: posterolateral, mediolateral, and anterolateral. Each of the ventral lobes contains two rays. The disposition and configuration of these rays are used in classification and identification of strongylids. In typical members of the superfamilies Strongyloidea and Ancylostomatoidea, dorsal and lateral lobes are about equally developed (Figure 4-69; see also Figure 4-60); in Trichostrongyloidea, lateral lobes predominate (see Figure 4-68); and in Metastrongyloidea, the bursa tends to be reduced in size (Figure 4-70). In some metastrongyloids (e.g., *Filaroides* species), the bursa is completely absent (Figure 4-71).

The **spicules** of males of the superfamilies Strongyloidea and Ancylostomatoidea tend to be long, thin, and flexible (see Figures 4-60 and 4-69), whereas those of the Trichostrongyloidea tend to be shorter and substantially stouter (Figure 4-72; see also Figure 4-68). In the Metastrongyloidea, spicules vary so widely in size and shape that generalization is unprofitable.

The strongylid uterus has two horns and is equipped with a well-developed muscular ovijector (see Figure 4-61). In typical trichostrongyloids and ancylostomatoids, the vulva is located near the middle of the body, and the two horns of the uterus extend in opposite directions **(amphidelphic).** In strongyloids and metastrongyloids, the vulva is typically located close to the anus, and both horns of the uterus extend anteriorly **(prodelphic).**

Life History

The life histories of superfamilies Strongyloidea, Trichostrongyloidea, and Ancylostomatoidea are typically direct, with free-living microbivorous first and second larval stages and an infective third larval stage (Figure 4-73). Females of all three superfamilies lay typical **strongyle eggs** (i.e., eggs with smooth-surfaced, ellipsoidal shells that contain an embryo in the morula stage of development when laid and passed out with the feces). Such eggs are produced by all members of the order Strongylida, except certain genera in

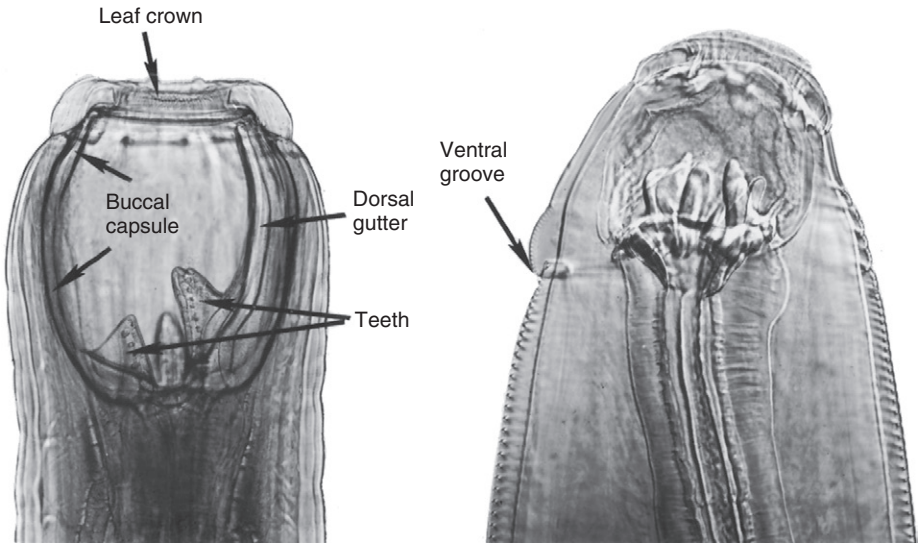


FIGURE 4-65. Superfamily Strongyloidea. *Left, Strongylus equinus. Right, Ternidens deminutus.*

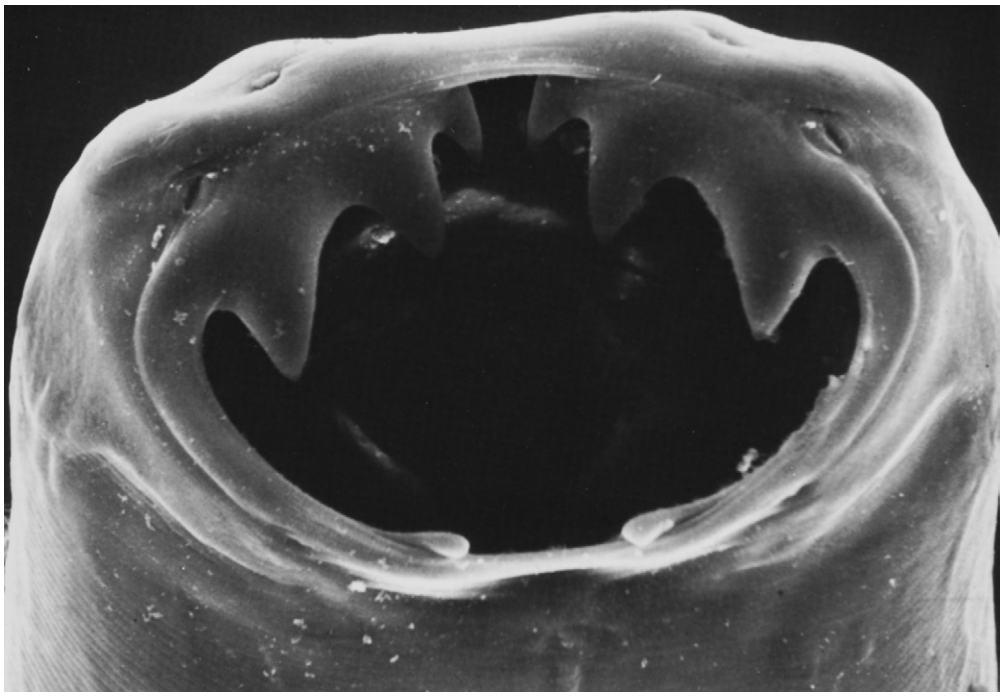


FIGURE 4-66. Superfamily Ancylostomatoidea. Dorsal aspect of the buccal capsule of *Ancylostoma caninum,* the common hookworm of the dog. The three pairs of pointed teeth are at the ventral margin of the stoma.

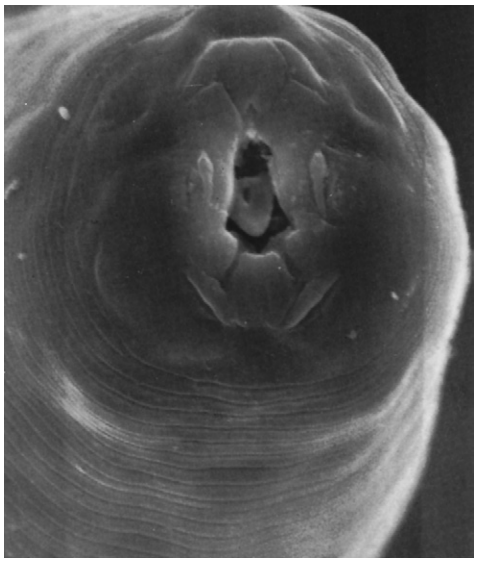


FIGURE 4-67. Superfamily Trichostrongyloidea. En face view of the stoma of *Haemonchus contortus,* the stomach worm of sheep. This voracious bloodsucking nematode uses its lancet to puncture the mucous membrane of the abomasum. (Courtesy Dr. Marguerite Frongillo, Cornell University, Ithaca, New York.)

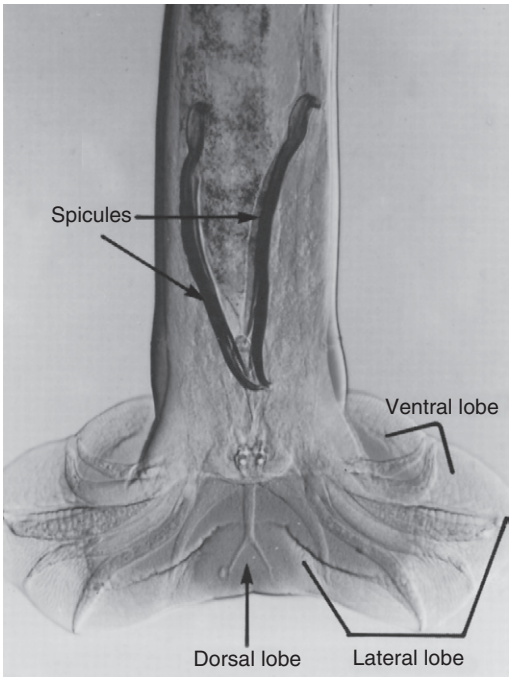


FIGURE 4-68. Superfamily Trichostrongyloidea. Bursa and spicules of *Teladorsagia circumcincta,* an abomasal parasite of sheep.

the superfamily Metastrongyloidea, and are therefore properly termed *strongylid eggs.* However, "strongyle" conveys the same meaning to most and is commonly used. Often in ruminants, where eggs of the trichostrongyloids predominate, such eggs are called "trichostrongyle eggs," even though it is clear that some of the eggs might be those of the rarer strongyloids present in these hosts. Similarly, in dogs and cats such eggs are often called "hookworm eggs" because they are the predominant strongylid worms present in these hosts.

Typically, in the developing eggs, the morula develops into a first-stage larva that hatches from the egg within a day or two. After feeding, this larva undergoes its first molt to become a second-stage larva. Both first- and second-stage larvae remain in the feces, where they feed on bacteria. In the second molt, the cuticle of the second stage is temporarily retained as a protective sheath about the infective third-stage larva and will not be shed until this larva enters a suitable host. In about a week, these sheathed third-stage larvae begin to migrate out of the fecal mass and into the water film

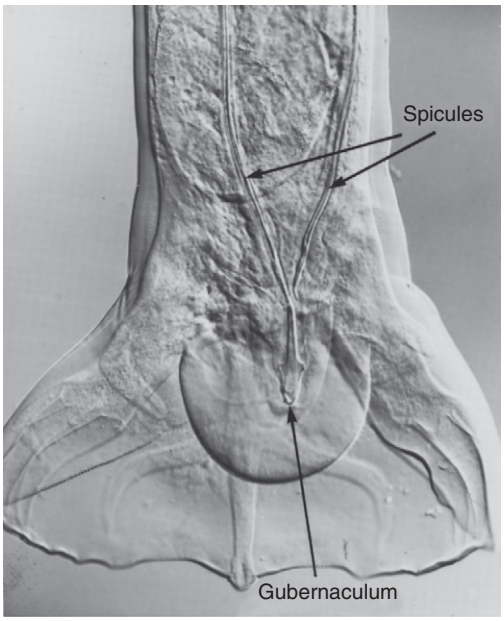


FIGURE 4-69. Superfamily Ancylostomatoidea. Bursa and spicules of *Placoconus lotoris,* a hookworm of the raccoon, *Procyon lotor*.

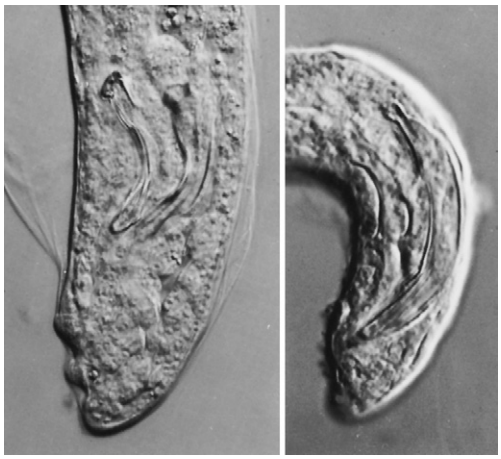


FIGURE 4-71. Superfamily Metastrongyloidea. Caudal ends of male *Filaroides hirthi (left)* and *Filaroides milksi (right),* showing reduction of bursal structures to mere papillae. The spicules of *F. hirthi* are shorter, are broader in relation to their length, and have broader knobs than the spicules of *F. milksi* for attachment of retractor muscles.

FIGURE 4-70. Superfamily Metastrongyloidea. Bursa and spicules of *Protostrongylus rufescens*.

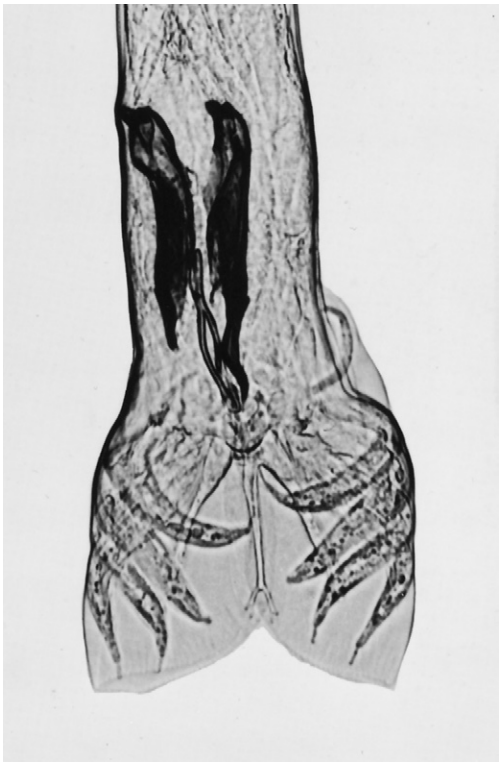


FIGURE 4-72. Superfamily Trichostrongyloidea. Bursa and spicules of *Trichostrongylus axei,* a parasite of the abomasum of ruminants and of the stomach of horses.

covering the surrounding soil particles and vegetation. Infection occurs when these sheathed larvae are ingested by grazing animals. Variations on this basic life history pattern are discussed later in connection with the several genera.

Various representatives of the superfamily Metastrongyloidea lay eggs in all stages of development from a single cell (e.g., *Aelurostrongylus* species) to an egg containing a first-stage larva (e.g., *Filaroides* species). However, sufficient development occurs within the host that the form found in the feces may be a first-stage larva or an egg containing a first-stage larva. Metastrongyloids typically require a molluskan or annelid intermediate host for development from the first stage to the infective third stage, and infection of the definitive host occurs through ingestion of snails, slugs, or earthworms containing infective third-stage larvae. *Filaroides osleri* and *Filaroides hirthi,* both directly infective to the dog in the first larval stage, are important exceptions to this rule.

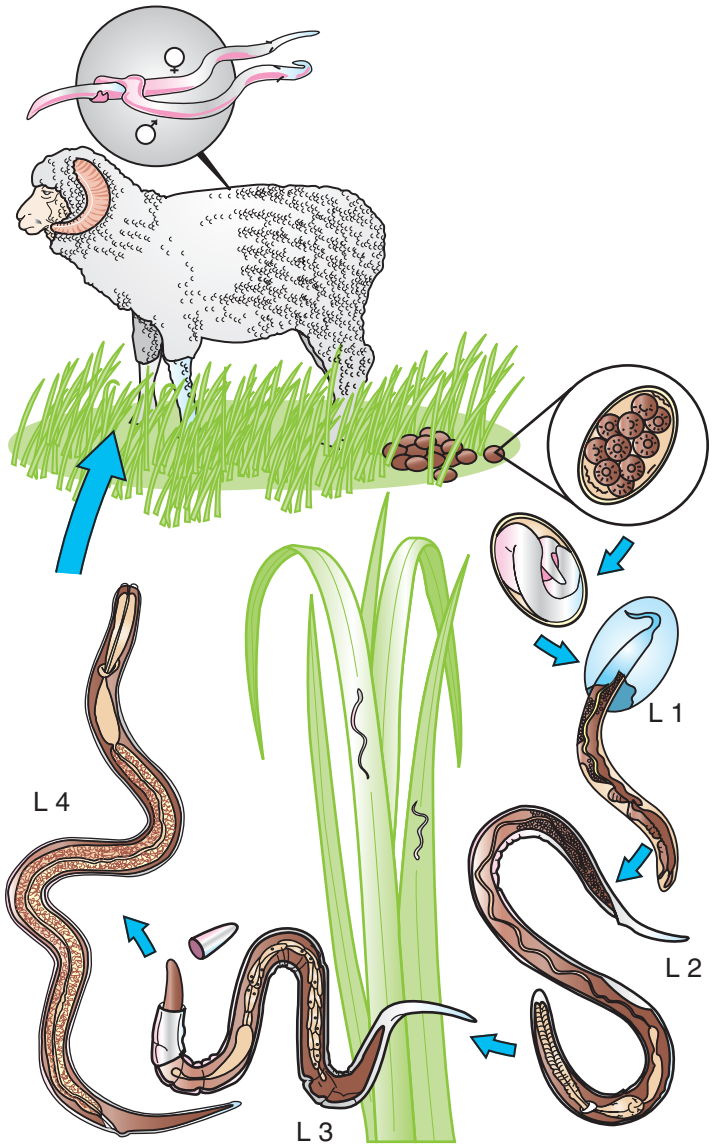


FIGURE 4-73. Life history of a typical strongylid nematode, *Haemonchus contortus.* Eggs are shed in the feces in the morula stage of development. First-stage larvae develop and hatch in a day or two to feed on microorganisms in the feces. After a molt, the resulting second-stage larva also feeds on microorganisms. The second molt is started but not completed in the external environment, so the infective third-stage larva remains encased in the cuticle of the second stage until it is ingested by a sheep. The sheath is cast off in the abomasum of the sheep, and the now parasitic third-stage larva undergoes a molt to the fourth stage. The fourth stage sooner or later molts to the fifth or adult stage, depending on whether it enters a period of arrested development.

Superfamily Trichostrongyloidea

Trichostrongyloid nematodes are especially common and pathogenic in grazing ruminants, but swine, horses, cats, and birds also host important species. The abomasum and the small intestine are the usual locations in ruminants, but one aberrant genus, *Dictyocaulus,* reaches maturity in the air passages. It is sufficient, for practical purposes of effective treatment and control, to identify trichostrongyloids at the generic level of the older classification schemes (Yorke and Maplestone, 1926).

Trichostrongylus

IDENTIFICATION. These very small, hairlike worms are less than 7 mm long, without cephalic inflations, and virtually without a buccal capsule; spicules are short, twisted, and usually pointed (Figure 4-74; see also Figure 4-72). *Trichostrongylus axei* parasitizes the simple stomach or abomasum of a wide range of hosts including ruminants, horses, and leporids. Other species are parasites of the small intestine of ruminants and display a higher order of host specificity. Even heavy infection with *Trichostrongylus* will be overlooked on necropsy examination unless care is taken to thoroughly examine washings or scrapings of the stomach and the first 6 m of the small intestine, preferably with a hand lens or a stereoscopic microscope. *Trichostrongylus* species are most likely to be confused with *Strongyloides* species or with the smaller species of *Cooperia.*

LIFE HISTORY. The infective third-stage larvae of *Trichostrongylus* species survive the winter on pasture, and ruminants are exposed to infection when they are turned out to pasture in spring. As the weather becomes warmer, the infective larvae die off, and by summer the overwintering generation is essentially gone. However, egg production from new infections rapidly recontaminates the pasture and continues well into fall to produce the next season's overwintering population of *Trichostrongylus* organisms.

IMPORTANCE. Although *Trichostrongylus* infections are often asymptomatic, when present in large numbers (10,000 to 100,000 or more), these parasites are capable of producing protracted and debilitating watery diarrhea, especially in stressed or malnourished sheep, cattle, and goats. At first the feces remain semisolid, but soon they become watery and dark green in color ("black scours"), staining the fleece of the hindquarters. Some of the feces accumulate in pea- to egg-sized masses ("dingleberries," "dags") that dangle from the fleece and grow by accretion as fluid feces continue to pour over and dry on their surfaces. The resulting foul condition tends to attract blowflies, such as *Lucilia cuprina* in Australia, and to result in myiasis. Egg counts rarely exceed 5000 eggs per gram because *Trichostrongylus* organisms are very small worms that lay few eggs, and because the feces are greatly diluted with water. Necropsy examination reveals a wasted carcass without obvious lesions even in the affected small intestine; the parasites themselves are easy to overlook because they are so small. Protracted diarrhea is sufficient to account for the weakness and emaciation typically observed in trichostrongylosis, but it is important to remember that less than massive burdens of *Trichostrongylus* organisms do not usually cause serious illness in well-nourished, unstressed ruminants. Therefore it may be important to consider the quality of the environment and animal husbandry in identifying the ultimate causes of particular outbreaks.

Ostertagia and *Teladorsagia*

IDENTIFICATION. *Ostertagia* and *Teladorsagia* are indistinguishable by the criteria outlined as follows; however, *Teladorsagia* are parasites of sheep and goats (e.g., *Teladorsagia circumcincta*) whereas *Ostertagia* are parasites of cattle (e.g., *Ostertagia ostertagi*). Usually less than 14 mm long and brownish in color, with a short, broad buccal cavity (see Figure 4-74) and short, two- or three-pronged spicules (Figure 4-75; see also Figure 4-68), parasites of these genera are found in the abomasum of ruminants. The tip of the mature female's tail is usually annulated (Figure 4-76); the eggs in the amphidelphic ovijector are typical strongylid eggs; and the vulva is guarded by a cuticular expansion called a *vulvar flap.*

LIFE HISTORY. *Ostertagia*- and *Teladorsagia*-infective third-stage larvae resemble those of *Trichostrongylus* in overwintering on northern pastures and in thus infecting ruminants during the early grazing season. However, arrested development of parasitic larvae

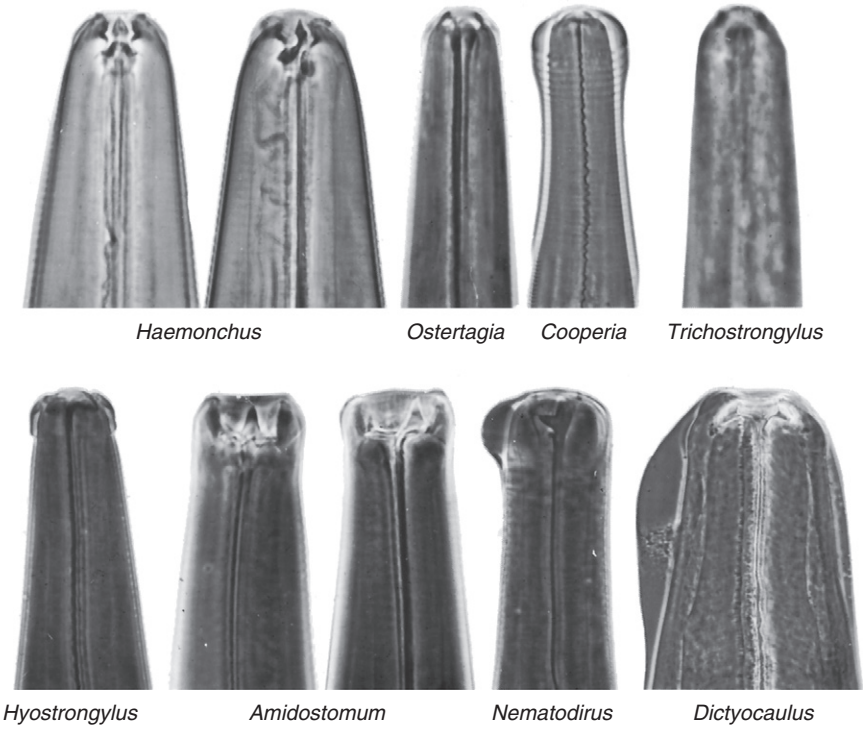


FIGURE 4-74. Stomas of eight genera of the superfamily Trichostrongyloidea. *Amidostomum* is a parasite of geese and ducks but not of mammals; its large, toothed buccal capsule is not typical of trichostrongyloids. (From Whitlock JH: *Diagnosis of veterinary parasitisms,* Philadelphia, 1960, Lea & Febiger.)

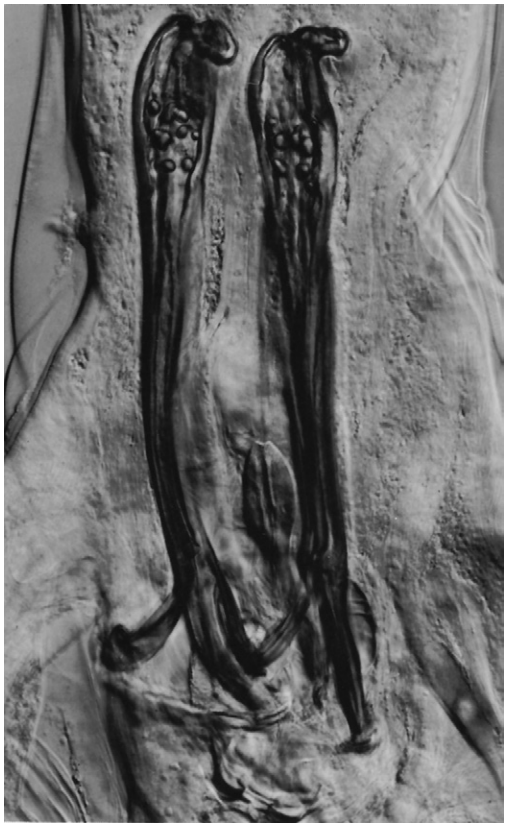


FIGURE 4-75. Spicules of *Ostertagia ostertagi.*

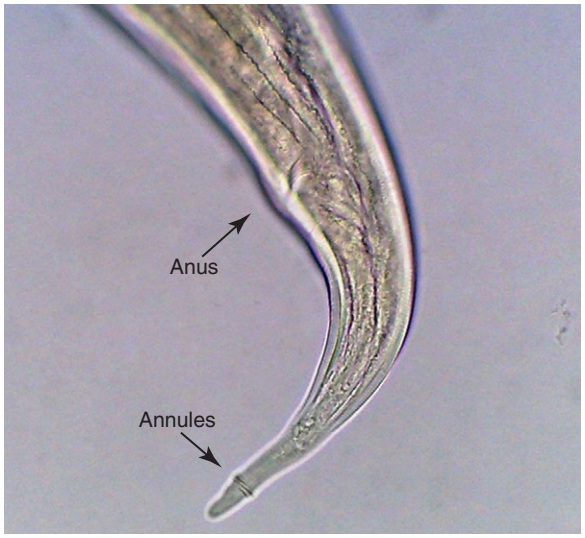


FIGURE 4-76. Tail of female *Teladorsagia* nematode. (Photograph courtesy J. Avula, Department of Pathobiology, University of Guelph.)

is also very well established in *Ostertagia* species, and this is of both epidemiologic and pathologic importance. **"Type I"** or **"summer" ostertagiosis** usually occurs in pastured young cattle, the worms maturing without first passing through a developmental arrest (i.e., hypobiotic or latent phase). By contrast, **"type II"** or **"winter" ostertagiosis** typically occurs in late winter, when larvae that have remained in arrested development since fall once again become metabolically active and proceed to develop into adults. Such behavior is part and parcel of the normal mechanism used by *Ostertagia* species and certain other trichostrongyloids for overwintering. However, when mistimed or overdone to such an extent as to overcome the compensatory mechanisms of the host, it leads to winter ostertagiosis.

IMPORTANCE. *O. ostertagi* causes chronic abomasitis in young cattle, a disease marked by profuse watery diarrhea, anemia, and hypoproteinemia manifested clinically as submandibular edema. The animal is typically hidebound and emaciated. The appetite

remains intact, which seems paradoxical in view of the advanced pathologic changes taking place in the abomasum. The hydrogen ion concentration of the gastric juice approaches neutrality. Necropsy examination reveals a wasted carcass with depletion of fat deposits typical of extreme malnutrition. The rumen, reticulum, and omasum may be full of good feed, but when the alimentary tract from the cardia onward is virtually empty owing to malfunction of the abomasums, the animal has starved to death in the midst of plenty. The "Morocco leather" appearance of the abomasal mucosa is pathognomonic; the whole mucosa is studded with grayish white, pinhead- to pea-sized nodules with a worm protruding from a small opening at the summit of each (see Figures 7-69, 8-76, and 8-77). *O. ostertagi* is the most important helminth parasite of cattle in the United States. Young cattle infected with large numbers of this parasite waste away and die in a matter of weeks. Those infected with sublethal parasite burdens fail to achieve their full potential for growth and development or require substantially more time to do it. Either situation is economically disadvantageous. *Teladorsagia* species of sheep and goats may also cause serious endemic disease in certain localities.

Haemonchus

IDENTIFICATION. Up to 30 mm in length, these parasites of the abomasum of ruminants have a buccal cavity armed with a **lancet** (see Figure 4-65). Species in the United States include *Haemonchus contortus, Haemonchus placei,* and *Haemonchus similis.* The male has an asymmetric dorsal ray in its bursa (Figure 4-77) and short, wedge-shaped spicules. The white, egg-filled uterus of the female spirals around the blood-filled gut, giving rise to the so-called barber pole appearance. The vulva is located about a quarter body length from the tail and may or may not be guarded by variously shaped cuticular inflations (vulvar flaps). The prevalence of various vulvar flap configurations varies among species and subspecies of *Haemonchus* (Figure 4-78).

IMPORTANCE. The disease haemonchosis is characterized by anemia. At peak infection, naturally acquired populations of *Haemonchus contortus* may remove one fifth of the circulating erythrocyte volume per day from lambs and may remove an average of one tenth of the circulating erythrocyte volume per day over the course of nonfatal infections lasting 2 months. These are round numbers drawn from observations of a flock of 100 to 175 lambs with erythrocyte loss estimated by the whole-body radioiron retention technique (Georgi, 1964; Georgi and Whitlock, 1965). The pathogenic effects of *H. contortus* result from the inability of the host to compensate for blood loss. If the amount of loss is small and restitution by the host complete, no measurable illness results.

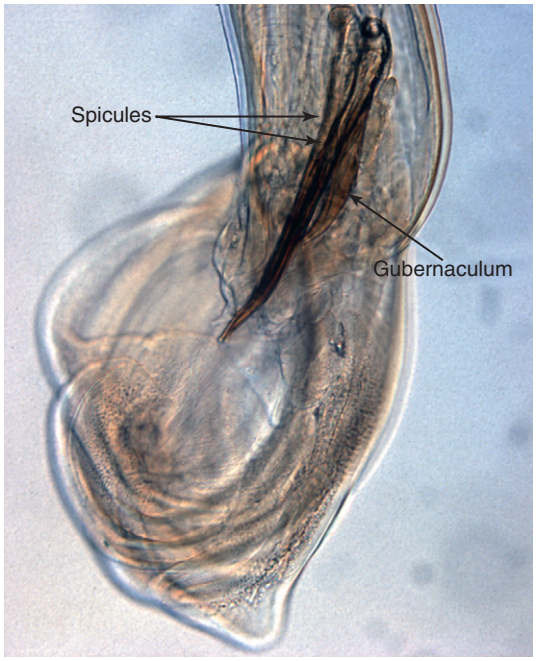


FIGURE 4-77. Spicules of *Haemonchus contortus.*

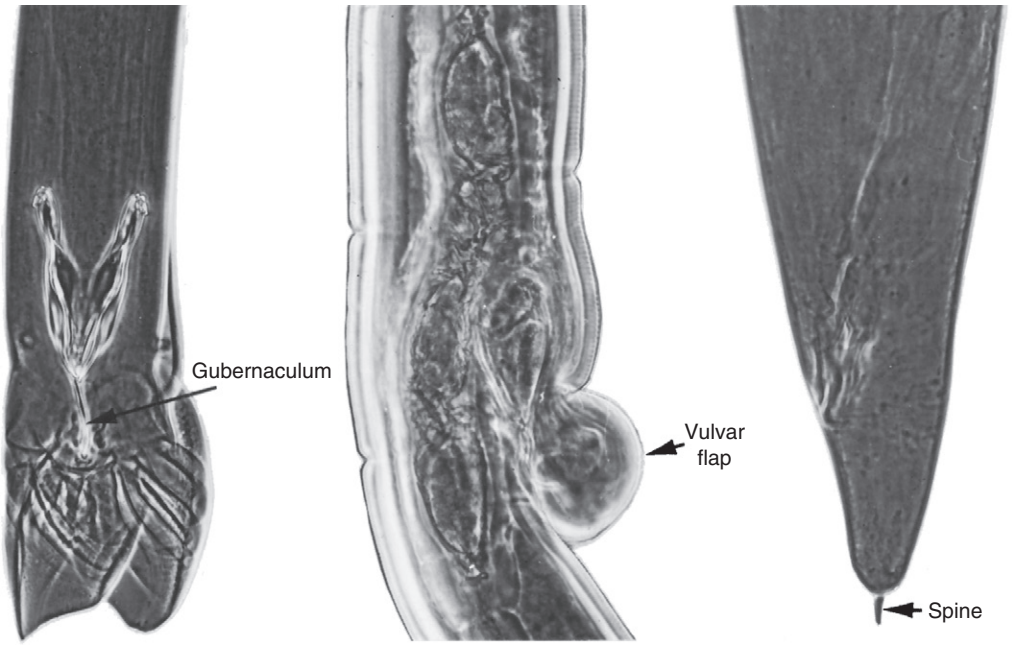


FIGURE 4-78. Three genera of the superfamily Trichostrongyloidea. (From Whitlock JH: *Diagnosis of veterinary parasitisms,* Philadelphia, 1960, Lea & Febiger.)

"It is doubtful, indeed, whether in such circumstances (i.e., satisfactory nutrition) infection with up to 500 worms has any effect on growth or wool production" (Clunies Ross and Gordon, 1936). However, if the rate of blood loss exceeds the host's hematopoietic capacity, because the challenge is overwhelming, or because the response is handicapped by poor nutrition, defective phenotype, or stress, progressive anemia leads rapidly to death. The cardinal sign of haemonchosis is pallor of the skin and mucous membranes. A hematocrit reading of less than 15% is always accompanied by extreme weakness and shortness of breath and warrants a grave prognosis. A simple means of measuring anemia due to haemonchosis in sheep and goats, along with determining which animals require treatment, is the use of a FAMACHA chart, which shows images of eyes of animals with different hematocrit levels with an indication of which should be treated (Kaplan et al, 2004a). Loss of plasma protein results in anasarca, which frequently manifests externally as submaxillary edema (bottle jaw). The appetite typically remains good, and in acute outbreaks affected animals may not lose appreciable weight. Feces are well formed, diarrhea occurring only in infections complicated by the presence of such species as *Trichostrongylus* and *Cooperia*. Lambs are often the most seriously affected members of a flock, but older sheep under stress also may have fatal anemia. Individual older ewes may succumb in late spring to the overwhelming challenge imposed by hordes of larvae simultaneously emerging from developmental arrest. High egg counts of 10,000 or more eggs per gram are typical of haemonchosis.

Mecistocirrus

IDENTIFICATION. *Mecistocirrus* species are parasites of the abomasum of ruminants and the stomach of pigs in Central America, India, and the Far East. They are similar in morphology to *Haemonchus* species, except that the vulva is close to the anus and the spicules are long and thin (Figure 4-79).

Cooperia

IDENTIFICATION. As parasites of the small intestine of ruminants, species of *Cooperia* are less than 9 mm long. The cuticle of the stomal region is transversely striated and slightly inflated, the buccal cavity is very small, the spicules are short and blunted at their tips, and the dorsal ray of the bursa is lyre-shaped (Figures 4-80 and 4-81; see also Figure 4-74). *Cooperia* species in the United States, including *Cooperia punctata* and *Cooperia oncophora*, are most likely to be confused with *Trichostrongylus* or *Strongyloides* species because of similarity in size and location within the host.

IMPORTANCE. The relationship of *Cooperia* species to disease production is similar to that presented for *Trichostrongylus* species; however, *Cooperia* species are now the most prevalent parasites in cow/calf operations in the United States, probably because they were the rate-limiting nematode for dose determination when the macrocyclic lactones were brought to market (Stromberg et al, 2012). In a 60-day-long study in 2009 in which a Wisconsin strain of *Cooperia punctata* resistant to macrocyclic lactones was used in groups of 80 experimentally infected and 80 uninfected control calves, it was shown that the uninfected calves gained weight 7.4% more rapidly than those that were infected (Stromberg et al, 2012).

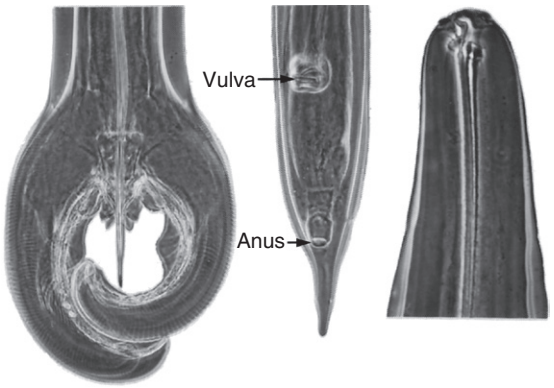


FIGURE 4-79. *Mecistocirrus* spp. (From Whitlock JH: *Diagnosis of veterinary parasitisms,* Philadelphia, 1960, Lea & Febiger.)

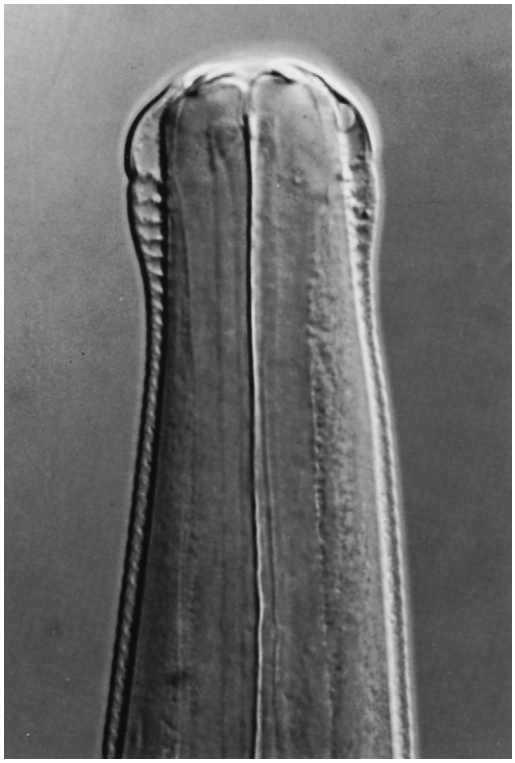


FIGURE 4-80. Stomal end of *Cooperia*.

FIGURE 4-81. Spicules of *Cooperia*.

As was expected with the resistant isolate, at the end of 60 days, when half the infected animals were treated with injectable doramectin and the other half with fenbendazole, the labeled dose of doramectin had little to no effect on worm burdens in these animals, and fenbendazole was efficacious.

Nematodirus

IDENTIFICATION. Species of *Nematodirus*, which among the dozen or so species in the United States includes *N. spathiger, N. filicollis, N battus,* and *N. helvetianus,* vary considerably in size; the largest grows to a length of 25 mm. The cuticle of the stomal region is transversely striated and may be inflated; the stoma is armed with a dorsal, triangular tooth (see Figure 4-74). The neck is usually coiled, the spicules are long and thin, the uterus contains very large eggs, and the female has a spine at the tip of her tail (see Figure 4-78).

LIFE HISTORY. The life history and epidemiology of *Nematodirus* species infecting domestic ruminants are distinctly different from those of most other trichostrongyloids. The larva develops to the infective third stage within the eggshell, and hatching depends on extrinsic stimuli, at least in certain species. For example, the infective larva of *Nematodirus battus* usually must be subjected to freezing followed by warmer weather before it will hatch. This property tends to concentrate hatching of infective larvae in the spring, limiting reproduction to one generation per year and generating a single wave of infection and disease in late spring. As a result, the severity of infection is typically directly proportionate to the previous year's pasture contamination, and timing of the outbreak depends on weather favorable for mass hatching of eggs. However, a second wave of larvae on pasture and consequent infection of sheep have been observed to occur in the fall (Gibson and Everett, 1981; Rodger, 1983; McKellar et al, 1983; Hollands, 1984; Hosie, 1984). Development and hatching of the infective larvae of *Nematodirus spathiger* and *Nematodirus filicollis* tend not to be seasonally constrained in this manner, and these species are common parasites of sheep.

IMPORTANCE. Although *Nematodirus* species infections usually are not associated with clinical disease, *N. battus* causes a specific strongylosis characterized by very restricted seasonal incidence and by extremely severe and debilitating diarrhea. Most of the lamb flock displays a sudden loss of thrift quickly followed by profuse diarrhea. Deaths begin from 2 days to 2 weeks after onset of clinical signs and continue for several weeks, after which survivors gradually recover; mortality may reach 30%. Egg counts average 600 and rarely exceed 3000 eggs per gram of feces. Necropsy reveals a dehydrated carcass, enlarged pale edematous mesenteric lymph glands, and mild catarrhal enteritis, but very little else in the way of lesions. A count of 10,000 *N. battus* worms is considered significant (Thomas and Stevens, 1956). Originally described as from Great Britain (Crofton and Thomas, 1951, 1954), *N. battus* appeared in Oregon in 1985 (Hoberg, Zimmerman, and Lichtenfels, 1986) and has since been identified in sheep fecal samples from Washington, New York, Vermont, and Maryland (Zimmerman et al, 1986).

Hyostrongylus

IDENTIFICATION. A parasite of the stomach of swine, *Hyostrongylus rubidus* is less than 9 mm long and has a small, annular buccal collar, short spicules with two points, and a long narrow gubernaculum (see Figures 4-74 and 4-78). *Hyostrongylus kigeziensis* is a parasite of the mountain gorilla (Durette-Desset et al, 1992).

LIFE HISTORY AND PATHOGENESIS. *H. rubidus* is a typical trichostrongyloid nematode somewhat resembling *Ostertagia* species in its habits. The adult worms parasitize the stomach and produce typical strongylid eggs that closely resemble those of the *Oesophagostomum* species that infect swine. Ensheathed third-stage larvae develop within a week under optimum conditions; these larvae are infective when swallowed by swine. Like *Ostertagia* species, *H. rubidus* invades the gastric glands, where the third and fourth molts take place. *H. rubidus* evokes a catarrhal, sometimes diphtheritic, gastritis with ulceration and secretion of tenacious mucus. Clinical signs include anemia and inappetence with occasional melena as evidence of gastric hemorrhage. Hyostrongylosis is mainly a disease of adult pigs at pasture, but transmission can be markedly reduced during dry summers (Roepstorff and Murrell, 1997). It has been shown, however, that transmission can occur under confinement conditions (Bladt-Knudsen et al, 1994).

ANTHELMINTIC MEDICATIONS. Fenbendazole, ivermectin, and doramectin are approved for treatment of or have been shown to successfully treat infection in pigs with *H. rubidus*.

Ollulanus

IDENTIFICATION. A parasite of the stomach of the pig, dog, cat, and other felids including the cougar, cheetah, and tiger, *Ollulanus tricuspis* is minute (less than 1 mm long). The anterior end is rolled up, the vulva is near the anus, the female tail terminates in three or more sharp points, and the spicules of the male are short, equal, and bifurcated (Figure 4-82). These worms can be diagnosed using endoscopy specimens (Cecchi et al, 2006).

LIFE HISTORY. *O. tricuspis* is **ovoviviparous** (the eggs develop and hatch within the uterus of the female), and the larvae develop to maturity in the stomach of the host. It is a rare example of a nematode capable of completing its life history within a single host. Ingestion of vomitus from an infected host is the most likely means of transmission of *O. tricuspis*.

IMPORTANCE. In cats these worms are capable of causing chronic gastritis that can prove fatal (Hänichen and Hasslinger, 1977). Chronic gastritis also has been observed in a tiger (Breuer et al, 1993) and in captive cheetahs (Collett et al, 2000). In

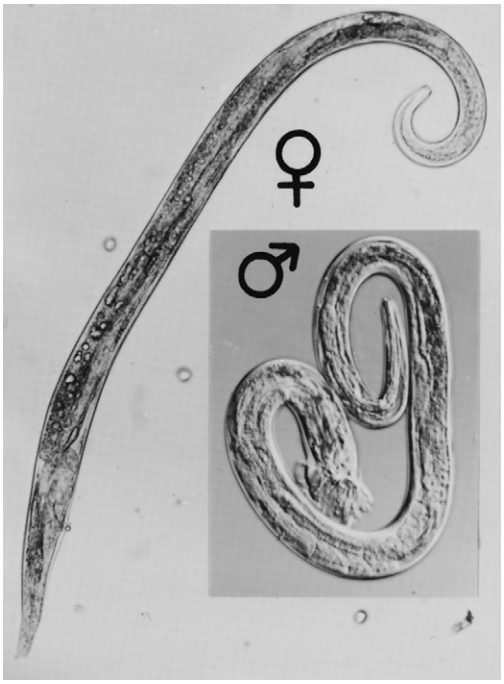


FIGURE 4-82. *Ollulanus tricuspis* from a leopard. Diagnosis is usually based on finding adult specimens of this viviparous species in vomitus.

stomachs of infected cats, a significant increase in mucosal fibrous tissue and mucosal lymphoid aggregates is noted (Hargis, Prieur, and Blanchard, 1983).

ANTHELMINTIC MEDICATION. It has been reported that tetramisole (a 2.5% formulation administered at 5 mg/kg) has proved efficacious without side effects (Hasslinger, 1984).

Dictyocaulus

IDENTIFICATION. Up to 80 mm long, white adult *Dictyocaulus* worms are found in the respiratory passages of ruminants and horses: *Dictyocaulus viviparus* in cattle, *Dictyocaulus filaria* in sheep, and *Dictyocaulus arnfieldi* in equids. The buccal cavity is small; the bursa is somewhat reduced; the spicules are short, dark, and granular in appearance; the vulva is near the middle of the body; and the egg contains a first-stage larva when laid (Figure 4-83; see also Figure 4-74).

LIFE HISTORY. Adult *Dictyocaulus* organisms live in the lumen of the bronchial tree, where they cause chronic bronchitis and localized occlusion of the bronchial tree with atelectasis. *Dictyocaulus viviparus* is the only nematode that reaches maturity in the lungs of cattle. The freshly laid egg contains a vermiform embryo that usually hatches before it is eliminated in the feces (see Figure 7-62). The free-living stages probably derive their energy from stored food materials instead of ingested bacteria because they can develop to the doubly ensheathed infective stage in aerated clean water, and because the characteristic "food granules" in the intestinal cells of the first-stage larva become less conspicuous and finally disappear as development proceeds. Development to the infective stage requires about 5 days under optimum conditions. When ingested, the infective larvae migrate by way of the mesenteric lymph nodes and thoracic duct and arrive in the lungs about 5 days later (Jarrett et al, 1957). Egg-laying starts about 4 weeks after infection.

IMPORTANCE. Light infections with *D. viviparus* are borne without obvious physiologic embarrassment; calves cough occasionally and may breathe slightly faster than normal. Heavier infections lead to partial or complete obstruction of the air passages, and clinical disease develops in proportion to the degree of obstruction. A progressive increase in respiration rate starts at about the fifth day, after ingestion of several thousand infective larvae, and the animal coughs occasionally. During the third week, respirations become forced and reach a rate of 100 per minute. Auscultation reveals harsh bronchial sounds and occasional crepitation. Until the fourth week, no larvae are shed in the feces, and the diagnosis rests entirely on the history and clinical signs. During the fourth week, first-stage larvae appear in the feces, and the severity of the clinical signs reaches a maximum. The respiratory rate exceeds 100 per minute, coughing is frequent, crepitation and harsh bronchial sounds can be heard, and air hunger becomes acute. The calves do not feed because they cannot spare the time needed for breathing. Clinical improvement can be noted in survivors after the fifth week.

D. filaria in sheep and goats has a life history similar to that of *D. viviparus* (Daubney, 1920). However, unless unusually large infections are acquired, the clinical signs are usually mild. Most cases of severe clinical illness associated with *D. filaria* are complicated by the presence of less obvious but more pathogenic parasites in the alimentary tract.

D. arnfieldi is a relatively well-adapted parasite of donkeys *(Equus asinus)* but tends to be quite pathogenic in horses. Where this parasite is endemic, it is hazardous to pasture horses and donkeys together.

FIGURE 4-83. Bursa and spicules of *Dictyocaulus*.

Ecology and Epidemiology of Strongylid Infections of Ruminants

The following discussion refers principally to ruminants because the ecology and epidemiology of ruminant strongylids have been subjects of intensive research for the best part of a century. The lessons learned from sheep can be applied at least qualitatively to horses. The typical strongylid life history as outlined in Figure 4-84 is generally applicable to members of the superfamilies Trichostrongyloidea, Strongyloidea, and Ancylostomatoidea. Important embellishments on this scheme, such as the skin penetration of hookworm infective larvae and the atypical larval development of *Dictyocaulus* species, do not significantly alter the qualitative ecologic and epidemiologic relationships portrayed:

1. The rate of environmental contamination with eggs is in direct proportion to the degree of infection of the host population with adult worms.
2. Development and survival of the infective stage depend on prevailing conditions of temperature and moisture. Optimum requirements vary distinctly among worm species.
3. Host resistance varies as a function of age, vigor, genetic constitution, presence or absence of an already established infection, and, in some instances, acquired immunity.
4. The maturation of fourth-stage larvae may be held temporarily in abeyance by as yet poorly understood influences. Populations of arrested larvae may be harbored for months before some unknown stimulus restarts their final development.

Adult Worm Populations

Although some infective larvae may survive for weeks or months under suitable environmental conditions, it is the carrier host that often perpetuates strongylid infections from year to year. The infection may be maintained as a small population of adult worms, as a latent population of histiotropic larvae, or as both. Strongylids, like cold viruses and daffodils, display marked seasonal variations. The worm population normally is regulated in a way that spares the host and perpetuates the parasite. Only when this regulation breaks down do outbreaks of disease occur.

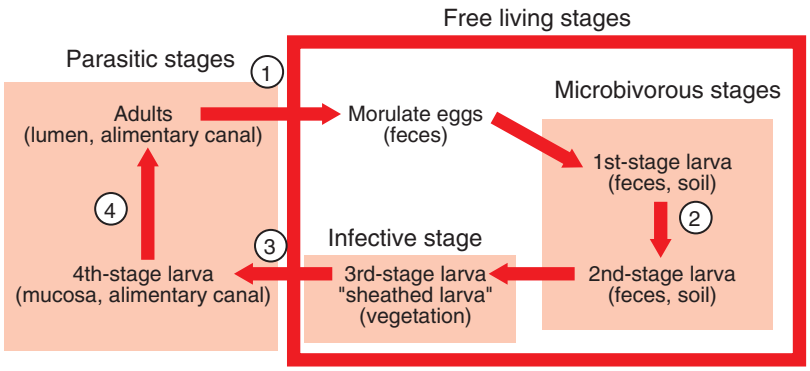


FIGURE 4-84. A typical strongyloid life history. Stages 1 through 4 are explained in the text.

During their first season at pasture, calves, lambs, and kids acquire strongylid burdens rapidly by ingesting third-stage larvae as they graze. If the vegetation is heavily contaminated with pathogenic species (e.g., *O. ostertagi, H. contortus*), disease and death may occur among these young and inexperienced hosts. The accumulation of infection is manifested by a corresponding increase in fecal egg output and by further contamination of the pasture. With sufficient warmth and moisture for larval development, the number of infective stages on vegetation will tend to increase exponentially, at least during the early part of the grazing season. However, the hosts now begin to develop resistance to further infection. The principal component of this developing resistance is a peculiar phenomenon called **premunition:** "a state of resistance to infection which is established after an acute infection has become chronic and which lasts as long as the infecting organisms remain in the body" (*Dorland's Illustrated Medical Dictionary,* ed 27, Philadelphia, 1988, Saunders). The mechanism of premunition is unknown, but the phenomenon can be readily demonstrated by a variety of simple experiments. For example, if we decide to impose a severe *H. contortus* burden on a sheep that is already harboring a moderate population of these parasites, we must first remove the already established population by anthelmintic medication. Otherwise, part or all of the dose of larvae that we administer experimentally will fail to take. As premunition and other forms of host resistance develop, individual strongylid burdens reach a peak and then begin to decline. Normally the calf, lamb, or kid enters its first winter with a substantially reduced population of adult strongylids.

What becomes of the infective larvae that the now-premunized host continues to ingest as it grazes? There are three possibilities: Such larvae may be rejected, may replace established adult worms, or may become arrested in their development as fourth-stage larvae, but the total number of adult worms tends to remain at a plateau. The **arrested larvae** (also referred to as **latent, inhibited,** or **hypobiotic larvae**) remain in the alimentary mucous membranes until some stimulus related to the coming of spring, to the reproductive cycle of the host, or to both, restarts their development. For example, in spring a substantial increase in the output of strongylid eggs is observed in the feces of ewes, rams, and wethers. A more pronounced rise also commonly occurs in lambing ewes from 2 weeks before until 8 weeks after parturition at any season. Both "**spring rise**" and "**periparturient rise**" (Crofton, 1954) in fecal egg counts are related principally to maturation of the larvae that have overwintered as arrested fourth stages in the alimentary mucosae of adult sheep (Herd et al, 1983). "The production of a large number of eggs about two months after parturition ensures that infective stages will be available in large numbers at a time when the sheep population is not only enlarged by lambing but also has a high proportion of susceptible individuals which have not been exposed to infection previously" (Crofton, 1963). The periparturient rise in fecal egg counts can be abrogated by protein supplementation of the ewe (Donaldson, van Houtert, and Sykes, 1997).

In summary, calves, lambs, and kids tend to carry large parasite burdens, whereas adult cattle, sheep, and goats usually harbor lighter infections. One peak of strongylid reproductive activity is observed during the grazing season. This occurs in both mature and growing ruminants but tends to be more marked and pathogenic in the latter. A second peak occurs in mature females a few weeks after parturition and is marked by the "postparturient rise" in egg output. This increase is most marked in ewes lambing in spring, at which season a modest spring rise also is observed in wethers and barren ewes.

The **biotic potential** or **reproductive capacity** of strongylids depends jointly on the rate of production of fertile eggs and on the **generation time** (i.e., the time required for these eggs to develop into egg-producing adults). The normal degree of realization of the biotic potential tends to maintain stable worm populations that display marked periodicity but neither explode nor fade away to extinction. Normally the probability of any individual strongylid egg reaching reproductive age is only one in thousands, so the worms must compensate by producing enormous numbers of eggs. *Haemonchus* species are the most fecund, with *Oesophagostomum, Chabertia, Bunostomum, Ostertagia, Cooperia, Trichostrongylus,* and *Nematodirus* species following roughly in that order. The species with low per-individual reproduction rates tend to compensate by maintaining larger adult populations (*Trichostrongylus* and *Cooperia* spp.) or by producing eggs more resistant to inclemencies of the external environment (*Nematodirus* spp).

Development and Survival of the Infective Stage

Most strongylids are capable of developing and maintaining significant populations of infective larvae over considerable ranges of temperature and moisture. Minimum conditions are of interest because they dictate the point at which the environment ceases to harbor significant infection, and optimum conditions are of interest because it is during periods favorable for development and survival of preparasitic stages that outbreaks of clinical strongylosis usually occur.

No strongylid life history can be completed in totally arid environments, and parasitism with strongylids is correspondingly rare in desert regions. Even under apparently dry conditions, however, microhabitats may exist that contain enough moisture to allow survival, if not development of eggs and larvae.

The temperature necessary for development varies with the species, and in each case the rate of development varies with the

temperature. With the very significant exceptions of *N. filicollis, N. battus,* and *Ostertagia* species, which appear to be well adapted to cold climates, the egg and larva populations of most strongylids experience marked reductions or even disappear from northern pastures during winter. Such pastures become recontaminated in spring. *Nematodirus*-infective larvae develop and remain viable in the eggshell during winter in climates about as harsh as possible for the profitable practice of cattle, sheep, or goat husbandry. *Ostertagia* overwinters both as infective larvae on pasture and as arrested larvae in the host population; pasture larvae begin to die off as warmer and dryer conditions supervene.

Host Resistance

AGE. A general increase in resistance to strongylid infection with age is well marked in cattle, slightly less so in sheep, and least in goats. Age resistance may break down in the face of overwhelming challenge or as a secondary result of malnutrition or disease. Old ewes may succumb to strongylidosis when their teeth fail them, and limited milk production by ewes predisposes their nursling lambs (Whitlock, 1951). Examination of the teeth and udders of ewes should accompany any investigation of parasitic disease in sheep (Love and Biddle, 2000).

PHENOTYPE. Whitlock (1955b, 1958) reported an inherited resistance to trichostrongylidosis in sheep. The progeny of a ram called Violet harbored smaller populations of worms and suffered less reduction in hematocrit than did the progeny of other rams. Unfortunately, one dark and stormy night, the electric transmission lines fell on Violet and blew him to glory. Years later, when he retired and turned over his Zeiss photomicroscope, Dr. Whitlock had a brass plate engraved in Violet's memory and mounted on the microscope. Currently the process has been used in Australia and New Zealand, and the resistance status of rams is included in their records. Thus this aspect of genetics is currently being applied on a regular basis to aid in preventing nematode-related disease in sheep.

PREMUNITION. The presence of a stable population of adult strongylids in the alimentary canal tends to inhibit further infection or, at least, further maturation of larvae. Removal of this stable adult population by anthelmintic medication vacates an ecologic niche that is promptly filled through maturation of arrested larvae, uninterrupted development of recently ingested infective larvae, or both. Whatever the underlying reason for premunition—that is, ecologic or immunologic—a ruminant with a subclinical strongylid infection should not be treated with anthelmintics unless an uncontaminated environment can be provided after treatment. Loss of premunition resulting from removal of the stable and established infection will permit rapid reinfection, perhaps with a heavier parasite load than before.

The following seeming paradox lends a measure of symmetry to this argument. If sheep are removed during peak exposure from an *H. contortus*–infested pasture to a parasite-free environment, they will develop more serious infection than if left on the pasture. Interrupting the flow of larvae apparently throws the regulation out of balance in some way. Inhibition of larval development by adult worms is manifested as premunition. It appears that the larvae in turn exercise a measure of control over the adults. At any rate, the practical advice to be gleaned from this is as follows: Be sure to administer an anthelmintic to *H. contortus*–infected sheep before transferring them to an uncontaminated environment, at least during the parasites' normal period of rapid population growth.

Although host immunity is often credited for the state of premunition, it might also be due to interactions between the parasites. An ecologic explanation of premunition might be kin selection—that is, once established, worms exploit the chosen niche and somehow directly or through manipulation of the host make the niche inhospitable to other worms from different parental stocks. When sheep are infected first with one group of worms and then with brothers and sisters or cousins after the first group has matured, evidence suggests that the genetic relatedness of existing and incoming populations has an effect on the number of worms that will develop to adulthood (Ketzis et al, 2001).

SELF-CURE. Few examples show immunity that protects the host against reinfection after the initial strongylid population is gone. Stoll (1929) reported an experiment "in which two helminth-free lambs, upon fenced-in pasturage permitting natural repeated infection, during the summer developed, following an initial dose of *Haemonchus contortus* larvae, first an accumulation of parasites and then a self-cure which expelled the worms and protected the animals thereafter against any significant amount of further infestation with this stomach worm." Thus was born the celebrated phenomenon called *self-cure*.

Stewart (1950) observed seven periods of self-cure within 18 months in a flock of grazing sheep, demonstrated that an identical response could be elicited by giving large doses of infective *H. contortus* larvae, and concluded that self-cure taking place after periods of rain could be attributed to the intake of large numbers of *H. contortus*–infective larvae. He subsequently related rejection of the previously established adult worm population to an acute hypersensitivity reaction in the alimentary mucous membrane.

An edematous change was evident in the mucous membrane of the abomasum or small intestine, depending on the site of attachment of the adults, on the day on which a rise of blood histamine occurred after the administration of larvae. The intake of *H. contortus* larvae produced this change only in the abomasum of a sheep that had been infested with *H. contortus* and only in the small intestine of a sheep that had been infested with *Trichostrongylus* spp. (Stewart, 1953).

The lack of permanent protection against reinfection observed by Stewart does not necessarily invalidate Stoll's observations, but examples of functional acquired sterile immunity are rare where *H. contortus* is concerned. Drs. Georgi and Whitlock had no difficulty reinfecting lambs of the New York State Veterinary College flock with *H. contortus cayugensis* after their naturally acquired worm burdens had been removed by anthelmintic therapy. Similar results are commonly observed in other parts of the world with other subspecies of this parasite.

At least one definite practical consequence of self-cure has been noted. Sheep or goats may die in the throes of evicting their worms, which may confuse the diagnosis by findings of "uninfected" at necropsy when the clinical signs and history correctly pointed to haemonchosis. Profound anemia in grazing sheep or goats indicates haemonchosis unless positive evidence of another cause (e.g., acute radiation sickness) can be produced. The absence of *H. contortus* worms from the abomasum of an anemic sheep or goat in no way rules out the diagnosis of haemonchosis.

Active Immunity

A durable sterile immunity is conferred in cattle by infection with the lung nematode *D. viviparus,* and considerable success has been achieved by means of artificial immunization with irradiated larval vaccines (see the review by Poynter, 1963). The practical application of vaccines is of course limited to areas of endemic dictyocaulosis, and although *D. viviparus* infection is cosmopolitan in distribution, clinical parasitic disease tends to be sporadic. Clinical dictyocaulosis is common in the British Isles, and this is where the vaccine has found ready acceptance and effective application.

Delayed Maturation of Larvae

Arrested development of larvae not only helps perpetuate certain strongylids from year to year but spares the host during the period of winter (or dry season) stress, when energy invested in the reproduction of worms with free-living larvae would be a losing proposition biologically. Normally these larvae mature the following spring. However, outbreaks of severe strongylidosis may result from the unseasonable maturation of arrested larvae during winter and early spring. It is important to recognize the parasitic cause of such outbreaks despite their unseasonable incidence.

Treatment and Control of Strongylid Infection in Ruminants

The first step in dealing with an outbreak of strongylidosis in a herd of cattle, sheep, or goats is to identify the source of infection and to separate the animals from it. For purposes of observation and nursing, it is usually more convenient to confine the herd in a barn or drylot; restriction of activity may help prevent losses precipitated by exertion. Never hurry patients that are acutely ill with haemonchosis; they may drop dead at your feet. Segregate all animals showing anemia, diarrhea, weakness, or depression to facilitate therapy and to prevent their being bullied to death by their stronger fellows, but do not separate nurslings from their dams unless the owner is willing and able to cosset them.

Administration of an anthelmintic may hasten the death of very sick animals, and the owner should be forewarned of possible further losses precipitated by drenching. However, the benefit of an effective anthelmintic drench in primary haemonchosis is usually dramatic. Strongylid nematodes continue to infect our cattle, sheep, and goats despite the plethora of safe and efficacious anthelmintic drugs. The use of anthelmintic drugs should be based on thorough knowledge of the biology of the worms and the area's climatic conditions. The entire herd may be treated at regular "strategic" intervals in the hope of preventing buildup of infective larvae in the pastures, thus preventing outbreaks of clinical strongylosis. When contamination is particularly severe, strategic treatments preceding parturition and turnout to pasture, at midsummer and in fall, may need to be supplemented by "tactical" treatments at times when infection pressure may be particularly severe, for example, after a period of moist, warm weather particularly favorable for larval development.

Strongylids of the Alimentary Canal

Ruminant anthelmintics include fenbendazole, albendazole, ivermectin, doramectin, moxidectin, eprinomectin, levamisole, and morantel (other products are available outside the United States). All of these drugs are available in a variety of pharmaceutical forms to suit all types of farm and feedlot management systems.

Abomasal parasites such as *Haemonchus* species, *Ostertagia* species, and *T. axei* tend to be more susceptible to anthelmintic medication than related parasites of the small intestine such as *Trichostrongylus, Cooperia,* and *Nematodirus* species. Normally these latter genera tend to concentrate in the first quarter of the small intestine, and only a few specimens are found lower down. It is thought that poisoned small intestinal parasites have a greater opportunity to recover and reestablish infection lower down in the small intestine, whereas poisoned abomasal parasites have left the abomasum before they have had a chance to recover. Therefore unless experiments designed to evaluate the efficacy of anthelmintics against parasites of the small intestine are based on postmortem examination of the entire small intestine, reported results are likely to be biased in favor of the anthelmintic (Bogan et al, 1988).

Fall or early winter treatment ideally should be carried out with anthelmintic drugs active against the immature, arrested parasitic stages of *Ostertagia* species (Armour, Duncan, and Reid, 1978; Duncan et al, 1976; Williams et al, 1977). In northern temperate, nonarid areas of the United States, treatment of ewes with a larvicidal anthelmintic at the time they are put indoors in fall prevents periparturient rise, at least in fall- and early spring–lambing ewes (Herd et al, 1983).

Resistance

A population of parasites under more or less continuous chemical attack must alter its genetic composition through selection or mutation or be driven to extinction. Increased resistance of the parasites to the chemical, the more frequent outcome, is most common when antiparasitic chemicals are most needed and therefore most frequently used. Purchased livestock also may introduce resistant strains of parasites. However, it must be borne in mind that most cases of apparent anthelmintic failure are due to continued exposure to infective larvae or to errors in selection and administration of an appropriate anthelmintic chemical (Coles, 1988). *H. contortus, T. circumcincta,* and *Trichostrongylus colubriformis* of sheep and goats in widely scattered parts of the world have displayed resistance to ivermectin, benzimidazoles, and levamisole/morantel. Resistance to anthelmintics has been slower to appear in relation to cattle parasites, but it seems that sporadic cases of resistance to benzimidazoles or macrocyclic lactones may occur (McKenna, 1996; Vermunt, West, and Pomroy, 1995). The genus most typically incriminated in the case of cattle is *Cooperia,* but it appears that *Ostertagia* and *Trichostrongylus* may also sometimes be involved.

Resistance to different antiparasitics in the United States is of greatest concern in goats, but reports have also described resistance in sheep and cattle. Resistance to ivermectin was first reported in the United States for *H. contortus* in Angora goats (Craig and Miller, 1990). Resistance of this same parasite was also observed in cattle in Texas (DeVaney, Craig, and Rowe, 1992). Resistance has also been seen in *Haemonchus* and *Trichostrongylus* in goats in the southern United States (Kaplan et al, 2007). It seems that resistance of the gastrointestinal nematodes of goats is now a very common event in the United States; resistance has been seen with respect to albendazole, levamisole, ivermectin, and moxidectin (Mortensen et al, 2003), and in some cases, the same population of worms is resistant to all three anthelmintic classes (Williamson et al, 2011).

Dictyocaulosis

Clinical outbreaks of dictyocaulosis are treated with fenbendazole, ivermectin, doramectin, levamisole, oxfendazole, or albendazole. These are highly efficacious against both adult and immature stages of *Dictyocaulus* species.

Subclinical Parasitism of Adult Dairy Cattle

The decision whether to treat adult dairy cattle for the helminths that may be present still remains an open-ended question. Herd et al (1983) compared 26 trials in which milk production was examined and found no change in 14 trials; in seven trials an increase was noted after treatment, and in five trials an increase was observed in the control group. In a large trial of 9721 lactations examined in Britain over a 305-day lactation period, a 42-kg gain in milk production was reported (Michel et al, 1982). The authors thought that this was not a cost-effective increase, whereas others have interpreted the gain as cost-effective (Theodorides and Free, 1983).

In a New Zealand trial, half of 5556 cows on 47 dairies were treated twice with oxfendazole when dry (Bisset, Marshal, and Morisson, 1987). During the next 251-day lactation, treated cows produced an average of 2.24 kg more butterfat or 52.9 kg more milk. A positive response was seen in 36 of 47 treated herds, but only one herd showed a significant increase. The authors noted a greater response to treatment in cows that had been grazed previously on pastures that had been occupied with calves and with cows that were historically higher milk producers.

Two trials were performed in the Netherlands (Ploeger et al, 1989, 1990). In the first trial, 285 of 527 dry cows were treated with ivermectin. The milk yield over a hypothetical 305-day lactation increased an average of 205 kg among treated cows. In this trial, 17 of 31 treated herds had a positive response, and again greater responses were noted in cows that had historically higher milk yields. In the second trial, 676 of 1385 cows in 81 herds were treated with albendazole within a week of calving. The milk yield during the hypothetical 305-day lactation of treated cows increased by 133 kg, and 49 of the 81 herds had a positive response.

In an Australian trial, half of 498 cows in five pasture-fed herds were treated with ivermectin when dry (Walsh, Younis, and Morton, 1995). The milk yield increased by 74 L during the first 100 days of lactation, whereas the yield over the entire lactation was 86 L. All herds had a positive response, but the increase was significant in only one herd. No increased response was observed among cows that previously had been noted to have a high lactation production index. No difference in the cows was noted as to time from calving to first service, but calving to conception time was reduced among treated cows by 2 to 8 days. As is typical of lactating dairy cows, few eggs were present in the feces of the Australian cows, and no correlation was noted between egg reduction and observed increases in milk production (see the excellent review by Reinemeyer, 1995).

Eprinomectin, an avermectin that can be applied to lactating dairy cattle, has been examined for its effects on adult dairy cattle in several trials, where it was administered at the time of calving. In the case of pastured dairy cattle in Canada, the treatment did seem to produce an economic increase in milk production (Ndtvedt et al, 2002). In a similar trial in Canada looking at breeding parameters, a marginally significant improvement in the calving to conception interval was observed, but not in the calving to first service interval; a reduction in the number of breedings to conception was reported in treated animals (Sanchez et al, 2002). In two studies in cattle in Canada and the United States of cattle having limited outdoor exposure, there were no apparent advantages to treatment at calving either in milk production or in reproduction parameters (Sithole et al, 2005, 2006). These studies suggest that when cattle are at risk of continued infection pressure from pasture, treatment may be warranted but likely will be of little value in most confinement systems.

Methods are being developed that might lead to the strategic deworming of dairy cattle without the need for routine collection of fecal samples. Great strides have been made with the use of ELISA technology to sample bulk milk from dairies for antibodies to *Ostertagia* and *Dictyocaulus* (Schunn et al, 2012; Vanderstichel et al, 2012). These methods allow determination of the herd level of exposure to these nematodes and provide the ability to monitor herd status for prevalence of infection and effects of treatment on various production parameters. Although the practice has not yet fully stood the test of time, it seems that ELISAs on bulk-tank milk might be sufficient to diagnose moderate to severe outbreaks of dictyocaulosis on dairy farms in the Netherlands (Ploeger et al, 2012), and they were successful in showing the usefulness of deworming cattle every 4 weeks on one of three pasture-based, seasonally calving dairy farms in New Zealand (Mason et al, 2012). If the use of these methods can be characterized as to their usefulness and value, they are a useful adjunct to the other production parameters used in the dairy industry.

Young Cattle: Dairy Replacement Heifers and Beef Stocker Calves

Unlike the case for treating adult dairy cattle, agreement is fairly unanimous among parasitologists that treatment of dairy replacement heifers, beef stocker calves, and other yearlings and 2-year-olds is a profitable undertaking (see a second excellent review by Reinemeyer, 1990). Cattle at these ages tend to suffer significantly from parasitism. Parasitized replacements grow more slowly and often fail to reach their full growth potential. Such performance results in real financial loss, of which the producer may well be completely unaware.

Subclinical Parasitism of Sheep

The effects of moderate parasitism in lambs were investigated by administering 5000, 10,000, or 20,000 infective *T. colubriformis* larvae and comparing weight gains and feed efficiency of these artificially infected lambs with the performance of uninfected controls. Although about one half of the larvae administered became adult worms, and group average fecal egg counts of 536 to 2236 eggs per gram were observed, these levels of infection apparently caused no significant differences in average daily gain or feed efficiency (Bergstrom, Maki, and Kercher, 1975).

Integrated Control of Ruminant Strongylid Infections

Much has been written about prevention and control of strongylidoses. Every scheme has its proponents and detractors, but no unique formula applies in all situations.

Parasitism should be considered as a year-round game among the livestock, the strongylids, and the stockman. Certain moves at propitious times are capable of biasing the game in the stockman's favor, but these moves must not violate the rules of the game, or the results may be disappointing or even disastrous. The ultimate criterion for success in any control effort is the net profit that accrues, not the number of worms fatally poisoned. The purchase of a livestock scale, as suggested by Whitlock (1955a), and the maintenance of adequate production records provide objective measures of success.

Control efforts may be classified under selective breeding for resistant stock, rotational grazing, and anthelmintic medication. The first of these has been used the longest. Long before worms were recognized as disease agents, shepherds selected productive livestock for breeding, and worms claimed the lives of weaklings (against the shepherd's wishes perhaps, but to his eventual benefit) (Whitlock, 1966). There exist in many parts of the world and under certain systems of husbandry, cattle, sheep, and goats capable of thriving without help from science and technology. These animals have parasites and handle them effectively as a population. Individual animals occasionally die of parasitism, just as individuals occasionally get killed by predators, hung up in fences, or drowned in watering places, but the effect of minor losses such as these on the general population is minimal. On the other hand, there are parts of the world and systems of husbandry in which the economic production of food and fiber requires intelligent intervention to suppress strongylid populations. Host resistance continues to be of paramount importance here, even though conscious selection of resistant stock is seldom part of the breeding program. The reason is that resistant hosts contribute less to growth of the parasite

populations than do more susceptible animals, and their presence thus tends to benefit the flock as a whole.

In theory, rotational grazing seeks to prevent or limit the intake of infective larvae, by permitting animals to graze on a particular area of pasture no longer than a week, so that eggs passed in the feces do not have time to develop into infective larvae, and then not allowing the animals to return until all the larvae have died off. The considerable investment in fence construction required by rotational grazing schemes usually discourages strict observance of the rules, so the theoretic ideal is seldom realized in practice. However, any practicable rotation scheme undoubtedly increases the productivity of the pasture and may prolong the parasite generation, if only slightly (Levine and Clark, 1961).

Modern anthelmintics are efficient and comparatively nontoxic. In some places in the world, efficient livestock production is virtually impossible without them, and they are of undoubted benefit in increasing productivity wherever significant parasite losses occur. However, there are limitations, hazards, and expenses that we cannot afford to ignore. No anthelmintic can overcome excessive exposure to infection, just as no amount of bailing can overcome too large a leak. Crofton (1958) concluded that periodic treatment with interim reinfection merely delayed attainment of the full parasite potential. He suggested concentrating treatments early in the pasture season to obtain maximum delay in the parasite population increase, because an adult worm in spring is a potential forebearer of a whole series of generations that season.

Currently it is widely believed that, at least in temperate climates, only one generation of ruminant trichostrongylids capable of causing disease is produced (Herd et al, 1984). However, Crofton's basic premise is supported by Herd, Parker, and McClure (1984), who found that "prophylactic treatments in the spring were just as effective as suppressive treatments throughout the entire grazing season and resulted in significant ($P < 0.001$) increases in weight gain." The prophylactic treatments used by Herd, Parker, and McClure (1984) consisted of four doses of ivermectin (0.02 mg/kg) administered 3, 6, 9, and 12 weeks after spring turnout. In New York State, we freely admit, 12 weeks after turnout is getting pretty close to fall.

Probably the most important type of host resistance is premunition. The development of premunition in a grazing flock tends to truncate the growth curve of the parasite population by preventing the maturation of new waves of larvae, thus in effect prolonging the generation time. Although interference with the development of premunition is obviously to be avoided, periodic anthelmintic medication may have precisely this effect.

FAMACHA and Refugia

It has been strongly suggested that a way to prevent resistance within *Haemonchus contortus* from developing in a flock of sheep is to treat only those animals needing therapy. This leaves the remainder of the sheep shedding feces into the environment containing eggs from worms that are still fully susceptible to whatever drug or drugs have been used. In the case of haemonchosis, the relationship between worm burden and anemia is well established (Whitlock et al, 1966). The FAMACHA technique combines the ability to detect anemia in sheep (and goats) using the mucous membranes around the eyes with the need to treat sheep that have apparent anemia to reduce their burdens of *Haemonchus contortus* (Vatta et al, 2001). Thus, in warmer areas, where haemonchosis is typically the major disease threatening sheep and goats, the FAMACHA chart system provides a means of easily recognizing those sheep in need of treatment. It must be remembered that this system is used in areas where haemonchosis is the major helminth causing disease in a sheep flock; the system is not designed to work where the major parasites are *T. circumcincta* or species of *Trichostrongylus, Cooperia,* or *Nematodirus.*

Superfamily Strongyloidea

Strongyloids tend to be larger and stouter-bodied than trichostrongyloids, and most of them have a large buccal cavity surrounded by a sclerotized wall (buccal capsule) that usually is rigid but may be jointed or thin and flexible. The stomal structures of strongyloids are sufficiently distinct to permit identification of species with occasional reference to other characters. Greater dependence must be placed on these other characters when it is impossible to examine both dorsal and lateral aspects of the stoma, as is the case with permanently mounted specimens.

The **buccal cavity** of strongyloids is large and directed anteriorly (see Figure 4-65). The stomal opening is surrounded by a row or two of what appear to be leaves or palings of a stockade, depending on the imagination of the observer. These are called **leaf crowns** (corona radiata), and much is made of them in the taxonomy of strongyloids. In some species, the duct of the dorsal esophageal gland is carried to the rim of the buccal capsule in a sclerotized ridge (**dorsal gutter;** see Figure 4-65) on the inner wall of the buccal capsule. In other species the dorsal gutter is absent (Figure 4-85). Teeth, when present, lie at the base of the buccal cavity, where they lacerate the plug of mucous membrane that is drawn into the buccal cavity by the sucking action of the muscular esophagus. The copulatory bursa is well developed, the spicules long and thin. The vulva is close to the anus, and the uterus is prodelphic in most strongyloids.

Strongyloid life histories are typical of the order Strongylida—that is, they are direct with an infective third-stage larva—but significant variations occur in certain groups. Most are acquired by the ingestion of larvae from pasture, but some, for example, *Stephanurus dentatus,* the kidney worm of swine and the various gapeworms, *Syngamus* species, and domestic and wild birds, use earthworms as paratenic hosts.

Family Strongylidae; Subfamily Strongylinae

IDENTIFICATION. Members of the subfamily Strongylinae, often referred to as "large strongyles," are chiefly parasites of the large intestine of equines *(Strongylus, Triodontophorus, Oesophagodontus,* and *Craterostomum),* elephants *(Decrusia,* Equinurbia, and *Choniangium),* macropodid marsupials *(Macropicola* and *Hypodontus),* and ostriches *(Codiostomum).* Identification of genera and species of strongylin parasites of horses is a matter of comparing the microscopic appearance of the stomal region of specimens with the series of illustrations of equine parasites found in Chapter 7. Two leaf crowns are present, but because the elements of each are similar in size and number, the two crowns appear as one.

IMPORTANCE. *Strongylus vulgaris, Strongylus edentates,* and *Strongylus equinus* are among the most destructive parasites of the horse. All three are bloodsuckers as adult worms in the cecum and colon, but more important, their larvae undergo migrations that inflict even greater damage, especially in foals and yearlings. *Triodontophorus* organisms appear, by the ferocious teeth at the base of their buccal cavities (see Figure 7-83), to be bloodsucking parasites. Clusters of *Triodontophorus tenuicollis* worms cause localized ulceration of the colonic mucous membrane.

LIFE HISTORY OF *STRONGYLUS VULGARIS*. The extrahost development of *S. vulgaris* is typical of strongylids in general (see Figure 4-73). Development to the infective stage requires adequate moisture and temperatures in the range of 8°C to 39°C; the time required is inversely related to temperature (e.g., about 8

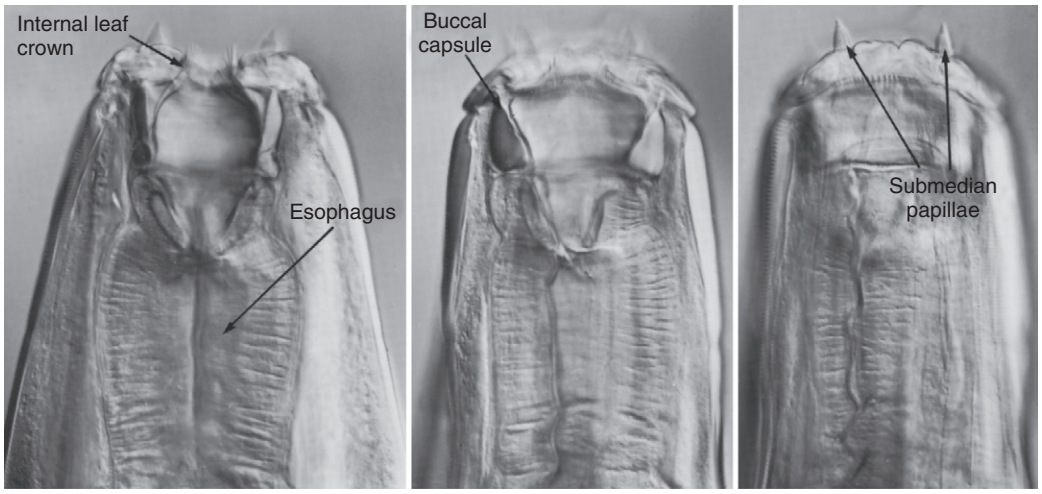


FIGURE 4-85. *Murshidia dawoodi* (Strongylidae: Cyathostominae) from an African elephant.

to 10 days at 18°C, 16 to 20 days at 12°C). In arid regions, scattering the droppings with a tractor and harrow reduces strongylid larva populations by breaking up the manure and causing it to dry out before the larvae have reached the desiccation-resistant third stage. However, in more humid regions, the interior of even scattered manure remains sufficiently moist long enough for development to the third stage. Once *S. vulgaris* larvae have arrived at the third stage, they are very resistant to cold and desiccation and can survive on pasture through a northern winter or in stored dry hay for many months. The longevity of *S. vulgaris* third-stage larvae depends mainly on the food reserves in their intestinal cells; the greater the activity of the larvae, the more rapidly these reserves become exhausted. However, it is imprudent to depend on *S. vulgaris* to wear itself out no matter how warm and humid the weather may be. Any pasture that has held a horse within a year can be assumed to be contaminated with *S. vulgaris*–infective larvae.

In 1870 Otto Bollinger hypothesized that occlusion of the intestinal arteries by verminous thrombi and emboli could account for most equine colic cases, both fatal and nonfatal. Since then, the causative relationship between *S. vulgaris* and colic has been extensively debated and somewhat investigated, although not to an extent commensurate with its scientific and practical importance.

The meticulous experimental observations and well-thought-out conclusions of Enigk (1950b, 1951) provide the basis for the following outline. For the reader interested in greater detail than can be presented here, Dr. Georgi published an English translation of Enigk's papers (Georgi, 1973), and all serious students of equine medicine and pathology should study the review by Ogbourne and Duncan (1977).

When ingested by a horse, the infective third-stage larvae of *S. vulgaris* cast off their sheaths in the lumen of the small intestine and enter the wall of the cecum and ventral colon. Here the larvae penetrate to the submucosa, where they undergo the third molt, which is completed by the seventh to eighth day after infection. Leaving their third-stage cuticles surrounded by round cells, the fourth-stage larvae penetrate nearby small arterioles that lack an internal elastic lamina and wander into the intima of these vessels and progressively larger branches of the cranial mesenteric artery.

Enigk observed that *S. vulgaris* cannot penetrate the internal elastic lamina, which thus confines the larvae to the intima and helps keep them on their proper course. Thus constrained, the rapidly migrating larvae reach the colic and cecal arteries by the eighth to the fourteenth day after infection, and the cranial mesenteric artery by the eleventh to the twenty-first day (Enigk, 1950b; Duncan and Pirie, 1972). Some of the larvae push on into the aorta and its branches, where they may cause important pathologic changes (see Figure 7-95). However, larvae proceeding beyond the cranial mesenteric artery are probably lost to their species because of the improbability of their finding their way back to the cecum and ventral colon to breed.

After 2 to 4 months of migrating in the intima, fourth-stage larvae that have not gone astray or become trapped deep in thrombi are carried by the bloodstream to the small arteries in the subserosa of the intestinal wall. The larvae, now grown large, occlude these small arteries, whose walls then become inflamed and in due course are destroyed. Larvae thus liberated from the arterial tree then enter the surrounding tissue and become encapsulated in pea- to bean-sized nodules, wherein the final molt occurs. Some larvae complete the final molt even before returning to the intestinal wall. According to Duncan and Pirie (1972), most of the larvae found in the cranial mesenteric lesions at 4 months after infection have molted to the fifth stage, although the fourth-stage cuticle is still retained as a sheath. This sheath is cast off before these immature adults return to the intestinal wall. Finally, the immature adults enter the lumen of the cecum and ventral colon, mature, and commence reproductive activity at about 6 months after infection. It is rare to find more than 100 or 200 adult *S. vulgaris* worms in a horse, and their egg production usually constitutes 10% or less of the total strongylid output.

The migrations of fourth-stage *S. vulgaris* larvae cause arteritis, thrombosis, and embolism of the cranial mesenteric artery and its branches. Although these arterial lesions exist to some degree in almost every horse, and principal branches are frequently completely occluded by them, fatal infarction of the bowel wall is relatively infrequent. This seeming paradox suggests a Darwinian interpretation. Of all domestic animals, the horse has by far the most elaborate system of anastomoses in the arterial supply to the large intestine. The colic vessels are particularly well supplied with the means for rapidly establishing effective collateral circulation (Dobberstein and Hartmann, 1932). In an evolutionary context, this may be interpreted as evidence that *S. vulgaris,* which has no direct counterpart in other domestic animals, probably has been occluding horses' intestinal arteries and thus exerting selection pressure for ages.

However, despite this exceptional adaptation, obstruction of the intestinal arteries does occasionally lead to fatal infarction of the

bowel. Even temporary curtailment of blood flow pending establishment of collateral circulation may account for a high proportion of clinical colic cases from which the patient recovers. Furthermore, the fatal intestinal displacements often interpreted at necropsy examination to be the cause of colic symptoms are more likely to be the result of abnormalities of intestinal tone and motility brought about by verminous thromboembolism and the horse's violent efforts to obtain relief.

After the larvae have migrated back to the intestinal lumen, the arterial lesions heal (Duncan and Pirie, 1975; Pauli, Althaus, and Von Tscharner, 1975). These lesions also heal dramatically after destruction of the larvae by medication with any of several newer anthelmintics, including ivermectin (Holmes et al, 1990). Development and resolution of verminous arteritis can be studied radiographically in young foals by injecting contrast medium through a catheter that has been introduced into the aorta by way of a peripheral artery (Slocombe et al, 1977). Two such radiographs are shown in Figure 4-86. The upper radiograph of a 2-month-old pony foal was taken 1 month after 500 *S. vulgaris* larvae were administered through a nasogastric tube. The cranial mesenteric and ileocecal arteries are enlarged, and blood flow through the colic arteries is greatly diminished, as evidenced by the lack of contrast medium flowing through them. The lower radiograph of the same foal was taken 1 month after albendazole therapy. Now the stem arteries have returned to nearly normal size, and the contrast medium clearly outlines the colic arteries, indicating greatly increased flow through those vessels (Rendano et al, 1979b).

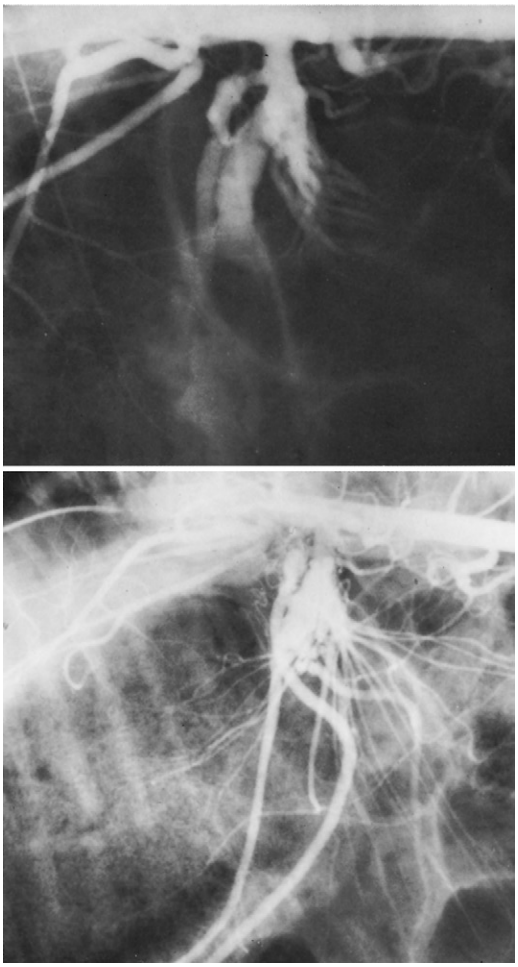


FIGURE 4-86. Resolution of equine verminous arteritis after larvicidal therapy with albendazole. The ramifications of the cranial mesenteric artery are made visible by contrast arteriography. The upper radiograph was taken 1 month after infection with 500 *Strongylus vulgaris* larvae, and albendazole therapy was started immediately afterward. The lower radiograph was taken 1 month after albendazole therapy.

LIFE HISTORIES OF *STRONGYLUS EDENTATUS* AND *STRONGYLUS EQUINUS*. Adult *S. edentatus* and *S. equinus* are about twice as large as *S. vulgaris,* probably twice as bloodthirsty, and considerably more difficult to remove with anthelmintic drugs, but their larvae are not quite as pathogenic. The migration routes followed by larvae of *S. edentatus* and *S. equinus* have been elucidated by Wetzel (1940b), Wetzel and Kersten (1956), and McCraw and Slocombe (1974, 1978).

The third-stage larvae of *S. edentatus* burrow into the wall of the large intestine and reach the liver through the portal veins. Enclosed in nodules in the hepatic parenchyma, they molt to the fourth stage in about 2 weeks. The fourth-stage larvae then wander about in the hepatic tissue for about 2 months, growing larger as they go. Leaving the liver by way of the hepatic ligaments, the larvae wander for months in the parietal retroperitoneal tissues and eventually make their way to the base of the cecum and thence to the bowel lumen. The prepatent period usually is cited as 11 months but may be as short as 6 months (McCraw and Slocombe, 1978).

The third-stage larvae of *S. equinus,* similar to those of *S. vulgaris,* undergo their third molt in nodules in the wall of the cecum and colon. About 11 days after infection, the newly molted fourth-stage larvae leave their intestinal nodules, cross the peritoneal space, and enter the right half of the liver, which in the living horse lies in contact with the cecum. These larvae wander about in the hepatic tissue for 2 months or longer before emerging and entering the pancreas or abdominal cavity, where they complete their development to the fifth stage. The fourth molt occurs about 4 months after infection. Finally these adult worms penetrate the wall of the large intestine and reenter the lumen to mate. The prepatent period of *S. equinus* is 9 months.

ANTHELMINTIC MEDICATIONS—ADULTS. Adult *S. vulgaris, S. edentates,* and *S. equinus* are susceptible to febantel, fenbendazole, ivermectin, moxidectin, oxibendazole, and pyrantel pamoate. Coles, Brown, and Trembath (1999) reported on the discovery of *S. edentatus* that were resistant to pyrantel. Strongyle eggs were collected from the feces of three horses that had high numbers of eggs after treatment, and they were found by in vitro methods to be apparently resistant. A second treatment of one horse had very little effect on fecal egg counts after treatment. This appears to be the first report of large strongyle resistance to any anthelmintic.

ANTHELMINTIC MEDICATIONS—MIGRATING LARVAE. Larvae of *S. vulgaris* migrating in the cranial mesenteric artery and its ramifications are accessible to attack by several anthelmintics. Ivermectin is highly effective in a single dose at 0.2 mg/kg (Klei et al, 1984; Lyons, Drudge, and Tolliver, 1982; Slocombe and McCraw, 1980, 1981; Slocombe et al, 1983). Moxidectin was highly efficacious against fourth-stage larvae at single doses of 0.3, 0.4, and 0.5 mg/kg (Monahan et al, 1995b). Fenbendazole may be administered in a single dose of 30 to 60 mg/kg (Duncan et al, 1977) or in five daily doses of 7.5 to 10 mg/kg (Duncan, McBeath, and Preston, 1980). Oxfendazole is effective at a dose of 10 mg/kg (Duncan, McBeath, and Preston, 1980; Kingsbury and Reid, 1981; Slocombe et al, 1986).

POTENTIAL REEMERGENCE OF LARGE STRONGYLES. Concern has arisen that small strongyle treatment programs may have allowed an increase in the prevalence of large strongyles. For about 20 years, parasitologists have been recommending strategic deworming of horses to slow the development of resistance by

small strongyles, but the current concern is that perhaps the presence of large strongyles was overlooked as these recommendations were being promulgated (Nielsen et al, 2012). Parasitologists agree that frequent anthelmintic treatments lead to the development of resistance; therefore they have been routinely recommending that the frequency of treating animals should be reduced to forestall the appearance of resistance (Duncan and Love, 1991; O'Meara and Mulcahy, 2002; Kaplan, 2002). This has led to the recommendation that for cyathostomin control on horse farms, all horses should be tested, and only those with a fecal egg count above a preset value should be treated; in Denmark this was codified into law in 1999, wherein on horse farms a prescription-only restriction on anthelmintic administration requires that a parasitologic diagnosis must be reached before a drug can be prescribed (i.e., prophylactic treatments are not allowed; Nielsen et al, 2012). Similar legislation has been put forth in several other European Union countries. However, because of concern that selective therapy might be giving the less numerous adult large strongyles, particularly *S. vulgaris,* an opportunity to increase in prevalence, a study was undertaken to examine the prevalence of *S. vulgaris* among Danish horses. In this examination of larvae from fecal cultures using samples from 42 Danish horse farms with 662 horses, it was revealed that *S. vulgaris* was present in 12.2% of the horses and on 64.3% of the farms (Nielsen et al, 2012). The authors of this study very carefully state the following in the discussion of their findings: "It is important to emphasize that this study should not disqualify selective therapy as a treatment principle, but it does suggest that some modification to the Danish approach should be considered," and "… now *S. vulgaris* may constitute a reemerging threat to equine health. The results of this study suggest that adequate equine parasite control cannot rely on surveillance by fecal egg counts alone, and underlines the need for more research within this area."

Family Strongylidae; Subfamily Strongylinae; *Triodontophorus*

Triodontophorus species (and the 40-odd species of cyathostomes) do not migrate far beyond the mucous membrane of the colon; therefore the pathogenic effects of their larvae are considerably less dramatic than those inflicted by larvae of *Strongylus* species. However, *T. tenuicollis* adults are frequently observed clustered in ulcerated areas in the large intestine. These helminths appear susceptible to most of the equine anthelmintics that target the large strongyles.

Family Strongylidae; Subfamily Cyathostominae

IDENTIFICATION. These "small strongyles" are parasites of the large intestine of horses, elephants, pigs, marsupials, and turtles, and a multitude of them have been identified. About 40 species of cyathostomes parasitize the cecum and colon of horses, and it is commonplace to find as many as 15 to 20 of these species infecting an individual host at the same time. Cyathostomins have somewhat smaller buccal cavities than strongylins. All have distinct inner and outer leaf crowns, the elements of which differ in size and number (see Figure 4-85). In some species, the inner leaf crown elements are inconspicuous and can be seen only in well-cleared specimens. Identification of species of equine cyathostomes can be accomplished by comparing dorsal and lateral aspects of the buccal regions of fresh or cleared, fixed specimens with the photomicrographs of strongylins and cyathostomins portrayed in Chapter 7. All of the more common species are represented in that collection.

IMPORTANCE. From 75% to 100% of the eggs passed in the feces of naturally infected horses are produced by the small strongyles (Cyathostominae) because these greatly outnumber the large strongyles (Strongylinae) both in numbers of species and in numbers of individuals. Cyathostomin larvae do not migrate beyond the mucous membrane of the cecum and colon, so their pathogenic effects are usually less dramatic than those inflicted by the larvae of *Strongylus* species. However, infection by large numbers of arrested cyathostomin larvae causes a distinct clinical disease that usually is observed in late fall, winter, or early spring (Mirck, 1977). This form of cyathostominosis is characterized by watery diarrhea associated with severe inflammation of the mucous membrane of the cecum and colon, and often terminates fatally. Affected horses display persistent diarrhea, progressive emaciation, and marked hypoalbuminemia sometimes attended by anasarca. The feces may be negative for strongylid eggs, and the history often includes regular and vigorous anthelmintic medication without effect (Church, Kelly, and Obwolo, 1986; Jasko and Roth, 1984). Many more larvae are present than can be accommodated as adult parasites, and as they mature, many are swept out with the manure. Lesions consist of granulomatous colitis, and masses of cyathostomin larvae are embedded in the mucous membrane (Figure 4-87). Massive invasions of the bright red fourth-stage larvae of *Cylicocyclus insigne* riddling the mucosa of the large intestine are particularly impressive in this regard. Most of the worms are immature, and egg counts are therefore misleadingly low. Anthelmintic therapy has no influence on the course of the disease, although continued treatment will reduce the numbers of worms being passed in the feces (Deprez and Vercruysse, 2003). Church, Kelly, and Obwolo (1986) diagnosed their two cases heroically by taking full-thickness biopsies of the jejunum and cecum or ventral colon,

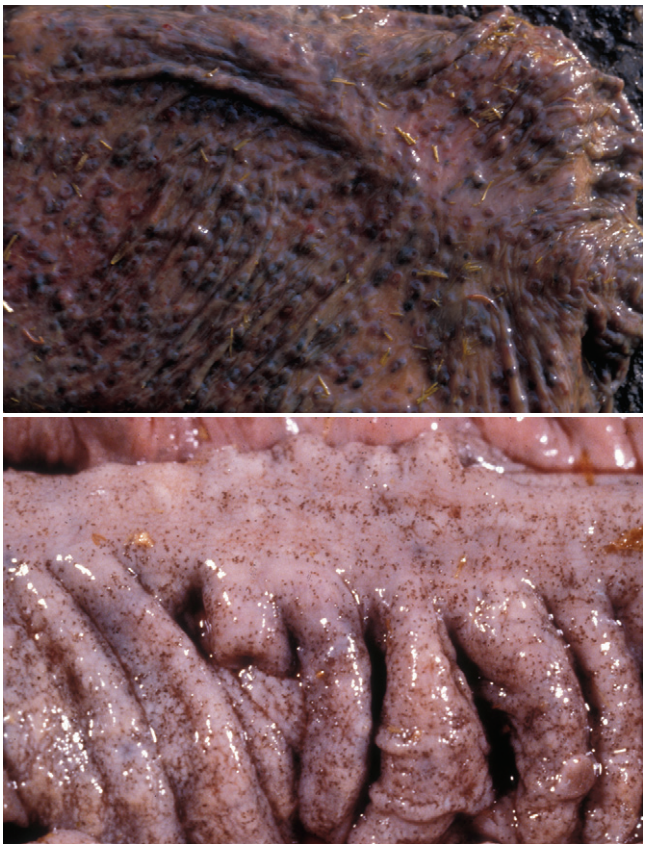


FIGURE 4-87. Fourth-stage larvae and juvenile adult "small strongyles" (Cyathostominae) in the colonic mucosa of a horse. Massive invasions such as this usually cause severe diarrhea.

and cured both patients with steroid therapy directed at the inflammatory reaction. In one case, dexamethasone (20 mg) was administered intramuscularly each day for 4 days and on alternate days thereafter, with the dose reduced by 4 mg every fourth day. In the second case, dexamethasone (20 mg) was administered intramuscularly for 10 days. In both cases, response to steroid therapy was dramatic, with improvement in fecal consistency noted within 24 hours and return to normal serum albumin levels within 1 week.

TREATMENT. The cyathostomin larvae encysted in the mucosa are basically unaffected by routine pyrantel, fenbendazole, or oral ivermectin at 0.2 or 1.0 mg/kg (Klei et al, 1993). Moxidectin at 0.3 or 0.4 mg/kg has effects against the more mature encysted larvae but is less efficacious against the younger third-stage larvae (Bairden et al, 2006; Xiao, Herd, and Majewski, 1994). Fenbendazole at a dose of 10 mg/kg/day for 5 days has been labeled as effective against encysted early third-stage larvae and against older encysted third-stage and fourth-stage larvae.

ANTHELMINTIC RESISTANCE AND THE CYATHOSTOMINAE. Phenothiazine, thiabendazole, cambendazole, mebendazole, fenbendazole, oxfendazole, and febantel are no longer as effective against small strongylids as they were when first introduced (Drudge and Elam, 1961; Drudge and Lyons, 1965; Drudge, Lyons, and Tolliver, 1977, 1979; Hagan, 1979; Slocombe et al, 1977). Drudge, Lyons, and Tolliver (1979) identified five species *(Cyathostomum catinatum, Cyathostomum coronatum, Cylicocyclus nassatus, Cylicostephanus goldi,* and *Cylicostephanus longibursatus)* that exhibited cross-resistance to cambendazole, fenbendazole, mebendazole, oxfendazole, and thiabendazole. However, all of these worms were highly susceptible to 10 mg/kg of oxibendazole, a 2-amino substituted benzimidazole. Later trials after repeated dosing of the herd for 14 years with oxibendazole showed that these five species of worms were resistant to other benzimidazoles but were still affected by ivermectin and piperazine (Lyons et al, 1996). It has also been found that pyrantel is no longer fully effective in clearing small strongyle infections from horses (Kaplan et al, 2004b; Traversa et al, 2009a and 2009b). In the United States, where pyrantel tartrate is available for daily feeding, pyrantel resistance has been found to be even more widespread (Kaplan et al, 2004b). Thus, all that remains for cyathostomin control is the use of a benzimidazole administered with piperazine or a macrocyclic lactone.

Remarkably, and to the great advantage of the horse, macrocyclic lactone resistance has not yet been documented among any of the small strongyles. Recent work has revealed a possible trend toward resistance in some populations, but no definitive evidence indicates that resistance is present (von Samson-Himmelstjerna, 2012). Lack of avermectin resistance in the equine cyathostomes remains a mystery to most parasitologists. Possible theories include absence of resistance gene(s) to be selected by treatment pressure (considered the least likely by most); avermectins as dosed having only a minimal "tail" effect (a period when worms are surrounded by less than curative doses of drug); ivermectin having no effect on encysted cyathostomes, leaving them intact as an untreated refugia (it is thought that all benzimidazoles may have had some effect on the encysted forms); and maybe just luck. We can hope that this continues to be the case forever, but even with all the evidence to the contrary, it is expected that sooner or later, the small strongyles of the horse will become resistant to this class of compounds.

Pasture Management for Strongyle Control

Dr. Georgi used to say something to the effect that "the king's horses probably had fewer worms." The reason was simply that fecal matter was always immediately picked up after deposition—that is, with sufficient manpower, it is theoretically possible to completely break the life cycle of common horse parasites. This is exactly the concept behind the development by Herd of both a mechanical pasture vacuum and a pasture sweeper (Herd, 1986). Horses will often refuse to graze in areas where they defecate, dividing a pasture into areas called *roughs* and *lawns*. It has been suggested that this may be a means by which most horses reduce their intake of strongyle larvae, although this may not be true in smaller pastures (Medica et al, 1996). Dragging and harrowing pastures when occupied will reduce roughs but may spread infected feces over the entire pasture, increasing the chance for a horse to ingest larvae. Composting of horse manure before spreading will kill any parasite eggs that are present.

Family Chabertiidae: Subfamilies Oesophagostominae and Chabertiinae

IDENTIFICATION. A transverse fold of cuticle ("ventral groove"; see Figure 4-65) is present on the ventral side of the body just posterior to the buccal cavity. This buccal cavity varies in size from small (e.g., *Oesophagostomum columbianum;* Figures 4-88 and 4-89) to very large (e.g., *Chabertia ovina;* Figures 4-90 and 4-91). Oesophagostomins are parasites of the large intestines of ruminants (*O. columbianum, Oesophagostomum venulosum, Oesophagostomum radiatum,* and *C. ovina*), swine *(Oesophagostomum dentatum, Oesophagostomum brevicaudum),* and primates (*Conoweberia* species and *Ternidens deminutus*).

IMPORTANCE. Oesophagostomins are called *nodular worms* because their parasitic larvae tend to become encapsulated by a somewhat excessive reactive inflammation on the part of the previously sensitized host. Acute inflammation may lead to clinical disease characterized by fetid diarrhea that may be fatal. The nodules later caseate and calcify, and severe involvement may interfere mechanically with normal intestinal motility. Clinical signs in ruminants and swine usually are associated with these reactions to

FIGURE 4-88. *Oesophagostomum columbianum,* dorsoventral view of buccal and anterior esophageal regions.

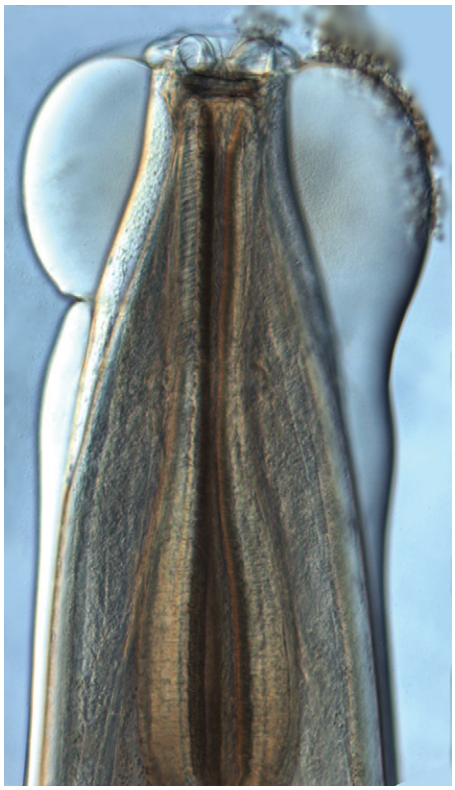


FIGURE 4-89. *Oesophagostomum columbianum,* lateral view of buccal and anterior esophageal regions.

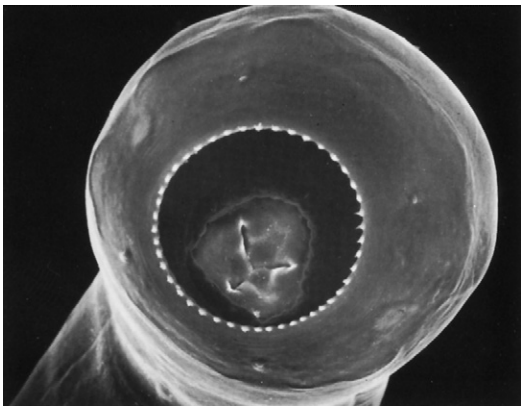


FIGURE 4-90. *Chabertia ovina,* head-on. The oral end of the esophagus with its triradiate lumen is visible at the base of the buccal cavity.

the larval stages in the wall of the bowel, not to adult worms in the lumen. Therefore clinical disease is likely to be associated with nonpatent infection, and diagnosis must depend on correct interpretation of clinical signs or postmortem findings. The feces are watery, dark, and very fetid. Weakness is marked, and emaciation rapid. Necropsy examination conducted during an outbreak of nodular worm disease reveals an inflamed intestine studded with active nodules filled with creamy pus, each containing a living larva (Figure 4-92). Caseated and calcified nodules should not be held accountable for current acute parasitic enteritis but occasionally may cause intussusception or another mechanical abnormality.

The most important effect of *Oesophagostomum* species in swine is the formation of nodules in the gut wall by developing third-stage larvae. Fourth-stage larvae emerge from these nodules as early as 2 weeks after infection, or they remain for several months.

FIGURE 4-91. *Chabertia ovina,* lateral view of buccal cavity and anterior esophageal regions.

Nodule formation may be accompanied by catarrhal enteritis and spoils sausage casings; it probably interferes with maximum growth of young swine. A rise in egg output by sows peaks at 6 or 7 weeks after farrowing and then drops off rapidly. This could be an important epidemiologic factor in situations favorable for the development of infective larvae.

Conoweberia apiostomum, Conoweberia stephanostomum, and *T. deminutus* are pathogenic, especially in recently captured primates with the unaccustomed stresses of confinement and transportation (see Figures 8-83 and 8-84). Acute and chronic disease syndromes caused by *C. stephanostomum* occurred in gorillas from the thirteenth to the fortieth day after capture (Rousselot and Pellissier, 1952). The chronic syndrome consisted of intermittent diarrhea, paleness of the mucous membranes, and the presence of eggs in the feces. In the acute form, the gorilla refuses to eat or nibbles a little and suffers some diarrhea, but very soon passes only small quantities of glairy mucus streaked with blood, much like that observed in acute amoebic dysentery of humans. The gorilla remains lying down or sitting with both hands on its head in an attitude of human desperation.

ANTHELMINTIC MEDICATION. Many different products are approved for treating infection with the adults of *Oesophagostomum* and *Chabertia* species in cattle and sheep and of *Oesophagostomum* species in swine. However, recent reports have described cases in Brazil in which *O. radiatum* was resistant to moxidectin treatment (Condi, Soutello, and Amarante, 2009).

Family Stephanuridae, Subfamily Stephanurinae

IDENTIFICATION. *Stephanurus dentatus,* the kidney worm of swine, is a stout (up to 2 by 40 mm) parasite of the hepatic, renal, and perirenal tissues, axial musculature, and spinal canal of swine and sometimes of cattle. The buccal cavity is cup-shaped and is directed straightforward with 6 to 10 triangular teeth at its base (Figure 4-93). The gut is convoluted, the spicules equal and short, and the bursa reduced.

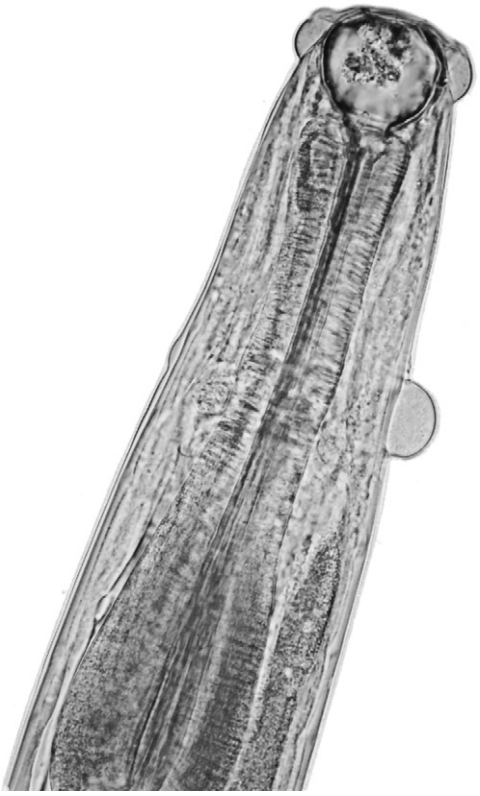


FIGURE 4-92. *Oesophagostomum radiatum* fourth-stage larva from a nodule in the intestinal wall of a calf. *Oesophagostomum* species fourth-stage larvae are unusual in that they have buccal cavities that are relatively larger than those in the adult stage.

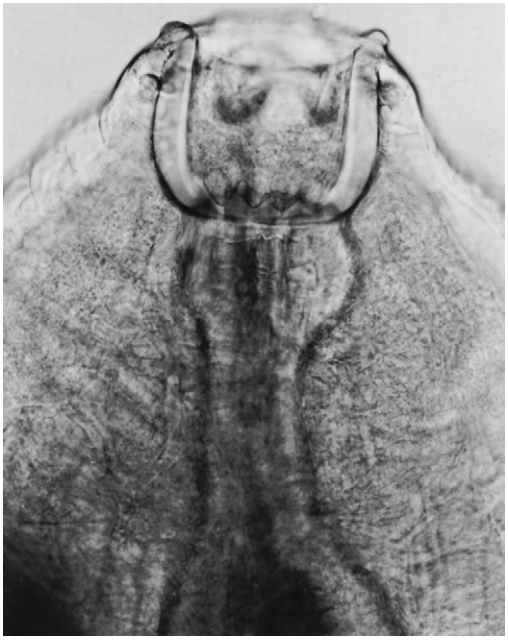


FIGURE 4-93. *Stephanurus dentatus.*

Earthworms serve as intermediate hosts. The life history may be direct or could involve earthworms as facultative intermediate hosts or infection occurring by ingestion or skin penetration of third-stage larvae or by ingestion of infected earthworms. Once in the body of the pig, the larvae enter the liver and spend 4 to 9 months wandering destructively there. Some are trapped by an

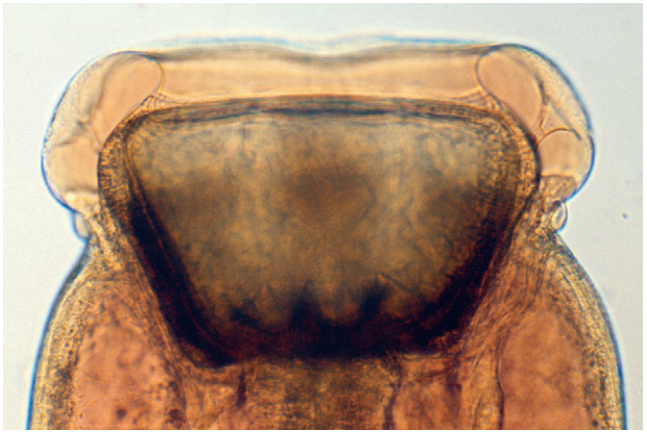


FIGURE 4-94. *Cyathostoma* (family Syngamidae) buccal capsule.

FIGURE 4-95. *Syngamus tracheae,* five male and female pairs. The inset on the left shows the anterior ends of a female *(left)* and the attached male *(right).*

encapsulating tissue reaction, but the rest migrate to the retroperitoneal tissues surrounding the kidneys and ureters. Eggs appear in the urine 9 to 16 months after infection and persist for 3 years or longer. Piglets may become infected in utero (Batte, Harkema, and Osborne, 1960; Batte, Moncol, and Barber, 1966).

S. dentatus larvae migrate abortively in other hosts (e.g., cattle) and frequently lose their way in pigs. Not only liver and kidney but also choice loin chops are frequently condemned because of these destructive larvae. Although migration of *S. dentatus* larvae in the spinal cord may cause posterior paralysis, the clinical signs of infection are otherwise not distinctive. Extensive liver damage may lead to emaciation and death.

ANTHELMINTIC MEDICATION. Doramectin injectable (0.2 mg/kg body weight) and fenbendazole (oral; 9 mg/kg body weight for 3 to 12 days) are approved anthelmintics for the treatment of *S. dentatus* infection in the United States.

Family Syngamidae

The subfamily Syngamidae includes the genera *Syngamus* and *Cyathostoma* (not *Cyathostomum*) in birds and *Mammomonogamus* in mammals (see Figure 7-59). All three have large buccal capsules (Figure 4-94), and all are parasites of the upper respiratory tract. Males and females of *Syngamus* and *Mammomonogamus* species are fused permanently in copula (Figure 4-95). Earthworms serve as paratenic hosts for *Syngamus*. *Syngamus trachea* infections have caused the deaths of farmed rheas, and in these birds, treatment

with fenbendazole at 25 mg/kg was successful therapy (de Witt, 1995). Ivermectin also is highly effective in the treatment of *Syngamus* infection. In a fecal survey of 100 stray cats in St. Kitts, West Indies, 45% were shedding the eggs of *Mammomonogamus* in their feces (Krecek et al, 2010), and in 500 slaughtered cattle in Colombia, *Mammomonogamus laryngeus* was found in 14.8% of the cattle and in 47% of the animals 2 to 2.5 years old (Echeverry et al, 2011).

Superfamily Ancylostomatoidea

Family Ancylostomatidae

IDENTIFICATION. Adult hookworms are parasites of the small intestine. Some species such as *Ancylostoma caninum* cause the loss of large quantities of blood from the hosts, whereas others such as *Uncinaria stenocephala* remove very little. Fresh specimens of *A. caninum* tend to be dark in color, whereas those of *U. stenocephala* are quite pale. All hookworms have a large buccal cavity directed obliquely dorsally, so the anterior end of the worm is more or less "hooked," but again, this trait is variably developed, as can be appreciated from a comparison of *Bunostomum* (Figure 4-96) and *Globocephalus* species (Figure 4-97). The male hookworm, provided with a well-developed bursa, is often found in copula with the female, the two worms forming a T because the vulva is located some little distance from the caudal extremity. The female lays typical strongylid eggs, and these appear in the feces during the morula stage of development.

Two subfamilies are distinguished: Ancylostomatinae and Bunostominae. "Carnivorous hosts are parasitized only by the Ancylostomatinae, herbivorous hosts by the Bunostominae, and omnivorous hosts by both subfamilies" (Lichtenfels, 1980).

The subfamily Ancylostomatinae includes the genera *Ancylostoma, Uncinaria, Globocephalus,* and *Placoconus.*

The most common hookworms of the dog and cat are species of *Ancylostoma* and *U. stenocephala*. Species of *Ancylostoma* have buccal cavities with sharp teeth, whereas those of *Uncinaria* have cutting plates (Figure 4-98). The ventral margin of the stoma of *Ancylostoma* is armed by one *(Ancylostoma braziliense),* two *(Ancylostoma duodenale),* or three *(A. caninum*, *Ancylostoma tubaeforme)* pairs of sharp teeth. *A. braziliense* matures in dogs and cats, *A. duodenale* in humans, *A. caninum* in dogs (see Figures 4-66 and 4-98), and *A. tubaeforme* in cats (Figure 4-99). The ventral margin of the stoma of *Globocephalus urosubulatus* of swine has neither plates nor teeth (see Figure 4-97). The buccal capsule of *Placoconus lotoris* of raccoons is formed of five articulating plates (Figure 4-100). The Companion Animal Parasite Council (CAPC) has been collecting data on the prevalence of the results of fecal samples nationally by county. These maps, which include samples from dogs (n = 2,622,470) (Figure 4-101) and cats (n = 558,707) (Figure 4-102) submitted by veterinarians show that there remain many counties in the United States where more than 10% of canine and feline samples from pets seeing veterinarians remain positive for hookworm eggs.

The subfamily Bunostominae includes the genera *Bunostomum* of ruminants (see Figure 4-96), *Necator* of humans, *Bathmostomum* of elephants, and *Grammocephalus* of elephants and rhinoceroses.

LIFE HISTORY. Infection typically occurs through ingestion or skin penetration by infective larvae, which then undergo more or less extensive migrations through the tissues of the host before developing into adult hookworms in the small intestine (Figure 4-103; also see Figure 7-41). Hookworms in sea lions, seals, and dogs are capable of infecting neonates through transmammary transmission; transmammary transmission does not appear to occur with the hookworm of the cat.

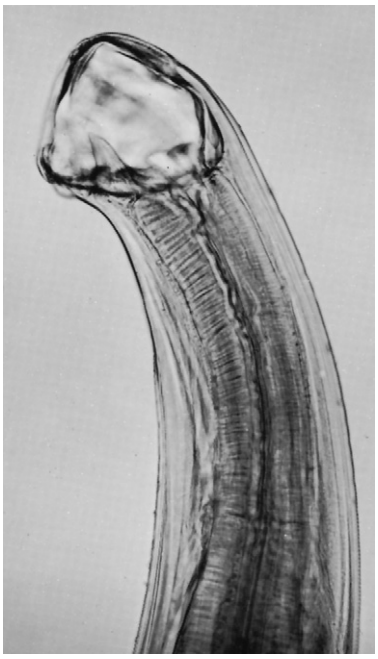
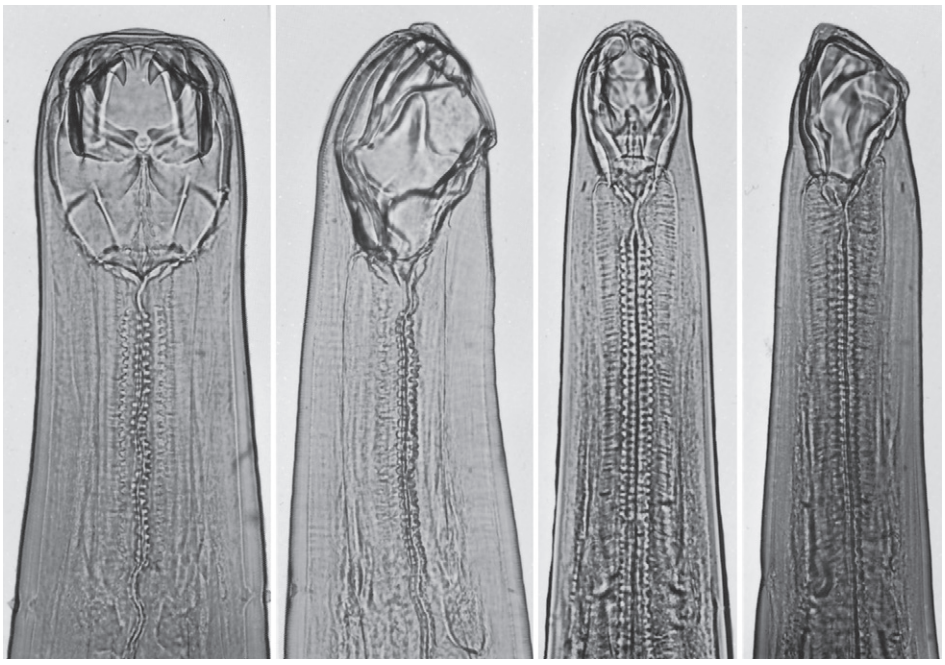


FIGURE 4-96. *Bunostomum* sp.

FIGURE 4-97. *Globocephalus urosubulatus,* a hookworm of swine; dorsal *(left)* and lateral *(right)* aspects. (Courtesy Dr. E. I. Braide.)

ANTHELMINTIC MEDICATION. In ruminants, hookworms can be treated with avermectins, levamisole, or various benzimidazoles. In pigs, treatment is usually performed using an avermectin. Cats can be treated with labeled products containing ivermectin, selamectin, moxidectin, milbemycin oxime, pyrantel pamoate, emodepside, or febantel. Dogs can be treated with many available products that contain pyrantel pamoate, febantel, fenbendazole, milbemycin oxime, and moxidectin.

In Australia the concern is that the hookworm of the dog, *A. caninum,* is becoming resistant to treatment with pyrantel. This was first suggested by a report from New Zealand of a dog imported from Australia that had a hookworm infection that would not clear with pyrantel (Jackson et al, 1987). Larvae grown from eggs in the feces of this dog were used to infect two other dogs that could not be cleared of their infection with a 5× dose of pyrantel. Since then, other reports of reduced efficacy have been received from Australia (Hopkins and Gyr, 1991; Hopkins, Gyr, and Schimmel, 1998).



Ancylostoma caninum *Uncinaria stenocephala*

FIGURE 4-98. Dorsoventral and lateral aspects of the buccal and esophageal regions of *Ancylostoma caninum* and *Uncinaria stenocephala*.

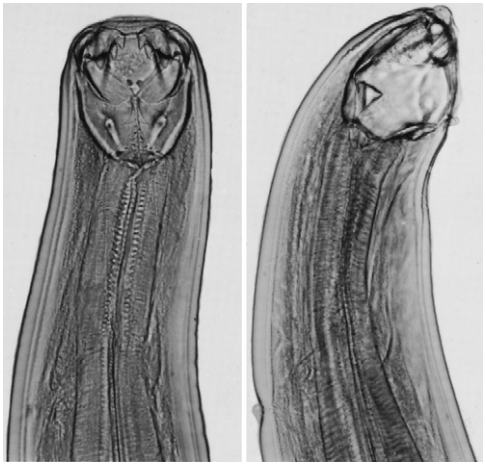


FIGURE 4-99. *Ancylostoma tubaeforme*. At left is the dorsoventral aspect of the stoma, and at right, its lateral aspect.

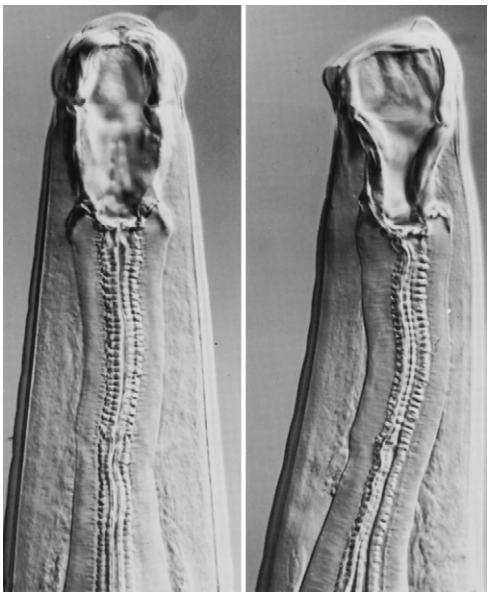


FIGURE 4-100. *Placoconus lotoris,* hookworm of the raccoon; dorsoventral *(left)* and lateral *(right)* aspects of the buccal and esophageal regions.

Most recently, a controlled trial in Australia showed poor efficacy against worms in experimentally infected dogs (Kopp et al, 2007). This appears to provide additional rationale for practitioners to order posttreatment fecal examinations to monitor the effects of therapy.

Hookworm Disease of Dogs

The principal importance of hookworms is associated with their ability to cause anemia. Hookworm disease varies in severity from asymptomatic infection to rapidly fatal exsanguination, depending on the magnitude of the challenge and the resistance of the host. Magnitude of challenge is determined by the virulence and the number of hookworms. Virulence depends on the species of hookworm involved. *A. caninum* is much more pathogenic for dogs than is *A. braziliense* or *U. stenocephala* because it causes much greater blood loss per worm. The number of hookworms infecting a particular host depends very heavily on the degree of exposure to infective larvae. Exposure, in turn, depends on the extent to which infected hosts have contaminated the environment by shedding eggs in their feces, and on the suitability of the substrate (gravel and sand are ideal), temperature, and moisture for development and survival of infective larvae.

Infection of nursling pups with *A. caninum* occurs through the mammary gland via **transmammary transmission** (Kotake, 1929a, 1929b; Stone and Girardeau, 1966, 1968). Transplacental transmission, if it occurs at all, is overshadowed by transmammary infection (Stoye, 1973). A bitch exposed to only one substantial oral or percutaneous infection will shed *A. caninum* larvae in her milk for

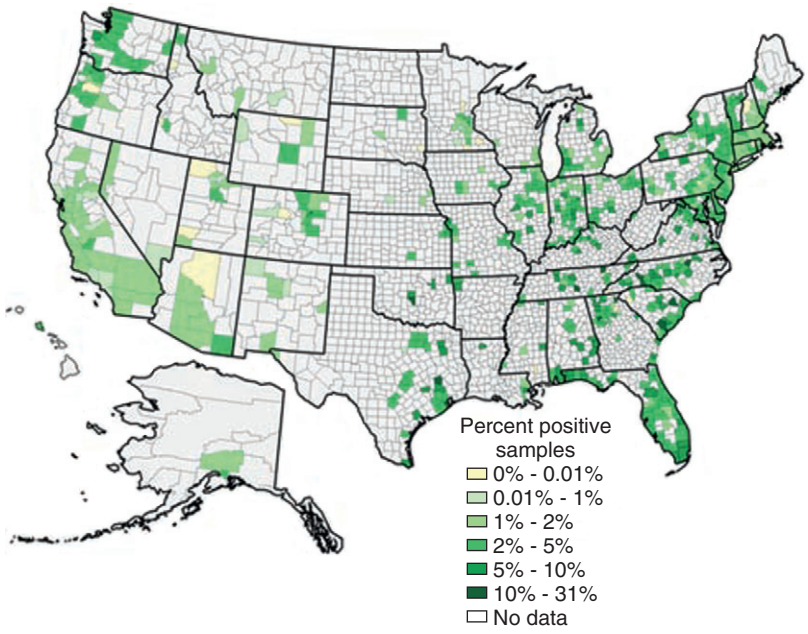


FIGURE 4-101. Map of prevalence by county of hookworm egg–positive samples from dogs (n = 2,622,470) in data collected by the Companion Animal Parasite Council (CAPC) for the year 2011; only counties with more than 20 examined samples are included on the map.

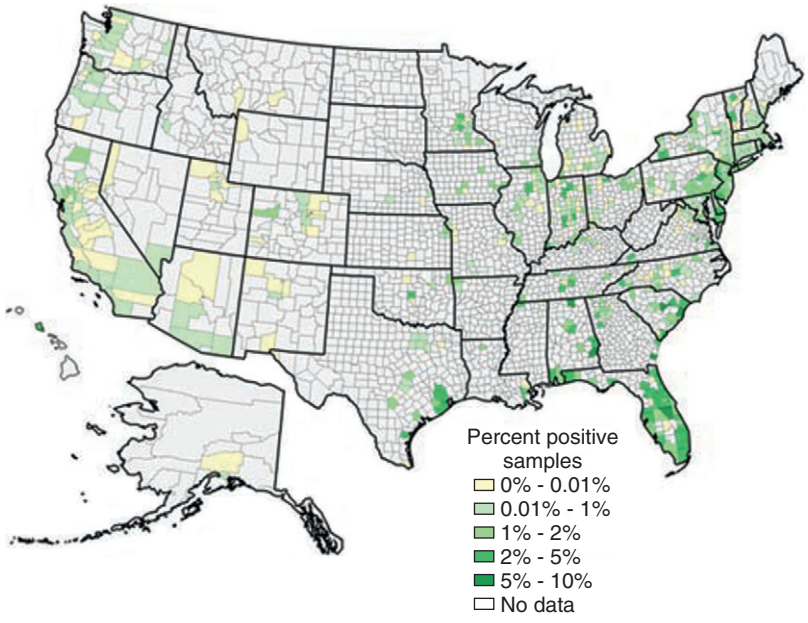


FIGURE 4-102. Map of prevalence by county of hookworm egg–positive samples from cats (n = 558,707) in data collected by the Companion Animal Parasite Council (CAPC) for the year 2011; only counties with more than 20 examined samples are included on the map.

the next three lactations, although larval output will diminish with each lactation. Currently available anthelmintics administered at dosages to treat and control adult hookworm infections lack significant efficacy against hookworm larvae arrested in the tissues. The arrested larvae of *A. caninum* may be present in dogs receiving monthly treatment with a combination heartworm preventive; thus they are still available to move through the mammary gland to the intestines of the nursling pups (see Figure 4-103).

Host resistance can be resolved into two abilities: (1) The ability to limit the number of hookworms maturing in the small intestine is influenced by age, premunition, and acquired immunity. As dogs grow older, they become more resistant to hookworms, whether or not they experience infection. Immunity acquired from previous infection confers increased resistance, but this is difficult to disentangle from the influences of advancing age and from the marked inhibition of further infection exerted by a residual population of hookworms (premunition). (2) The ability to compensate for blood loss caused by hookworms is influenced by the hematopoietic capacity and the state of nutrition of the individual, and by the presence or absence of other stresses.

CLINICAL FORMS OF DISEASE. Four different forms of canine hookworm disease can be distinguished. Peracute disease

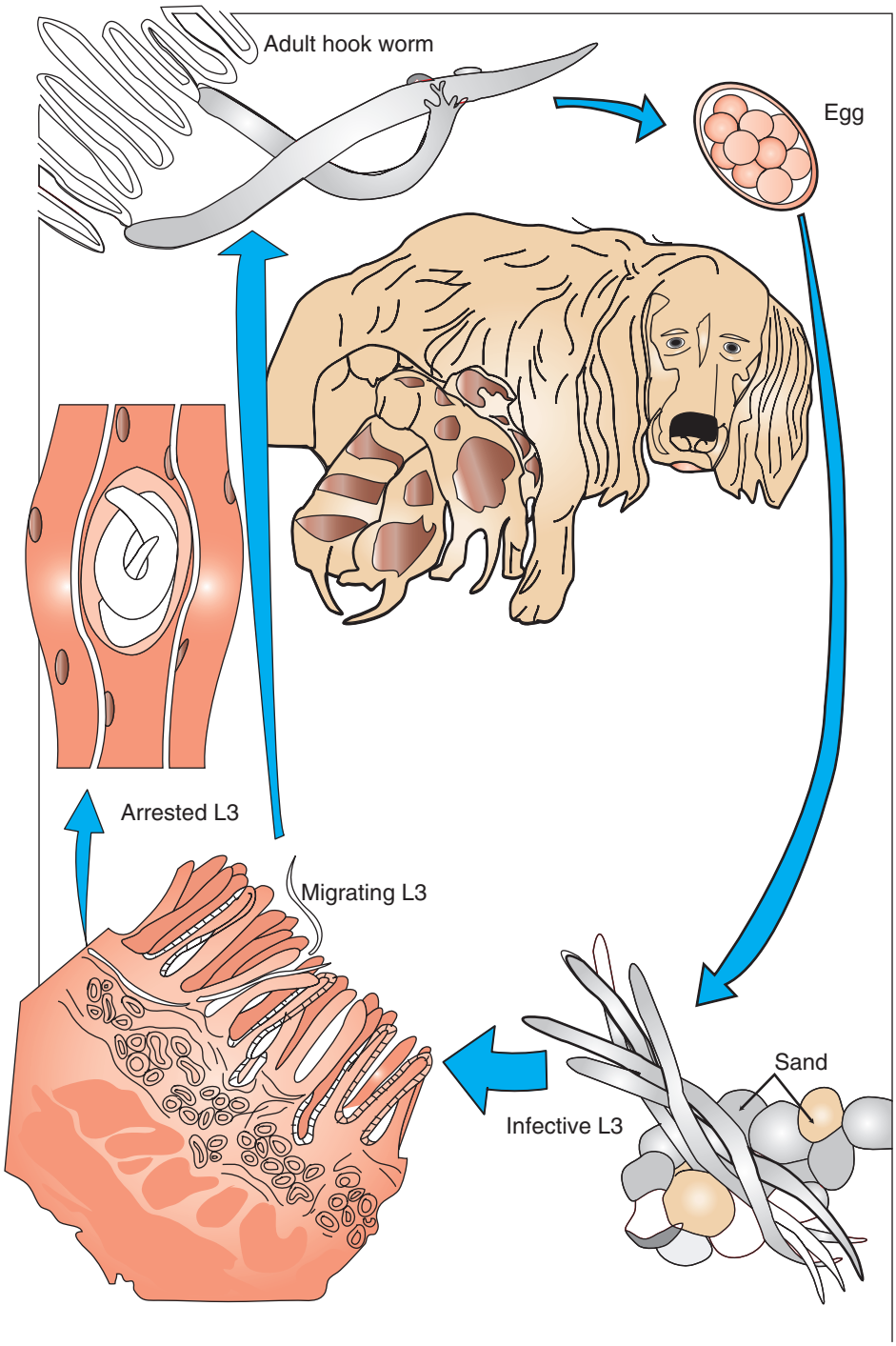


FIGURE 4-103. Life history of *Ancylostoma caninum*. The actively motile sheathed larva develops in 2 to 8 days. Shaded, well-drained soils, warmth, and humidity provide optimum conditions for development and survival of this stage, which may infect the host on being swallowed or by penetrating its skin. Eggs are shed in the feces about 2 weeks after ingestion of larvae and about a month after penetration of skin by larvae. However, not all larvae mature. Some invade skeletal muscle cells (Little, 1978) or gut wall (Schad, 1974, 1979) and enter an arrested state of development. Arrested larvae later become reactivated in response to obscure cues and migrate to the small intestine, where they mature, or to the mammary glands, where they are shed in the milk and infect the pups. Arrested larvae are regularly reactivated during the last 2 weeks of pregnancy.

occurs in the neonatal pup. Acute disease occurs in older pups and mature dogs. Chronic hookworm infection is not uncommon in adult dogs and may or may not be associated with clinical signs.

Peracute hookworm disease results from the passage of infective larvae from the dam to nursing pups in the milk. Transmammary infection of very young pups with as few as 50 to 100 adult *A. caninum* may prove fatal. Typically, the pups appear healthy and sleek the first week, then sicken and deteriorate rapidly the second week. The visible mucosae are very pale, and the soft to liquid feces are very dark in color because the blood shed from the lesions made by the hookworms in the small intestine has been partially digested on the way out. The worms do not lay eggs until the sixteenth day

of infection, so diagnosis must rest on the clinical signs of disease. Prognosis is guarded to poor with or without treatment.

Treatment is often to little avail in peracute neonatal hookworm disease. Blood transfusion is essential to keep affected pups alive long enough for anthelmintic medication to take effect, and anthelmintic medication must be administered immediately to stop the loss of blood as soon as possible. On no account should anthelmintic therapy be delayed. It is impracticable to attempt replacement of hookworm blood losses by transfusion for any appreciable length of time.

Routine cage, pen, and run sanitation and periodic anthelmintic medication of all adult dogs are essential to reduce the level of environmental contamination with hookworm larvae. When neonatal losses have already been experienced, it is essential to examine the visible mucosae of each pup daily from about the seventh day of life until weaning, and to administer an anthelmintic at the first sign of anemia. Currently, it is recommended that dogs in the United States begin heartworm prevention with broad-spectrum parasite control as near birth as possible, and if the product or product that is to be used is not labeled for dogs as young as 2 weeks of age, that they be treated with pyrantel every 2 weeks until they can be placed on appropriate lifelong hookworm control products.

Bitches that have lost litters may be treated with fenbendazole, 50 mg/kg per day, from the fortieth day of gestation to the fourteenth day of lactation, to prevent further losses (Burke and Roberson, 1983; Düwel and Strasser, 1978). This treatment attacks the reactivated larvae and is effective but rather expensive. It has been shown that ivermectin treatment of the bitch (0.5 mg/kg body weight administered 4 to 9 days before whelping, followed by a second treatment 10 days later) can also prevent puppies from being infected by larvae passed in the milk (Stoye, Meyer, and Schneider, 1987). Treatment of four bitches with a single 1-mg/kg subcutaneous injection of doramectin failed to prevent transmammary infection of all puppies, with 5 of 23 puppies, representing three of the four litters, becoming infected (Schnieder et al, 1996). When three bitches were treated topically 56 days after conception (i.e., 3 to 7 days before parturition) with imidacloprid and moxidectin (4 to 4.6 mg/kg), neither of 2 puppies from each litter born to the treated dogs had adult worms or hookworm larvae in their tissues (Kramer, Epe, and Mencke, 2009). Unfortunately few worms were present in dogs born in the three untreated control litters, but a few hookworm adults were seen in some of the 6 puppies examined, and they were also found to have a few hookworm larvae in their tissues. It would appear that this use of moxidectin would prove beneficial, if not completely protective.

Acute hookworm disease results from sudden exposure of susceptible older pups to large numbers of infective larvae. Even mature dogs may be overwhelmed if exposure is sufficiently great. Usually many eggs will be found in the feces of affected animals, but clinical signs may precede the appearance of eggs by about 4 days, particularly in heavy infection. In acute hookworm disease and in chronic (compensated) hookworm infection, response to simple anthelmintic therapy is usually dramatic. Supportive therapy beyond provision of an adequate diet is unnecessary.

Chronic (compensated) hookworm infection usually occurs without signs. Diagnosis rests on the presence of hookworm eggs in the feces and measurable reductions in erythrocyte count, blood hemoglobin, or packed cell volume. Occasionally, however, incomplete adjustment between parasite and host produces a state of chronic ill health.

Secondary (decompensated) hookworm disease usually involves older dogs that have more ailing them than just hookworms. The cardinal sign again is profound anemia, usually in a malnourished or even emaciated animal. The hookworms may indeed kill the dog, but it is important in this case to recognize that they play a secondary role. An accurate diagnosis, for example, of "malnutrition with secondary hookworm infection" logically leads to effective therapy. The efficacy of mebendazole and fenbendazole was dramatically reduced in iron- and protein-deficient rats infected with *Nippostrongylus brasiliensis* (Duncombe et al, 1977a, 1977b). Clinical experience indicates that protein sufficiency is essential for efficient anthelmintic action against hookworms and other parasites. Malnourished dogs that have secondary hookworm disease and dogs that seem adequately nourished but fail to respond to anthelmintic medication should first be given a course of supportive therapy (e.g., high-protein diet, ferrous sulfate orally or parenteral iron injections, vitamins, and, if necessary, blood transfusion) and then be remedicated with a suitable anthelmintic.

ARRESTED LARVAE AND THE REFRACTORY EGG SHEDDER. Arrested *A. caninum* larvae are found in the intestinal wall and skeletal muscle tissue of adult dogs; these arrested larvae are not killed by routine treatment. Little (1978) found that larvae of *A. caninum* are continually migrating from the muscles to the intestine through the lungs. When adult worms were already present in the intestine, few if any of these larvae developed to maturity, but when adult worms were eliminated by treatment, larvae from the muscles were able to mature and to start producing eggs in about 4 weeks. A second course of treatment eliminated the new adults, and these in turn were replaced by additional larvae from the muscles. Schad found that if infective larvae were chilled before they were administered orally to dogs, they became arrested in the gut wall. When reactivated, these larvae were able to become established in the intestine in the presence of adults, and neither removal of adult worms with anthelmintic nor immunosuppression with prednisolone initiated resumption of development of the arrested *A. caninum* larvae (Schad, 1974, 1979; Schad and Page, 1982). Thus, besides serving as a source of infection for nursing puppies, arrested larvae repopulate the intestine with adults that contaminate the environment. Practicing veterinarians frequently encounter dogs with hookworm infections that refuse to "clean up" even after repeated treatment with a variety of drugs over the course of many months. This "larva leak" phenomenon provides a plausible explanation for these refractory cases.

ENVIRONMENTAL CONTAMINATION. Because hookworm infection is common and because females are prodigious egg-layers, populations of infective larvae are likely to bloom whenever the weather becomes favorable for their development and survival. Therefore most frank hookworm disease cases occur during late spring, summer, and early autumn in temperate climates, particularly when mild weather is accompanied by adequate rainfall. The infective challenge may become overwhelming in carelessly managed kennels and pet shops where feces are allowed to accumulate long enough to permit infective larvae to develop. Unpaved runs are particularly favorable for perpetuation of the parasite because feces mix with the soil. This makes sanitation difficult and provides more favorable cultural conditions, especially when the soil is light, open textured, and well drained.

From 2 to 8 days is required for the morula in the hookworm egg to develop into an infective third-stage larva. Shirt-sleeve temperatures (23°C to 30°C) and a moderately moist, well-aerated medium are optimal. Thus hookworm larvae develop well in shaded areas of well-drained soils but not in heavy, waterlogged soils, or where they are exposed to direct sunlight and desiccation. *Ancylostoma* eggs and larvae are destroyed by freezing,

whereas those of *Uncinaria* are very resistant to cold. *A. caninum* larvae will not develop to the infective stage at temperatures consistently below 15°C. Above the optimum temperature for development (30°C), larvae rapidly develop to the infective stage. It can be reached in 48 hours at 37°C—the highest temperature compatible with development (McCoy, 1930). Thus, compared with *Toxocara* eggs, soil pollution by hookworm infective larvae may be viewed as a temporary problem that a good hard freeze will probably solve.

People are always looking for ways to kill the larvae on soil or lawns, but no good method is known. During mild weather, sodium borate, broadcast at the rate of 10 lb/100 sq ft (0.5 kg/m^2) and raked in, will destroy hookworm larvae in gravel- or loam-surfaced runs. This treatment destroys vegetation as well as hookworm larvae and therefore is unsuitable for lawns. Resinated dichlorvos, an organophosphate, was reported to interfere with the development of first- and second-stage larvae of *A. caninum* (Kalkofen, 1971). Paved surfaces, cages, and the like should first be cleaned thoroughly and then mopped or sprayed with 1% sodium hypochlorite solution (Clorox). This solution kills the larvae or at least induces them to cast off their sheaths, after which they are more susceptible to drying and other unfavorable environmental stresses. Large commercial dog-rearing operations make extensive use of wire-bottomed cages and pens to effect the physical separation of dogs from the bulk of their feces.

In most situations, environmental protection is provided by the routine treatment of pet dogs and cats. Anthelmintic medication may be used to reduce the output of hookworm eggs in the feces, thus limiting the degree of contamination of the environment with infective larvae. Therapeutic dosages may be administered monthly, periodically, or when indicated by positive fecal examinations. Most monthly heartworm preventives also do an excellent job protecting the environment from hookworm eggs.

Cutaneous Larva Migrans

"Creeping eruption" (human cutaneous larva migrans) is a linear, tortuous, erythematous, and intensely pruritic eruption of the human skin usually caused by migration of a nematode larva (Kirby-Smith, Dove, and White, 1926). *A. braziliense* larvae are most frequently involved in typical protracted cases, especially in the coastal regions of the southeastern United States (White and Dove, 1926). Accidental sporadic or experimental cases involving *A. caninum, U. stenocephala, Bunostomum phlebotomum, Strongyloides stercoralis,* and *Gnathostoma* spp. have also been reported, and the larvae of those species that normally mature in man *(A. duodenale, Ancylostoma ceylonicum,* and *Necator americanus)* produce a transient but otherwise typical creeping eruption in previously sensitized individuals. It should be noted that larvae of *Gasterophilus* and *Hypoderma* species also migrate in human skin (James, 1947), producing a clinical condition properly termed *cutaneous larva migrans*. There is every reason to believe that after the larvae disappear from the skin, they will enter deeper tissues, where they will persist for extended periods (see Figure 8-86).

> Probably no nematode larva capable of penetrating the skin is above suspicion in individual cases, but the epidemiologic importance of any particular species depends on many influences beyond its intrinsic capabilities. For example, the etiologic prominence of *A. braziliense* may have much to do with the defecation behavior of dogs and cats, as may be surmised from the following description of circumstances surrounding infection, lesions, and symptoms by Kirby-Smith, Dove, and White (1926).
>
> At least 50% of cases of creeping eruption seen by the senior author are believed to have originated at the beach, the probable origin being traced to the soft damp sand in front of beach buildings at points slightly above the high water mark. Such patients reported with lesions varying in numbers. They were not the most extensively infected ones. Persons with hundreds of lesions definitely attributed the origin of their infection to contact with damp sand when they were wet with perspiration while working: repairing an automobile, doing brick work, making plumbing connections underneath houses, and the like.
>
> The most recent visible lesion is a very narrow erythematous formation along the course traveled by the worm. Soon a slightly raised line representing the location of the burrow can be palpated. This line becomes visibly elevated and is more or less continuous and vesicular. Sometimes bullae are formed. The surface of the lesion dries, resulting in a thin crust. When the parasite travels, it moves from a fraction of an inch to several inches a day, advancing as a rule more rapidly at night.
>
> For some patients, the itching sensation resulting from infection is almost intolerable, whereas others endure it with less suffering. The severity of the lesions, too, is more pronounced in some than in others.
>
> Severity and persistence of the lesions are related at least in part to hypersensitivity resulting from previous exposure. The lungs may be invaded, but intestinal infection with mature worms ensues only in cases involving those species that are normal parasites of humans.

Human Enteric Infections With *Ancylostoma caninum*

Prociv and Croese (1996) reported on a series of human cases of eosinophilic enteritis from northern semitropical Queensland, Australia. Most of these cases came from typical suburban housing developments. An adult *A. caninum* was recovered at colonoscopy from the terminal ileum of one patient, and an unidentifiable adult hookworm was found on a portion of ileum resected from a second patient. In additional cases reported from Australia and the United States since that time, adult *A. caninum* have been recovered, and cases have showed signs and serology suggestive of *A. caninum* infection (Prociv and Croese, 1996; Khoshoo et al, 1995). Signs of infection have included obscure abdominal pain that may or may not be associated with an increased level of circulating eosinophils. Worms are not observed in most seropositive patients. It appears that these people became infected with infective-stage larvae by the cutaneous route as they went about parks and yards without shoes and socks. These cases provide yet another good reason why veterinary practitioners must insist that clients submit fecal samples from their pets for annual evaluation and work with their clients to practice hookworm prevention and control.

Superfamily Metastrongyloidea

Metastrongyloids are parasites of the respiratory, vascular, and nervous systems of mammals. Most species whose life histories have been investigated require a snail or slug intermediate host. However, *Metastrongylus* species develop to the infective stage in earthworms, and *F. osleri* and *F. hirthi* infect their definitive hosts directly. The copulatory bursa is of the basic strongylid pattern but has suffered varying degrees of reduction in the evolution of different families. For example, the bursa is best developed in the family Metastrongylidae (Figure 4-104) but is reduced to mere papillae in the family Filaroididae. The vulva is close to the anus except in the family Crenosomatidae, in which it is located in the midregion of the body. The diversity of structure and biology displayed by members of the superfamily Metastrongyloidea makes further generalization precarious.

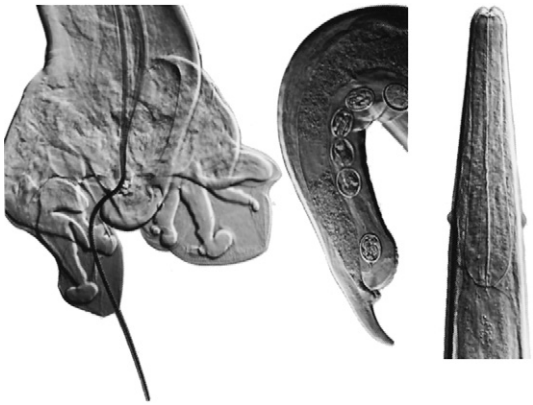


FIGURE 4-104. *Metastrongylus apri.*

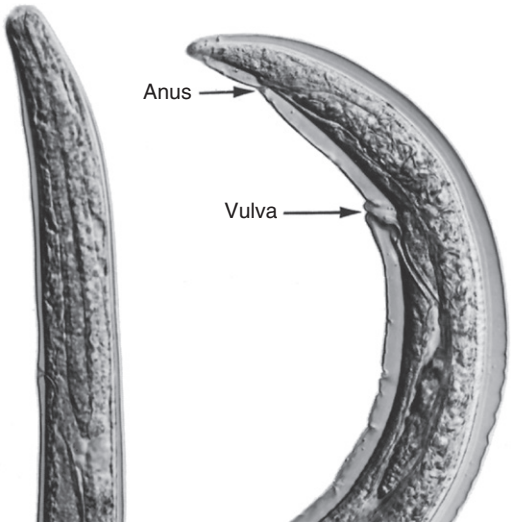


FIGURE 4-105. *Muellerius capillaris* female.

Family Metastrongylidae

The family Metastrongylidae contains only one genus, *Metastrongylus*, all species of which are large white parasites of the bronchi and bronchioles of swine.

IDENTIFICATION. The mouth is flanked by a pair of trilobed lips. The spicules are long and thin, the bursa is well developed, and the vulva is near the anus (see Figure 4-104). When passed in the feces of infected swine, the egg contains a larva.

LIFE HISTORY. Oviparous females lay eggs containing first-stage larvae. The standard view is that these eggs do not hatch or develop into infective larvae unless they are ingested by an earthworm. However, continued high prevalence (50%) in Iowa swine despite confinement rearing and improved sanitation indicates that the earthworm may not be an obligatory intermediate host of *Metastrongylus* species (Ledet and Greve, 1966).

Metastrongylus species are of only modest pathologic and economic importance. It was once supposed that they acted as vectors of swine influenza virus, but substantial proof for this idea is lacking (Wallace, 1977).

ANTHELMINTIC MEDICATION. Fenbendazole, levamisole, ivermectin, and doramectin are approved anthelmintics with activity against swine lungworms.

Family Protostrongylidae

IDENTIFICATION. Protostrongylids have a well-developed bursa, spicule, and spicule guide, and the vulva is near the anus (Figure 4-105; see also Figure 4-70).

LIFE HISTORY. The oviparous protostrongylid females deposit unsegmented eggs in the surrounding lung, vascular, or neural tissues. These eggs develop into first-stage larvae before they appear in the feces. If these first-stage larvae are ingested by any of a wide range of snails and slugs, they develop in these intermediate hosts into doubly ensheathed third-stage infective larvae. All of the protostrongylids considered here are parasites of sheep and goats.

PROTOSTRONGYLUS. *Protostrongylus rufescens* lives in the smaller bronchioles of sheep and goats, where they may cause localized lesions. Males of this brownish red species can be distinguished from *D. filaria* by their longer, comblike spicules (see Figure 4-70). The female *Protostrongylus* organism is prodelphic, whereas the female *Dictyocaulus* organism is amphidelphic. Fenbendazole-medicated salt has been used successfully to control protostrongylid lungworms in free-ranging Rocky Mountain bighorn sheep in Montana (Jones and Worley, 1997).

MUELLERIUS. *Muellerius capillaris* (see Figure 4-105) is a tiny species so deeply embedded in lung tissue or reactive nodules in the lungs of sheep and goats that specimens are extremely difficult to dissect out intact. Antemortem diagnosis is less difficult because the active first-stage larvae are easily separated from the host's feces by the Baermann technique and are not difficult to distinguish from those of *Protostrongylus* and *Dictyocaulus* species (see Figure 7-65). *Muellerius* species are usually nonpathogenic at the levels of infection normally encountered in nature and agriculture, but heavy infections may have serious consequences, especially in goats.

TREATMENT. *M. capillaris* infection has been treated successfully in sheep with moxidectin (1% injectable solution at 0.2 mg/kg) and in goats with topical eprinomectin (0.5 mg/kg) (Geurden and Vercruysse, 2007; Papadopoulos et al, 2004). Also, levamisole, fenbendazole, albendazole, and ivermectin have been used to treat *M. capillaris* infections in sheep and goats, but for all these products, results have not been the ones that clinicians hoped to attain.

PARELAPHOSTRONGYLUS. *Parelaphostrongylus tenuis* is normally a parasite of the meninges of the white-tailed deer, *O. virginianus,* in which species it rarely if ever causes disease (Figure 4-106). However, in abnormal hosts, such as sheep, goats, llamas, camels, moose, caribou, reindeer, wapiti, fallow deer, and mule deer, *P. tenuis* tends to invade the nervous tissue proper, causing serious or fatal neurologic disease (Baumgärtner et al, 1985; Krogdahl, Thilsted, and Olsen, 1987; Mayhew et al, 1976; Nichols et al, 1986) (see Figures 8-93 and 8-94). Because *P. tenuis* rarely matures in these hosts, larvae are not shed in the feces. Therefore diagnosis is presumptive and is based on the appearance of neurologic signs in ruminants that share their pastures with white-tailed deer. Cattle are now known to also succumb to infection with this parasite; at least two cases have been reported (Duncan and Patton, 1998; Mitchell et al, 2011). Six horses had neurologic signs apparently associated with parelaphostrongylosis, and worms were seen in the nerve tissue of two of these animals (Biervliet et al, 2004). A 6-month-old colt from New York State developed severe encephalitis, was humanely killed, and was found to be infected with a worm that was consistent in morphology with *P. tenuis* (Tanabe et al, 2007). A male *P. tenuis* was removed from the anterior chamber of the eye of a horse in Wisconsin (Reinstein et al, 2010).

Family Crenosomatidae

IDENTIFICATION. Crenosomatids have well-developed bursae with a large dorsal ray, the uterus is amphidelphic with a prominent ovijectoral sphincter, and the cuticle is thrown into crenated folds, especially anteriorly (Figure 4-107). *Crenosoma vulpis* is less than

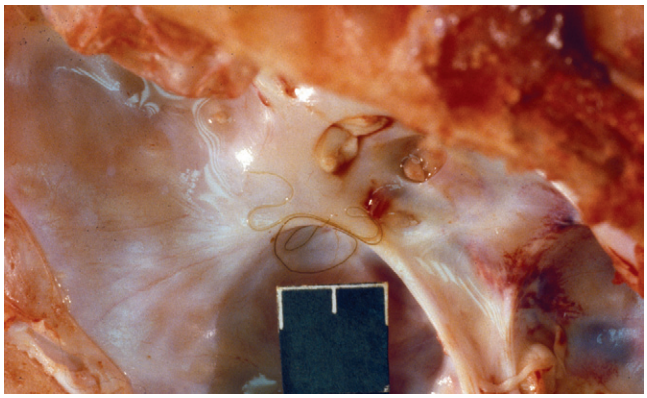


FIGURE 4-106. Adult *Parelaphostrongylus tenuis* in the brain cavity of a deer. The end of the scale is 2 cm across.

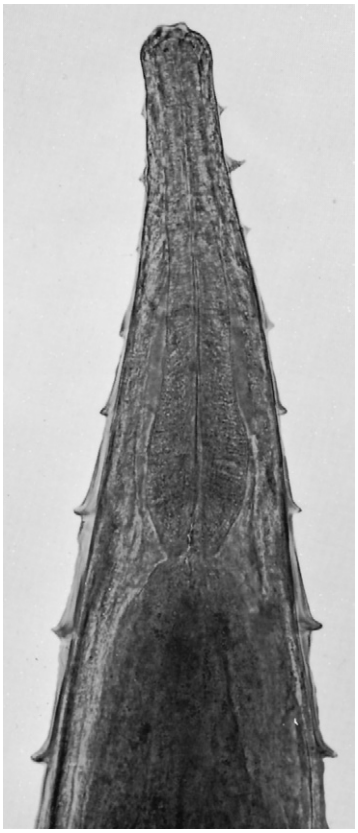


FIGURE 4-107. *Crenosoma* sp. from the lung of a bear.

16 mm long and is found in the bronchi and bronchioles of foxes *(Vulpes vulpes),* wolves *(Canis lupus),* raccoons *(Procyon lotor),* and dogs. *Troglostrongylus* species are parasites of Felidae, and cases of *Troglostrongylus brevior* and *Troglostrongylus subcrenatus* from domestic cats in Spain and Italy have been reported (Jefferies et al, 2010; Brianti et al, 2012).

LIFE HISTORY. In *C. vulpis,* the ovoviviparous females deposit first-stage larvae or thin-shelled eggs containing first-stage larvae. These ascend the trachea and descend the alimentary tract to exit in the host's feces (see Figure 7-26) and develop into infective third-stage larvae in snails and slugs. The definitive host becomes infected by ingesting infected mollusks; the prepatent period is 19 days (Wetzel, 1940a). In the case of *T. brevior,* Gerichter (1949) showed that the larvae also developed in snails, that mice fed larvae from snails could serve as paratenic hosts, and that the prepatent period in an experimentally infected cat was 28 days.

TREATMENT. Fenbendazole (50 mg/kg daily for 3 days) was apparently successful in curing an infection of *C. vulpis* in a Labrador retriever (Peterson et al, 1993). A survey of 55 afebrile dogs with chronic cough in Prince Edward Island, Canada, revealed that 15 (27.3%) were infected with *C. vulpis* (Bihr and Conboy, 1999). The dogs were successfully treated with a course of fenbendazole therapy (50 mg/kg daily for 3 to 7 days). A single treatment with milbemycin oxime cleared 32 naturally infected dogs of their *C. vulpis* infection (Conboy, 2004). Treatment of eight experimentally infected dogs with patent *C. vulpis* infection using topical moxidectin (2.5 mg/kg) and imidacloprid (10 mg/kg) was 100% efficacious compared with treatment in eight control dogs that had 58 to 87 worms in their lungs (Conboy et al, 2009).

The high percentage of dogs positive for *C. vulpis* in Canada with the only sign being chronic cough indicates the need to carefully consider this worm in the differential for such a sign in regions where this worm is prevalent. As foxes become more and more abundant in North America because of reduced hunting, it is expected that this infection will become more prevalent.

Crenosoma species require a molluskan intermediate host. Control depends on preventing the dog's access to these intermediate hosts.

Family Angiostrongylidae

The angiostrongylid bursa may be somewhat reduced, but the rays conform to the typical strongylid pattern and are well defined. The vulva is near the anus, and the uterus is prodelphic. *Aelurostrongylus abstrusus* is a parasite of the lung parenchyma in cats, *Gurltia paralysans* is a parasite of the leptomeningeal veins in South American cats, and *Angiostrongylus vasorum* is a widely distributed parasite of the pulmonary arterial tree of foxes and dogs in western Europe. Recently this worm was found for the first time to occur in dogs in North America in Newfoundland, Canada (Conboy, Whitney, and Ralhan, 1998). *Angiostrongylus cantonensis* is found in the pulmonary arteries of rats, whereas *Angiostrongylus costaricensis* is found in the mesenteric arteries of rodents. Both *A. cantonensis* and *A. costaricensis* can also cause disease in other mammalian hosts such as dogs and primates, including humans. Some place the species *A. cantonensis* and *A. costaricensis* in the genus *Parastrongylus*.

AELUROSTRONGYLUS ABSTRUSUS

LIFE HISTORY. The oviparous female of *A. abstrusus* deposits unsegmented eggs in "nests" in the lung parenchyma (see Figure 8-87). These appear as small, grayish white subpleural nodules. It is hard to tease intact worms out of tissues, but the males have fairly stout spicules (Figure 4-108). In histologic sections or squash preparations of such nodules, all degrees of development from one-celled eggs to hatched first-stage larvae are in evidence. The first-stage larvae are carried up the tracheobronchial tree and swallowed, appearing later in the cat's feces (Figure 4-109). These larvae are very active and can be readily demonstrated by the Baermann technique, which was found to detect 18 of 20 cases of infection with this parasite (Willard et al, 1988); the larvae can also be found using centrifugal zinc sulfate flotation. Further development occurs only if these first-stage larvae enter any of a wide variety of snails and slugs (Blaisdell, 1952; Hobmaier and Hobmaier, 1935). Two molts without cuticle shedding occur in the mollusk's foot tissues, so that the infective larvae, which develop in 2 to 5 weeks, are enclosed in two sheaths. Cats may be infected experimentally by being fed snails containing third-stage larvae, but the natural mode of infection is probably predation of paratenic hosts that normally

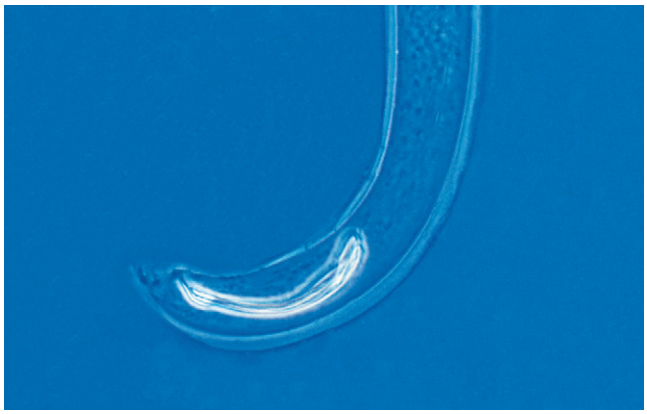


FIGURE 4-108. *Aelurostrongylus abstrusus,* posterior end of a male tail showing the spicules.

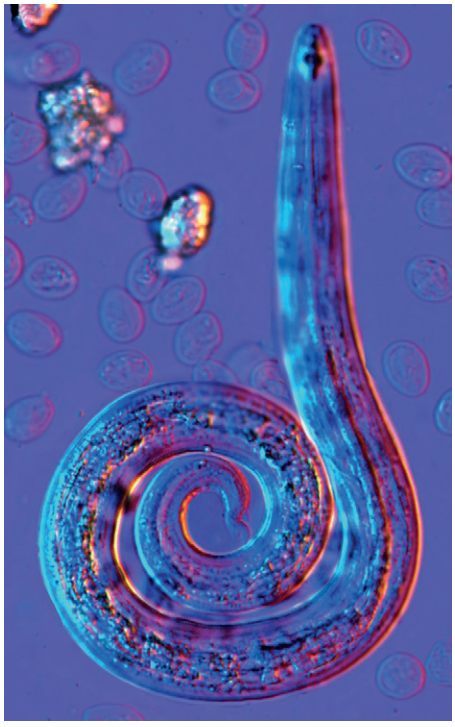


FIGURE 4-109. A living larva of *Aelurostrongylus abstrusus* collected using zinc sulfate centrifugal flotation; the image also includes a number of cysts of *Giardia*.

eat snails. Mice and possibly birds may serve as paratenic hosts. The third-stage larvae merely encyst in their tissues and undergo no further development until they are ingested by a cat. Larvae appear in the cat's feces 5 to 6 weeks after infection.

Infection with *A. abstrusus* usually involves individual rural cats that like to hunt. Control consists of preventing the cat's access to infected intermediate hosts. Unfortunately, we cannot specify what these might be, except for a wide range of snails and slugs that few cats would deign to eat anyway. Probably cats get their *A. abstrusus* infective larvae from paratenic hosts such as mice and voles, but our knowledge of the epidemiology of *A. abstrusus* and other carnivoran metastrongyloids is incomplete.

IMPORTANCE. With use of the fairly insensitive methods of centrifugal zinc sulfate and sugar flotation, 6.2% of 1322 cat fecals from shelters in upstate New York were found positive for *A. abstrusus* (Lucio-Forster and Bowman, 2011). Although many cats with *A. abstrusus* infection are free of clinical signs, coughing and anorexia may be associated with moderate infection. Severe infections are manifested by cough, dyspnea, and polypnea, all of which may terminate fatally (Blaisdell, 1952). In cats from New York State that died of anesthetic-associated death while part of a spay-neuter program, 9% were found to be infected with *A. abstrusus* at necropsy (Gerdin et al, 2011). A cat with severe aelurostrongylosis in the Netherlands had marked pulmonary hypertension that resolved after the infection was successfully treated with milbemycin oxime (Dirven et al, 2012).

TREATMENT. Kirkpatrick and Megella (1987) successfully treated a case of *A. abstrusus* infection with a single parenteral dose of ivermectin (0.4 mg/kg); however, of two cats in Turkey treated with this regimen, only one cleared (Burgu and Samehmetoglu, 2004), and in another case this treatment was not efficacious (Grandi et al, 2005). In Germany, two treatments of an infected cat with topical selamectin (6 mg/kg) a month apart resulted in successful resolution (Reinhardt et al, 2004), but the same treatment failed in two out of three cats in Italy (Grandi et al, 2005). Fenbendazole (50 mg/kg daily for 3 days) has proved efficacious in the treatment of a cat infected with *A. abstrusus* (Schmid and Düwel, 1980) and in four of four cats treated at 50 mg/kg daily for 15 days (Grandi et al, 2005). Prednisone (1 mg/kg orally twice a day for 5 days) is thought to perhaps alleviate some of the clinical signs during recovery. In naturally infected cats treated with moxidectin (1 mg/kg) in a 1% moxidectin/10% imidacloprid formulation, or with fenbendazole at 50 mg/kg for 3 days, the percentage of efficacy was based on posttreatment fecal larval counts of 100% for moxidectin and 99.3% for fenbendazole (Traversa et al, 2009a). In a similarly designed study with 12 cats per group comparing topical emodepside 3 mg/kg (in a formulation of 2.1% emodepside and 8.6% praziquantel) versus 50 mg fenbendazole orally for 3 days, the resulting efficacies were 99.4% and 99.3%, respectively (Traversa et al, 2009b).

ANGIOSTRONGYLUS VASORUM

LIFE HISTORY. First-stage larvae shed in the feces of infected dogs resemble those of *A. abstrusus*. These larvae invade a wide range of molluskan intermediate hosts and develop to the infective third stage, but the practical epidemiology of canine angiostrongylosis has yet to be worked out in detail. After ingesting the mollusk, the larvae migrate to the visceral lymph nodes, where they molt to the adult stage before making their way into the lungs and the pulmonary arteries, where they mature and live (see Figure 8-88). The prepatent period is around 7 weeks.

IMPORTANCE. The parasite has made its way from Europe into the Atlantic coastal provinces of Canada. Previously, cases were present in imported dogs, as in a fatal case reported in a greyhound from Ireland, with extensive pulmonary thrombosis and interference with clotting leading to multiple subcutaneous hemorrhages (Williams et al, 1985). In a survey of dogs from coastal Canada, 202 dogs from the provinces of New Brunswick, Newfoundland, Nova Scotia, and Prince Edward Island were examined; *A. vasorum* was found in only 16 of 67 dogs from the Avalon peninsula of Newfoundland (Conboy, 2004). This parasite has also been found in a coyote from the Avalon peninsula (Bourque, Whitney, and Conboy, 2005). Besides causing lung disease from the deposition of eggs and larvae into the lungs, these infections induce clotting disorders that can manifest, as in the imported greyhound, with subcutaneous hemorrhages or with more deadly intracranial hemorrhage (Garosi et al, 2005).

TREATMENT. The 16 dogs that were diagnosed as naturally infected in Newfoundland were treated with four weekly oral doses of 0.5 mg of milbemycin oxime per kilogram. In 14 of the dogs,

the clinical signs resolved and shedding of larvae ceased, whereas one dog with severe signs died during the course of treatment; one dog was reported to have improved clinical signs, but a posttreatment fecal sample was not available (Conboy, 2004). Fifty naturally infected dogs in Denmark were treated with a single topical application of 0.1 mL of imidacloprid 10%/moxidectin 2.5% per kilogram of body weight (27 dogs), or with 25 mg of oral fenbendazole per kilogram of body weight for 20 days (23 dogs), and 85.2% and 91.3% of the dogs, respectively, were cleared of larvae in the feces (Willesen et al, 2007). *A. vasorum* has also been treated with ivermectin at 0.2 mg/kg (Martins et al, 1993; Migaud, Marty, and Chartier, 1992), fenbendazole at 20 mg/kg twice daily for 2 or 3 weeks (Migaud, Marty, and Chartier, 1992; Patteson et al, 1993), or levamisole at 7.5 mg/kg for 2 consecutive days, followed by 10 mg/kg for 2 days; if the infection does not clear, each of the individual regimens is repeated (Bolt et al, 1994).

ANGIOSTRONGYLUS CANTONENSIS

LIFE HISTORY. First-stage larvae are shed in the feces of infected rats and invade molluskan intermediate hosts, where they develop to the infective stage. When the mollusk is eaten by a rat, the liberated third-stage larvae make their way to the brain of the rat, where they molt and grow to young adults that are about 1 cm long. They then enter a vein and are carried to the heart and pulmonary arteries, where they mature and mate, and the female lays eggs that embryonate and hatch. Paratenic hosts involved include crustacea and amphibians.

IMPORTANCE. If people, dogs, or other mammals ingest snails or paratenic hosts, the worms may undergo their migration into the brain, causing eosinophilic meningitis and encephalomyelitis. During the past few decades, this worm has spread across the Pacific with one of its intermediate hosts, the giant African snail, *Achatina fulica*. Infection is typically acquired by eating infected raw snails or slugs or freshwater prawns, which serve as paratenic hosts (Alicata, 1988). In a series of 55 natural cases of canine neural angiostrongylosis from Brisbane, Australia, infection was characterized by ascending paresis involving the tail and urinary bladder and lumbar hyperalgesia. Three grades of clinical illness were characterized. Grade 1 consisted of caudal paresis and ataxia of one or both pelvic limbs and pain on deep pressure over the lumbar muscles. Grade 2 began as grade 1, but posterior paresis and inability to stand unaided developed quickly. Manual expression of urine was necessary. Dogs with both grade 1 and grade 2 illness responded satisfactorily to nursing care and immunosuppressive corticosteroid therapy. However, when the anthelmintics levamisole and mebendazole were administered in grade 1 and grade 2 cases, either alone or in combination with corticosteroids, a death rate of 75% ensued. Clearly, anthelmintic medication is contraindicated in canine neural angiostrongylosis. Grade 3 illness was characterized by rapidly developing ascending paralysis and extreme hyperalgesia. The prognosis was very poor, and all seven dogs were euthanized (Mason, 1987).

In 1986 and 1987, rats in New Orleans, Louisiana, were found to be infected with *A. cantonensis* (Campbell and Little, 1988). A few years later, a howler monkey in the New Orleans zoo had fatal cerebral disease and was ultimately diagnosed as having been infected with this worm (Gardiner et al, 1990). In 1995 a nonfatal case was reported from New Orleans in an 11-year-old boy who ate a snail on a dare (New, Little, and Cross, 1995). In 1996 a miniature horse in Baton Rouge, Louisiana, had meningoencephalitis and was euthanized (Costa et al, 2000). At necropsy, the horse was found to be infected with *A. cantonensis*. As of 1997, around one quarter of rats, *Rattus norvegicus,* examined in Baton Rouge were found to be infected with this parasite. The worm has been found in additional animals in Louisiana—a lemur, a wood rat, and opossums (Kim et al, 2002)—and it has killed a white-handed gibbon in a zoo in Miami (Duffy et al, 2004). Most recently, human cases of *A. cantonensis* have been reported in Hawaii, with 24 cases reported on the islands of Hawaii, Oahu, Maui, and Lanai (Hochberg et al, 2007, 2011). What is most surprising is that no reports have described cases in dogs in Hawaii; because of their gustatory habits, dogs would seem likely to have eaten some infected snails and developed neurologic disease, especially considering the numerous reports of angiostrongylosis in dogs in Australia (and a few cases in people).

TREATMENT. Treatment seems to be mainly supportive, with immunosuppression to prevent reaction to the migrating worms. In Australia, an ELISA has been developed for detection of the infection; this allows diagnosis to take place before therapy is begun (Lunn et al, 2003).

ANGIOSTRONGYLUS COSTARICENSIS.

A. costaricensis is a parasite of rodents in Central and South America, where adult worms live in the mesenteric arteries; it was reported once from cotton rats in Texas (Ubelaker and Hall, 1979). Eggs are laid by the females, and first-stage larvae occur in the feces of the rodents. Snails are the intermediate hosts. People have become infected with this worm on ingestion of snails; they develop pain in the lower right abdomen, fever, and often vomiting. It has been reported that infection with this worm caused the death of two Ma's night monkeys *(Aotus nancymaae)* and the surgical resection of a portion of the intestine of a siamang *(Hylobates syndactylus)* housed in zoos in Florida (Miller et al, 2006). Raccoons and opossums trapped around the zoos were also found to be infected.

Family Filaroididae

The Filaroididae family of worms differs from the others within the superfamily Metastrongyloidea in that these worms are without a bursa. (Do not confuse the Filaroididae family of Metastrongyles with the very distantly related superfamily Filarioidea, the superfamily that contains the mosquito-transmitted canine heartworm, *Dirofilaria immitis*.) There are species within the Filaroididae in a number of carnivores that use snail intermediate hosts (*Filaroides martis* and *Filaroides rostratus*). There is also a species, *Filaroides decorus,* in the lungs of the California sea lion that uses fish as the intermediate host. The two best-known species in veterinary medicine are canine parasites that have direct life cycles (i.e., *F. osleri* and *F. hirthi;* Figure 4-110). Some place the species *F. osleri* and *F. rostratus* in the genus *Oslerus;* although accepted by some and possibly correct, this placement is not accepted by all.

IDENTIFICATION. The bursal lobes are reduced to mere papillae (see Figure 4-71). The spicules are short and arcuate, the vulva is preanal, the uterus is prodelphic, and the body cuticle is inflated to form a diaphanous teguminal sheath. *F. osleri* occurs in nodules within the epithelium of the trachea and bronchi. *F. hirthi* lives threaded through the lung parenchyma.

FILAROIDES OSLERI

LIFE HISTORY. Adult *F. osleri* occur in nodules in the trachea and bronchi of dogs and in certain wild canids such as the Australian dingo (Figure 4-111; see also Figures 8-91 and 8-92). The females deposit delicate, thin-shelled eggs containing first-stage larvae that hatch before they are voided in the host's feces (see Figure 7-26). The first-stage larvae are directly infective, and development through all five stages is completed in the lung tissue of the dog. Infection is acquired through ingestion of regurgitated stomach contents, lung tissue, or feces of infected dogs. John Dorrington, a South African veterinary practitioner, was the first to succeed in transmitting *F. osleri* infection to dogs by feeding

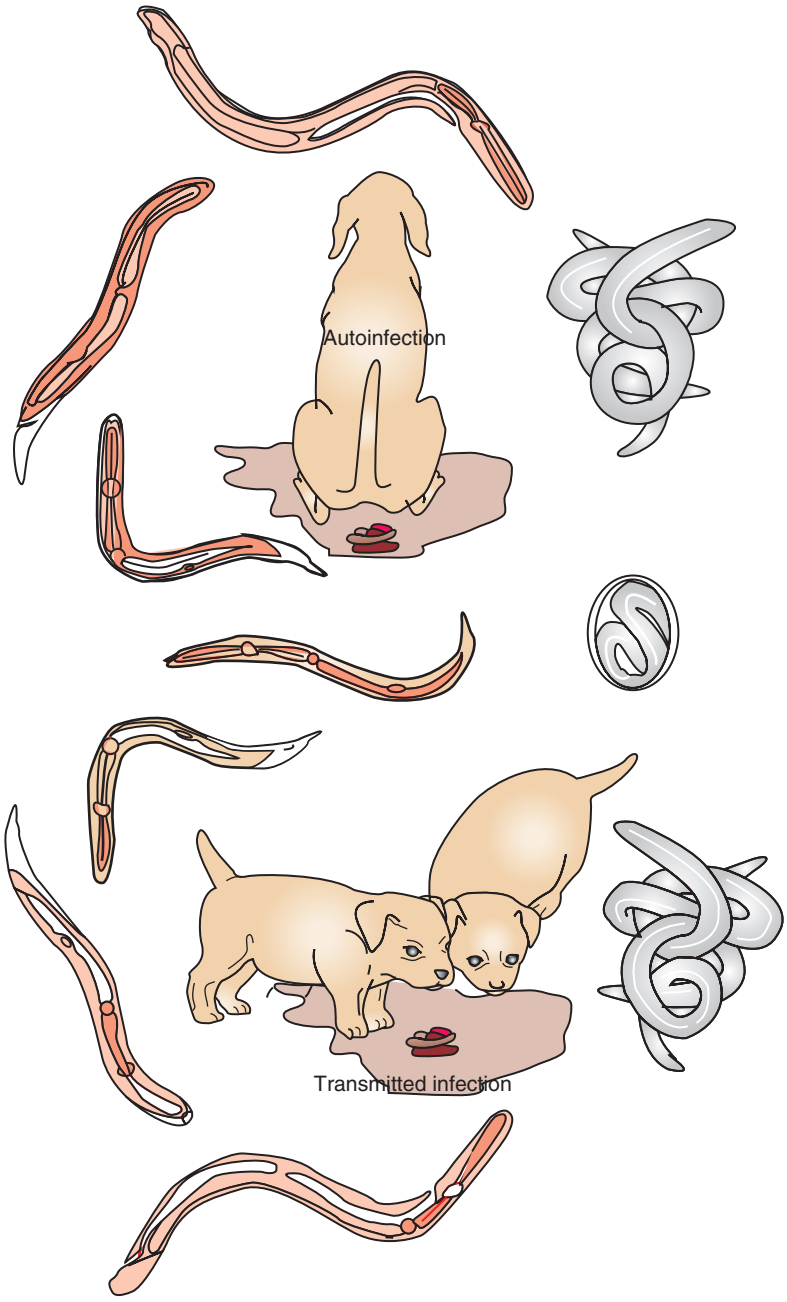


FIGURE 4-110. Life history of *Filaroides hirthi*. The female worm in the lung parenchyma of the dog lays eggs containing infective first-stage larvae. Because these larvae are released within the host, autoinfection is inevitable and the degree of resulting infection is apparently governed solely by the host's immune reactions. First-stage larvae pass up the trachea and out with the feces, and transmission of *F. hirthi* infection occurs principally by coprophagy. Cannibalism and regurgitative feeding serve as other mechanisms.

them first-stage larvae obtained from female worms (Dorrington, 1968). It has been postulated that transmission of *F. osleri* occurs directly from bitches to their pups by salivary contamination during licking (Dorrington, 1968), and from parent dingoes to their pups during the period of regurgitative feeding (Dunsmore and Spratt, 1976).

F. osleri infection develops slowly. Nodule formation can be detected with the bronchoscope at about 2 months, and larvae can first be demonstrated in the feces by zinc sulfate flotation at 6 to 7 months after experimental feeding of larvae.

IMPORTANCE. Milks (1916) summarized the clinical signs manifested in his three cases of *F. osleri* infection as follows:

> The only common symptom … was the spasmodic attack of a hard, dry cough which could be started by exercise or exposure to cold air. These attacks could not be started by pressure upon the larynx as in most cases of bronchitis. The dogs would cough several times and finally retch after which the attack would usually cease … the disease runs a very chronic course and does not materially interfere with the health of the animal until the knots become so numerous as to seriously obstruct the air passages.

F. osleri displays rather low prevalence in spite of its worldwide distribution. It tends to become entrenched in breeding stock and resists all efforts to expel it. Public knowledge that *F. osleri* is present in a kennel can destroy the kennel's reputation.

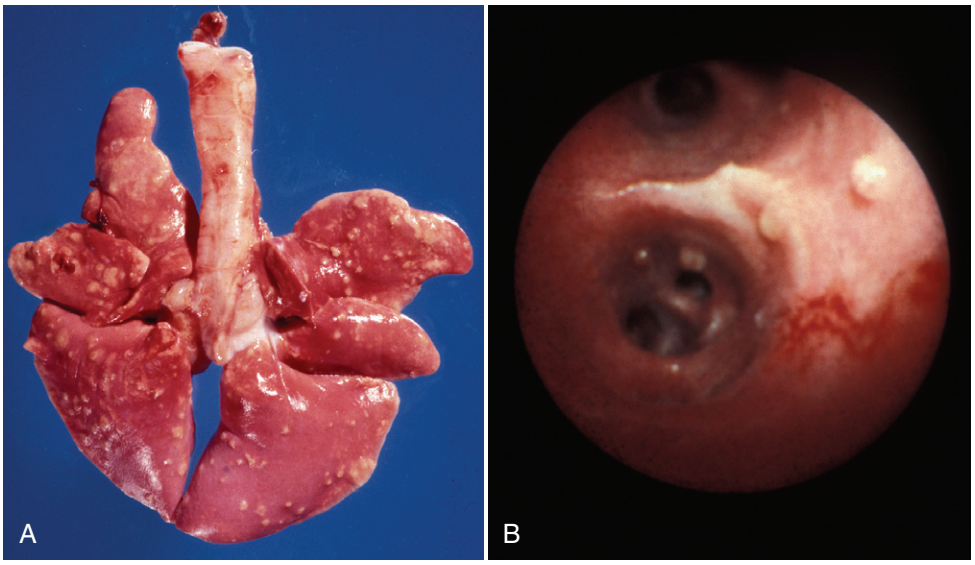


FIGURE 4-111. Lesions of *Filaroides* species. **A,** Lung of a dog with *Filaroides hirthi* infection. Foci of inflammatory reaction to dead and dying worms are scattered over the lungs. Live *F. hirthi* worms excite little if any tissue reaction and, because they are so very small, are scarcely visible to the unaided eye. **B,** Early *Filaroides osleri* nodules near the tracheal bifurcation of a dog photographed through a fiberoptic endoscope. (Courtesy Dr. James Zimmer.)

TREATMENT AND CONTROL. Criteria for successful chemotherapy of *F. osleri* infection include (1) disappearance of cough and air hunger on exercise, (2) resolution of tracheal and bronchial nodules as demonstrated by bronchoscopy, and (3) cessation of fecal larval output. These criteria have rarely been satisfied, and authors disagree as to the efficacy of various treatments. Some treatments that have been used include fenbendazole, ivermectin, and doramectin. Fenbendazole (50 mg/kg daily for 7 days) was reported to stop coughing in a dog with an *F. osleri* infection (Lamb, 1992). Also, ivermectin has been reported to clear dogs of the signs of *F. osleri* infection (Boersema, Baas, and Schaeffer, 1989; Valet-Picavet, 1991). Two reports from India have described individual dogs successfully treated with a single injection of doramectin (0.2 mg/kg) (Gahlod, Kolte, and Kurkure, 2002; Jana, 2002).

FILAROIDES HIRTHI

LIFE HISTORY. *F. hirthi,* similar to *F. osleri,* is infective in the first larval stage and requires no period of development outside the host (Georgi, 1979a; see Figure 4-110). Transmission has been shown to occur among cagemate puppies through ingestion of first-stage larvae in freshly passed feces, and it has been hypothesized that transmission from brood bitches to their litters occurs by the same mechanism after the fourth or fifth week of the nursing period (Georgi et al, 1979a). First-stage larvae arrive in the lungs as early as 6 hours after oral infection, traveling by way of the hepatic portal circulation, the mesenteric lymphatic drainage, or both. Molts occur at 1, 2, 6, and 9 days in the lung tissue, and larvae can be demonstrated in the feces by zinc sulfate flotation at 32 to 35 days after infection (Georgi, Georgi, and Cleveland, 1977; Georgi et al, 1979b) (see Figure 7-26).

IMPORTANCE. *F. hirthi* is important because the lesions it induces in the lungs of dogs used in toxicologic research interfere with interpretation of experiments (see Figures 4-111, 8-89, and 8-90). In 1973, Hirth and Hottendorf described pathologic changes in commercially reared beagle dogs that were associated with *F. hirthi*. The presence of these minute lungworms in the alveoli and bronchioles evoked a focal granulomatous reaction and other pulmonary changes, including some that resembled drug-induced and neoplastic lesions. Research dogs still appear on occasion with *F. hirthi* in their lungs (Bahnemann and Bauer, 1994; Vajner, Vortel, and Brejcha, 2000).

Usually *F. hirthi* infection is not attended by clinical signs of disease, and antemortem diagnosis is based on demonstration of first-stage larvae in the feces (see Figure 7-26), although very severe infection may be suspected from radiographic changes (Rendano et al, 1979a). However, fatal cases of hyperinfection with this parasite have developed in severely stressed and immune-deficient animals (August et al, 1980; Craig et al, 1978). Massive hyperinfection with *F. hirthi* was observed in two beagle pups experimentally treated with prednisolone at a dosage rate of 4 mg/kg/day for longer than 4 months (Genta and Schad, 1984). Dr. Georgi encountered several other cases of fatal *F. hirthi* hyperinfection in dogs experimentally maintained on corticosteroids for long periods. However, because these occurred in commercial pharmaceutical laboratories observing strict proprietary secrecy, the particulars were unavailable.

TREATMENT AND CONTROL. For treatment of *F. hirthi* infection, albendazole administered orally at a dosage of 25 mg/kg body weight twice daily for 5 days is highly effective (Georgi, Slauson, and Theodorides, 1978). Fenbendazole, 50 mg/kg daily for 2 weeks, did not clear a dog of its *F. hirthi* infection, whereas a single subcutaneous injection of ivermectin (0.05 mg/kg) given at a later time appeared to clear the dog of its infection (Bourdeau and Ehm, 1992). Treatment of 40 dogs with subcutaneously administered ivermectin once at 1 mg/kg or with ivermectin twice at 1 mg/kg a week apart reduced infection with *F. hirthi* by 44.8% and 74.1%, respectively, as revealed by necropsy (Bauer and Bahnemann, 1996). Fecal examination of these treated dogs revealed that only 5% to 10% of the dogs were shedding larvae in their feces, although higher percentages of the dogs still had worms in their lungs.

ORDER RHABDITIDA

The order Rhabditida is a very large group of small nematodes with a rhabditoid or rhabditiform esophagus consisting of corpus, isthmus, and bulb (Figure 4-112). Many species are free-living inhabitants of soil or parasites of lower vertebrate or invertebrate

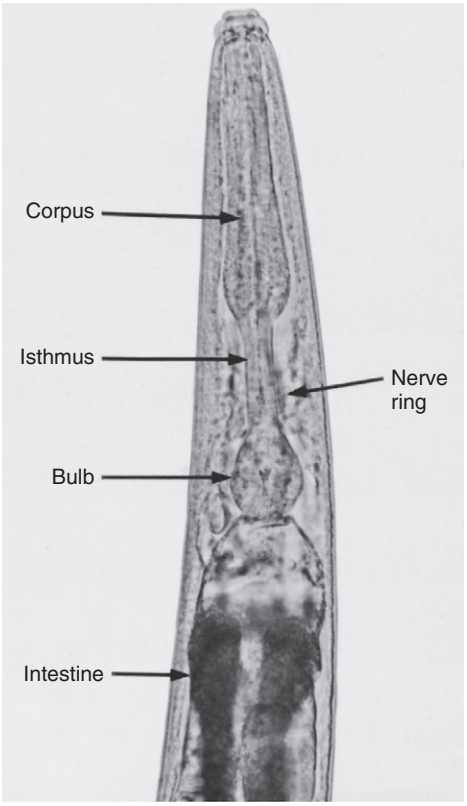


FIGURE 4-112. Anterior end of a *Strongyloides papillosus* free-living adult with a typical rhabditiform esophagus.

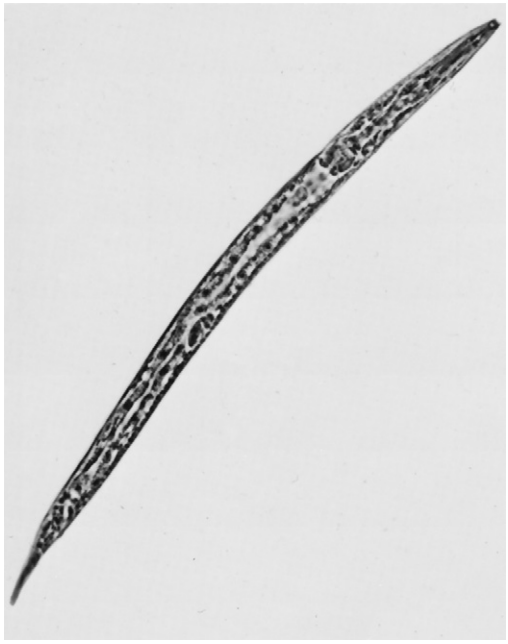


FIGURE 4-113. *Rhabditis strongyloides* rhabditiform larva from a nutrient agar culture. The culture was grown from scrapings of an acute erythematous dermatitis affecting a dog.

animals. The most famous member of this group is the free-living, model genetic organism, *Caenorhabditis elegans*. Only three genera in this order Rhabditida parasitize domestic animals: *Rhabditis* (syn., *Pelodera*), *Halicephalobus* (syn., *Micronema*), and *Strongyloides*.

Rhabditoidea

There is only a single superfamily, the Rhabditoidea, in this order in the CIH classification used in the text. The genera discussed below appear in the families Rhabditidae (genus *Rhabditis*), Panagrolamidae (genus *Halicephalobus*), and Strongyloididae (genus *Strongyloides*). As stated earlier, in the cladistic taxonomy that has been presented (see Figure 4-64), the groups containing *Halicephalobus* and *Strongyloides* appear in Clade IV, while *Rhabditis* appears in Clade V. These nuances are probably way beyond the needs of the clinical veterinary parasitologist, so it is easiest just to call them all Rhabditids or Rhabditoids.

Rhabditis (Pelodera)

Rhabditis (Pelodera) strongyloides is a free-living inhabitant of decaying organic matter that occasionally produces a pruritic, hyperemic dermatitis of cattle, swine, dogs, horses, people, and rodents that have been exposed to an excess of the nematode's normal habitat. Damp straw bedding has been incriminated repeatedly in canine dermatitis caused by this parasite and was associated with larvae-associated lesions in 11 hounds in Finland (Saari and Nikander, 2006). Similarly, damp and dirty straw with high humidity was responsible for the lesions in a large number of heifers on a dairy in Israel (Yeruham and Perl, 2005). Diagnosis is based on finding nematode larvae with a rhabditiform esophagus in skin scrapings or histologic sections (Figure 4-113; see also Figure 8-72); sometimes adults are also present. If *R. strongyloides* larvae are placed on nutrient agar, they will develop into adults in a day or so. These adults are 1 to 2 mm long and will promptly fill the Petri dish with their offspring. Ivermectin was used to treat a number of the hounds in Finland with success.

In cattle, especially in the tropics, a parasitic otitis externa can develop that is caused by a nematode described as *Rhabditis bovis.* Once the infection is established in the ear canal, destruction of the ear epithelium appears to result in ulcerations (Msolla, 1989). These ulcerations predispose the ears to secondary bacterial infection. The cattle have a condition that appears as chronic wasting. On farms of Gyr cattle in Colombia, 63% of the cattle had detectable nematodes (Cardona, Gonzalez, and Alvarez, 2012). Treatment of Gyr cattle with oral albendazole and with topical ivermectin did not significantly reduce ear infection (Verocai et al, 2009a).

Halicephalobus

Halicephalobus gingivalis (syns. *Halicephalobus deletrix* and *Micronema deletrix*) is tiny (250 to 450 by 15 to 20 µm) and has a rhabditoid esophagus and only one egg in its uterus. A male of this species has yet to be reported. The other seven species of *Halicephalobus* apparently are all free-living in soil, manure, or humus, but *H. gingivalis* is a highly pathogenic facultative parasite of horse and man (Anderson, Linder, and Peregrine, 1998; Nadler et al, 2003) (see Figure 8-73). *H. gingivalis* also has been seen in a section of skin from the scrotum of a bull. *H. gingivalis* was first observed in a nasal swelling in a horse (Anderson and Bemrick, 1965) and in the nasal and maxillary sinuses, gums, jaws, kidneys, heart, brain, spinal cord, and meninges in 12 equine cases reported subsequently (Blunden, Khalil, and Webbon, 1987). Cases in horses from around the world keep appearing at a fairly alarming rate; since the last edition of this text, there have been reports of infected horses often with a fatal outcome from Canada, Brazil, Turkey, Austria, the United Kingdom, and France (Ferguson et al, 2008; Ondrejka et al, 2010; Reiser et al, 2011; Hermosilla et al, 2011; Eydal et al, 2012; Deniau et al, 2012). Three fatal human infections with this

nematode have been reported (Gardiner, Koh, and Cardella, 1981). The first reported human case of fatal meningoencephalomyelitis caused by *H. gingivalis* involved a 5-year-old boy who sustained extensive injuries heavily contaminated with manure when he fell into a running manure spreader and passed through its mechanism (Hoogstraten, Connor, and Neafie, 1976).

Strongyloides

Strongyloides is an unusual genus in terms of morphology and life history. (Be careful to avoid confusing the genus name *Strongyloides* with the species name of *R. strongyloides* or with the superfamily name Strongyloidea. Also be warned that the adjective "strongyloid" as used by many authors is more likely to refer to properties of members of the superfamily Strongyloidea than to those of the genus *Strongyloides.* The ubiquitous prefix derives from the Greek word *strongylos,* meaning round and compact, and apparently has great appeal to taxonomists of every stripe. *Strongyl-* has not been restricted to the christening of worms but has been applied to such diverse animals as sponges *[Strongylophora],* bugs *[Strongylodemas],* and fishes *[Strongyliscus],* among others.)

IDENTIFICATION. The tiny **parthenogenetic** parasitic female lies deep in the mucosal crypts of the alimentary tract, particularly the small intestine (see Figure 8-74); parasitic males do not exist. The esophagus of the female is nearly cylindric and is at least one fourth as long as the body (Figure 4-114); the elongate shape of the esophagus is the reason why the female is termed "**filariform**." Other small nematodes in this location include members of the superfamily Trichostrongyloidea, which have a very much shorter esophagus, and species of *Trichinella* and *Capillaria,* both of which have a stichosome esophagus. The embryonated egg, the **rhabditiform** larva (so called because it has the typical corpus, isthmus, and bulbus of the Rhabditida), and the infective **filariform** third-stage larva (with a long esophagus) are the stages most important in diagnostic procedures. Of the significant species of *Strongyloides* in veterinary medicine, only those of dogs (also humans) and cats produce eggs that routinely hatch before leaving the body, such that first-stage larvae rather than embryonated eggs are found in the feces. The free-living adults (see Figures 4-106 and 4-108) frequently develop in cultures of feces from *Strongyloides*-infected animals.

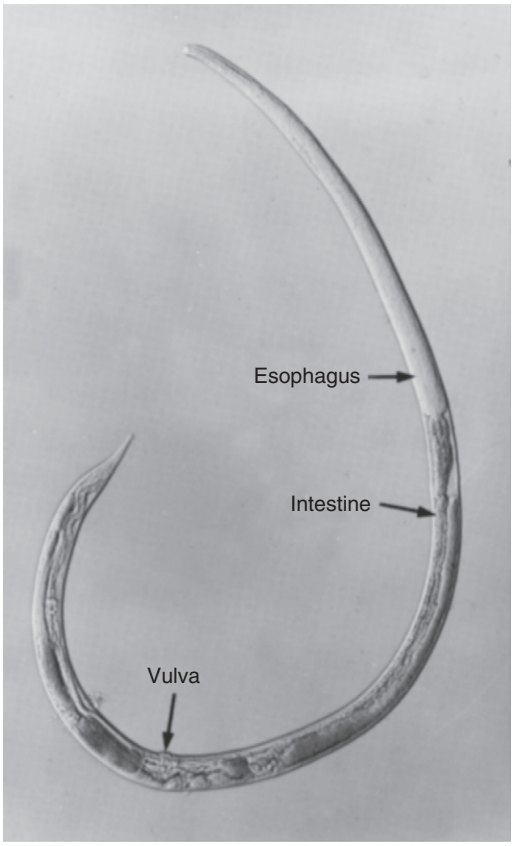


FIGURE 4-114. *Strongyloides stercoralis* parasitic female.

Prominent species of *Strongyloides* parasitizing domestic animals and humans include *S. stercoralis* of humans and dogs; *Strongyloides westeri* of horses; *Strongyloides papillosus* of ruminants (it has been suggested that based on the ability to molecularly separate the forms in sheep and cattle, and on the fact that the cattle form has not been detected in sheep, the cattle form should be considered a separate species—*Strongyloides vituli*) (Eberhardt et al, 2008). Other species include *Strongyloides ransomi* of swine; *Strongyloides fuelleborni* of African and Asian primates and of humans; *Strongyloides cebus* of American primates; and *Strongyloides ratti* and *Strongyloides venezuelensis* of rats. Cats in Australia and India are parasitized by *Strongyloides felis,* and on rare occasions in the southeastern United States, cats are infected with *Strongyloides tumefaciens,* which is probably naturally a parasite of the bobcat and causes fibrotic lesions in the colon. Thus all species of domestic animals have a species of *Strongyloides,* as do many species of wild mammals and birds (Little, 1966a, 1966b).

LIFE HISTORY. The genus *Strongyloides* is unique among parasites of domestic animals in having alternate free-living and parasitic generations. The filariform parasitic female produces eggs by mitotic parthenogenesis, and the larvae from these eggs are termed **homogonic** offspring to distinguish them from the **heterogonic** offspring of the free-living, sexual generation. Homogonic rhabditiform larvae in the external environment may develop through two molts into infective filariform larvae, or through four molts into free-living males and females, in which all stages have rhabditiform esophagi. If the third-stage filariform larva enters a suitable host, usually by penetrating its skin, development proceeds through third and fourth molts to the filariform parasitic female. The free-living rhabditiform males and females mate to produce heterogonic rhabditiform larvae that, with minor exceptions, develop only into infective filariform larvae (Basir, 1950; Triantophyllou and Moncol, 1977). The life history of *Strongyloides* species is portrayed in Figure 4-115.

The major mode of transmission of *Strongyloides* species in mammals appears to be transmammary. This occurs in dogs, horses, pigs, and ruminants. After an initial infection has been established, additional larvae tend to migrate to deeper body tissues, from which they are then passed to offspring in the colostrum and milk; this **transmammary transmission** has important implications for disease induction and control.

IMPORTANCE. *Strongyloides* infections are moderate and asymptomatic in most individuals of all domestic species; when disease does occur, it usually is confined to massively challenged neonates and nurslings. The other exception has been seen in immunocompromised or immunosuppressed animals.

DOGS. *S. stercoralis* infection may be asymptomatic, or it may cause any grade of clinical illness. Serious cases involve signs of bronchopneumonia and severe watery or mucous diarrhea that may easily be confused with the generalized viral diseases of puppyhood. In massive invasions, the lungs of young pups may be sprinkled with petechial and ecchymotic hemorrhages caused by migrating larvae breaking out of the alveolar capillaries. Cases of disseminated strongyloidiasis in dogs often appear after the administration of

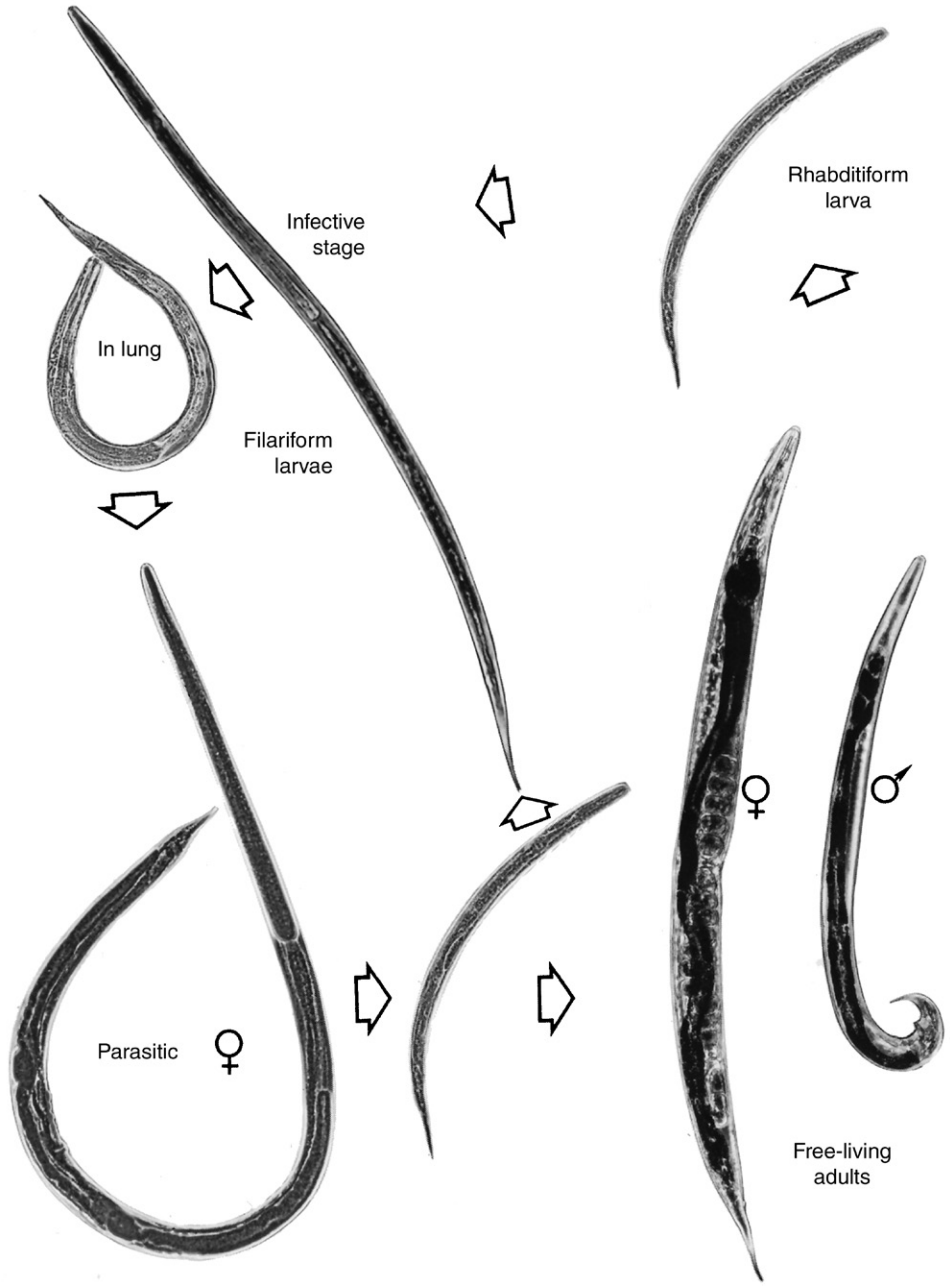


FIGURE 4-115. Life stages of *Strongyloides stercoralis.* Not to the same scale.

corticosteroids, as in a case from Italy (Stancampiano et al, 2011). The prepatent period in a direct infection is about 1 week. *S. stercoralis* routinely appears in kennels, and infection of pups can be life threatening (Dillard, Saari, and Anttila, 2007).

S. stercoralis infection in man is unique in its chronicity (Gill et al, 2004). This infection may persist for decades or for life owing to the development of the infective filariform larval stage within the patient's digestive tract. These infective larvae may reinvade the host by penetrating the bowel wall **(internal autoinfection)** or the perianal skin **(external autoinfection).** Autoinfection accounts for the extreme chronicity of infection and, in part, for the explosive development of massive disseminated infection **(hyperinfection)** that may overwhelm patients with depressed cell-mediated immunity. Hyperinfection with *S. stercoralis* has caused the death of many persons who have immunodeficiency diseases or are undergoing immunosuppressive therapy or immunosuppression for transplantation (Dwork, Jaffe, and Lieberman, 1975); the worm has even been transplanted in the donor organ (Patel, Arvelakis, and Sauter, 2008). Dogs can also develop autoinfection when immunosuppressed (Schad, Hellman, and Muncey, 1984). The ability of *S. stercoralis* to undergo internal autoinfection is probably due in part to the passage of larvae rather than eggs, as occurs with other species of *Strongyloides* parasites in domestic animals.

S. stercoralis is a parasite that is zoonotic, and infections may be shared between dogs and people. Galliard (1951) had no difficulty producing durable infections in dogs by using 19 strains of *S. stercoralis,* 11 obtained from Europeans infected in different regions of French Indochina (Vietnam) and eight from natives of Tonkin, but he found dogs to be quite refractory to strains imported from the West Indies and Africa. The epidemiologic role of the dog in human *S. stercoralis* infection has actually been documented by only one report of natural transmission from dog to man (Georgi and

Sprinkle, 1974). A survey of kenneled dogs and kennel workers in Brazil found some dogs infected but none of the workers infected, although some workers were serologically positive for antibodies to *S. stercoralis* on ELISA (Gonçalves et al, 2007). Another epidemiologic examination of potential cross-infection on the Amami Islands of Japan found that 2.8% of 660 people and 10% of 55 dogs were infected with *S. stercoralis,* but owners who had infected dogs were not infected, and owners who had parasites had dogs free of parasites, suggesting that no cross-transmission was taking place (Takano et al, 2009). Unfortunately, in kennels the infections often go unnoticed, are maintained through transmammary transmission and skin-penetrating larvae, and should always be considered a potential zoonotic agent for kennel employees if a diagnosis of infection in the animals they are caring for is discovered.

HORSES. *S. westeri,* as with other members of the genus, develops rapidly in passed feces to the infective filariform stage, which usually enters the host by penetrating its skin or oral mucous membranes. *S. westeri* eggs are encountered almost exclusively in suckling and weanling foals; the dam of an infected foal sheds no *S. westeri* eggs even though she is the source of infection via the mammary gland (Lyons, Drudge, and Tolliver, 1969, 1973). The foals will begin to shed eggs in their feces at 10 days to 2 weeks after birth. Diarrhea rather frequently afflicts foals between the ninth and thirteenth days of life, thus occurring coincidentally with the first postparturient estrus of the mare. Enigk, Dey-Hazra, and Batke (1974) presented convincing evidence that this so-called foal-heat diarrhea is caused by *S. westeri* and is not related to any alteration in the chemical composition of the mare's milk. Heavy infection in foals persists for 10 weeks; lighter infections may last two or three times as long. Occasionally, very light infections are observed in yearlings and older horses. These may represent percutaneous infection in hosts that were not exposed as sucklings (Enigk, Dey-Hazra, and Batke, 1974). Fortunately, the use of ivermectin has probably markedly reduced the presence of *S. westeri* on many farms; a recent survey in Kentucky thoroughbred foals found a prevalence of only 1.5%, whereas decades ago in the same area, the prevalence was greater than 90% (Lyons and Tolliver, 2004). However, in Brazil, six foals were weaned at 4 months of age, dosed with ivermectin, and placed on a hectare paddock with sparse paster; 15 days later, five of the foals showed diarrhea and weight loss and were again treated with ivermectin, along with IV fluids and antibiotics (Lucena, Fighera, Barros, 2012). Massive numbers of *S. westeri* eggs were seen in the feces, three of the foals died, and a fourth was euthanatized; at necropsy, severe damage to the duodenal mucosa was noted, along with massive numbers of worms present in the intestinal mucosa.

RUMINANTS. *S. papillosus* has long been considered to behave typically as a commensal or at the least to cause significant disease only when present in very large numbers. In a recent report on a series of studies performed in the late 1960s and early 1970s, it was shown that even relatively light infection with this parasite can cause severe disease in goats (Pienaar et al, 1999). In these studies of 89 goats infected with various dosing regimens, some kids died after three infections with as few as 2000 to 5000 larvae per exposure. The most susceptible age group consisted of kids 6 weeks to 6 months of age, although older goats, 6 to 12 months of age, also succumbed. Death typically occurred within 9 to 30 days of receiving 75,000 larvae. Clinical signs included dehydration, inappetence, emaciation, weakness, cachexia, diarrhea, anemia, respiratory distress, and abnormal stools. Fever was not seen in any of the animals. Nervous signs were exhibited from day 43 after exposure onward, and about 22% of the goats that died had histopathologic lesions in the brain and spinal cord. Sudden death from hepatic rupture occurred in 6% of the goats. In a different set of studies (Nakamura et al, 1994), it was shown that inoculation of live parthenogenetic females into the duodenum of susceptible lambs produced continuous sinus tachycardia immediately after inoculation, and the result was death due to cardiac arrest. Thus the effects of adults in one study and of numerous lesions seen in varied tissues of goats in the other set of studies would suggest that *S. papillosus* may be more pathogenic than was previously considered.

PIGS. The *S. ransomi* female lies deeply embedded in the mucous membrane of the small intestine. The larvae in eggs shed in feces develop into infective third-stage filariform larvae in 2 or 3 days that infect the next host via penetration of the skin or oral mucosae. They may follow a tracheal migration route to maturation in about 6 days or a somatic migration route to accumulate as arrested larvae in adipose tissues, especially those of the mammary area. Tracheal migration and maturation are the usual outcome in piglets and occur to some extent in older pigs. Mature gilts tend instead to store *S. ransomi* larvae in their adipose tissues and to shed them later in the colostrum and in milk. Third-stage larvae in the colostrum and milk are said to be "advanced" as compared with the third-stage larvae that originally infected the gilt because they are slightly larger; their genital primordia are longer, wider, and more conspicuous; and they mature in suckling pigs in only 2 to 4 days instead of 6 days. Transmammary infection is the key to the epidemiology of *S. ransomi* infection. Piglets separated at birth from their dam and reared artificially were free of *S. ransomi* infection, whereas piglets allowed to nurse began to shed eggs in their feces 2 to 4 days after birth (Moncol and Batte, 1966). This initial transmammary infection thus serves to contaminate the environment of the sow and litter, thereby augmenting the mature worm burdens of the piglets and rebuilding the sow's tissue store of arrested larvae for subsequent litters (Moncol, 1975).

Strongyloidosis of piglets is an acute enteritis with bloody diarrhea (dysentery), rapid emaciation, anorexia, anemia, and stunting. Death losses may occur, but from an economic standpoint, these may be economically less significant than the retarded growth of survivors.

TREATMENT. Ivermectin seems to be the treatment of choice for almost all species of *Strongyloides,* including those affecting dogs and humans (Lindo et al, 1996; Mansfield and Schad, 1992); in people it is marketed as Stromectol (tablets containing 3 mg ivermectin). In dogs with experimental infection of *S. stercoralis,* treatment with ivermectin at 0.8 mg/kg body weight failed to remove larvae from the tissues (Mansfield and Schad, 1992). *S. ransomi, S. papillosis,* and *S. westeri* are also treated with ivermectin (in some cases, other avermectins are also so labeled). It has been shown that treatment of mares at foaling with ivermectin can prevent infection of suckling foals (Ludwig et al, 1983), but the report (described previously) of dying foals with massive numbers of *S. westeri* after two ivermectin treatments is worrisome. *S. westeri* in horses can also be treated with oxibendazole (15 mg/kg). *Strongyloides* in pigs can be treated with levamisole and doramectin.

ORDER OXYURIDA

Although the order Oxyurida is named for *Oxyuris equi,* the common and unusually large pinworm of the horse, most pinworms are very much smaller than *O. equi*. The oxyurid esophagus has a more or less spheric bulb immediately anterior to its junction with the intestine; this bulb often has a valve in its lumen (Figure 4-116). One or both sexes have a long, tapering tail, and it is for this that they are called *pinworms*. All oxyurids are highly host-specific parasites of the large intestine.

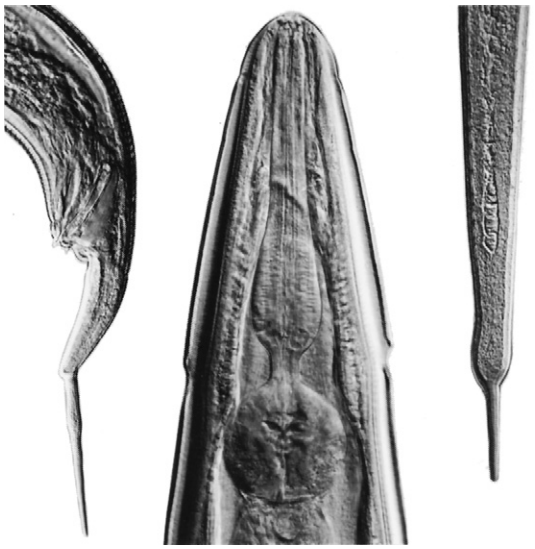


FIGURE 4-116. *Passalurus ambiguus* (a pinworm of the rabbit). Tail of male *(left)*, stomal end *(center)*, and tail of female *(right)*.

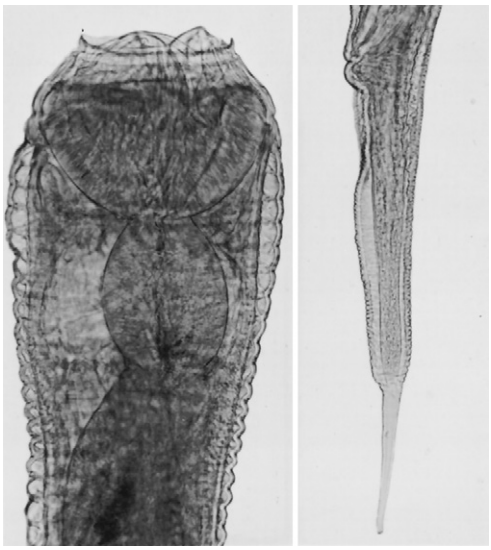


FIGURE 4-118. *Oxyuris equi* fourth-stage larva. *Left,* The anterior end shows the temporary buccal capsule–like modification of the esophageal corpus that permits attachment to the mucous membrane. *Right,* The tail.

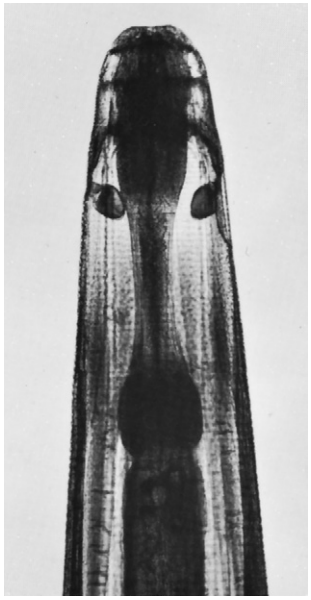


FIGURE 4-117. *Oxyuris equi* anterior end showing the esophageal bulb.

Oxyuris equi

Adult *O. equi* (Figure 4-117; see also Figure 7-82) are found principally in the small colon, although occasional specimens may be found in the large colon. Instead of simply discharging her eggs in the fecal stream, the gravid female *O. equi,* which may measure anywhere from 40 to 150 mm long, migrates down the colon and rectum and out through the anus to cement her eggs in masses to the skin of the anus and its immediate surroundings. These egg masses consist of a tenacious yellowish gray fluid containing 8000 to 60,000 eggs. The eggs develop to the infective stage in 4 or 5 days, during which the cementing fluid dries, cracks, and detaches from the skin in flakes. These flakes, which contain large numbers of infective eggs, adhere to mangers, water buckets, walls, and the like, thus contaminating the environment of the stable. Paper towels or disposable cloths are preferred for cleansing the perineum of horses because any nondisposable object, such as a sponge or a towel, will inevitably become heavily contaminated with *O. equi* eggs. Then when the sponge or the towel is applied to a horse's muzzle after a workout or is used to clean the bit, the future brightens up for *O. equi*. The prepatent period is 5 months.

Severe infection with third- and fourth-stage *O. equi* (Figure 4-118) may produce significant inflammation of the cecal and colonic mucosa, manifested by vague signs of abdominal discomfort. However, the most common affliction perpetrated by *O. equi* on the horse is pruritus ani caused by the adhesive egg masses deposited on the perianal skin by the female worm. In its efforts to relieve the itching, the horse will persistently rub its tail against posts, mangers, and the like, until the tail head becomes disheveled, bare of hair, or even scarified.

TREATMENT. *O. equi* is an easy parasite to control. All of the available equine anthelmintics are highly effective against both immature and adult large pinworms. Ivermectin appears to continue to work very well (Klei et al, 2001). Pinworms also are controlled by the daily administration of pyrantel tartrate. An examination of 14 horses naturally infected with *O. equi* and treated with pyrantel pamoate (7 horses) or ivermectin (7 horses) revealed that both of these products provided efficacy above 90% against adults and greater than 99% against fourth-stage larvae (Reinemeyer et al, 2010).

Probstmayria vivipara

Probstmayria vivipara is a tiny (less than 3 mm long) pinworm parasite of horses (related species occur in the posterior bowel of tapirs, pigs, and Old World primates) that gives birth to infective larvae and therefore is capable of completing its life history within the confines of its host's large intestine (Figure 4-119 and see Figure 7-120).

Skrjabinema

Skrjabinema ovis and *Skrjabinema caprae,* harmless parasites of sheep and goats, respectively, are 8 to 10 mm long. The genus name is pronounced "Skreeyabinema."

Enterobius vermicularis

Enterobius vermicularis is a small (up to 13 mm long) pinworm of humans and great apes that still has an extensive distribution

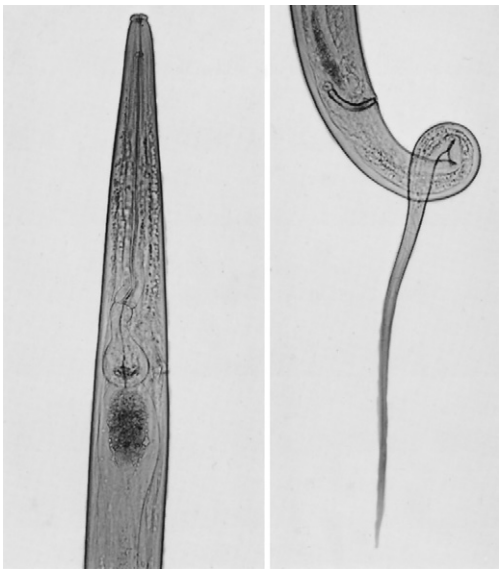


FIGURE 4-119. *Probstmayria vivipara* adult male anterior end *(left)* and tail *(right)*.

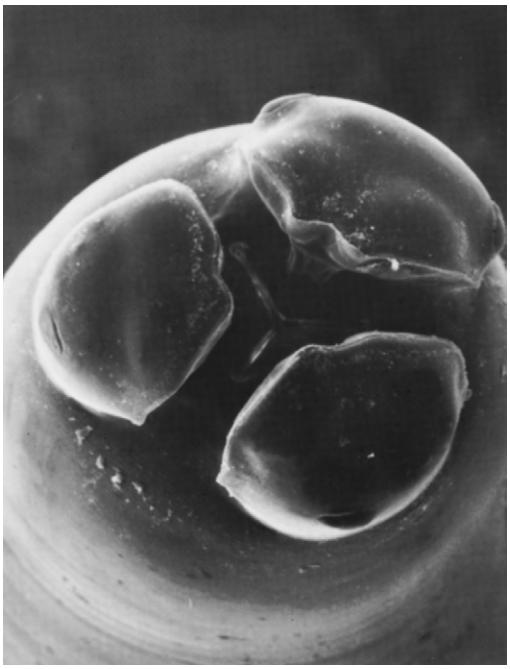


FIGURE 4-120. *Ascaris suum* lips and stoma.

among civilized man despite cooking and washing—the nemeses of many other parasites (see Figure 7-118). Infection rates range up to 40%, depending on age and race. White elementary school children display the greatest intensity and prevalence of infection. The gravid *E. vermicularis* female migrates through the anal opening to cement her eggs to the host's perianal skin. The eggs develop to the infective stage within hours and are ready to reinfect the host by contamination of the hands, to infect other individuals by contamination of bedclothing or other fomites, or to become airborne on dust particles.

Infection may be suspected in children who have pruritus ani and insomnia. Diagnosis is reached by observing the female worm in the act of depositing her eggs on the perianal skin or by demonstrating the eggs. This can best be accomplished by momentarily pressing the adhesive side of a piece of cellophane tape against the anus and then sticking the tape to a slide to prepare it for microscopic examination. Conventional fecal examination techniques almost uniformly fail to demonstrate the eggs of *Enterobius* species and many other pinworms (e.g., *Oxyuris* species). The important practical point for veterinarians is that *E. vermicularis* is a parasite of humans and apes (apes have other species of *Enterobius* as well), but never of dogs or cats. Occasionally a physician prescribes removal or euthanasia of the family pet to help control pinworms. The finest degree of tact is required in dealing with this situation.

Infection of apes with species of *Enterobius* is usually asymptomatic. However, sporadic cases of fatal ulcerative enteritis with extensive invasion of the intestinal submucosa and even of the mesenteric lymph nodes by the adult pinworms have been reported in chimpanzees (Holmes, Kosanke, and White, 1980; Keeling and McClure, 1974; Schmidt and Prine, 1970). Both *Enterobius anthropopitheci,* a natural parasite of apes, and *E. vermicularis* of humans have been implicated.

ORDER ASCARIDIDA

Ascarids are among the largest and most familiar of nematode parasites infecting the intestinal tract of domestic animals. The worms found in domestic animals range from several inches up to 2 feet in length. The mouth is surrounded by three fleshy lips, one dorsal and two subventral (Figure 4-120), and the tail of the male is usually curved ventrally. Some genera have lateral cervical alae that make the anterior end of the worm resemble an arrowhead, thus such generic names as *Toxocara* and *Toxascaris*.

Development to the infective stage differs only in detail for the various ascarid genera. The single cell develops into an infective larva inside the eggshell within several days or weeks, depending on the species of worm and the ambient temperature. Many genera of ascaridoid nematodes parasitize aquatic vertebrates (e.g., fish, crocodilians, birds, sea mammals); these genera typically have free-swimming larval stages initially and various required intermediate hosts. The ascaridoids found in domestic animals have adapted to their terrestrial existence by changing the typical life history pattern. Thus the life cycles of the ascaridoids in domestic animals are direct with or without various migrations within the body of the host or through transplacental or transmammary pathways. Another adaptation to the terrestrial environment has been the development of an eggshell capable of withstanding the extremes of harsh environments. Ascarid eggs are remarkably resistant to chemical and physical insults. The single most important fact to remember in relation to the epidemiology of ascariasis is that the eggs remain infective in soil for many years. Various ascarid genera display remarkable differences in patterns of intrahost development; however, for terrestrial species, almost without exception, it is now accepted by most that part of the adaptation to the terrestrial environment has been the incorporation of two molts within the eggshell, so that the larval stage hatching from the egg of these ascaridoids is a third-stage larva.

Identification

For the purposes of practical identification, adult ascarids are quite host-specific. Thus *Ascaris suum* infects swine, *Parascaris equorum* infects horses, *Toxocara vitulorum* infects cattle, *Toxocara canis* infects dogs, and *Toxocara cati* infects cats. (In Southeast Asia, cats are also host to another species, *Toxocara malaysiensis,* which resembles *T. canis* more than it does *T. cati*.) Dogs and cats share a second ascarid, *Toxascaris leonina,* which must be distinguished from their respective species of *Toxocara* (see Figures 7-24 and 7-51).

FIGURE 4-121. *Ascaris suum,* adult worms collected from naturally infected pigs.

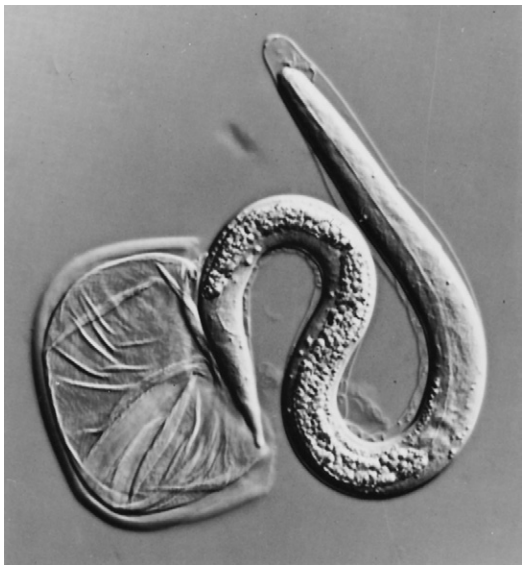


FIGURE 4-122. *Ascaris suum,* mechanically hatched infective larva with retained cuticle of previous stage.

Ascarid eggs are relatively thick walled, contain a single cell when passed in the feces, and are usually sufficiently distinctive to permit identification of the species (see Figures 7-7 to 7-9, 7-25, 7-51, 7-77, and 7-99).

Ascaris

A. suum is a ubiquitous and pathogenic parasite of swine. The adult worms are about 30 cm long and white to cream colored, with three large lips typical of the ascaridoids (Figure 4-121; see also Figure 4-120). Long considered a variety of the morphologically indistinguishable human ascarid *Ascaris lumbricoides, A. suum* is considered a distinct species by most contemporary authors. However, *A. lumbricoides* can mature in swine, and *A. suum* can mature in humans. Typically, however, these two species maintain separate cycles, with the swine species staying in swine and the human species in humans even when both hosts live very close together (Anderson, 1995; Anderson, Romero-Abal, and Jaenike, 1993).

Although the eggs of both species will hatch and their larvae will migrate extensively in a wide range of hosts, the infective egg in polluted soil or stuck to the mammary skin of the sow is the key element in the epidemiology of *A. suum* infection. The infective egg hatches in the stomach and the small intestine (Figure 4-122), releasing the third-stage larva (Geenen, Bresciani, and Boes, 1999), which enters the wall of the cecum and colon and proceeds to the liver, arriving there in a matter of hours by way of the portal vein (Murrell et al, 1997). After tunneling about in the liver for several days, the larva arrives in a pulmonary capillary by way of the caudal vena cava, heart, and pulmonary artery. At this point, the larva may remain in the circulation to be carried to the somatic tissues, or it may lodge temporarily in the pulmonary capillary and then break out into an alveolus. In the case of *A. suum,* the latter course appears to be much more probable because the larva typically will proceed up the bronchial tree and trachea to the pharynx, there to be swallowed, then will arrive once again in the small intestine, where it will mature.

In their migrations through various tissues, ascarid larvae at first inflict only mechanical damage, but hypersensitivity rapidly develops, and allergic inflammation with eosinophilic inflammation characterizes the host reaction to subsequent invasions. In pig livers, the inflammation heals by fibrosis, giving rise to the so-called milk spot lesions (see Figure 7-92) that cause the organ to be condemned by meat inspectors as unfit for human consumption.

The lesions of early migrations in the lungs are likewise mechanical in nature, and once again the initial focal hemorrhages are followed by hyperemia, edema, and eosinophilic infiltration as hypersensitivity develops. In young pigs, extensive lung lesions give rise to severe respiratory embarrassment. Breathing is rapid, shallow, and marked by audible expiratory efforts ("thumps") and coughing; pigs may die. A report from Norway, where 40 pigs purchased for fattening and placed in a room containing highly contaminated litter died or were killed owing to acute respiratory disease related to *A. suum* migration, highlights the continued need for vigilance against this infection (Gjestvang, 2005).

The pathologic effects of adult *A. suum* infection in the small intestine are less dramatic than those of the larval migrations, but they are undoubtedly significant. Diarrhea may occur, but the most important effect is interference with proper nutrition and normal growth. Heavily infected pigs fail to make economically profitable gains. Occasional bizarre accidents such as occlusion of the bile duct or perforation of the bowel wall result from the tendency of ascarids to wander.

Diagnosis of clinical ascarosis frequently depends on clinical and necropsy findings because the main pathologic events occur during the prepatent stage. Clinical signs of severe respiratory distress in a group of growing pigs and the discovery of extensive petechial and ecchymotic pulmonary hemorrhages and edema contribute to a diagnosis of acute ascarosis. Pieces of lung tissue should be minced and placed in a Baermann apparatus for demonstration of the migrating larvae. Less acute cases are marked by respiratory distress, varying degrees of malnutrition, and lesions of interstitial pneumonia. Chronic ascarosis is marked by stunting, emaciation, a copious outpouring of *A. suum* eggs in the feces, and lesions of chronic interstitial pneumonia and hepatic fibrosis. Such pigs are hopeless from an economic point of view.

Anthelmintic Medication

A. suum, which is economically the most important nematode of swine, continues to menace the swine industry despite its susceptibility to hygromycin B, piperazines, dichlorvos, fenbendazole, levamisole, ivermectin, doramectin, and pyrantel tartrate. It is obvious that drugs alone are not successful in controlling this ubiquitous parasite. However, treating and cleaning sows with soap

and warm water 2 weeks before moving them to the farrowing crates will materially reduce the contamination to which the piglets will be exposed. Treating again at weaning with continuing attention to the hygienic conditions of the premises should keep the growing pigs reasonably free of *A. suum.* Continuous provision of feeds containing pyrantel tartrate prevents the migration and establishment of *A. suum*. Pyrantel tartrate is the only approved drug that kills the infective larva immediately after it hatches in the small intestine.

In summary, control efforts should be directed at preventing infection of pigs during the first few weeks of life. Anthelmintic medication of the sow before farrowing, careful sanitation at farrowing time, and avoidance of exposure of young pigs to contaminated soils all serve to limit early infection. A method has been described for moving pigs into a new breeding facility without movement of their parasites (Epe and Blomer, 2001). The described method included using pigs known to have a low level of *A. suum* infection; providing treatment with ivermectin 2 weeks before and on the day of transport in a clean trailer to a disinfection platform, where each pig was washed using a high-pressure sprayer for 10 minutes with tap water and for 10 minutes with a 2% Venno Oxygen wash (a combination of 2-[2-butoxyethoxy]-ethanol, a nonionic surfactant [isotridecanol ethoxylates in an emulsifier, sulfochlorinated paraffin oil]); providing transport in another clean trailer to the facility; and leading through a bath of 2% Neopredisan solution. A total of 1203 fecal samples examined 4, 6, and 10 weeks after transfer were negative for *A. suum*.

Parascaris

P. equorum, the very large ascarid parasite of the horse, can be up to 2 feet long and has large distinctive lips (Figures 4-123 and 4-124). *P. equorum* resembles *A. suum* both epidemiologically and with respect to the route adopted by its larvae in migrating through the tissues. When the infective egg of *P. equorum* is swallowed by a foal, the larva hatches, burrows into the wall of the small intestine, and is carried to the liver by the portal vein. After migrating about in the hepatic tissues, the larva enters a hepatic vein and is carried by the caudal vena cava, heart, and pulmonary artery to the lungs, where it enters an alveolus. After completing a molt in the lungs, the larva ascends in the expectorant mucus of the tracheobronchial tree and returns by way of the lumen of the esophagus and stomach to the intestine, where it completes a final molt and matures.

The first waves of invading larvae inflict mainly mechanical injury, and little more than petechial hemorrhages can be observed. However, as the host becomes sensitized to *Parascaris* antigens, the tissues respond to the presence of larvae with infiltrations of eosinophilic leukocytes and other inflammatory cells. The damage done to the liver and lungs eventually heals, but the chronic reduction in functional capacity suffered during what normally is a period of rapid growth leaves its mark on the yearling. It never will be what it could have been.

The durable infective egg is the key element in the epidemiology of *P. equorum* infection. These eggs accumulate as a growing reservoir of infection in polluted soils, and they adhere by their sticky shell covering to the teats and udder of the brood mare and wait there for the foal to be born.

Heavy infection with adult ascarids causes moderate enteritis and subnormal growth through interference with digestion and absorption of nutrients. Ascaridosis produces a malnourished, undersized, sickly individual with little stamina and reduced resistance to disease: Its haircoat is dull, its skin dry and leathery, and its abdomen too large for its frame. It is not unusual to find a

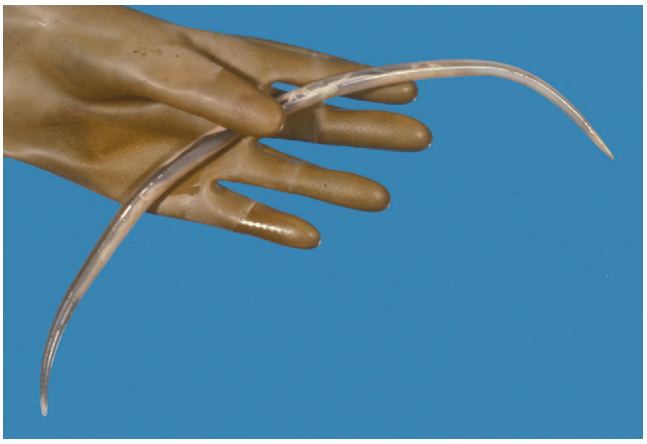


FIGURE 4-123. Adult *Parascaris equorum.*

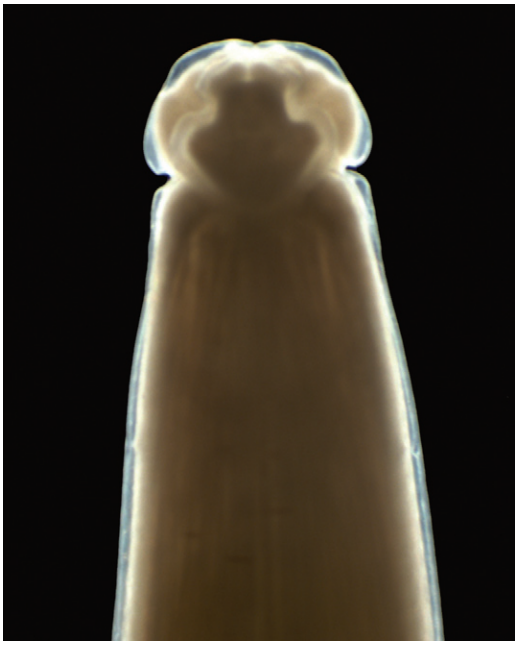


FIGURE 4-124. *Parascaris equorum,* anterior end of adult female showing the shape of the characteristic lips.

half-pail of *P. equorum* in the small intestine of a foal—a sufficient mass of parasites to compete with the host for nutrients. Occasionally, adult *P. equorum* perforate the bowel wall and cause fatal peritonitis. Administration of anthelmintics that tend to paralyze ascarids (e.g., pyrantel pamoate, piperazine, and ivermectin) to a foal with a heavy *P. equorum* burden may occasionally cause impaction or complete obstruction of the bowel (Cribb et al, 2006; Schusser, Kopf, and Prosl, 1988).

Control

The thick eggshell of *P. equorum* protects the egg from temperature extremes and ultraviolet irradiation and makes the egg resistant to desiccation and most chemical disinfectants. The epidemiology of *P. equorum* infection therefore differs considerably from that of strongylids with their free-living infective larvae. Therefore effective stall sanitation for control of ascarids involves weekly removal of all manure and bedding and thorough cleaning of all surfaces with a high-pressure cleaner or steam jenny. Most horsemen find such

a program excessively laborious and rely instead on anthelmintics to suppress production and environmental contamination with the eggs of *P. equorum*. However, because of the extraordinary longevity and hardihood of ascarid eggs, contamination, however gradual, tends to be cumulative, and thorough cleaning at least of the foaling stall and of the mare's udder and teats before foaling is well worth the effort.

Anthelmintic Medication

Piperazine compounds (100 mg/kg), fenbendazole (10 mg/kg), pyrantel (6.6 mg/kg), ivermectin (0.2 mg/kg), moxidectin (0.4 mg/kg), and a number of other anthelmintics, both current and obsolete, are highly effective against the intestinal stages of *P. equorum*. Pyrantel tartrate used as a feed additive prevents ascarid infection in horses.

Over the past few years, reports from around the world have described the ineffectiveness of macrocyclic lactones in the treatment of *P. equorum* in infected horses (von Samson-Himmelstjerna, 2012). In these trials horses were not cleared of their infection by treatment with a macrocyclic lactone at the labeled dose. For the purpose of clearing horses of their infection, it was necessary to use pyrantel pamoate or fenbendazole (in one case [Craig, Diamond, and Ferwerda, 2007] a 2× dose of pyrantel pamoate was required for clearance). Thus, it is fairly well accepted that *P. equorum* has developed resistance against ivermectin and moxidectin, and if resistant worms are present on a farm, this can result in drastic consequences for foals if they are not cleared of their infection.

Development of *Strongylid, Ascarid,* and *Strongyloides* Infections in Foals

Around 60 years ago, Ann F. Russell (1948) reported on her study of sequential changes in the composition of worm populations in 26 foals from seven different thoroughbred studs. She performed fecal egg counts and identified infective larvae developing in fecal cultures of samples collected from these foals every week from the age of 4 weeks to at least 6 months and, in a few cases, for longer than 1 year. These studies remain of interest because they indicate what happens without the pressure of modern anthelmintics. The interesting thing is that the curves would probably still appear about the same; the only thing that might change would be that the number of eggs per gram would be less, and probably almost no larvae of *S. vulgaris* would be recovered recovered from the cultures. However, these two graphs and their interpretation remain an excellent primer in equine parasitology.

In Figure 4-125, egg counts are plotted against age for *S. westeri,* for *P. equorum,* and for the family Strongylidae collectively. Note that *S. westeri* infection reached a maximum early in life, then rapidly dropped to a low level and finally disappeared at about 5 months of age. This aligns perfectly with what we now know about mammary transmission of *S. westeri*.

P. equorum eggs first appeared at about 12 weeks of age, after which egg counts rose steeply to a peak and then rapidly fell but, instead of disappearing completely, persisted at a low level indefinitely. The 12-week delay in appearance of *P. equorum* eggs corresponds closely to the prepatent period of this parasite, and we may deduce from this that the infection was acquired soon after birth. Thus anthelmintic medication of the pregnant mare, careful bathing of her udder and teats, and thorough cleaning of the foaling box are logical measures for the prevention of significant infection of foals with *P. equorum*. The persistence of infection at a low level in horses of all ages and the extraordinary resistance of the egg to the rigors of the external environment make *P. equorum* a difficult parasite to control.

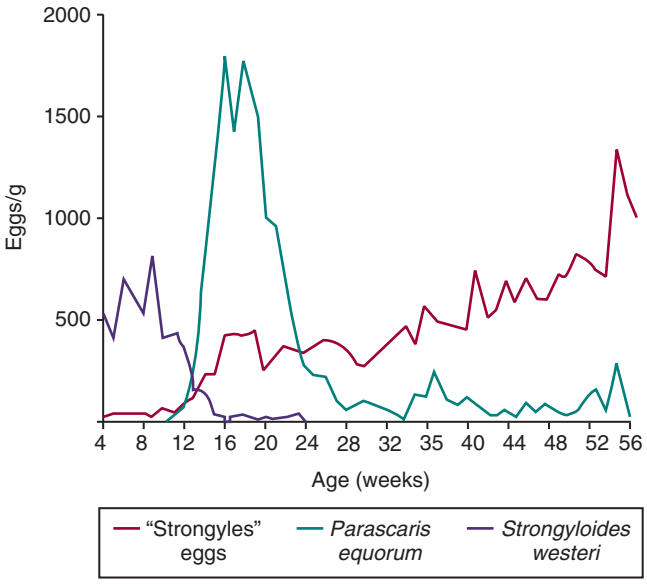


FIGURE 4-125. Average number of eggs of *Parascaris equorum,* "strongyles," and *Strongyloides westeri* counted per gram of manure. Data obtained from weekly observations of 26 foals. (Modified from Russell 1948; reproduced from Evans JW, Barton A, Hintz HF, et al: *The horse,* New York, 1977, WH Freeman.)

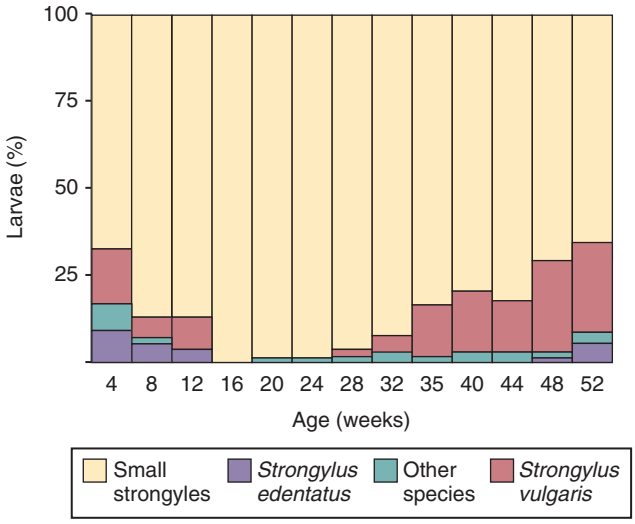


FIGURE 4-126. Percentages of larvae of different species of strongyles in fecal cultures. Data obtained from weekly observations of 26 foals. (Modified from Russell 1948; reproduced from Evans JW, Barton A, Hintz HF, et al: *The horse,* New York, 1977, WH Freeman.)

The third and most important curve shown in Figure 4-125 represents a gradual increase in composite strongylid egg counts during the first year of life. To interpret this curve, one must take into account the relative abundances of *S. vulgaris, S. edentatus,* and the "small strongylids," as determined by fecal culture and identification of infective larvae. These findings are portrayed in Figure 4-126, which shows that the eggs of the small strongylids always predominated, representing, at various ages, between 80% and 100% of the total strongylid eggs shed in the feces of these foals. This is to be expected in view of the 6- to 11-month prepatent periods of *Strongylus* species and the general predominance of cyathostomes in horses. It is curious therefore that small numbers of *S. vulgaris* and *S. edentatus* eggs appear in fecal samples of foals

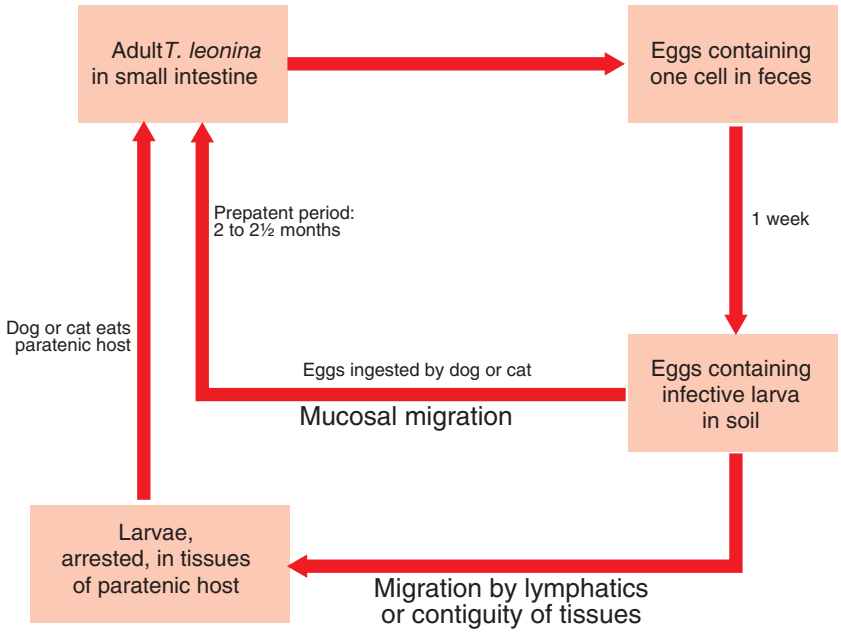


FIGURE 4-127. Alternative life histories of *Toxascaris leonina*.

up to 12 weeks of age. Russell (1948) observed this phenomenon in every one of 26 foals studied and interpreted it as evidence of coprophagia. This ingestion of feces by foals is probably related to the normal process of "seeding" the cecum and colon with beneficial microorganisms essential for the digestion of cellulose, but it also presents a clear opportunity for invasion by parasites.

As Figures 4-125 and 4-126 show, strongylid egg output increases steadily, and *S. vulgaris* and *S. edentatus* eggs appear on schedule at 6 months and 11 months, respectively. This clearly indicates that strongylid infection begins shortly after birth of the foal and proceeds without interruption thereafter. Because young foals are much more susceptible than older horses to the pathogenic effects of these parasites, it follows that the greatest efforts should be directed toward preventing excessive exposure, especially during the first months of life.

Toxascaris

T. leonina is a parasite of cats and dogs in cooler climates of the world. The adult female can be 10 or so cm long. The egg of *T. leonina* develops rapidly, usually reaching the infective stage in about a week. If the egg is ingested by a rodent or an animal other than the final host, the larva hatches and invades the wall of the intestine, where it remains for about a week before proceeding to other tissues, where it encysts and remains arrested in the infective stage. When the infective egg or an infected rodent is ingested by a dog, cat, or other suitable definitive host, the larva invades the mucosa of the small intestine. There it develops and molts before returning to the lumen of the intestine to mature. Cats and dogs can thus acquire *T. leonina* infection by ingesting infective eggs or rodents with infective larvae encysted in their tissues (Figure 4-127).

The eggs of *T. leonina* develop to the infective stage in only 1 week as compared with 4 weeks for *Toxocara* species (see Figure 7-8). This rapid development might explain the persistence of *T. leonina* infection in reasonably well-sanitized cage colonies of dogs. The life cycle of *T. leonina* with its rapid infective-larval development and ability to use mice as paratenic hosts is such that this ascaridoid also often becomes a problem of felids or canids housed in zoologic gardens.

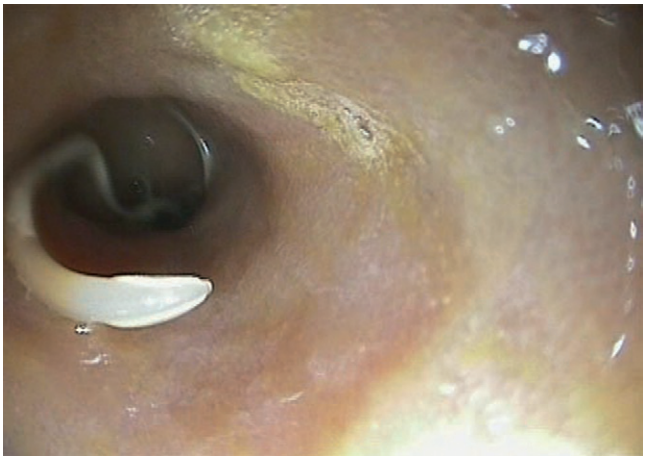


FIGURE 4-128. *Toxocara canis* male viewed by video endoscopy showing the large lateral alae on the cephalic end of the worm.

Toxocara

Toxocara is a genus of rather large ascaridoids that as adults are parasites in the small intestine of various mammals. The worms have three large lips and a glandular esophageal bulb (the ventriculus) located at the junction of the esophagus and the intestine. They tend to have cervical alae, and their eggs have pitted surfaces. *T. canis* and *T. cati* are two of the most commonly observed parasites of the dog and cat, respectively. *T. vitulorum* of calves is commonly seen in developing parts of the world, and the egg still appears in feces from calves in the United States. Other species of *Toxocara* include those found in elephants, hippopotami, bats, civet cats, rats, coati mundis, and mongooses.

Toxocara canis

The *Toxocara canis* worm is commonly seen in puppies during the first few months after birth. Adults tend to be 10 to 15 cm long, have cervical alae, and are cream colored, with the internal reproductive organs appearing white when viewed through the cuticle in fresh worms (Figures 4-128 and 4-129). Sometimes when

worms are passed in the feces, the gut tends to appear rather gray or black, and the worms appear darker than when still quite lively. Infections do occur in adult dogs, and these dogs do shed eggs in their feces.

IMPORTANCE. In spite of the long-term existence of anthelmintics that are capable of killing *T. canis,* these worms remain highly prevalent across the United States, and in a good number of counties, one or more out of every 10 canine fecal samples submitted by a veterinarian to a diagnostic laboratory will contain the eggs of this parasite (Figure 4-130). Heavy prenatal *T. canis* infections cause severe abdominal discomfort in nursling pups. The pups whimper and shriek almost continuously and adopt a peculiar straddle-legged posture of the hindlimbs when standing or walking. Alarming numbers of immature and adult worms may appear in the feces or vomitus. Death may result from rupture or obstruction of the intestine as the ascarids, reacting to some irritant, thrash about and become tangled into knots. Obstruction of the bile or pancreatic duct occasionally provides prize exhibits for pathology museums.

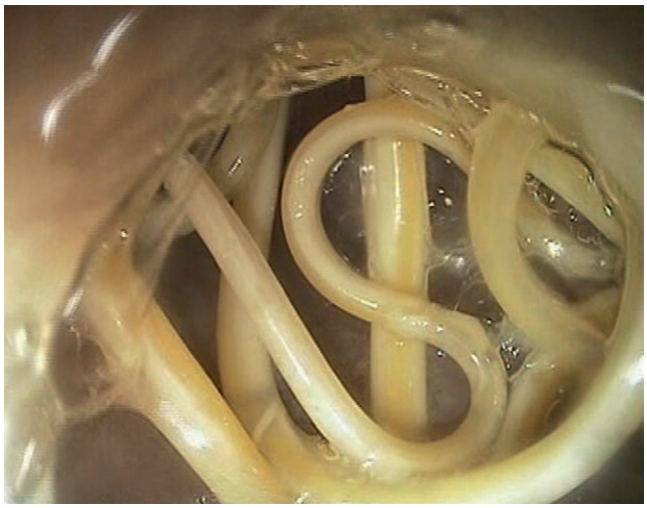


FIGURE 4-129. A frame from a video endoscopic image of a mass of *Toxocara canis* in the ileum maintaining their position in the lumen by pressure applied to the surrounding intestinal wall.

LIFE HISTORY. The adolescent wanderings of nematode larvae are influenced not only by their intrinsic capabilities for penetrating tissues and responding to various chemical and physical stimuli, but also by the suitability of the host invaded. If a *T. canis* egg hatches inside a dog's stomach, the larva invades the bowel wall and arrives in a pulmonary capillary by the same route outlined earlier for *A. suum*. Unlike *A. suum,* however, the *T. canis* larva is considerably more prone to remain in the circulation than to break into the alveolus, especially if its host is a mature dog. If the larva fails to enter the alveolus, it will be returned to the heart by the pulmonary veins and carried away by the systemic circulation, perhaps to lodge in a kidney or some other somatic tissue, where it will encyst as an arrested infective larva.

The direction taken at the alveolus is crucial in determining whether the larva will undergo tracheal migration and develop to sexual maturity or a somatic migration to remain arrested as an infective larva in that particular dog. The probability of tracheal migration is high in a newborn puppy. However, by the time the pup is 1 or 2 months old, the probability that a newly hatched *T. canis* larva will develop into an adult ascarid in that particular pup has fallen to a very low level and remains so indefinitely. During the same period of the pup's life, the probability of somatic migration progressively increases, and arrested infective larvae accumulate in the tissues.

Somatic migration also accounts for the accumulation of arrested infective *T. canis* larvae in the tissues of a wide range of other paratenic intermediate hosts, including rodents, sheep, pigs, monkeys, humans, and earthworms (see Figures 7-106, 7-115, and 8-99). If a mouse with arrested infective larvae in its tissues is eaten by a dog, somatic migration is not observed, and in some instances at least, development proceeds to maturity in the alimentary tract (Sprent, 1958). The mouse not only has saved the larvae but

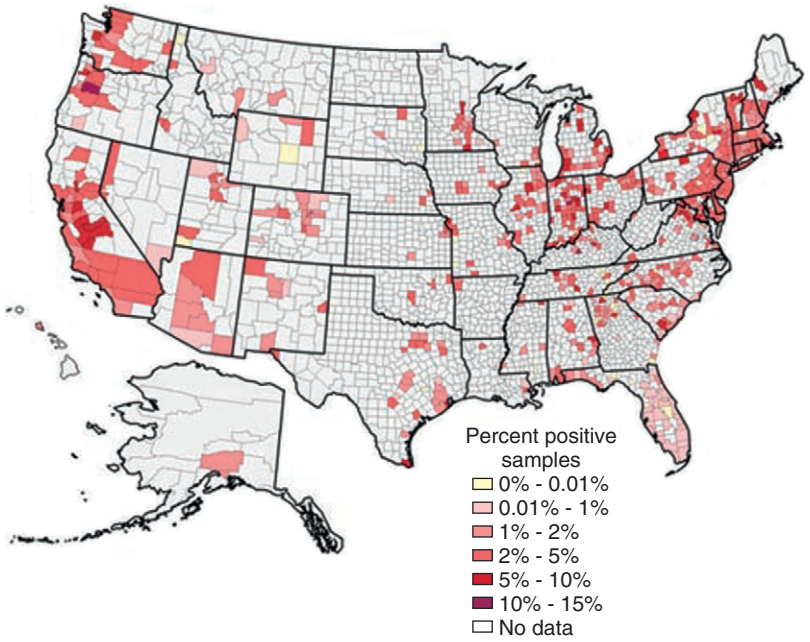


FIGURE 4-130. Map of prevalence by county of *Toxocara* egg–positive samples from dogs (n = 2,622,470) in data collected by the Companion Animal Parasite Council (CAPC) for the year 2011; only counties with more than 20 examined samples are included on the map.

apparently has changed them, too. Migration and encystment in paratenic hosts and exploitation of the prey-predator relationship constitute an epidemiologic norm for carnivoran ascarids in general. Both *T. cati* and *T. leonina* can be transmitted in this manner, as can ascarid parasites of certain wild carnivorans such as *Baylisascaris procyonis* of the raccoon *P. lotor*.

It should be remembered that adult dogs can be infected with *T. canis*. In a national survey of fecal samples from dogs in shelters around the United States, although the lowest level of roundworm infection occurred in dogs older than 7 years of age, more than 5% of dogs in this age group were infected (Blagburn et al, 1996). It has also been shown that adult dogs can be infected with *T. canis* routinely, as well as repeatedly after anthelmintic clearance, if they are given only a relatively few infective eggs—100 to 200—at once (Dubey, 1978; Fahrion et al, 2008; Maizels and Meghji, 1984). No studies have investigated whether larvae in paratenic hosts may more successfully develop in adult dogs than in larvae in infective eggs.

From the perspective of the dog and the veterinarian, the most important arrested larvae of *T. canis* are those found in the tissues of the female dog (see Figure 7-48). Transmission of infection from bitch to pups occurs almost exclusively by way of **transplacental transmission.** During the last trimester of pregnancy, arrested larvae are reactivated and migrate from the tissues of the bitch to the pups in utero (Fülleborn, 1921). After parturition, small numbers of reactivated larvae also may be shed in the milk, but this is a minor form of transmission for this parasite. Alternative life histories of *T. canis* are summarized in Figure 4-131.

TREATMENT. Owing to transplacental transmission, unless heroic measures have been taken to prevent infection, pups may be

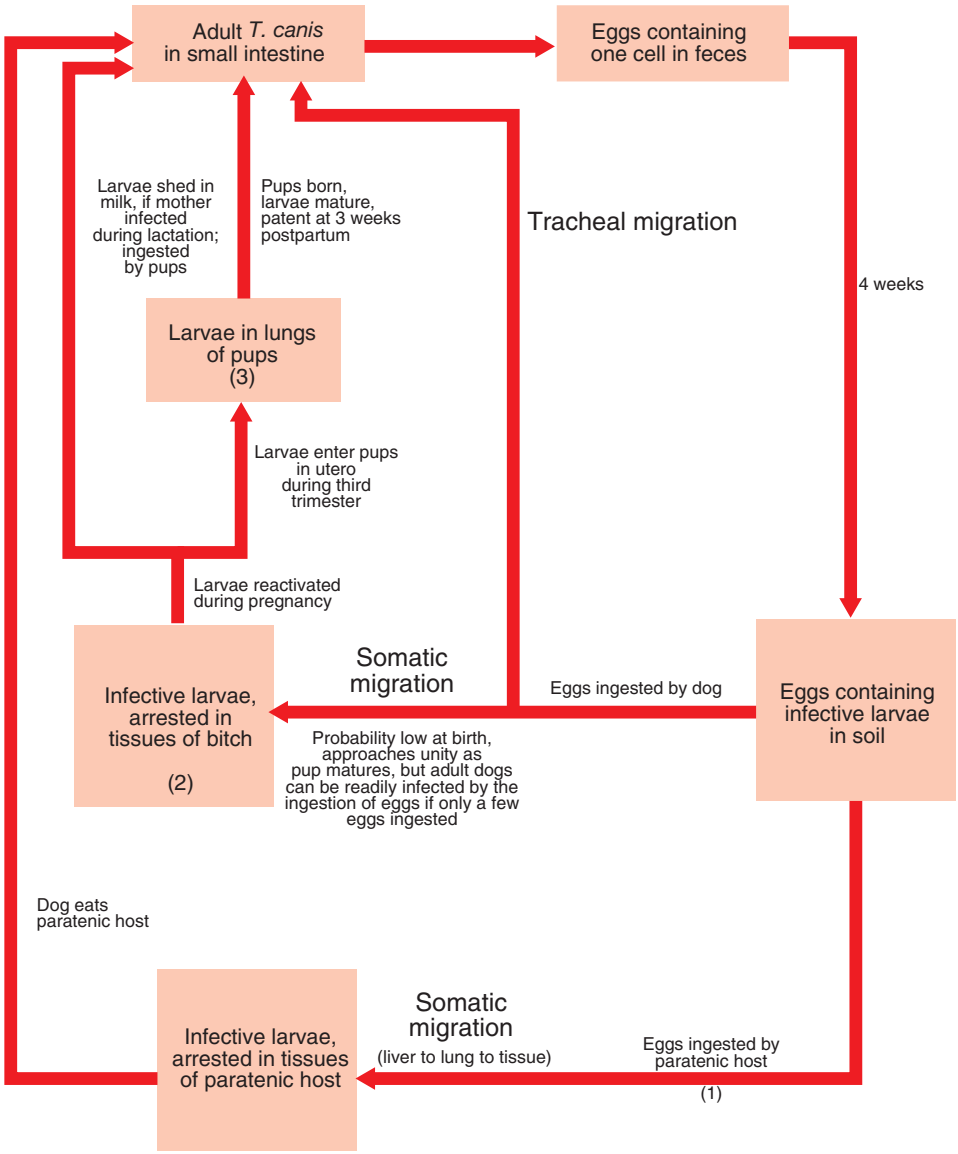


FIGURE 4-131. Alternative life histories of *Toxocara canis*. *1,* A paratenic host is any in which a larval parasite may survive and remain infective for its definitive host without undergoing development. Any of a wide range of animal species including rodents, sheep, pigs, monkeys, humans, earthworms, and adult dogs may serve as paratenic host for *Toxocara canis* larvae. *2,* Arrested infective larvae are also found in the tissues of male dogs, but these are supposed to be of little if any epidemiologic importance. *3,* The larvae that have entered the pups through the placenta molt once in the fetuses but defer further development until after birth. (Data from Sprent JFA: Observations on the development of *Toxocara canis* [Werner, 1782] in the dog, *Parasitology* 48:184, 1958.)

assumed to be infected. About the only anthelmintic labeled for the treatment of 2-week-old puppies is pyrantel pamoate. Medication should start routinely as early as the second week of life and should be repeated every 2 weeks until the pup is 3 months old. Young puppies are also regularly treated with piperazine compounds (110 mg piperazine base per kilogram), which is considered safe and highly effective against ascarids in the lumen of the alimentary tract and therefore ideally suited to removing *T. canis* as they arrive and develop in the intestinal lumens of perinatally infected pups. Many of the labels for piperazine products, however, state that they should not be used in puppies younger than 6 weeks of age. Drontal Plus (febantel, praziquantel, and pyrantel pamoate) is labeled for use in puppies older than 3 weeks and weighing more than 2 pounds. Milbemycin oxime (with or without lufenuron) is labeled for puppies older than 4 weeks of age and weighing 2 pounds. Puppies older than 6 weeks of age can be treated with fenbendazole or ivermectin with pyrantel pamoate. At 7 weeks of age, puppies can be treated topically with moxidectin and imidacloprid. At 8 weeks the formulation of ivermectin with pyrantel pamoate and praziquantel is labeled for use in puppies.

The question is often asked whether the puppy placed on a monthly heartworm preventive also needs to be treated every 2 weeks, as per Centers for Disease Control and Prevention (CDC) guidelines (http://www.cdc.gov/parasites/zoonotichookworm/resources/prevention.pdf), which state, "In areas where both ascarids and hookworms are common, begin treating both puppies and their mothers with an age-appropriate anthelmintic at 2, 4, 6, and 8 weeks of age. Some recommend extending this to 12 weeks and then treating monthly until the pet is 6 months old. To treat for ascarids alone, begin by 2½-3 weeks and treat every 2 weeks for at least three additional treatments." In one study in the United Kingdom, 104 puppies from three kennels where *T. canis* was common were given milbemycin oxime with lufenuron (Sentinel) or febantel, pyrantel pamoate, and praziquantel (Drontal Plus), beginning at 2 weeks of age; the Sentinel dogs were treated monthly until the dogs were 26 weeks old, and the Drontal Plus dogs were treated every other week for 12 weeks, and then again when the dogs were 26 weeks old (Schenker, Cody, and Strehlau, 2006). Very little difference was noted in the amount of egg shedding between the two groups, and the Sentinel-treated dogs in this study actually shed slightly fewer eggs and had more negative fecal samples. Two additional points need to be considered relative to this question. First, in light of the CDC recommendation, it may be wisest to treat between the first few monthly treatments of a puppy to stay in compliance. Second, it should be remembered that monthly products are safety tested by the U.S. Food and Drug Administration (FDA) as though they are going to be given once a month for the life of the pet, whereas other products are tested as though they are going to be given once per indication of infection. So, the question remains a complicated one.

In breeding situations, the role of the bitch in the epidemiology of *T. canis* is paramount because she harbors the better part of the reservoir of infection not contained in the soil. Clients should be advised that bitches bestowing pathogenic *T. canis* burdens on their litters will likely repeat the performance once or twice again, even after the uptake of infective eggs has ceased. Clients should be made aware that the environment of a bitch with a litter of nurslings is likely to contain veritable clouds of eggs from 3 weeks postpartum onward, and it is during this period that anthelmintic medication and sanitation can be applied most effectively and efficiently. Rather heavy patent infections are regularly observed in nursing bitches for a short period beginning about 1 month after parturition. This has been explained as follows (Sprent, 1961). Some reactivated larvae fail to establish themselves in the pups' intestines and are passed with their feces. Brood bitches eat their pups' feces to clean the nest and, in so doing, afford these jettisoned larvae a second chance to mature.

TREATING TO CLEAR ARRESTED LARVAE. The phrase "*Toxocara canis*–free dogs" implies that the dogs are devoid of both adult and larval parasites. However, it is nearly impossible to detect small numbers of arrested larvae in the tissues of even a small pup, so the status "*T. canis*–free" is always to be taken with a grain of salt. The sort of measures required to produce *T. canis*–free dogs are usually beyond the resources (and requirements) of commercial breeders.

Griesemer and Gibson (1963) obtained *T. canis*–free pups from colostrum-deprived bitches raised in isolation that had been maintained on wire through several gestations without anthelmintic medication. The somatic larval burden apparently was eliminated through the placenta over the course of several pregnancies.

Bitches with *T. canis* and *A. caninum* infections were medicated daily with fenbendazole from the fortieth day of gestation to the fourteenth day of lactation at a dosage rate of 50 mg/kg. Their pups were found free of both parasites (Düwel and Strasser, 1978). Burke and Roberson (1983) obtained 89% fewer ascarids and 99% fewer hookworms in pups from dams subjected to the same regimen. The timing of medication coincided with the period of reactivation and migration of arrested *T. canis* larvae in these parturient females.

Ivermectin administered during gestation has been shown to cause marked reductions in the number of *T. canis* organisms in puppies born to experimentally infected bitches (Shoop et al, 1988). Treatment of 1 mg/kg body weight on days 20 and 42 or 0.5 mg/kg body weight on days 38, 41, 44, and 47 of gestation caused a marked reduction in the number of worms recovered from puppies of treated bitches. These dosages are well above the level of ivermectin used in heartworm prophylaxis. A single treatment of four pregnant dogs subcutaneously with 1% moxidectin at a dosage of 1 mg/kg on days 40 and 55 after conception and oral inoculation with 20,000 *T. canis* eggs prevented egg shedding in the feces by both dams and their puppies, and all were free of ascarid adults and larvae (Kramer et al, 2006).

Toxocara cati

The worm *T. cati* is slightly smaller than *T. canis,* with females up to 12 cm long, and has very elegant cervical alae (Figure 4-132; also see Figure 7-56). When the fresh worm is observed, the ventral curvature of the anterior end along with the large cervical alae gives the front end of the worm a cobra-like appearance. These worms are commonly delivered to practitioners after they have been observed in vomitus by owners. If in doubt about the worm's identity, the practitioner can always break the worm open about one third of the body length behind the head and look for the more familiar *Toxocara* eggs with a microscope. This will work, of course, only if the worm presented is a female.

LIFE HISTORY. The migration patterns of *T. cati* differ qualitatively from those of *T. canis* in that (1) prenatal infection through the placenta does not occur, and (2) the probability of tracheal migration in egg infection remains high throughout the cat's life (Figure 4-133). Neonatal infection through the mammary glands had been considered an important route of infection in kittens (Swerczek, Nielsen, and Helmbolt, 1971); however, more recent work has shown that transmammary transmission does not occur in cats with chronic infection, although it can occur if cats are infected acutely during the last part of pregnancy (Coati, Schnieder, and Epe, 2004). Infected paratenic hosts unquestionably represent

FIGURE 4-132. *Toxocara cati,* stomal end showing the broad cervical alae.

an important reservoir of infection for adult cats, at least those with well-developed predatory habits. In larvae from cats infected acutely during pregnancy and from paratenic hosts, migration in the cat and arrested development in the paratenic host appear in some way to satisfy the larval wanderlust; although a small proportion of the larvae may wander as before, most develop to maturity after a sojourn in the wall of the stomach (i.e., a mucosal migration; Sprent, 1956).

IMPORTANCE. Infection of cats does not appear to be beset with the same lethal consequences as infection of dogs, particularly puppies, with *T. canis*. However, cats are a potential source of eggs that are capable of using humans as paratenic hosts, and the fact that people are infected by *T. gondii* via oocysts would suggest that they are also getting infected with *T. cati* eggs via a similar route. Again, the map produced by CAPC for the percentage of fecal samples positive for *T. cati* eggs shows that in many counties in the United States, up to one out of every four fecal samples submitted by a veterinarian to a diagnostic laboratory contains the eggs of this parasite (Figure 4-134). This is just another excellent reason why cats should be under the care of a veterinarian and should routinely receive year-round parasite control.

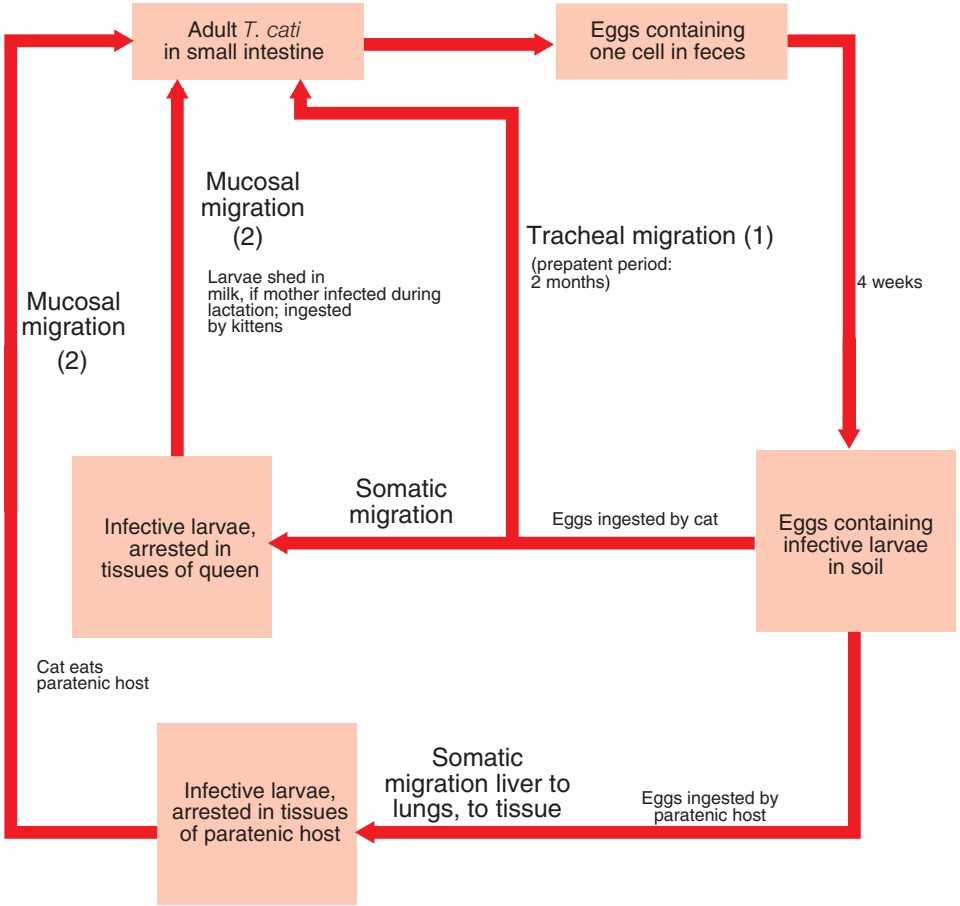


FIGURE 4-133. Alternative life histories of *Toxocara cati*. *1,* The probability that ingestion of infective eggs will lead to patent infection remains substantial throughout the life of the cat. *2,* Larvae that have already undergone somatic migration in a paratenic host, including the queen, satisfy their histotrophic requirements with a mucosal migration. Transmammary transmission seems to occur only if the queen is acutely infected during late pregnancy. The relative epidemiologic importance of these alternatives will depend on the kind of environment, the abundance of suitable paratenic hosts, and the sex and habits of the cats. (Redrawn from Coati N, Schnieder T, Epe C: Vertical transmission of *Toxocara cati* Schrank 1788 [Anisakidae] in the cat, *Parasitol Res* 92:142, 2004; Sprent JFA: The life history and development of *Toxocara cati* [Schrank, 1788] in the domestic cat, *Parasitology* 46:54, 1956; Swerczek TW, Nielsen SW, Helmbolt CF: Transmammary passage of *Toxocara cati* in the cat, *Am J Vet Res* 32:89, 1971.)

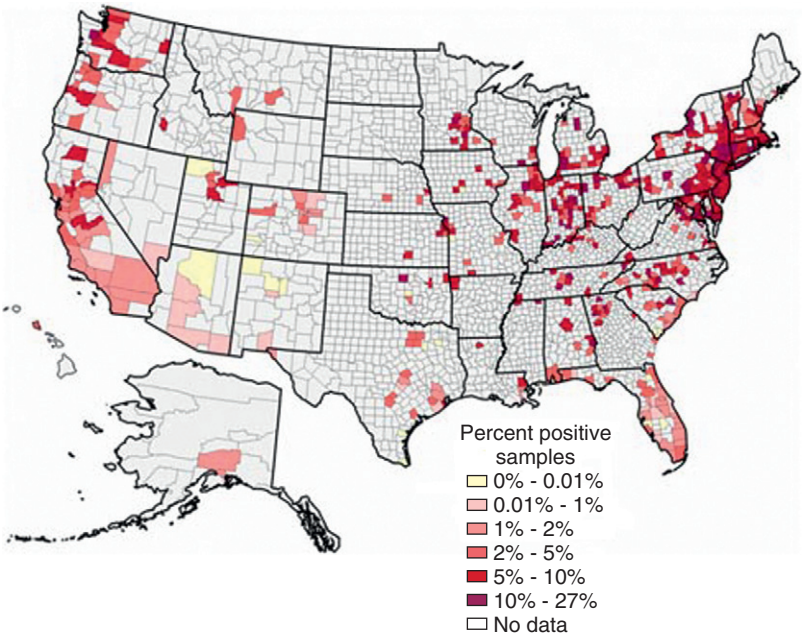


FIGURE 4-134. Map of prevalence by county of *Toxocara* egg–positive samples from cats (n = 558,797) in data collected by the Companion Animal Parasite Council (CAPC) for the year 2011; only counties with more than 20 examined samples are included on the map.

TREATMENT. The fact that cats get neither hookworms nor roundworms routinely from their mother by transmammary or transplacental transmission means that treatment of the very young kitten is not as critical as early treatment of young puppies. The only product in the United States labeled for 2-week-old kittens is pyrantel pamoate; when this is formulated with praziquantel (Drontal), the age limit on the label is 4 weeks and the weight requirement is 1½ pounds. Piperazine in various formulations is often administered to young kittens, although as with the dog, many of the labels on these products say they should not be given to kittens younger than 6 weeks of age. The other products that are approved for cats have labels that are quite conservative relative to the dosing of kittens, with some approved for treatment beginning at 6 weeks of age (ivermectin and milbemycin oxime), 8 weeks (selamectin), and 9 weeks (emodepside and praziquantel; moxidectin with imidacloprid); some of these have weight restrictions as well.

Environmental Control of Dog and Cat Roundworms

SOIL POLLUTION. *Toxocara* and *Toxascaris* eggs are highly resistant to environmental extremes and remain infective for years, especially in poorly drained clay and silt soils, hence their accumulation in soil and filth and the threat they pose to successful dog-rearing progress with time. A reasonable explanation for the heavy ascarid infections so frequently encountered in hound pups might be sought in the common practice of chaining the hounds almost permanently to doghouses—a practice particularly conducive to soil pollution. Because the infective eggs are virtually immune to any reasonable measures taken to destroy them, the most effective measure is to entomb them under a concrete or bituminous asphalt slab. Once the slab is installed, and provided that feces are not allowed to accumulate for longer than a week at a time, the probability of the confined dog ingesting infective ascarid eggs becomes quite small. The next best way of decontaminating polluted soil is to replace the top foot or so with fresh gravel.

CONTAMINATED KENNEL AREAS. All surfaces must first be made physically clean. High-pressure washers like those in car washes are very effective, and inexpensive mobile units are quite satisfactory. Wood and wire construction is difficult to clean properly with any kind of equipment or amount of effort. After surfaces are physically clean, they may be mopped or sprayed with 1% sodium hypochlorite (3 cups Clorox per gallon of cool water) to strip off the outer protein coat of the ascarid eggs so they will not stick to surfaces and can be rinsed away. The preliminary cleaning is absolutely essential because any appreciable amount of residual organic matter will neutralize the sodium hypochlorite and render it ineffective in stripping the ascarid eggs. Notice that nothing has been said about killing the ascarid eggs. The preceding treatment does not kill ascarid eggs; it just knocks them loose. Ascarid eggs are killed by heat. Raising the temperature of a cage or bedding to above 60°C (140°F) for 5 minutes will kill all eggs, but this temperature may be difficult to reach under many circumstances when different housing structures are involved.

Paratenic Hosts

Mice and other small paratenic hosts may play a significant role in the epidemiology of *Toxocara* and *Toxascaris* infection, especially with regard to predacious cats. If you dissect the mice, voles, moles, shrews, and snakes that your cat drags in, you will probably find *Toxocara* larvae encysted in many of them. A survey in rural England of brown rats found larvae of *Toxocara* in 15% of the rats examined (Webster and Macdonald, 1995). In a rural setting, probably little can be done about this source of infection, except to confine dogs and cats indoors. Rodents are attracted to the abundance of food in kennels and catteries and are not put off by the presence of their ferocious predators; a mouse is quite willing to risk its life for a kibble. There seems to be little information about the importance of rodents in transmitting ascarids and other parasites to dogs and cats confined to buildings and outdoor enclosures. However, given the facts gathered here, an investment in rodent control could be partly written off against the cost of controlling parasites.

Human Toxocarosis (Visceral Larva Migrans; Larval Toxocariasis)

The widespread distribution of dog feces and the prevalence of *T. canis* eggs therein led Fülleborn (1921) to wonder about the pathologic significance in man of nodules containing larvae of this parasite. These nodules occurred principally in the liver, lungs, kidneys, and brain. Beaver et al (1952) recognized the causative role of *T. canis* larvae in cases of sustained eosinophilia (above 50%), pneumonitis, and hepatomegaly in children younger than 3 years of age and dubbed the condition *visceral larva migrans*. As a horrible sequela occurring at 3 to 13 years, the larvae may produce granulomatous retinitis. Misdiagnosis of *T. canis*–induced granulomatous retinitis as retinoblastoma has prompted the unnecessary enucleation of children's eyes in at least 36 reported cases.

The typical epidemiologic situation around symptomatic cases involves a toddler eating soil heavily contaminated with infective *T. canis* eggs. Such soil is likely to be found wherever dogs habitually defecate and, in particularly high concentration, in the nests of maternal bitches and their litters. The soil of public parks in cities tends to be heavily contaminated with infective *T. canis* eggs (Dubin, Segall, and Martindale, 1975; Woodruff and Burg, 1973). Although dirt eating is often considered to be a manifestation of depraved appetite (i.e., pica) resulting from dietary deficiency or emotional insecurity, even well-nourished, well-adjusted babies should not be trusted to forgo whatever delicacies may be at hand. Children must not be allowed to play where dogs habitually defecate, and dog feces must never be used to fertilize vegetable gardens.

A vast majority of infections in humans around the world are without recognized symptoms. People act like other paratenic hosts, and larvae can persist in the tissues of primates for at least 10 years (Beaver, 1966). A survey of human sera in the United States from people older than 6 years of age (n = 20,395) revealed that some 13.9% were serologically positive for the infection, and because of the biology of the larvae (Won et al, 2007), it is highly likely that this means that they are currently infected. *T. cati* appears somewhat less important than *T. canis* as a cause of human infection, and some cases are diagnosed serologically (Petithory and Beddock, 1997; Virginia et al, 1991), but as yet no consensus has been reached as to whether specific infections can be reliably distinguished serologically. For people, there is no logical source of infection in most cases other than infective eggs in the environment, and in the United States, because of the lack of other common intestinal parasites in people, concern about cross-reaction with antibodies to other parasites is considered minimal. Thus it seems that people are getting infected through ingestion of eggs that have embryonated in soil after having been passed in the feces of dogs and cats. This means that the veterinary profession has a clear responsibility to identify and eliminate *T. canis* and *T. cati* infection at every opportunity, and to provide the public with objective scientific information about the epidemiology and prevention of human toxocarosis.

People, being good paratenic hosts, can get infected if they eat another paratenic host, and thus they may also suffer from larval toxocariasis after ingestion of raw meat, but most typically they infect themselves by ingestion of raw liver. These cases seem to more typically be symptomatic. Cases resulting from the ingestion of raw or rare beef, lamb, rabbit, duck, chicken, and ostrich liver have been reported (Noh et al, 2012; Hoffmeister et al, 2007). As seen on computed tomography (CT) scans, after infection occurs, the larvae can continue to move about in the human's liver for months (Lim, 2010). Most cases have occurred in Japan and Korea, but the infected lamb liver was eaten in the United States and the rabbit liver in Switzerland.

Cases have been reported of children infected with adult *T. cati* (Eberhard and Alfano, 1998), but it is believed that these children may have ingested intact adult worms recovered from litter boxes.

Visceral Larva Migrans in Nonhuman Hosts

In veterinary medicine, it should not be forgotten that other hosts besides people and other primates can develop disease as a result of migrations of the larvae of *T. canis* (and *T. cati*) within their tissues. Cats infected with *T. canis* develop elevated eosinophil counts and massive eosinophilic granulomas in their kidneys and livers, and have lungs with severe medial hypertrophy of the pulmonary vessels (Parsons et al, 1988). A long list of other hosts can develop disease due to *T. canis,* including sheep, pigs, and tortoises (Parsons, Bowman, and Grieve, 1989). The larvae of *T. canis* and *T. cati* can cause white-spot disease in the livers of pigs, similar to those caused by *A. suum* (Ronéus, 1966).

Toxocara vitulorum

This species of *Toxocara* is a threat to cattle and buffalo in the developing world; calves are infected via their mother's milk and potentially develop massive infections that can lead to impaction and death. However, it seems that this parasite is making a comeback in the developed world among beef cattle. In recent reports from a bison herd in western Canada, sucking beef calves in the Netherlands, and suckling Simmental cattle in the United Kingdom, and in a closed cow-calf beef operation in north central Florida, 17.6% of calves younger than 3 months of age were infected (Woodbury et al, 2012; Borgsteede et al, 2012; Jones et al, 2009; Davila, Irsik, and Greiner, 2010). The larvae passed in the milk of the mother are fairly large—0.75 to 1.5 mm in length. The eggs passed in the feces of the cow look very similar to the eggs of *T. canis* (Figure 4-135). When a cow ingests embryonated eggs, the larvae undergo a liver-lung migration before they make their way to the cow's tissues, where they have been shown to persist for at least 5 months (Anderson, 2000). Because this has not been seen as a problem in the United States in the past 50 or more years, no products have been approved for its treatment.

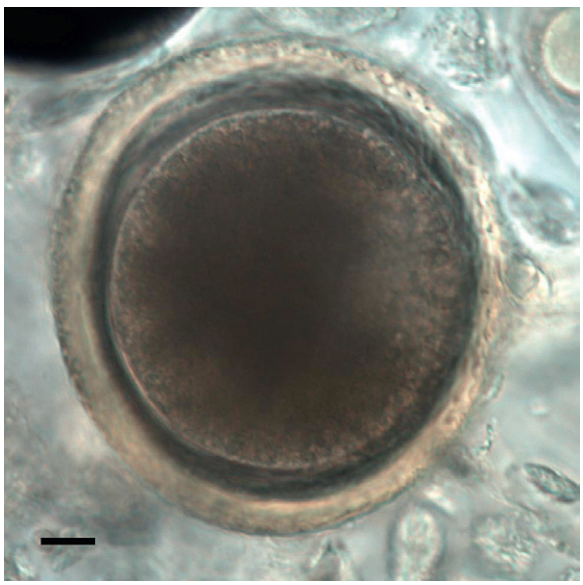


FIGURE 4-135. Egg of *Toxocara vitulorum* from a calf in New York State. Bar = 10 µm.

Baylisascaris

Species of *Baylisascaris* common in North American wildlife include *B. procyonis* of the raccoon, *Baylisascaris columnaris* of the skunk, *Baylisascaris transfuga* of bears, and *Baylisascaris laevis* of the woodchuck. The raccoon has been introduced into Europe, where it has proliferated quite successfully, and the raccoon roundworm is now present in Europe. *B. procyonis* causes a particularly serious form of visceral larva migrans in a wide range of hosts including humans (Kazacos, 2001), and zoonotic infections have occurred in Europe as well (Küchle et al, 1993). Unlike *Toxocara* larvae, the larvae of *B. procyonis* grow larger as they migrate. However, they resemble *T. canis* larvae in that they tend to invade the central nervous system of paratenic hosts, and because they grow as they migrate (see Figure 8-100), only one to three *B. procyonis* larvae in the brain may prove fatal. These properties render them very pathogenic to more than 100 species of animals, including woodchucks, rabbits, ground squirrels, chickens, turkeys, partridges, pigeons, cockatiels, chukar partridges, emus, quail, and humans (Kazacos, 2001; Kazacos et al, 1983; Myers, Monroe, and Greve, 1983; Roth et al, 1982). Unfortunately, human cases continue to occur; one of the most recent was reported in New York City's Borough of Brooklyn (Saffra et al, 2010), and it is imperative that veterinarians be aware of the risk posed by raccoons being held in captivity or within a community. Raccoons infected with *B. procyonis* can be treated with most of the anthelmintics active against *T. canis* (Bauer and Gey, 1995).

Hay, straw, and other feedstuffs and bedding materials contaminated with raccoon feces are often found to be the source of infective eggs (Figure 4-136) of this parasite. Haylofts and attics may be attractive places for children to play during inclement weather, but such areas should be inspected beforehand to make sure that raccoons have not been nesting in them. Ground-feeding birds such as doves, pigeons, and robins are particularly at risk when they feed on nondigested seeds in dried raccoon feces (Evans and Tangredi, 1985).

Dogs can be hosts of the adult worms. Greve and O'Brien (1989) diagnosed infection with adult *B. procyonis* in a 5-month-old Labrador retriever (patent) and a 6-month-old Golden retriever (nonpatent) by administering piperazine and identifying the adult and juvenile worms when they were passed in the feces. Eggs of this worm also have been observed in the feces of dogs in Minnesota, Indiana, Michigan, and Prince Edward Island (Conboy, 1996; Kazacos 2001). Dogs naturally infected with adult worms have been treated for their *B. procyonis* infection using milbemycin oxime (Bowman et al, 2005). The eggs of this worm are slightly smaller than those of *Toxocara canis* and *Trichuris vulpis* (Figure 4-137).

FIGURE 4-136. Infective egg of *Baylisascaris procyonis.*

ORDER SPIRURIDA

The order Spirurida contains two suborders: Camallanina and Spirurina. Members of both suborders require an arthropod, either a crustacean or an insect, intermediate host for development to the infective stage. The definitive host acquires spirurid infections by ingesting infected arthropods or paratenic hosts that have fed on such arthropods. The suborder Spirurina also includes the superfamily Filarioidea, for which the intermediate host is a blood-feeding arthropod that becomes infected while taking its blood meal, and that vectors the parasite when taking another blood meal.

Suborder Camallanina

Dracunculus

The suborder Camallanina contains only one genus of veterinary significance—*Dracunculus,* a parasite of the subcutaneous tissues of carnivorans and man (Figure 4-138; also see Figure 7-46). The female *Dracunculus* is very large (up to 120 cm), and the male is smaller (up to 40 mm). When a female has been fertilized, the anus and the vulva atrophy, and a shallow ulcer is formed in the host's skin at the location of the anterior end of the worm. When water wets this ulcer, the female projects her body and prolapses a length of uterus, which then bursts to discharge a horde of larvae

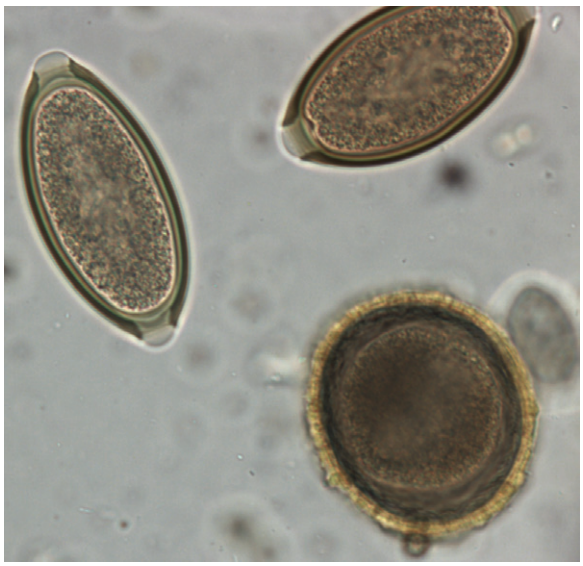


FIGURE 4-137. An egg of *Baylisascaris procyonis* and two eggs of *Trichuris vulpis* in the feces of a naturally infected dog.

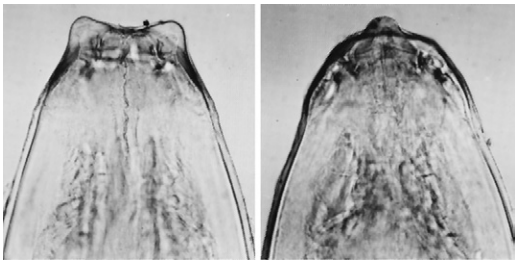


FIGURE 4-138. *Dracunculus insignis* from the axillary connective tissue of a dog. *Left,* The lateral aspect of the stomal end. *Right,* The dorsoventral aspect.

FIGURE 4-139. *Dracunculus insignis* first-stage larvae.

(Figure 4-139). Her body then slowly moves toward the opening to await the next wetting.

Veterinarians in the United States are liable to come across *Dracunculus insignis,* a parasite of the raccoon and other carnivorans, on fairly rare occasions in dogs and cats in North America (see Figures 7-46 and 8-108). *Dracunculus* species are also sometimes seen in snakes and snapping turtles in the United States. In the life cycle of these species of *Dracunculus*, the larvae liberated from the female that are slowly inching out of the body will become infective if ingested by a copepod of the genus *Cyclops*. Development in the copepods takes about 3 weeks. The definitive host becomes infected by ingesting these *Cyclops* organisms in drinking water. It appears that in the case of *D. insignis,* frogs can serve as paratenic hosts (Eberhard and Brandt, 1995); this increases the chance for dogs to become infected through ingestion of frogs.

Humans are infected with their own species of *Dracunculus*—*Dracunculus medinensis,* better known as the Guinea worm. This parasite is on the ropes in its bout with extinction owing to a massive international campaign aimed at its eradication. At the end of 2012, fewer than 1000 cases will exist—a little over 500 in South Sudan, and less than a dozen each in Chad, Mali, and Ethiopia.

Suborder Spirurina

The suborder Spirurina contains 10 superfamilies; six are of interest as parasites of domestic animals. The stoma and surrounding structures of spirurins are distinctive. Comparison of specimens with the illustrations of this section should suffice for generic identification. The one exception here is the Filarioidea, which for the most part has a very plain and simple stoma.

Superfamily Gnathostomatoidea

Gnathostoma species have a doughnut-shaped inflation on the anterior end that is covered with spines (Figures 4-140 and 4-141). Adult specimens are found in cystic nodules in the stomach walls of wild and domestic carnivores. Eggs are passed in the one- to

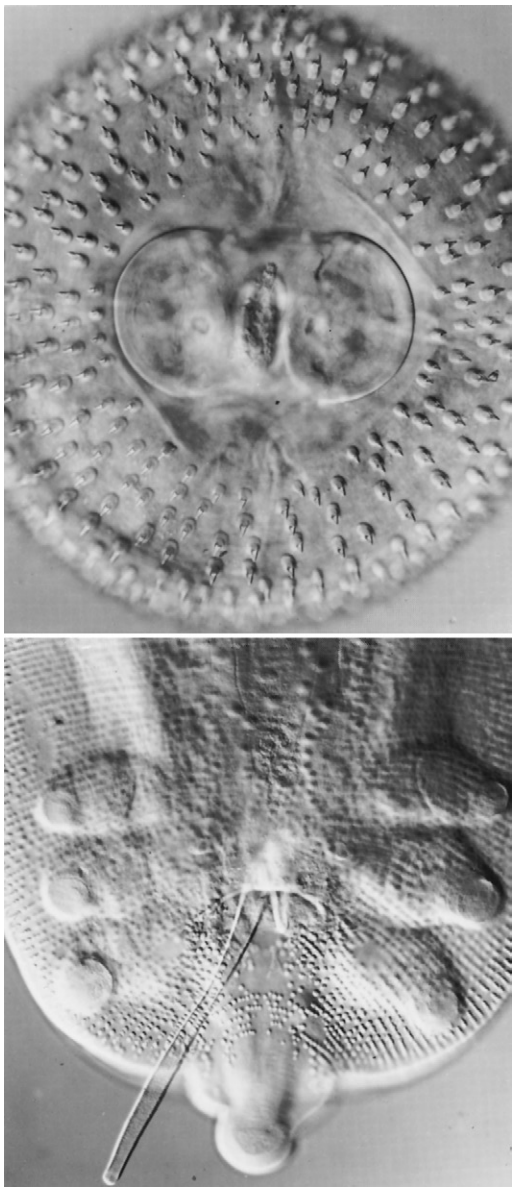


FIGURE 4-140. *Gnathostoma* stomal end *(upper)* and caudal extremity of the male *(lower)*.

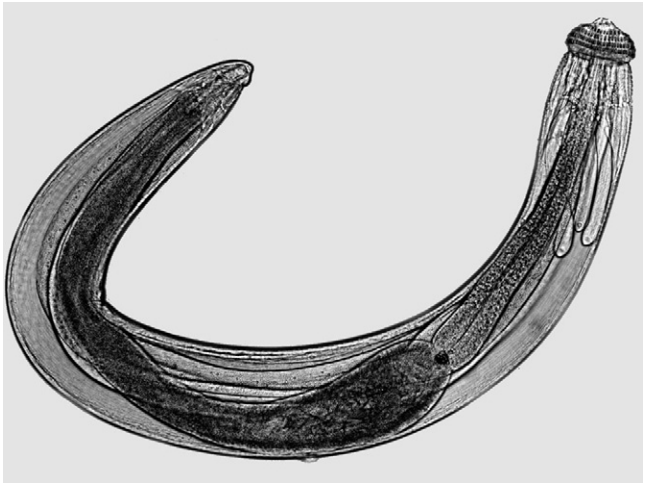


FIGURE 4-141. Lateral view of *Gnathostoma* larva showing inflation and spines on the anterior end.

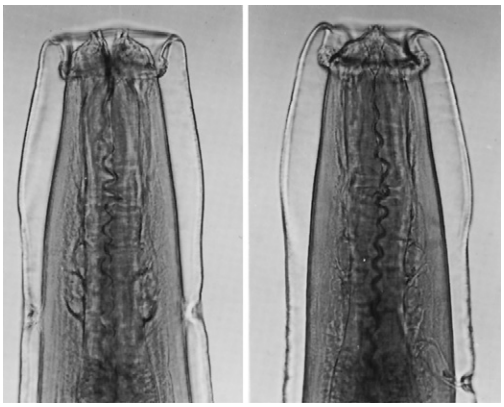


FIGURE 4-142. *Physaloptera* sp. *Left,* The dorsoventral aspect of the anterior extremity. *Right,* The lateral aspect of the anterior extremity.

two-cell stage and develop to the second larval stage in water. These larvae hatch and develop to the infective third stage only if ingested by copepods *(Cyclops)*. A variety of amphibians, snakes, and fish may serve as paratenic hosts to convey the gnathostome from the copepod to the definitive host. In the final host, the larvae undergo an extensive and destructive tissue migration before they return to the stomach. The cystic nodules in the stomach that house the mature adult may break open into the peritoneal cavity with a fatal outcome. In Asia, *Gnathostoma spinigerum* is a species more commonly found in the stomachs of dogs and cats; it is responsible for most of the larval infections that occur in human beings. In people who acquire the infection by ingesting copepods or raw fish, the larvae tend to wander aimlessly without maturing, sometimes for nearly a decade.

Superfamily Physalopteroidea

IDENTIFICATION. *Physaloptera* species are parasites of the stomach of carnivorans. The mouth is flanked by pseudolabia and surrounded by a cuticular collar (Figures 4-142 and 4-143). The adult worms are white or pinkish in color and tend to live with the anterior end embedded in the mucosa (see Figure 7-55). In the dog the adult worms often are present also in the very anteriormost portion of the duodenum at the level of the gastric valve. Infections with these worms in dogs and cats often are associated with vomiting, and the adults are often viewed during endoscopy (Jergens and Greve, 1992).

LIFE HISTORY. The female worm lays small, thick-walled, larvated eggs. The larvae in the eggs will develop to the infective stage in various coprophagous beetles, crickets, and other insects. The larvae will also use various cold-blooded vertebrates as paratenic hosts.

TREATMENT. Dogs have been treated with fenbendazole at 50 mg/kg for 3 days (Jergens and Greve, 1992). Infected cats have been treated with ivermectin at 0.2 mg/kg (Gustafson, 1995) and with two doses of pyrantel pamoate at 5 mg/kg given 3 weeks apart (Santen, Chastain, and Schmidt, 1993). A summary of *Physaloptera* infections in 29 dogs and six cats in Iowa concludes with the recommendation that animals be given a trial course of pyrantel pamoate at 20 mg/kg that may be repeated if signs of vomiting do not cease (Campbell and Graham, 1999). These authors also suggest that the different anthelmintics used in their series of cases (fenbendazole, pyrantel pamoate, and pyrantel pamoate, praziquantel, and febantel) all appeared efficacious, but some required elevated doses or longer treatment times than are suggested for typical labeled use.

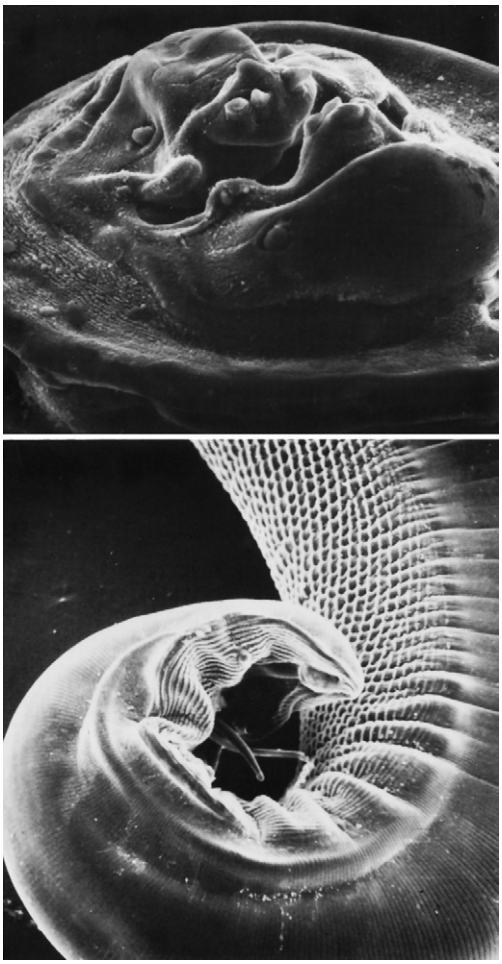


FIGURE 4-143. *Physaloptera* sp. stoma *(upper)* and caudal extremity of male *(lower)*.

Superfamily Thelazioidea

FAMILY PNEUMOSPIRURIDAE. Pneumospirurids are parasites of the lungs of wild carnivorans and appear occasionally in domestic dogs and cats. *Pneumospirura* and *Metathelazia* are representative genera.

FAMILY THELAZIIDAE. *Thelazia* species (Figure 4-144) are parasites of the conjunctival and lachrymal sacs of domestic animals. North American species include *Thelazia lacrymalis* in horses, *Thelazia skrjabini* in cattle and horses, *Thelazia gulosa* in cattle, and *Thelazia californiensis* in dogs, sheep, and various wild mammals. Slightly less than half of the horses surveyed in Kentucky were found to be infected with *T. lacrymalis* (Lyons et al, 1986). *Thelazia* species apparently do little harm to cattle and horses in North America, but exceptional cases requiring treatment may arise.

LIFE HISTORY. The female *Thelazia* worm deposits thin-shelled eggs containing larvae that develop to the infective stage in the face fly, *Musca autumnalis*. The Oriental face fly, *Musca hervei*, serves as intermediate host of *Thelazia* species in Japanese cattle (Shinonaga et al, 1974). A great deal of work over the past few years, carried out mainly in China and Italy with a canine and human Eurasian species of *Thelazia, Thelazia callipaeda,* has revealed that the vectors of this species are drosophilid fruit flies of the genera *Phortica* and *Amiota* (Shen et al, 2006). *T. californiensis* has been considered as being vectored by the muscoid latrine flies, *Fannia canicularis* and *Fannia benjamini,* but the fruit flies may explain ocular cases that have occurred in the western United States

FIGURE 4-144. *Thelazia* sp. from the conjunctival sac of a horse.

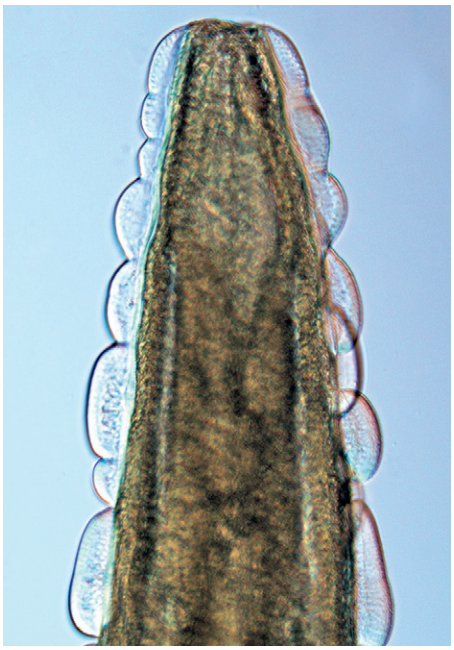


FIGURE 4-145. *Gongylonema pulchrum,* anterior end of worm showing bosses on cuticle.

that have been associated with people getting gnats in their eyes (Kirschner, Dunn, and Ostler, 1990).

TREATMENT. For *Thelazia* in cattle, doramectin at 0.2 mg/kg given subcutaneously or intramuscularly has been approved for treatment and control. A single dose of tetramisole subcutaneously at 12.5 to 15 mg/kg produced rapid clinical recovery in infected cattle. Levamisole at a rate of 5 mg/kg administered subcutaneously or 1% aqueous solution as an eye lotion was also effective (Aruo, 1974; Corba, Scales, and Froyd, 1969; Vassiliades et al, 1975).

In dogs, successful treatment of *T. callipaeda* infection has been provided with subcutaneous injections of 0.2 mg ivermectin per kilogram body weight (Rossi and Peruccio, 1989), direct instillation of 1 or 2 drops of 1% moxidectin into each eye (Lia et al, 2004), or topical application to the back of the neck of topical moxidectin (2.5%) with imidacloprid (10%), providing a dose of moxidectin of 2.5 to 6.5 mg/kg (Bianciardi and Otranto, 2005). The treatment of dogs and cats naturally infected with *T. callipaeda* with oral milbemycin oxime and praziquantel tablets one time or twice, a week apart, revealed that within 2 weeks of the first or only treatment, the worm reduction in the dogs was 97% and that in the cats was 80% (Motta et al, 2012). There are indications that treatment of dogs with sustained-release moxidectin will also provide successful protection against infection for a full fly season (Rossi et al, 2007).

Brooks, Greiner, and Walsh (1983) successfully treated conjunctivitis in a Senegal parrot caused by *Oxyspirura* sp. by instilling one drop of 0.125% demecarium bromide, a cholinesterase inhibitor, into the conjunctival sac and subsequently flushing three paralyzed worms with sterile saline solution.

Superfamily Spiruroidea

GONGYLONEMA. *Gongylonema* species are parasites of cattle and other ungulates, also sometimes appearing in pigs and bears. The cuticle is covered with wartlike cuticular bosses (Figure 4-145), especially near the anterior end, and the nematode can usually be found woven into a remarkably regular sinusoidal tract in the mucous membrane of the host's esophagus *(Gongylonema pulchrum)* or rumen *(Gongylonema verrucosum)* (Figure 4-146). Eggs containing first-stage larvae are passed on the host's feces and, if ingested by a dung beetle or a cockroach, develop to the infective stage in about a month. The definitive host becomes infected by ingesting the infected insect. *Gongylonema* species are usually harmless unless present in massive numbers.

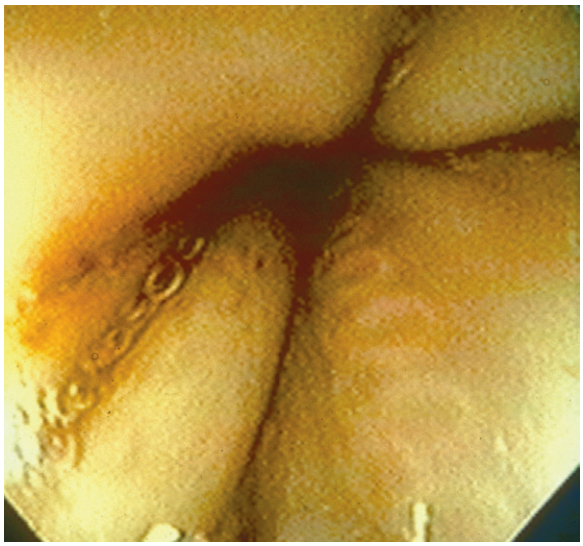


FIGURE 4-146. *Gongylonema pulchrum.* Sinusoidal worm under esophageal mucosa as viewed with an endoscope. (Courtesy Dr. Thomas Divers, College of Veterinary Medicine, Cornell University, Ithaca, New York.)

SPIROCERCA

IDENTIFICATION. *Spirocerca lupi,* a parasite of canids, is found in fibrous nodules in the wall of the esophagus or stomach (see Figures 8-103 to 8-105). The very small (12 × 30 µm) egg contains a vermiform embryo when shed in the feces (see Figures 7-24 and 8-105). If ingested by a coprophagous beetle, this vermiform embryo develops into a larva capable of infecting dogs and a broad range of paratenic hosts, including lizards, chickens, and mice.

When infective larvae are ingested by a dog, they migrate in the adventitia of the visceral arteries and aorta to the walls of the esophagus and stomach. Some go astray and encyst in ectopic locations, but reproductive adults are normally found in cystic nodules that communicate with the lumen of the esophagus or stomach through fistulas. Dysphagia and vomiting, esophageal neoplasia, aortic aneurysm or rupture, and secondary pulmonary osteoarthropathy may be associated with chronic *S. lupi* infection.

TREATMENT. In the treatment of five dogs with naturally acquired *S. lupi* in St. Kitts, West Indies, with milbemycin oxime (11.5 mg per 12 to 25 kg dog on days 0, 7, and 28, and monthly thereafter), no eggs were detected in fecal samples collected from any dogs on or after 31 days from the first treatment, and the esophageal nodules disappeared in all dogs between 95 and 186 days after the first treatment (Kelly et al, 2008). Dogs with naturally acquired *S. lupi* have also been treated with doramectin given subcutaneously every other week for 4 to 6 total treatments at 0.2 or 0.4 mg/kg, with additional treatments given monthly until lesions had resolved (subcutaneously at 0.4 mg/kg) or orally (0.5 mg/kg daily) for an additional 6 weeks (Berry, 2000; Lavy et al, 2002). The treatment appears efficacious, and the lesions in the esophagus resolve.

PREVENTION. Studies have been performed on the prevention of *S. lupi* infection using both moxidectin in a topical application with imidacloprid and oral milbemycin oxime. When topical moxidectin was applied per label instructions to dogs starting at 2 to 4 months of age on the French island of Réunion off the coast of Madagascar, one of 58 puppies receiving treatment developed an esophageal lesion and 19 of 54 untreated dogs developed esophageal lesions (Le Sueur, Bour, and Schaper, 2010). Dogs experimentally infected with *S. lupi* and treated with milbemycin oxime in a formulation containing praziquantel 30 days later showed 80% efficacy against the establishment of nematodes within the esophagus; treatment given repeatedly every 2 or 4 weeks completely prevented the establishment of *S. lupi* in the esophagus of experimentally infected dogs (Kok et al, 2011). When milbemycin oxime in a formulation with praziquantel was used in a preventive fashion to protect puppies from infection in an endemic area, being administered every 28 days for a total of 6 treatments, efficacy in preventing esophageal nodules was 80% and efficacy in reducing the mean number of worms present was 90% (Kok et al, 2010).

OTHER SPIRUROIDS. Other examples of spiruroids are *Ascarops* and *Physocephalus* species (Figure 4-147), parasites of swine, and *Streptopharagus* species (see Figure 7-118), all of which are parasites of primates.

Superfamily Habronematoidea

IDENTIFICATION. *Draschia megastoma, Habronema muscae,* and *Habronema microstoma* are parasites of the equine stomach, where the adult worms stay remarkably close to the margo plicatus. *D. megastoma* is about 13 mm long and has a funnel-shaped buccal cavity, whereas *Habronema* species are larger (22 to 25 mm) and have cylindric buccal cavities (Figure 4-148). The left spicule of *H. muscae* is five times as long as the right one, whereas only a twofold disparity exists between the spicules of *H. microstoma*. *D. megastoma* excites the formation by the host of fibrous nodules riddled with intercommunicating galleries filled with a creamy puslike material in which the worms live (see Figure 7-80). *Habronema* species are not associated with nodules.

LIFE HISTORY. Larvae hatch from the tiny eggs (see Figure 7-77) soon after they are laid, and larvae or eggs may be present in the feces. If larvae are ingested by maggots *(Musca domestica* for *D. megastoma* and *H. muscae; Stomoxys calcitrans* for *H. microstoma),*

FIGURE 4-147. *Physocephalus sexalatus.*

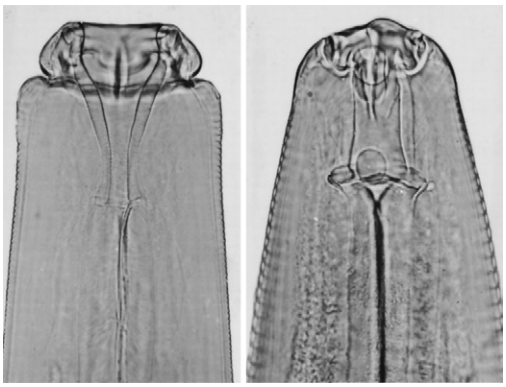


FIGURE 4-148. *Draschia megastoma* and *Habronema muscae.*

they develop to infective third-stage larvae in a little more than a week. The infective larvae migrate to the head of the fly and collect within the labium. When a fly alights on a warm, moist surface such as the muzzle, the ocular conjunctiva, or cutaneous wounds of a horse, the larvae change hosts. Those larvae that are swallowed presumably complete their life histories, whereas those that enter wounds have probably reached an impasse. However, from a veterinary standpoint, these aberrant larvae are extremely important because of the granulomas they induce.

IMPORTANCE. Although *Draschia* and *Habronema* species are unimportant as stomach parasites, their larvae are responsible for persistent cutaneous granulomas called *cutaneous habronemiasis* and a variety of colloquial names ("swamp cancer," "bursatti," "summer sores," "esponja"). These granulomas develop in minor wounds and in areas of skin subjected to more or less continuous wetting. In pastured horses the skin adjacent to the medial canthus of the eye may be drenched in tears stimulated by the presence of flies and very attractive to them. Typical cutaneous habronemiasis lesions are characterized by an initial rapid production of granulation tissue that steadfastly refuses to resolve during fly season, by the subsequent appearance of caseocalcareous nodules in this granulation tissue, and by the presence of *Draschia* or *Habronema* larvae.

Pruritus is intense, and secondary injury may result from the horse's efforts to find relief. Habronemic conjunctivitis usually assumes the form of an ulcerated nodule containing caseocalcareous foci and situated near the medial canthus. Such nodules tend to abrade the cornea and must be removed surgically to prevent or alleviate keratitis (Underwood, 1936; Rebhun et al, 1981).

TREATMENT. Ivermectin and moxidectin are the treatments of choice for adult *Habronema* and *Draschia* species. Ivermectin is approved for the treatment of summer sores caused by larvae of *Habronema* and *Draschia* species. Infections, although rather rare, still occur in the United States, with 63 of 12,720 horses entering the Equine Field Service of the University of California–Davis Veterinary Medical Teaching Hospital between January 1988 and June 2002 (Pusterla et al, 2003). These horses were treated by surgical excision (seven) or medically (56); all were also treated with ivermectin. Gritty masses on conjunctival membranes must be excised to prevent injury to the cornea. In the past few years, cases have been reported from the United Arab Emirates, Israel, as well as a truly impressive one from the United Kingdom (Schuster et al, 2010; Yarmut et al, 2008; Down, Hughes, and Henson, 2009). The case in the United Kingdom appears to be the first reported there in 20 years. The large lesion, a 3-inch hemispheric mass on the dorsal pastern of the right foreleg, was treated by surgical excision.

Superfamily Filarioidea

The superfamily Filarioidea contains two families: One group contains forms that are familiar to most people with an interest in veterinary or human medicine, as well as a second group that is of interest to zoologists and a few veterinary professionals. The better known group is the family Onchocercidae. This group contains the dog heartworm, *D. immitis,* the filarioid of greatest importance in veterinary medicine. It also includes some of the most important nematode parasites of man in tropical climates. *Wuchereria bancrofti* and *Brugia malayi* cause the acute lymphangitis and chronic elephantiasis of bancroftian filariasis, and *Onchocerca volvulus* causes the ophthalmitis of "river blindness." The other group is the family named the Filariidae. (Yes, this is horrible: There is the Filarioidea, known colloquially as the "filarids" or to some as the "filarioids," meaning the superfamily containing the heartworm genus, *Dirofilaria,* and then there is this family the Filariidae, and its subfamily Filariinae, and a genus *Filaria,* which is a parasite of badgers and other carnivores; and then we have the Metastrongyle family Filaroididae containing the lungworm genus *Filaroides*. So, if you say "filaroid," you have a lungworm, and if you say "filarioid," you have a group of worms that includes heartworm, but if you say "filariid," you have a group of filarioids that does not include heartworms. One is never certain where to put one's i's, ii's, id's, oid's, or did's.)

Within the Filariidae are two genera of importance in veterinary medicine: *Parafilaria* and *Stephanofilaria*. These filariids differ from onchocercids in that the adults are near the surface of the skin, where they induce bloody lesions that attract dipteran intermediate hosts to feed. *Parafilaria* of the subfamily Filariinae produces larvated eggs rather than microfilariae, and the transmission is similar to what occurs with *Thelazia; Stephanofilaria,* subfamily Stephanofilariinae, produces lesions as well, but it produces microfilariae rather than larvated eggs. You typically find the adults of the Filariids near the lesions where they are depositing their offspring.

The Onchocercidae, in contrast to the Filariidae, can be a long way from the surface of their host because the microfilariae make their way into the blood or are generally dispersed in the skin far from where the adults are found. The Onchocercidae tend to be rather long and thin white- to cream-colored worms. They are found typically in tissue spaces and body cavities, or sometimes within the vasculature or lymphatic system. They tend to be without marked cuticular ornamentation or lips and have almost no buccal capsule. Often the tail of the male has a spiral flexure. All filarioids are transmitted by bloodsucking insects in which vermiform embryos called **microfilariae** develop into infective third-stage larvae. The microfilariae either circulate in the blood of the definitive host (e.g., *Wuchereria, Brugia, Dirofilaria, Acanthocheilonema, Setaria* species) or accumulate in the dermal connective tissues (e.g., *Onchocerca, Elaeophora,* and *Cercopithifilaria* species). In either case the microfilariae are ingested and the infective larvae deposited when the insect feeds on the definitive host. The subfamilies of the Onchocercidae that will be discussed here are the Onchocercinae, the Dirofilariinae, and the Setariinae.

DIROFILARIA; SUBFAMILY DIROFILARIINAE

DIROFILARIA IMMITIS. *Dirofilaria immitis,* the inexorable dreaded thread, is one of the most deadly parasites of dogs wherever it is found. It probably arrived in the Americas with Columbus or soon thereafter and settled in as a major threat to dogs throughout the Americas; it is now found throughout the United States. In spite of marvelous control potential since the approval of ivermectin as a preventive 25 years ago, the threat remains. Concerns have recently arisen as to the possibility that there could be resistance to the macrocyclic lactones that are the mainstay of prevention (Bowman, 2012). It must be hoped that this is not the case, but if it is the case, it is imperative that it be recognized and the resistant phenotypes contained if at all possible.

IDENTIFICATION. These worms are parasites of the pulmonary arteries. The adult males are 12 to 20 cm long, and the females are 25 to 31 cm long (10 to 12 inches). The faces of these large (up to 30 cm long) white worms are very plain indeed (Figure 4-149). The dog and its close relatives are the natural hosts, but infection also occurs in cats (Calvert and Mandell, 1982; Dillon et al, 1982) and ferrets *(Mustela putorius furo)*. As few as five adult *D. immitis* may prove lethal to a ferret (Campbell and Blair, 1978; Miller and Merton, 1982; Moreland, Battles, and Nease, 1986; Parrott, Greiner, and Parrott, 1984). Human infection is abortive and results in radiographic changes referred to as "coin lesions," which have been misinterpreted as representing neoplasia and can lead to unnecessary thoracic surgery (Theis, 2005).

LIFE HISTORY. The life history, as outlined in Figure 4-150, may involve many different species of mosquitoes as intermediate hosts. Today, mosquito-borne human diseases such as malaria and filarial infections are popularly viewed as tropical diseases, but not too long ago malaria accompanied every summer in the United States. Malaria disappeared when the population density of suitable mosquitoes fell below the level necessary for transmission.

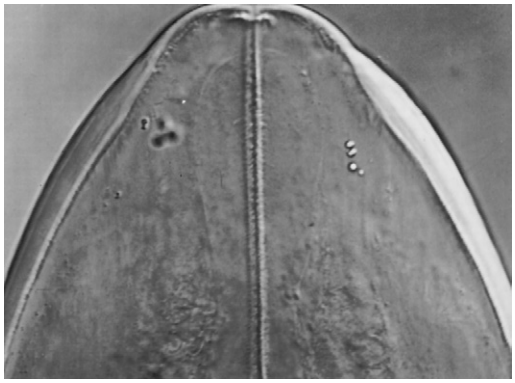


FIGURE 4-149. *Dirofilaria immitis,* stomal end.

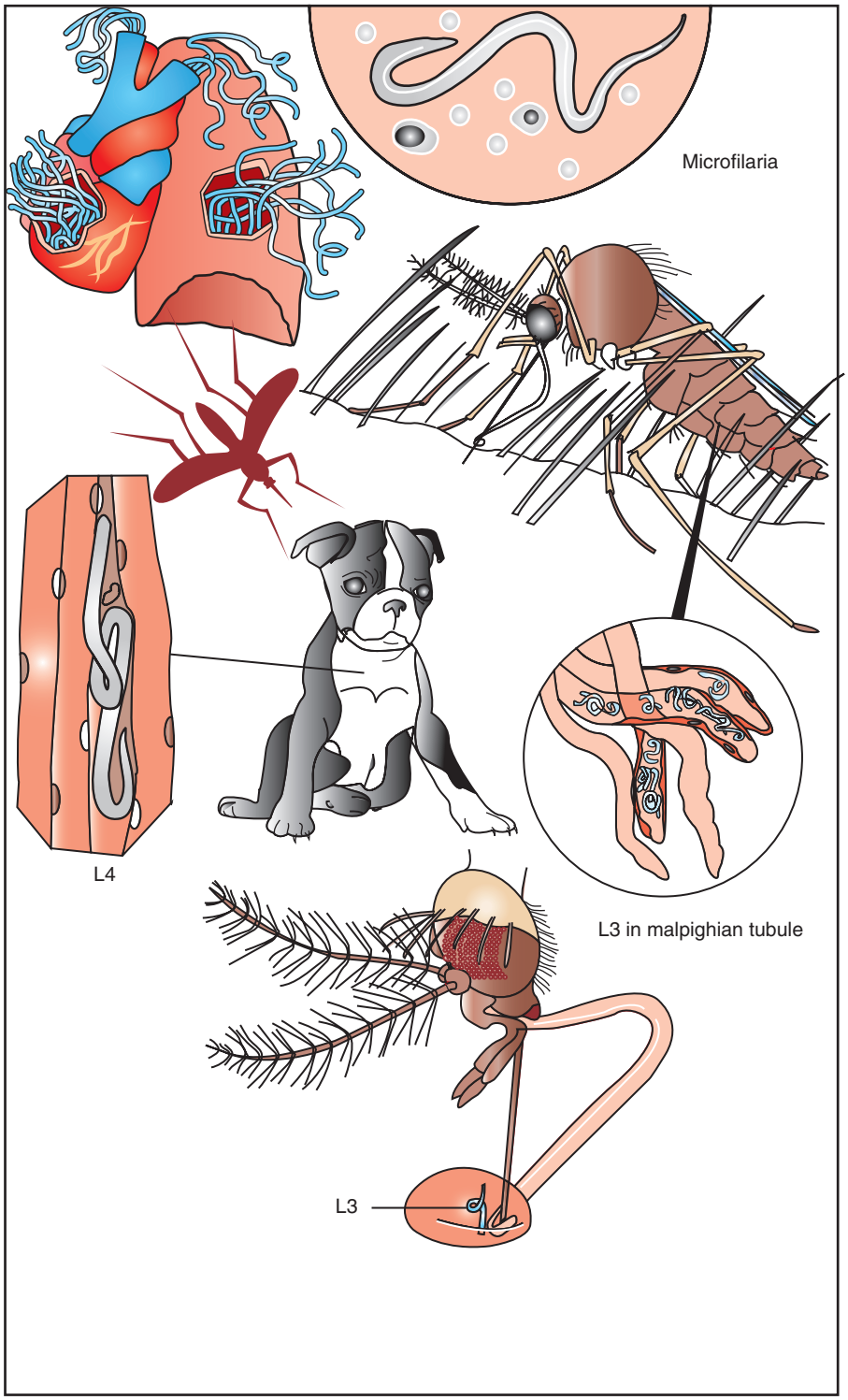


FIGURE 4-150. Life history of *Dirofilaria immitis,* the canine heartworm. Adult heartworms may survive and produce microfilariae for as long as 5 years. Microfilariae circulate in the blood, where they may be ingested by a feeding mosquito. About half of the species of North American mosquitoes are possible intermediate hosts, but significant vector roles have been demonstrated for only a few. Larval development occurs in the malpighian tubules, after which infective third-stage larvae migrate to the salivary glands of the mosquito. Third-stage larvae enter the bite wound when the mosquito feeds on a dog. The molt from third-stage larva to fourth-stage larva occurs within 3 days after the bite of the infecting mosquito. Fourth-stage larvae remain in the connective tissues for several months, with the molt from fourth-stage larva to young adult occurring 2 to 3 months after infection. After the final molt, immature adults (fifth stage) migrate to the pulmonary arteries, apparently by way of the venous circulation. After reaching the right side of the heart, the young adults mature and start to produce microfilariae at 6 to 9 months after infection.

FIGURE 4-151. *Dirofilaria immitis*. Microfilaria **(A)** in an unstained Knott's preparation (the red blood cell ghosts are visible), and infective third-stage larvae from a mosquito **(B)**.

Reduction in mosquitoes came with the drainage of swamps for agricultural purposes, with the construction of roads, and with intentional efforts at mosquito abatement. Heartworm manages to remain endemic and even to spread to regions where malaria has disappeared, possibly because this parasite is less discriminating in its choice of mosquito hosts. Mosquito control, although invisible to the public and most veterinarians, still plays a large part in preventing human disease and probably plays a major role in keeping heartworm infection levels lower than they would be otherwise.

The life cycle of *D. immitis* is initiated when the dog is bitten by an infected mosquito. The cycle is summarized in detail in the excellent review of Abraham (1988). The microfilaria (Figure 4-151) is taken up by a female mosquito with her blood meal. The larva develops to the infective third stage in the mosquito (see Figure 4-151). When the mosquito takes another blood meal, the third-stage larva leaves the mouth parts and enters the bite wound and takes up residence in the skin (Figure 4-152). The third-stage larva that enters the bite wound molts to a fourth-stage larva within 3 days after infection. The young fourth-stage larvae are about 1.5 mm long at this time. The fourth-stage larvae reside in the subcutaneous connective tissues and muscles of the abdomen or thorax for the next 2 to 3 months after infection. Orihel (1961) reported that the molt from the fourth-stage larva to adult occurred 60 to 70 days after infection. Lichtenfels et al (1985) reported that the molt occurred at 50 to 58 days after infection.

The worms are 12 to 15 mm long when they molt to become juvenile adults. The worms enter the pulmonary arteries and heart after they have been in the dog for 70 days (Kotani and Powers, 1982). When worms first reach the right side of the heart and pulmonary arteries, they are 20 to 40 mm (about an inch) long (Orihel, 1961). By 85 to 120 days after infection, they reach lengths of up to 3.2 to 11 cm (Kume and Itagaki, 1955).

FIGURE 4-152. *Dirofilaria immitis* third-stage larvae protruding from the end of the proboscis of an infected mosquito.

Fertilized females appear by 120 days after infection of the dog, and they contain fully developed microfilariae within the sixth month after infection (Orihel, 1961). Microfilariae typically are not found in the peripheral blood for several more weeks. Thus the prepatent period (i.e., the period between infection and the first appearance of microfilariae in the blood) is between 6 and 9 months long. Once worms begin to produce microfilariae, they can continue to do this for over 5 years. The microfilariae circulate in the blood of the dog and are capable of living for up to 2½ years (Underwood and Harwood, 1939).

Mosquitoes are infected when they bite an infected dog. The microfilariae, after remaining in the midgut of the mosquito for a day, make their way to the malpighian tubules, where they penetrate the cytoplasm of the primary cells. Under optimal conditions, the larvae reenter the lumen of the malpighian tubules about 5 days

after infection and molt to second-stage larvae about 10 days after infection and to third-stage larvae by 13 days after infection. The infective third-stage larvae then migrate through the body of the mosquito to the cephalic spaces in the head and proboscis, where they await the chance of gaining entry into a new canine host.

IMPORTANCE. The canine heartworm, *D. immitis,* is by far the most important filarioid parasite of domestic animals in North America. Adult heartworms normally are found in the pulmonary arteries. In heavy infection, worms may be found in the right side of the heart. Worms probably are more common in the right side of the heart at necropsy than in living dogs because of the reduced pressure that occurs as blood stops flowing into the pulmonary arteries. When defunct, the worms are carried deeper into the lungs, where they occlude the pulmonary arterial branches and produce infarcts. Endemic areas exist in all parts of the United States (Rothstein, 1963). Heartworm infection is particularly common along the Atlantic and Gulf Coasts, where salt marsh mosquitoes are prevalent, and in some localities, half the dogs not on preventives that are examined will be found to be infected. Prevalence is also increased along the course of the Mississippi River and its major tributaries such as the Ohio and Missouri Rivers. A lower prevalence is encountered in the midwestern and north-central states. Heartworm is present and transmitted in the western United States (Bowman, Torre, and Mannella, 2007). Heartworm transmission is also occurring in southern Canada (Klotins et al, 2000; Slocombe and Villeneuve, 1993). In the United States, heartworm is a reportable disease in Washington, Utah, and Los Angeles County, in California.

The 6- to 7-month prepatent period is free of any evidence of infection, and the developing and migrating worms cause no disturbance. The patent period, when microfilariae (see Figure 7-38) may be detected in the circulating blood, is the time of clinical illness. In the conventional view, the physiologic burden imposed on the host is attributed in part to the physical obstruction of vessels, heart chambers, and valves by adult worms, and in part to the development of a progressive pulmonary endarteritis and obstructive fibrosis leading to pulmonary hypertension and right-sided heart failure (Adcock, 1961). A remarkable villous proliferation occurs on the endothelium of the pulmonary arteries that grossly causes the surface of the vessel to appear as though it is covered with a lawn of villi (Figure 4-153). Repeated embolisms of the finer arterial branches by defunct adults with infarction and inflammatory response eventually lead to permanent damage to the vascular bed. However, obstruction of capillaries by microfilariae may also play a part in the pathogenesis of heartworm disease.

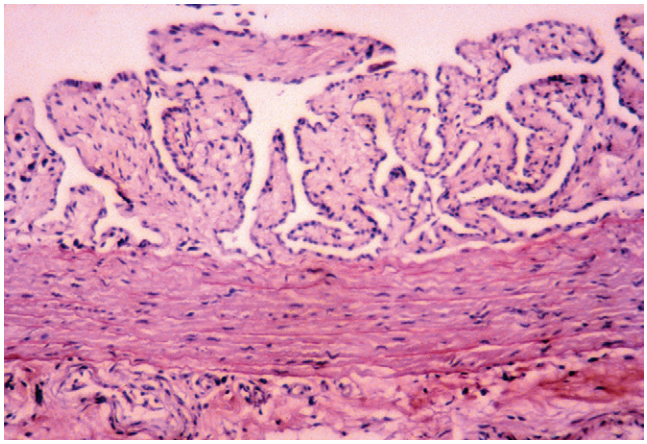


FIGURE 4-153. Histologic section (hematoxylin and eosin [H&E] stained) of the pulmonary artery of a dog infected with *Dirofilaria immitis* showing the villous proliferation present on the endothelium.

Jackson et al (1966) found that dogs with no signs of disease harbored an average of 25 worms, and that about 50 worms were associated with moderate to severe heartworm disease. In dogs with signs of acute hepatic failure, about 100 worms were concentrated in the venae cavae and right atrium. Dogs with typical heartworm disease fatigue easily, cough, and appear unthrifty. Decompensation of the right side of the heart leads to chronic venous congestion, with hepatic cirrhosis and ascites. Pulmonary embolism precipitates acute episodes of respiratory distress, during which blood and worms from ruptured vessels may be coughed up. Postcaval occlusion causes sudden collapse followed by death within a few days from acute hepatic insufficiency. A surgical procedure has been devised for relieving caval occlusion by way of a jugular vein (Jackson et al, 1977; Jackson, von Lichtenberg, and Otto, 1962).

Based on a survey of U.S. veterinarians (12,173 reporting clinics), more than 250,000 dogs in the United States tested positive in 2004 for heartworm (Guerrero, Nelson, and Carithers, 2006). This was up a bit from the 2001 survey (244,000 positive dogs), and it is believed that the survey underestimated the actual prevalence of infection. There are 50 million or so dogs in the United States, and this would mean that about 0.5% of the dogs each year (one in 200) are being diagnosed as infected. The CAPC map from 2011 shows that many dogs are diagnosed with heartworm throughout the United States each year (Figure 4-154). This is a preventable disease.

DIAGNOSIS. Infection in dogs with heartworms can be diagnosed with various antigen detection tests or by a finding of microfilariae in the blood. Antigen will appear in the blood about 5 months after inoculation of third-stage larvae. In the normal course of events, microfilariae of *D. immitis* first appear in the circulation about 6½ months after exposure of the dog to the bites of infected mosquitoes. Thus, during the rather long prepatent period, no microfilariae can be detected in blood samples from an infected dog.

In the young dog, even in areas with very high background prevalence in dogs not on prevention, there is very little to be gained by an examination for heartworm infection before it is 6 or so months of age. Thus dogs younger than 6 months old should just be started on monthly prevention, and if there is concern that a dog may have been infected before beginning prevention, it can be checked after 6 months of preventive therapy rather than waiting a year.

Adult dogs that have not been on preventive therapy should be examined first to verify that they are heartworm negative. This can be done with an antigen test. I and others have seen the occasional dog that is antigen negative with very high microfilarial counts (50,000 to 100,000 per milliliter). Thus, if an adult dog is from an area with very high background prevalence, or if its background is unknown, it would probably be wise to also examine a drop of blood under a microscope to look for microfilariae. In this case there is no need to perform a concentration method, because one is looking for the dog that is antigen negative and has very high microfilarial counts to ensure that it does not react to the death of the microfilariae when the preventive is administered. If microfilariae are found in the blood of a North American dog, they most likely belong to either *D. immitis* or *Dipetalonema reconditum;* dogs infected with *D. reconditum* will be antigen negative and typically have fairly low microfilarial counts. (Differentiation of microfilariae of these two species is discussed in Chapter 7.)

If a dog is on a preventive regimen year-round, testing should be performed yearly. With year-round prevention, there is no

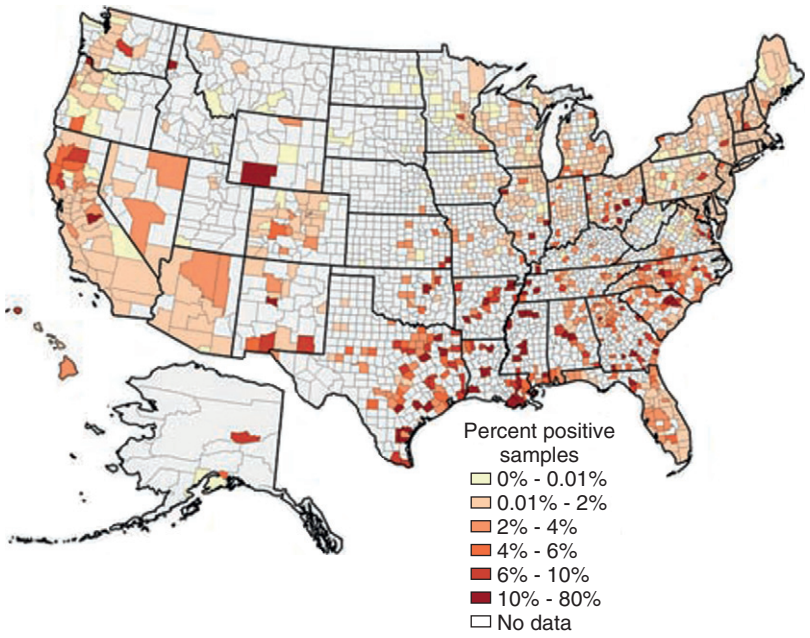


FIGURE 4-154. Map of prevalence by county of *Dirofilaria immitis* antigen–positive samples from dogs (n = 2,356,126) in data collected by the Companion Animal Parasite Council (CAPC) for the year 2011; only counties with more than 20 examined samples are included on the map.

reason why testing cannot be performed at any time during the year, and in these cases and with puppies that started year-round prevention soon after birth, heartworm testing can just become part of the annual examination. It must be remembered that macrocyclic lactone products are anthelmintics, and there is always the potential for resistance. Only by regular checking of dogs on a preventive program can vigilance be guaranteed that would identify and prevent the spread of a resistant form of the parasite if one ever did appear.

A dog receiving monthly preventives only part of the year must be retested before the initiation of therapy each year. Unfortunately, as is the case with all tests, there is a greater chance of a false-positive result when the population tested is nearly certain to be all negative (Peregrine, 2005). In this context, it must be remembered when one is performing an antigen test on a large population of uninfected dogs (by definition, all dogs on preventive regimens should be without infection) that there are going to be false-positive results no matter how good the test is (sensitivity and specificity of 99.9% translate to one false-positive test result in every 1000 tests performed). Thus, if a dog on a preventive regimen tests positive for heartworm, it should be retested and, if it still tests positive, carefully examined for clinical signs that support the diagnosis of heartworm infection. The treated dog is going to receive a large dose of arsenic, so there is fairly good reason to proceed toward treatment with a bit of trepidation.

TREATMENT. Different anthelmintics are used to attack three different parasitic stages of *D. immitis:* adult worms in pulmonary arteries and the right side of the heart, microfilariae in the circulating blood, and larvae from mosquitoes migrating through the tissues on their way to the heart. Treatment of a dog with patent heartworm infection consists of first removing the adult parasites with an arsenical and then later eliminating the circulating microfilariae with ivermectin or milbemycin oxime. Drugs that target the larvae migrating through the tissues are used for prevention.

Melarsomine dihydrochloride has been approved for the treatment of dogs infected with adult heartworms. In dogs with mild to moderate clinical signs, treatment consists of two intramuscular injections (2.5 mg/kg) given 24 hours apart. This treatment can be repeated 4 months later if necessary. Dogs with more severe disease should receive a single intramuscular injection of 2.5 mg/kg followed 1 month later by two such treatments 24 hours apart. Melarsomine dihydrochloride treatment seems to be more effective than the older intravenously administered thiacetarsemide with no increase in the severity of posttreatment hypertension and thromboembolism (Rawlings et al, 1993).

After arsenical therapy has been used, heartworms die slowly over a period of days or weeks and are carried by the pulmonary arteries to the lungs, where they lodge and temporarily obstruct the circulation. Eventually the dead worms are removed by phagocytosis. Probably, if the worms were killed rapidly and simultaneously, the treatment would prove more lethal than the heartworms. However, even with the slow kill, the lungs are gravely insulted during the 4 to 6 weeks after arsenical therapy, and the dog must not be subjected to stress during this period. Occasionally a dog vomits or has fever and respiratory distress after treatment. If these reactions are more than transitory, arsenical medication should be discontinued and supportive therapy, administration of steroids, and enforced rest initiated.

For removal of microfilariae from the circulation after adulticide therapy, dogs can be given a microfilaricidal dose of ivermectin (0.05 mg/kg body weight), ivermectin at the preventive dose of 0.006 mg/kg, or milbemycin oxime at the prophylactic dose of 0.5 mg/kg body weight. These products are not labeled for this use, but because of the lack of drugs approved for the removal of circulating microfilariae, the American Heartworm Society has included these treatments in its recommendations. It seems that in some cases microfilarial clearance is now nearly impossible to attain using macrocyclic lactones alone (see later).

There are those who had recommended that adult heartworms be removed by placing infected dogs on year-round ivermectin therapy for a number of years (*2005 Guidelines for the Diagnosis, Prevention, and Management of Heartworm* [Dirofilaria immitis] *Infection in Dogs,* American Heartworm Society). Fortunately, this is no longer the position of the American Heartworm Society.

Instead of using the preventives as therapeutics, the FDA's label claims for the monthly products should be followed, and dogs should be started on monthly therapy only after they have been cleared of infection with adult worms. If dogs with patent heartworm infections are started on monthly prophylaxis with an avermectin, some 10% to 20% or more of dogs, depending on the product chosen, will continue to have circulating microfilariae in the blood for up to a year, or perhaps longer (Bowman et al, 1992; Bowman and Torre, 2006a, 2006b). If one wanted to create a scenario in which one was selecting for resistant microfilariae, one would take a dog with a high microfilarial count and give it an avermectin for months so that most of the microfilariae in circulation would be "resistant." The conservative approach is to place only dogs that are heartworm free on avermectin preventive therapy.

Heartworms and many other filarioids are hosts to endosymbiotic bacteria of the genus *Wolbachia*. The bacterium is passed transovarially from the female to her offspring (Kozek, 1977). These bacteria are also present in *D. immitis,* in *Onchocerca volvulus,* and in the species that causes lymphatic filariasis in humans. It has been suspected that if these endosymbionts are required for survival, or if their breakdown products are toxic for the nematode host, they might be used as targets for chemotherapy. Cattle infected with the related filarioid *Onchocerca ochengi* were cleared of their adult worms in nodules by treatment with ocytetracycline (Langworthy et al, 2000). Unfortunately, the results of trials with *D. immitis* have not been as dramatic. However, it has been shown in one long-term trial that treating dogs with doxycycline prevented the development in mosquitoes of the few microfilariae available for transmission studies (McCall, 2007). It is possible that doxycycline may be having a direct effect on the worms rather than through the *Wolbachia* (Smith and Rajan, 2000), but whatever the cause, this seems an excellent reason to provide doxycycline therapy to dogs undergoing adulticide therapy, preventing potential secondary infection in the lungs around dying worms, and preventing transmission of any residual microfilariae until they are removed with an avermectin.

PREVENTION. Prevention of heartworm infection currently involves the monthly oral or topical administration of a macrocyclic lactone or the injection every 6 months of a slow-release formulation of a macrocyclic lactone (moxidectin) to all dogs exposed to attacks of infectious mosquitoes. A vast majority of dogs on preventive therapy are receiving products that are administered once per month. The prolonged injectable heartworm preventive with moxidectin in a slow-release carrier is currently unavailable in the United States.

A spectrum of avermectin/macrocyclic lactone products is available that when regularly given monthly to dogs will kill any heartworms less than 30 days old. These include ivermectin, milbemycin oxime, selamectin, and moxidectin. Although some of the molecules themselves at heartworm preventive doses have activity against internal parasites, many products have been combined with agents that provide additional internal parasite control or have activity against ectoparasites, mainly fleas. Thus, practitioners can now choose from several different products that will provide dogs with protection against heartworms and will also treat or control internal and external parasites, making it fairly easy to develop a useful program for a pet within any given geographic area or with a specific lifestyle.

A commonly asked question is whether dogs should undergo a heartworm prevention program year-round, for 6 months, or for even shorter periods in regions where the potential transmission cycle may be less than 6 months. Dr. Slocombe and colleagues (Slocombe, Bhactendu-Srivastava, Surgeoner, et al, 1995) and Drs. Knight and Lok (1995) presented isolines for the mean start and end dates of heartworm transmission in Canada and the United States. These isolines are based on a model that includes the average life of a mosquito, the times when mosquitoes are likely to take their first and last blood meals each year, the amount of time required at different temperatures for an ingested microfilaria to become an infectious third-stage larva, and temperature data collected at different national weather collection stations. Thus, by examination of the maps presented, the period of transmission for the locale in which they practice can be determined. The model proposed by Knight and Lok (1995) indicates that there are probably no parts of the continental United States where transmission occurs throughout the year. Thus treatment might be given for 3 months in parts of Canada and for 10 months in parts of Florida, with different starting and stopping dates in various locations from south to north. This model has received support recently by work done in Florida and Louisiana, where mosquitoes (a total of 109,597) were examined year-round with a polymerase chain reaction (PCR) assay for *D. immitis* DNA (Watts et al, 2001). No infected mosquito heads were detected in Gainesville, Florida, or Baton Rouge, Louisiana, in the months of December, January, February, and March.

The practical advantage of applying this model is a reduction in prescriptions for unnecessary preventive treatment in areas where it is not required. If the model is to be applied, other factors must be taken into consideration. First, there are likely to be microclimatic fluctuations (large bodies of water that stabilize temperatures, decaying manure or vegetable matter that raises temperatures, heated industrial effluents, or heat-absorbing natural and artificial surfaces) that allow mosquitoes to feed longer, perhaps much longer, in certain areas within given isolines. Also, several species of mosquito overwinter as adults in some very cold places in North America, and the contained infective-stage larvae may overwinter within diapausing adult female mosquitoes. Second, it is likely that many dogs will travel with their owners, with the effect that isolines will be crossed by many pets during the course of a year. It also is unlikely that most patients will see their veterinarian often enough for the discovery and initiation of preventive therapy to work in all cases. Third, the availability of products that control infection or have been combined with anthelmintics active against intestinal helminths complicates the desire to stop therapy for the pets of some clients during periods when there may be no heartworm transmission. *Toxocara canis, T. leonina,* and *T. vulpis* are all capable of being transmitted even in the coldest months of the year if soil containing infective eggs is disturbed, and *A. caninum* larvae in sequestered sites in the body are known to migrate periodically back to the intestine, where they develop. Finally, the addition of a flea control product to the heartworm preventive adds another reason for consideration of year-round prevention. In a household, it is highly possible that the temperatures will remain such that fleas can continue to cycle throughout the year, even if they are worse in the summer.

With currently available products, there is currently no good reason why any dog under the care of a veterinarian should become infected with heartworm. Thus it is imperative that the practitioner carefully consider the area in which the practice is located, the individual client, and the stated and suspected behaviors of the pet when formulating a plan for each individual going on a preventive program. However, in the design of specific programs for individuals, it is important to remember that clients do converse with one another, and difficulties will arise when all clients and pets are not treated equally if the reasons behind the specific recommendations are not made very clear.

PERSISTENT MICROFILARIAE AND ELEVATED MACROCYCLIC LACTONES. Although anecdotal reports had described dogs being treated for heartworm having persistent microfilaremias in spite of being placed on monthly therapy, this was not documented until a detailed case report was made on a Katrina rescue dog that was adopted in Canada (Bourguinat et al, 2011). This dog was infected with heartworms and was treated with melarsomine dihydrochloride twice 5 months apart. The dog was microfilaremic after the first treatment in spite of having been placed on a monthly heartworm preventive. Eight months after the first adulticide treatment, the dog was antigen negative, but it still had circulating *D. immitis* microfilariae after multiple treatments with macrocyclic lactones, including two doses of ivermectin at 0.2 mg/kg. The dog was then treated every other week with milbemycin oxime at 1.1 mg/kg, then daily for 7 days at 2 mg/kg, then a month later again daily for 8 days at 2 mg/kg. The dog remained antigen negative and microfilariae positive for longer than 2 years after the second adulticidal treatment.

PREVENTIVE FAILURES WITH THE MP3 ISOLATE. The macrocyclic lactones developed for heartworm prevention include ivermectin (in Heartgard and Heartgard Plus, and in Iverhart Max), milbemycin oxime (in Interceptor, Sentinel, Trifexis, and the approved but not yet released Sentinel Spectrum), selamectin (in Pfizer's Revolution), and moxidectin (in Bayer's Advantage Multi and Pfizer's ProHeart 6). Several generic formulations of Heartgard Plus (e.g., Iverhart Plus, PetTrust Plus, Tri-Heart Plus, Worm Shield) are also available. Most of these products are designed for monthly administration, either orally or topically, the one exception being ProHeart 6, which is designed to provide a slow release of moxidectin to protect dogs against heartworm for 6 months. Persistence of moxidectin in the body of the dog may be noted after the application of Advantage Multi; when hookworm larvae were given to dogs and cats a month after their last of 4 or 5 monthly Advantage Multi treatments, they were protected against the incoming hookworm challenge (Cruthers et al, 2008).

New Animal Drug Application (NADA) reports to the FDA have typically included heartworm products being tested in two trials where dogs have been experimentally infected with heartworms; typically these studies have been performed by two different investigators using larvae from different source animals to infect the dogs. In some instances, the source of the blood with microfilariae to grow the larvae was used only once (i.e., every time a new infection was needed, mosquitoes were fed on a different dog). In other cases, the infections were maintained in experimentally infected dogs so that there was always a source available. When this was done, the worms from these dogs have been called an *isolate* (a *strain* is an isolate that has been reared through passages in animals in the laboratory for longer than 10 years), and the isolate has typically been assigned a name or number to identify it, and to help identify in which trials it had been used. Thus, some studies were done with maintained isolates and some with "wild-caught" isolates used only once. The FDA has required that the details on isolates be "identified as to source, length and method of storage, and the number of animal passages after cryopreservation before infecting canines/felines." MP3 is a heartworm isolate from Athens, Georgia, that has been used in several studies related to efficacy determination. In a publication showing that with this isolate neither Heartgard Plus nor Interceptor were 100% protective following a single treatment, it became apparent that there was something different about this isolate because it was expected that these products would be 100% effective, as they had been during development.

It is now known that MP3 was used in a number of trials where test results showed heartworm infection after dogs had been given a single preventive dose 30 days after they were given the infective third-stage larvae of heartworms. Again, the products do not protect all month (with the exception of ProHeart 6) but instead work by killing any incoming larvae that are 30 days old or younger when the monthly dose of preventive is given. This is the reason for testing these products using a single dose. The 30-day postinfection test is used to show that the products are able to kill any incoming third-stage heartworms as old as 30 days; good evidence suggests that these products become less efficacious the older the worms get (McCall, 2005). In the first published report with MP3, a single dose of neither Heartgard Plus nor Interceptor was 100% protective (Snyder et al, 2011b). Trifexis was shown to be 100% efficacious against MP3 after three doses, but not after only one or two (Snyder et al, 2011a). Sentinel Spectrum was not found to be 100% protective against MP3 with a single dose or with two consecutive doses, but it was 100% protective when given monthly for 6 months; 3, 4, or 5 monthly treatments were not tested (FDA report NADA141-333).

Through published articles, NADAs, and proprietary information from Bayer Animal Health, nine documented studies have demonstrated that some heartworm preventives were not 100% effective after a one-dose treatment of 30-day-old MP3 larvae. The drugs found to be ineffective with one treatment included Heartgard Plus (two studies), Trifexis, Interceptor (two studies), Sentinel Spectrum, Revolution, and an oral formulation of ivermectin at 1× and 1.5× the Heartgard Plus dose being developed by Bayer. In two cases, it appears that products—Trifexis and Sentinel Spectrum—were not effective after doses given both 30 and 60 days after MP3 third-stage larvae were given to dogs. Three of these reports of lack of efficacy after a single trial constituted a product comparison study wherein Heartgard Plus, Interceptor, Revolution, and Advantage Multi were given to dogs as a single treatment 1 month after they had been inoculated with 100 third-stage larvae of MP3 (Blagburn et al, 2011). In this study, Advantage Multi was the only product that was 100% protective in 8 dogs after 1 dose; 7 out of 8 of the other dogs had between 1 and 9 worms (an average of 3.3 worms per infected dog).

A practical application of all this work with MP3 is that dogs on heartworm preventives need to remain on heartworm preventives year-round. Also, veterinarians need to be rigorous in testing dogs before they start prevention, as is stated on the label, and they need to require that dogs on preventives are tested annually to ensure that they are being protected as expected. Again, preventive products need to be reserved for prevention, and treatment of adult heartworms should be provided with melarsomine dihydrochloride. Also, now after treatment, it is critical for the purpose of protecting other dogs to check and see whether the microfilariae have cleared. If after the dog has been moved onto a monthly preventive, the microfilariae have not cleared, they need to be cleared using elevated doses of macrocyclic lactone with the possible addition of doxycycline to the treatment regimen.

FELINE HEARTWORM. Awareness of infection with *D. immitis* in cats has increased; in 1995, the American Heartworm Society first published *Guidelines for the Diagnosis, Treatment, and Prevention of Heartworm* (Dirofilaria immitis) *Infection in Cats,* and current guidelines can be obtained at the Society's website (www.heartwormsociety.org). The cat differs from the dog in several major respects relative to heartworm infection. First, cats tend to harbor very few adult worms and to remain amicrofilaremic. Thus examination of blood through concentration methods usually is not a reliable detection method, and circulating antigen may not be

sufficient for detection by the different antigen detection assays. Antigen and antibody tests are available for use in cats. Antibodies will simply show exposure, and antigen may be negative if cats are infected with few worms. Second, cats can develop severe disease owing to the migration of young adult heartworms in their lungs even if they do not develop patent infection; this syndrome is called HARD, for heartworm-associated respiratory disease (Blagburn and Dillon, 2007). Third, cats can have heartworms migrate to ectopic sites and can die suddenly as a result of aberrantly migrating heartworms. Fourth, adulticide therapy in cats usually is reserved for animals in stable condition that nevertheless continue to have clinical signs not controlled by empiric therapy. Fifth, surgical removal of the worms is considered a potential option in cats. Several products that will prevent heartworm and other internal parasites, including ivermectin, milbemycin oxime, selamectin, and moxidectin with imidacloprid, can be topically or orally administered to cats monthly. As with similar products for use in dogs, these products all have slightly different spectra of activity, giving the veterinarian the opportunity to pick a product that best fits the practice.

DIROFILARIA REPENS. *Dirofilaria repens* is a parasite of dogs in Eurasia and Africa. This species of *Dirofilaria* lives threaded through the subcutaneous tissues; males are about 6 cm long and females are 10 to 17 cm. The microfilariae are in the blood, and transmission occurs via mosquitoes. It has typically been found in southern Europe, but its range has spread in recent years. It is now found in Hungary, the Czech Republic, Slovakia, northern Serbia, and Romania, and autochthonous *D. repens* infections in dogs have recently been reported from northern Germany, Austria, and the Netherlands (Genchi, Kramer, and Rivasi, 2011). More than a thousand human cases of ocular and dermal dirofilariasis have been described in Europe as being caused by *D. repens* (Simon et al, 2012).

***ONCHOCERCA;* SUBFAMILY ONCHOCERCINAE.** Members of the subfamily Onchocercinae are characterized as having a long tail without caudal alae, as are found on male specimens of the Dirofilariinae. *Onchocerca* adults, although large, are likely to escape notice because they are intricately woven into the deep connective tissues. Once found, they are virtually impossible to isolate intact, so specimen bottles tend to contain many fragments of the midsection and very few ends.

Onchocerca cervicalis adults are found in the nuchal ligament of the horse (see Figures 8-111 and 8-112), and the microfilariae (see Figure 7-79) are widely distributed in the dermis and other connective tissues including those of the ocular conjunctiva. Infection is transmitted between horses by species of *Culicoides*. Before the days of ivermectin, in a random survey of pastured horses in Tompkins County, New York, 8 of 12 horses yielded from 1 to 3000 *O. cervicalis* microfilariae per biopsy specimen, a piece of skin weighing about 15 mg (Georgi, 1976b). Microfilarial pityriasis, summer mange, equine dhobie itch, and plica polonica are colloquial names for an intensely pruritic dermatitis conventionally ascribed to microfilariae of *O. cervicalis*.

Herd and Donham (1983) successfully treated 40 horses with dermatitis, alopecia, and pruritus in association with microfilariae of *O. cervicalis* with a single intramuscular injection of 0.2 mg ivermectin per kilogram body weight. Twenty-four hours after medication, the ventral abdomen of four of the horses became edematous. However, this reaction to dead microfilariae subsided over the next few days, and marked clinical improvement followed in all horses 2 to 3 weeks after treatment. Moxidectin at 0.3 to 0.5 mg/kg also will eliminate these microfilariae from the blood of infected horses (Monahan et al, 1995a).

In North American cattle, *Onchocerca gutturosa* adults are found in connective tissues about the nuchal ligament, and *Onchocerca lienalis* are found in connective tissue between the spleen and the rumen. Both species also may be found on occasion in other connective tissue locations. Microfilariae of both species are found in the dermis (see Figure 7-79). The intermediate hosts of bovine *Onchocerca* species are species of *Simulium* and *Culicoides*.

Onchocerca lupi has been associated with ocular lesions in dogs in the southwestern United States and Europe (Sreter and Szell, 2008). Cases can present as conjunctivitis, exophthalmos, periorbital swelling, photophobia, discomfort, lacrimation, and discharge, and fragments of worms are typically removed by forceps from the conjunctiva. In chronic cases, the worms are embedded in pea-sized subconjunctival granulomatous nodules or in cysts in various periocular tissues. Male worms are about 5 cm long, and females, often broken during extraction, are capable of being at least 16 cm long. The microfilariae are found in the skin are very small—only 100 µm long. At this time, the vector is not known. Human cases have now been reported from Turkey and Tunisia (Otranto et al, 2012b). One report has described *O. lupi* being diagnosed in two cats from a shelter in southern Utah; one cat had lived previously in southern Nevada, the other in southern California (Labelle et al, 2011).

***ACANTHOCHEILONEMA;* SUBFAMILY ONCHOCERCINAE.** Members of the genus *Acanthocheilonema* occur in a variety of hosts, including canids, seals, insectivores, and rodents, and it seems that they have a variety of non-nematoceran vectors, fleas, mallophagan and anopluran lice, louse flies, and hard and soft ticks. Adult specimens of the related genus *Dipetalonema* can be encountered as parasites of the peritoneal cavity of American monkeys, in which they are very common (Figure 4-155).

ACANTHOCHEILONEMA RECONDITUM. This is a parasite as the adult in subcutaneous tissues and fascia of dogs and other canids around the world. The adults, as the name "reconditum" suggests, have been viewed by few humans because of their location in the host, their small size, and the fact that they are often few in number. The microfilariae, on the other hand, are rather commonly seen (see Figures 7-36 and 7-37) and are easy to confuse with those of *D. immitis*. *D. reconditum* is nonpathogenic to dogs. Its clinical importance attaches only to confusion of its microfilariae with those of *D. immitis* (Lindemann, Evans, and McCall, 1983).

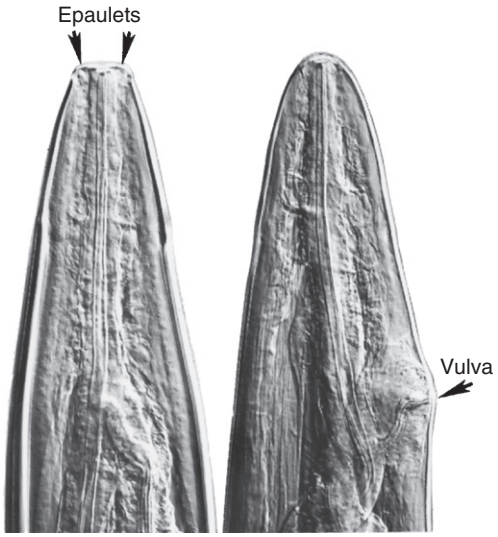


FIGURE 4-155. *Dipetalonema* sp. from the peritoneal cavity of a monkey. *Left,* The dorsoventral aspect of the stomal end. *Right,* The lateral aspect of the stomal end.

LIFE HISTORY. *A. reconditum* develops to the infective stage in the flea *Ctenocephalides felis* and in the amblyceran louse *Heterodoxus spiniger*. Microfilariae taken in with the blood meal develop into infective third-stage larvae in 7 to 14 days. When injected into a dog, these third-stage larvae develop into adult worms in 2 to 3 months (Farnell and Faulkner, 1978; Lindemann and McCall, 1984).

DIAGNOSIS. The small adults of *D. reconditum* cause no pathologic changes to betray their presence but may be seen in a sufficiently lean cadaver by scanning the loose subcutaneous fascia of the limbs and back with a stereoscopic microscope (Nelson, 1962). About 90% of the adults are located in subcutaneous tissues, but a small percentage can be found in the peritoneal cavity (Mello, Maia, and Mello, 1994). The microfilariae circulate in the blood, usually at low densities. However, substantial microfilaremias are occasionally observed. It is not safe to assume that because many microfilariae are present, it must necessarily follow that their parents are heartworms. The microfilariae of *D. reconditum* are distinguished from those of *D. immitis* by their more slender body, lack of taper at the anterior extremity, and the presence of a very much larger cephalic hook in the former species. (Differentiation of these two species of microfilaria is considered in detail in Chapter 7.)

Patton and Faulkner (1992) found that microfilariae in about 50% of 805 microfilaria-positive dogs in eastern Tennessee were the microfilariae of *D. reconditum,* and these authors warn practitioners about the need to make an accurate diagnosis before initiating heartworm adulticide therapy. Most antigen detection tests used in diagnosing *D. immitis* infection are capable of distinguishing between infections with these two parasites.

ACANTHOCHEILONEMA DRACUNCULOIDES. This is a parasite of the abdominal cavity of canids in Africa, southern Europe, and Asia. The males are 1.5 to 3 cm long; the females are 3 to 6 cm long. The microfilariae circulate in the blood. It appears that the parasite can be transmitted by louse flies, *Hippobosca longipennis,* or by the brown dog tick, *Rhipicephalus sanguineus* (Rani et al, 2011; Olmeda-Garcia, Rodriguez-Rodriguez, and Rojo-Vazquez, 1993). Again, as with *A. reconditum,* its major importance is currently seen in the confusion it can cause in making a diagnosis of heartworm or *Dirofilaria repens* infection.

***CERCOPITHIFILARIA;* SUBFAMILY ONCHOCERCINAE.** This parasite gets its name from the fact that it was originally isolated from baboons, a primate in the Cercopithecidae. Other species are found in canids, bovids, American opossums, porcupines, and rabbits. When the life cycles were examined, they were found to be transmitted by hard ticks, the Ixodidae.

It recently came to light that the microfilaria of a yet not completely described *Cercopithifilaria* species is very common in skin samples from dogs in southern Europe (Otranto et al, 2012a). Microfilariae were seen in 22% of the skin samples from dogs in La Vera, Spain; 4% of the dogs in Xanthi, Greece; and 12% and 13% of dogs from Basilicata and Sicilia, Italy, respectively. It has also been shown that this filariid is transmitted by the brown dog tick, *Rhipicephalus sanguineus,* and that in these same areas, developing stages in the different cities were seen in some 5% to 17% of 633 ticks collected from 192 sampled dogs. Two additional *Cercopithifilaria* have been described from dogs: *Cercopithifilaria grassii* from central Italy, which has a microfilaria more than three times as large as those of this species, and *Cercopithifilaria bainae* from a dog in Brazil.

***ELAEOPHORA;* SUBFAMILY ONCHOCERCINAE.** Microfilariae of *Elaeophora schneideri,* the arterial worm of deer, elk, and domestic sheep, produce patches of moist, exudative dermatitis with crust formation on the polls and faces of sheep sent to summer range above 6000 feet (1828 m) in New Mexico, Arizona, and Colorado. Adults up to 120 mm long are found in the carotid, iliac, and mesenteric arteries. It has been reported that nearly 50% of moose in Wyoming are infected (Henningsen et al, 2012). Tabanids are cyclodevelopmental intermediate hosts.

***SETARIA;* SUBFAMILY SETARIINAE.** The worms typically are medium to large and are often found in the abdominal cavities of artiodactyls and equines. *Setaria labiatopapillosa* (Figure 4-156) and *Setaria equina* (Figure 4-157) are large white parasites of the serous membranes of cattle and horses, respectively. The infection is transmitted between hosts by mosquitoes. Sheathed microfilariae (microfilariae inside the stretched eggshell) of *Setaria* species show up on blood smears (see Figure 7-79), and adult parasites are likely to be encountered during abdominal surgery or on the killing floor or necropsy table (Figure 4-158).

Migrating *Setaria* larvae occasionally invade the central nervous system and cause serious neurologic disease, especially when they find themselves in other than their normal host species. Actively motile *Setaria* adult worms are occasionally observed in the anterior eye chamber of horses. Jemelka (1976) described surgical removal of a 4.38-cm-long *Setaria digitata* adult from the anterior eye chamber of a horse suffering from corneal opacity and hypopion.

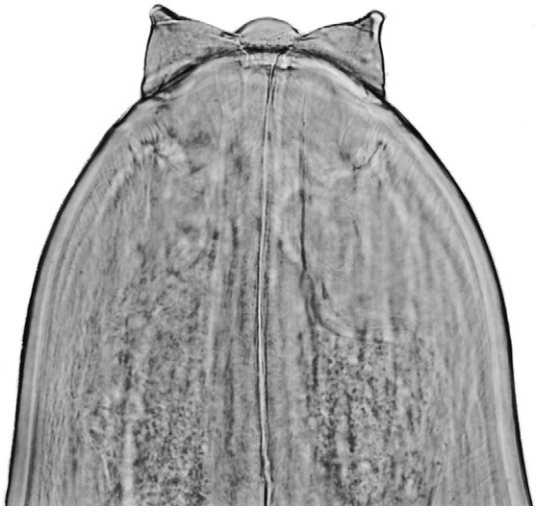


FIGURE 4-156. *Setaria labiatopapillosa,* stomal end.

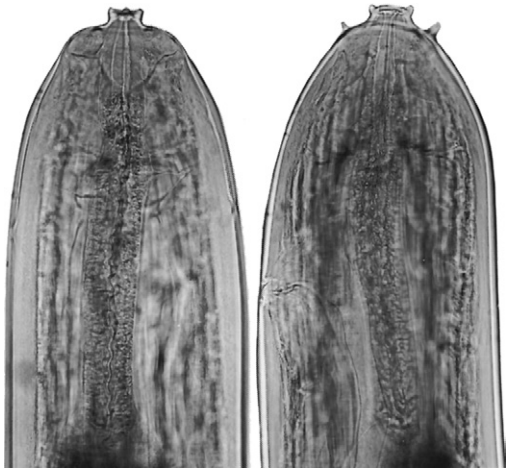


FIGURE 4-157. *Setaria equina,* dorsoventral *(left)* and lateral *(right)* aspects of the stomal end.

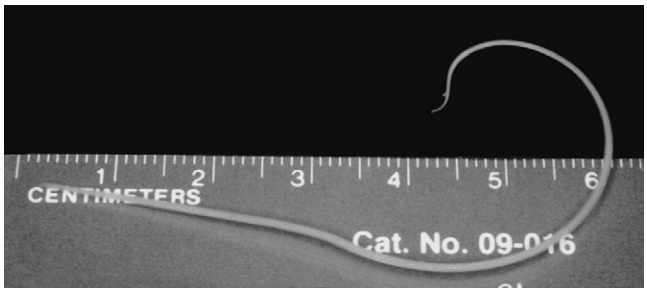


FIGURE 4-158. *Setaria labiatopapillosa,* complete worm, recovered at surgery from a cow.

***PARAFILARIA;* FILARIIDAE, SUBFAMILY FILARIINAE.** Parafilariosis ("summer bleeding") occurs only outside of North America and is caused by *Parafilaria multipapillosa* in horses and *Parafilaria bovicola* in cattle. These parasites live in the subcutaneous and intermuscular connective tissues and, when sexually mature, produce crops of pea-sized nodules that bleed through a tiny pore. The blood escapes in fine drops, runs off in streaks along the hairs, and dries in brown crusts. Eggs and microfilariae of *Parafilaria* species may be demonstrated in this material but never in samples from the circulation. Active bleeding occurs only during daylight hours, especially when horses are exposed to direct sunshine. Baumann (1946) reported that bleeding in affected horses would, as a rule, immediately stop when they were brought into the stable, only to start again when they were led back out into the sunshine. He rarely observed bleeding during cool weather. The activity of the lesions observed by Baumann suggests an adaptation on the part of *Parafilaria* to the habits of flies that feed on blood; they are active in warm weather and avoid shade. It has been shown that *P. multipapillosa* develops in the fat body of *Haematobia atripalpis* (Gnedina and Osipov, 1960).

P. bovicola causes dermal bleeding and subcutaneous bruiselike lesions in cattle in the Philippines, India, Tunisia, Morocco, the former Soviet Union, Rwanda, Burundi, Romania, Bulgaria, South Africa, and Sweden (Bech-Nielsen, Sjögren, and Lundquist, 1982). For a good while, the only place in Europe to really worry about *Parafilaria* was Sweden, where it appeared in the 1970s, but recently, *Parafilaria* has been appearing more widely in Europe. It is in France and in French animals shipped to the Netherlands and Canada, it is in Belgium, and it has been reported in Italy, Austria, and southern Germany (Galuppi et al, 2012). The subcutaneous lesions result in substantial trim losses at slaughter. In South Africa, three vectors have been identified: *Musca lusoria, Musca fasciata,* and a third as yet undescribed *Musca* species. Transmission probably occurs there throughout the year (Nevill, 1975, 1985). These dung-breeding *Musca* species ingest first-stage larvae in the bloody discharge from skin perforations made by adult *P. bovicola* female worms lying in the subcutaneous tissues. Larvae develop to the infective third stage in the body of the fly and are probably deposited in the eyes of cattle when the infected fly feeds on the lachrymal secretions (Nevill, 1975).

***STEPHANOFILARIA;* FAMILY FILARIIDAE; SUBFAMILY STEPHANOFILARIINAE.** Adults and microfilariae of *Stephanofilaria stilesi,* a very small (less than 6 mm long) filarioid, are found in dermatitic lesions on the ventral abdomen of cattle. The infective larvae of *S. stilesi* develop in the horn fly, *Haematobia irritans*.

In India, *Stephanofilaria assamensis* causes a serious dermatitis called *humpsore* in cattle *(Bos indicus).* Lesions may occur on other parts of the body, but the major sites are the hump, neck, and legs.

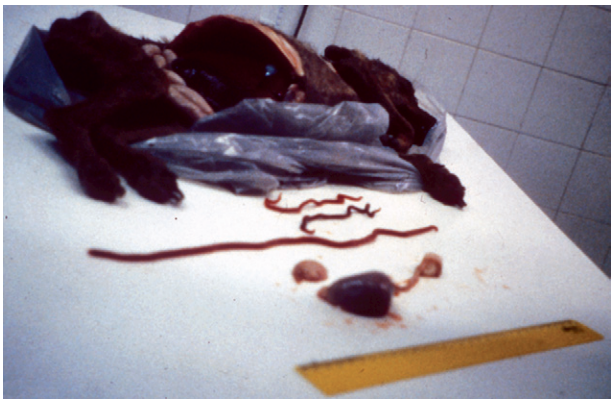


FIGURE 4-159. Three specimens of *Dioctophyme renale* recovered at necropsy from the abdominal cavity of a dog in Brazil. The ruler in the figure is 30 cm long. (Courtesy Dr. Suzanne Wolfson.)

ADENOPHOREAN NEMATODES

ORDER ENOPLIDA

The nematodes in the order Enoplida differ markedly from all the other nematodes discussed to this point. Some classifications consider the Enoplida part of a different class within the phylum Nematoda called the Adenophorea. In the system used here, all the other orders discussed up to here—Strongylida, Rhabditida, Oxyurida, Ascaridida, and Spirurida—would be placed within the Class Secernentea. The Enoplida, discussed here, differ from the Secernentea in two major respects. They do not have tails (i.e., the anus is terminal), so the posterior end of the worm looks like a snipped-off piece of tubing, and if present, there is only a single spicule. Also, the first-stage larvae of all these genera have a little stylet called an *onchiostyle*. For the Secernentea, the final host is almost invariably infected by a third-stage infective larva, whether on pasture, in an egg, or coming out of a mosquito. For the Trichinelloidea, the final host is always infected by eating a first-stage larva, even if a paratenic host is involved. In the case of the Dioctophymatoidea, infection of the final host is similar to that of the Secernentea, at least in the most familiar example, *Dioctophyma renale,* in that the infective stage is the third stage. In some classifications, Adenophorea would be replaced with Enoplea, and Secernentea with Chromadorea; also, there would be potential changes in how the words would end. Things do change: Twenty years ago, the Secernentea were the Phasmidia, and the Adenophorea were the Aphasmidia.

One other worm is included in this section, *Haycocknema perplexum*. These are members of the family Robertdollfusidae, which is a family of parasites related to the Trichinelloidea with a few species in mammals.

Superfamily Dioctophymatoidea

Dioctophyme

Dioctophyme renale, the giant kidney worm of carnivorans, swine, and sometimes humans, is one of the largest species of nematodes (Figure 4-159). Mink are the principal definitive hosts. The female *D. renale,* which may reach 1 m in length and 1 cm in diameter, produces brownish, thick-shelled eggs (68 × 44 µm) with bipolar plugs. Males are somewhat smaller (less than 400 mm) and have a terminal bell-shaped copulatory bursa and one spicule. Eggs are passed in the urine in the one- or two-cell stage (see Figure 7-47), and they they develop, in water, to the first larval stage in a month or longer. Larvated eggs are infective to oligochaete annelid worms,

in which they develop to the infective third larval stage. If infected oligochaetes are ingested by fish or frogs, the larvae invade the tissues of these paratenic hosts but do not undergo development. However, if the infected oligochaete (or paratenic host) is ingested by a dog, the *D. renale* larvae mature and complete the cycle (Karmanova, 1968). In the dog, *D. renale* may be found in the pelvis of the right kidney or free in the abdominal cavity; the latter type of infection is nonpatent. The first confirmed case of parasitism of a domestic cat was reported from Brazil, where an adult male worm was removed from the abdominal cavity upon necropsy after the cat that had presented in prostration expired (Verocai et al, 2009b).

Superfamily Trichinelloidea

The superfamily Trichinelloidea contains some very common parasites of domestic animals. Members of this superfamily are distinguished by their stichosome esophagus, which consists of a capillary tube surrounded by the bodies of a single-file column of gland cells called *stichocytes* (Figure 4-160). There are four genera, *Trichinella, Trichuris, Trichosomoides,* and *Anatrichosoma*, and a group of genera that are here called capillarids that may be seen by most veterinarians. Of these five genera, all lay eggs but *Trichinella,* and the laid eggs have bipolar plugs. Also, the males of the genera in the superfamily, other than adult male *Trichinella,* have a single spicule or at least a spicular sheath, which is often spinate.

Trichinella

The genus *Trichinella* contains nine species and three unnamed genotypes (Krivokapich et al, 2012). The species everyone thinks about most commonly is *Trichinella spiralis,* the species that was domesticated along with the domestic pig; the other species are seen mainly in wildlife. The characters that define the species are molecular, along with other factors, such as cross-breeding to see whether species mixtures produce fertile offspring, resistance to freezing, reproductive capacity in domestic pigs, host range, other biological aspects, and so forth. Six of the nine species and the three unnamed genotypes (T6, T8, and t9) are similar in that they infect only mammals; these *Trichinella* species are: *T. spiralis, T. nativa, T. nelsoni, T. britovi, T. murrelli,* and *T. patagoniensis*. In these species and genotypes, the stage in the muscle fibers of the meat is within a capsule. The other three species have larvae that remain unencapsulated, and they infect mammals and other host types: mammals and birds in the case of *T. pseudospiralis*, and mammals and reptiles in the case of *T. papuae* and *T. zimbabwensis*. Again, the species *T. spiralis* is of greatest concern because it does very well in domestic swine, and great efforts and success have been made of clearing swine of this infection around the world. *T. nativa* is the arctic species; it is able to withstand significant freezing. *T. nelsoni* is the species of sub-Saharan Africa. *T. britovi* is found in Eurasia and North Africa, *T. murrelli* in the Nearctic south of *T. nativa*, and *T. patagoniensis* in South America. As the result of a lot of hard work by swine producers, government agencies, and governments, *T. spiralis* is very rare currently; so now, when people become infected with *Trichinella* and develop trichinosis, they are typically infected with *T. britovi* or *T. murrelli*. Infections in people, especially in the developed world, typically result from ingestion of meats other than domestic pork, but *T. britovi* and *T. murrelli* can have sylvatic cycles that can spill over into and onto swine farms, typically small ones, if the conditions for transmission are available.

IDENTIFICATION. The tiny adults of *Trichinella spiralis* are found embedded in the mucosa of the small intestine of swine, carnivorans, and man (see Figure 8-115); the other species are all very similar in morphology. The male is 1.4 to 1.6 mm long, lacks spicule or spicular sheath, and presents two small knobs over the cloaca. The female is 3 to 4 mm long, with vulva in the midesophageal region and anus terminal. She deposits prelarvae directly into the host's intestinal mucosa (Figure 4-161).

LIFE HISTORY. Predation has provided an efficient channel for the evolutionary development of many parasites. In most instances, the larval parasite lies encysted in the tissues of the prey, and the reproductive adults inhabit the alimentary tract of the predator. Thus in most systems, the predator becomes infected by eating the prey, and the prey becomes infected by ingesting eggs passed in the feces of the predator. However, in the unique life history of *T. spiralis,* both adult and larval stages occur in sequence

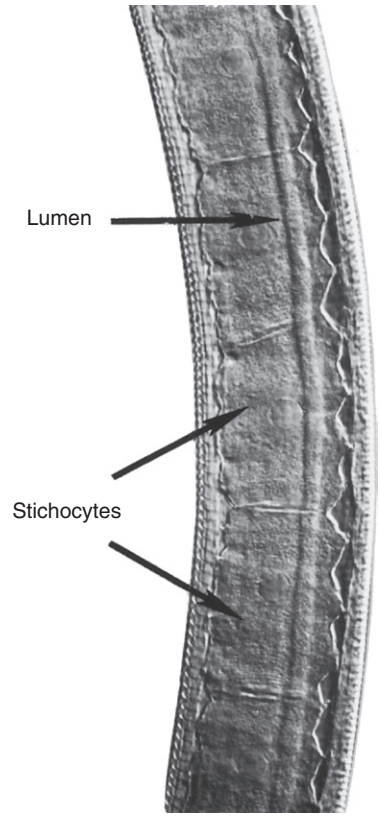


FIGURE 4-160. A portion of the stichosome esophagus of *Trichuris giraffae*.

FIGURE 4-161. *Trichinella spiralis* adult male *(left)* and female *(right)* from the small intestine of an experimentally infected rat; a prelarva is exiting the vulva of the female. (Courtesy Dr. Judy Appleton.)

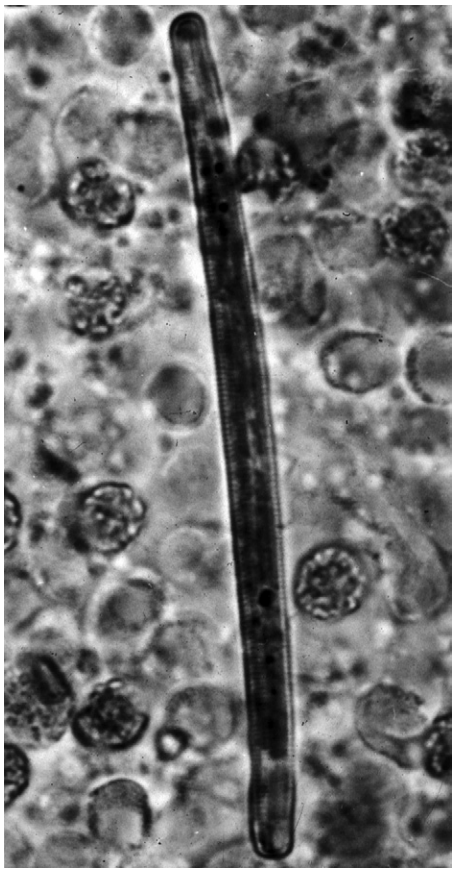


FIGURE 4-162. *Trichinella spiralis* prelarva demonstrated in the blood of a cat by the Knott technique.

in the same host, the tiny adults lying within the villi of the small intestine and the larvae they produce becoming curled up in cysts in the striated muscle (see Figure 8-116). In this sense, for the *Trichinella* life cycle to work, the predator has to become prey.

First-stage larvae of *T. spiralis,* liberated from their cysts by digestive enzymes of the host (see Figure 7-100), invade the intestinal mucosa. Both sexes reach maturity about 2 days after the infected meat is eaten. At 5 days after infection, the viviparous females are giving birth to prelarvae (Figure 4-162), which enter the lymphatics and later the bloodstream to be transported to the muscles (Ali Kahn, 1966). After these prelarvae invade striated muscle cells, they at first lie parallel to the long axes of the fibers and are quite easily overlooked. After 2 or 3 weeks, they have developed into first-stage larvae and roll up in spirals, or like pretzels become enveloped in cysts and are then infective (Figure 4-163; see also Figures 7-93 and 8-116). Old cysts containing defunct larvae calcify.

The intestinal (adult) phase of *T. spiralis* infection varies in duration from a little more than a week in dogs to 3 or 4 months in humans. Immunosuppressant therapy, often instituted to ameliorate the tissue reaction to invading larvae, may prolong the lives of the adult female worms. Fortunately, these are accessible to anthelmintic attack. Almost all mammals can be experimentally infected with *T. spiralis,* but carnivores and omnivores are more likely to become naturally infected. Infection occurs through predation, cannibalism, and carrion feeding. The larvae encysted in muscles are exceptionally resistant to external conditions, including extreme putrefaction.

IMPORTANCE. Human trichinosis usually results from eating raw or undercooked pork or bear. In the United States, outbreaks

FIGURE 4-163. *Trichinella spiralis* larvae in muscle press.

of human clinical trichinosis most often involve small groups of people who have shared uncooked sausage, an undercooked roast from a locally slaughtered pig, or undercooked bear meat. Almost all of these cases have been found to be due to *T. murrelli*. However, in one Illinois outbreak, in which 23 of 50 members of an extended Dutch-German family became ill, the source of the *T. spiralis* larvae in the homemade sausage was USDA-inspected pork (Potter et al, 1976). Occasionally, individuals eat completely raw ground meat ("cannibal sandwich")—a habit more prevalent among beef lovers than pork lovers. However, hamburger often contains a considerable amount of ground pork, whether it is supposed to or not. Outbreaks of trichinellosis in France and other European countries have been traced to consumption of horsemeat. It seems that horses are more willing to eat meat scraps than people expected, and that they are fed table scraps more commonly than expected (Murrell et al, 2004). When the horsemeat or the larvae from human biopsies of cases has been identified, the species involved have been *T. spiralis* and *T. britovi*.

It has been estimated that for humans, ingestion of five *T. spiralis* larvae per gram of body weight is fatal; for hogs, 10; and for rats, 30 (Chandler and Read, 1961). Human trichinosis sufferers may display periorbital edema, myalgia, fever, gastroenteritis, conjunctivitis, pruritus, and skin eruption. Eosinophilia usually exceeds 20%.

Clinical trichinosis in domestic animals may result from both insult inflicted on the intestinal mucosa by the adult worms and the host's reaction to invasion of skeletal muscles by the larvae. A case of trichinosis in a rural Massachusetts cat caused transient hemorrhagic enteritis, during which adult *T. spiralis* worms were found in the feces, and prelarvae were identified in the blood (see Figure 4-149). The phase of muscle invasion was without clinical signs, but eosinophilia persisted for 3 months (Holzworth and Georgi, 1974). A second case in a 3-month-old kitten is typical of the phase of muscle invasion: The kitten was lying helplessly on its side with limbs extended, showed pain on handling, salivated, breathed superficially, and cried constantly (Hemmert-Halswick and Bugge, 1934). Case reports of trichinosis in dogs and cats are few, but how often it may be overlooked or misdiagnosed remains a question.

TREATMENT. *T. spiralis* infection is infrequently diagnosed in cats and dogs, but because both of these hosts frequently consume uncooked meat in the form of scraps and prey, and because the dog displays such a predilection for eating carrion, it stands to reason that canine and feline *Trichinella* infection must in fact be rather common. Treatment is experimental. Cats and dogs experimentally infected with *T. spiralis* have been found to have reduced numbers of muscle-stage larvae after treatment with albendazole 50 mg/kg body weight twice daily for 7 days (Bowman et al, 1993).

CONTROL. Properly cooked trichinae are quite harmless, but a sojourn in the oven does not guarantee that the parasites in the center of a large roast will be made more than uncomfortable unless raised to a uniform internal temperature of 60°C. Some methods of rapid cooking in microwave ovens do not kill all of the encysted *T. spiralis* at 60°C, apparently because the meat does not heat uniformly (Kotula et al, 1983); even roasts that appear well done may contain live larvae when prepared in a microwave oven (Zimmermann, 1983). Until recently, the USDA was recommending that pork products be cooked to an internal temperature of 160°C F or 71°C. In 2011, the recommended temperature was lowered to 145°C (63°C) with a 3-minute rest time before serving, which "will result in a product that is both microbiologically safe and at its best quality."

Freezing of pork products for several weeks (e.g., at –15°C for 20 days) has long been considered adequate to kill *T. spiralis.* However, this approach cannot safely be applied to the sylvatic sibling species, *Trichinella native,* found in bears and other holarctic wildlife, which can withstand storage at –20°C for 6 months (Pozio et al, 1992). In certain countries (e.g., Germany) where the public demands uncooked pork products, meat inspection includes microscopic examination for trichinae in diaphragm muscle squash preparations of every carcass. In the United States the traditional policy has been instead to persuade the public to cook fresh pork thoroughly and to require manufacturers of "ready-to-eat" products to cook or freeze them according to specifications that ensure the destruction of trichinae.

Trichuris

IDENTIFICATION. Adult capillarids are found in mammals and other vertebrates, but adults of the genus *Trichuris* are found only in mammals. The adult body is whip-shaped; the anterior end fine, hairlike, and embedded in the wall of the large intestine (see Figure 8-113); and the posterior end stout and lying free in the lumen (Figure 4-164; see also Figures 7-44 and 8-114). The egg is lemon-shaped with a distinct plug at each pole and contains a single cell when passed in the feces (Figure 4-165; see also Figures 7-24, 7-50, 7-61, and 7-99); the male has a spinate, spicular sheath (Figure 4-166).

LIFE HISTORY. Eggs passed in the feces contain only a single cell and are not infective. An infective first-stage larva develops inside the egg in about 1 month but does not hatch unless swallowed by a suitable host. The infective egg is highly resistant, so animals confined in contaminated environments tend to become reinfected after treatment. Once eggs are ingested, all development occurs within the epithelium of the intestine (i.e., there is no extraintestinal migration). The prepatent period of *Trichuris vulpis* in the dog is slightly less than 3 months, in cattle about 3 months, and in swine about 45 days.

IMPORTANCE. Most canine whipworm infections are symptom free, but heavy infections cause bouts of diarrhea alternating with periods during which normal stools are passed. When 100 to 200 worms or so are present, they are found mainly in the cecum (Figure 4-167), but as the numbers increase, the worms will be found in the wall of the colon. The diarrheal feces often contain much mucus and may be flecked with blood. This infection remains common among dogs in the United States (Figure 4-168).

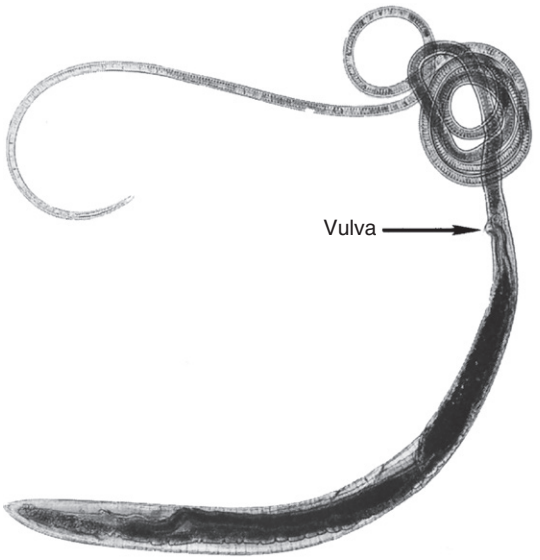


FIGURE 4-164. *Trichuris* sp. from a cat from Puerto Rico.

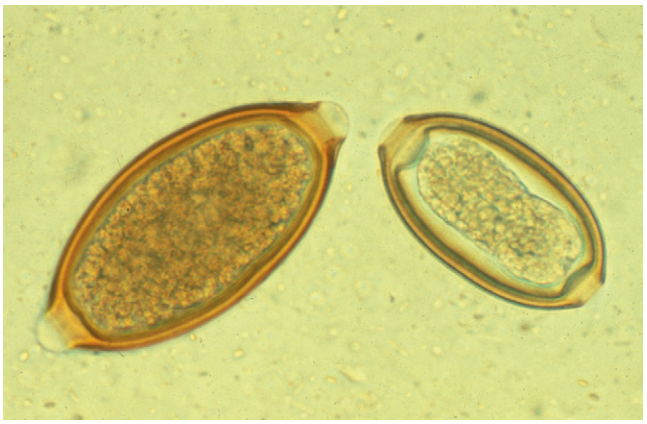


FIGURE 4-165. *Trichuris vulpis* and *Eucoleus boehmi* eggs in a fecal preparation from a dog.

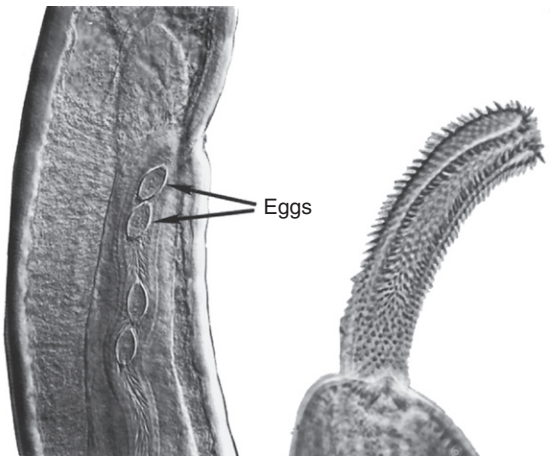


FIGURE 4-166. *Trichuris discolor*. *Left,* Four eggs are seen in the vagina of a female. *Right,* The spinate spicular sheath of the male is protruded.

Trichuris infection is rare and unimportant in cats in the United States but interesting for its novelty (see Figures 4-164 and 7-50). However, *Trichuris* infections seem fairly common in the Caribbean, being reported in 71% of 100 stray cats in St. Kitts, the West Indies (Krecek et al, 2010). The species infecting these cats probably needs to be determined and redescribed. Potential species that have been described from the Caribbean and from Central and South America include *Trichuris felis, Trichuris campanula,* and *Trichuris serrata*.

Ruminants are frequently infected but only occasionally made ill by their respective species of *Trichuris*. Individual young cattle with extraordinarily heavy *Trichuris discolor* infection may suffer massive, sometimes fatal, hemorrhages into the lumen of the cecum (Georgi, Whitlock, and Flinton, 1972). Such cases tend to be isolated and rare. When a bona fide case of bovine whipworm disease is diagnosed, all other members of the herd may be free of clinical signs. Clinically affected individuals may be those that practice peculiar habits favoring ingestion of soil containing *T. discolor* eggs, or they may be afflicted with a hemorrhagic diathesis that magnifies the cost of the minor trauma inflicted on the cecal wall by the parasites.

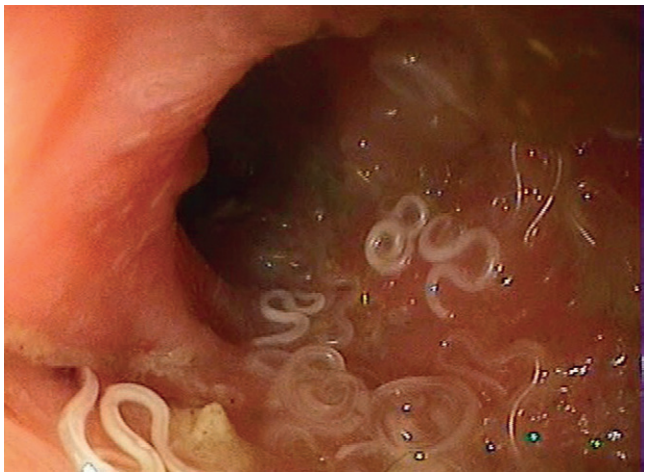


FIGURE 4-167. *Trichuris vulpis* in the cecum of a dog as viewed by video endoscopy.

Very severe *Trichuris suis* infections in young swine cause catarrhal enteritis with clinical signs of diarrhea, dehydration, anorexia, and retardation of growth (Batte et al, 1977). It has been shown that pigs experimentally infected by the feeding of *T. suis* eggs in the presence of antibiotics will have significantly reduced lesions compared with pigs that are simply infected by the whipworms (Mansfield and Urban, 1996). The authors suggest that the complex pathogenesis of necrotic proliferative colitis in pigs may be linked to worm-induced suppression of mucosal immunity to resident bacteria. Control of *T. suis* infection depends on separating swine from the source of infective eggs, which usually is contaminated soil or filthy housing.

TREATMENT AND CONTROL. *Trichuris* infections in beef cattle can be treated with ivermectin, eprinomectin, or doramectin pour-on with 5 mg/10 kg body weight, or with injectable doramectin with 0.2 mg/kg body weight. Ivermectin can be used as a drench in sheep for treatment of *Trichuris ovis* at 0.2 mg/kg body weight.

T. suis infections in swine are susceptible to dichlorvos (Atgard) fed in meal-type feed at 11.2 to 21.6 mg/kg body weight. *T. suis* infections are also susceptible to fenbendazole (9 mg/kg for 3 to 12 days).

Infective *T. vulpis* eggs survive in soil for a long time, and dogs kept in contact with contaminated soils tend to become reinfected after treatment. Lasting success in removing these parasites depends on separating the patient from these eggs. However, in the emphasis on the need for sanitation, an important possibility may be overlooked. If it is assumed that the developing parasitic larvae are

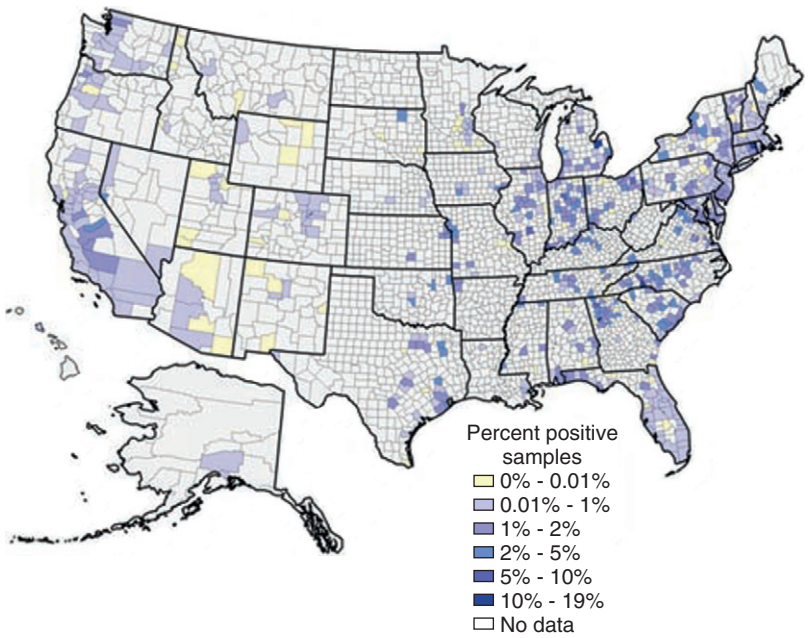


FIGURE 4-168. Map of prevalence by county of *Trichuris vulpis* egg–positive samples from dogs (n = 2,625,642) in data collected by the Companion Animal Parasite Council (CAPC) for the year 2011; only counties with more than 20 examined samples are included on the map. The laboratories processing the samples routinely use zinc sulfate specific gravity of 1.18 for sample processing in the centrifugal flotation; therefore these numbers very likely represent an underestimation of the prevalence in many areas caused by the greater relative density of whipworm eggs compared with other eggs, such as hookworms and roundworms, in canine fecal samples.

more resistant than the adult worms to anthelmintic action, it follows that patent infection is almost certain to recur through maturation of immature forms that have survived a dose of anthelmintic. Most common canine intestinal nematode parasites require only a few weeks to mature, so a second dose of anthelmintic administered 2 or 3 weeks after the first theoretically rids the host of the worms that were unaffected by the first treatment. *T. vulpis* differs from the others in requiring about 3 months to mature, so medication should be routinely repeated three times at monthly intervals to destroy the worms as they mature and prevent them from contaminating the environment.

In the United States, the preferred drugs for treatment of *T. vulpis* infection are fenbendazole (Panacur), milbemycin oxime (Interceptor or Sentinel), febantel (with praziquantel and pyrantel pamoate in Drontal Plus), and moxidectin (with imidacloprid in Advantage Multi). The rare case of *Trichuris* infection in the cat must be handled on an experimental basis because no drug has been cleared specifically for this purpose, although fenbendazole or febantel is probably suitable.

Capillarids

The genus *Capillaria* has been divided by taxonomists into a number of genera on several occasions (Moravec, 1982; Moravec, Prokopic, and Shlikas, 1987). The capillarids comprise a very large group of worms parasitic in all classes of vertebrates, and it would seem that differences in morphology and life cycles would warrant such a division of the group, although not all systematists working in the field agree with some or all the divisions that have been made. Because it is such a large group, the genus *Capillaria* has been divided into a large number of smaller genera with names unfamiliar to most (e.g., *Eucoleus, Hepaticola, Skrjabinocapillaria, Thominx,* and upward of a dozen others), and most of us would be incapable of distinguishing the adults of the different genera.

The adult worms typically are associated with certain epithelial surfaces of their hosts. The veterinary practitioner almost never sees the worms themselves, that is, unless the worms are associated with visible epithelia, allowing their tracts to be observed, as when the worms are in the skin of the African clawed frog (Wade, 1982) or the frontal sinuses of the fox (Supperer, 1953). Thus in most cases, the practitioner sees only eggs passed in the feces. The species found in dogs and cats have been placed into three genera: (1) *Eucoleus* for those found in the airways, (2) *Aonchotheca* for worms found in the intestinal tract, and (3) *Pearsonema* for those that occur in the bladder. The worms found in the liver of rats and in a few other hosts have been placed in the genus *Calodium*. It is possible to differentiate these few capillarid eggs with relative ease, and it seems that this division, at least, is workable.

IDENTIFICATION. The adult body is small and, although it is not whip-shaped, otherwise somewhat resembles that of *Trichuris* species, lying partially embedded in mucous membranes (e.g., bronchial, alimentary, vesical) or buried in tissue (e.g., liver; see Figure 8-117). The eggs differ from those of *Trichuris* species only in detail and are described well by Campbell (1991).

NASAL CAPILLARIASIS. *Eucoleus* (*Capillaria*) *boehmi* was described as a parasite of the frontal sinus mucosae of the fox (Supperer, 1953). This report was largely overlooked, and for a long time it was assumed that capillarids found in the nasal and paranasal sinuses were the same as those found in the bronchi (i.e., *Eucoleus aerophilus*). The eggs of *E. boehmi* can be distinguished from those of *E. aerophilus* by careful microscopic inspection of their surfaces. The surface of *E. boehmi* is covered with tiny pits like those of a thimble, whereas the surface of *E. aerophilus* is a network of branching and anastomosing ridges (Supperer, 1953). Also, the eggs of E. *boehmi* when passed in the feces have undergone a number of cell divisions (see Figure 4-165), whereas the eggs of *E. aerophilus* are passed containing a single cell. A fecal specimen from a dog that had been treated repeatedly over a period of a year for purported intractable whipworm infection was found to contain the eggs of *E. boehmi,* not *T. vulpis,* and the reason for the repeated therapeutic failures became clear. Evinger, Kazacos, and Cantwell (1985) reported success in treating nasal capillariasis with a single oral dose of ivermectin 0.2 mg/kg.

BRONCHIAL CAPILLARIASIS. The life history of *E. (Capillaria) aerophilus* may be direct, or it may involve earthworms as facultative intermediate hosts. Infection of dogs and cats is rarely responsible for more than a slight cough, but foxes on fur farms may harbor pathogenic burdens. Hanson (1933) described the disease in foxes as insidious and chronic, characterized by a rattling and wheezy respiration with spells of coughing and weakness, and by poor growth, unthrifty fur, failure to shed properly, and death due to bronchopneumonia in heavy infections. Low-grade *E. aerophilus* infection is common in cats and dogs. Diagnosis is based on identifying the rather plump, often asymmetric bipolar eggs in the feces or tracheal mucus (see Figures 7-24, 7-50, and 7-61). However, cats and dogs infrequently develop the severe degree of infection observed in captive foxes confined to earthen runs. Cats naturally infected with *E. aerophilus* and treated with topical moxidectin (1 mg/kg) and imidacloprid (10 mg/kg) one time had a 99.8% reduction in the number of eggs in the feces 2 to 3 months after treatment (Traversa et al, 2012).

INTESTINAL CAPILLARIASIS. *Aonchotheca (Capillaria) putorii,* a parasite of the small intestine of bears, hedgehogs, raccoons, swine, bobcats, and various mustelids, is occasionally found in the domestic cat (see Figure 7-50), in which it causes little if any harm. However, the eggs present a differential diagnostic problem with respect to those of other capillarid species found in cats (Greve and Kung, 1983).

Ruminants also host several species of capillarids that fall within the genus *Aonchotheca* (Pisanu and Bain, 1999), none of which are of importance in producing disease in these hosts.

HEPATIC CAPILLARIASIS. Adult *Calodium (Capillaria) hepaticum* worms live in the liver of rats, muskrats, woodchucks, other rodents, and a wide range of occasional hosts, including humans. Eggs deposited by the female worms are trapped in the hepatic tissues (see Figure 8-117), where, for lack of sufficient oxygen, they remain undeveloped until the host is eaten or otherwise dies and disintegrates. Only then do the eggs develop to the infective first larval stage.

URINARY CAPILLARIASIS. *Pearsonema (Capillaria) plica* adults weave the anterior portions of their bodies into the mucous membrane of the urinary bladder and other parts of the urinary tract of dogs, foxes, and wolves. The eggs contain one cell when passed in the urine. The first-stage larva develops in a little more than a month but does not hatch unless ingested by an earthworm, which serves as paratenic host. The definitive host becomes infected by eating earthworms with first-stage larvae in their tissues, and eggs first appear in the urine about 2 months later. Enigk (1950a) claimed that *P. plica* infection caused growth impairment in young foxes, but dogs and cats appear to bear their usually modest worm burdens without inconvenience. Kirkpatrick and Nelson (1987) reported apparent success in treating a case of symptomatic urinary capillariasis in a border terrier with a single dose of ivermectin 0.2 mg/kg, injected subcutaneously.

Pearsonema (Capillaria) feliscati is a parasite of the urinary bladder of the cat and resembles *P. plica* in its biologic properties (see Figure 7-52). An 8-month-old cat with a a distended painful

bladder and urinary blockage was found to have eggs and some 20 larval nematodes in urine sediment; the cat was treated with fenbendazole at 25 mg/kg twice daily for 10 days, and the signs and the capillarid disappeared (Rossi et al, 2011).

Trichosomoides

Trichosomoides crassicauda is a parasite of the urinary bladder of rats. The tiny male *T. crassicauda* lives inside the uterus of its mate (Figure 4-169; see also Figure 8-120). Infection usually is transmitted from mother rats to their offspring before weaning. *T. crassicauda* has been treated in laboratory rats with ivermectin subcutaneously at 0.2 mg/kg or orally at 3 mg/kg (Findon and Miller, 1987; Summa et al, 1992) and in pet hooded rats (Bowman, Pare, and Pinckney, 2004).

Anatrichosoma

Anatrichosoma species are 25 × 0.2-mm-long capillarid-like worms that burrow within the stratified squamous epithelium of the nasal passages of African monkeys and in the buccal mucosa of the American opossum, *Didelphis virginiana*. The female worms deposit 76 × 58-µm bipolar eggs in these burrows. The fully embryonated eggs reach the surface in the normal course of regeneration and desquamation. Antemortem diagnosis is based on demonstration of the eggs on nasal swabs or in skin biopsies (see Figures 7-121, 8-118, and 8-119). *Anatrichosoma cutaneum* gives rise to subcutaneous nodules and edema about the joints of the extremities and to serpiginous blisters of the palms and soles of monkeys.

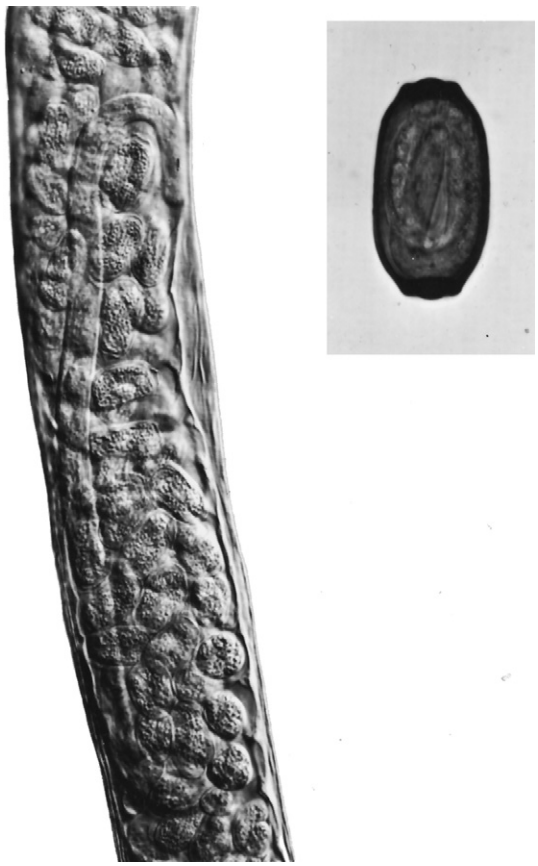


FIGURE 4-169. *Trichosomoides crassicauda* male in the uterus of a female *T. crassicauda (left).* S. H. Weisbroth, who provided this specimen, has described a Millipore filtration procedure for demonstrating the eggs of *T. crassicauda (right)* in rat urine.

Superfamily Muspiceoidea

Haycocknema perplexum

H. perplexum is a nematode that has been seen in three people in or from Tasmania who became seriously ill with a worm that has adults and larvae within muscle fibers (Spratt, 2005). It was described as a member of the superfamily Muspiceoidea in the family Robertdollfusidae. This group of worms has poorly known species in the subcutaneous tissues of mice and bats, the anterior chamber of the eye of cervids, the brain of falconids, the portal and intracardiac veins and epicardial lymphatics of kangaroos and wallabies, the pulmonary arteries of koalas and brushtail possums, and the subcutaneous capillaries of the ears of reindeer (Spratt et al, 1999).

A 14-year-old horse imported from Ireland to Switzerland had masseter atrophy and severe chronic myositis caused by numerous immature and mature female nematodes (Eckert and Ossent, 2006). The authors felt that the infection was due to something that appeared *Haycocknema*-like, but they could not rule out the possibility of *H. gingivalis* because of the degeneration of many of the worms. Attempts were made to compare isolated DNA with that of *Trichinella* and *Halicephalobus*, but there was no amplification.

MISCELLANEOUS WORMS

Thorny-headed worms and leeches are not related to the nematodes, nor are they related to one another. They are lumped together here for want of a logical and convenient alternative.

PHYLUM ACANTHOCEPHALA

The Acanthocephala, or thorny-headed worms, are a small phylum of highly specialized parasites of the vertebrate digestive tract (Figures 4-170, 4-171, and 4-172). There are separate sexes. The body is normally white and flattened in situ but becomes more or less cylindric when placed in water, which is the indispensable first step in preparing specimens for identification. The resulting osmotic turgor forces the retractable, spiny attachment organ or proboscis out of the body, so that the shape and number of spines can be ascertained and the specimen thereby identified (see Figure 4-171). Once the proboscis (and male copulatory bursa) is well protracted, the specimen can be fixed in hot alcohol-formaldehyde-acetic acid (AFA) solution (85 parts of 85% ethanol, 10 parts of stock formalin, 5 parts of glacial acetic acid; if interested in collecting DNA, do not use formaldehyde or acetic acid). These technical details are stressed here because unless specimens are properly prepared, even a specialist may not be able to identify them.

Identification

Acanthocephalans consist of a body and a retractable spiny proboscis by which the parasite attaches itself to the intestinal wall of its host. There is no digestive tract. Nutrients are absorbed through the tegument.

Life History

When the egg is laid, it contains a fully developed larva called an *acanthor* (Figure 4-173). If the egg is ingested by a suitable arthropod intermediate host, the acanthor develops through an acanthella stage (Figure 4-174) into an encysted infective larva called a *cystacanth* (Figure 4-175). The cystacanth is capable of reencysting in a range of vertebrate paratenic hosts, should they ingest the infected arthropod. Frequently the cystacanth even reencysts in its normal definitive host instead of developing to maturity. For example,

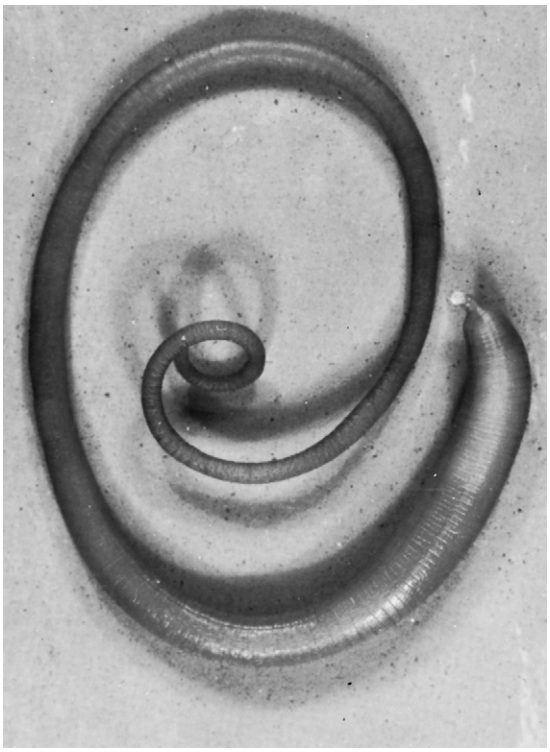


FIGURE 4-170. *Macracanthorhynchus hirudinaceus* (three fourths natural size). This worm typically is white but with fixation appears dark in this photograph.

FIGURE 4-171. *Macracanthorhynchus ingens* proboscis.

Prosthenorchis elegans adults may be found in the intestinal lumen of a monkey, and cystacanths of the same parasite may be found encysted in the peritoneal membranes.

Macracanthorhynchus

Macracanthorhynchus hirudinaceus is a parasite of the small intestine of swine (see Figure 4-155). The body is white, flattened, and transversely wrinkled, which occasionally causes this parasite to be mistaken for a tapeworm. The males are about 10 cm long, whereas the females can be 35 cm long. Development to the cystacanth stage infective for pigs occurs in May beetles, dung beetles, or water beetles in about 3 months. Pigs acquire *M. hirudinaceus* infection when rooting for beetle grubs, but the infected adult beetle is also a source of cystacanths. The prepatent period is 2 or 3 months. Pigs may display no outward signs of *M. hirudinaceus* infection, or

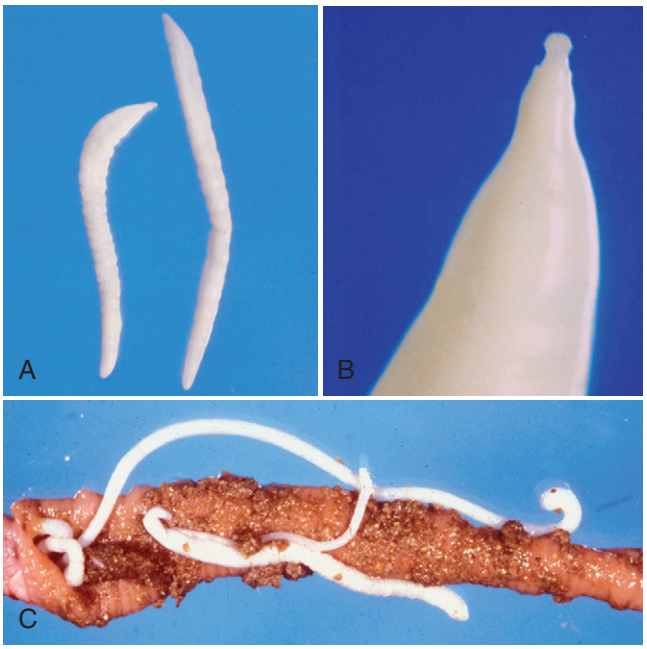


FIGURE 4-172. Adult *Macracanthorhynchus ingens*. Two adults **(A),** anterior end with proboscis **(B),** adult worms in situ in dog intestine **(C).**

FIGURE 4-173. *Macracanthorhynchus ingens* egg containing acanthor larva.

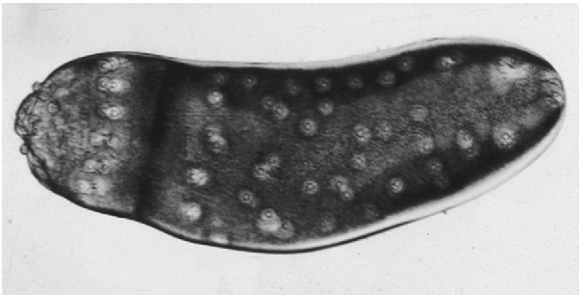


FIGURE 4-174. *Macracanthorhynchus ingens* acanthella from a *Narceus* millipede.

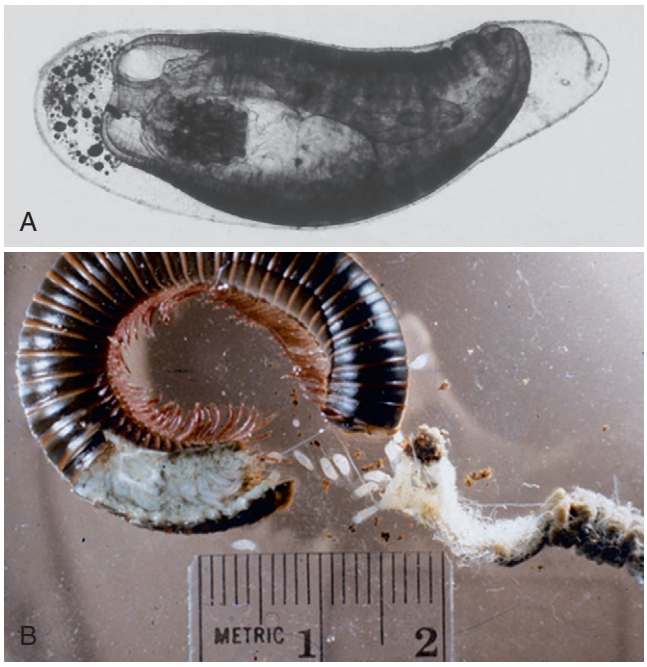


FIGURE 4-175. *Macracanthorhynchus ingens* cystacanth infective larvae **(A)** and as they appear when recovered from a broken *Narceus* millipede **(B).**

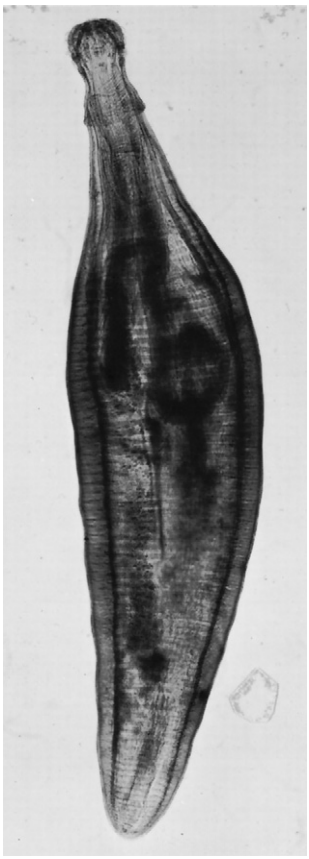


FIGURE 4-176. *Oncicola* sp. from an Arizona coyote, *Canis latrans*. (Courtesy Dr. Frances Phillips.)

diarrhea and emaciation may be seen along with evidence of acute abdominal pain, depending on how deeply the proboscis is embedded in the intestinal wall.

Treatment

No treatment has been approved for *M. hirudinaceus* infection. Benzimidazole anthelmintics may be tried. An in-feed formulation of ivermectin (0.1 or 0.2 mg/kg body weight for 7 days) resulted in 100% removal of adult *M. hirudinaceus* from pigs (Alva-Valdes et al, 1989). Doramectin at 0.3 mg/kg also proved very good in removing *M. hirudinaceus* from pigs (Yazwinski et al, 1997).

Macracanthorhynchus ingens (see Figure 4-72), even larger than *M. hirudinaceus,* is a parasite of the raccoon *(P. lotor)* and the black bear *(Ursus americanus)* and uses millipedes of the genus *Narceus* as intermediate hosts. These parasites occasionally infect dogs that eat the infected millipedes. To eat a millipede requires extraordinary cunning, frightful taste, great excitement, or utter boredom on the part of the dog because the millipedes give off a potent defensive secretion. The raccoon gets around the problem by rolling the millipede about in the dust to exhaust its supply of defensive secretion, but few dogs have learned that trick. Cases in dogs have been treated with ivermectin (Pearce et al, 2001).

Prosthenorchis

Prosthenorchis species are up to 55-mm-long, pink acanthocephalan parasites of primates. *Prosthenorchis* organisms propagate very successfully in monkey colonies by using cockroaches and certain beetles as intermediate hosts. Monkeys become infected when they eat a cockroach containing the cystacanth larvae of *Prosthenorchis* species.

Both chronic and acute disease syndromes have been described for *Prosthenorchis* infection. The chronic course is marked by watery diarrhea of several months' duration, with weakness and progressive emaciation. The appetite remains normal until a day or so before death. The acute course is of less than 1 day's duration and is caused by acute bacterial peritonitis resulting from perforation of the intestinal wall by the proboscis.

Treatment of caged marmosets *(Saguinus mystax)* infected with *P. elegans* has shown that fenbendazole (20 mg/kg body weight for 7 days) was effective in removing these parasites (Demidov et al, 1988). After treatment with 0.2 mg/kg ivermectin failed to treat a white-footed tamarin *(Sanguineus leucopus)*, the acanthocephalan was surgically removed with full recovery (Pérez, Ramírez, and Hernández, 2008).

Moniliformis

Common parasites of wild rodents, *Moniliformis* species use cockroaches as intermediate hosts. The great length (up to 32 cm) and pseudosegmentation of the body invite misidentification of this acanthocephalan as a tapeworm.

Oncicola

Oncicola canis (Figure 4-176), less than 14 mm long, is a parasite of the dog, coyote, and other canids. It uses the armadillo as paratenic host for cystacanths.

PHYLUM ANNELIDA

Class Hirudinea

Leeches are predatory or parasitic worms of the phylum Annelida, which includes the free-living earthworms. Leeches have terminal suckers for locomotion and attachment and move by looping movements, like those of an inchworm. They usually are dark or black in color. Bloodsucking species fasten to the skin or oropharyngeal mucous membrane by means of their powerful suckers, pierce the epidermis, and suck blood. A salivary enzyme, hirudin, acts as an

anticoagulant and ensures a copious flow of blood. In some localities, surface waters abound with bloodsucking leeches that attach to the oropharyngeal or laryngeal mucous membrane when imbibed by the unwary person or animal. Their presence in these locations may cause severe bouts of coughing and choking, during which blood is ejected by the victim. Infection may last several weeks and occasionally causes death. Treatment consists of mechanical removal of the leech.

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CHAPTER 5

Vector-Borne Diseases

Susan E. Little

The term **vector-borne disease** refers to any of a broad array of infectious diseases caused by pathogens that are transmitted by arthropods or other invertebrate, biologic intermediaries. Vector-borne diseases have become increasingly important in recent years as the result of dramatic geographic expansion of the pathogens and the arthropod populations that transmit them, as well as the emergence and increased recognition of novel agents transmitted by vectors. West Nile virus was introduced into New York in 1999 and rapidly adapted to local bird and mosquito populations to spread across the North American continent in only 4 years (Enserink, 2002). Approximately 1.8 million people were infected and 360,000 were made ill in the decade that followed. Increases have also been seen in reported cases of Lyme disease and Rocky Mountain spotted fever in recent years as tick populations have flourished. Although the risk of disease due to West Nile virus in horses or Lyme borreliosis in dogs can be reduced through vaccination, vaccines are not available to protect people from these infections in the United States. Controlling vector populations remains the mainstay of protecting human and veterinary health from the morbidity and mortality caused by vector-borne disease agents.

Transmission of vector-borne pathogens usually occurs on blood feeding by an infected insect or acarine parasite. For example, flaviviruses such as West Nile virus are transmitted rapidly when infected mosquitoes feed on naïve hosts, and warmer ambient temperatures are needed for efficient transmission (Reisen et al, 2006). Historically, transmission of *Borrelia burgdorferi,* the bacterium that causes Lyme disease, has been thought to require as long as 24 to 48 hours of tick feeding; efficiency of transmission increases with increased tick attachment time (Little et al, 2010; Piesman et al, 1987). Other tick-borne agents, such as *Rickettsia rickettsii,* the causative agent of Rocky Mountain spotted fever, may be transmitted in as little as 6 hours of tick attachment (Nicholson et al, 2010). However, infection with vector-borne agents can also result when a vertebrate host ingests a vector, or on contamination of a wound by infectious organisms in the feces of the arthropod intermediary. Ingestion of infected ticks during grooming leads to transmission of *Hepatozoon americanum* and *H. canis* to dogs (Allen et al, 2011), and ingestion of vectors or contaminated food can also result in *T. cruzi* infection (Bern et al, 2011). Wound contamination leads to transmission of *Bartonella henselae* to people when bacterial-laden flea feces are introduced into the skin through a cat scratch (Chomel and Kasten, 2010); this same route is responsible for transmission of the rickettsial agents that cause murine typhus and epidemic typhus from fleas and lice, respectively (Badiaga and Broqui, 2012; Eisen and Gage, 2012). Metacyclic trypomastigotes of *T. cruzi* are deposited in vector feces and then enter the host through the skin wound caused by the feeding bug or through mucous membranes (Bern et al, 2011). Regardless of the means of transmission, the vector, a critical component in disease transmission, engages in a lifestyle that is at least partially parasitic and that somehow contributes to its ability to both acquire and serve as a source of infection to animals.

Diseases transmitted by arthropods have held a central role in veterinary medicine in general, and veterinary parasitology in particular, for over a century. In 1889, Drs. Theobald Smith, Frederick Kilborne, and Cooper Curtice completed their description of transmission of *Babesia bigemina,* causative agent of Texas cattle fever, by *Rhipicephalus (Boophilus)* spp. ticks, and then used that knowledge to design and implement a successful eradication program in the United States (Logue, 1995). Their discovery was the first confirmation of arthropod transmission of an infectious agent, and it paved the way for the elucidation of numerous other vector-pathogen relationships, including recognition of mosquito transmission of *Plasmodium* spp. by Dr. Ronald Ross in 1897 (CDC, 2012), and confirmation in 1901 by Dr. Walter Reed and colleagues, at the suggestion of Dr. Carlos Finlay, a Cuban physician, that yellow fever was also mosquito transmitted (Downs, 1982). In recent years, knowledge about the relative importance and diversity of vector-borne diseases in both veterinary medicine and public health has dramatically expanded, particularly in North America, where a number of new or emerging disease agents have been described, including West Nile virus across the continent, an *Ehrlichia muris*–like agent of people and dogs in the upper midwestern United States, and *R. rickettsii* transmission by brown dog ticks in the southwestern region of the United States (Demma et al, 2005; Murray et al, 2010; Pritt et al, 2011).

The apparent increase in the frequency with which veterinarians and physicians encounter vector-borne diseases has been attributed to several factors, including increasing vector populations as a result of spread to new areas or point introductions of new species of vectors, expanding habitat and increasing wildlife reservoir host populations, and relatively recent biogeographic and climate changes that favor vector populations and may increase transmission rates (Gratz, 1999). Another likely explanation for increased awareness of vector-borne disease agents is increased recognition of these organisms due to improved detection methods that use molecular rather than purely classic microbiologic approaches. Several of the organisms discussed in this chapter have been described primarily by microscopic description augmented by nucleic acid sequence data but have yet to be isolated in culture.

Arthropod vectors transmit disease agents from almost every major class of pathogens, including viruses, rickettsia and other bacteria, protozoa, and helminths. Many of these organisms gain entry to the host via blood feeding, but vector-borne disease agents are by no means limited to the circulation and on initial infection may go on to establish and cause disease in virtually any organ system. Arthropods may serve as **mechanical** transmitters of pathogens, in which the vector harbors a transient infection on, for example, contaminated mouthparts, or the arthropod may be a true **biologic** vector of the disease agent, remaining infected with the organism long term, and in many instances even may be a required part of the life cycle of a given pathogen. When there is a long-standing evolutionary relationship, biologic vectors may become intimately associated with the pathogen and may maintain the infection **transstadially** as they molt from an immature to a mature form, or they may **transovarially** pass the organism from the female to the offspring. In addition, some disease agents can be transmitted horizontally within vector populations through sexual contact between arthropods or via simultaneous cofeeding on a vertebrate host.

Classically, arthropod vectors acquire infection via feeding on an infected vertebrate **reservoir host.** Therefore the disease agent requires both an active vector population and an infected reservoir host system to persist in nature. In some systems, vertebrates may become only transiently infected and the disease agent instead is maintained in chronically infected arthropods and/or is passed transovarially to the next arthropod generation. In these systems, the infected arthropods may infect a vertebrate **amplifying host,** which can develop a short-lived infection capable of infecting the rest of the vector population. In other systems, infection in the vertebrate reservoir hosts may be maintained by transmission by a species of arthropod that does not feed on domestic animals or people. In these instances, a distinct, often related species of arthropod is required to serve as a **bridge vector** to actually bring the infection from the wildlife reservoir host to companion animals, livestock, or people.

Vectors and the organisms they transmit develop intimate associations over evolutionary time. The species of arthropod that can effectively serve as a biologic intermediary for a given pathogen is often limited to one or a few closely related organisms that serve as the **primary vector** for that disease agent. However, in some cases other **secondary vectors** that have the ability to transmit at least some strains of the same species of pathogen are also found. Although these secondary vectors may have a somewhat reduced competency for transmission, they can be regionally important and may facilitate the spread of a given disease agent or allow the persistence of an organism on introduction into a new area. Similarly, a given arthropod species may be infected with and capable of transmitting several distinct agents. Exposure to a vector population that harbors several different pathogens creates a risk of **co-infection,** which may exacerbate the disease state in the animal (Thomas et al, 2001).

The **transmission rate** of a pathogen is defined as the number of new infections that occur per unit time. For vector-borne diseases, characteristics of the vector, the reservoir host, and the pathogen itself all influence the transmission rate. In addition to direct variables such as longevity and home range of vector species and longevity and persistence of infection in the reservoir host, the interactions among vector, reservoir, and pathogen will affect the ultimate transmission rate of a given vector-borne disease agent. For example, the **extrinsic incubation period** required for the pathogen to develop in the vector to the infectious stage will directly influence how rapidly the vector can transmit the organism after acquisition; the length of this extrinsic incubation period may be influenced by ambient temperature. Similarly, the **intrinsic incubation period** required for a vertebrate reservoir to develop a patent infection that can go on to infect subsequent vectors will affect prevalence of infection in the vector population (Reisen, 2002).

To effectively serve as a reservoir host, a given vertebrate species must be not only susceptible to infection and able to infect vectors that are likewise competent to go on to transmit the infection, but it also must share a common niche with the competent vector that allows frequent interactions. For example, the reservoir and the vector must be active in the same habitat, at the same time of day, and at the same time of year for acquisition and transmission to occur. Without frequent ecologic interactions, vector-borne diseases are unlikely to persist even when ample vertebrate reservoir hosts, arthropod vectors, and pathogens are present in a given area.

VIRAL PATHOGENS TRANSMITTED BY ARTHROPODS

A number of important viral pathogens are transmitted by arthropods (Table 5-1). Viral disease agents transmitted by arthropod biologic vectors are commonly referred to as **arboviruses** and include members of Togaviridae, Flaviviridae, Bunyaviridae, and Reoviridae. Mosquitoes are by far the most common vectors of arboviruses, but biting midges (*Culicoides* spp.) and ticks also play an important role in transmission of these agents. A majority of known arboviruses are zoonotic, with wild birds, wild rodents, and, in some cases, domestic animals serving as reservoir hosts and amplifying hosts to infect arthropods and create the risk of infection to both animals and people. Other arboviruses of both veterinary and public health importance are transmitted mechanically by fleas, mosquitoes, or other biting flies such as stable flies, deerflies, and black flies.

TOGAVIRUSES

Arboviruses within the group IV Togaviridae are referred to as alphaviruses. This group is composed of the equine encephalitides, well known to veterinarians and public health officials in North America, as well as viruses more important on other continents, including Chikungunya virus in Africa and Southeast Asia; Babanki virus in Africa; Sindbis virus in Africa, the Middle East, and part of Australia; Barmah Forest virus and Ross River virus in Australia; and Mayaro virus and Una virus in South America. All of these agents are maintained in mosquito vectors and either mammalian or avian reservoir hosts. However, Chikungunya virus is considered urbanized as it is well adapted to direct transmission via a human-mosquito-human cycle. The closely related Mayaro virus does not appear to have yet adapted to the human reservoir, and thus cases are strongly associated with exposure to forest environments where nonhuman primate reservoirs are found. Like the other arboviruses, many alphaviruses have emerged in new geographic locations in recent years to create dramatic epidemics; a Chikungunya outbreak on the Indian continent resulted in an estimated 1 to 6 million human cases and a higher than average number of deaths (Weaver and Reisen, 2010).

The equine encephalitides are the best known of the veterinary alphaviruses in the Western Hemisphere, with a wide distribution throughout North, Central, and South America (Paessler and Pfeffer, 2008). This complex is composed of a large group of related serovars of **Eastern, Western, and Venezuelan equine encephalitis** virus, which are commonly referred to as EEE, WEE, and VEE

TABLE 5-1 Vector-Borne Viral Diseases of Veterinary Importance

Disease	Agent	Virus Genus	Vector	Reservoir Host	Species Affected
TOGAVIRIDAE					
Equine encephalitides	Eastern equine encephalitis virus (EEEV)	Alphavirus	Mosquitoes	Passerine birds	Horses, birds, dogs, pigs, people
	Western equine encephalitis virus (WEEV)	Alphavirus	Mosquitoes	Passerine birds	Horses, people
	Enzootic Venezuelan equine encephalitis virus (enVEEV)	Alphavirus	Mosquitoes	Rodents primarily; also birds, opossums, bats	Horses, people
	Epizootic Venezuelan equine encephalitis virus (epVEEV)	Alphavirus	Mosquitoes	Birds, horses	Horses, people
FLAVIVIRIDAE					
West Nile	West Nile virus (WNV)	Flavivirus	Mosquitoes	Birds	Horses, people, dogs
Japanese encephalitis	Japanese encephalitis virus (JEV)	Flavivirus	Mosquitoes	Birds, horses, pigs	Horses, pigs, people
Tick-borne encephalitis complex (louping ill, Powassan encephalitis, Omsk hemorrhagic fever)	Tick-borne encephalitis viruses	Flavivirus	*Ixodes, Dermacentor, Haemaphysalis*	Various	People, sheep, cattle, horses, dogs, pigs, and others
BUNYAVIRIDAE					
Rift Valley fever	Rift Valley fever virus (RVFV)	Phlebovirus	Mosquitoes	Ruminants	Cattle, goats, sheep, people
Cache Valley fever	Cache Valley virus	Orthobunyavirus	Mosquitoes	Sheep	Sheep
Akabane	Akabane virus	Orthobunyavirus	*Culicoides*	Ruminants	Cattle, goats, sheep
Schmallenberg	Schmallenberg virus	Orthobunyavirus	*Culicoides*	Ruminants	Cattle, goats, sheep
Crimean-Congo hemorrhagic fever	Crimean-Congo hemorrhagic fever virus	Nairovirus	*Hyalomma*	Rabbits, rodents; domestic ruminants	People
REOVIRIDAE					
Bluetongue, epizootic hemorrhagic disease (BT, EHD)	Reoviridae virus Epizootic hemorrhagic disease virus	Orbivirus	*Culicoides*	Ruminants	Sheep, goats, deer
African horse sickness (AHS)	African horse sickness virus	Orbivirus	*Culicoides*	Wild equids, horses	Horses, mules, donkeys
Colorado tick fever	Colorado tick fever virus	Coltivirus	*Dermacentor*	Rodents	People
ASFARVIRIDAE					
African swine fever	African swine fever virus	Asfivirus	*Ornithodorus* species, *Stomoxys calcitrans*	Ticks, wild suids, pigs	Pigs
POXVIRIDAE					
Fowlpox	Fowlpox, canarypox, pigeonpox virus	Avipoxvirus	Mosquitoes, fleas	Birds	Birds
Myxomatosis	Myxoma virus	Leporipoxvirus	Fleas, mosquitoes, *Simulium* species	Rabbits, hares	European rabbits
RETROVIRIDAE					
Equine infectious anemia	Equine infectious anemia virus	Lentivirus	*Stomoxys, Chrysops*	Horses	Horses, other equids

virus, respectively. The serovars of VEE virus are further subdivided into endemic and epizootic types. Related alphaviruses such as Everglades virus, a type of VEE, and the Highlands J virus are often also considered as part of this group of organisms. The equine encephalitis viruses are maintained in bird or rodent reservoir hosts, and infection of people or horses occurs as the result of spillover from this natural cycle.

EEE virus and WEE virus are maintained in passerine bird reservoir hosts and strictly ornithophilic mosquitoes. However, outbreaks can occur when bridge vectors emerge to carry virus from birds to horses and people. Enzootic VEE virus is maintained largely in rodent reservoir hosts and is transmitted by culicine mosquitoes. The maintenance cycle of epizootic VEE virus is less well understood but appears to involve avian reservoir hosts, a large number of mosquito vectors, and, it is important to note, equine amplifying hosts; horses develop high viremia on infection with epizootic VEE virus, providing a ready source of infection to mosquitoes during outbreaks. Although susceptible to infection and disease with many of the viruses in this complex, horses are a source of mosquito infection only during outbreaks of epizootic VEE.

Vaccines are widely available and are commonly used to protect horses from infection with the equine encephalitis viruses (Tabamo and Donahue, 1999; Weaver et al, 2004). In the United States, vaccination is recommended to protect horses from disease associated with EEE and WEE virus infection; annual revaccination before the vector season in the spring is also recommended. Of the two, EEE is both more common and more severe, with mortality in horses approaching 90%. Vaccination against VEE virus in areas of Central and South America where the virus cycles protects both equine and public health as it prevents infection in horses and thus reduces their ability to serve as an amplifying host in the event of an outbreak. The historic use of inactivated VEE virus in vaccine preparations apparently led to amplification of virus in vaccinated equids following escape of residual live virus from incomplete inactivation (Paessler and Weaver, 2009). Subsequent inactivated vaccines, including those available currently in the United States, are based on attenuated strains of VEE virus. Nonetheless, administering inactivated, attenuated VEE virus–based vaccines to horses in the United States is considered controversial for a number of reasons, namely, that disease due to VEE virus has not been identified in this region for several decades, that vaccination may confound critical test results in the event of an outbreak, and that available inactivated vaccines are relatively ineffective compared with the modified live vaccine that would be distributed during an outbreak.

FLAVIVIRUSES

Other important mosquito-borne veterinary viral diseases include those caused by flaviviruses, such as West Nile, Japanese, St. Louis, and Murray Valley encephalitis. All of these viruses are maintained in cycles involving bird reservoir hosts and mosquito vectors. Spillover to humans and other animals results in development of severe, potentially fatal encephalitis. Infection of horses, people, and, rarely, dogs with **West Nile** virus can lead to development of a febrile disease that in severe cases can progress to encephalitis and death. West Nile virus is also fatal to wild birds, particularly crows and American robins. This virus, which was originally described in Africa, was introduced to North America in 1999 and is now well established across the United States. During some years, apparently as the result of climatic conditions and reservoir host population shifts, dramatic increases are seen in the number and severity of West Nile cases reported in people and horses in a given region (Nolen, 2012). Horses do not serve as a source of mosquito infection, but vaccines are available and are widely used to protect horses from the severe disease associated with infection with West Nile virus (Dauphin and Zientara, 2007).

Japanese encephalitis is the most frequent arboviral cause of encephalitis in people worldwide (Weaver and Reisen, 2010). Outbreaks of Japanese encephalitis in Asia usually involve pigs as well as horses and people, and pigs have been shown to serve as an amplifying host for this virus (Wu, Huang, and Chien, 1999). St. Louis encephalitis and Murray Valley encephalitis cause disease in people in the United States and Australia, respectively; wild birds serve as reservoir hosts, but domestic animals are not involved in transmission cycles. Other viruses in this group include dengue and yellow fever, both of which use people as the primary virus host.

BUNYAVIRUSES

Diseases caused by bunyaviruses, like **Rift Valley fever,** are also transmitted by biting flies. Rift Valley fever is endemic in areas of Africa and is maintained in a cycle between mosquito vectors and ruminant reservoir hosts. Infection results in dramatic, widespread abortion storms. Huge epidemics involving hundreds of thousands of cases in ruminants and people have occurred. Although Rift Valley fever virus is zoonotic, with people becoming infected via direct contact with infected animals as well as through mosquito bites, human disease is usually characterized by high morbidity but low mortality (Gerdes, 2004). Other mosquito-transmitted bunyaviruses include La Crosse virus and California encephalitis virus, which cause encephalitis in people, and **Cache Valley fever** virus, which causes congenital abnormalities in sheep in North America. *Culicoides*-transmitted members of this group include **Akabane** virus in Australia and southeast Asia and a novel orthobunyavirus referred to as **Schmallenberg** virus described from Europe; both Akabane and Schmallenberg viruses cause congenital malformations in cattle, sheep, and goats, particularly upon geographic spread to new areas.

REOVIRUSES

Bluetongue and **epizootic hemorrhagic disease** (EHD) are caused by closely related members of the Reoviridae and are transmitted to ruminants via biting midges (*Culicoides* species). *Culicoides variipennis* is considered the principal vector of both bluetongue virus and EHD virus in North America; *Culicoides brevitarsis* is more important in Australia, and *Culicoides imicola* is the major vector in southern Europe, Africa, and the Middle East. More than 25 serotypes of bluetongue are known worldwide and at least 10 serotypes of EHD. Although many ruminants are susceptible, disease caused by bluetongue virus is most common in sheep, is occasionally seen in goats, and is considered rare in cattle (Barratt-Boyes and MacLachlan, 1995). Disease is characterized by ulcerative lesions in the mouth, around the muzzle, on the coronary band, and between the toes. In severe cases, respiratory compromise due to pleural effusion and hemorrhage is manifested as cyanosis, which gives a bluish cast to the tongue. Hemorrhage is also a prominent finding in EHD, which in North America most commonly infects and causes disease in deer. Cattle and sheep are susceptible to EHD virus, but most infections in domestic ruminants appear to result in subclinical disease. The **Ibaraki virus,** which is considered to be a member of the EHD virus group, does cause a febrile disease in cattle that results in both oral ulceration and striated and skeletal muscle degeneration (Inaba, 1975).

African horse sickness (AHS) virus is another *Culicoides*-borne member of the Reoviridae and is transmitted between equids by *C. imicola* and *Culicoides bolitinos*. This virus causes a severe, often fatal

disease of horses and other equids in sub-Saharan Africa; outbreaks have also been reported in the Middle East and in southern Europe. During epidemics, which occur after periods of drought followed by heavy rains, mechanical transmission by other biting flies may occur. Dogs can become infected with AHS virus but do not play a role in the epidemiology of disease. Infected horses develop a fever that may be followed by respiratory distress and/or pronounced facial edema. In susceptible populations of horses, mortality rates from AHS virus infection range from 50% to 95%. Mules and donkeys develop less severe disease with lower mortality, and death is rarely seen in zebras (Mellor and Hamblin, 2004).

TICK-TRANSMITTED VIRUSES

Viruses may also be transmitted by ticks. For example, **Colorado tick fever virus,** a reovirus that causes a febrile disease in people, is transmitted from rodent reservoir hosts to people via *Dermacentor andersoni*. Disease is most commonly seen in the western United States and in Canada and develops as soon as 4 to 5 days after a tick bite, when affected individuals develop a nonspecific febrile flulike illness; the fever is often biphasic. Similar diseases of people and animals include those caused by flaviviruses in the **tick-borne encephalitis** (TBE) complex, such as louping ill, Powassan encephalitis, TBE, and Omsk hemorrhagic fever; all are transmitted via hard ticks, including members of the genera *Ixodes, Dermacentor,* and *Haemaphysalis* (Dumpis, Crook, and Oksi, 1999; Emmons, 1988). Some authors suspect that members of the TBE complex may also be transmitted by fleas, but these relationships are not well defined. **Louping ill** is primarily a disease of sheep, although cattle, horses, pigs, and humans may also be affected; infected individuals develop a febrile illness followed by progressively worsening neurologic disease that in sheep is often characterized by gait abnormalities (Gritsun, Nuttall, and Gould, 2003). Powassan encephalitis is reported primarily from people in the western United States, western Canada, and the former Soviet Union, whereas TBE and Omsk hemorrhagic fever are more commonly seen in people in Europe and northern Asia (Gritsun, Nuttall, and Gould, 2003). All mammals are susceptible to infection with TBE viruses, and neurologic disease has been described in a number of species. Dogs are commonly infected with TBE virus. Although the subsequent febrile, neurologic disease is rare in dogs, it is usually fatal (Pfeffer and Dobler, 2011). In addition to infection from tick bites, human disease can follow ingestion of unpasteurized dairy products, particularly those prepared from goat milk (Dumpis, Crook, and Oksi, 1999).

Crimean-Congo hemorrhagic fever is a severe disease of people in Africa caused by a tick-borne bunyavirus. The virus in maintained in wildlife reservoirs, including rabbits and rodents, and is transmitted to ruminant livestock via *Hyalomma* spp. ticks. Illness generally does not develop in cattle or small ruminants, but people develop flulike symptoms followed by a severe hemorrhagic illness within 1 week after tick bite or exposure to infected blood or tissues of livestock; fatality occurs in approximately 30% of people 2 weeks after infection. In 2009, a novel tick-borne bunyavirus in the phleboviridae, commonly referred to as **heartland virus,** was described from two human patients in northwestern Missouri (McMullan et al, 2012).

African swine fever (ASF) virus, currently classified with other ASF-like viruses as an Asfarviridae, is directly or indirectly transmissible between pigs but can also be maintained in *Ornithodoros* species soft ticks transstadially, transovarially, and sexually for years, and is transmitted to pigs whenever the opportunity arises for feeding to occur (Plowright, 1981). Biting flies, including *Stomoxys calcitrans,* are also able to transmit ASF virus mechanically between pigs (Mellor, Kitching, and Wilkinson, 1987). Infection of pigs results in high fever, anorexia, hemorrhage, and rapid death; with the most virulent strains, mortality approaches 100% (Mebus, 1988). Less virulent strains produce chronic ASF and may result in weight loss, respiratory disease, and enlarged lymph nodes in infected pigs (Mebus, 1988). Treatment and vaccines for ASF are not available.

MECHANICAL TRANSMISSION OF VIRUSES BY ARTHROPODS

Mechanical transmission of viruses by arthropod vectors also occurs. Although **iatrogenic transmission** via needle inoculation can allow transmission, for some of these disease agents the presence of arthropod vectors greatly facilitates transfer within a population. For example, myxoma virus of rabbits causes **myxomatosis** and is mechanically transmitted between rabbits by a number of blood-feeding arthropods, including mosquitoes and fleas; the organisms may survive and remain infectious in a flea for several months. Native rabbits in the Americas develop only mild fibromas when infected, but when the virus is transmitted to European rabbits, a severe and usually fatal infection characterized by high viremia and progressively enlarging skin lesions ensues—characteristics that led this virus to be used in attempts at biologic control of rabbits in both Australia and Europe. However, over time, populations of European rabbits develop resistance to strains of myxoma virus, making control efforts largely ineffective (Kerr and Best, 1998).

Equine infectious anemia virus is another example of a virus that is transmitted mechanically by arthropods. Infection is readily spread between horses in relatively close proximity by blood-feeding flies, particularly horseflies and deerflies. These large biting flies deliver irritating, painful bites, and defensive activity by the horses results in frequent interrupted feeding. The flies quickly return, however, to complete their blood meal on the same horse or another horse in the same vicinity, resulting in mechanical transmission (Issel et al, 1988). Most infected horses do not develop clinical disease. However, in some horses, acute infection can result in high fever and death within 2 to 3 weeks. Others may develop chronic disease associated with intermittent fever, depression, anemia, and petechial hemorrhages. Regardless of the presence of clinical disease, almost all infected horses remain so for life, serving as a reservoir of infection (Coggins, 1984). An agar immunodiffusion test (Coggins test) is widely used to identify these carriers so they can be segregated from noninfected horses and thus transmission can be prevented.

Fowlpox of birds, including poultry, canaries, pigeons, and a variety of wild birds, is caused by several avian pox viruses that can be transmitted mechanically by mosquitoes or through direct contact between infected and naïve birds. Infections have also been associated with the presence of the sticktight flea, *Echidnophaga gallinarum,* on poultry (Gustafson et al, 1997). The viral infection induces the development of hyperplastic cutaneous lesions on unfeathered regions of the skin (beak, cere, legs) that often become hemorrhagic. Occasionally, perhaps owing to infection after inhalation or ingestion of contaminated material, lesions form in the oral cavity or respiratory tract. Infection results in decreased growth and production, but most affected animals survive. Mortality, when it occurs, is associated with severe oral or respiratory tract lesions or, in the case of wild birds, large lesions on the legs, feet, or periocular region that impair mobility or vision, resulting in predation or starvation. No treatment is available once lesions have developed. However, vaccines are available to prevent disease in poultry.

RICKETTSIAL PATHOGENS TRANSMITTED BY VECTORS

The term *rickettsia* refers to any of a large number of obligate intracellular gram-negative bacteria within the order Rickettsiales. Two major families are currently defined within the order Rickettsiales: the **Rickettsiaceae,** which includes the genera *Rickettsia* and *Orientia,* and the **Anaplasmataceae,** which includes the genera *Anaplasma, Ehrlichia, Neorickettsia,* and *Wolbachia;* phylogenetic reorganization of the latter group in 2001 resulted in a number of taxonomic changes, particularly at the genus level (Dumler et al, 2001). Survival of these organisms and transmission between animals are dependent on invertebrate vectors. Ticks are by far the most common vector of rickettsial agents, but some rickettsial organisms, including members of the genera *Wolbachia* and *Neorickettsia,* use helminth vectors (Table 5-2).

Many rickettsial organisms have long been recognized as agents of veterinary and human disease. In recent years, evidence continues to amass that underscores the significance and importance of rickettsia as pathogens (see Table 5-2). Species of rickettsia differ in the primary vector responsible for transmitting infection, the reservoir host(s) important for maintaining a source of infection in nature, and the cell type infected, but all are susceptible to tetracycline antibiotics. Because of this shared susceptibility, tetracycline, specifically doxycycline, is considered the treatment of choice for rickettsial infections in both veterinary and human medicine (Raoult and Drancourt, 1991). To date, with the exception of Potomac horse fever, no commercial vaccines are widely available that reliably protect against infection with rickettsial agents; accordingly, stringent attention to control and avoidance of ticks and other vectors remains the best means of preventing disease.

THE RICKETTSIACEAE

Rocky Mountain Spotted Fever

The best known member of the family Rickettsiaceae in the Americas is *Rickettsia rickettsii*, causative agent of Rocky Mountain spotted fever. In the sylvatic cycle in North America, the organism is transmitted between rodent reservoir and amplifying hosts and to dogs and people via ticks. *Dermacentor* species are the most important vectors of sylvatic *R. rickettsii* in North America and maintain the infection within the tick population transovarially as well as transstadially, thus serving as both reservoir and vector of the pathogen (McDade and Newhouse, 1986). *Rhipicephalus sanguineus* has long been established, along with *Amblyomma cajennense,* as an important vector of *R. rickettsii* in Mexico, Central America, and South America. In 2004, *R. sanguineus* was identified as responsible for an outbreak of Rocky Mountain spotted fever in humans and dogs in the southwestern United States (Demma et al, 2005). Subsequent work has shown that this domestic cycle for Rocky Mountain spotted fever, in which *R. sanguineus* transmits *R. rickettsii* to people and dogs, occurs in several areas in the southwestern United States and is associated with high populations of both dogs and brown dog ticks. Home and kennel infestations with *R. sanguineus* are commonly reported in the southern United States.

Rodents are widely considered the most important vertebrate reservoir amplifying hosts in nature for *R. rickettsii;* however, the involvement of *R. sanguineus,* which prefers to feed on dogs in all life stages, in transmitting infection to dogs and people in some regions suggests that other vertebrate hosts may also play a role in maintaining a source of infection. The full role of other ticks historically implicated as vectors of *R. rickettsii,* including *Amblyomma americanum* or *Haemaphysalis leporispalustris,* warrants further investigation (Breitschwerdt et al, 2011). Understanding

TABLE 5-2 Vector-Borne Rickettsial Diseases of Veterinary Importance

Disease	Agent	Primary Vector	Reservoir Host
Rocky Mountain spotted fever	*Rickettsia rickettsii*	*Dermacentor* spp.	Rodents
Epidemic typhus	*Rickettsia prowazekii*	*Pediculus humanus*	Humans, flying squirrels
Endemic typhus; murine typhus	*Rickettsia typhi*	*Xenopsylla cheopis; Ctenocephalides felis*	Rodents, opossums, dogs, cats, other mammals
Murine typhus–like disease	*Rickettsia felis*	*Ctenocephalides felis*	Dogs, other mammals
Rickettsialpox	*Rickettsia akari*	*Liponyssoides* mites	Rodents
Scrub typhus	*Orientia tsutsugamushi*	*Leptotrombidum* mites	Rodents
Canine, equine, and human anaplasmosis	*Anaplasma phagocytophilum*	*Ixodes* spp.	Rodents, ruminants
Bovine anaplasmosis	*Anaplasma marginale*	*Dermacentor* spp. *Tabanus* spp.	Cattle
Canine cyclic thrombocytopenia	*Anaplasma platys*	*Rhipicephalus sanguineus**	Dogs
Canine ehrlichiosis; tropical canine pancytopenia	*Ehrlichia canis*	*Rhipicephalus sanguineus*	Dogs
Granulocytic ehrlichiosis	*Ehrlichia ewingii*	*Amblyomma americanum*	Dogs, white-tailed deer
Human monocytic ehrlichiosis	*Ehrlichia chaffeensis*	*Amblyomma americanum*	White-tailed deer
Heartwater	*Ehrlichia ruminantium*	*Amblyomma* spp.	Ruminants
Salmon poisoning	*Neorickettsia helminthoeca*	*Nanophyetus salmincola*	Salmonid fish
Potomac horse fever	*Neorickettsia risticii*	*Acanthatrium oregonense*	Bats, caddisflies

*Transmission by *Rhipicephalus sanguineus* is suspected but has not yet been fully confirmed.

the epidemiology of *R. rickettsii* is complicated by the presence of a variety of closely related *Rickettsia* species, which cross-react on serologic assays (Brouqui et al, 2007). In 2010, in recognition of the difficulty involved in accurately interpreting serologic test results in patients, the Centers for Disease Control and Prevention (CDC) changed reporting practices for human cases to standardize reports of infection in people as spotted fever rickettsioses (including Rocky Mountain spotted fever).

Infection with *R. rickettsii* causes a febrile illness that is often quite severe. Disease is most commonly seen in dogs and people, although Rocky Mountain spotted fever has also been reported in cats and horses. The organisms infect and damage endothelial cells, resulting in a progressive necrotic vasculitis; thrombocytopenia is also commonly seen. People with Rocky Mountain spotted fever often develop a nonpruritic rash ("spots") that characteristically appears first on the forearms, wrists, and ankles 3 to 4 days after initiation of fever (Thorner et al, 1998). Most human patients rapidly progress to the classic triad of fever, rash, and severe headache. Fatalities follow severe meningitis or meningoencephalitis and, when antibiotic treatment is not instituted, exceed 20%. Petechial and/or ecchymotic hemorrhages may develop in some dogs, but a rash is not evident; fatalities are also common in dogs.

A number of tick-transmitted spotted fever rickettsia with potential public health importance continue to be described (Azad and Beard, 1998). For example, *R. parkeri* is transmitted by *A. maculatum* ticks, resulting in the formation of a focal necrotic area, termed an *eschar,* at the tick attachment site, as well as a mild spotted fever–like disease (Paddock, 2005). Another organism, *R. amblyommii,* is found commonly in *A. americanum* ticks. Although not known to cause actual disease, *R. amblyommii* does appear to induce antibody responses in patients that may result in confusion on indirect assays for antibodies to *R. rickettsii* due to cross-reactivity (Apperson et al, 2008). In Europe and Africa, *R. sanguineus* transmit *R. conorii,* which causes the relatively milder disease of **Mediterranean spotted fever** (Boutonneuse fever, Marseilles fever) in people. Most patients develop a self-limiting febrile illness characterized by widespread rash and a dark eschar at the tick attachment site, but neurologic complications have been described (Aliaga et al, 2009). Dogs are considered reservoir hosts of *R. conorii*.

Other *Rickettsia*

Other *Rickettsia* species of public health importance include *Rickettsia typhi,* a flea-transmitted organism that causes **endemic or murine typhus,** and *Rickettsia prowazekii,* causative agent of **epidemic typhus** in people and primarily transmitted between people by body lice. Both of these organisms cause a disease in people similar to spotted fever. Historically, large typhus epidemics are associated with war, famine, and periods of mass migration (Raoult et al, 2004). In 1847, almost 16,000 people, most of them recent immigrants fleeing famine in Europe, died from epidemic typhus in Canada; a similar outbreak albeit on a smaller scale occurred that same year in New York City (Gelston and Jones, 1977). Antibiotics can be used to treat *R. prowazekii* infection but must be administered promptly—a need that is often complicated by infrastructure challenges associated with wide-scale epidemics. This pathogen is profoundly dangerous when antibiotics are not available; both Dr. Ricketts and Dr. Prowazek succumbed to the infection while researching the organism in 1910 and 1915, respectively (Raoult et al, 2004). At present, only specialized laboratories with federal approval are allowed to conduct research with this agent.

Murine typhus caused by *R. typhi* is maintained in a rodent-flea cycle and is transmitted to people through wound contamination with flea feces; flea bite has also been suggested as a route for infection. Endemic areas of *R. typhi* transmission are supported by flea infestations on dogs, cats, and opossums (Boostrom et al, 2002; Williams et al, 1992). Human infection results in fever, headache, myalgia, confusion, and, in approximately half of patients, a diffuse rash (Civen and Ngo, 2008). The disease is considered endemic in parts of Hawaii, California, and Texas, and is sporadically reported from other areas (Adjemian et al, 2010). A related organism, *R. felis,* is considered an agent of **flea-borne spotted fever** and is also transmitted by *C. felis* (Parola, 2011). **Sylvatic typhus** in North America is also caused by *R. prowazekii* but is associated with exposure to the reservoir host, the southern flying squirrel *(Glaucomys volans);* an arthropod vector has not yet been identified (Chapman et al, 2009). Cases of sylvatic typhus tend to be less severe than epidemic typhus, with patients developing fever, headache, and malaise, but no rash. Sporadic relapse of previous *R. prowazekii* infection due to waning immunity may lead to **Brill-Zinsser disease** in some patients (Faucher et al, 2012; McQuiston et al, 2010).

Mites transmit some rickettsial pathogens as well. For example, *Rickettsia akari,* causative agent of **rickettsialpox,** a nonfatal febrile disease of people that is predominantly seen in urban areas, is transmitted between mice and to humans through the bite of *Liponyssoides sanguineus,* a murine mite. Infection with *R. akari* results in a febrile disease accompanied by eschar and a diffuse papulovesicular rash (Paddock et al, 2006). Another mite-transmitted disease is scrub typhus, which is caused by *Orientia tsutsugamushi*. Predominantly seen in Asia and Australia, *O. tsutsugamushi* is transmitted to people by trombiculid mites (Boyd, 1997; Chattopadhyay and Richards, 2007). Classically, patients with scrub typhus present with fever, rash, and eschar, although fever of unknown origin is also described (Koh et al, 2010).

THE ANAPLASMATACEAE

The family Anaplasmataceae includes a wide variety of pathogens, including organisms such as *Anaplasma marginale* and *Ehrlichia canis,* which have been known to be important in veterinary medicine for many decades, as well as a number of recently recognized important zoonotic and veterinary pathogens. Novel organisms from this family continue to be described from a variety of vertebrate hosts, including people, dogs, deer, and cattle (Hegarty et al, 2012; Dawson et al, 1996; Gajadhar et al, 2010; Pritt et al, 2011; Yabsley et al, 2008). Although different species tend to infect different cell types and tend to cycle in nature using distinct reservoir hosts and tick vectors, all respond to therapy with doxycycline, and, with the exception of the *Neorickettsia* and *Wolbachia* species, all are primarily transmitted by ixodid ticks. Direct mechanical transmission of some blood-borne agents in this group by biting flies or via intentional or accidental blood sub-inoculation has also been shown to occur (Kocan et al, 2010; Reine, 2004).

Anaplasma Species

Major *Anaplasma* species or species groups important in veterinary medicine include *A. phagocytophilum, A. marginale,* and *A. platys*. *Anaplasma phagocytophilum,* originally referred to as *Ehrlichia equi,* and the human granulocytic ehrlichiosis (HGE) agent in North America and *Ehrlichia phagocytophila* in Europe, causes an acute, febrile disease in people, horses, dogs, and ruminants (Dumler et al, 2005). The organisms primarily infect granulocytes, and the disease produced is referred to as **granulocytic anaplasmosis,** or, in the case of ruminants in Europe, **tick-borne fever.** Dogs with disease due to *A. phagocytophilum* develop lethargy and fever; lameness, vomiting, diarrhea, anorexia, and, occasionally, bleeding diatheses

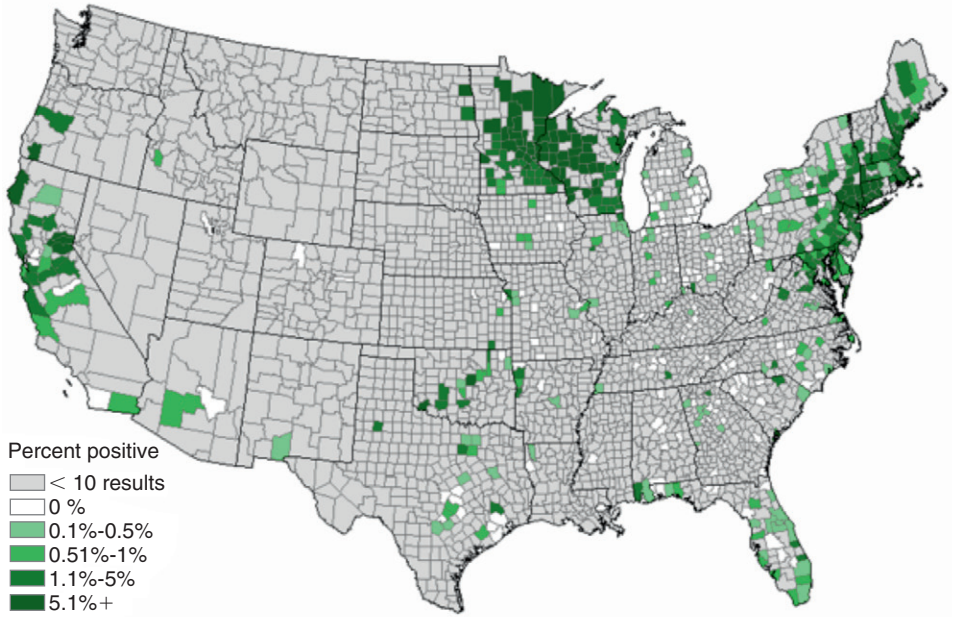

FIGURE 5-1. Distribution of antibodies to *Anaplasma* spp. in domestic dogs in the United States. (Reprinted with permission from Bowman D, Little SE, Lorentzen L, et al: Prevalence and geographic distribution of *Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia canis,* and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey, *Vet Parasitol* 160:138, 2009.)

are also reported (Carrade et al, 2009). Although most canine infections appear self-limiting, *A. phagocytophilum* may persist in some dogs, and recrudescence after apparent resolution has been documented (Egenvall et al, 2000). In the United States, infection with *A. phagocytophilum* is most common in the northeastern United States, in the upper Midwest, and along the West Coast (Figure 5-1).

Although synonymized in 2001, strains of *A. phagocytophilum* have distinct disease characteristics, host affinities, and geographic distribution patterns (Foley et al, 2008; Woldehiwet, 2010). The organisms are maintained in a variety of vertebrate reservoir hosts, including a number of different rodents, deer, and birds (Woldehiwet, 2010). Infection with *A. phagocytophilum* is transmitted by *Ixodes* spp. ticks, with *I. scapularis* considered most important in the eastern United States, *I. pacificus* on the West Coast, and *I. ricinus* in Europe (Woldehiwet, 2010). Commonly available patient-side canine antibody tests for *A. phagocytophilum* will also detect antibodies to *A. platys,* an organism more commonly found in dogs in areas where *R. sanguineus* ticks predominate, such as the southern United States (Bowman et al, 2009).

A. marginale, which causes hemolytic anemia in cattle, is transmitted among cattle by *Dermacentor* and *Rhipicephalus (Boophilus)* species ticks. *A. marginale* may also be transmitted mechanically between cattle via biting flies, including *Tabanus* spp., *Stomoxys* spp., and a number of mosquito species (Ewing, 1981; Hawkins, Love, and Hidalgo, 1982; Kocan et al, 2010), although mechanical transmission by stable flies has been shown to be less efficient than tick transmission (Scoles et al, 2005). Mechanical transmission by contaminated fomites, such as needles, ear tagging devices, or castration equipment, also occurs (Kocan et al, 2010).

Infection with *A. marginale* results in fever, abortion, icterus, and weight loss; mortality also occurs (Kocan et al, 2010). A related agent, *A. ovis,* is associated with hemolytic anemia in sheep and goats, although many infections are subclinical and outbreaks are considered rare, particularly in sheep (Stuen and Longbottom, 2011). Morulae of *A. marginale* (Figure 5-2) and *A. ovis* are readily found in erythrocytes of acutely affected cattle and sheep, respectively. *Anaplasma (Ehrlichia) platys,* on the other hand, infects

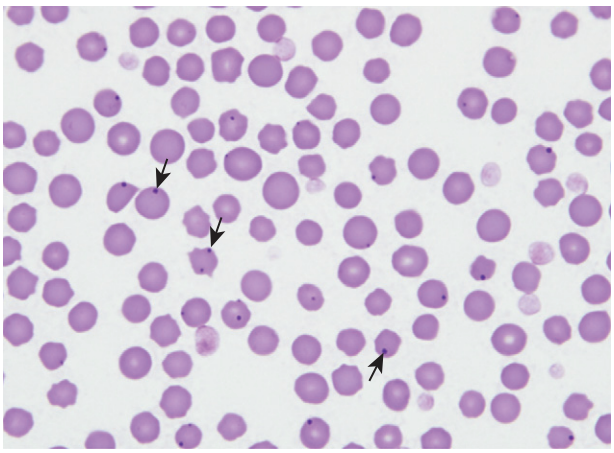

FIGURE 5-2. *Anaplasma maginale (arrows)* in bovine erythrocytes. (Courtesy K. Kocan, Oklahoma State University.)

platelets of dogs. Although not yet definitively shown, this organism is thought to be transmitted to dogs by *R. sanguineus* and can cause a mild febrile disease characterized by cyclic thrombocytopenia in some dogs, which may be exacerbated on co-infection (Gaunt et al, 2010). *A. marginale, A, ovis,* and *A. platys* have not been shown to be zoonotic.

Ehrlichia Species

Ehrlichiosis is a common, important infection of dogs and people in the Americas, and canine infections are commonly reported from Europe, Africa, and Asia. Serologic surveys of dogs demonstrate that ehrlichial infections are particularly common in the southern United States, where coexistence of high populations of vector ticks and ample infected reservoir hosts maintains a source of the organisms in nature (Figure 5-3). The species important in veterinary medicine and public health include *E. canis,* causative agent of canine monocytic ehrlichiosis (Figure 5-4); *Ehrlichia ewingii,* which primarily infects neutrophils (Figure 5-5) and, occasionally, eosinophils; and *Ehrlichia chaffeensis,* the causative agent of human

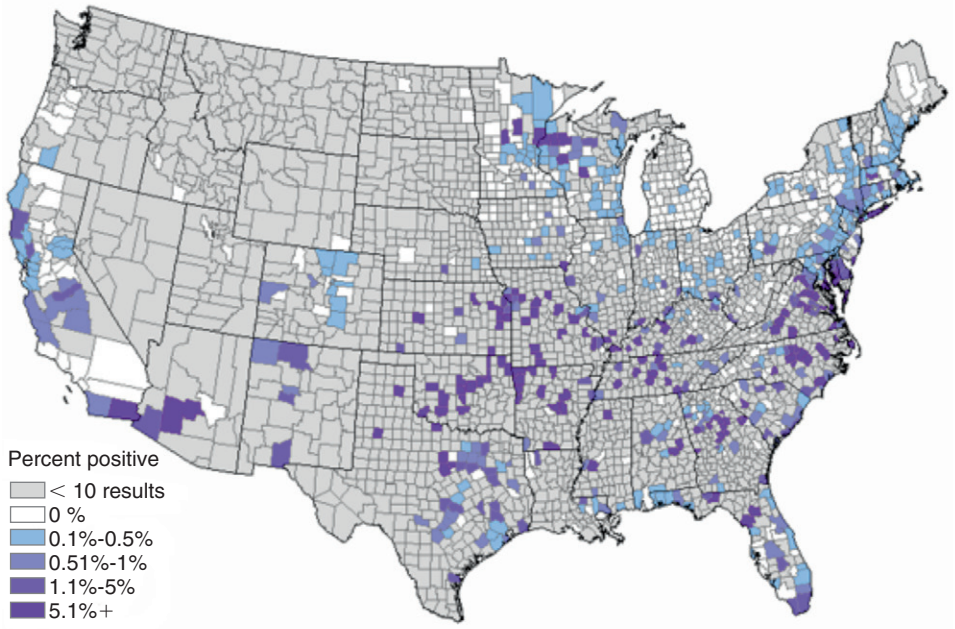

FIGURE 5-3. Distribution of antibodies to *Ehrlichia* spp. in domestic dogs in the United States. (Reprinted with permission from Bowman D, Little SE, Lorentzen L, et al: Prevalence and geographic distribution of *Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia canis,* and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey, *Vet Parasitol* 160:138, 2009.)

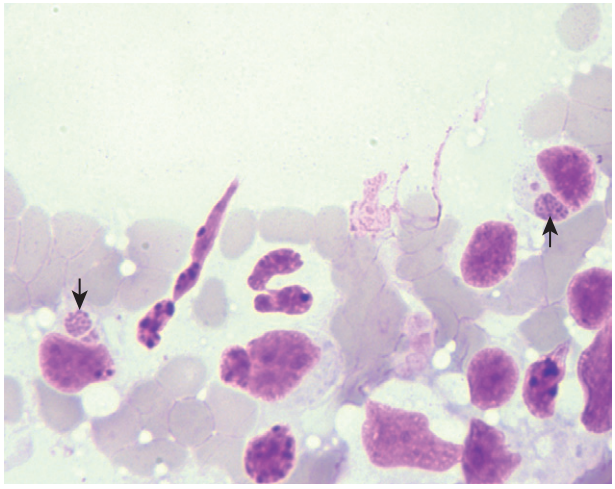

FIGURE 5-4. Morulae *(arrows)* of *Ehrlichia canis* within a circulating monocyte. (Courtesy E. Johnson, Oklahoma State University.)

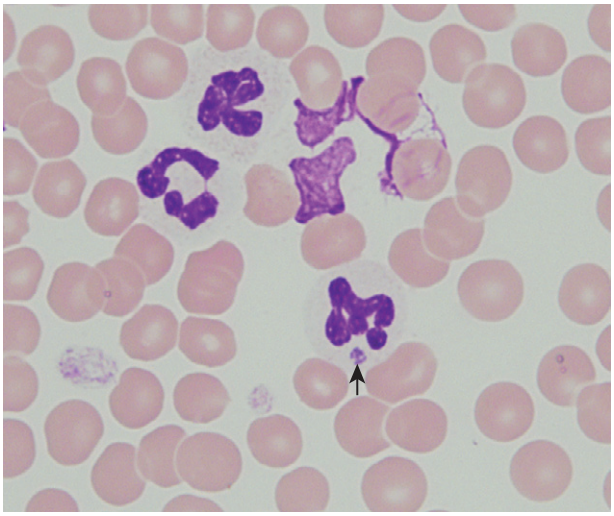

FIGURE 5-5. Morula *(arrow)* of *Ehrlichia ewingii* inside a neutrophil.

monocytic ehrlichiosis, which has also been reported from dogs. All three of these organisms are now known to be zoonotic, with people becoming infected after a tick bite (Parola, Davoust, and Raoult, 2005). Although *E. canis* is best known as a disease agent of dogs, in North America, canine infection with *E. ewingii* has been shown to occur most commonly, particularly in regions of the southern United States where there are large populations of *Amblyomma americanum* ticks (Beall et al, 2012; Liddell et al, 2003). Serologic assays for *Ehrlichia* spp., including both diagnostic laboratory-based immunofluorescent antibody tests and commercial in-clinic enzyme-linked immunosorbent assays (ELISAs), commonly detect antibodies to all three agents but do not distinguish between the individual organisms responsible for eliciting the antibody response.

E. canis causes a severe, febrile disease in dogs, characterized by thrombocytopenia, lymphadenomegaly, ocular signs, and bleeding diatheses. Chronic infection may result in emaciation and hypoplastic bone marrow, leading to pancytopenia. Infections appear to occur most commonly in the southern United States, including areas of Texas, Louisiana, Oklahoma, Arizona, and southern California (Beall et al, 2012). The organism is maintained in dog populations and is transmitted between dogs by *R. sanguineus; Dermacentor variabilis* has also been shown capable of transmitting *E. canis* (Johnson et al, 1998). Although morulae are occasionally seen in circulating monocytes of infected dogs, rickettsemia is often low and the absence of identified morulae should not preclude treatment of clinical ehrlichiosis when infection is suspected.

Canine infection with *E. ewingii* appears to result in a less severe, febrile disease, although both neurologic signs, including head tilt and ataxia, and lameness due to a neutrophilic polyarthritis are reported. Infections with *E. ewingii* are most commonly seen in the middle southern United States from North Carolina across to Oklahoma, including Arkansas, where as many as 44% of dogs

tested had antibodies to *E. ewingii* (Beall et al, 2012). Both dogs and deer may be able to serve as a source of infection to vector ticks (Anziani, Ewing, and Barker, 1990; Yabsley et al, 2002).

E. chaffeensis infection in dogs also occurs but appears to only rarely result in overt clinical disease. However, human monocytic ehrlichiosis caused by *E. chaffeensis* is considered the most common tick-borne disease of people in many areas of the southern United States (Salinas et al, 2010). *E. chaffeensis* is maintained in nature in a cycle involving *A. americanum* as vector tick and white-tailed deer as primary reservoir host (Lockhart et al, 1997); both people and dogs become infected when bitten by infected ticks. Organisms are present in monocytes, but, as with *E. canis*, low levels in circulation often make detection by microscopy alone difficult (Dawson et al, 2005).

Novel *Ehrlichia* spp. continue to be described, including a novel *Ehrlichia* sp. infecting cattle in Saskatchewan (Gajadhar et al, 2010), and an *Ehrlichia muris*–like (EML) agent causing disease in people in Minnesota and Wisconsin (Pritt et al, 2011). Published serologic surveys had shown evidence of canine exposure to a novel *Ehrlichia* sp. in this region, and later work confirmed canine infection with the EML agent in a dog from Minnesota (Bowman et al, 2009; Hegarty et al, 2012). Other *Ehrlichia* spp. are likely to be discovered in the future.

Heartwater Disease

Another ehrlichial agent, *Ehrlichia ruminantium* (formerly *Cowdria ruminantium*), causes heartwater, or cowdriosis, in ruminants in Africa as well as in areas of the Caribbean where the organism and vector ticks, particularly *A. variegatum,* have been introduced and established. This organism, which is transmitted by a variety of *Amblyomma* ticks, may also cause disease in dogs and people (Allsopp and Allsopp, 2001; Allsopp, Louw, and Meyer, 2005). A variety of wild ruminants, including blesbok and wildebeest, serve as reservoir hosts for *E. ruminantium*. On introduction by a feeding tick, the organisms invade and multiply in endothelial cells, resulting in a febrile disease characterized by vasculitis; the common name of the disease refers to the development of pericardial effusion in acute cases.

An endemic cycle of heartwater is not known to have been established in the continental Americas, but occasional introductions occur, and native ticks and wildlife have been shown to be competent vectors and reservoir hosts, respectively (Burridge et al, 2002; Uilenberg, 1982). Transmission of *E. ruminantium* by *A. maculatum*, but not by *A. americanum*, has been confirmed experimentally (Mahan et al, 2000). In addition, a novel *E. ruminantium*–like organism from *A. americanum* collected from the southern United States has been reported to cause disease in a goat (Loftis et al, 2006). This agent, referred to as the Panola Mountain *Ehrlichia* (PME) agent is widespread in deer and ticks in the southern United States and has been implicated in human infection (Reeves et al, 2008; Yabsley et al, 2008). Previous exposure to the PME agent or to other *Ehrlichia* spp. may confound serologic tests for *E. ruminantium* in naturally exposed ruminants in North America (Katz et al, 1996).

Neorickettsia Species

Members of the genus *Neorickettsia* are unusual among the rickettsial pathogens in that they are transmitted by trematodes rather than arthropod vectors; accordingly, infection is associated with consumption of intermediate hosts harboring trematode metacercaria infected with the rickettsia rather than infestations with ticks or other ectoparasites. Control programs for disease due to *Neorickettsia* spp. should focus on preventing ingestion of materials containing infected metacercaria, as well as prompt recognition and appropriate treatment of infected animals to limit morbidity and mortality associated with these pathogens.

Neorickettsia helminthoeca is vectored by *Nanophyetus salmincola,* a trematode of dogs and other carnivores, and causes **salmon poisoning disease** in domestic dogs. The rickettsiae invade the tissues of the fluke and are passed along with the immature trematode stages through snails to salmonid fish intermediate hosts. When a dog ingests a fish harboring metacercariae, it becomes infected with both the trematodes and the rickettsia. A highly fatal disease, commonly referred to as salmon poisoning, ensues and is characterized by severe gastroenteritis, profuse diarrhea, enlarged lymph nodes, and a very high fever that decreases to hypothermia shortly before death (Sykes et al, 2010). Affected dogs are anorexic and rapidly lose weight. Although many vertebrates, including humans, may develop infection with *N. salmincola,* salmon poisoning caused by *N. helminthoeca* appears to occur only in dogs and wild canids. Salmon poisoning in dogs is largely limited to areas of the Pacific Northwest in the United States and British Columbia in Canada, where the vector trematode cycles in nature, although canine disease caused by *N. helminthoeca* has been reported from Brazil (Headley et al, 2009, 2011).

Neorickettsia risticii is a related rickettsia that causes **Potomac horse fever,** also referred to as **equine monocytic ehrlichiosis,** sporadically in many areas of North America; this disease has also been reported from Europe. Infections are associated with aquatic habitats. Horses acquire the infection on ingestion of caddisflies parasitized with metacercariae of *Acanthatrium oregonense,* a trematode of bats (Pusterla et al, 2003), or ingestion of trematode stages free in water. Infection results in an acute, febrile disease that can be severe; depression, anorexia, dehydration, abortion, diarrhea, and laminitis also occur (Madigan and Pusterla, 2000). In infected horses, the organisms are primarily found in monocytes. Infection with *N. risticii* also has been occasionally described in dogs and, experimentally, in cats (Dawson et al, 1988; Kakoma et al, 1994; Ristic et al, 1988). Although *N. risticii* has not been reported to infect people, a related organism, *Neorickettsia sennetsu,* is well established as the causative agent of Sennetsu fever in Japan and Malaysia. The life history and maintenance cycle for *N. sennetsu* are unknown (Rikihisa, 2006).

Wolbachia Species

Members of the genus *Wolbachia* have been reported as endosymbionts from a variety of helminths and arthropods (Bowman, 2011; Fenn et al, 2006). *Wolbachia* species are found in close association with several filarid worms, including *Dirofilaria immitis,* the causative agent of heartworm disease (Sironi et al, 1995). Some evidence suggests that *Wolbachia* species may play a role in inflammation during heartworm infection (Genchi et al, 1998; Kramer et al, 2005; Kramer et al, 2011). However, not all dogs treated with doxycycline alone, which presumably decreases *Wolbachia* populations, show reduced lesion scores, and the presence of *Wolbachia* does not consistently correlate with severity of disease in dogs and cats (Dingman et al, 2010; Kramer et al, 2005; Kramer et al, 2011). Exploring the role of *Wolbachia* species in filarid worm survival and pathogenesis is an area of ongoing research.

OTHER BACTERIAL PATHOGENS TRANSMITTED BY VECTORS

Several other bacterial pathogens, in addition to rickettsia, are also transmitted by arthropod vectors. Some of these diseases are of

TABLE 5-3 Other Bacterial Vector-Borne Diseases of Veterinary Importance

Disease	Agent	Primary Vector	Reservoir Host
Lyme borreliosis	*Borrelia burgdorferi;* also *Borrelia afzelii* and *Borrelia garinii* in Europe	*Ixodes scapularis,* other *Ixodes* species	Mice, other rodents
Avian spirochetosis	*Borrelia anserina*	*Argas persicus*	*Argas persicus*
Tick-borne relapsing fever	Various *Borrelia* species	*Ornithodorus* species	Rodents
Louse-borne relapsing	*Borrelia recurrentis*	*Pediculus humanus*	People
Bovine borreliosis	*Borrelia theileri*	*Rhipicephalus* species	Cattle
Trench fever	*Bartonella quintana*	*Pediculus humanus*	People
Cat scratch disease	*Bartonella henselae,* other *Bartonella* spp.	Fleas *(Ctenocephalides felis)*	Cats
Canine bartonellosis	Various *Bartonella* species	Ticks suspected	Unknown
Feline hemoplasmosis	*Mycoplasma haemofelis, Mycoplasma haemominutum*	Fleas suspected	Cats
Canine hemoplasmosis	*Mycoplasma haemocanis*	*Rhipicephalus sanguineus*	Dogs
Tularemia	*Francisella tularensis*	Various ticks, mosquitoes	Rabbits, other mammals
Plague	*Yersinia pestis*	*Xenopsylla cheopis;* other fleas	Rodents
Q fever	*Coxiella burnetii*	*Amblyomma* spp., other ticks	Various mammals
Infectious bovine keratoconjunctivitis	*Moraxella bovis*	*Musca autumnalis*	Cattle

considerable consequence and concern to pet and livestock owners. Important vector-borne bacterial genera associated with veterinary and human disease include *Borrelia, Bartonella, Mycoplasma*, and *Yersinia* (Table 5-3).

BORRELIA SPECIES

The best known and most commonly diagnosed arthropod-transmitted bacterial disease in the United States is *Borrelia burgdorferi,* the causative agent of **Lyme borreliosis** or **Lyme disease** in North America. In Europe, borreliosis in people and dogs may be caused by *B. burgdorferi, Borrelia garinii,* or *Borrelia afzelii*. The annual number of cases of Lyme disease reported in people in the United States has increased in recent years—a change largely attributed to increased vector tick populations resulting in greater exposure risk. *Borrelia burgdorferi* is maintained in nature in a cycle involving rodent reservoir hosts and *Ixodes* species vectors. The most important vector of *B. burgdorferi* in the eastern United States is *I. scapularis,* whereas *I. pacificus* is responsible for the majority of infections seen on the West Coast. Other *Ixodes* species can transmit *B. burgdorferi* in nature but rarely feed on humans or dogs (Oliver et al, 2003). Deer are important as a host for the adult ticks and thus serve to maintain large tick populations in an area, but deer are not considered an important reservoir host for *B. burgdorferi* (Telford et al, 1988).

Historically, endemic transmission of Lyme borreliosis in North America has been largely limited to areas of the northeastern, upper midwestern, and West Coast states. Laboratory-confirmed cases of **autochthonous infection** (an infection transmitted locally or indigenously, as opposed to imported) with *B. burgdorferi* in eastern states south of Maryland or Virginia, and in many areas of the southern, midwestern, and western United States, are considered rare (Wormser et al, 2006). However, geographic spread of infection has been documented, and increasing cases of infection in wider areas than those traditionally considered enzootic for disease have been reported in recent years (Bacon et al, 2008; Bowman et al, 2009; Hamer et al, 2010).

Geographic distribution of infection in dogs parallels that seen in humans (Figure 5-6), with the great majority of infected dogs found in areas where transmission of *B. burgdorferi* is historically endemic or hyperendemic (Bowman et al, 2009). In published surveys using specific assays, most dogs that test positive for *B. burgdorferi* in nonendemic areas have a history of travel to an area where disease is endemic (Bowman et al, 2009; Duncan et al, 2004). Nonetheless, because dogs do travel or relocate with their owners, large numbers of dogs in nonendemic areas, particularly those where relocation is common, such as retirement or vacation communities, college towns, and military bases, may test positive for antibodies to *B. burgdorferi*. Diagnostic evidence of past infection may be clinically relevant in a dog with evidence of disease, but should not necessarily be interpreted as confirming local transmission, particularly when a travel history is reported.

Polyarthritis is the most common presenting complaint in dogs with Lyme borreliosis. Affected dogs may also develop fever, anorexia, and lymphadenopathy (Little et al, 2010). Although uncommon, protein-losing nephropathy associated with infection may result in edema, weight loss, vomiting, and diarrhea—a condition referred to as Lyme glomerulonephritis, which is potentially fatal in dogs (Dambach et al, 1997). Despite the severity of illness seen in some individuals, the great majority of dogs with antibodies to *B. burgdorferi* on specific serologic assays will not develop any clinical disease associated with the infection (Littman et al, 2006). Antibody tests for *B. burgdorferi* vary in specificity; those assays with poor specificity or for which external validation data are not available should be interpreted with particular caution. Vaccines are available and are widely used in endemic areas to protect dogs from infection with *B. burgdorferi*.

In people, acute Lyme borreliosis is characterized by headache, fever, muscle and joint pain, and, in approximately 70% of patients, an expanding circular rash (>5 cm diameter), termed *erythema migrans,* which develops at the primary tick bite or as a secondary lesion (Wormser et al, 2006); erythema migrans is not recognized in dogs (Little et al, 2010). If not treated in the acute phase, people may experience chronic, disseminated disease that can result in arthritis, carditis, or neurologic disease; it is not clear whether cardiac or neurologic disease is associated with *B. burgdorferi* infection in dogs (Littman et al, 2006; Wormser et al, 2006).

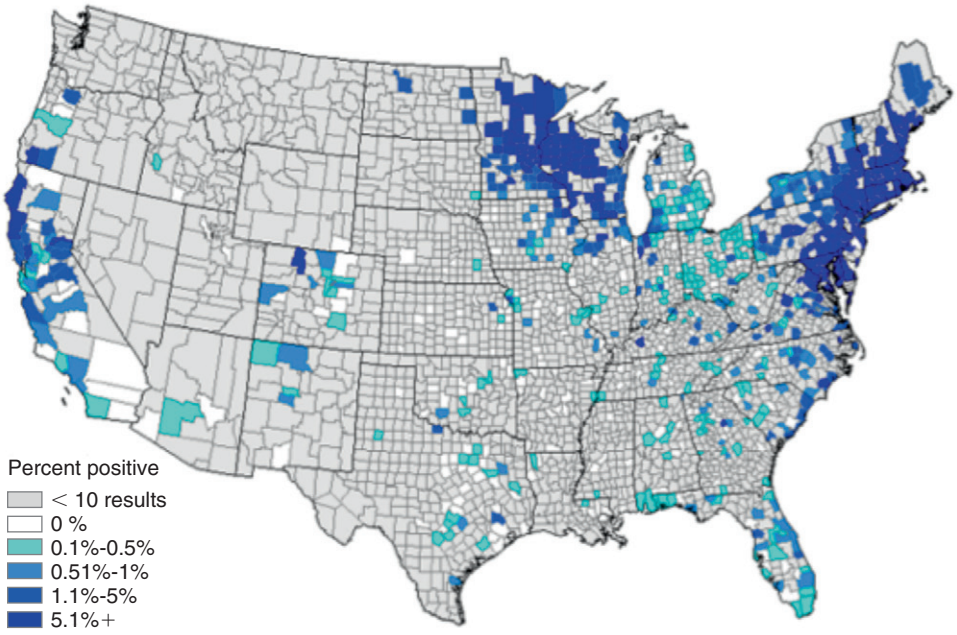

FIGURE 5-6. Distribution of antibodies to *Borrelia burgdorferi* in domestic dogs in the United States. (Reprinted with permission from Bowman D, Little SE, Lorentzen L, et al: Prevalence and geographic distribution of *Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia canis,* and *Anaplasma phagocytophilum* in dogs in the United States: results of a national clinic-based serologic survey, *Vet Parasitol* 160:138, 2009.)

Other Diseases Caused by *Borrelia* Species

Other diseases caused by *Borrelia* species include avian spirochetosis, relapsing fever, and bovine borreliosis.

Avian spirochetosis due to infection with *Borrelia anserina* causes disease in turkeys, chickens, geese, pheasants, and other birds. Affected birds become febrile and cyanotic. Infection is transmitted to birds via the feces of the soft-tick vectors, *Argas persicus* and related species; infection can also be maintained long term in soft-tick populations through transovarial transmission (Zaher, Soliman, and Diab, 1977).

Tick-borne relapsing fever is caused by a large number of soft-tick–transmitted *Borrelia* species, such as *Borrelia hermsii, Borrelia turicata,* and *B. parkeri,* each of which is transmitted by a corresponding *Ornithodoros* soft tick (Barbour and Hayes, 1986). Tick-borne relapsing fever borreliosis is present in Asia, Europe, Africa, and the Americas; in North America, disease is most commonly seen in people in the western United States (Dworkin, Schwan, and Anderson, 2002).

A related agent, *Borrelia coriaceae,* is found in *Ornithodoros coriaceus* ticks in western North America and was considered for a time to be a putative etiologic agent of **epizootic bovine abortion** (EBA), or foothill abortion. However, subsequent work has shown that EBA is more likely caused by a proteobacter in the order Myxococcales commonly found in *O. coriaceus* in endemic areas in California, Oregon, Idaho, and Nevada (King et al, 2005; Teglas et al, 2011). Infection of cattle results in significant production loss due to late-term abortions and/or the birth of unhealthy calves (Howarth et al, 1956).

Louse-borne relapsing fever is caused by *Borrelia recurrentis* and is transmitted by the human body louse, *Pediculus humanus*. Infection with *B. recurrentis* occurs only in people, with epidemics developing in times of famine, war, or mass migration; animals are not involved as reservoir hosts (Raoult and Roux, 1999).

Bovine borreliosis caused by *Borrelia theileri* induces a relatively mild disease in cattle, sheep, and horses; infection is transmitted by *Rhipicephalus* ticks, including *Boophilus* subspecies. Also referred to as *tick spirochetosis,* bovine borreliosis caused by *B. theileri* has been reported from Africa, Australia, and central and southern North America (Smith et al, 1985). Other related *Borrelia* species include *Borrelia miyamotoi* and *Borrelia lonestari* (Figure 5-7), both of which are also hard-tick–transmitted spirochetes that infect both ticks and mammals (Fukunaga et al, 1995; Moyer et al, 2006). Human infection with *B. miyamotoi* has been reported from Russia (Platonov et al, 2011). *Borrelia lonestari* was considered a putative agent of **southern tick–associated rash illness** (STARI), a Lyme disease–like illness in people from the southern United States associated with the bite of *A. americanum,* the lone star tick (Masters et al, 2008). However, a review of patient tissues failed to confirm this etiology (Wormser et al, 2005).

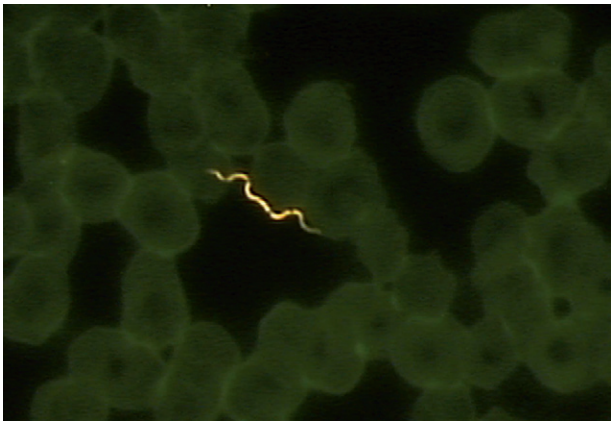

FIGURE 5-7. Relapsing fever–like *Borrelia* species *(Borrelia lonestari)* on a blood smear from a white-tailed deer.

BARTONELLA SPECIES

Several vector-borne *Bartonella* species also infect and cause disease in people, dogs, and cats. **Trench fever,** a moderate to severe febrile disease of people characterized by marked splenic enlargement, is

caused by *Bartonella quintana* and is transmitted to people via infected body lice, *P. humanus*. Trench fever, so named because of the widespread illness recognized in soldiers during the First World War, is not a zoonotic disease, and people rather than animals serve as reservoir hosts (Maurin and Raoult, 1996). In contrast, other forms of **bartonellosis,** such as those caused by *Bartonella henselae, B. clarridgeiae,* or *B. kohlerae,* are directly zoonotic, with people most often infected when bitten or scratched by a bacteremic cat (hence the traditional name **cat scratch disease**) that harbors infectious *Bartonella* sp. bacteria on teeth or claws (Chomel and Kasten, 2010). The agents of cat scratch disease and other forms of bartonellosis are not known to be transmitted to people by arthropod vectors; however, *B. henselae* can be transmitted from infected to naïve cats, particularly kittens, through fleas as well as through direct contact, and controlling flea infestation is considered important to limit bacteremia in cats (Foil et al, 1998; Foley et al, 1998).

Infection of immunocompetent people with *B. henselae* following the bite or scratch of a cat results in a classic disease characterized by regional lymphadenopathy and mild fever; most cases are described in children (Brietschwerdt, 2008; Chomel and Kasten, 2010). In recent years, bartonellosis due to infection with a number of *Bartonella* spp. has been linked to other disease manifestations in people, including granulomatous hepatitis, myalgia, neurologic disease, and arthropathies (Breitschwerdt et al, 2011; Maggi et al, 2011). Infection with *Bartonella* spp. is considered by some to be a potential occupational risk in veterinary medicine and in other fields with frequent animal contact (Breitschwerdt et al, 2010). In addition, infection with both *B. quintana* and *B. henselae* can induce potentially fatal bacillary angiomatosis in immunocompromised patients (Koehler et al, 1997). Although the relationship between *Bartonella* spp. and disease in cats is not completely understood, feline bartonellosis has been associated with clinical disease ranging from a self-limiting, febrile episode to uveitis, endocarditis, persistent lymphadenitis, and gingivitis (Guptill, 2010).

B. quintana and *B. henselae,* along with other *Bartonella* species such as *Bartonella vinsonii* subsp. *berkhoffii* and *Bartonella elizabethae,* have also become increasingly recognized as canine pathogens in recent years. These organisms have been associated with endocarditis, myocarditis, and granulomatous lymphadenitis in dogs (Kelly et al, 2006; Morales et al, 2007). Although the arthropod(s) responsible for transmitting these *Bartonella* infections to dogs, if any, have not yet been confirmed, ticks are suspected to play a role, and these agents are likely to increase in importance as canine vector-borne pathogens in the future (Angelakis et al, 2010). Human infection and associated disease have been reported with some of the canine-associated *Bartonella* species (e.g., *B. vinsonii* subsp. *berkhoffii*); transmission routes to people are not clear, but direct transmission from an infected dog to a person via a bite or a scratch is suspected to be a potential route for exposure (Chomel et al, 2006).

MYCOPLASMA SPECIES

Other important vector-associated bacterial infections include the hemoplasma species of *Mycoplasma* (formerly *Haemobartonella*), which appear as small pleomorphic bacteria attached to the surface of erythrocytes on stained blood smears. *Mycoplasma haemocanis* is known to be transmitted to dogs by ticks *(R. sanguineus)*, and infections with *M. haemocanis* are maintained in tick populations both transstadially and transovarially (Seneviratna et al, 1973). *Mycoplasma haemofelis* (Figure 5-8) is widely thought to be transmitted to cats by fleas, and bacteria have been detected in fleas collected from infected cats, but this route of infection has yet to be experimentally confirmed, leading some to suspect that transmission may occur through bite wounds during fighting (Lappin et al, 2006; Sykes, 2010; Woods, Wisnewski, and Lappin, 2006). Infection with *Mycoplasma* spp. is more commonly seen in male cats and in those with concurrent immunosuppressive viral infection (Sykes et al, 2008).

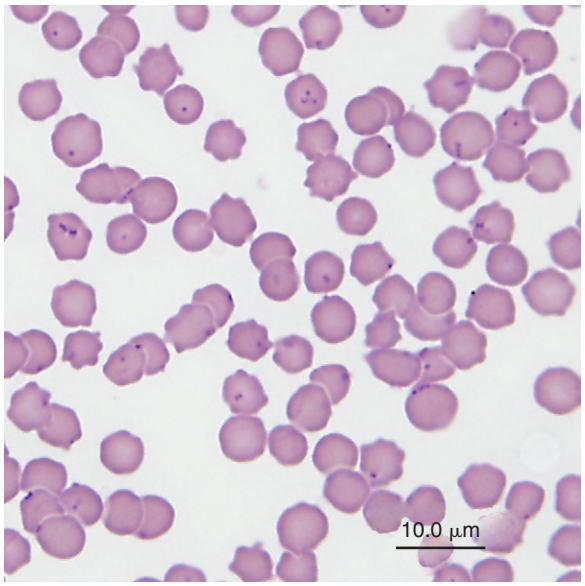

FIGURE 5-8. *Mycoplasma haemofelis (arrows)* on erythrocyte of an infected cat. (Courtesy R. Allison, Oklahoma State University.)

In cats, infection with *M. haemofelis* and the related *Mycoplasma haemominutum* may be clinically inapparent. However, *M. haemofelis* can cause mild to severe clinically apparent anemia, anorexia, and lethargy; infected cats often present with splenomegaly, enlarged lymph nodes, icterus, and respiratory distress. Blood donors should be evaluated for *M. haemofelis* infection (Hackett et al, 2006). Disease is more common in cats immunocompromised by concurrent immunosuppressive viral infections such as feline leukemia virus (FeLV) but can also be seen in cats without concurrent FeLV infection (George et al, 2002; Harrus et al, 2002). Disease due to infection with *M. haemocanis* is considered rare in spleen-intact dogs. Another small haemotropic mycoplasma, *Candidatus M. haematoparvum,* is also occasionally described in dogs (Sykes et al, 2005).

TULAREMIA

Arthropod vectors can be important in the transmission of disease agents considered to have a potential role in bioterrorism, including the causative agents of tularemia and plague. In North America, infections with *Francisella tularensis,* the causative agent of tularemia, are acquired directly from contact with infected carcasses, particularly rabbits. However, transmission by ticks and biting flies is also considered an important route of infection, and a number of tick species in the genera *Dermacentor, Amblyomma, Ixodes,* and *Haemaphysalis* may be responsible for transmitting infection between animals in nature. The American dog tick, *D. variabilis,* may be particularly important as a bridging vector of infection to people in some areas of North America (Reese et al, 2011). Mosquitoes are also involved in transmission of some biovars of *F. tularensis* (Petersen and Schriefer, 2005). Clinical disease in animals is most commonly seen in cats, presumably after ingestion of infected prey (Woods et al, 1998). Transmission of *F. tularensis* to people directly via bites or scratches of infected cats, although possible, is considered rare.

PLAGUE

Plague caused by *Yersinia pestis* is transmitted between animals and to people via fleas; infection with *Y. pestis* is rare in North America, but a natural focus of transmission is maintained in a cycle involving fleas and prairie dogs in the western United States (Anderson and Williams, 1997). Animals infected with *Y. pestis* may develop fever and enlarged lymph nodes; cats appear particularly susceptible to the disease (Gage et al, 2000). Infected cats can serve as a source of infection directly through bites and scratches or through aerosolization of bacteria; cats and dogs also may support populations of fleas, which are then able to transmit the infection to people (Gould et al, 2008). Both flea control and prevention of ingestion of prey species are critical to preventing infection with *Y. pestis* in cats and dogs.

Q FEVER

Another bacterial agent, *Coxiella burnetii,* causes Q fever in people and a variety of animals. Transmission of *C. burnetii* by ticks can occur, but most cases in people are thought to be acquired by inhalation of organisms in contaminated dust (Terheggen and Leggat, 2007). Zoonotic infections have also been associated with human exposure to infected ruminants, especially during lambing, kidding, or calving.

MECHANICAL TRANSMISSION OF BACTERIA BY ARTHROPODS

In addition to their role in biologic maintenance and transmission of disease agents, arthropods can serve as important mechanical transmitters of bacteria. For example, transmission of *Moraxella bovis,* the causative agent of infectious bovine keratoconjunctivitis (pink eye) in cattle, is facilitated by the presence of the face fly, *Musca autumnalis,* which efficiently moves the organism between animals housed together on pasture (Alexander, 2010; Gerhardt et al, 1982). Disease is more commonly seen in pastured cattle in summer and early fall, when face fly populations are well established and exposure to ultraviolet light, another risk factor for infection, is at its peak (Lepper and Barton, 1987). Vaccines and effective antibiotic treatments are available, but face fly control remains a critical component of preventing infection with *M. bovis* in cattle.

VECTOR-BORNE PROTOZOA

In addition to viral and bacterial pathogens, a number of protozoal agents, many of which cause serious, potentially fatal diseases of domestic and wild animals, are transmitted via arthropod and, occasionally, nematode vectors (Table 5-4). Primary arthropod transmitters of protozoan parasites include sandflies, mosquitoes, reduviid bugs, and ticks. The pathogens transmitted by these vectors and the diseases they cause are described in detail in Chapter 3, but here we provide an overview of the vector-borne transmission patterns responsible for maintaining the sources of infection to animals and people.

The *Leishmania* spp. (Figure 5-9) that cause visceral, mucocutaneous, and cutaneous **leishmaniasis** in dogs, people, and other animals are transmitted primarily by sandflies (*Lutzomyia* spp. in the Americas and *Phlebotomus* spp. in Africa, Asia, and Europe). A number of species have been described, each of which tends to cause different forms of the disease. Vector-borne transmission of *Leishmania* spp. is suspected but is not documented in North America, where infections among foxhounds occasionally occur. Treatment of dogs with pyrethroid repellents has been shown to decrease infection rates with *Leishmania* spp. in areas where vector-borne transmission predominates, presumably by discouraging feeding by the phlebotomine vectors (Ferroglio et al, 2008; Otranto et al, 2010). Because dogs are a major reservoir of *Leishmania* spp. in endemic areas, maintaining dogs on repellents also serves a public health role in decreasing the number of infected sandflies available to transmit the parasite to people. Transmission of *Leishmania infantum* by *R. sanguineus* has been suggested but not yet confirmed with experimental feeding studies (Solano-Gallego et al, 2012).

Transmission of *Trypanosoma cruzi* (Figure 5-10), the agent of **American trypanosomiasis,** or **Chagas' disease,** in people and dogs, is achieved through triatomine insects, commonly referred to as *kissing bugs* or *assassin bugs*. The classic vector-borne transmission pattern is stercorarian with metacyclic trypomastigotes passed in the feces of the bug as it feeds on a vertebrate host, entering the bite wound directly after it is rubbed or scratched by the host, or when transferred to conjunctival membranes. Infection with *T. cruzi* is considered to cause the highest burden of any human parasitic disease in the Americas, and vector-borne transmission causes the great majority of infections in endemic areas (Bern and Montgomery, 2009; Bern et al, 2011). However, additional routes are described, including congenital transmission, blood transfusion, organ donation, and infection following ingestion of contaminated fruit juice (Bern et al, 2011). In the United States, most human cases of Chagas' disease are seen in immigrants, but locally acquired vector-borne infections do occur and are likely under-recognized in people (Bern and Montgomery, 2009; Cantey et al, 2012).

Major vectors of *T. cruzi* in Latin America include a number of different species of *Triatoma* and *Rhodnius*. In North America, several *Triatoma* spp. vectors of *T. cruzi* have also been described, with common species such as *T. sanguisuga* and *T. gerstaeckeri* considered potentially important as sources of autochthonous infection in the southern United States. Cases of *T. cruzi* in dogs are most commonly reported from Texas and Oklahoma, although a number of other southern states also report cases in dogs. A wide variety of mammalian hosts can serve as reservoirs for infection, including domestic dogs, and all mammals are considered susceptible to infection. Opossums and armadillos are important reservoirs throughout the Americas (Bern et al, 2011). In the western United States, wood rats are considered the major reservoir host, whereas raccoons, opossums, armadillos, and skunks are important sources of infection in the eastern United States (Bern et al, 2011; Brown et al, 2010; Charles et al, 2012).

Other *Trypanosoma* spp. of medical significance include *T. brucei* in Africa, which is transmitted via the bite of a number of different species of tse-tse fly, *Glossina* spp. Subspecies of *T. brucei* cause **nagana** in cattle *(T. brucei brucei)* and human African trypanosomiasis, or **sleeping sickness,** in people (*T. b. gambiense* and *T. b. rhodesiense*), and the two subspecies responsible for human disease differ markedly in geographic distribution and maintenance cycles. For example, *T. b. gambiense* is transmitted by flies in the *G. palpalis* group and the main reservoir host is people; infections result in chronic, endemic disease in people in central and western Africa. In contrast, *T. b. rhodesiense* is transmitted by flies in the *G. morsitans* group and both people and ruminants serve as reservoir hosts, including cattle and some wildlife species such as antelopes. Infections with *T. b. rhodiense* cause acute, epidemic disease in southern and eastern Africa (Malvy and Chappuis, 2011).

African animal trypanosomiasis, or nagana, is mainly caused by infection with *T. b. brucei, T. congolense,* and *T. vivax,* which infect a wide variety of domestic and wild animals. Other species,

TABLE 5-4 Representative Vector-Borne Protozoal Diseases of Veterinary Importance

Disease	Cause	Primary Vector	Reservoir Host
Leishmaniasis (visceral leishmaniasis; cutaneous leishmaniasis; mucocutaneous leishmaniasis)	*Leishmania* species	Sandflies (*Lutzomyia* spp., *Phlebotomus* spp.)	Rodents; other small mammals; domestic dogs
Chagas' disease	*Trypanosoma cruzi*	Triatomine bugs (*Triatoma* spp. and *Rhodnius* spp.)	Rodents; raccoons, opossums, armadillos, skunks; domestic dogs
Human African trypanosomiasis, sleeping sickness	*Trypanosoma brucei gambiense, T. b. rhodesiense*	Tsetse flies *(Glossina palpalis, G. morsitans)*	People *(T. b. gambiense);* people, cattle, wild ruminants *(T. b. rhodesiense)*
African animal trypanosomiasis, nagana	*T. b. brucei, T. congolense,* and *T. vivax*	Tsetse flies (*Glossina morsitans, G. palpalis,* and *G. fusca*); *Tabanus* spp. *(T. vivax)*	Cattle, wild ruminants
Canine hepatozoonosis	*Hepatozoon canis*	*Rhipicephalus sanguineus*	Domestic dogs
American canine hepatozoonosis	*Hepatozoon americanum*	*Amblyomma maculatum*	Domestic dogs, other wildlife?
Cytauxzoonosis	*Cytauxzoon felis*	*Amblyomma americanum, Dermacentor variabilis*	Domestic cats, bobcats
Canine babesiosis	*Babesia canis, B. vogeli, B. rossi, B. gibsoni*	*Dermacentor reticulatus, Haemophysalis, Rhipicephalus sanguineus*	Domestic dogs
Bovine babesiosis	*Babesia bovis, Babesia bigemina, Babesia divergens*	*Rhipicephalus* spp., *Dermacentor* spp., *Ixodes ricinus*	Cattle, wild ruminants
East Coast fever	*Theileria parva*	*Rhipicephalus* spp.	Cattle and wild ruminants, especially African buffalo
Tropical theileriosis	*Theileria annulata*	*Hyalomma* spp.	Cattle, water buffalo, yak, camel
Equine piroplasmosis	*Babesia caballi* *Theileria equi*	*Rhipicephalus* spp., *Dermacentor* spp., *Amblyomma cajennense*	Horses and other equids
Avian malaria	*Plasmodium* spp.	Mosquitoes	Birds
Other avian hemoprotozoa	*Leukocytozoon, Haemoproteus*	Black flies (*Simulium* spp.), *Culicioides* spp.; mosquitoes, *Culicoides* spp., tabanid flies, hippoboscid flies	Birds

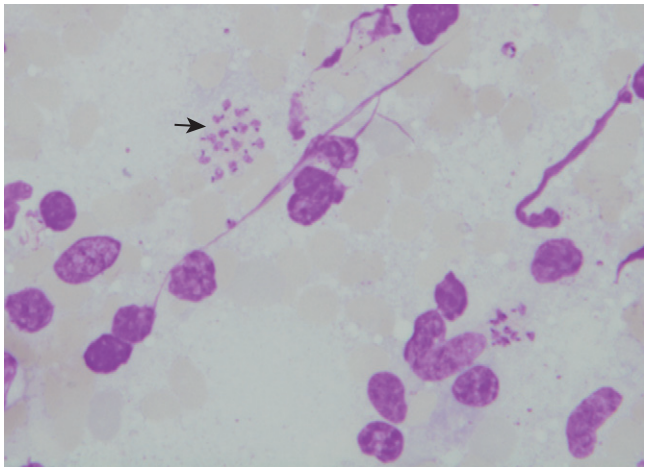

FIGURE 5-9. *Leishmania* amastigotes *(arrow)* within macrophages.

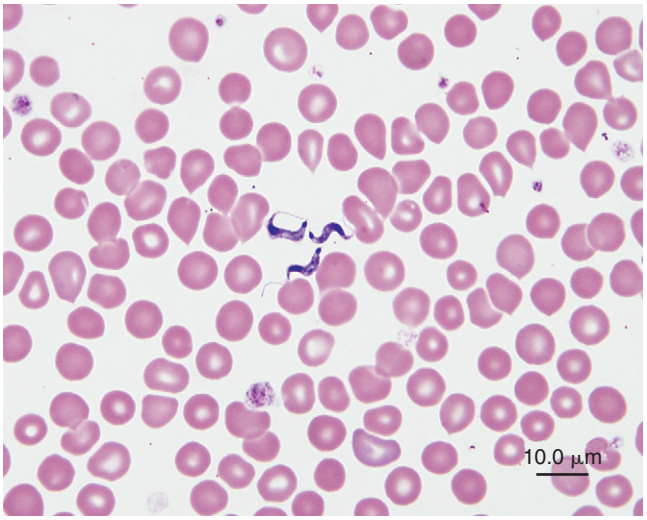

FIGURE 5-10. Trypomastigotes of *Trypanosoma cruzi* on a blood smear from an infected dog. (Courtesy R. Allison, Oklahoma State University.)

such as *T. simiae* and *T. godfreyi,* are also associated with animal disease but are more commonly found in pigs (Spickler and Roth, 2006). Disease is most commonly seen in cattle, the preferred host for the main vectors, but it has been reported from sheep, goats, horses, and pigs. Both cattle and wild ruminants serve as important reservoirs of infection (Brown, 2008b). People are not susceptible to infection with the agents of nagana. The trypanosomes are transmitted by *Glossina* flies, primarily *G. morsitans, G. palpalis,* and *G. fusca* (Brown, 2008). Other biting flies, including tabanids, can also spread *T. vivax* between animals; this route, in addition to mechanical transmission via contaminated fomites such as needles, is particularly important in transmission of animal trypanosomiasis South America (Spickler and Roth, 2006). Some nonpathogenic trypanosomes of ruminants are also vector-borne. Trypomastigotes of *T. theileri* are commonly found in the blood of cattle worldwide and are transmitted between cattle by tabanid flies (Schafler, 1979). A related megatrypanum in North America, *T. cervi,* is commonly found in white-tailed deer and other cervids, including mule deer and elk, and may be transmitted by tabanid or hippoboscid flies (Böse and Petersen, 1991; Kingston, 1981).

The agents of **canine hepatozoonosis** are unusual in that they are transmitted to dogs via ingestion of tick vectors, most likely during grooming, rather than tick bite. *Hepatozoon canis,* which is present worldwide, is transmitted to dogs upon ingestion of infected *R. sanguineus* ticks containing oocysts with mature sporozoites; dogs also serve as the reservoir for *H. canis,* infecting immature ticks with gamonts present in circulating blood (Allen et al, 2011; Baneth et al, 2011). As such, these infections can be maintained in kennels or other groups of dogs so long as ample *R. sanguineus* populations are also present. **American canine hepatozoonosis** caused by *H. americanum* (Figure 5-11) has been described primarily from the southern United States and is also transmitted to dogs via ingestion of infected vector ticks, namely, *A. maculatum,* the Gulf Coast tick (Mathew et al, 1998). However, infection with *H. americanum* can also occur following ingestion of rodent or rabbit tissue containing cystozoite stages (Johnson et al, 2009). Indeed, dogs that develop American canine hepatozoonosis often have a history of ingestion of prey species, including rodents and rabbits, and may have been infected via ingestion of either cystozoites in tissues or ticks feeding on prey species in nature (Johnson et al, 2009). In areas where *H. americanum* is enzootic, coyotes are commonly infected, but other species, such as wild rodents, may serve as important maintenance hosts in nature (Little et al, 2009; Starkey et al, 2012).

Feline cytauxzoonosis, present throughout much of the southern and eastern United States, is caused by *Cytauxzoon felis* (Figure 5-12) and is transmitted to cats via tick bite. Historically, the disease agent was considered to be maintained in bobcat reservoir hosts and transmitted by *Dermacentor variabilis* ticks from bobcats to domestic cats, leading to development of a severe, almost universally fatal disease following infection (Meinkoth and Kocan, 2005). However, although fatalities are still often seen, in recent years many cats have been shown to be capable of surviving infection, and persistently infected domestic cats are now considered a potential important reservoir of *C. felis,* able to repeatedly transmit the organism to ticks (Brown et al, 2010; Meinkoth et al, 2000; Reichard et al, 2010). Additionally, experimental studies have confirmed that *Amblyomma americanum,* the lone star tick, is a competent, dependable vector of *C. felis* (Reichard et al, 2010). This was a particularly important finding in that the distribution of *A. americanum* corresponds well to enzootic areas for cytauxzoonosis in North America (Mueller et al, 2013). Other *Cytauxzoon* spp. are occasionally described from wild and domestic felids and presumably are also transmitted by ticks (Carli et al, 2012; Millán et al, 2009; Reichard et al, 2005).

Canine babesiosis is caused by a number of different large and small piroplasms and is found in dogs worldwide. The large piroplasms were previously referred to as subspecies of *Babesia canis* but are now known to be distinct species with differing geographic distribution, preferred tick vectors, and disease manifestations (Irwin, 2010). *Babesia canis* is found in Europe and Asia and is transmitted between dogs by *Dermacentor reticulatus; B. rossi* (Figure 5-13) is described from Africa, although occasionally reported from other areas, and is transmitted by *Haemaphysalis elliptica;* and *B. vogeli* is found worldwide, including in the Americas, and is transmitted by *R. sanguineus* (Solano-Gallego and Baneth, 2011). Novel large *Babesia* spp. have been described from immunocompromised dogs in the United States and from the United Kingdom (Allison et al, 2011; Birkenheuer et al, 2004; Holm et al, 2006; Sikorski et al, 2010). The small piroplasms of dogs include *B. gibsoni* (Figure 5-14), which is found in dogs worldwide; *B. conradae* reported from California; and a *B. microti*–like organism reported from southern Europe and occasionally other areas (Camacho et al, 2001; Irwin, 2009; Kjemtrup et al, 2006; Yeagley et al, 2009). Transmission patterns and vectors for the small *Babesia* spp. are not well established.

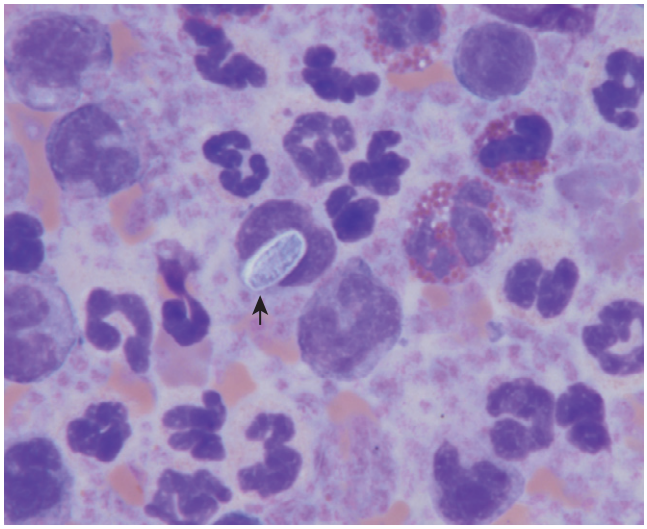

FIGURE 5-11. *Hepatozoon americanum* gamont *(arrow)* within a canine leukocyte. (Courtesy E. Johnson, Oklahoma State University.)

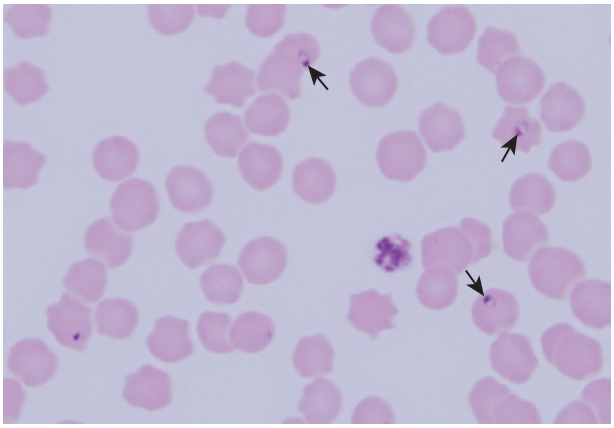

FIGURE 5-12. *Cytauxzoon felis* merozoites *(arrows)* within feline erythrocytes. (Courtesy M. Reichard, Oklahoma State University.)

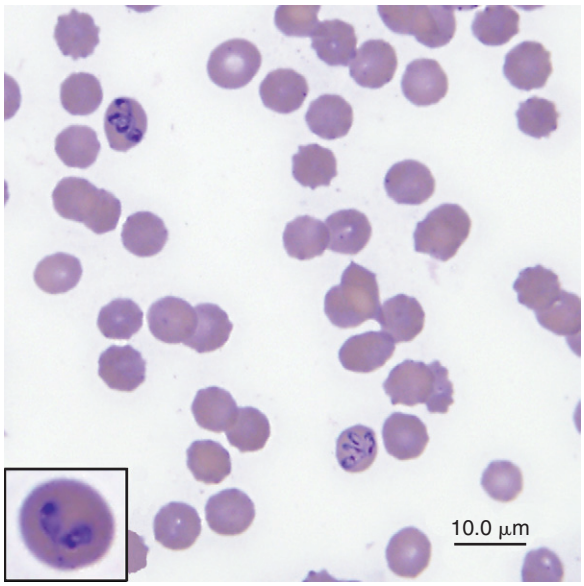

FIGURE 5-13. *Babesia rossi,* a large piroplasm canine babesiosis agent, in erythrocyte of naturally infected dog. (Courtesy R. Allison, Oklahoma State University.)

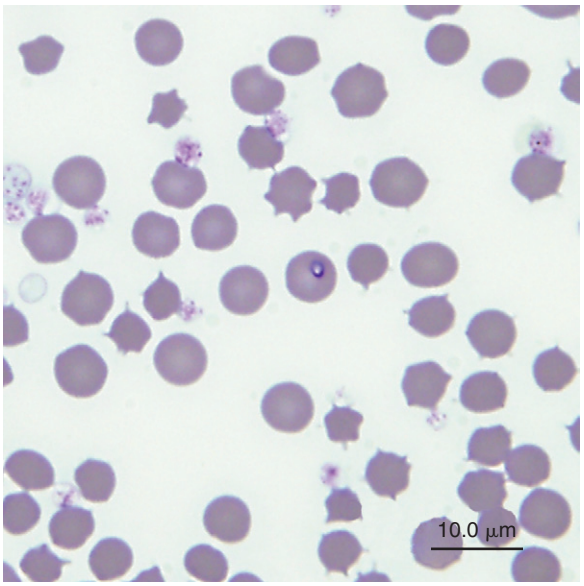

FIGURE 5-15. *Theileria* sp. merozoite in erythrocyte of a naturally infected cow. (Courtesy R. Allison, Oklahoma State University.)

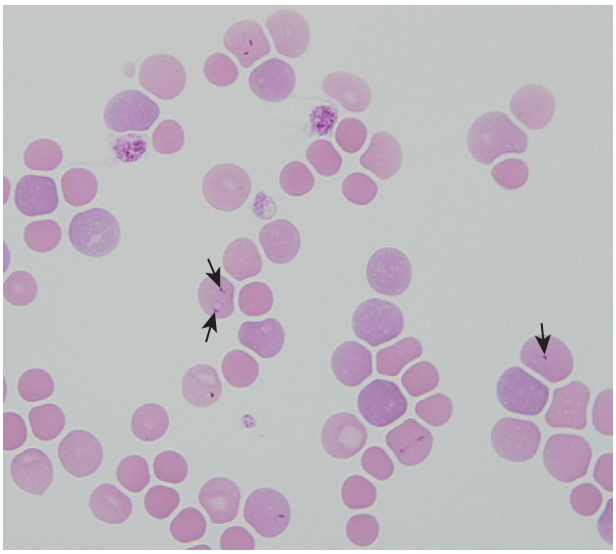

FIGURE 5-14. *Babesia gibsoni (arrows)* within canine erythrocytes. (Courtesy R. Allison, Oklahoma State University.)

In Asia, *B. gibsoni* appears to be transmitted by ticks (*Haemaphysalis* spp.), but direct dog-to-dog transmission, usually through fighting, is thought to be the primary route of transmission in North America (Irwin, 2010). Dogs serve as the main reservoir for infection with canine babesiosis agents.

Bovine babesiosis, historically referred to as **Texas cattle fever** before eradication from the southern United States, is caused by tick-borne infection with *Babesia bovis* and *B. bigemina* and remains prevalent in many areas of the world, including Africa, Asia, Australia, Latin America, and southern Europe (Barros and Fighera, 2008; Hunfeld et al, 2008). A number of different *Rhipicephalus* spp. ticks, including *R. annulatus* and *R. microplus,* transmit *B. bovis* and *B. bigemina* between cattle. Infections are maintained in ticks transovarially as well as transstadially, allowing for efficient transmission by these one-host tick species, although the epidemiologic significance of different stages of each tick species varies somewhat (Hunfeld et al, 2008). Infections with *B. bovis* and *B. bigemina* are almost entirely limited to cattle, which, together with ticks, are the primary reservoir of infection, and only occasionally are reported in wild ruminants. Persistently infected cattle serve as an ongoing source of infection to the vector ticks for months or years following initial infection (Barros and Fighera, 2008). A related organism, *B. divergens,* infects cattle in Europe and is transmitted by *I. ricinus*. Transovarial and transstadial transmission also occurs in the *B. divergens/I. ricinus* system, and populations of ticks may harbor infection for several years even in the absence of exposure to infected cattle (Hunfeld et al, 2008; Zintl et al, 2003). Unlike *B. bovis* and *B. bigemina, B. divergens* is potentially zoonotic, particularly for immunocompromised people (Zintl et al, 2003).

Cattle also acquire the agents of bovine theileriosis from ticks. **East Coast fever** is a severe, febrile disease of cattle caused by *Theileria parva* infection in sub-Saharan Africa and is transmitted among cattle and wild ruminants, such as water buffalo, by *Rhipicephalus appendiculatus* ticks (Bishop et al, 2004; Mahan, 2008). **Tropical theileriosis** is caused by *T. annulata* infection of ruminants in northern Africa, the Middle East, and some coastal Mediterranean areas including southern Europe. Infections with *T. annulata* are primarily seen in cattle, water buffalo, yaks, and camels, and the organism is transmitted by a number of different *Hyalomma* spp. (Brown, 2008a). *Hyalomma* spp. ticks also serve as vector for *T. lestoquardi,* an agent of a severe form of theileriosis in sheep and goats reported from Asia, the Middle East, Africa, and Europe (Brown, 2008a). Clinical cases of theileriosis in ruminants are considered rare in North America. However, autochthonous infection of cattle with *T. buffeli,* a theileriosis agent with a wide geographic distribution, has been reported from the central United States (Figure 5-15; Stockham et al, 2000). In addition, *T. cervi,* a common parasite of white-tailed deer transmitted by *A. americanum* ticks in the southern United States and considered to be largely nonpathogenic, has occasionally been associated with clinical disease in fawns (Kingston, 1981; Yabsley et al, 2005).

Equine piroplasmosis is caused by infection with *Babesia caballi* and *Theileria equi* and is enzootic in many regions, including Africa, Asia, the Caribbean, southern Europe, the Middle East, and Central and South America, with the exception of the

southernmost areas of Argentina and Chile. This disease is not considered enzootic in Australia, Canada, England, Iceland, Ireland, Japan, New Zealand, and the United States (Rothschild and Knowles, 2007). In most regional surveys of infected horses, *T. equi* is more common than *B. caballi* (Rothschild and Knowles, 2007). A large number of different tick species have been implicated in transmitting these agents between horses, including members of the genera *Dermacentor, Hyalomma, Rhipicephalus,* and *Amblyomma* (USDA, 2010). *Babesia caballi* is able to disseminate in tick tissues, infecting the ovaries of adult female ticks, and thus can be transmitted transovarially as well as transstadially within tick populations (Uilenberg, 2006). In contrast, *T. equi* is not maintained by transovarial transmission in most tick species. However, intrastadial transmission, in which ticks acquire infection and then move between horses to transmit the infection without requiring a molt, has been described for *T. equi* (Ueti et al, 2008).

Potential vectors of *T. equi* and *B. caballi* in the United States include *D. nitens, D. albipictus, D. variabilis,* and *R. microplus* (USDA, 2010). A recent outbreak of *T. equi* that originated in southern Texas ultimately led to identification of more than 400 infected horses in more than 20 states (Scoles et al, 2011). Although perhaps augmented in some cases by sub-inoculation through the use of contaminated needles or blood products, the source of the infection on the original premise has been attributed to transmission by the cayenne tick, *Amblyomma cajennense*—the first time this tick has been implicated as a vector of this agent (Scoles et al, 2011). Horses and other equids are the only known vertebrate reservoirs for the equine piroplasmosis agents (USDA, 2010). Equine infection with *B. caballi* and *T. equi* persists for years, even with treatment, allowing infected horses to serve as a source of future infection to ticks (Rothschild and Knowles, 2007). Transovarial transmission of *B. caballi* by some tick species, including *D. nitens,* allows ticks to also serve as a reservoir of infection even in the absence of infected horses (Rothschild and Knowles, 2007).

Avian vector-borne protozoal parasites include *Plasmodium* spp., which cause **avian malaria,** and the closely related hemosporidian genera, *Leukocytozoon* and *Haemoproteus*. More than 60 different species of *Plasmodium* have been isolated from hundreds of different species of birds, and all appear to be transmitted by mosquitoes (Braga et al, 2011). One notable species, *P. relictum,* has been implicated as a cause of death in penguins in zoologic collections and has contributed to the decline of native species in sensitive ecosystems such as that in Hawaii (Atkinson et al, 1995; Cranfield et al, 1994). Increasing temperatures and other changes in climate have been linked to a rise in the number of cases of avian malaria, presumably due to higher mosquito populations (Garamszegi, 2011). Members of the genus *Leucocytozoon* are transmitted by blackflies (*Simulium* spp.) and, rarely, by biting midges (*Culicoides* spp.). All described species of *Leucocytozoon* are found in birds (Forrester and Greiner, 2008). A majority of members of the related apicomplexan genus *Haemoproteus* are also found in birds, although some *Haemoproteus* spp. are described from reptiles and amphibians. Infections with *Haemoproteus* spp. are transmitted between hosts by a variety of blood-feeding arthropods including mosquitoes, biting midges (*Culicoides* spp.), and tabanid and hippoboscid flies (Atkinson, 2008; Bennett et al, 1994).

VECTOR-BORNE HELMINTHS

A number of helminth parasites of both small and large animals are also transmitted via arthropod vectors and thus are considered vector-borne infections (Table 5-5). These parasites, their transmission patterns, and the diseases they cause are described in greater detail in Chapter 4. Although infection with vector-borne helminths, like other vector-borne infections, may be reduced by efforts to control vector populations, vector control alone is not considered an effective means of preventing infection or disease in animals. Nonetheless, understanding the arthropod sources of any parasitic infection is a critical component in achieving effective control, particularly as anthelmintic resistance continues to be recognized in populations of some vector-borne helminths.

Mosquito transmission is the only natural means of infection of dogs and cats with third-stage larvae of *Dirofilaria immitis,* which can go on to develop into adult heartworms. Scores of mosquito species have been identified as potential vectors of *D. immitis,* although it is likely that a much more limited number is actually of enzootic significance in a given area (Ferasin and Knight, 2005; Ludlam et al, 1970). The vectors important in a given area may influence not only seasonal transmission dynamics but also infection pressure, as mosquito species vary in their host feeding preferences and willingness to enter homes (Ledesma and Harrington, 2011). Fleas *(Ctenocephalides felis)* are the most important vector of *D. caninum,* a common cestode of dogs and cats, although chewing lice have also been shown capable of serving as intermediate hosts supporting development of the cysticercoids (Conboy, 2009). Dogs

TABLE 5-5 Representative Vector-Borne Helminth Diseases of Veterinary Importance

Disease	Cause	Primary Vector	Reservoir Host
Heartworm	*Dirofilaria immitis*	Mosquitoes	Dogs, wild canids
Dipylidiasis	*Dipylidium caninum*	*Ctenocephalides felis;* also chewing lice	Dogs, cats
Habronemiasis; swamp cancer; summer sores	*Habronema* species, *Draschia* species	Muscid flies	Horses
Onchocerciasis	*Onchocerca* species	*Culicoides* species; *Simulium* species	Horses, cattle
Setariasis	*Setaria* species	Mosquitoes	Cattle, horses
Parafiliariasis; summer bleeding	*Parafilaria multipapillosa*	*Haematobia* species	Horses
	Parafilaria bovicola	Muscid flies	Cattle
Elaeophorosis; sorehead	*Elaeophora schneideri*	Tabanid flies	Mule deer
Bovine stephanofilariasis; hump sore	*Stephanofilaria stilesi, Stephanofilaria assamensis*	*Haematobia irritans, Musca conducens*	Cattle
Eyeworm	*Thelazia* species	Muscid flies	Various mammals

and cats become infected during grooming, which results in ingestion of the infected arthropod vectors. Fleas remain an important vector for *Acanthocheilonema reconditum,* a subcutaneous filarid of dogs (Brianti et al, 2012), and brown dog ticks *(R. sanguineus)* appear to transmit *Cercopithifilaria* sp. between dogs (Otranto et al, 2012).

Eyeworms (*Thelazia* spp.) are transmitted between animals by fly intermediate hosts feeding on mammalian lachrymal secretions. For example, drosophilid flies, including *Phortica* spp., serve as vector and intermediate hosts for *Thelazia callipaeda;* human infections are occasionally reported (Otranto and Dutto, 2008; Otranto and Traversa, 2005; Otranto et al, 2006). Blood-feeding flies transmit a large number of filarid nematodes between large animals, including *Elaeophora, Setaria, Onchocerca, Stephanofilaria,* and *Parafilaria* spp., and muscid flies transmit *Habronema* spp. and *Draschia* spp. between horses (see Table 5-5).

Preventing infection with vector-borne helminths, like controlling all vector-borne diseases, relies on controlling the arthropods that serve as the source of infection. Changing weather patterns, expanding geographic distribution, and growing evidence of resistance to insecticides and acaricides by arthropod populations worldwide have made vector control more difficult to achieve and have increased the threat of vector-borne infections for humans and other animals. Ultimately, understanding the arthropods and reservoir hosts responsible for creating the risk of these infections is critical to the success of our efforts to interrupt transmission and protect animal and human health.

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CHAPTER 6

Antiparasitic Drugs

Tad B. Coles and Randy C. Lynn

A *parasiticide* is a poison that is more toxic to parasites than to their hosts. This is the principle of selective toxicity. The degree of discrimination is sometimes small, sometimes considerable, but never complete, so that application of parasiticides always entails some hazard to the host. As a matter of fact, it is sometimes easier to explain the deleterious effects that parasiticides frequently exert on the host than to explain how they kill parasites.

This chapter is divided into three sections: insecticides, antiprotozoals, and anthelmintics, although not every drug fits nicely into just one of these sections. Consider endectocides, named as such because just like endoparasiticides, they kill internal parasites, and just like ectoparasiticides, they also kill external parasites. Examples are ivermectin, selamectin, doramectin, and eprinomectin, which have some insecticide activity but are covered in the anthelmintic section. Another agent, fenbendazole, is also covered in the anthelmintic section, has some antiprotozoal activity, too.

The literature on insecticides and antiparasitic drugs is voluminous. In the interests of both economy and readability, we have tried to list the few references that will guide the veterinarian who needs more specific information about these agents.

DEVELOPMENT

Stages in the development of a typical insecticide or anthelmintic proceed approximately as follows. First, many thousands of compounds must be screened before one is found that shows promise. The screening procedure, in the case of an anthelmintic, could require the demonstration of in vivo activity against some convenient parasite (e.g., *Nematospiroides dubius, Nippostrongylus brasiliensis, Syphacia obvelata*, or *Hymenolepis nana* of laboratory rodents; *Ascaridia galli* or *Heterakis gallinarum* of chickens). In vitro assays have been developed that allow rapid screening of large numbers of potential agents (Londershausen, 1996). A preliminary estimate of mammalian toxicity is also obtained from experiments on rats and mice.

The activity screening tests and preliminary toxicity studies greatly reduce the list of suitable candidates, but are of little value in predicting the effect of a particular drug either on a particular species of domestic animal or on its customary assemblage of parasites. Responses of various species and strains of parasites and their hosts to antiparasitic agents are sometimes quite selective. Thus ascarids are very sensitive to piperazines, whereas hookworms are quite refractory. Most canine and bovine breeds tolerate judicious applications of organophosphate insecticides, whereas Brahman cattle, Greyhounds, and Whippet dogs are likely to be fatally intoxicated by such treatment (Riviere and Papich, 2009). The necessary information can be obtained only through experiments on domestic animals and the parasites for which the anthelmintic is intended.

When a commercial manufacturer files a New Animal Drug Application with the Food and Drug Administration (FDA), it must submit complete information on its chemistry, manufacturing process, and quantitative assay methods. Results of all experiments conducted to establish the safety and efficacy of the new product and all relevant published reports must also be submitted. Drugs intended for food animals must be accompanied by data on tissue residues and the route and rate of excretion of the parent compound and its major metabolites. The amount and the structure of the longest-lasting tissue residue must also be determined, and if the substance has similarities to known carcinogenic chemicals, 2-year toxicity experiments are required in rats and mice.

The Environmental Protection Agency (EPA) requires an environmental impact analysis of the new agent. Phytotoxicity and effects on fish and other lower animals also must be vigorously studied. A thorough analysis must be conducted to establish any potential effects on users who apply the product. Worker safety must be addressed, so that the appropriate safety measures (e.g., gloves, safety glasses) can be written into the instructions.

Before a new anthelmintic or any new parasiticide can be approved, well-controlled experiments must be carried out involving the sacrifice of test animals and determination of residual parasite burdens after treatment. Several independent laboratories must conduct confirmation experiments with a series of field tests in different geographic regions of the United States.

The package label is required by law to bear all the necessary cautions and to notify the user about all adverse reactions that have been discovered. The manufacturer is required to report any adverse reactions that have come to light to the appropriate regulatory agency (FDA or EPA) and to either add appropriate notices to the label or withdraw the product from the marketplace. As a result, the label (aka package insert or product insert) has become one of the most objective and current sources of information on parasiticides.

In the early phases of new product development, a code number (e.g., S-147) usually identifies the agent. This is to keep the hundreds of thousands of potential products separate and to avoid the trouble of naming each one. Once a product clears the early hurdles of activity and safety, it is given a nonproprietary or generic name.

The nonproprietary name is used in the scientific literature worldwide to identify the molecule. Thus S-147 becomes milbemycin oxime. As product development proceeds, the marketing staff develops a trade name. This name will be trademarked and applied to a specific formulation. At this point milbemycin oxime becomes identified under the registered trademark as INTERCEPTOR.

One molecule may have several different trade names that correspond to different formulations or to different countries. For example, in the United States milbemycin oxime is marketed and sold as INTERCEPTOR for internal parasite control, is sold as MILBEMITE for treatment of ear mites, and is sold in combination with lufenuron as SENTINEL. These trade names will be used in the advertising and promotion of the product. It is not unusual for the same trade name to be used for a variety of types of products, which, in addition to being easier to market because of name recognition, may be confusing for owners, especially those who have difficulty reading or comprehending what they read. As an example, "HARTZ ULTRAGUARD" brand name is part of the trade name for products that are formulated as a flea and tick spot-on, cat spray, dog spray, dog collar, cat collar, shampoo, home spray, and home fogger.

When products with vastly different characteristics have similar brand names, it is easy for people to become confused. Use of similar brand names for dissimilar products may improve sales and, as such, are favored by marketing departments, but may also lead to increased adverse events when people do not read labels carefully. As an example, spot-ons marketed with the "VECTRA" brand name include some with permethrin that are toxic to cats, and some that have no permethrin and are perfectly safe to use in cats. The "HARTZ ULTRAGUARD" and "VECTRA" brand names are included here as examples, but many others could have been highlighted because using similar brand names on dissimilar products is pervasive throughout the pharmaceutical industry. Spot-on products, which are discussed in-depth in the insecticide section, can be particularly dangerous. See the permethrin section for more about spot-on labeling changes required by regulatory agencies in the United States and Canada.

In this text nonproprietary names are used to identify products, and one or more trade names may be mentioned in small caps, usually parenthetically. No discrimination is intended and no endorsement is implied when brand or trade names appear.

RESISTANCE TO PARASITICIDES

Regular application of antiparasitic drugs to populations of parasites often results in the development of resistant parasite populations through selection of resistant phenotypes. Eventually the once-effective drug ceases to work and must be replaced by another.

Unfortunately, the replacement also may fail against the resistant strain, especially if it is a chemical congener of the original. This has happened often enough to serve as a warning. We need to develop better ways of controlling parasites than to lash away at them crudely and blindly with one chemical after another. In general, principles of evidence-based medicine should be used to identify the best product to use in each particular situation. Resistance of specific parasites may be mentioned in the section specific to each parasiticide. One of the most troublesome developments of resistance concerns anthelmintics, especially helminth-resistant strains in goats and sheep and cyathostome-resistant strains in horses. At the end of the chapter, after all parasiticides have been discussed, you will find an overview of how resistant parasites are affecting certain animal species.

CONSULTATIONS AND REPORTING REACTIONS

Treatment of antiparasitic drug overdose or insecticide toxicity is a complex subject that, for the most part, lies outside the primary focus of this chapter. A few general comments, however, are included for many of the drugs. Some signs associated with parasiticide overdose or toxicity of animals may be included along with some discussion of treatment of affected veterinary patients. Because of recent advancements in the treatment of avermectin toxicity, this topic is covered with a bit more vigor. Likewise, some clinical signs associated with human exposure to certain products may be mentioned, but the signs listed should not be considered all-inclusive. The veterinarian should seek guidance elsewhere for specific up-to-date parasiticide toxicity and treatment information.

It is important to note where to call when potential adverse reactions or problems arise. For emergencies, calling the National Capitol Poison Center number (800-222-1222) anytime day or night, from anywhere in the United States (and Puerto Rico), will automatically connect you to a local human poison center at no charge. Availability of veterinary information is not the primary focus of human poison centers and varies with locale. For veterinary information, the American Society for the Prevention of Cruelty to Animals (ASPCA) Animal Poison Control Center (http://www.aspca.org/pet-care/poison-control/) is well staffed and has a large database for consultation support. This group primarily covers the United States and Canada and infrequently takes international calls. They charge a $65 consultation fee for each case and can be reached at 800-548-2423 or 888-426-4435 day or night. In addition, the Pet Poison Helpline (http://www.petpoisonhelpline.com/) is available in the United States, Canada, and the Caribbean, 24 hours a day. This group can be reached at 800-213-6680 and charges a $39 consultation fee per incident.

The National Pesticide Information Center (NPIC; http://npic.orst.edu/), formed by a cooperative agreement between Oregon State University and the EPA, provides a wide variety of pesticide information at no charge. This group has information available online, promptly answers email requests (npic@ace.orst.edu), and answers phoned-in questions (800-858-7378) from 7:30 AM to 3:30 PM PST, Monday through Friday. Online service includes information about pesticide safety, regulations, toxicity, and ingredients. The group encourages pesticide users to call or email with specific questions about pesticides, such as predicting pesticide degradation in the environment or on a premise. It also provides information about where to report pesticide concerns, spills, and emergencies, along with contact information for manufacturers and state and federal authorities. In addition, an online "vet portal" allows veterinarians to report adverse pesticide incidents (http://pi.ace.orst.edu/vetrep/). NPIC forwards these reports to the EPA.

The manufacturer of the product can also be consulted. They are often helpful and may provide assistance in investigating causality and treating patients suffering from an adverse event. Drug manufacturers are required by law to notify federal authorities concerning all adverse reactions, including lack of efficacy. They may be willing to pay for diagnostic costs in order to confirm product causality or clear the product name. Reporting adverse events improves the quality of label information the longer a product is on the market.

INSECTICIDES

Since insecticides are poisons that are more toxic to the target than to the host, risks are involved in their application. The World Health Organization (WHO) classifies pesticides by hazard as presented in Table 6-1, which helps quantify risk to those applying insecticides.

As indicated in Table 6-2, the EPA categorizes products according to specific risk in each of the following types of studies: oral median lethal dose (LD_{50}), dermal LD_{50}, inhalation, eye irritation, and skin irritation. Different signal words and warning statements are used in labeling products that fall into each specific category for each type of study.

The International Agency for Research on Cancer (IARC) has classified some insecticides according to their human carcinogenic potential as indicated in Box 6-1.

When particular insecticides are described, WHO classes, EPA categories, and/or IARC groups may be referenced.

In the United States, pesticide users bear a legal responsibility for knowing which chemicals they are currently permitted to use, and for using these chemicals only in strict accordance with the indications and directions on package labels. Current information on pesticides should be sought from the EPA, the pesticide coordinator, and the extension entomologist with livestock responsibility, or from the extension veterinarian appointed by the state agricultural extension services and land grant colleges.

BOX 6-1 IARC Human Carcinogenic Classification

Group 1	Carcinogenic to humans
Group 2A	Probably carcinogenic to humans
Group 2B	Possibly carcinogenic to humans
Group 3	Not classifiable as to its carcinogenicity to humans
Group 4	Probably not carcinogenic to humans

Adapted from International Agency for Research on Cancer (IARC): *Agents classified by the IARC monographs, volumes 1-105,* 2012.

TABLE 6-1 World Health Organization (WHO) Classification of Pesticides by Hazard

WHO Class		LD_{50} FOR THE RAT (MG/KG BODY WEIGHT) Oral	Dermal
Ia	Extremely hazardous	<5	<50
Ib	Highly hazardous	5-50	50-200
II	Moderately hazardous	50-2000	200-2000
III	Slightly hazardous	2000-5000	2000-5000
U	Unlikely to present acute hazard	>5000	>5000

Adapted from World Health Organization: *The WHO recommended classification of pesticides by hazard and guidelines to classification 2009*, 2010.

In the United States, the Federal Environmental Pesticide Control Act (FEPCA) of 1972 is administered by the EPA, which controls the distribution, sale, and use of pesticides within each state and between states. This Act specifies what penalties may be imposed for the misuse of pesticides. State governments may establish even stricter standards than those set by FEPCA. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) was amended in 1988 to accelerate the reregistration of products with active ingredients registered before November 1, 1984, and was amended again by the Food Quality Protection Act of 1996 (FQPA) and the Pesticide Registration Improvement Act of 2003 (PRIA), to set time frames for the issuance of Reregistration Eligibility Decisions (RED). FIFRA calls for the development and submission of data to support the reregistration of an active ingredient, as well as a review of all data submitted to the EPA. Reregistration involves a thorough review of the scientific database underlying a pesticide's registration. The purposes of the agency's review are to reassess potential hazards arising from currently registered uses of a pesticide, to determine the need for additional data on health and environmental effects, and to determine whether or not the pesticide meets the "no unreasonable adverse effects" criterion of FIFRA. Data included in the RED and in other EPA materials will be cited and referenced as indicated.

The diversity of structure, biologic activity, and toxicity among insecticides is exceeded only by the number and variety of insects, mites, and ticks that we try to control. The label of every insecticide container must be read carefully and understood before the contents are applied to an animal. As previously stated, the label is the most up-to-date and consistently available authoritative source of information on insecticides (North American Compendiums, 2012). In addition, several good review texts discuss the chemistry, mode of action, and toxicity of insecticides (Krieger, 2010; Riviere and Papich, 2009; Stenersen, 2004; Yu, 2008). The Insecticide Resistance Action Committee (IRAC) organizes all known insecticides by their mode of action and routinely updates its classification scheme to keep up with ongoing research (Insecticide Resistance Action Committee [IRAC], 2012). IRAC mode-of-action data will be cited and referenced as needed.

TABLE 6-2 EPA Toxicity Category (Signal Word)

Study	Category I (DANGER)	Category II (WARNING)	Category III (CAUTION)	Category IV (optional)
Acute oral LD_{50}, mg/kg	≤50	>5000-500	>500-5000	>5000
Acute dermal LD_{50}, mg/kg	≤200	>200-2000	>2000-5000	>5000
Acute inhalation LC_{50}, mg/L*	≤0.05	>0.05-0.5	>0.5-2	>2
Eye irritation	Corrosive (irreversible destruction of ocular tissue) or corneal involvement or irritation persisting for >21 days	Corneal involvement or other eye irritation clearing in 8-21 days	Corneal involvement or other eye irritation clearing in ≤7 days	Minimal effects clearing in <24 hours
Skin irritation	Corrosive (tissue destruction into the dermis and/or scarring)	Severe irritation at 72 hours (severe erythema or edema)	Moderate irritation at 72 hours (moderate erythema)	Mild or slight irritation at 72 hours (no irritation or slight erythema)

*Four-hour exposure.
Adapted from U.S. Environmental Protection Agency: *Label review manual*, 2012a.

BOTANIC AGENTS

The botanic insecticides are derived from plant materials. Ground plant parts (e.g., flowers, leaves, stems, roots) or their extracts may be combined in a variety of formulations. Essential oils from plants are often used as insect attractants or repellents. Botanic insecticides, particularly pyrethrins, have excellent toxic effects against a variety of crop and animal insect pests, very short persistence in the environment, and relatively low toxicity to animals. Pyrethroids are synthetic pyrethrin-like compounds with superior potency and knockdown activity.

Rotenone

Rotenone is an insecticidal product obtained from plant roots. It is the insecticidal component of derris root, cube root, and several other leguminous shrubs. Long ago, natives in South America used rotenone to paralyze fish, which then surfaced and were easily caught. In the 1800s it was first used to control leaf-eating caterpillars.

It acts as an inhibitor of mitochondrial respiratory enzymes. Rotenone is insoluble in water but is very soluble in alcohols, acetone, carbon tetrachloride, chloroform, and many other organic solvents. It decomposes on exposure to light and air. Rotenone is a WHO class II, moderately hazardous insecticide. In humans it can cause confusion, cough, and other clinical signs on inhalation, gastrointestinal (GI) signs on ingestion, and redness of eyes and skin on contact. The oral LD_{50} of rotenone for rats is 133 mg/kg and for white mice, 350 mg/kg. Rotenone is highly irritating to rabbit skin and is toxic to fish and aquatic life.

Historically, rotenone, alone or synergized, was the main insecticidal ingredient in several ear mite solutions (e.g., GOODWINOL OINTMENT). It was applied to cats and dogs as an ointment, solution, or shampoo for the control of a variety of arthropod parasites including localized demodicosis in dogs and ear mites *(Otodectes cynotis)* in cats, dogs, and rabbits. In 2006, the EPA reviewed rotenone during reregistration (a process previously described in the first paragraph of the Insecticide section), and as a result rotenone is being phased out for all uses (including livestock, residential and home owner uses, and domestic pet uses) except for use as a piscicide (fish-kill agent) (U.S. Environmental Protection Agency, 2007b). In 2011, a study showed that rotenone use in farm workers was associated with Parkinson's disease (Tanner et al, 2011). Goodwinol Shampoo is available, but the name is confusing because rotenone is not one of its ingredients. Currently rotenone is only available for veterinary use as the insecticidal ingredient in GOODWINOL OINTMENT.

WARNING: Kittens younger than 4 weeks of age and suckling puppies should not be treated with rotenone products. Rotenone is toxic to swine, fish, and snakes. It should not be applied to these animals. Cats and dogs may vomit after licking rotenone from their coats.

Limonene

Limonene was first registered as an insecticide in the United States in 1958. D-Limonene (common isomer) is a cyclic terpene obtained from citrus fruit. Limonene is one of the pesticide active ingredients with a reduced set of generic data requirements for registration or reregistration. Limonene is naturally occurring, has been established as inert, is exempt from the requirement of a tolerance study, and is recognized as safe by the FDA. It is not carcinogenic or mutagenic, and is not considered a developmental toxin (U.S. Environmental Protection Agency, 1994). Limonene has low acute oral toxicity. It is listed in the Code of Federal Regulations as "generally recognized as safe" for flavoring and can be found in common food items. Limonene is a dermal irritant in high concentrations and may cause dermal sensitization.

The primary toxicologic concerns for domestic animals are adverse reactions in a small percentage of animals, cats in particular, that develop transient signs when exposed to limonene in flea and tick spray, shampoo, or dip products (U.S. Environmental Protection Agency, 1994). Limonene is available as the active ingredient in a 5% formulation in a flea and tick shampoo (ADAMS D-LIMONENE FLEA & TICK SHAMPOO) for dogs and cats (do not use in puppies or kittens younger than 12 weeks of age) and in a 4% formulation insecticide premise spray (SENTRY NATURAL DEFENSE BRAND HOUSEHOLD SPRAY). It is also available in several shampoo formulations, two of which—D'LIMONENE FRAGRANCE DIP & SHAMPOO ADDITIVE and D'LIMONENE SHAMPOO—are labeled as not containing insecticides, with product insert language focusing instead on the "natural" nature of the ingredients.

Pyrethrins

The flower head of the pyrethrum plant, *Chrysanthemum cinerariaefolium,* contains six closely related insecticidal substances (pyrethrin I and II, cinerin I and II, jasmolin I and II) that are known as *pyrethrins.* Pyrethrins are rapidly degradable in the presence of moisture, air, and light and are also rapidly biodegradable. They are highly soluble in kerosene but insoluble in water. They are considered WHO class II—moderately hazardous—and the oral LD_{50} of pyrethrins for rats is 200 to 1500 mg/kg, depending on the purity of the product. The dermal LD_{50} for rats is >1800 mg/kg and for rabbits is >2000 mg/kg. Pyrethrins are not considered dermal irritants, but they do induce hepatic microsomal activity and cause tumors in rats and mice. Pyrethrins may produce some inhalation problems in rats, but regular aerosol applications should not produce any adverse reactions in domestic animals. Because they are toxic to fish, pyrethrin aerosols should not be used near fish tanks.

Pyrethrins rapidly knock down, paralyze, and kill arthropods by affecting sodium and potassium ion transport in nerve membranes, thus disrupting neurotransmission along the axon and at the synapse (Krieger, 2010). Residues of pyrethrins are sometimes repellent. Pyrethrins are usually combined with a synergist such as piperonyl butoxide, or *N*-octyl bicycloheptene dicarboximide (MGK 264). Synergists increase the insecticidal activity 10 to 20× (Plapp, 1991). Synergists poison the mixed function oxidases, which detoxify insecticides in the insect (Kahn, 2005).

Because of the safety and rapid knockdown effect of natural pyrethrins, they are widely used in the home and in agriculture. Natural pyrethrins have more uses approved by the EPA than any other insecticide. Many commercially available insecticides formulated as aerosols, dips, fogs, otic solutions, powders, repellents, roll-ons, shampoos, and mists contain a mixture of pyrethrins and a synergist (e.g., ADAMS PYRETHRIN DIP, BIO SPOT CARPET POWDER, ENDURE ROLL-ON FOR HORSES, FLYS-OFF INSECT REPELLENT, MITA-CLEAR, MYCODEX PET SHAMPOO, PROZAP DAIRY CATTLE SPRAY).

Pyrethrins are one of the active ingredients in many aerosols, fogs, sprays, and powders that control face flies, horseflies, deer flies, horn flies, house flies, stable flies, gnats, moths, bedbugs, mites, mosquitoes, fleas, ticks, lice, and many other insects. Pyrethrins are registered for application to dogs, cats, horses, cattle (beef and lactating dairy), swine, sheep, and goats, and in a wide variety of premises (e.g., cattle, horse, poultry, and swine quarters, including stables, dairy barns, milk houses, milk parlors, horse barns, loafing sheds, holding lots, poultry barns, hog barns, poultry roosts, nests,

cages; food processing plants; restaurants). They are not persistent insecticides, so regular and repeated application is necessary.

WARNING: Pyrethrins should not be applied to kittens younger than 4 weeks of age or to suckling puppies. In case of ingestion, the most toxic component is usually the solvent. Therefore inducing emesis is contraindicated. Administer activated charcoal and supportive therapy. In case of dermal exposure, the animal should be bathed with detergent.

PYRETHROIDS

Pyrethroids are synthetic pyrethrin-like substances. These chemicals are more potent and possess a greater knockdown effect than do the plant pyrethrins. While pyrethroids are biodegradable when exposed to air and light, they are sufficiently stable that weekly or biweekly applications provide excellent control of insects. Toxicity and LD_{50} data for pyrethroids are variable depending on isomer ratios, the vehicle used for oral administration, and husbandry (i.e., fasting) of the animals tested. Pyrethrins and pyrethroids are among the safest of the ectoparasiticides with a safety factor (oral LD_{50} in rat/contact LD_{50} in flies) >1000 compared with organophosphates, which have a safety factor <100 (Adams, 1995). Pyrethrins and pyrethroids are included in >3500 EPA-registered products. Their use has increased in the past decade as the use of organophosphates, which are more toxic, has declined.

Pyrethroids have a greater insecticidal effect when the temperature is lowered. In chemists' language, they have a negative temperature coefficient. Thus insects, with a lower body temperature than mammals, are more susceptible to pyrethroid toxicity. Pyrethroids initially stimulate and then depress nerve cell function and eventually cause paralysis. The fast knockdown of flying insects is the result of rapid muscular paralysis. Pyrethroids have low mammalian toxicity, but some pyrethroids provoke sensation of the skin or mucosa. Pyrethroids, like pyrethrins, are toxic to fish. Both pyrethrins and pyrethroids are considered safe for use in birds unless the product's propellants and carriers are hazardous upon inhalation (Poppenga and Oehme, 2010).

Research into pyrethroid chemistry has resulted in many products. For one to make some sense of this profusion of products, it is best to divide them by generation (Ware and Whitacre, 2004). The first generation is represented by D-trans-allethrin, which is a synthetic duplicate of cinerin I, a component of natural pyrethrin. The second-generation pyrethroids include tetramethrin and phenothrin. They are more potent than pyrethrin in knockdown potency, but decompose rapidly on exposure to air and sunlight. The third-generation pyrethroids are appreciably more potent than earlier generations and are photostable for several days in full sun. They are represented by esfenvalerate and permethrin. The fourth-generation pyrethroids are represented by cypermethrin and fluvalinate.

The fifth-generation pyrethroids are the newest available and are represented by beta-cyfluthrin, an isomer subset of cyfluthrin. They are more photostable and more potent than the previous generation. The disadvantage of increased persistence in the environment is the development of insect resistance. In fact, insect resistance to synthetic pyrethroids has been documented (Plapp, 1991) and is spreading in cattle ticks (Rosario-Cruz et al, 2009). In the following discussion, synthetic pyrethroid products commonly used on domestic animals are listed according to generation.

First-Generation Pyrethroids

The first-generation synthetic pyrethroid, allethrin, appeared in 1949 (Ware and Whitacre, 2004). The D-trans isomer form of allethrin (D-trans-allethrin) is a synthetic duplicate of the natural pyrethrin, cinerin I. No more potent or stable than natural pyrethrin, it is rapidly degraded by light and air. D-Trans-allethrin, the first-generation pyrethroid, is a mixture of several optical isomers. With a WHO II classification as moderately hazardous insecticides, allethrins have low mammalian toxicity and are not mutagenic, carcinogenic or embryotoxic. D-Trans-allethrin is formulated as shampoos to kill fleas and ticks on dogs (e.g., HARTZ ULTRAGUARD PLUS FLEA & TICK DOG SHAMPOO) and to control fleas and ticks on dogs, puppies, cats, and kittens (i.e., MYCODEX SENSICARE FLEA & TICK SHAMPOO).

Second-Generation Pyrethroids

The second-generation synthetic pyrethroids were the first step forward from the natural pyrethrins. They have increased knockdown potency 10 to 50× greater than that of the natural products, but they are not much more stable in sunlight than the natural pyrethrins.

Phenothrin

Phenothrin is a second-generation pyrethroid used for flea and tick control in pets. The acute oral LD_{50} of phenothrin in rats is >5000 mg/kg, and the LD_{50} for dermal exposure in rats is >10,000 mg/kg, hence its WHO "U" classification—unlikely to present acute hazard to humans (World Health Organization, 2010). Racemic phenothrin was first synthesized in 1969. D-Phenothrin has been in use since 1977, mainly to control household insects and to protect stored grain, either alone or in combination with other insecticides. Phenothrin is an ingredient of several premise sprays (e.g., ADAMS PLUS INVERTED CARPET SPRAY). It was formulated into an array of Hartz over-the-counter spot-on products for control of fleas in dogs and cats, but feline use was stopped in 2005 because of adverse events including death. Hartz continues to market a multitude of flea and tick spot-on products for dogs that contain phenothrin (e.g., HARTZ INCONTROL FLEA & TICK DROPS FOR DOGS). The product is applied as a spot-on every month to treat and control fleas and ticks on dogs and has instructions not to use on pups younger than 12 weeks of age, cats, or kittens. Hartz also markets several flea and tick shampoos for dogs with phenothrin. The only feline phenothrin products still on the market are several Sentry and Sergeant's flea and tick collars that are to be replaced after 6 months.

Tetramethrin

Tetramethrin is a second-generation synthetic pyrethroid originally developed in Japan, first synthesized in 1964, and marketed in 1965. Its acute oral and dermal LD_{50} in rats is >5000 mg/kg, making it the second insecticide discussed thus far that has a WHO "U" classification and is "unlikely to present acute hazard" to humans (World Health Organization, 2010). Tetramethrin's EPA categories are IV for oral and dermal LD_{50} and III (Caution) for skin and eye irritation (U.S. Environmental Protection Agency, 2010b). Tetramethrin is one of the ingredients in several premise sprays and foggers. With all foggers, be certain to follow the directions, which include covering food, leaving the area, avoiding contact with pilot lights and open flames, and airing out the premises thoroughly after treatment. Tetramethrin in combination with etofenprox (a pyrethroid ether insecticide) is available in a handheld fogger to kill flying and crawling insects outside (i.e., VETKEM SIPHOTROL X-TEND HANDHELD YARD & PATIO FOGGER). The product should be used judiciously outdoors as it is very toxic to bees. Tetramethrin is one of several ingredients (pyrethrins and synthetic pyrethrins) included in a topical horse insect repellent

wipe or spray that is also marketed as a premise insecticide (i.e., ABSORBINE ULTRASHIELD RED INSECTICIDE & REPELLENT).

Third-Generation Pyrethroids

The third-generation synthetic pyrethroids became available in the 1970s. Photostability is the hallmark of this class. For the first time, increased potency and photostability were available in the same molecule.

Esfenvalerate

Esfenvalerate replaced fenvalerate in the United States; the latter was the first of the third-generation pyrethroids to be commercially successful. The only difference between esfenvalerate and fenvalerate is the relative proportion of the four isomers. Esfenvalerate is preferred because it contains a much higher percentage of the insecticidal isomer, requires lower applications rates, is less chronically toxic, and is a more powerful insecticide. It is very photostable, relatively stable to hydrolysis, and highly toxic to fish and bees. Esfenvalerate was not found to be carcinogenic or genotoxic to rodents. Esfenvalerate, a WHO class II insecticide, has an oral LD_{50} of 90 mg/kg in rats and a dermal LD_{50} of >5000 mg/kg in rats and >2000 mg/kg in rabbits (World Health Organization, 2002). It can cause red skin upon contact with human skin. Formulated for long residual insecticide activity, it is available in premise foggers and sprays for the house, kennel, and yard (e.g., SERGEANT'S HOUSEHOLD FLEA & TICK SPRAY). People and pets should not be allowed in treated areas until spray has dried. Obviously, it should not be applied directly to animals.

Permethrin

Permethrin, a third-generation pyrethroid, is an extremely active insecticide with rapid knockdown against a variety of insects. Although it has a WHO class II (moderately hazardous, <2000 mg/kg LD_{50}) classification (World Health Organization, 2010), the EPA lists the acute oral LD_{50} for rats as 3580 mg/kg (male)/2280 mg/kg (female) and the dermal LD_{50} for rabbits at >2000 mg/kg for an EPA III (Caution) toxicity category (U.S. Environmental Protection Agency, 2006b). The EPA eye irritation category is III and the skin irritation category is IV. Although no human data were found on human carcinogenicity of permethrin, the EPA has classified it as likely to be carcinogenic upon ingestion (Toynton et al, 2009). This classification was based on reproducible permethrin studies that resulted in benign lung and liver tumors in mice (U.S. Environmental Protection Agency, 2006b). Conversely, the IARC classified permethrin in Group 3 (Not classifiable as to its carcinogenicity to humans).

Like esfenvalerate, permethrin is very toxic to fish. It is photostable, with effective residues lasting 4 to 7 days on crop foliage. Permethrin is the most ubiquitous of the pyrethroids approved for use on or around animals. About 2 million pounds of permethrin is used annually—more than a half million pounds agriculturally and almost 1.5 million pounds nonagriculturally (U.S. Environmental Protection Agency, 2006b). It is even available incorporated into clothing. The repellency of permethrin is emphasized in EPA-registered insect-repellent cloth (e.g., BUZZ OFF INSECT SHIELD, INSECT SHIELD INSECT REPELLENT GEAR, GUARDIAN GEAR INSECT SHIELD BANDANA) that is used in clothing for people and clothing, blankets, crates, cots, and bandanas for dogs. This cloth not only repels fleas and ticks, it also provides knockdown of 80.8% to 96.7% for mosquitoes and 95.8% to 100% for ticks through 70 launderings (Insect Shield, 2010). One result of such prolific use is that house fly resistance to permethrin is increasing (Zhu et al, 2008).

Permethrin is registered in a wide variety of formulations as a premise treatment for animal quarters (dairy barns, feedlots, stables, poultry houses, swine, and other animal housing) to control house flies, stable flies, many other manure-breeding flies, gnats, mosquitoes, and a multitude of other insects including cockroaches, ants, silverfish, spiders, crickets, mites, weevils, beetles, mealworms, moths, and bedbugs.

For use on animals, it is available in wide variety of concentrations (0.1% to 65%) as wipe-on, pour-on, spot-on, back rub, paste (for dermal application, not oral), spray, dip, shampoo, ear tag, and dust formulations for use on dogs, cats, horses, cattle, sheep, goats, and swine to kill fleas, ticks, and lice, and to control many of the same pests listed previously for premise products. Some premise products are registered for use directly on animals as well, emphasizing the importance of careful reading of the label before use.

Permethrin is both lethal (knockdown) and repellent. It interferes with the parasitic ability to attach and feed, which is important, not only because of elimination of irritation associated with feeding, but also because it may prevent transmission of vector-borne diseases (Blagburn, 2003).

Care must be taken when permethrin is used on or around cats. Cats exposed to permethrin may develop hyperexcitability, depression, ataxia, vomiting, anorexia, tremors, convulsions, or death. Signs can begin within a few minutes or up to 3 days after exposure (Toynton et al, 2009). A report on 11 cats treated by owners intentionally, although not maliciously, with 45% to 65% permethrin products described the following clinical signs after exposure: muscle tremors, seizures, incoordination, agitation, and death (Meyer, 1999). When seizures developed, they occurred within 2 to 24 hours of exposure. In one additional case, a cat developed signs including agitation, tremors, seizures, and ataxia, 18 and 24 hours after being near two recently permethrin-treated dogs. Permethrin-exposed animals may drool or smack their lips, probably as a result of licking at the application site, possibly caused by taste or an oral tingling sensation.

Spot-on products with permethrin can be particularly dangerous. A surge of permethrin spot-on complaints in 2008 sparked EPA interest. Total spot-on incident report numbers (including permethrin and non-permethrin spot-on products) to the EPA by year are as follows (U.S. Environmental Protection Agency, 2012c):

- 2008 = 44,465
- 2009 = 38,073
- 2010 = 27,539
- 2011 = 21,158

Although adverse events associated with their use declined from 2009 to 2010, in 2011 the EPA asked spot-on manufacturers to make labeling clearer by using enlarged fonts and images of animals, and, among other things, to add a cat prohibition icon to the lower right corner of canine flea and tick packaging for products that are toxic to cats (Selinger and Fiala, 2012; U.S. Environmental Protection Agency, 2011).

The spot-on problem is not unique to the United States. In Canada, the Pest Management Regulatory Agency has reported that 75% of flea and tick pesticide adverse events involved spot-on products (Turner et al, 2011). Similarly, in a survey of Canadian small animal veterinarians about adverse events involving flea and tick pesticides conducted in 2007-2009, most involved spot-on products (Turner et al, 2011). As a result of Health Canada's analysis, label changes were required in 2011 for all spot-on products containing permethrin to include a pictogram on the primary label panel indicating that the product should not be used in cats (Turner et al, 2011).

That said, some low-concentration permethrin products are labeled for cats. A 0.05% permethrin (plus pyrethrin) spray is formulated for use on cats only (i.e., SERGEANT'S FLEA AND TICK SPRAY FOR CATS). A review of the Compendium of Veterinary Products revealed 0.25% permethrin dust formulations (i.e., HORSE LICE DUSTER III and PROZAP INSECTRIN DUST) and a permethrin (plus pyrethrin and piperonyl butoxide) spray formulation (i.e., PROZAP FLY-DIE EQUINE SPRAY) marketed primarily for livestock use, but also labeled for use in cats. The latter product includes 0.05% permethrin and is labeled for use as a spray or a dip in dogs, but only as a spray, not a dip, in cats.

Some permethrin products have a concentrated form (≈45% permethrin) for direct application to dogs to kill and repel fleas, ticks, and mosquitoes; one product (i.e., PROTICALL INSECTICIDE FOR DOGS) has a 65% (wt/wt) concentration formulation. In addition to synergists, many permethrin-containing products have other active ingredients, such as insect growth regulators (e.g., pyriproxyfen, (S)-methoprene), neonicotinoid insecticides (e.g., imidacloprid, dinotefuran), or oxadiazine insecticides (e.g., indoxacarb). These will be discussed in greater depth in the sections that focus on those ingredients.

Warning: Cats are sensitive to permethrin. High concentrations of permethrin (>0.5%) are not approved for and should not be used on cats (Blagburn, 2003). A novel technique for treating certain toxicoses, lipid rescue, has been used successfully to treat cats with permethrin toxicity (Bruckner and Schwedes, 2012; Haworth and Smart, 2012). This technique is discussed more fully in the section on treating avermectin toxicity.

Fourth-Generation Pyrethroids

The fourth-generation pyrethroids are more potent and longer lasting than earlier generations. The class is represented by cyfluthrin, cypermethrin, deltamethrin, and lambdacyhalothrin in an increasing variety of formulations.

Cyfluthrin

Cyfluthrin is a fourth-generation pyrethroid, a mixture of eight possible isomers. Beta-cyfluthrin consists of four of the more potent isomers of cyfluthrin and is detailed below as a fifth-generation pyrethroid. The oral rat LD_{50} of cyfluthrin is <100 mg/kg (U.S. Environmental Protection Agency, 2010a). The WHO applies a class Ib—highly hazardous—designation to it (World Health Organization, 2010).

For premise treatments, cyfluthrin is available as a 20% wettable powder and a 1% dust (i.e., TEMPO 20 WP INSECTICIDE and TEMPO 1% DUST INSECTICIDE) used to provide residual pest control for a wide variety of flying and crawling insects and spiders.

For treatment of beef and dairy cattle (including lactating) to control horn flies, face flies, biting lice, and sucking lice, cyfluthrin is available in insecticide dust and pour-on formulations. In addition, cyfluthrin is available in a gel cap (i.e., AIM-C GELCAPS) that is to be used only with a device that is similar to a paint-ball gun (i.e., VETCAP APPROVED APPLICATOR DEVICE) with specific safety instructions and directions to reapply in 3 weeks for lice, and no more often than every 3 weeks for flies.

Cypermethrin/Zeta-cypermethrin

Cypermethrin is a potent fourth-generation synthetic pyrethroid. Zeta-cypermethrin is an S-enantiomer enriched formulation of cypermethrin that is not distinguishable from cypermethrin by the analytical enforcement method. The toxicologic endpoints are the same for both cypermethrin and zeta-cypermethrin. Its acute oral LD_{50} for rats is 247 mg/kg (male) and 309 mg/kg (female) (EPA Category II). Technical grade cypermethrin has moderate acute dermal toxicity (EPA Category III) and is not a strong skin irritant (EPA Category IV) (U.S. Environmental Protection Agency, 2006c). Human skin sensations, reported during field studies, generally lasted only a few hours and did not persist for more than one day after exposure.

Cypermethrin is available as the sole ingredient in two products approved to protect horses and ponies from horse flies, house flies, stable flies, face flies, horn flies, deer flies, gnats, and mosquitoes (*Culex* spp.) (i.e., ABSORBINE ULTRASHIELD SPORT INSECTICIDE & REPELLENT and OUTLAST FLY AND MOSQUITO INSECTICIDE/REPELLENT). It is also available as a spray or a lotion, and in roll-on formulations in combination with other insecticides, some labeled for only horses and ponies and others formulated to use on horses, ponies, llamas, alpacas, mules, and donkeys. Zeta-cypermethrin is available in dust and ear-tag formulations. No cypermethrin or zeta-cypermethrin products are available for dogs or cats.

Deltamethrin

Deltamethrin is a fourth-generation pyrethroid. The acute oral LD_{50} in rats is 30 mg/kg (oily vehicle) to >5000 mg/kg (aqueous vehicle) for deltamethrin, and the acute dermal LD_{50} in rabbits is >2000 mg/kg (Johnson et al, 2010). Deltamethin has a WHO II classification (World Health Organization, 2010). It is available as a water-resistant flea and tick collar for dogs (i.e., SCALIBOR PROTECTOR BAND FOR DOGS). This product has reasonable efficacy against fleas but is noteworthy for 6-month efficacy against ticks in dogs (van den Bos and Curtis, 2002). The efficacy of the collar is not affected by exposure to water. In Europe these collars are used to prevent leishmania in dogs (Foglio Manzillo et al, 2006). The deltamethrin in this odorless collar is transferred to the dog's skin and carried by the skin's natural oils over the whole body, a process that takes 1 to 2 weeks to achieve an efficacious level and up to 3 weeks to achieve maximum efficacy. Degreasing shampoo may remove deltamethrin from the skin, but the collar continues to deliver the active ingredient, which reaches efficacy levels again in 1 to 2 weeks.

Lambdacyhalothrin

Lambdacyhalothrin is a fourth-generation synthetic pyrethroid that is highly toxic to bees and rats with an acute oral LD_{50} for male rats of only 79 mg/kg. It is formulated into premise sprays (e.g., GRENADE ER INSECTICIDE, OXYFLY INSECTICIDE) to control insects in and around livestock housing structures. The product is available alone or with piperonyl butoxide as a pour-on for use on beef cattle and calves to control lice and horn flies. Lambdacyhalothrin is also formulated in combination with an organophosphate, pirimiphos, in ear tags (i.e., DOUBLE BARREL VP INSECTICIDE EAR TAGS) labeled for up to 5 months' control of horn flies and face flies, or in combination with piperonyl butoxide (i.e., SABER EXTRA INSECTICIDE EAR TAGS) for up to 5 months' control of horn flies and 4 months' control of face flies. The ear tags are approved for use on beef cattle and nonlactating dairy cattle and calves.

Prallethrin

Another fourth-generation pyrethroid is prallethrin, which is the common name for the WHO class II pyrethroid insecticide and repellent also known by the brand name ETOC (World Health Organization, 2010). It is a racemic mixture of eight stereoisomers with an LD_{50} of 460/640 mg/kg (female/male) in the rat. The

dermal LD_{50} in rabbits is >5000 mg/kg. It is not available as a sole ingredient, but only in combination products, which include premise sprays and horse sprays.

Cyphenothrin

Cyphenothrin is a fourth-generation synthetic pyrethroid with WHO II classification (World Health Organization, 2010) and EPA category II for acute oral and inhalation toxicity (rat acute oral LD_{50} of 318/419 mg/kg male/female and LC_{50} >1.850 mg/L). It is in EPA category III for dermal toxicity (LD_{50} >5000 mg/kg) and category IV for primary eye and dermal irritation (rabbit). Cyphenothrin is not considered a dermal sensitizer per guinea pig testing (U.S. Environmental Protection Agency, 1996a; U.S. Environmental Protection Agency, 2006a). It was first registered in 1991, so it was not subject to an EPA Reregistration Eligibility Decision (RED).

Cyphenothrin formulations are available only in combination with fipronil or pyriproxyfen. These products, which are used on dogs, not cats, are discussed subsequently in the fipronil and pyriproxyfen sections.

Flumethrin

Flumethrin is not WHO classified but is in EPA category II (label caution) with mild toxicity via oral, dermal, and inhalation routes of exposure, and category IV (label optional) for eye and skin exposure (U.S. Environmental Protection Agency, 2012b). It has been registered in EU member states as an acaricide for use on companion and food-producing animals since 1986 (Stanneck et al, 2012b) and has been used outside the United States as a dip or a spray to treat tick infestations on poultry, dogs, horses, and cattle. Flumethrin was registered with the EPA in March 2012, for use in pet collars. It is not available in any U.S. products as the sole active ingredient. Flumethrin is available in pet collars only in combination with imidacloprid (Seresto). These products have 4.5% flumethrin and 10% imidacloprid as active ingredients. The collars are labeled for prevention and treatment of ticks and fleas on dogs and cats and are covered in detail subsequently in the imidacloprid section.

Fifth-Generation Pyrethroids

The fifth-generation pyrethroids are at the cutting edge of pyrethroid development. They are the most potent and the longest lasting, but the only one available is beta-cyfluthrin.

Beta-cyfluthrin

Beta-cyfluthrin consists of four of the more potent isomers of cyfluthrin (see fourth-generation pyrethroids). The oral rat LD_{50} of beta-cyfluthrin is 960 mg/kg (male) and 1150 mg/kg (female) (U.S. Environmental Protection Agency, 2010a). The WHO applies a class Ib—highly hazardous—designation to beta-cyfluthrin (World Health Organization, 2010).

For premise treatments, beta-cyfluthrin is available as an 11.8% concentrate and spray (i.e., Tempo SC Ultra Pest Control Concentrate and Tempo SC Ultra Premise Spray) to provide residual pest control for a wide variety of flying and crawling insects and spiders. Beta-cyfluthrin ear tags are available alone (e.g., CyGuard) or with piperonyl butoxide (i.e., CyLence Ultra Insecticide Cattle Ear Tag) for treatment of beef and dairy cattle (including lactating) to control face flies, horn flies, Gulf Coast ticks, and spinose ear ticks. The ear tags remain effective for up to 5 months. Like other insecticide ear tags, continuous use of one agent can lead to insect resistance. To help delay resistance, one should rotate the class of insecticide from season to season. Cyfluthrin ear tags should be removed at the end of fly season and before slaughter.

ETOFENPROX

Etofenprox is an insecticide with an action similar to pyrethroids. With an oral LD_{50} >10,000 mg/kg, it is classified by WHO as "U," or unlikely to present acute hazard (World Health Organization, 2010).

It is marketed in a multitude of spot-on products, either as the sole ingredient (e.g., Sergeant's Silver Squeeze-On for Cats and Kittens) labeled to kill fleas and deer ticks and repel mosquitoes, or with other active ingredients such as fipronil (e.g., Spectra Sure Plus for Cats). When etofenprox is combined with insect growth regulators such as (S)-methoprene (e.g., Bio Spot Defense Flea & Tick Spot On for Cats) or pyriproxyfen (e.g., Sentry Pro Toy & Small Breed Flea & Tick Squeeze-On for Dogs, Triforce Feline Squeeze-On), the product will also kill flea eggs and larvae. Etofenprox combined with piperonyl butoxide and the synergist, MGK 264 (e.g., Hartz UltraGuard Topical Flea & Tick Prevention for Dogs & Puppies), is labeled to start killing fleas within 15 minutes, to kill brown dog ticks and deer ticks for 30 days, and to repel mosquitoes. The same label claims are made for this combination of ingredients (etofenprox, piperonyl butoxide, MGK 264) plus pyriproxyfen (e.g., Hartz InControl Advanced Flea & Tick Topical Drops for Dogs and Puppies).

A spray formulation of etofenprox, piperonyl butoxide, and (S)-methoprene (e.g., Bio Spot Defense Flea & Tick Spray for Cats & Kittens) kills fleas, flea eggs, and ticks, and repels mosquitoes. A shampoo formulation of the same ingredients (e.g., Vet-Kem Ovitrol X-Tend Flea & Tick Shampoo for Dogs and Cats) kills fleas and ticks, and prevents flea eggs from hatching for 1 month.

A feline monthly spot-on flea and tick product with etofenprox, fipronil, and (S)-methoprene that has recently been introduced to a limited market (Frontline Tritak for Cats) is covered in the fipronil section.

Etofenprox is one of several ingredients in premise sprays, foggers (e.g., Vet-Kem Siphotrol X-Tend Carpet Aerosol, Adams Flea & Tick Indoor Fogger).

INDOXACARB

Indoxacarb is an oxadiazine insecticide with activity against lepidopteran pests developed by DuPont for agricultural uses (DuPont, 2006). Indoxacarb is in WHO class II with an oral LD_{50} of 1730 mg/kg in male and 268 mg/kg in female rats and a dermal LD_{50} >5000 mg/kg. It has two properties that provide unique action against fleas. Indoxacarb is actually a pro-insecticide which has limited activity against insects, but once ingested by the flea the pro-insecticide is rapidly metabolized to an active moiety, decarbomethoxylated indoxacarb, which blocks the voltage-gated sodium ion channel. No other flea-control product is known to act at this site in the flea neuron (Lapied et al, 2001; Lavialle-Defaix et al, 2010; Wing, 2000). The pro-insecticide parent molecule has low mammalian toxicity and is metabolized in mammals to molecules with low toxicity. Since the active metabolite acts at a site that is dissimilar for all other flea-control agents, there is evidence that it will perform well in flea populations that have developed tolerance to widely used flea products (Flochlay-Sigognault et al, 2011). Indoxacarb is also effective against flea eggs and larvae, so it is effective in breaking the flea life-cycle without the need of an insect growth regulator (Dryden, 2013). It is available in spot-on formulation as the sole active ingredient

(e.g., ACTIVYL SPOT-ON FOR CATS) to control fleas in kittens, cats, puppies, and dogs, and combined with permethrin (i.e., ACTIVYL TICKPLUS FOR DOGS AND PUPPIES) to control fleas and ticks in puppies and dogs.

CARBAMATES AND ORGANOPHOSPHATES

Carbamates and organophosphates are commonly used insecticides. These insecticides are toxic because they inhibit acetylcholinesterase (AChE), an important nervous system enzyme that inactivates synaptic acetylcholine. The organophosphate insecticides bind and inactivate AChE irreversibly. Carbamates, on the other hand, are reversible inhibitors of AChE. Over a period of several hours, carbamates are metabolized and AChE inhibition ceases. With both organophosphates and carbamates, the end result is the same; acetylcholine accumulates at the neural synapse because AChE is nonfunctional (Whitford, 2002). If acetylcholine is not removed, nerve stimulation continues.

The accumulation of acetylcholine results in signs of acute poisoning, which are principally the result of acetylcholine's muscarinic effects at autonomic effector organs (salivation, anorexia, vomiting, diarrhea, lacrimation, miosis or mydriasis, dyspnea, frequent urination, and bradycardia or tachycardia) and its nicotinic effects at the neuromuscular junction (rapid involuntary muscle twitching and scattered fasciculations, followed by severe weakness and paralysis) (Brunton, 2006; Talcott, 2009). Mnemonic devices to remember the clinical signs are SLUD (salivation, lacrimation, urination, defecation) and DUMBBELS (diarrhea, urination, miosis, bronchospasm, bradycardia, emesis, lacrimation, salivation) (Talcott, 2009). Many organophosphate insecticides produce a chronic neurotoxicity pattern with degeneration of long axons in the spinal cord and peripheral nerves (e.g., sciatic nerve). Pancreatitis has also been associated with organophosphate exposure.

Death is usually due to respiratory failure, so artificial respiration may be required in severe cases of carbamate and organophosphate poisoning. Induction of emesis, gastric lavage, and topical decontamination via bath and rinse should be considered.

Atropine administered parenterally and repeated as needed to control salivation and other signs is the preferred antidote for both carbamate and organophosphate poisoning. Pralidoxime (2-PAM), on the other hand, can be used to reverse organophosphate poisoning, but not carbamate toxicity. The use of 2-PAM in carbamate toxicity is contraindicated (Whitford, 2002) or is considered at best controversial (Talcott, 2009). The principal action of 2-PAM is to reactivate organophosphate-inhibited AChE, which in turn destroys the accumulated acetylcholine so that the synapses and neuromuscular junctions can regain normal function. But 2-PAM by itself inhibits AChE and will exacerbate signs caused by carbamate toxicity (Whitford, 2002). 2-PAM is relatively short acting, so repeated administration is usually required (Papich, 2007).

To reiterate, atropine is used to block carbamate- or organophosphate-caused overstimulation of acetylcholine receptors until the clinical signs can be alleviated. Then 2-PAM is administered to reactivate organophosphate-inhibited AChE, but is contraindicated with carbamate toxicity (Whitford, 2002).

Carbamates and organophosphates should not be used in conjunction with other cholinesterase inhibitors or other insecticides because the effect of these chemicals on cholinesterase reserves is cumulative, especially organophosphates. Cats and young, lean animals are more susceptible to cholinesterase inhibition. Sighthounds (e.g., Greyhounds, Whippet dogs) and certain breeds of cattle (e.g., Chianina, Charolais, Gelbvieh, Simmental, Brahman) have idiosyncratic reactions to organophosphates, which are contraindicated in these breeds. Application of organophosphates to cattle currently infested with *Hypoderma* larvae may lead to a host-parasite reaction, resulting in bloat, salivation, ataxia, and posterior paralysis. See the *Hypoderma* treatment and control section in Chapter 2 for more information.

Carbamates

Carbamates are reversible inhibitors of AChE. Over a period of several hours, carbamates are metabolized and AChE inhibition ceases. The antidote of choice is atropine; 2-PAM is contraindicated for carbamate toxicity.

Propoxur is the only carbamate currently used in veterinary medicine. Another carbamate, carbaryl, was commonly used in veterinary medicine, but not since EPA review of carbaryl for reregistration. An EPA lawsuit, which was filed by the Natural Resources Defense Council (NRDC), probably contributed to the decision by the manufacturer to withdraw carbaryl from the veterinary market, although it is still used agriculturally.

Propoxur

Propoxur is an older carbamate that was introduced in 1959. It has a quick knockdown action and affords residual effects for several weeks. Propoxur is a WHO class II (World Health Organization, 2010); oral EPA category II; and dermal, inhalation, and eye irritation EPA category III insecticide (U.S. Environmental Protection Agency, 1997b). The oral LD_{50} of propoxur for rats is ≈100 mg/kg. It is very highly toxic to birds and is highly toxic to honey bees, but it can be used safely on and around domestic animals. The NRDC is currently putting pressure on the EPA to get propoxur removed from the market.

Propoxur is commonly used in flea and tick collars for dogs and cats. Propoxur is available in collars as the sole active ingredient (e.g., SCRATCHEX COLOR-FULL FORMULA 5 FLEA & TICK COLLAR FOR CATS); with the insect growth regulator (IGR) (S)-methoprene (e.g., ADAMS FLEA & TICK COLLAR FOR CATS & KITTENS); with phenothrin and the synergist MGK 264 (e.g., BANSECT FLEA & TICK COLLAR FOR CATS); or with phenothrin, MGK 264, and the IGR pyriproxyfen (e.g., SENTRY PRO FLEA & TICK COLLAR FOR DOGS).

Organophosphates

The organophosphates are synaptic poisons that work by inactivating AChE. Toxicity from organophosphate insecticides is usually a medical emergency requiring treatment with activated charcoal and bathing to decrease absorption, 2-PAM to reverse binding to AChE, and atropine to decrease the clinical signs of acetylcholine excess (Kahn, 2005) as was previously discussed in detail in the Carbamates and Organophosphates section.

Many organophosphates are available for use on and around animals. For the long list of available organophosphates to be less imposing, they will be divided into three groups by chemical structure: aliphatic derivatives, phenyl derivatives, and heterocyclic derivatives. The aliphatic derivatives were the first to be developed. They have a simple linear structure, without complex rings. Because their structure is simple, they are rapidly broken down in the animal and the environment. The phenyl derivatives have a benzene ring and were the second class of organophosphates to be developed. They are longer lasting than the aliphatic derivatives. The last group to be developed, the heterocyclic derivatives, has ring structures in which at least one carbon atom is replaced by an oxygen, nitrogen, or sulfur atom. The members of this group are the longest lasting of the organophosphates.

Many of the organophosphates available in the past have disappeared from the scene, either from losing market share to newer

products or from reregistration issues with the EPA. The current list is shorter than that found in earlier editions of this volume.

Aliphatic Derivatives

The aliphatic derivatives were the very first organophosphate products to be commercially available. Dichlorvos is the only aliphatic derivative still used on animals. Because of their simple straight-chain structures, they are readily broken down.

DICHLORVOS. Insecticidal use of dichlorvos is discussed herein; anthelmintic use is discussed later in the chapter. Dichlorvos (DDVP) is an aliphatic organophosphate first registered for use in 1948, widely used since the early 1960s, and known by a multitude of trade names (e.g., VAPONA). Its acute oral LD_{50} for rats is approximately 50 mg/kg, garnering it WHO Ib classification (highly hazardous) and EPA II (warning) categorization for oral toxicity. Its acute dermal LD_{50} is 107 mg/kg in male rats and ≥75 mg/kg in female rats for EPA category I, the most dangerous, hence the EPA signal word on the label is "danger" regarding human dermal exposure (U.S. Environmental Protection Agency, 2006e; World Health Organization, 2010).

But the danger of formulated or resinated dichlorvos is much less, in the range of 6 to 20× safer than unformulated dichlorvos. In dogs, the oral LD_{50} of unformulated dichlorvos is 28 to 45 mg/kg, whereas formulated dichlorvos is of lower toxicity, with an oral LD_{50} of 387 to 1262 mg/kg (Courtney and Roberson, 1995).

A unique property of dichlorvos is its high vapor pressure, which makes it an excellent agent for killing insects in a closed space, hence its use as fumigant. It was marketed in 1963 as an impregnated resin strip, the well-known SHELL NO-PEST STRIP, and was the first product to be incorporated into an effective flea collar, although dichlorvos is not currently available in a flea collar formulation. It has quick knockdown insecticidal action as a contact and fumigant agent, but little residual effect. Its half-life in neutral aqueous media is about 8 hours. Rapid hydrolysis also is noted in the mammalian body.

Dichlorvos is available as an impregnated resin strip (e.g., PROZAP INSECT GUARD) to kill flying and crawling insects in enclosed premises such as animal buildings, milk rooms, and reptile houses; places where people will not be present for extended periods of time, especially children.

A ready-to-use 1% formulation (PROZAP BEEF & DAIRY SPRAY RTU) is available to spray on cattle, goats, horses, sheep, and swine and is labeled to control flies, gnats, and mosquitoes. A concentrate formulation (PROZAP VAPONA 400E) is available with instructions to dilute to a 0.5% to 1% water or diesel oil solution for a variety of uses, such as fogging or spraying premises or animals (beef or dairy cattle). There is a specific label precaution not to treat Brahman and Brahman-cross cattle as they may have organophosphate hypersensitivity.

Dichlorvos is combined with pyrethrin, piperonyl butoxide, and MGK 264 in several products for use on cattle and their premises (e.g., SUPER II DAIRY & FARM SPRAY). Food animals should not be treated within 1 day of slaughter. See earlier discussion for standard precautions to follow when dealing with organophosphate insecticides.

Phenyl Derivatives

Phenyl derivatives are structurally more complex organophosphates than aliphatic derivatives (such as dichlorvos) because they have a benzene ring in their structure. They were the second major class of organophosphate developed. Phenyl derivitaves are structurally different than aliphatic derivatives and last longer in the environment. They are represented by tetrachlorvinphos.

TETRACHLORVINPHOS. Tetrachlorvinphos is a phenyl derivative organophosphate with low mammalian toxicity. The oral LD_{50} of tetrachlorvinphos for rats is 4000 to 5000 mg/kg, resulting in WHO III, slightly hazardous, classification (World Health Organization, 2010). Regardless, the NRDC is currently putting pressure on the EPA to get tetrachlorvinphos removed from the market.

It is available as powder to dust cattle, swine, and poultry and to apply to poultry premises for control of flies, lice, and mites (PROZAP DUST'R) and in an array of sprays, powders, and collars for dogs and cats, a vast majority of which are marketed by Hartz. Tetrachlorvinphos is formulated as the sole active ingredient in sprays to kill fleas and ticks on dogs and cats (e.g., HARTZ ULTRAGUARD FLEA & TICK SPRAY); in powders to kill fleas, ticks, and lice on dogs and cats for up to 7 days (e.g., HARTZ ULTRAGUARD FLEA & TICK POWDER); and in collars to kill fleas and ticks on dogs, puppies, cats, and kittens for up to 5 months (e.g., HARTZ INCONTROL 5 MONTH FLEA & TICK COLLAR). Tetrachlorvinphos is also formulated in combination with (S)-methoprene (an IGR) as sprays and collars for more effective control of the flea life cycle; for killing fleas and ticks for 7 days and flea eggs and larvae for 1 month in sprays (e.g., HARTZ INCONTROL FLEA & TICK SPRAY); and for providing 7-month flea and tick protection in collars for dogs (e.g., ADAMS FLEA & TICK CONTROL COLLAR FOR SMALL DOGS) and cats (e.g., HARTZ ULTRAGUARD FLEA & TICK COLLAR FOR CATS AND KITTENS).

Heterocyclic Derivatives

Heterocyclic derivatives are the last group of organophosphates that have been developed. Chemically they have a ring structure in which at least one of the atoms in the ring is oxygen, nitrogen, or sulfur. The heterocyclic ring may consist of three, five, or six atoms. Heterocyclic derivatives are the longest lasting of all the organophosphates. They are used widely on animals and are represented by chlorpyrifos, coumaphos, diazinon, phosmet, and pirimiphos.

CHLORPYRIFOS. Chlorpyrifos (Dursban) is moderately persistent in the environment and serves well for the control of mosquito larvae, fly larvae, fire ants, and termites. Its acute oral LD_{50} in rats is 163 mg/kg, and its acute dermal LD_{50} in rabbits is 2000 mg/kg for a WHO II classification (World Health Organization, 2010).

Chlorpyrifos is formulated as a dog dip to kill fleas, ticks, and sarcoptic mange mites, with instructions to wear gloves when applying and to use at half strength on dogs weighing less than 25 lb (HAPPY JACK ENDURACIDE DIP II). It is also available in combination with diazinon as an ear tag for beef and nonlactating dairy cattle to control flies, lice, and ticks (Y-TEX WARRIOR INSECTICIDE CATTLE EAR TAGS).

COUMAPHOS. Coumaphos is a heterocyclic derivative organophosphate insecticide and acaricide of moderate to high acute toxicity in mammalian laboratory animals. Mice are very sensitive to coumaphos (oral LD_{50} is 55 mg/kg), whereas the oral LD_{50} for rats is 90 to 110 mg/kg, resulting in the WHO Ib classification (highly hazardous) (World Health Organization, 2010). For humans, the EPA considers it highly toxic by oral and inhalation routes of exposure (Categories I and II, respectively) and moderately acutely toxic by the dermal route of exposure (Category III). Technical coumaphos causes only mild eye and dermal irritation (Categories III and IV, respectively) and is nonsensitizing (U.S. Environmental Protection Agency, 2008). Coumaphos does not cause organophosphate-type delayed neurotoxicity and is not mutagenic or suspected to be carcinogenic in humans (U.S. Environmental Protection Agency, 1996b).

Coumaphos hydrolyzes slowly under alkaline conditions, but rapid degradation occurs in the liver of cattle.

Coumaphos is available as a "restricted use pesticide" (for application by certified pesticide applicators only) in a 42% concentrate (CO-RAL FLOWABLE INSECTICIDE, Restricted Use Pesticide), to be used (a) as a dip to control scabies on cattle, (b) as a spray to control horn flies and lice, (c) as a dip or spray to control ticks, and (d) as a spray to control screwworms on beef and nonlactating dairy cattle, and horses. It is also available as a 11.6% concentrate restricted-use pesticide (CO-RAL EMULSIFIABLE LIVESTOCK INSECTICIDE, Restricted Use Pesticide) to control horn flies, face flies, lice, and ticks. The latter product is labeled to control specific insects on specific livestock species; as an example, on swine it is labeled to control lice only.

Coumaphos is also available as a 6.15% concentrate (CO-RAL FLY AND TICK SPRAY), which is not a restricted use pesticide. Whether restricted use certification is required to apply coumaphos or not, care must be taken when planning application of any of these products. There are specific detailed instructions to follow for use, as an example, on lactating versus nonlactating dairy cattle and for use on horses not intended for slaughter. The 6.15% concentrate is labeled for use in backrubbers for beef and lactating dairy cattle. All three of these products have a label notice to veterinarians regarding symptomatic treatment, but not atropinization of patients with host-parasite reactions that present with bloat, excessive salivation, and posterior paralysis. Although contraindicated for host-parasite reactions, atropine is antidotal for coumaphous overdose. Frequent urination and defication, muscle twitching, and watering eyes are the initial signs of overdose followed by salivation, diarrhea, and muscle weakness.

Coumaphos is available as a 1% dust to control lice on swine and horn flies on horses (PROZAP ZIPCIDE DUST) and to control horn flies and lice on beef and dairy cattle, reduce face flies on beef and dairy cattle, and control lice on swine (i.e., Y-TEX CO-RAL LIVESTOCK DUST).

Coumaphos is formulated in combination with diazinon (see next section) in two ear tag products. The first is approved for use on beef and nonlactating dairy cattle to control horn flies, Gulf Coast ticks, and spinose ear ticks, and as an aid for control of face flies (CO-RAL PLUS). In the second product, it is used with FYBERTEK, a fiber technology that allows for maximum insecticide holding capacity (contains 50% organophosphate) and even dispersion of insecticide (CORATHON). These ear tags are for use on beef and nonlactating dairy cattle to control face flies, horn flies (including pyrethroid- and chlorinated hydrocarbon-resistant horn flies), Gulf Coast ticks, and spinose ear ticks for up to 5 months.

DIAZINON. Diazinon, a heterocyclic organophosphate, was previously one of the most widely used pesticides in the United States, but in 2000 the EPA announced the elimination of all indoor uses; in 2004 all residential outdoor use ended; and in 2007 certain agricultural uses were cancelled. Its oral LD_{50} in rats is ≈1250 mg/kg, and its dermal LD_{50} in rabbits is >2020 mg/kg for an EPA III category and WHO II classification (U.S. Environmental Protection Agency, 2006d; World Health Organization, 2010).

Diazinon is currently available for use on animals only in ear-tag formulation. It is the only active ingredient in Y-TEX OPTIMIZER INSECTICIDE CATTLE EAR TAGS, which are for use on beef and nonlactating dairy cattle (a) in the summer to control horn flies (including pyrethroid-resistant populations), lice, Gulf Coast ticks, and spinose ear ticks and to aid in control of face flies and (b) in the winter to control cattle biting lice and little blue *(Solenopotes capillatus)* cattle lice and to aid in control of long-nosed *(Linognathus vituli)* and short-nosed *(Haematopinus eurysternus)* cattle lice.

Diazinon is used in combination with chlorpyrifos in Y-TEX WARRIOR INSECTICIDE CATTLE EAR TAGS for use on beef and nonlactating dairy cattle to control horn flies (including pyrethroid-resistant populations), biting lice, sucking lice, Gulf Coast ticks, and spinose ear ticks, and to aid in control of face flies, stable flies, and house flies. As described in depth in the previous section, diazinon is also formulated in ear tags in combination with coumaphos.

PHOSMET. Phosmet is a heterocyclic organophosphate insecticide with WHO II classification and is in EPA category II for oral (rat oral LD_{50} = 1113 mg/kg) and inhalation exposure, and category III for dermal exposure (rabbit dermal LD_{50} >5000 mg/kg) (U.S. Environmental Protection Agency, 2006f; World Health Organization, 2010). It is formulated for use in a sprayer or backrubber (VET-KEM PARAMITE L.A. INSECTICIDAL SPRAY & BACKRUBBER) on beef and nonlactating dairy cattle to control flies, lice, sarcoptic mange mites, and ticks; or on swine to control lice and sarcoptic mange mites. Cattle treated may be slaughtered 3 days after treatment and swine 1 day after treatment. Do not apply to sick, diseased animals or to calves younger than 3 months old. Do not treat dairy animals within 28 days of freshening and milk must be not be used for human consumption. Do not apply to sick pigs and do not apply directly to nursing pigs.

PIRIMIPHOS. Pirimiphos is a heterocyclic organophosphate. The WHO considers it obsolete as a pesticide and does not classify it (World Health Organization, 2010). Its acute oral LD_{50} for male rats is 1450 mg/kg (World Health Organization, 1983). It is formulated into a 20% cattle ear tag (DOMINATOR INSECTICIDE EAR TAGS) that is approved for use on beef and nonlactating dairy cattle and on calves for up to 5 months of horn fly control. It also aids in control of face flies for 5 months. Pirimiphos is also formulated in combination with the synthetic pyrethroid lambdacyhalothrin to form ear tags (DOUBLE BARREL VP INSECTICIDE EAR TAGS) approved for use on beef and nonlactating dairy cattle and on calves for up to 5 months of horn fly and face fly control.

FORMAMIDINES

The formamidines are a promising group of acaricidal compounds, of which amitraz is the only one of note for veterinary use.

Amitraz

Amitraz is the only formamidine approved for animal use in the United States, where it is used on dogs, cattle, and swine. Amitraz works as an octopamine receptor agonist in insects (Insecticide Resistance Action Committee [IRAC], 2012) and is a monamine oxidase (MAO) inhibitor in mammals (Papich, 2007; Boothe, 2012). The WHO classifies amitraz in Class II regarding human toxicity. It has caused liver tumors in female mice (Pharmacia and Upjohn Company, 1998). In acute toxicity studies, amitraz is moderately toxic by the dermal route with rabbit dermal LD_{50} >200 mg/kg; and the EPA has placed it in Toxicity Category II for this effect. It is slightly toxic by oral and inhalation routes with an oral rat LD_{50} of 515 to 531 mg/kg and an inhalation LC_{50} of 2.4 mg/L, resulting in EPA Category III classification for these effects. Animal studies indicate that amitraz is nonirritating to the eyes and skin, and the EPA places it in Category IV in that regard (U.S. Environmental Protection Agency, 1996c). Although EPA documentation indicates that amitraz does not cause skin sensitization or cholinesterase inhibition, the reader should refer to the product labels carefully regarding warnings and human risks associated with use and application products containing amitraz. The human acute oral amitraz dose of 0.125 mg/kg is considered the no observable

effects limit (NOEL), and 0.25 mg/kg is the lowest observed effects limit (LOEL), which is the dose at which sedation, disorientation, and hypothermia were noted (U.S. Environmental Protection Agency, 1996c).

Amitraz—Dogs

Amitraz is the sole ingredient in two canine products: a liquid concentrate 19.9% solution (MITABAN) indicated to treat generalized demodicosis, and a collar (PREVENTIC TICK COLLAR FOR DOGS) that prevents ticks from attaching to dogs and kills ticks within one day. Amitraz is also available as a 22.1% solution in a combination spot-on treatment (CERTIFECT FOR DOGS) that includes fipronil and (S)-methoprene for control of ticks, fleas, and chewing lice in dogs.

MITABAN liquid concentrate is supplied in a 10.6-mL bottle that contains 19.9% amitraz, which is diluted to a 0.025% (250 ppm) solution for the treatment of generalized demodicosis in dogs (Pharmacia and Upjohn Company, 1998). The MITABAN package insert states that amitraz should not be used for treatment of localized demodicosis or scabies, but it is efficacious for scabies and also ticks—indications that are extralabel (Boothe, 2012). The contents of one bottle are mixed with 2 gallons of warm water for each of three to six treatments spaced 14 days apart. Clipping and shampooing is recommended before treatment of dogs with long hair or dense coats to improve acaricidal contact with the mite (Boothe, 2012; Curtis, 2004). The label suggests that treatment should be continued until no viable mites are found in skin scrapings made at two successive treatments, or until six treatments have been applied. It also advises practitioners to discontinue treatment of nonresponsive dogs. But for refractory cases, higher doses have been used extralabel. Concentrations of 0.025%, 0.05%, and 0.1% have been applied once or twice weekly. In extremely refractory cases, a dose of 0.125% (1250 ppm) has been applied to half the dog one day and the other half the next day, repeating this alternating schedule every day for 4 weeks to 5 months to achieve a cure (Mueller, 2004; Papich, 2007). An even higher dose, 1.25%, has been successfully used in a small number of extremely refractory dogs that were premedicated with both atipamezole and yohimbine (Hugnet et al, 2001; Mueller, 2004).

Adverse reactions may be observed after MITABAN treatment. In clinical trials, transient sedation (duration 1 to 3 days) occurred within 2 to 6 hours of initial treatment in 8% of demodicosis patients. Sedation occurred less frequently on subsequent treatments. Amitraz sedation is caused by agonist activity on alpha$_2$-adrenergic receptors. This side effect can be reversed by yohimbine or atipamezole (Papich, 2007). Other side effects include lethargy, pruritus, bradycardia, hypothermia, hypotension, hyperglycemia, and hyperexcitability, the latter is uncommon (Boothe, 2012). According to dermal toxicity studies noted on the product insert, side effects and adverse events increase when applied at concentrations higher than the labeled dose.

After healthy dogs were given a single 250-ppm or 1250-ppm treatment, transient sedation was observed within 8 hours posttreatment in 1 of 6 dogs at 250 ppm and in all of the 1250-ppm treated dogs. Significant depression of rectal temperatures was noted at 4 hours posttreatment in the 1250-ppm group. Blood glucose values were elevated at 4 hours posttreatment in the 250-ppm female group, and in both sexes at 1250 ppm. All dogs returned to normal within 1 day (Pharmacia and Upjohn Company, 1998).

Another study was performed on healthy Beagles, which were dipped with 250 ppm, 750 ppm, 1250 ppm, or 2500 ppm of amitraz at 14-day intervals for 12 weeks. Blood glucose values were elevated in the 750-ppm group 4 hours posttreatment after three of six treatments, and in the 1250-ppm group after five of six treatments. Blood glucose values returned to normal within a day in the 750-ppm group, but not in the 1250-ppm group, in which glucose remained elevated at 24 hours and after three of six treatments (Pharmacia and Upjohn Company, 1998).

Caution should be used when treating diabetic patients (Curtis, 2004). MITABAN safety has not been established for dogs younger than 4 months of age and pregnant dogs, and it is unknown whether MITABAN impairs fertility (Pharmacia and Upjohn Company, 1998). MITABAN should not be used in pregnant or nursing bitches or puppies younger than 3 months of age (Curtis, 2004). Amitraz is a MAO inhibitor and should not be used in conjunction with other MAO inhibitors such as deprenyl (Anipryl) (Papich, 2007), tricyclic antidepressants (clomipramine and amitriptyline), and serotonin reuptake inhibitors (e.g., fluoxetine) (Line, 2000).

MITABAN concentrate is flammable, but not in treatment dilution. Wear rubber gloves when preparing and applying dilutions. Diabetic people should use extra caution when applying MITABAN because dermal contact and exposure to vapors can cause transient hyperglycemia (Curtis, 2004). Avoid handling dogs immediately after treatment. Both the concentrate and the dilution may cause eye or skin irritation in sensitive individuals. Contact with treated dogs may cause skin irritation for a few days in sensitive people (Pharmacia and Upjohn Company, 1998).

Amitraz is also available in a collar, PREVENTIC, which kills and detaches ticks on dogs for 3 months. It has no effect on fleas. The collar contains enough amitraz to cause illness if ingested, so it should not be used in dogs that chew on each other's collars, and children should not be allowed to play with it or with pieces of the collar that are cut to fit. The collar must be fitted properly to prevent it from coming loose and being ingested. Ingestion of PREVENTIC collars, a diagnosis that can be confirmed by radiograph, is becoming more common in dogs. Clinical signs associated with amitraz toxicity may be noted within an hour of collar ingestion and may include bradycardia, mydriasis, shock, hypotension, hypovolemia, hypothermia, hyperthermia, respiratory depression, vomiting, gastrointestinal stasis, ileus, gastric dilatation, sedation, depression, disorientation, ataxia, hyperglycemia, polyuria, urinary incontinence, tremors, coma, and seizures (Manning, 2000; Talcott, 2000). If a dog ingests the PREVENTIC collar, induction of vomiting or removal by endoscopy, gastrotomy, or enterotomy should be considered. If surgery is necessary, xylazine and other alpha$_2$-agonists should be avoided. Other treatment considerations should include administration of a nonoily laxative, activated charcoal, a cathartic (e.g., magnesium sulfate), and an enema. Amitraz is not a cholinesterase inhibitor, so atropine and 2-PAM are contraindicated. Either yohimbine at 0.1 mg/kg IV or atipamezole at 0.05 mg/kg (50 mcg/kg) IM is recommended to treat dogs that are intoxicated and severely depressed (Talcott, 2000). Administration of one of these alpha$_2$-antagonists should reverse bradycardia and hypovolemia (Manning, 2000). If hypovolemic, intravenous fluids should help. The amitraz collar must not be used on sick, convalescing animals or on puppies younger than 12 weeks of age.

Last, amitraz is available in a spot-on combination, CERTIFECT, with fipronil (a novel insecticide) and (S)-methoprene (an IGR), both of which are discussed in depth later in this chapter. CERTIFECT has amitraz on one side and fipronil and (S)-methoprene on the other side of the spot-on container, both of which are broken open when the product is applied. It is labeled for the control of ticks, fleas, and chewing lice and kills all stages of ticks within 6 hours of application. It is effective against ticks

for 1 month and against fleas for 3 months. The product aids in the control of sarcoptic mange when applied monthly for at least 2 months. CERTIFECT can be used to treat breeding, pregnant, and lactating bitches. It remains effective on the dog even after water immersion, bathing, and exposure to sunlight.

Do not use CERTIFECT on cats, rabbits, or other animals. Do not use this product concurrently with other MAO inhibitors or on dogs with diabetes or heart problems. Avoid use in debilitated, aged, or obese dogs. People administering this product should use extra care if they are diabetic or are taking an MAO inhibitor.

Amitraz—Cats

Amitraz is not labeled for use in cats, but has been used extralabel to treat 13 cases of feline demodicosis at a concentration of 0.0125% to 0.025% twice weekly to every other week (Mueller, 2004). Eleven of those cats were cured after 2 to 4 weeks. Side effects included ptyalism and sedation. If administering to cats, use caution (Curtis, 2004).

Amitraz—Cattle and Swine

Amitraz is available in a 12.5% emulsifiable concentrate (TAKTIC EC) for use against ticks, mange mites, and lice on beef cattle, dairy cattle, and swine. The product should be used within 6 hours of mixing. For use against cattle ticks and lice, the product is diluted 760 mL/100 gal of water and is applied as a spray or dip. For lice a second treatment in 10 to 14 days is recommended to kill recently hatched lice since it does not kill lice eggs. For use against scabies and mange mites in cattle and lice in swine, the product is diluted 760 mL/50 gallons of water and is used as a spray or dip. For scabies, a second treatment must be applied after 7 to 10 days.

No slaughter withdrawal is required for cattle, and no milk withdrawal is required for dairy cattle. Swine must not be treated within 3 days of slaughter. Do not use on animals more than 4× per year. This product should not be used on horses or dogs.

WARNING: Horses must not be treated with amitraz, or fatal colon impaction may result.

NEONICOTINOIDS

The neonicotinoids represent a heterogenous class of insecticides that work by binding to nicotinic acetylcholine receptors (nAChR), serving as agonists. They represent the newest major class of insecticides being used against arthropod pests of domestic animals. The neonicotinoids have low toxicity to mammals, birds, and fish (Tomizawa and Casida, 2003; Tomizawa and Casida, 2005). As of 2010, there were no records of cross-resistance of insect nAChR agonists with other ectoparasiticides (such as carbamates, organophosphates, or pyrethroids) (Vo et al, 2010). Considering the flea's role in transmitting disease, the rapid flea killing action of the neonicotinoids is particularly advantageous compared with slower-acting flea adulticides (such as fipronil and selamectin) (Dryden et al, 2005).

Imidacloprid

Imidacloprid is a chloronicotinyl insecticide. It irreversibly binds at nAChR sites. This receptor is a subtype that is apparently essential for insect neurologic function, but it is different in pharmacology and tissue distribution from all known mammalian nicotinic receptors (Buckingham et al, 1997; Griffin et al, 1997b; Liu and Weller, 1996; Tomizawa and Casida, 2003; Tomizawa and Casida, 2005). Its acute oral LD_{50} in rats is 450 mg/kg (World Health Organization, 2010). In 1991 it was the first neonicotinoid introduced to the market. Although flea resistance to imidacloprid has not yet been reported, studies indicate that resistance genes exist in high frequency in some populations of the whitefly (Tomizawa and Casida, 2003; Vo et al, 2010). Imidacloprid has very low toxicity regarding dermal LD_{50} and eye and skin irritation, but some pet owners have reported contact dermatitis after using it on their pets (Gervais et al, 2010; World Health Organization, 2001).

Although imidacloprid is not effective in preventing fleas from feeding, it is a very effective flea adulticide (Dryden, 2009). Imidacloprid is available in a 9.1% topical spot-on formulation (ADVANTAGE) for use in dogs, cats, puppies, and kittens for control of fleas. The product provides very effective flea control in laboratory and field use (Arther et al, 1997; Cunningham and Everett, 1997; Ritzhaupt et al, 2000b), killing fleas on animals within 12 hours (Cruthers and Bock, 1997). Fleas that reinfest are killed within 2 hours. Protection against further flea infestation should last for up to a month, but in cases of severe environmental flea loads, retreatment may be needed sooner. Do not retreat more than once weekly. Imidacloprid is waterproof and remains effective following shampoo treatment, swimming, or after exposure to rain or sunlight (Cunningham et al, 1997a). Safety testing has revealed no concerns when the product is used according to the label (Griffin et al, 1997a). Do not use it in puppies 7 weeks or younger, in kittens 8 weeks or younger, or in sick or debilitated animals.

Imidacloprid is formulated in combination with permethrin, a synthetic pyrethroid covered in a prior section, for use in dogs. The combination product (K9 ADVANTIX) is registered for use against fleas, ticks, and mosquitoes. The spot-on formulation is applied once every 30 days. Do not use on puppies younger than 7 weeks old. Do not use on cats, which are very sensitive to permethrin.

Imidacloprid is also formulated in combination with pyriproxyfen (ADVANTAGE II) for use in dogs and cats to kill fleas (all life stages), control existing flea infestations, and prevent further infestations. The product has 9.1% imidacloprid and 0.46% pyriproxyfen, kills existing fleas within 12 hours, and kills reinfesting fleas within 2 hours. It is waterproof, remains effective after shampooing, and also kills chewing lice. Do not allow the product to get into a cat's mouth or eyes. Salivation will occur if the cat licks the treatment area, so it is best to apply it to the base of the skull in cats. Do not use in puppies younger than 7 weeks of age or in kittens younger than 8 weeks of age.

One of the newer imidacloprid combination products is only for use in dogs as a spot-on formulation of 8.8% imidacloprid, 44% permethrin, and 0.44% pyriproxyfen (K9 ADVANTIX II). Pyriproxyfen is an IGR that is discussed more fully in a subsequent section. K9 ADVANTIX II has activity against fleas, ticks, mosquitoes, biting flies, and lice. It repels and kills fleas, ticks, and mosquitoes. K9 ADVANTIX II kills fleas on dogs within 12 hours. Fleas that reinfest are killed within 2 hours. Protection against further fleas and ticks should last for up to a month, but in cases of severe environmental flea or tick loads, retreatment may be needed sooner. Do not retreat more often than once weekly. The formulation is waterproof and remains effective after bathing, swimming, or exposure to rain or sunlight.

The newest imidacloprid combination to hit the market includes flumethrin, a pyrethroid, in pet collars (SERESTO) for kittens, cats, puppies, and dogs. These collars have 4.5% flumethrin and 10% imidacloprid impregnated in a polymer for slow release and are efficacious against fleas and ticks for 8 months. Slow release of the active ingredients has been demonstrated by collar weight loss of 15% to 20% over an 8-month period (Stanneck et al, 2012a). Removal of the collar for bathing or swimming is not necessary. The formulation takes advantage of the effect that imidacloprid has on fleas and the effect that flumethrin has on ticks. In addition, these active ingredients are synergistic in action (Stanneck et al,

2012a). Fleas are killed within 24 hours of collar application. Reinfesting fleas are killed within 2 hours, thus killing and repelling fleas before they lay eggs. This can help prevent tapeworm *(Dipylidium caninum)* infection, bartonellosis, and rickettsiosis in dogs and cats, and feline infectious anemia in cats. Tick infestations are prevented within 48 hours of application. Deer ticks, American dog ticks, Brown dog ticks, and Lone Star ticks are repelled and killed, helping to prevent Lyme disease, anaplasmosis, Rocky Mountain spotted fever, cytauxzoonosis, ehrlichiosis, and babesiosis in cats and dogs, and canine hemoplasmosis (previously called haemobartonellosis) in dogs. In addition, these collars can aid in the treatment and control of Sarcoptic mange (Fourie et al, 2012; Stanneck et al, 2012b; Stanneck et al, 2012c). Do not use in puppies younger than 7 weeks of age or in kittens younger than 10 weeks of age.

Two other imidacloprid combination products have 10% imidacloprid and 1% moxidectin for cats (ADVANTAGE MULTI FOR CATS) or 2.5% moxidectin for dogs (ADVANTAGE MULTI FOR DOGS). These products are formulated for topical use in the dog and cat for external and internal parasites. The cat formulation kills fleas; treats and controls ear mites, roundworms, and hookworms; and prevents heartworm disease. The dog formulation kills fleas; treats and controls roundworms, whipworms, and hookworms; and prevents heartworm disease. Moxidectin is covered in depth later in the anthelmintic section of this chapter.

Imidacloprid is also available as the sole active ingredient in 21.4% and 42.8% concentrated solutions to control beetles and mealworms in poultry buildings. Combination formulations of imidacloprid and (Z)-9-tricosene, a female fly pheromone that attracts male flies, are available as a fly bait, as a fly bait strip, and in sprays to control flies.

Dinotefuran

Dinotefuran is a furanicotinyl insecticide in the third generation of neonicotinoids (Wakita et al, 2003). It was discovered via a research program that started with acetylcholine as a lead compound, with a goal of finding a new, original structured neonicotinoid (Wakita et al, 2003; Wakita, 2011). Dinotefuran has excellent activity against fleas (Wakita, 2005). The WHO has not classified dinotefuran for its human toxicity potential. The EPA categorizes dinotefuran as having low acute human toxicity potential (Category IV) by the oral, dermal, and inhalation routes. Dinotefuran is not a dermal sensitizer. It causes a low level of skin irritation (Category IV) and moderate eye irritation (Category II) in animal studies (U.S. Environmental Protection Agency, 2004). Labeled products advise people to avoid skin contact and warn that substantial, but temporary, eye injury may result if the product gets in the eye.

There are two basic dinotefuran formulations of topical spot-on combinations; one has permethrin and cannot be used in cats. The other formulation has no permethrin. The products without permethrin (FIRST SHIELD, VECTRA FOR CATS, VECTRA FOR CATS & KITTENS, and VECTRA FOR DOGS & PUPPIES) have 22% dinotefuran and 3% pyriproxyfen (DP). These products kill fleas within 6 hours and provide monthly control of adult fleas and flea eggs, larvae, and pupae.

The dog-only formulation (FIRST SHIELD TRIO AND VECTRA 3D) has 4.95% dinotefuran, 0.44% pyriproxyfen, and 36.08% permethrin (DPP). Dinotefuran provides knockdown against fleas; pyriproxyfen interrupts the flea life cycle; and permethrin provides activity against ticks (deer tick, *Ixodes scapularis;* brown dog tick, *Rhipicephalus* spp.; American dog tick, *Dermacentor variabilis;* and Gulf Coast tick, *Amblyomma maculatum*) and mosquitoes (*Culex* spp., *Ochlerotatus* spp., and *Aedes* spp.). A single topical dose has onset of activity within 2 hours, kills 96% of fleas within 6 hours, and provides effective control of fleas, ticks, and mosquitoes for at least 30 days.

A study of dogs infested with a strain of *Ctenocephalides felis* that had been laboratory maintained without exposure to pesticides since 1990 revealed that DPP efficacy ranged from 96.2% to 100% when evaluated 48 hours after infestation within the 30 first days posttreatment. Then DPP efficacy decreased progressively, to 93.2% (day 37), 87.8% (day 44), 65.8% (day 51), 58.9% (day 58), and 54.6% (day 65) (Bouhsira et al, 2012). A study of dogs exposed to *Aedes aegypti* mosquitoes revealed 94% repellency for a month after DPP treatment. The insecticidal effect was 96% for 3 weeks and dropped to 87% the last week of the month after treatment (Franc et al, 2012). When investigators applied the appropriate formulation (DP spot-on formulation for cats and DPP for dogs) to heavily infested pets in Florida residences, excellent flea control was achieved with >95% reduction of pet area flea counts and pet flea burden after just two monthly applications (Dryden et al, 2011).

Use of dinotefuran-containing products is not without risk. A warning on the label states that sensitivity may occur and may cause red skin at the application site. Manufacturers advise bathing the pet should this happen.

Most important, applying one of the permethrin-containing (DPP) products to cats can be a deadly mistake. Veterinarians need to take every opportunity to emphasize such warnings when selling insecticides to owners. The fact that both permethrin (DPP) and non-permethrin (DP) spot-on products carry the same brand name calls for veterinarians and staff to be particularly diligent, pointing out that the DPP canine product (with permethrin) is absolutely not safe to use on cats. This is a concern with DPP and DP products that are sold under VECTRA or FIRST SHIELD brand names. For more on this topic, spot-on adverse events, and the regulatory response in Canada and the United States, see the permethrin section.

Nitenpyram

Nitenpyram is a neonicotinoid, nAChR-agonist insecticide administered PO as a rapid-acting flea adulticide. It has unique characteristics of rapid oral absorption and low toxicity to dogs and cats. As a result of this activity, a single oral dose provides extremely rapid knockdown of flea populations (Schenker et al, 2003). Studies have shown activity against fleas within 30 minutes after oral administration. Posttreatment efficacy was 64% at 3 hours and 97.7% at 8 hours in cats compared with 83.6% at 3 hours and 99.1% at 8 hours in dogs (Food and Drug Administration, 2000). There was >98% reduction in blood consumption of fleas placed on cats treated PO with nitenpyram compared with fleas placed on control cats (Dryden, 2009). Nitenpyram has a short half-life and is quickly cleared from the body unchanged in the urine. Daily administration in dogs and cats will not result in bioaccumulation.

Nitenpyram is available in tablet form (CAPSTAR). The small tablet contains 11.4 mg and is labeled for use in cats and dogs up to 25 pounds in body weight. The large tablet contains 57 mg and is for use in dogs weighing 25.1 to 125 pounds. The wide dosage range is a testament to the favorable margin of safety, which includes labeled use in pregnant or nursing dogs and cats. That said, post-marketing surveillance revealed birth defects, fetal loss, and neonatal loss associated with treating pregnant or lactating animals. In addition, post-marketing surveillance revealed increased adverse events in animals at less than 2 pounds of body weight or younger than 8 weeks of age, and in those in poor condition (Novartis Animal Health, 2011). Pets that are heavily infested with fleas may begin scratching after treatment with nitenpyram, probably as a reaction to dying fleas rather than a reaction to the drug itself

(Chatellier, 2001; Dryden et al, 2001; Schenker et al, 2001a; Schenker et al, 2001b; Witte and Luempert, 2001). Those affected may show transient signs of hyperactivity, panting, vocalization, and excessive grooming/licking.

In 2003 concurrent use of nitenpyram and lufenuron (an IGR reviewed in a subsequent section) was approved as a flea management system (Food and Drug Administration, 2003). Concurrent use of these products was effective in field studies (Dryden et al, 2001).

Anecdotal reports indicate efficacy of rectally administered nitenpyram, such as when fleas are discovered during a surgical procedure (Boothe, 2012). Nitenpyram has also been used, off label, to treat dogs with screwworm *(Cochliomyia hominivorax)* myiasis (Correia et al, 2010).

Spinosad

Spinosad is structurally classed as a spinosyn, which is a nonbacterial tetracyclic macrolide. Introduced initially into the agricultural market to control insect pests, it has WHO III classification (World Health Organization, 2010). Spinosad is a mixture of two naturally occurring macrocyclic lactones (spinosyn A and spinosyn D), not a true neonicotinoid insecticide, but it is still an nAChR allosteric activator, activating these receptors in the insect (Insecticide Resistance Action Committee [IRAC], 2012; Vo et al, 2010). The nAChR binding site for spinosad is separate and distinct from that of other neonicotinoids and from fipronil, milbemycins, avermectins, and cyclodienes. Spinosad-treated insects show involuntary muscle contractions and tremors from activation of motor neurons. Prolonged exposure results in paralysis and death of the flea. Selective toxicity in the flea versus the vertebrate host is conferred by the differential sensitivity of the nicotinic receptors between flea and host (Snyder et al, 2007).

Spinosad is available as the sole active ingredient in a chewable tablet formulation that was first introduced to the market in 2007 for dogs. In 2012, the label was changed when spinosad was marketed for cats and smaller dogs, too (COMFORTIS CHEWABLE TABLETS FOR DOGS AND COMFORTIS CHEWABLE TABLETS FOR CATS). For dogs, six different chewable tablet sizes are formulated to deliver the minimum dose of 30 mg/kg of body weight. For cats, four different chewable tablet sizes are formulated to deliver a higher dosage to cats than dogs, with a minimum dose of 50 mg/kg. These products are approved for redosing every 30 days for the prevention and control of flea infestations.

Laboratory studies in dogs demonstrate 53% activity against fleas within 30 minutes of oral administration, and 100% efficacy within 4 hours (Elanco Animal Health, 2012b). Laboratory studies also reveal that spinosad confers long-lasting protection, providing 100% efficacy at 21 days and >95% efficacy 30 days after a single oral dose, with elimination of flea egg production even in the face of heavy challenge (Blagburn et al, 2010; Snyder et al, 2007). One laboratory study (comparing oral spinosad with a fipronil/(S)-methoprene topical product) revealed good spinosad efficacy the first 2 weeks, but disappointing spinosad efficacy thereafter, with 62.5% of dogs being flea-free 3 weeks after treatment and only 25% of dogs being flea-free 4 weeks after treatment (Beugnet et al, 2011). Other studies stand in stark contrast. A study of client-owned dogs (comparing spinosad with selamectin) found that 1 month after the last of three consecutive monthly spinosad treatments, a significant reduction in pruritus was noted, along with >99% reduction in geometric mean flea counts, and 95% of dogs were flea-free (Robertson-Plouch et al, 2008). Another similar study of client-owned dogs (comparing spinosad with fipronil/(S)-methoprene) revealed that three consecutive monthly spinosad treatments alleviated pruritus in 95% of dogs and resulted in 95% of dogs being flea-free at the end of the study; in addition, geometric mean flea counts were reduced by 99.9% (Dryden et al, 2012).

In feline laboratory studies, spinosad started to kill fleas within 30 minutes of administration and has 98% efficacy in 4 hours, 100% efficacy the first day after treatment, and >90% efficacy 1 month later (Elanco Animal Health, 2012a). In a field study of cats, flea count was reduced by 97.5% one month after the first treatment and by 99.3% after three monthly treatments.

The canine product had a wide safety margin when tested in laboratory and clinical studies, but post-marketing surveillance revealed adverse reactions that included vomiting, decreased appetite, and lethargy. If vomiting occurs within 1 hour of administration, it is safe to repeat treament with a full dose. Spinosad use in breeding male dogs has not been evaluated, and caution is advised when treating breeding female dogs. Safety studies in breeding bitches showed increased lethargy, weakness, and dehydration in pups of bitches given a high dose (4.4×) of spinosad (Elanco Animal Health, 2012b). Spinosad is excreted in the milk of lactating bitches. Pups with the highest levels of spinosad in their milk had the most severe signs of an adverse reaction.

In 2008 the FDA issued a safety warning about the use of COMFORTIS in combination with high doses of ivermectin to treat nonresponsive demodectic mange because some dogs treated in this manner developed ivermectin toxicity (Food and Drug Administration, Center for Veterinary Medicine, 2008). As a result, the manufacturer recommended not administering Comfortis to dogs treated with extralabel doses of ivermectin, some of which may receive up to 100× the label dose (Eli Lilly and Company, 2008). The company also funded a study revealing that administration of 5× spinosad concurrently with 10× milbemycin to Collies with the multidrug resistance gene *(MDR1)* mutation did not cause avermectin toxicosis (Sherman et al, 2010). The pharmacokinetic basis of this interaction is uncertain. Dunn et al demonstrated that although ivermectin does not alter spinosad pharmacokinetics, spinosad causes a pharmacokinetic interaction with ivermectin. They suspected that spinosad probably inhibits P-glycoprotein (Dunn et al, 2011), but a recent study found that spinosad did not inhibit P-glycoprotein function 48 hours after administration (MacKay et al, 2012). To reiterate, the manufacturer advises not using spinosad in dogs on extralabel doses of ivermectin and using caution if spinosad is used concomitantly with extralabel ivermectin doses in cats.

Spinosad has been used in Australia to control blowflies and lice on sheep (Kirst et al, 2002). Spinosad is available in a 44.2% solution to be diluted and applied as a premise spray to control a variety of beetles and flies and the northern fowl mite *Ornithonyssus sylviarum*.

Spinosad is available combined with (Z)-9-tricosene in a granular fly control formulation. Spinosad is also available combined with milbemycin (TRIFEXIS) as a monthly flea, heartworm, and gastrointestinal parasite preventive; a product that will be reviewed in detail subsequently in the anthelmintic section. Spinosad is available for human use as a 0.9% topical suspension (NATROBA) pediculicide for the treatment of head lice infestation (Villegas and Breitzka, 2012).

NOVEL INSECTICIDES

Benzyl Benzoate

Benzyl benzoate is an insecticide with an unknown mode of action. It is effective against most ectoparasites, but is used only on dogs infested with sarcoptic mange. Benzyl benzoate is marketed as a

29% preparation (HAPPY JACK SARDEX II or NO-BITE MANGE REMEDY). For the treatment of generalized forms of sarcoptic and demodectic mange, the hair is first clipped from affected areas, if necessary from the entire body, and the dog is bathed to remove all crusts. The product is then applied after the dog is dry, with care taken to avoid getting any product in the dog's eyes. Benzyl benzoate has no residual effect. Therefore repeated applications are required every 7 days until the condition clears up. Do not apply to dogs younger than 12 weeks of age or to pregnant or nursing bitches. Do not use on cats.

Fipronil

Fipronil is a phenylpyrazole insecticide that was developed in the mid-1990s (Narahashi et al, 2007). It is a potent antagonist of the gamma-aminobutyric acid (GABA)-gated chloride channel (Insecticide Resistance Action Committee [IRAC], 2012; Narahashi et al, 2007). The acute oral LD_{50} of fipronil for rats is 100 mg/kg, hence it has WHO II classification (World Health Organization, 2010). A large volume of literature is available regarding the mechanism of action, clinical efficacy, and safety of fipronil when used against fleas in dogs and cats. Although it does not prevent fleas from feeding, fipronil is very effective in killing adult fleas (Dryden, 2009).

It was first available as a 0.25% spray (Postal et al, 1995). As a sole ingredient, it is currently approved in a 0.29% spray (FRONTLINE SPRAY TREATMENT FOR CATS AND DOGS) and as a 9.7% spot-on (FRONTLINE TOP SPOT) for use on dogs, cats, puppies, and kittens.

The spray formulation is effective against ticks, chewing lice, and sarcoptic mange mites (Curtis, 1996; Hunter et al, 1996a; Merial LTD, 2011a). To apply, mist until hair is damp, using 1 to 2 pumps/lb of body weight. Although it is reported and labeled to be effective against fleas even after bathing (Jeannin et al, 1994; Merial LTD, 2011a), Rozencrantz recommends not bathing dogs for 2 days before or after treatment (Rosenkrantz, 2012). The spray kills new fleas, ticks, and chewing lice for at least 30 days, and may protect dogs against fleas for up to 90 days. It should not be reapplied within 30 days and should not be used in puppies and kittens younger than 8 weeks of age. The spray is alcohol based, and cats may object during application, causing owners to apply an insufficient amount (Rosenkrantz, 2012).

The spot-on formulation is available from many manufacturers for dogs and for cats (e.g., FRONTLINE TOP SPOT) and effectively spreads fipronil through the sebum covering the hair and skin with minimal systemic absorption (Birckel et al, 1996; Weil et al, 1997). The cat product is effective against ticks for 30 days and fleas for as long as 45 days (Ritzhaupt et al, 2000a). The dog product is effective against ticks for at least 30 days and fleas for as long as 90 days (Cunningham et al, 1997b; Cunningham et al, 1997c; Hunter et al, 1996b; Postal et al, 1996; Ritzhaupt et al, 2000b). It is effective against chewing lice in cats. It is also effective after exposure to rain or bathing (Everett et al, 1997). Laboratory and field safety studies reveal no concerns when the product is used according to the label (Arnaud and Consalvi, 1997a; Arnaud and Consalvi, 1997b). It is effective in controlling flea allergy dermatitis in client-owned cats (Medleau et al, 2002). Although the spot-on formulation package inserts state that it will control fleas in cats for up to 6 weeks and in dogs for up to 3 months, the manufacturer also states that in cases of flea allergy dermatitis, it may be reapplied as often as monthly (Merial LTD, 2011b; Merial LTD, 2011c). Rosenkrantz routinely uses the spot-on formulation on dogs and cats extralabel every 2 to 3 weeks to get more complete control of flea allergy signs (Rosenkrantz, 2012). Do not use it in puppies younger than 10 weeks old or in kittens younger than 8 weeks old. Wear rubber gloves when applying the product. Some reports indicate that fipronil is effective against ear mites (Vincenzi and Genchi, 1997).

Fipronil is now available in combination with several other active ingredients. The first fipronil combination product was a convenient spot-on formulation that used (S)-methoprene, an IGR, which will be discussed in detail subsequently in the IGR section. The formulations that are currently available have 9.8% fipronil with 11.8% (S)-methoprene in the cat product and 8.8% (S)-methoprene in the dog product, sold under the trade name FRONTLINE PLUS. For dogs and cats it is effective against fleas, ticks, and chewing lice for 30 days. The combination of an adulticide and an IGR provides activity against the immature and adult life stages of the flea, thus breaking the life cycle of the pest. When investigators applied the fipronil/(S)-methoprene formulation to heavily infested dogs and cats in Florida residences, excellent flea control was achieved with >95% reduction of pet area flea counts and pet flea burden after just two monthly applications (Dryden et al, 2011). Do not use in puppies or kittens younger than 8 weeks old.

When the fipronil patent expired, other flea- and tick-killing combination products hit the market. A combination product used in dogs has 9.8% fipronil and 5.2% cyphenothrin (i.e., MARTIN'S PREFURRED PLUS FOR DOGS, PARASTAR PLUS, SENTRY FIPROGUARD MAX FOR DOGS, SERGEANT'S PRONYL OTC MAX FOR DOGS, and SPECTRA SURE PLUS FOR DOGS). Cyphenothrin characteristics were discussed in the pyrethrin section. Formulated for monthly use to kill fleas, ticks, and chewing lice, these products start to kill fleas and ticks within an hour of application and are waterproof. Fipronil/cyphenothrin products should not be used in puppies younger than 12 weeks of age or in cats.

A combination product used in cats has 9.8% fipronil and 15% etofenprox (e.g., SENTRY FIPROGUARD MAX FOR CATS) and is available from several manufacturers. Etofenprox, a previously discussed insecticide, has action similar to pyrethrins but can be used in cats. Formulated for monthly use to kill fleas, ticks, and chewing lice, these products start to kill fleas and ticks within an hour of application and are waterproof. Fipronil/etofenprox products should not be used in kittens younger than 12 weeks of age.

One of the newest fipronil combination product includes (S)-methoprene and amitraz (CERTIFECT FOR DOGS). The formulation has 9.8% fipronil and 8.8% (S)-methoprene in one container and 22.1% amitraz in the other. Both containers are opened at the same time and the product is applied for a resultant 6.4% fipronil, 5.8% (S)-methoprene, and 7.6% amitraz concentration of ingredients. The product is labeled to control ticks, fleas, and chewing lice on dogs. It kills all stages of ticks within 6 hours of application. It is effective against ticks for 1 month and against fleas for 3 months. It remains effective on the dog even after water immersion, bathing, and exposure to sunlight. CERTIFECT is waterproof, aids in the control of sarcoptic mange, and can be used on pregnant, breeding, or lactating bitches. The addition of amitraz, an acaricide, the characteristics of which were previously discussed in the formamidines section, makes this product more effective against ticks on dogs, but it should not be used on cats, and its use with other MOA inhibitors is contraindicated. CERTIFECT has a very strong odor that lingers for about a day after application.

A new combination monthly spot-on feline product with 9.8% fipronil, 15% etofenprox, and 11.8% (S)-methoprene (FRONTLINE TRITAK FOR CATS) is being sold in a test market of some select states where it is marketed with claims of activity against both fleas and ticks. It is not available throughout the United States. As with

other fipronil/etofenprox products, it should not be used in kittens younger than 12 weeks of age.

A new combination monthly spot-on canine product with 9.8% fipronil, 5.2% cyphenothrin, and 8.8% (S)-methoprene (FRONTLINE TRITAK FOR DOGS) is being sold in a test market of some select states where it is marketed with claims of activity against both fleas and ticks. It is not available throughout the United States.

Fipronil had good efficacy when used as a 1% pour-on to prevent and treat screwworm myiasis in cattle in Brazil, but such a product is not labeled for use in the United States (Alexander, 2006; Lima et al, 2004).

REPELLENTS

A vast majority of currently marketed veterinary products labeled with repellent activity contain one or more of the pyrethroids (e.g., permethrin, cypermethrin, tetramethrin) that have already been discussed. In this section, repellents that do not kill insects are reviewed. Repellents are compounds that prevent or discourage pests from approaching a treated area or that induce them to leave soon after approaching. The most intensive research in this area has been to protect humans from flying insects. In general these products are rather volatile and are regarded as having little toxicity for the host animal (Hayes and Laws, 1991).

DEET

DEET is the official nonproprietary name for *N,N*-diethyl-3-methylbenzamide or *N,N*-diethyl-*m*-toluamide. Its oral LD_{50} for rats is 2000 mg/kg. DEET is used in humans as a repellent for mosquitoes, gnats, flies, fleas, ticks, and chiggers. For continuing protection, frequent applications are necessary. At this time, no DEET-containing products are labeled for veterinary use, but it is included here because it is the standard against which all repellents are compared, it is readily available for human use, and owners sometimes apply it to dogs and cats. Neurologic signs including seizures may result, especially in cats.

Di-*N*-Propyl Isocinchomeronate

Di-*N*-propyl isocinchomeronate is a relatively safe insect repellent, with an oral LD_{50} for rats of 5200 to 7200 mg/kg. The WHO classifies it as unlikely to be hazardous (Pesticide Action Network [PAN], 2010). The chemical is also known as dipropyl isocinchomeronate and by its proprietary name, MGK Repellent 326. Di-*N*-propyl isocinchomeronate is a nearly ubiquitous ingredient in veterinary repellent products. It is formulated with a wide variety of insect repellents, insecticides, and synergists for use on pets and livestock and is available as an ingredient in more than 100 products listed in the Compendium of Veterinary Products (North American Compendiums, 2012).

Butoxypolypropylene Glycol

The mode of action of butoxypolypropylene glycol (BPG) is unknown. With an acute oral LD_{50} >5000 mg/kg in rats, it is in EPA toxicity category IV for oral exposure. The acute rabbit dermal LD_{50} >2000 mg/kg puts it in category III for dermal exposure. It is also in category III for eye and dermal irritation (U.S. Environmental Protection Agency, 2007a).

BPG is used to repel flying and crawling insects. It was first registered for use in 1960 and is not intended for use in food animals. Approximately 300,000 pounds of BPG is sold annually (U.S. Environmental Protection Agency, 2007a).

The EPA states that BPG can be directly applied to dogs, cats, or horses, or used on the bedding or premises where they live, but veterinary products containing it are by and large repellent sprays or wipes for horses and ponies. Spray examples include a formulation of 5% BPG, 1% di-*N*-propyl isocinchomeronate, 0.5% piperonyl butoxide, 0.2% pyrethrins, and 0.2% permethrin that contains two insecticides and two repellents to control face flies, stable flies, house flies, mosquitoes, gnats, mites, chiggers, and lice on horses (i.e., ADAMS FLY SPRAY AND REPELLENT) and to control flies, mosquitoes, gnats, mites, chiggers, lice, fleas, and ticks on puppies, dogs, foals, and horses (i.e., FLYSECT SUPER-7 REPELLENT SPRAY). A roll-on formulation with repellents BPG and di-*N*-propyl isocinchomeronate, insecticides cypermethrin and pyrethrins, and the synergist piperonyl butoxide promises 5- to 7-day protection of horses for a variety of flies, gnats, and no-see-ums (i.e., ENDURE ROLL-ON FOR HORSES). A fly repellent ointment formulation contains 10% BPG, 1% piperonyl butoxide, and 0.15% pyrethrins and is indicated to repel flies and kill ticks on the ears and between the toes of dogs and cats (i.e., VIP FLY REPELLENT OINTMENT). Several products combine pyrethrins, piperonyl butoxide, and BPG, one of which is advertised as a "Citronella Spray" (i.e., FLYSECT CITRONELLA SPRAY) without listing citronella as an active ingredient.

Picaridin

Picaridin has been used in Australian products since 1998 and is one of the most commonly used active ingredients in Europe and Australia (Katz et al, 2008). It is available in the United States as the sole active ingredient in an EPA-registered repellent spray for use on horses and people (CENTAURA INSECT REPELLENT FOR HORSE AND RIDER). The label indicates that it provides 12-hour protection against mosquitoes and ticks and up to 8-hour protection against flies, gnats, and chiggers.

Botanical Repellents

The EPA reviews and registers pesticides covered under section 25(b) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The following botanical oils—Citronella Oil, Lemon Eucalyptus Oil, Clove Oil, Peppermint Oil, Lemongrass Oil, Cedar Oil, Rosemary Oil, Thyme Oil, Olive Oil, Cinnamon Oil, Sesame Oil, Castor Oil, and others—have been incorporated into repellent solutions, sprays, and wipes, but they are not covered in this text. Interested practitioners are referred elsewhere to investigate indications, modes of action, efficacy, contraindications, and adverse effects of botanical repellents (Das et al, 2003; Katz et al, 2008; Maia and Moore, 2011).

INSECT GROWTH REGULATORS

An exciting area of recent advance in the struggle against insects is the advent of insect growth regulators (IGRs). The sheer number of insecticides covered in this chapter would suggest that insect problems are no longer a threat to the health and welfare of our domestic animals, but anyone who works in the field knows that this is far from the case. The central problem with most insecticides is that they are effective against only the adult insect—the one that bites and annoys.

Adulticidal products need to be applied thoroughly and often to control adult insect populations, but this is often unworkable. The applicator trying to stem the flow of adult insects often feels like the Dutch boy with his finger in the dike. IGRs provide relief from this approach by altering immature insects where they grow and develop, thus making them less viable, breaking the life cycle, and providing true relief from insect annoyance. IGRs typically are juvenile hormone mimics that bind to juvenile hormone receptors in the immature insect and prevent survival to the next stage of

development. (S)-Methoprene and pyriproxyfen are the best-known juvenile hormone mimics.

IGR products are the safest and most effective products available. Their safety lies in the fact that mammalian hosts have no juvenile hormones or juvenile hormone receptors (Londershausen, 1996). Therefore IGR products cannot have any biologic effect on the host. When used properly, they dramatically decrease the use of more toxic adulticides. It follows then that insect control programs with IGRs are often safer for the host and the environment when compared with adulticide-only programs.

Cyromazine

Cyromazine is a unique product that has IGR properties limited to the filth flies (e.g., blow flies, house flies, lesser house flies, stable flies, soldier flies). It has no effect on most of the other orders of beneficial insects. Cyromazine works by blocking the formation of new cuticle in the fly larvae. It is a molting disruptor; the fly larva molts from the first to the second instar stage, but it does not survive the molt (Insecticide Resistance Action Committee [IRAC], 2012). Cyromazine has WHO III classification and rat LD_{50} of 3300 mg/kg (World Health Organization, 2010).

For horses cyromazine is formulated into a 2.12% feed additive pellet (SOLITUDE IGR). The product should be fed as part of the daily ration to contain 300 mg per horse per day or 600 mg every other day. It is active in treated manure and is registered for use against house flies and stable flies in and around horses, barns, stables, paddocks, and racetracks. Take care when storing and disposing of cyromazine. Do not contaminate water, food, or feed with this product or apply it directly to water.

For chickens cyromazine is formulated as a feed premix (LARVADEX 1% PREMIX) and a 2% liquid concentrate (LARVADEX 2SL). The premix is approved for feeding to caged layers and broiler breeders at 1 pound of premix per ton of final feed. Cyromazine passes through the bird and is deposited in the manure, where it controls filth flies developing there. For premise use, the liquid concentrate is diluted to a 0.1% surface spray that is used to control fly larvae in breeding places such as feed spills, dead bird piles, and manure storage areas.

For premise use in cattle, hog, and poultry operations, a 2% water-soluble cyromazine dry granule (NEPOREX 2SG) can be applied by dry scattering or by diluting in water and spraying to eliminate fly larvae in breeding sites per product insert directions.

Cyromazine resistance has been detected in Australia in the Australian sheep blowfly *(Lucilia cuprina)* and in the United Kingdom in the house fly *(Musca domestica)* (Bell et al, 2010; Levot, 2012).

Diflubenzuron

Diflubenzuron was first registered in the United States as a pesticide in 1976 and is an inhibitor of chitin biosynthesis (Insecticide Resistance Action Committee [IRAC], 2012; U.S. Environmental Protection Agency, 1997a). It interferes with chitin deposition and thus prevents shedding of old skin, leading to the death of larvae or pupae. It also prevents egg hatching. Diflubenzuron does not bind to juvenile hormone receptors. In both acute and chronic studies in laboratory animals, diflubenzuron was well tolerated. With an oral rat LD_{50} >4640, it is in WHO class III (World Health Organization, 2010). It is absorbed through the skin and is in EPA dermal Toxicity Category III (the second lowest of four categories) and in EPA oral and inhalation Toxicity Category IV (the lowest of four categories) (U.S. Environmental Protection Agency, 1997a).

Diflubenzuron is formulated into 0.24% feed additive (SIMPLIFLY WITH LARVASTOP, EQUITROL II FEED-THRU FLY CONTROL) for use against stable flies and house flies in horses. The product is fed to horses daily to control filth fly larvae in the manure. The daily dose is 6.8 mg of diflubenzuron per 100 pounds of body weight. Do not use in horses intended for slaughter.

Lufenuron

Lufenuron is an IGR or insect development inhibitor that works by inhibiting chitin biosynthesis (Insecticide Resistance Action Committee [IRAC], 2012). Lufenuron is approved for use in dogs and cats for control of fleas (PROGRAM) and is approved for use in pets 4 weeks of age and older. It is given orally to dogs and cats every 30 days. Lufenuron is also available in an injectable formulation (PROGRAM 6-MONTH INJECTABLE FOR CATS) that is designed to allow application every 6 months for control of fleas. Adverse reactions after injection include pain on injection, injection site lumps/granulomas, vomiting, listlessness, lethargy, and anorexia. The drug is highly lipophilic, resides in the fat tissues of the pet, and redistributes into the bloodstream for at least 30 days. Adult fleas ingest lufenuron when they feed, and the drug is passed transovarially to the flea egg. Most flea eggs exposed to lufenuron fail to hatch, and the few flea larvae that do hatch die during their first molt. The action on the immature flea is thought to be due to disruption of chitin synthesis and deposition. Lufenuron is a convenient and effective agent for flea control in pets. It is known to be safe in pets of all ages, as well as in breeding dogs and cats.

Concurrent use of lufenuron and nitenpyram was discussed in the nitenpyram section. Lufenuron is also available in combination with milbemycin oxime (SENTINEL FLAVOR TABS) for control of fleas and internal parasites in dogs; see the combination products in the anthelmintics section of this chapter for more information.

(S)-Methoprene

(S)-Methoprene is an IGR with low toxicity in mammals. Its oral LD_{50} for rats is 34,600 mg/kg. (S)-Methoprene is a true IGR, acting as a juvenile growth hormone mimic that arrests larval development, which in turn results in death of the larva (Insecticide Resistance Action Committee [IRAC], 2012). (S)-Methoprene is sensitive to degradation by ultraviolet (UV) light.

(S)-Methoprene has had considerable commercial success against fleas. It is available in a wide variety of products (collars, sprays, spot-ons, shampoos, and premise sprays) formulated with (S)-methoprene alone or in combination with adult insecticides for control of fleas and other pests. (S)-Methoprene is ovicidal and larvicidal against fleas. Combination products are covered in the adulticide section.

(S)-Methoprene is formulated into feed premix (VITAFERM CATTLEMAN'S BLEND WITH IGR & CTC 350) for oral administration to beef cattle. It is registered for control of horn flies in the manure of treated cattle. It should not be used in sheep because of the amount of copper in the formulation.

Pyriproxyfen

Pyriproxyfen (NYLAR), which is also known as 2-[1-methyl-2-(4-phenoxyphenoxy) ethoxy] pyridine, is an IGR with a juvenile-hormone-mimic mode of action (Insecticide Resistance Action Committee [IRAC], 2012). The acute oral LD_{50} of pyriproxyfen in rats is greater than 5000 mg/kg, which demonstrates the very wide margin of safety (Anon, 1991). Secretion of juvenile hormone in the immature insect causes it to molt into the next life stage, but absence of juvenile hormone at the time of the molt allows maturation to occur. Pyriproxyfen interferes with both the larval-to-pupal

and pupal-to-adult molts. It also is deadly for insect eggs (Anon, 2012).

Pyriproxyfen is available in a wide variety of products formulated alone or in combination with adult insecticides for control of fleas and other pests. No products list pyriproxyfen as the sole ingredient. Two spot-on products with 2% pyriproxyfen and 20% to 30% cyphenothrin (SENTRY PRO XFT and SERGEANT'S EVOLVE) are used to treat fleas and ticks on dogs. These products should not be used on puppies younger than 12 weeks of age or on cats. Pyriproxyfen is also incorporated into a wide range of products that contain adult insecticides and synergists for application to dogs, cats, and premises to control fleas and other animal parasites. Other combination products are covered in the sections on the adulticides.

SYNERGISTS

Synergists are not considered toxic in their own right and have no direct effect in killing insects. They are used with insecticides to enhance insecticidal activity. They are most often used with pyrethroids, in which case they can increase pyrethroid potency tenfold to twentyfold (Plapp, 1991). The mode of action is to inhibit insect mixed-function oxidases—enzymes in the insect that metabolize foreign compounds. When the insect is inhibited from destroying the insecticide, the agent can kill the pest. Synergists are most commonly listed on the label by their chemical name, which is not particularly user friendly.

N-Octyl Bicycloheptene Dicarboximide (MGK 264)

N-Octyl bicycloheptene dicarboximide inhibits the microsomal detoxification of insecticides, thus maximizing their toxicity. It is also known as MGK 264. With an oral rat LD_{50} of 2800 mg/kg, it is in the WHO class III (World Health Organization, 2010). The drug is registered for application to beef and dairy cattle, sheep, goats, horses, swine, dogs, and cats, and to agricultural buildings and animal quarters for the control of annoying insects. It is often formulated with piperonyl butoxide and insecticides and is available as an ingredient in a wide variety of shampoos, spot-ons, dips, ointments, aerosols, sprays, foggers, and powders.

Piperonyl Butoxide

The synergist piperonyl butoxide is a pale yellowish liquid that is soluble in alcohols, benzene, freons, and other organic solvents. It is very safe for animals, with an oral LD_{50} for rats of >7500 mg/kg (National Pesticide Information Center, 2000). The insecticidal effect of chlorinated hydrocarbons, carbamates, organophosphates, and particularly pyrethroids and rotenone is boosted by piperonyl butoxide. The insecticidal activity of these compounds is enhanced because piperonyl butoxide inhibits degradation of the insecticide by the insect. Piperonyl butoxide is often formulated with MGK 264 and insecticides and is available as an ingredient in a wide variety of shampoos, spot-ons, dips, ointments, aerosols, sprays, foggers, and powders.

ANTIPROTOZOALS

This section will briefly describe the biologic activities of a few approved and some unapproved, but legally obtainable, antiprotozoal drugs. As with any drug, the information on the label or package insert must always be read and directions understood before antiprotozoal agents are administered. Some drugs do not fit nicely into the categories humans define. As an example, albendazole and fenbendazole, drugs with primarily anthelmintic activity, which are reviewed in the anthelmintics section, also treat animals with infections of *Giardia* spp. protozoa. This section is broken into subsections for nonsulfonamides and sulfonamides, with each drug listed alphabetically within that subsection. For more detailed information, the reader should consult detailed antiprotozoal reviews (Barr, 2006; Davis and Gookin, 2009; Lindsay and Blagburn, 2001; Schillhorn van Veen, 1986; Snyder et al, 1991; Speer, 1999; Wright, 2012). One of the best depots of practical information for veterinary practitioners is Section IV: Protozoal Diseases, in the text *Infectious Diseases of the Dog and Cat,* edited by Craig Greene, which provides excellent, in-depth information on this topic (Greene, 2012b).

NONSULFONAMIDES

Albendazole

Albendazole is more completely described later in the section on benzimidazole anthelmintics, where general mode of action, etc., is reviewed. It is included in this section for a discussion of its activity against *Giardia* organisms. Albendazole is 50× more effective against *Giardia* than metronidazole in vitro (Meloni et al, 1990). Albendazole causes structural changes to *Giardia* trophozoites, including damage to the adhesive disc and the internal microtubule cytoskeleton, but not to the flagella (Lindsay and Blagburn, 2001). Albendazole is available in an oral suspension and paste (VALBAZEN) containing 113.6 mg/mL. It is effective in treating humans, mice, dogs, and cattle with giardiasis (Davis and Gookin, 2009). Evidence in one study suggests that albendazole is very effective in treating giardiasis in dogs at 25 mg/kg twice a day for four doses, with 92% of treated dogs having subsequent negative testing for giardiasis compared with 4% recovery in the control group (Barr et al, 1993). It has also been shown to reduce *Giardia* cyst production by >90% when given to cattle at 20 mg/kg orally (PO) for 3 days (Davis and Gookin, 2009).

Unfortunately, evidence has shown that albendazole can cause significant adverse reactions in humans, dogs, and cats. It has been implicated in causing aplastic anemia in those species. The drug is known to be toxic in dogs and cats in clinical use (Meyer, 1998; Stokol et al, 1997). Reported toxicities included myelosuppression (anemia, leukopenia, and/or thrombocytopenia), abortion, teratogenicity, anorexia, depression, ataxia, vomiting, and diarrhea. Obviously, since albendazole is teratogenic, it should not be used in pregnant animals (Plumb, 2011b). Dogs treated with 50 mg/kg twice daily may develop anorexia, and cats treated extralabel with 100 mg/kg/day for 14 to 21 days showed weight loss, neutropenia, and mental dullness (Plumb, 2011b). Regarding its use in cats, although Vasilopulos lists a cat dose of 25 mg/kg twice daily for 3 to 5 days to treat giardiasis, he also notes that it may cause bone marrow suppression in dogs and cats; and Davis and Gookin state that they do not recommend using albendazole in cats (Davis and Gookin, 2009; Vasilopulos, 2006). Veterinarians are advised to use caution with extralabel use of this product in dogs and even more caution when using it extralabel in cats.

Amprolium

The coccidiostatic activity of amprolium is related to its mimicry of thiamine and competition for absorption of thiamine by the parasite. The activity occurs because of the structural similarity between thiamine and amprolium. The anticoccidial effect may by reversed by the feeding of excess thiamine. Amprolium is most effective against the first-generation schizont stage and thus is more effective as a preventive than as a treatment (Davis and Gookin, 2009). It is labeled for cattle and poultry for the

treatment and prevention of coccidiosis, particularly *Eimeria* spp. Amprolium has been used extralabel in dogs, swine, sheep, and goats for the control of coccidiosis. Overdoses have caused polioencephalomalacia in sheep and neurologic signs in dogs, but stopping treatment and administering thiamine may restore health while negating, of course, the coccidiostatic effect of amprolium (Plumb, 2011b).

Broilers, Layers, and Turkeys

Amprolium (e.g., AMPROL) is fed in poultry rations or drinking water to prevent or treat coccidiosis. Dosages vary widely depending on the severity of the outbreak, and referral to the package insert is recommended before a treatment or control program is instituted. Amprolium is given in the water for 3 to 5 days or up to 2 weeks at 0.012% (0.025% for severe outbreaks), then is given at 0.006% for another 2 weeks. It can be fed for a few days or continuously at a concentration range of 0.004% to 0.025% as a medicated feed. The package insert notes that some *Eimeria* spp. may be resistant to amprolium.

Cattle

For treatment of active coccidia, *Eimeria bovis,* and *Eimeria zuernii* infections in cattle, amprolium is formulated as a 9.6% drench solution (e.g., CORID ORAL SOLUTION), powder (e.g., CORID 20% SOLUBLE POWDER), or feed additive (i.e., CORID 25% TYPE A MEDICATED ARTICLE). Dosages vary widely depending on the severity of the outbreak, so referral to the package insert is recommended before a treatment or control program is instituted. For treatment of coccidiosis, administration of amprolium at an approximate dosage of 10 mg/kg for 5 to 21 consecutive days has been advised (Davis and Gookin, 2009; Plumb, 2011b). For prevention of coccidiosis caused by coccidia, *E. bovis,* and *E. zuernii,* a dosage of 5 mg/kg daily for 21 days is recommended. Other species of *Eimeria* are also susceptible to amprolium, but the drug label claims efficacy against only *E. bovis* and *E. zuernii*. Animals should not be medicated within 24 hours of slaughter.

Sheep and Goats

Amprolium used extralabel may protect lambs against coccidia when given PO at 55 mg/kg twice daily for 19 days (Plumb, 2011b).

Pigs

Coccidiosis caused by *Isospora suis* is occasionally a problem in swine. Pigs aged 5 to 10 days die without passing oocysts. Although not approved, amprolium therapy may be beneficial in preventing the disease (Sanford and Josephson, 1981). Recommended doses range from 25 to 65 mg/kg PO once to twice daily for 3 to 4 days to 100 mg/kg/day (frequency and duration not noted) (Plumb, 2011b).

Dogs

Treatment of dogs with amprolium is extralabel and requires adapting the approved formulations for small animal use. The target dose for treatment of dogs is 100 to 300 mg total dose (not mg/kg) by mouth daily in food or water once a day for 7 to 12 days (Plumb, 2011b). Dogs may be treated by mixing 30 mL of 9.6% amprolium with 1 gallon (3.8 L) of drinking water and offering it as the sole source of drinking water (Davis and Gookin, 2009; Greene, 2012a). Amprolium should be provided either in food or in water, but not both, for a period of 7 days. It may be given as a treatment for coccidia, or as a preventive for 7 days before puppies are shipped, or to bitches just before whelping.

Cats

Amprolium use in cats is extralabel. It may be used against coccidia at a dose of 60 to 100 mg total dose (not mg/kg) daily PO for 7 days, which may be accomplished best by direct oral administration in capsules (Greene, 2012a). Medication in food or water may be more unreliable in cats than in dogs because of their finicky tastes. Detailed food and water dosing recommendations can be found elsewhere (Davis and Gookin, 2009; Greene, 2012a; Plumb, 2011b).

Clindamycin

Clindamycin is a lincosamide antibiotic and a structural congener of lincomycin. The drug is well absorbed after oral administration and is widely distributed in most tissues. It readily crosses the placenta and is extensively bound to plasma proteins. Clindamycin is metabolized in the liver and excreted primarily in the urine and bile. It acts by binding to the 50S subunit of the bacterial (or parasitic) ribosome and blocking peptide bond formation (Plumb, 2011b).

Clindamycin is available in several veterinary formulations (e.g., ANTIROBE, CLINTABS TABLETS): capsules containing 25, 75, 150, or 300 mg; tablets containing 25, 75, or 150 mg; and an oral solution containing 25 mg/mL. Similar clindamycin formulations are available for use in people (CLEOCIN): 75, 150, and 300 mg oral capsules; 15 mg/mL oral pediatric suspension; and an injectable solution containing 150 mg/mL.

Clindamycin is currently considered the drug of choice for treating clinical toxoplasmosis in dogs and cats (Dubey and Lappin, 2012). Treatment of systemic toxoplasma infection in dogs can be accomplished with PO or IM clindamycin at 10 to 20 mg/kg twice daily for 4 weeks (Dubey and Lappin, 2012; Greene et al, 1985). Cats can be treated for systemic clinical infection with clindamycin PO or IM at 10 to 12.5 mg/kg twice daily for 4 weeks (Dubey and Lappin, 2012). This regimen is also useful for controlling the shedding of oocysts. To decrease zoonotic risk to susceptible humans and reduce the toxoplasma-shedding period, cats suspected of toxoplasmosis after fecal exam can be given clindamycin at:

- 25 to 50 mg/kg daily PO (Plumb, 2011b)
- 25 mg/kg every 12 hours PO or IM for up to 24 weeks (Dubey and Lappin, 2012)
- 50 mg/kg daily PO or IM for up to 24 weeks (Dubey and Lappin, 2012)

Gastrointestinal upset is sometimes reported in animals receiving clindamycin. Severe, even fatal, pseudomembranous enterocolitis has been reported in people on clindamycin, caused by overgrowth of *Clostridium difficile,* but appears not to be a significant risk in dogs or cats (Plumb, 2011b). If given PO in cats, administer a bit of food or water after pilling to avoid esophagitis and esophageal strictures that may result from dry pilling (Plumb, 2011b).

Clopidol

Clopidol is a pyridinol coccidiostat with a similar mode of action as the quinolone anticoccidial drugs, but it has no cross-resistance. It acts against the sporozoite stage, allowing host cell penetration without parasite development (Davis and Gookin, 2009).

Insoluble in water, it is available as a feed additive (Coyden 25%). The product is fed to chickens at 0.0125% or 0.025% to aid in the prevention of *Eimeria* spp. coccidiosis. It is also labeled to aid in the prevention of *Leucocytozoon smithi* in turkeys. It should not be fed to laying hens, to chickens older than 16 weeks of age, or within 5 days of slaughter (Davis and Gookin, 2009).

Decoquinate

Decoquinate is an approved coccidiostatic drug for the control of coccidial (*Eimeria* spp.) infection in chickens, cattle (ruminating and nonruminating), sheep, and goats. This quinolone product kills the sporozoite stage of the life cycle. It disrupts electron transport in the mitochondrial cytochrome system of the parasite (Plumb, 2011b). Decoquinate is indicated for prevention rather than treatment of coccidiosis.

Decoquinate is available as a medicated feed supplement for cattle (DECCOX), as a medicated powder to add to milk for calves (DECCOX-M), and as a medicated milk replacer for young goats (LAND O LAKES DOE'S MATCH KID MILK REPLACER DC MEDICATED). Decoquinate is indicated for prevention of coccidiosis caused by *Eimeria ninakohlyakinovae* and *E. christenseni* in kid goats and *E. bovis* and *E. zuernii* in ruminating calves and older cattle. It is fed to cattle at 0.5 mg/kg body weight per day for at least 28 days during periods of exposure to infective oocysts (Plumb, 2011b), but the package insert should be used to determine mixing to ensure proper dosing. Davis and Gookin advise using it in pregnant cows at 1.25 mg/kg/day in the feed for 30 days before and 8 days after parturition to prevent clinical signs of coccidiosis in calves (Davis and Gookin, 2009).

Decoquinate has been used extralabel to prevent coccidiosis in sheep and goats at 0.5 mg/kg/day for at least 28 days (Davis and Gookin, 2009). Withdrawal before slaughter is not necessary, but do not use in sheep or goats producing milk for human consumption. To prevent clinical relapses of *Hepatozoon americanum* in dogs, decoquinate 6% powder, which has 27.2 g of decoquinate/lb of premix, can be added to dog food at a rate of 0.5 to 1 TBS/10 kg twice a day (Davis and Gookin, 2009). Alternatively, decoquinate powder can be mixed with food at a rate of 1 tsp/10 kg and fed twice daily (Greene, 2012b). Treating with 10 to 20 mg/kg PO every 12 hours has also been recommended for a duration of 2 years after clinical signs of *H. americanum* as a way to prevent relapse because no drug effectively eliminates tissue stages of this organism (Greene, 2012b).

WARNING: Decoquinate should not be fed to laying hens, breeding animals, or lactating cows, sheep, or goats. Complete feeds containing decoquinate should be consumed within 7 days of manufacture. Bentonite should not be used in decoquinate feeds.

Diclazuril

Diclazuril is in the triazine class of antiprotozoals, which target the plastid body, an organelle present in members of the phylum Apicomplexa, but the actual mechanism of action is not clearly described (Plumb, 2011b). It is FDA approved in the United States as a coccidiostat (CLINACOX) in broiler chickens and growing turkeys, and as an antiprotozoal (PROTAZIL ANTIPROTOZOAL PELLETS) in horses to treat equine protozoal myeloencephalitis (EPM).

In poultry, a 0.2% diclazuril medicated feed (CLINACOX) is labeled for the prevention of coccidiosis in chickens caused by *Eimeria tenella, E. necatrix, E. acervulina, E. brunetti, E. mitis (mivati),* and *E. maxima*. Because it acts late in the life cycle of *E. maxima,* subclinical intestinal lesions may be present for a short time after infection, but diclazuril use results in reduced lesion scores and improved health and performance in birds challenged with *E. maxima*. It is also labeled for use in turkeys to prevent coccidiosis caused by *Eimeria adenoeides, E. gallopavonis,* and *E. meleagrimitis.* Do not use in breeding turkeys.

In horses, a 1.56% diclazuril pellet (PROTAZIL) is labeled for use as a top-dress in the horse's daily grain ration at a rate of 1 mg diclazuril per kilogram (0.45 mg/lb) of body weight for 28 days to treat EPM caused by *Sarcocystis neurona.* Although normalcy after treatment is possible, improvement is a more realistic goal. Safe use for breeding horses or during pregnancy or lactation has not been established, but the drug does have a wide margin of safety in horses, calves, and lambs. In horses a 50× overdose for 42 days resulted in decreased weight gain and increased blood urea nitrogen and creatinine. In calves and lambs, a 60× overdose did not cause any abnormalities (Plumb, 2011b).

While extralabel in the United States, a dose of 1 mg/kg is approved in the United Kingdom for use in treating coccidiosis in lambs and in controlling coccidiosis in calves (Plumb, 2011b). An extralabel dose of 25 mg/kg PO once has been advised for coccidiosis in dogs and cats (Greene, 2012b).

Fenbendazole

Fenbendazole (PANACUR) is more completely described later in the section on benzimidazole anthelmintics, where the general mode of action, and so forth, is reviewed. It is included in this section for a discussion of its activity against giardiasis. This is extralabel in the United States, but fenbendazole is labeled to treat canine giardiasis in Europe (paste) and the United Kingdom (granules). Fenbendazole does not have embryotoxic or teratogenic effects in rats, sheep, and cattle. In the rabbit, fenbendazole was fetotoxic but not teratogenic. It is generally considered safe to use in pregnancy in all species, thus making it the drug of choice for treating *Giardia* spp. in pregnant animals (Tams, 2007a). It is also the drug of choice for treating giardiasis in cats (Tams, 2007a).

In dogs and cats with giardiasis, fenbendazole is safe, and no contraindications for its use are known at a dose of 50 mg/kg orally once daily. To control *Giardia* spp., it is given for 3 days, but if upon retesting the infection has not cleared, the dosing regimen can be repeated for a longer duration of 5 to 7 days (Barr, 2006; Tams, 2007a; Vasilopulos, 2006).

In cattle, also extralabel, fenbendazole has been shown some efficacy against *Giardia* organisms in calves when given as a single oral dose of 10 mg/kg (O'Handley et al, 1997). A more recent study of 92 calves with clinical giardiasis revealed that oral treatment with 15 mg/kg daily for 3 days combined with movement of calves to thoroughly cleaned and disinfected (10% ammonia) pens resulted in near 100% efficacy based on fecal testing 3 to 4 weeks posttreatment (Claerebout et al, 2006). This is a good reminder of the importance of preventing reinfection by using disinfectants.

Imidocarb

Imidocarb is an aromatic diamidine antiprotozoal agent. It works by inhibiting nucleic acid metabolism in susceptible organisms (Papich, 2007). The product is oncogenic in rats. Imidocarb dipropionate is available in a sterile solution for SC or IM injection with 120 mg/mL (IMIZOL) labeled to treat dogs with clinical signs or diagnosis of babesiosis. The labeled dose in dogs is 6.6 mg/kg IM or SC repeated in 2 weeks for a total of 2 treatments. It is the best drug labeled in the United States to treat babesiosis in dogs (Holman & Snowden, 2009). It has been used extralabel to treat the following:

- Dogs with *Hepatozoon* infection
  - 5 to 6 mg/kg IM or SC every 2 weeks until blood smear gamonts clear (Allen et al, 2011)
- Cats with *Cytauxzoon* infection
  - 5 mg/kg IM, repeat in 1 to 2 weeks (pretreat with atropine or glycopyrrolate) (Davis and Gookin, 2009; Plumb, 2011b), or
  - 3 to 4 mg/kg IM, repeat in 7 days (Plumb, 2011b)
- Horses with piroplasmosis *(Babesia caballi, Babesia equi, Theileria equi)*
  - 1 to 2 mg/kg twice during 24-hour period (Davis and Gookin, 2009), or

- 2 mg/kg IM once a day for 2 days (Plumb, 2011b), or
- 4 mg/kg IM every 72 hours for a total of 4 doses (Davis and Gookin, 2009; Schwint, 2009; Grause, 2012)

- Cattle with babesiosis
  - 1 to 3 mg/kg IM or SC (Davis and Gookin, 2009)
- Sheep with babesiosis
  - 1.2 mg/kg IM, repeat in 10 to 14 days (Plumb, 2011b)

The safety of this product has not been established in puppies or breeding, pregnant, or lactating dogs. Side effects include parasympathetic, cholinergic signs (e.g., vomiting, weakness, lethargy, salivation), and pain at the injection site. Overdose toxicity target organs are liver and intestines. In cats, puppies, and debilitated dogs, pretreatment with atropine or glycopyrrolate has been advised (Davis and Gookin, 2009; Plumb, 2011b).

Warning: Do not give by intravenous injection because fatality may result.

Lasalocid

Lasalocid, an ionophore closely related to monensin, is produced by a streptomycete (Davis and Gookin, 2009). Like other ionophores, it forms complexes with sodium and potassium ions. This action renders the parasite membranes permeable to ions, and mitochondrial functions are inhibited. The trophozoite stage is most susceptible to lasalocid (Guyonnet et al, 1990). Lasalocid, the least toxic of the ionophores, is approved for use in cattle, sheep, rabbits, and poultry for control of coccidia and improvement of feed efficiency. It is available in a variety of products (AVATEC, BOVATEC, PRO-BAC-C) used to mix medicated feeds and is added to milk replacer.

Warning: Do not feed to horses; fatal reactions may result.

Cattle

Lasalocid is available in dry or liquid feed additives (BOVATEC). The product may be mixed into a complete feed for confined cattle or a feed supplement for pasture cattle to deliver a target dose of 1 mg/kg/day (360 mg/head maximum dose) (Davis and Gookin, 2009). It is effective against *Eimeria bovis* and *E. zuernii* in cattle. Feed continuously during exposure to coccidia. Do not feed to calves to be processed for veal.

Sheep

Lasalocid may be mixed into a complete feed for sheep fed in confinement. The feed should be mixed to provide a final concentration of 20 to 30 g of lasalocid per ton of complete feed, to deliver a dose of 15 to 70 mg/head/day (Davis and Gookin, 2009). This dose is effective against *Eimeria ovina, E. crandallis, E. ovinoidalis (E. ninakholyakimovae), E. parva,* and *E. intricata* in sheep. Feed continuously during exposure to coccidia.

Rabbits

Lasalocid is approved for use in rabbits for the prevention of coccidiosis caused by *Eimeria stiedae*. The product is formulated in a complete ration at a concentration of 113 g per ton and is fed to rabbits until 6.5 weeks of age.

Poultry

Lasalocid is approved in broilers, turkeys, and chukar partridges to prevent coccidiosis caused by *Eimeria tenella, E. necatrix, E. acervulina, E. brunetti, E. mivati, E. maxima, E. meleagrimitis, E. gallopavonis, E. adenoeides, E. stiedae,* and *E. legionensis tenella.* The product (AVATEC) is mixed into a complete ration for broilers and turkeys at a rate of 68 to 113 g/ton. Chukar partridges should be fed lasalocid at a dose rate of 113 g/ton.

Metronidazole

Metronidazole is one of the nitroimidazoles, which represent a very useful class of antibiotics that have broad-spectrum activity against trichomonads, amoebae, and *Giardia* organisms, as well as anaerobic cocci and bacillus species. Metronidazole is the prototypical nitroimidazole, Other drugs in the class include ipronidazole, tinidazole, dimetridazole, ronidazole, ornidazole, carnisazole, and benznidazole. Only metronidazole and tinidazole are currently available in the United States. None of the nitroimidazole drugs are approved for use in animals. The FDA strongly warns against their use in food-producing animals because this class of drug has been shown to produce tumors in laboratory rodents (Davis and Gookin, 2009).

Metronidazole (FLAGYL) is effective against the following anaerobic protozoa: *Trichomonas, Giardia, Entamoeba* (trophozoites), and *Balantidium* (Greene, 2012a). It is well absorbed from the gastrointestinal tract, has low protein binding, and is well distributed in the body. After entering the target cell, metronidazole interacts with the protozoal DNA, in which it causes loss of helical structure and strand breakage (Davis and Gookin, 2009). The liver extensively metabolizes the drug, and in humans hepatic transformation is responsible for 50% of the total elimination. Patients receiving cimetidine or phenobarbital may require adjustment in the dosage because of drug interaction; cimetidine increases metronidazole toxicity, conversely barbiturates reduce metronidazole therapeutic efficacy (Greene, 2012a). Metronidazole toxicity may be seen, especially in dogs. Neurologic toxicity includes ataxia, nystagmus, seizures, tremors, or weakness.

Numerous studies have demonstrated that metronidazole is an effective treatment for giardiasis (Barr, 2006; Kirkpatrick and Farrell, 1984; Zimmer and Burrington, 1986), although efficacy is rarely 100%. Conversely, Tams advises that metronidazole is only 67% to 74% effective in eliminating *Giardia* from dogs and that on confirming diagnosis, fenbendazole or febantel would also be reasonable to consider (Tams, 2007b). Albendazole may be more effective in clearing *Giardia*, but it is not as safe as metronidazole. Fenbendazole may also be more effective in clearing *Giardia* and it is safer than metronidazole.

Lappin recommends treating canine giardiasis with 15 to 25 mg/kg PO every 12 to 24 hours for 5 to 7 days; Barr recommends 15 to 30 mg/kg every 12 to 24 hours for 5 to 7 days; and Greene's formulary suggests a higher dose of 30 to 50 mg/kg every 24 hours for 5 to 7 days (Barr, 2006; Greene, 2012a; Lappin, 2006). For cats, Lappin recommends 15 to 25 mg/kg PO every 12 to 24 hours for 5 to 7 days; Barr recommends 10 to 25 mg/kg every 12 to 24 hours for 5 to 7 days; and Greene's formulary suggests a variety of doses including 8 to 10 mg/kg every 12 hours for 10 days and 10 to 30 mg/kg every 24 hours for 5 days (Barr, 2006; Greene, 2012a; Lappin, 2006). The commercially available product (FLAGYL) is formulated in 250- and 500-mg tablets. Parenteral formulations are also available, but their usefulness would seem questionable, given that the *Giardia* trophozoites live in the lumen of the gastrointestinal tract.

Metronidazole has also been used to treat dogs and cats with *Entamoeba histolytica* or *Pentatrichomas hominis* (Plumb, 2011b).

Monensin

Monensin was first approved for use in the United States in 1970. It is an antibiotic produced as a fermentation product of *Streptomyces cinnamonensis* and is used in cattle, goats, poultry, and quail for its coccidiostatic activity. Monensin forms ionophores with sodium and potassium in the host and in the parasite. When the parasite mitochondrial membrane is affected, it is rendered

permeable to potassium and sodium ions. Feeding monensin to horses or guinea fowl can be fatal.

Cattle

Monensin is available as a feed additive (RUMENSIN) for cattle for growth promotion and for prevention and control of coccidiosis. For control of coccidiosis due to *Eimeria bovis* and *E. zuernii,* the product should be mixed in the feed according to the package insert (Elanco Animal Health). It should be fed continuously during periods of exposure to coccidia, or when coccidia are likely to be a hazard. Do not feed to veal calves.

Goats and Sheep

Monensin is approved for use in confined goats (RUMENSIN) for the prevention of *Eimeria crandallis, E. christenseni,* and *E. ninakohlyakimovae* infection; the product should be mixed in the feed according to the package insert (RUMENSIN 90 PI). Do not feed to lactating goats. Monensin is not approved for use in sheep, but some authorities indicate that it is useful when fed at a rate of 1 mg/kg/day (McDougald and Roberson, 1988; Schillhorn van Veen, 1986).

Poultry

Monensin (COBAN) is fed at a rate of 90 to 110 g/ton of complete feed when used in broilers and pullets to prevent coccidiosis caused by *Eimeria necatrix, E. tenella, E. acervulina, E. brunetti, E. mivati,* and *E. maxima*. It is also approved for use in turkeys to prevent infection with *Eimeria adenoeides, E. meleagrimitis,* and *E. gallopavonis* when fed at 54 to 90 g/ton. Bobwhite quail can be fed monensin at 73 g/ton to prevent coccidiosis caused by *Eimeria dispersa* and *E. lettyae*.

Narasin

Narasin is a monovalent polyether ionophore coccidiostat produced by *Streptomyces aureofaciens* (Davis and Gookin, 2009) that was approved for use in the United States in 1986 (Lindsay and Blagburn, 2001). Similar in structure to salinomycin, it is available as a feed additive for use in broiler chickens only (Monteban 45) or for use in swine only (Skysis). The swine product is labeled for the indication of increasing weight gain, not as a coccidiostat. The chicken product is fed at a rate of 54 to 90 g/ton of feed for prevention of coccidiosis caused by *Eimeria necatrix, E. tenella, E. acervulina, E. brunetti, E. mivati,* and *E. maxima.* It should not be fed to other types of chickens, just broilers. No withdrawal period is required before slaughter. Its use may decrease egg production and quality. Concurrent use with tiamulin may interfere with narasin metabolism, resulting in decreased weight (Davis and Gookin, 2009). A combination product of narasin and nicarbazin is discussed subsequently.

Warning: Ingestion by adult turkeys, horses, or ponies may be fatal.

Nicarbazin

Nicarbazin is a synthetic coccidiostat that was approved for use in the United States in 1985. The mechanism of action is unknown (Lindsay and Blagburn, 2001). Nicarbazin is available as a 25% feed additive (NICARB 25%) and is approved for use at 0.0125% in the feed of broilers. It is not effective for treatment of coccidiosis but is effective in preventing cecal and intestinal coccidiosis caused by *Eimeria tenella, E. acervulina, E. maxima, E. necatrix,* and *E. brunetti*. It is best to avoid using this product during hotter months of the year as it enhances the effects of heat distress. Nicarbazin should not be fed to laying hens. It causes problems with egg production such as decreased egg weight and yolk mottling (Davis and Gookin, 2009). This product should not be fed within 4 days of slaughter.

Nicarbazin and Narasin

A combination narasin and nicarbazin product (MAXIBAN 72) was approved for use in the United States in 1989 for prevention of coccidiosis in broiler chickens only (Lindsay and Blagburn, 2001). When provided at 40 ppm of each active ingredient in the feed, it is effective in preventing (not treating) *Eimeria necatrix, E. tenella, E. acervulina, E. brunetti, E. mivati,* and *E. maxima*. This product should not be fed to laying hens; nor should it be used in the hotter months of the year or fed within 5 days of slaughter.

Ponazuril

Ponazuril is an antiprotozoal product (MARQUIS) that is approved for treatment of EPM, which is caused by *Sarcocystis neurona* (Food and Drug Administration, 2001; Lech, 2002).

The product has been tested at 5 mg/kg and 10 mg/kg. The approved dose is 5 mg/kg/day PO for 28 days. In the pivotal clinical study, 54% of horses with EPM improved at least one grade as judged by the attending veterinarian, and 58% of horses treated with 10 mg/kg improved at least one grade. In a smaller field study with seven horses, all seven improved when treated with 5 mg/kg. Safety studies demonstrated that administration at doses of 10 mg/kg or greater produced transient episodes of loose feces (Furr and Kennedy, 2001; Furr et al, 2001; Furr et al, 2006; Kennedy et al, 2001).

This drug has been used effectively extralabel to treat cattle infected with *Neospora caninum* at 20 mg/kg/day PO for 6 days (Davis and Gookin, 2009). Greene states ponazuril may be used extralabel to treat dogs and cats for neosporosis or toxoplasmosis at 50 mg/kg/day PO (duration as needed), but recommends compounding a reduced concentration product for ease and safety (Greene, 2012a). Directions for compounding a 5% suspension from the equine paste are provided in the formulary section of Greene's text. Marks successfully used the drug extralabel to treat kittens and cats with coccidiosis at 20 mg/kg/day PO for 3 days (Marks, 2009). Plumb has listed extralabel ponazuril doses for treating coccidiosis in bearded dragons, camelids, and rabbits and for treating *Cryptosporidium* respiratory disease in falcons (Plumb, 2011b).

Robenidine

Robenidine is a synthetic coccidiostat chemically similar to guanidine. It is an older drug (approved for use in the United States in 1972) with a history of developing resistant strains of coccidia, but it is now used to treat ionophore-resistant strains (Lindsay and Blagburn, 1995). Robenidine is available in a feed additive (ROBENZ) for use in broilers only. The product is fed at 30 g/ton of feed for prevention of coccidiosis caused by *Eimeria mivati, E. brunetti, E. tenella, E. acervulina, E. maxima,* and *E. necatrix*. It should not be fed to laying hens or within 5 days of slaughter. Meat and eggs from treated birds have an unpleasant taste if the withdrawal period is not followed (Davis and Gookin, 2009).

Salinomycin

Salinomycin was the third ionophore coccidiostat to enter the market in the United States. A monovalent polyether ionophore, it is a fermentation product of *Streptomyces albus* and is most active against the sporozoite stage. Salinomycin is available as a feed additive (BIO-COX, SACOX) for use in broilers, roasters, replacement breeders, replacement layers, pullets, and quail. It is fed at 40

to 60 g/ton (50 g/ton for quail) for prevention of coccidiosis caused by *Eimeria tenella, E. necatrix, E. acervulina, E. maxima, E. brunetti,* and *E. mivati* in chickens and coccidiosis caused by *E. dispersa* and *E. lettyae* in quail. Salinomycin does not adversely affect egg production or quality, but it should not be fed to laying hens producing eggs for human consumption (Davis and Gookin, 2009). No withdrawal period is required before slaughter. Concurrent use with tiamulin may interfere with salinomycin metabolism, resulting in decreased chicken weight (Davis and Gookin, 2009).

Warning: Salinomycin may cause fatalities if fed to adult turkeys or horses.

Semduramicin

Semduramicin is a monovalent polyether ionophore coccidiostat produced by *Actinomadura roseorufa*. It is available as a feed additive (AVIAX II) for use in broiler chickens only. The product is fed at 22.7 g/ton for prevention of coccidiosis caused by *Eimeria tenella, E. acervulina, E. maxima, E. brunetti, E. necatrix,* and *E. mivati*. Concurrent use of tiamulin does not cause the problems that occur with some other ionophore coccidiostats (e.g., salinomycin). Semduramicin does not adversely affect egg production or quality. It should not be fed to egg-laying chickens or to broilers within 5 days of slaughter.

SULFONAMIDES

Sulfonamides are a traditional group of antimicrobial compounds initially derived from the azo dye, prontosil. The first one to be used clinically was sulfanilamide. An improperly prepared sulfanilamide elixir caused a mass poisoning in the United States in 1937 that was responsible, in large part, for the enactment of the Food, Drug, and Cosmetic Act of 1938. Sulfonamides have been clinically useful since then, making them one of the oldest groups of antimicrobial compounds still in use today. As expected with decades of widespread use, resistance to many of the sulfonamides has been noted. For this reason, and because sulfonamides tend to crystallize in urine and its relatively acidic bladder environment, they are often given in combination with each other, taking advantage of the law of independent solubility, which describes the fact that each sulfonamide in a mixture of sulfonamides maintains its own solubility in solution (Spoo and Riviere, 1995).

Sulfonamides have been the treatment of choice for small-animal coccidia for a long time and are very useful for the treatment of large-animal coccidiosis as well. Sulfonamides combined with trimethoprim or ormetoprim are termed **potentiated sulfas** and perform synergistically (Papich and Riviere, 2009). These products have broader spectrum and greater antibacterial activity.

The sulfonamides are structural analogs of para-aminobenzoic acid (PABA) that competitively inhibit the dihydropterate synthetase step in the synthesis of folic acid, which is required for synthesis of RNA and DNA. Inhibition by sulfas impairs protein synthesis, metabolism, and growth of the pathogen. A vast array of sulfa agents has been created and described. The important differences among these agents are their solubility, duration of action, and activity against key pathogens. Fortunately, the sulfas included in this discussion demonstrate acceptable performance in all three categories: Solubility is adequate; they are given once or twice daily or in the feed; and they have a reasonably broad spectrum of action. Sulfa drugs are primarily effective against the schizont stages of the coccidia; therefore prolonged treatment may be required for the drug to effectively block the life cycle.

The diaminopyrimidine potentiators (trimethoprim, ormetoprim, pyrimethamine) act in concert with sulfonamides by blocking the next step (dihydrofolate reductase) in folic acid synthesis. These agents are highly selective inhibitors of dihydrofolate reductase. This sequential blockade of folic acid synthesis produces significant potentiation of activity and is a classic case of drug potentiation.

The sulfonamides are weak acids that are well absorbed from the gastrointestinal tract (except for sulfaquinoxaline) and are widely distributed in the body. Sulfadimethoxine and sulfamethoxazole have high serum protein binding, which provides decreased body clearance and long half-lives. They undergo metabolic alteration in the liver and subsequent renal clearance. Trimethoprim, ormetoprim, and pyrimethamine are well absorbed from the gut, widely distributed, then hydroxylated and excreted through the urinary tract.

The long history of sulfa use in veterinary medicine has resulted in a wide array of toxic and idiosyncratic reactions in animals. Historically, the most common and most avoidable reactions result from crystallization in the urinary tract, with secondary crystalluria, hematuria, and urinary obstruction. Recent reviews in human medicine indicate that improved solubility of modern preparations has greatly decreased the risk of crystalluria. Nevertheless, it is still prudent to ensure adequate water intake and proper hydration during sulfa therapy (Spoo and Riviere, 1995). The human literature also suggests that the sulfonamides may be directly nephrotoxic (Delanaye et al, 2011).

Adverse effects associated with sulfonamides include crystalluria, keratoconjunctivitis sicca, hypersensitivity (which may include glomerulopathy, polymyositis, polyarthritis, skin rash, skin eruptions, fever, hepatotoxicity, thrombocytopenia, neutropenia, and anemia), hepatic necrosis, hypoprothrombinemia, blood dyscrasias (anemia and thrombocytopenia), thyroid metabolic disorders, skin reactions, diarrhea, and carcinogenesis (Papich and Riviere, 2009). These adverse effects may occur more commonly in animals that are slow acetylators.

Veterinarians in the United States commonly use several simple sulfas and potentiated sulfas to treat animals with various protozoal infections: sulfadiazine with trimethoprim (TRIBRISSEN), sulfadiazine with pyrimethamine (REBALANCE), sulfadimethoxine (ALBON), sulfadimethoxine with ormetoprim (PRIMOR), sulfamethoxazole with trimethoprim (BACTRIM, SEPTRA), sulfaquinoxaline (SUL-Q-NOX), and the triple sulfa sulfamethazine/sulfamerazine/sulfaquinoxaline (POULTRYSULFA). Another potentiated sulfa, sulfamethazine and sulfachlorpyridazine (VETISULID), is commonly used, but to treat bacterial, not protozoal infection. Four sulfa products are commonly used in small-animal medicine: sulfadimethoxine, sulfadimethoxine with ormetoprim, sulfadiazine with trimethoprim, and sulfamethoxazole with trimethoprim. Sulfamethazine and sulfaquinoxaline are used in livestock, and sulfadiazine plus pyrimethamine (REBALANCE) is approved for use in horses for treatment of EPM.

Sulfadimethoxine

Sulfadimethoxine is a rapidly absorbed, long-acting sulfonamide. It is not acetylated in the dog and is excreted unchanged in the urine, decreasing the potential for drug-induced nephrotoxicity (Greene, 2012a). The drug is approved for treatment of coccidiosis in dogs, cats, cattle, chickens, and turkeys, and for treatment of strangles in horses. It has a wide margin of safety. No signs of toxicity were noted when dogs were dosed at 160 mg/kg PO daily for 13 weeks. Diarrhea was the only reaction seen in dogs given single oral doses of 3.2 g/kg (Agri Laboratories).

It is important that all treated animals receive adequate water intake to prevent dehydration and crystalluria, as well as to ensure proper nutrition during therapy for coccidiosis. Sulfadimethoxine

is available as an injectable (e.g., DI-METHOX INJECTION 40%); as 125-, 250-, and 500-mg tablets (ALBON TABLETS); as a pleasant-tasting suspension (ALBON ORAL SUSPENSION 5%), an oral solution (e.g., DI-METHOX 12.5% ORAL SOLUTION), or a soluble powder (e.g., SULFADIVED SOLUBLE POWDER); and in oral boluses (ALBON BOLUSES).

Dogs and Cats

Sulfadimethoxine is available as tablets or as a 5% oral suspension (ALBON), both of which are labeled for use in dogs and cats to treat a variety of infections including coccidiosis. The labeled dosage is to start at 55 mg/kg PO for one treatment with subsequent daily doses of 27.5 mg/kg PO and treatment duration of 3 to 5 days, with the caveat that treatment should continue until the animal is asymptomatic for 2 days (Pfizer Animal Health, 2008). But this dose may not be sufficient to clear coccidiosis.

For dogs with coccidiosis, Plumb cites extralabel references that recommend using the dose of 50 mg/kg/day for 10 to 14 days, or if used during the infant period, treating with 50 mg/kg the first day followed by 25 mg/kg/day PO, until symptoms regress (Plumb, 2011b). For cats with coccidiosis, Greene recommends initiating treatment as indicated on the label, but states that treating until 2 days after symptoms are resolved may take 14 to 29 days (Greene, 2012b); and Plumb's reference suggests using a dose of 50 mg/kg/day for 10 to 14 days. These should be very safe doses for dogs because, as was previously mentioned, when given 160 mg/kg/day PO for 13 weeks, no signs of toxicity were noted. There is not currently a sulfadimethoxine injectable product labeled for dogs and cats, but there was previously. The ALBON label for dogs and cats still refers to using the injectable product to initiate treatment, and Plumb cites a prior package insert that advised treatment by SC or IM injection for the first day at 55 mg/kg with subsequent daily doses of 27.5 mg/kg (Plumb, 2011b). The injectable product is labeled for IV use in cattle and may be used extralabel for IM, SC, or IV injection in the dog. That said, it seems reasonable that the oral route would be quite effective because coccidia are enteric pathogens and oral absorption is excellent in the dog, regardless of recent feeding or lack thereof.

Cattle

Sulfadimethoxine products labeled for cattle include injection, bolus, oral solution, and powder to add to drinking water or to use in making a drench. The injection is labeled for IV use. The recommended dosage is an initial dose of 55 mg/kg IV or PO for the first day and subsequent doses of 27.5 mg/kg/day IV or PO for a total of no more than 5 days. Diagnosis should be reevaluated if cattle are not asymptomatic by the second or third day of treatment. For the sustained-release bolus, give cattle one 12.5-g bolus PO per 200 pounds' body weight. Discard milk for 60 hours (five milkings) after the last treatment. Do not administer this drug within 7 days of slaughter. Consult the approved label for accurate dosage and withdrawal information, because there are differences depending on dosage form.

Horses

Currently no sulfadimethoxine products are labeled for horses, but the previous package insert dose was 55 mg/kg IV or PO initially, followed by 27.5 mg/kg/day IV (Plumb, 2011b).

Poultry

Sulfadimethoxine products labeled for poultry include an oral solution or powder to mix into drinking water to treat outbreaks of coccidiosis in broilers, replacement chickens, and turkeys. The usual dosage is 0.05% for chickens and 0.025% for turkeys for 5 days. Diagnosis should be reevaluated if birds are not asymptomatic by the second or third day of treatment. Do not use drug in chickens older than 16 weeks of age or in turkeys older than 24 weeks of age. Do not administer drug within 5 days of slaughter.

Sulfadimethoxine and Ormetoprim

Sulfadimethoxine with ormetoprim constitutes a rational combination that potentiates the action of both drugs by blocking two sequential steps in the synthesis of folic acid. Ormetoprim is a diaminopyrimidine potentiator with very low mammalian toxicity. As was previously mentioned, sulfadimethoxine is not acetylated in the dog and is excreted unchanged in the urine, decreasing the potential for drug-induced nephrotoxicity (Greene, 2012a). For a list of adverse events associated with treatment, see the previous introductory sulfonamide section.

Available tablets contain 100/20, 200/40, 500/100, or 1000/200 mg of sulfadimethoxine/ormetoprim (PRIMOR). The tablets are designated by the total weight of active ingredient in each tablet. Thus the PRIMOR 120 contains 100 mg of sulfadimethoxine and 20 mg of ormetoprim. The approved starting dosage for dogs is 55 mg/kg PO on the first day of treatment, then 27.5 mg/kg PO once per day for 14 to 21 days. Do not treat beyond 21 days.

It is interesting to note that one of the few controlled studies of coccidiosis therapy in dogs was conducted with this drug combination. In that study, 32.5 mg/kg or 66 mg/kg was given continuously in the food for 23 days subsequent to experimental oocyst infection. The higher dose of 66 mg/kg provided better results and did not produce any adverse reactions (Dunbar and Foreyt, 1985).

Poultry can be treated with the sulfadimethoxine and ormetoprim combination in a feed additive (ROFENAID 40), which is indicated to aid in the prevention of *Eimeria tenella, E. necatrix, E. acervulina, E. maxima, E. brunetti,* and *E. mivati* in chickens; prevention of *E. adenoeides, E. gallopavonis,* and *E. meleagrimitis* in turkeys; and prevention of *E. kofoidi* and *E. legionensis* in chukar partridges. Chickens are fed 0.0125% sulfa and 0.0075% ormetoprim. Turkeys are fed 0.00625% sulfa and 0.00375% ormetoprim. This product is also labeled to treat ducks and chukar partridges. See the package insert for feed mixing instructions (Alpharma). Do not feed drug within 5 days of slaughter. Do not feed to birds producing eggs for human consumption.

Sulfadiazine With Pyrimethamine

Sulfadiazine with pyrimethamine (REBALANCE ANTIPROTOZOAL ORAL SUSPENSION) is a rational drug combination that is approved for the treatment of horses with EPM caused by *Sarcocystis neurona*. It is provided in an oral suspension containing 250 mg of sulfadiazine per milliliter and 12.5 mg of pyrimethamine per milliliter. The approved oral dose is 4 mL/50 kg body weight, once daily. The duration of treatment is dependent on clinical response to treatment, but the usual course of therapy lasts from 90 to 270 days.

Horses undergoing treatment should be watched closely for worsening neurologic function (treatment crisis), which may occur during the first 5 weeks of treatment. The margin of safety is fairly narrow. When given a 2× overdose, signs of toxicity including transient anemia and loose stools resulted. Anemia, leukopenia, or bone marrow suppression may occur in some horses. Interruption of treatment or administration of dietary supplements with folinic acid may be indicated. In addition, folate supplementation, if needed, is not without risk in that such supplementation has been

associated with abortions and congenital defects (Bertone and Horspool, 2004).

Sulfadiazine With Trimethoprim

Sulfadiazine with trimethoprim is the potentiated sulfa with the greatest number of years of actual use in veterinary medicine. Currently the only FDA-approved product available with these ingredients is an antibacterial powder for use in horses (e.g., UNIPRIM), which contains 333 mg sulfadiazine and 67 mg trimethoprim per gram. The approved dosage in horses for bacterial infection is 3.75 g of powder per 50 kg (110 lb) of body weight once daily for 5 to 7 days. The recently available oral paste formulation (Tribrissen), which also contained 333 mg sulfadiazine and 67 mg trimethoprim per gram (Plumb, 2011b), is no longer on the market. When that product was available, the manufacturer recommended that it should not be given to horses with marked hepatic parenchymal damage, blood dyscrasias, or previous sulfonamide sensitivity. An equine injectable formulation containing 400 mg of sulfadiazine per milliliter and 80 mg of trimethoprim per milliliter (TRIBRISSEN 48% INJECTION), which was also previously available, is no longer being marketed in the United States.

This combination of ingredients has been recommended to treat coccidiosis in dogs and cats at 5 to 10 mg/kg + 25 to 50 mg/kg (trimethoprim + sulfadiazine) daily for 6 days to animals weighing >4 kg and half this dose for animals weighing <4 kg (Davis and Gookin, 2009), hence its inclusion herein. It has also been used to dogs with hepatozoonosis (Davis and Gookin, 2009).

Sulfamethazine

The sodium salt of sulfamethazine may be administered in water (SULMET) or by oral bolus (SULFA-MAX, SULMET) to cattle, swine, chickens, and turkeys, for the control of coccidiosis. The usual dose is ≈237 to 247 mg sulfamethazine per kilogram, which may be administered orally on the first day and followed by ≈123 mg/kg every day for 4 days (5 days total treatment). A sustained-release bolus (e.g., SUSTAIN III) is available for cattle, which delivers 32.1 g of sulfamethazine over 3 days. One bolus is given per 200 pounds of body weight. Animals should be provided with plenty of water when they are on sulfonamide medication. Withdrawal recommendations on the package insert should be followed for food-producing animals. Sulfamethazine is also available in combination with antibiotics such as penicillin, chlortetracycline, and tylosin.

Sulfamethoxazole and Trimethoprim

Sulfamethoxazole with trimethoprim is a readily available product approved for use in people (BACTRIM, SEPTRA). It is not currently approved for use in animals in the United States. Because of its similarity to veterinary potentiated sulfonamides, and because low-cost generics are available, this drug is widely used in veterinary medicine. Controversy surrounds the appropriate dosage regimen for this human-labeled product in animals, but many clinicians have achieved acceptable clinical results using the same dosage as for sulfadiazine. The dosage for bacterial infection and coccidiosis in dogs and cats is 30 mg/kg once or twice daily for 5 to 10 days and may be indicated in severe coccidial infections (Plumb, 2011b). Although some have recommended using this drug for longer durations for particularly persistent coccidial infections, it is important to realize that in dogs thyroid hormones may fall and hypothyroidism may even result. Normalization of thyroid hormones may take 8 to 12 weeks after stopping the product (Riviere and Papich, 2009).

A 5:1 fixed combination of sulfamethoxazole/trimethoprim is available as tablets and a pediatric suspension. Available single-strength tablets contain 400 mg and 80 mg, and double-strength tablets contain 800 mg and 160 mg, of sulfamethoxazole and trimethoprim, respectively. The pediatric oral suspension contains 40 mg of sulfamethoxazole per milliliter and 8 mg of trimethoprim per milliliter.

Sulfaquinoxaline

Sulfaquinoxaline is a sulfonamide approved for use in chickens, turkeys, and cattle for control and treatment of coccidia. It is not well absorbed from the gastrointestinal tract. Sulfaquinoxaline is available as a water medication (SUL-Q-NOX). It should be mixed according to the label, which provides cattle with a target dose of 6 mg/lb/day. In poultry, sulfa-medicated water is given for 2 to 3 days, followed by plain water for 3 days, alternating back and forth and repeating as needed to control signs; it is important to not change litter during treatment. The label provides solution concentrations and dosing schedules. Make a fresh solution every day. Do not give sulfaquinoxaline to lactating dairy cattle or to veal calves. It should not be used within 10 days of slaughter.

Extralabel use in rabbits and dogs has been described, but clinical use in these species is rare today (Papich and Riviere, 2009). Sulfaquinoxaline toxicity in animals is rare, but similar toxicity clinical signs and pathology have been reported in chickens and dogs. Poultry lesions included mildly enlarged pale livers; hemorrhages on heart, kidney, oviduct, and intestine; pale bone marrow; and gangrenous dermatitis. Dog lesions, in addition to those noted in chickens, included hypothermia, pale mucous membranes, and prolonged prothrombin time (Papich and Riviere, 2009).

Sulfamethazine, Sulfamerazine, and Sulfaquinoxaline

A combination of these three sulfonamides is available as a powdered drinking water additive for use in chickens and turkeys (POULTRYSULFA). It is used in turkeys for control of coccidiosis caused by *Eimeria meleagrimitis* and *E. adenoeides*. In chickens it may help control *Eimeria tenella* and *E. necatrix.* Sulfa-medicated water is given for 2 to 3 days, followed by plain water for 3 days, alternating back and forth and repeated as needed to control signs. Reevaluate if no improvement is noted in 72 hours. It is important to not change litter during treatment. The label provides solution concentrations and dosing schedules.

ANTHELMINTICS

Practicing veterinarians commonly use drugs to treat and prevent helminth infection in animals. This section will review anthelmintics commonly used in veterinary practice. The Compendium of Veterinary Products is an excellent collection of products approved by the FDA and available commercially in North America (North American Compendiums, 2012). An exhaustive review of the pharmacology, mechanism of action, pharmacokinetics, and efficacy of anthelmintics is outside the scope of this book. For those who wish to delve deeper, a few key citations were included in the text. For more exhaustive general information about anthelmintics and particulars, such as mechanism of action, the reader is advised to seek other references (Arundel et al, 1985; Campbell and Rew, 1985; Martin, 1997b; Riviere and Papich, 2009). It is hoped that new therapeutic agents will result from research into anthelmintic mechanisms of action, targets, and medicinal plants and fruits. Several reviews discuss these exciting areas of research (Behnke et al, 2008; Londershausen, 1996; Martin, 1993; Martin, 1997a; Martin et al, 2012).

Gastrointestinal parasites are among the most common infectious agents that veterinarians face in practice. A landmark parasite prevalence study evaluated more than 6000 canine fecal specimens from all 50 states and the District of Columbia (Blagburn et al, 1996). The results indicate that parasites are common in dogs in the United States. Nationwide, 36% of the samples tested were positive for roundworms *(Toxocara canis),* hookworms *(Ancylostoma caninum),* or whipworms *(Trichuris vulpis).* Even more surprising, 52% of samples from the southeastern United States were positive for at least one nematode. In a recent heartworm and fecal testing study in the western United States, the importance of annual testing and routine use of preventives was highlighted (Bowman, 2007). Clinics in 11 states were surveyed, and local dogs with no history of travel were diagnosed with heartworms in every state but Idaho and Wyoming. The prevalence of intestinal parasites in companion animals in Ontario and Quebec, Canada, during the winter was recently evaluated (Blagburn et al, 2008). The fact that 30% of feline and 39% of canine fecal samples were positive for gastrointestinal parasites prompted the authors to recommend that all veterinarians follow the Companion Animal Parasite Council (CAPC) guidelines (Companion Animal Parasite Council, 2013) regarding use of year-round broad-spectrum deworming protocols. Another reason for following CAPC guidelines, in this instance regarding routine heartworm testing and prophylaxis, is concern about animals moving from heartworm-endemic areas to those with limited heartworm exposure. These concerns were realized when Hurricane Katrina resulted in thousands of dogs and cats being shipped from Louisiana, where heartworm prevalence is quite high (Bowman et al, 2007) to shelters across the United States.

Although these parasites are important to the health of dogs, several are also important zoonotic pathogens. Ascarid larvae can migrate through human tissues, causing a variety of signs correlated with the location of the migration. These are primarily *Toxocara* species ascarids, but the raccoon ascarid, *Baylisascaris procyonis,* is being increasingly implicated as a cause of human disease in the United States (Murray and Kazacos, 2004). The Centers for Disease Control and Prevention (CDC) has published *Guidelines for Veterinarians: Prevention of Zoonotic Transmission of Ascarids and Hookworms of Dogs and Cats,* which is an excellent resource and is available online as a PDF download (see CDC website).

More than 60% of sheep producers identified stomach/intestinal nematodes as a major concern (National Animal Health Monitoring System [NAHMS], 1996). Obviously farm animals carry their share of helminth infections, some of which are zoonotic. Trichinosis is an example of a zoonotic disease caused by many species of the *Trichinella* genus that can result in illness of humans ingesting not only swine, but also horse, dog, and a variety of wild animals (Gajadhar et al, 2006). Another zoonotic farm animal concern is the potentially fatal neurocysticercosis, from which humans may suffer after eating cysticerci-infected pork or beef. Controlling the effects of disease caused by *Taenia* spp. relies heavily on education because, as an example, although anthelmintic treatment of livestock is effective, it is not economically feasible for cattle, and cysticerci are not quickly or reliably eliminated by anthelmintic treatment.

Worldwide, helminth infections are a major matter of animal and human health concern (Albonico et al, 2008), with hookworms infecting large numbers of people worldwide, especially those of low economic status (Hotez et al, 2005). In vast areas of South America and Asia, more than 30% of humans are infected with hookworms. More than half of the population is infected with hookworms in many southern areas of the African continent. Experts estimate that a billion people, more than a fifth of the planet's human inhabitants, harbor hookworms (Hotez and Pritchard, 1995). One study showed that nearly all dogs in a remote community of northeastern India were infested with one or more zoonotic gastrointestinal parasites (Traub et al, 2002). This study also demonstrated that dogs played a major zoonotic role both in transmitting parasites that use dogs as their host (definitive or paratenic) and in mechanically transmitting and spreading the dissemination range of an array of human-specific parasites. A recent feline study in metropolitan Rio de Janeiro revealed an 89.6% prevalence of overall gastrointestinal helminth parasites in cats (Gajadhar et al, 2006; Labarthe et al, 2004).

Through the prudent use of anthelmintics, the practicing veterinarian is in a unique position to positively affect not only patient health, but also public health. Resistance to anthelmintics, which started with *Haemonchus contortus* resistance to phenothiazine in sheep in the late 1950s (Kaplan, 2004), has become an increasingly hot topic, especially regarding heartworms of dogs and gastrointestinal parasites of horses and ruminants. Resistance of certain arthropods, protozoans, and helminths to particular agents has been reviewed in book sections dedicated to those parasites and will be addressed again at the end of this chapter. Anthelmintics approved by the FDA and commercially available are grouped together by class (macrocyclic lactones, benzimidazoles, imidazothiazoles, tetrahydropyrimidines, cyclic depsipeptides, piperazine, organophosphates, isoquinolones, arsenicals, miscellaneous, and finally, broad-spectrum combinations) and are listed alphabetically according to their generic names.

MACROCYCLIC LACTONES (AVERMECTINS AND MILBEMYCINS)

Macrocyclic lactones (or macrolides) have revolutionized the control of parasites in both man and animal. They consist of avermectins and milbemycins. Ivermectin is the best known agent in this class. They are generally regarded as the most effective and least toxic parasiticides yet developed. These products are all similar in that they are antibiotics produced by streptomycete microorganisms, and they have large macrocyclic structures. Although originally thought to act by disturbing GABA-mediated neurotransmission, it is now known that they bind with high affinity to a glutamate-gated chloride channel (Arena et al, 1991; Martin, 1993; Martin, 1997b; Shoop et al, 1995; Vercruysse and Rew, 2002; Wolstenholme and Rogers, 2005). Macrocyclic lactones bind to glutamate receptors that trigger chloride influx, which hyperpolarizes the parasite neuron and prevents initiation or propagation of normal action potentials. The net effect is paralysis and death of the target parasite.

Macrocyclic lactones have revolutionized the treatment of parasitic disease. In general, they are highly effective at low doses, are very safe, and provide true broad-spectrum activity against nematodes and arthropods. The dual activity of most macrolides, such as ivermectin and selamectin, against both endoparasites (such as helminths) and ectoparasites (such as fleas) gave rise to the term **endectocide.**

Macrolides are excreted in the feces as active drug. Drugs in this class, especially the avermectins, are toxic to aquatic animals and dung-feeding insects, but not to birds, plants, and earthworms. Elimination of coprophagous insects appears to delay processing of nutrients, but the overall environmental impact of this finding is unclear (USP, 2006). A recent literature review regarding the ecologic impact of macrolides clearly demonstrated that although many macrocyclic lactones are substances of high concern, particularly regarding their effects on larval instars of invertebrates, with

the exception of ivermectin (and to a lesser extent moxidectin and doramectin), knowledge available from the open literature is still sparse. By comparison, information on the ecological impact of other compounds such as eprinomectin and selamectin is relatively rare.

Commercially, ivermectin took the market by storm. Many conventional drugs that were direct competitors of this class were soon retired from common use and eventually were discontinued. Despite their beneficial activities, macrocyclic lactones have several flaws. They are ineffective against cestodes and trematodes and can be expensive. When the U.S. patent on ivermectin expired, generic competitors entered the market, which dramatically reduced the cost of treatment. Increased usage followed, as did increased resistance to macrocyclic lactones, which was reported in various parasites of several domestic animals, but started with parasites infecting small ruminants (Almeida et al, 2013; Geary et al, 2011; Howell et al, 2008; Kaplan, 2004; Kaplan and Vidyashankar, 2012; Traversa et al, 2009). Although macrolides are generally regarded as the most effective and least toxic parasiticides yet developed, toxicity may occur, especially in Collies, many of which are unusually sensitive to macrolide endectocides because they carry a mutant multidrug resistance gene (*MDR1* or *ABCB1*). This will be discussed in more depth in the following section. A recent review of macrolide treatment for *MDR1* mutant dogs is an excellent source of information for interested practitioners (Geyer and Janko, 2012).

The literature surrounding these products is overwhelming, but several good reviews pare the literature down to comprehensible levels (Bennett, 1986; Campbell, 1989; Shoop et al, 1995). For more information about macrolides, Vercruysse and Rew edited an excellent book specifically devoted to the topic (Vercruysse and Rew, 2002).

Avermectin Toxicity

It is important to not administer avermectins concurrently with drugs that could increase avermectin blood-brain barrier penetration, such as ketoconazole, itraconazole, cyclosporine, and calcium channel blockers (Papich, 2007). Ivermectin is the best known avermectin and will be discussed in detail first. Selamectin toxicity information is addressed in detail later, in the selamectin section.

Ivermectin Toxicity in Dogs

According to a popular veterinary pharmaceutical clinical text, signs of ivermectin toxicity in dogs, in order of frequency, are as follows: vomiting, ataxia, lethargy, tachycardia, hypersalivation, mydriasis, and seizures (Plumb, 2011b). Other signs include blindness, tremors, dehydration, depression, diarrhea, hyperthermia, bradycardia, sinus arrhythmia, coma, seizures, and death (Dorman, 1995; Paul and Tranquilli, 1989; Plumb, 2011b; Rumbeiha, 2009). A recent retrospective study of ivermectin toxicosis cases evaluated at a poison control center revealed clinical signs in the following order of frequency: ataxia, lethargy, tremors, mydriasis, and blindness (Merola et al, 2009).

The apparent LD_{50} of ivermectin in Beagles is 80 mg/kg (Paul and Tranquilli, 1989). The primary clinicopathologic sign in dogs is decreased serum iron values (Riviere and Papich, 2009). A common reference used by clinical veterinarians states that death could occur with doses above 40 mg/kg, tremors at 5 mg/kg, and mydriasis at 2.5 mg/kg, and that signs of toxicity rarely occur at doses below 1 mg/kg (Plumb, 2011b). But a recent retrospective poison control center study revealed that clinical signs may develop at between 0.2 and 2.5 mg/kg (Merola et al, 2009). At doses below 1 mg/kg a wide variety of signs were noted, including more severe signs like coma and seizure. In fact, death was noted in dogs that received doses of 1 to 2.5 mg/kg of ivermectin. It is possible that some of the dogs with signs evident at lower doses were ivermectin sensitive because they carried the *MDR1* mutation, which is also known as *ABCB1-1Δ* based on systematic nomenclature of the ATP-binding cassette transporter family. The *ABCB1-1Δ* term is the most current, but both terms are still being used in research reports, and the gene will be referred to by either terminology throughout this chapter.

A common presenting history is that of a dog that was in close proximity to horses during deworming and later started to show signs of toxicity. Dogs that develop clinical signs within 4 to 6 hours of ivermectin ingestion typically develop severe clinical signs, whereas dogs with signs developing 10 to 12 hours after exposure tend to have much milder clinical signs (Dorman, 1995). It is not uncommon for dogs with ivermectin toxicity to have seizures. When seizures are severe and uncontrolled for a considerable period, hemolytic anemia and muscle damage may occur. Some canine cases present with severe seizures and miosis; this warrants a poor prognosis since such presentation may be associated with severe brain damage (Gwaltney-Brant, personal communication, 2010).

***MDR1* MUTANTS.** When ivermectin was first used in dogs, it was found that some Collies were unusually sensitive to its toxic effects. Early studies indicated that some genetic lines of Collies developed severe adverse reactions when ivermectin was given at a dose of 0.1 to 0.2 mg/kg (16 to 32× the labeled heartworm preventative dose), producing mydriasis, ataxia, tremors, drooling, paresis, recumbency, excitability, stupor, and coma. At that time, Australian Shepherds, Border Collies, Shetland Sheepdogs, and Old English Sheepdogs were also reported to be sensitive to ivermectin. The lethal dose for some Collies was reported to be 1/200 of the lethal dose for Beagles (Pulliam et al, 1985).

After Collies were also found to be more sensitive to loperamide (Hugnet et al, 1996), *MDR1* was identified and was found to be mutated in ivermectin-sensitive Collies (Mealey et al, 2001). *MDR1* codes for P-glycoprotein, which is an integral part of the blood-brain barrier, is involved in active drug elimination by the liver and the kidney, and limits drug absorption in the gut. Other affected drugs include the following:

- Anticancer drugs (e.g., vinca alkaloids, paclitaxel, doxorubicin)
- Immunosuppressants (e.g., cyclosporine)
- Cardiac drugs (e.g., digoxin, verapamil, diltiazem, losartan)
- Opioids (e.g., morphine, loperamide, butorphanol, fentanyl)
- Steroid hormones (e.g., cortisol, dexamethasone)
- Antiparasitic agents (e.g., ivermectin, moxidectin, selamectin, milbemycin oxime)
- Antimicrobial agents (e.g., erythromycin, rifampicin, ketoconazole, levofloxacin)
- Many others including cimetidine, acepromazine, amitriptyline, and domperidone (Geyer and Janko, 2012; Mealey, 2004; Ohtsuki and Terasaki, 2007)

There are many excellent sources of information about the *MDR1*/*ABCB1-1Δ* gene mutation and mechanisms of ivermectin toxicity associated with increased GABA activity (Dorman, 1995; Geyer and Janko, 2012; Mealey, 2004; Rumbeiha, 2009).

The mutant *MDR1* allele was found in 35% of 40 Collies tested in one study—about the same percentage of Collies that are sensitive to ivermectin (Mealey et al, 2002). A survey of DNA from 4000 purebred dogs revealed that the *MDR1* mutation was present in seven breeds of Collie lineage and two sighthound breeds, although the mutation was not identified in all breeds known to have ivermectin sensitivity (Neff et al, 2004). It was found that the

potential for ivermectin sensitivity could be estimated by genotypic or polymerase chain reaction (PCR)-based testing for the *MDR1* mutation (Geyer et al, 2005). The mutant *MDR1* allele was found in the following (Geyer et al, 2005; USP, 2006):

- Australian Shepherds
- Miniature Australian Shepherds
- English Shepherds
- German Shepherd Dogs (white)
- Longhaired Whippets
- McNab Shepherds
- Old English Sheepdogs
- Shetland Sheepdogs
- Silken Windhounds
- Longhaired Whippet

Sensitivity to ivermectin was also noted in Australian Cattle Dogs, Bearded Collies, and Border Collies, but the mutant *MDR1* allele was not found in these breeds (Neff et al, 2004). More recently, the relationship with P-glycoprotein, the product of the *ABCB1* gene, was studied in depth by looking for the *ABCB1-1Δ* allele in a DNA study of 5368 dogs (Mealey and Meurs, 2008). The *ABCB1-1Δ* allele was found in Collies, Longhaired Whippets, Standard and Miniature Australian Shepherds, Shetland Sheepdogs, Old English Sheepdogs, Border Collies, Silken Windhounds, and German Shepherd Dogs.

The fact is that until a particular dog is tested, its susceptibility to ivermectin toxicity is unknown, that is, unless knowledge of the dog's heritage is certain and it is one of the following breeds: Bearded Collie, Anatolian Shepherd Dog, Greyhound, Belgian Tervuren, Kelpie, Borzoi, Australian Cattle Dog, and Irish Wolfhound, which are presumed to be completely free of *MDR1* mutation (Geyer and Janko, 2012). Washington State University, College of Veterinary Medicine, Veterinary Clinical Pharmacology Lab, provides genetic testing to determine the presence of the *MDR1* mutant gene. Box 6-2 was adapted from information available at the website of the aforementioned institution (Washington State University, 2010).

Other breeds noted in a recent review paper on this topic include the Waller (17% to 19%) and the White Swiss Shepherd (14%) (Geyer and Janko, 2012).

The general practitioner can use this information when presented with a dog that has had a known exposure to ivermectin to determine prognosis and the appropriate level of treatment. As noted previously, a common finding when history on ivermectin and moxidectin exposure cases is taken is that the dog was present while the owner was deworming a horse. It is not unusual for horses to spit out a small amount of dewormer, which is gobbled up by the dog. Ingestion of ≥0.12 mg/kg of ivermectin or ≥0.4 mg/kg of moxidectin is associated with clinical signs of avermectin toxicosis in *MDR1(–/–)* dogs (Geyer and Janko, 2012). Thus, if a 10-kg (22-lb) *MDR1(–/–)* dog ingests ≥1.2 mg ivermectin or ≥4 mg of moxidectin, signs of toxicity will occur.

Another presentation to consider is *MDR1(–/–)* dogs with the habit of eating horse feces. Obviously, the risk of severe toxicity is much greater with a Collie than with an unaffected breed. Dogs that are not ivermectin sensitive are probably not at risk, but an ivermectin-sensitive dog that eats the feces of a horse that was treated with ivermectin or moxidectin within the previous few days may have a severe reaction. Both ivermectin and moxidectin reach maximum fecal concentration of about 2.5 mg/kg at about 2 to 3 days after the horse is treated (Perez et al, 2001). Thus, a 10-kg (22-lb) *MDR1(–/–)* dog that eats ≥0.48 kg of feces with 2.5 mg/kg of ivermectin will ingest ≥1.2 mg of ivermectin and will have signs of toxicity. The same dog would have to eat ≥1.6 kg of 2.5 mg/kg of moxidectin feces to become toxic, which is possible although much less likely. The author (TC) has taken a first-hand report of an *MDR1(–/–)* dog with a fatal overdose of ivermectin-tainted horse feces. By 4 days posttreatment, 90% of ingested ivermectin had been excreted in the feces. Owners of ivermectin-sensitive, coprophagic dogs should treat feces deposited by ivermectin-treated horses from approximately the second through the fourth day after treatment as toxic waste, and should dispose of it in a manner that will prevent the dog from eating it.

If the amount ingested can be quantified, then Box 6-2 can help the veterinarian estimate prognosis and determine how aggressive to get with treatment. But often it is difficult to quantify the amount of drug ingested. Another prognostic indicator to consider is the time between ingestion and onset of clinical signs. Dogs with slow onset of clinical signs such as mydriasis, ataxia, and apparent blindness starting 4 to 8 hours after ingestion are typically dogs that will recover with time. Conversely, dogs with severe clinical central nervous system (CNS) signs that are rapidly deteriorating 1 to 2 hours after ingestion are typically dogs that have ingested very high doses of ivermectin relative to their *MDR1* status. The prognosis is poor for such dogs. They should be treated aggressively or their owners counseled to consider euthanasia, because even if treatment is successful, recovery will probably be a long, drawn out affair requiring intensive care (general supportive care, fluids, cardiopulmonary monitoring), that is, unless the fairly recently discovered lipid rescue procedure, as described in the section on treating avermectin toxicity, results in the sort of dramatic improvement that has been reported in some, but certainly not all, cases. Of course many cases have been reported of severely poisoned dogs that completely recovered after being comatose for weeks (Geyer and Janko, 2012).

BOX 6-2 Breeds Affected by *MDR1* Mutation

BREED	APPROXIMATE FREQUENCY
Collie	70%
Longhaired Whippet	65%
Australian Shepherd	50%
Australian Shepherd, Mini	50%
Silken Windhound	30%
McNab Shepherd	30%
Shetland Sheepdog	15%
English Shepherd	15%
German Shepherd Dog	10%
Herding Breed Cross	10%
Mixed Breed	5%
Old English Sheepdog	5%
Border Collie	<5%

Ivermectin Toxicity in Other Species

Dogs are about 10× as likely as cats to have ivermectin toxicity. Of the 318 exposures to ivermectin reported to the ASPCA Animal Poison Control Center (APCC) during 2008-2009, 282 were dogs (203 symptomatic), 24 were cats (15 symptomatic), 3 were cows (3 symptomatic), and 1 was a turtle, which was symptomatic. The remaining 8 cases (2 rodents, 2 sheep, and 4 horses) were asymptomatic. Clinical signs of ivermectin toxicity in cats were reported in the following order of frequency: ataxia, diarrhea, hypersensitivity, and vomiting. The common finding in cattle with ivermectin overdose is diarrhea (Plumb, 2011b).

Treating Avermectin Toxicity

No antidote is known for avermectins. Regarding treatment of ivermectin toxicity, although evidence suggests that intravenous administration of physostigmine may be of some benefit for dogs (Tranquilli et al, 1987) and neostigmine may help treat cats (Muhammad et al, 2004) suffering from severe ivermectin intoxication, adverse events associated with these treatments typically outweigh benefit, thus the mainstay of care given by most veterinarians is supportive and symptomatic (Paul and Tranquilli, 1989). Inducing emesis, giving activated charcoal, providing fluid therapy, supplying parenteral alimentation, monitoring cardiopulmonary status, and maintaining respiratory support and normal body temperature are essential. This supportive care may be needed for an extended time because in dogs the half-life of ivermectin is 2 days and the half-life of moxidectin is 19 days (Rumbeiha, 2009).

No antidote is known for avermectin toxicity, but veterinarians should consider lipid emulsion infusion, a promising therapy adapted from human medicine. Dr. Guy Weinberg initially described the use of an intravenous lipid emulsion (INTRALIPID) to treat local anesthetic toxicity (bupivacaine) in humans. He coined the term **lipid rescue.** One of the studies that support human use of lipid emulsion infusion was an experiment on dogs that were overdosed with bupivacaine and rescued from certain toxicity with intravenous lipid emulsion (Weinberg et al, 2003). Weinberg established a noncommercial website (www.lipidrescue.com) to disseminate information and foster discussion of cases, which has recently been updated (http://lipidrescue.squarespace.com/). Since then, lipid emulsion infusion has been used to treat nonbupivacaine toxicities in other species. Although support is certainly anecdotal, in 2008 a veterinary online contributor to the lipid rescue website described an ivermectin-overdosed dog that had clinical signs of toxicity and recovered nicely after activated charcoal, supportive care, and an intravenous lipid emulsion were administered. More recently, a case report of a puppy with moxidectin toxicosis was published describing the use of an intravenous lipid emulsion given as a bolus of 2 mL/kg, followed by 4 mL/kg/hr for 4 hours beginning 10 hours after exposure and repeated at 0.5 mL/kg/min for 30 minutes beginning 25.5 hours after exposure (Crandell and Weinberg, 2009). The 16-week-old dog presented with acute onset of seizures, paralysis, and coma soon after exposure to moxidectin. Diazepam, glycopyrrolate, and intravenous fluids were given, along with respiratory ventilation and other supportive care. The puppy improved dramatically within 30 minutes of the second dose of INTRALIPID. Although ideal dosages have not been thoroughly established, the typical recommendation is for bolus administration of 1.5 mL/kg of intravenous lipid emulsion, followed by constant rate infusion of 0.25 mL/kg/min for 30 to 60 minutes (Weinberg et al, 2003). It is best to have the product available ahead of time rather than try to acquire it in the midst of an emergency. Having a lipid rescue kit, such as that described on Weinberg's website (http://lipidrescue.squarespace.com/sample-lipidrescue-kit/) should be considered. Other brands of intravenous lipid emulsion, such as LIPOSYN II or LIPOSYN III, can also be considered.

LIPOSYN III was the product used successfully at the previously described dose in a recent overdose of a *MDR1(+/+)* (normal) Border Collie that ingested a toxic dose of ivermectin (6 mg/kg) (Clarke et al, 2011). Results of serial measurement of serum ivermectin supported the **lipid sink** mechanism of action in this case; lipid sink referring to lipid absorption facilitating metabolism and excretion of lipid-soluble drugs in circulation. Lipid emulsion infusion has been used successfully in cats, albeit to treat permethrin or lidocaine toxicoses (O'Brien et al, 2010; Bruckner and Schwedes, 2012; Haworth and Smart, 2012). It has also been used to treat toxicity of the following lipid-soluble compounds: local anesthetics (e.g., bupivacaine, lidocaine), tricyclic antidepressants (e.g., clomipramine), propranolol, bupropion, and baclofen (Lee, 2010). However, in a recent ivermectin toxicosis case of several *MDR1(–/–)* homozygous mutant Border Collies, lipid rescue at the same dose (1.5 mL/kg of intravenous lipid emulsion administered over 10 minutes, followed by constant rate infusion of 0.25 mL/kg/min for 60 minutes) was unsuccessful for unknown reasons (Wright et al, 2011). Weinberg's review of lipid emulsion infusion in human medicine with discussion of mechanism of action was recently published (Weinberg, 2012). Discussion of this technique in veterinary medicine is covered succinctly in the latest edition of *Plumb's Veterinary Drug Handbook* (Plumb, 2011a).

Doramectin

Doramectin is a fermentation product from a mutant strain of *Streptomyces avermitilis,* and its spectrum of action is similar to that of avermectin B_1, although it has an elimination half-life about twice that of ivermectin (Friis and Bjoern, 1997; Shoop et al, 1995).

Cattle

Doramectin is available in a 1% injectable solution (DECTOMAX INJECTABLE SOLUTION) and a 0.5% pour-on (DECTOMAX POUR-ON) for cattle. When injected subcutaneously in cattle, the 1% solution is formulated to deliver a dose of 0.2 mg/kg (200 mcg/kg) when given at 1 mL/50 kg (110 lb). Although labeled for either IM or SC use, directions indicate that SC injection is preferred per Beef Quality Assurance guidelines (Pfizer Animal Health, 2005). When applied topically, the 0.5% solution is formulated to deliver a dose of 0.5 mg/kg (500 mcg/kg) when given at 1 mL/10 kg (22 lb). Both products are effective against gastrointestinal roundworms, lungworms, eyeworms, grubs, sucking lice, and mange mites (Pfizer Animal Health, 2005; Eddi et al, 1993; Gonzales et al, 1993; Goudie et al, 1993; Hendrickx et al, 1993; Jones et al, 1993; Kennedy and Phillips, 1993; Logan et al, 1993; Moya-Borja et al, 1993a; Reinemeyer and Courtney, 2001a; Vercruysse et al, 1993; Weatherley et al, 1993; Wicks et al, 1993). According to product inserts, the pour-on product also has activity against biting lice *(Damalinia bovis),* but the injectable does not. Doramectin has extralabel activity against screwworms *(Cochliomyia hominivorax),* which is rather surprising in that other macrocyclic agents are ineffective in this regard (Moya-Borja et al, 1993b).

Both products should be used with caution in treating grubs (*Hypoderma* spp. larvae) because if treatment is provided when many larvae are present in the gullet, bloat may result, or if many larvae are present in the vertebral canal, staggering or paralysis may occur. These reactions can occur with any drug that kills cattle grubs; they are not specific to doramectin, but the result can be deadly for affected cattle. It is best to treat as soon as possible after the end of the heel fly season to avoid such reactions.

The injectable solution should not be used in cattle within 35 days of slaughter. The pour-on product should not be used in cattle within 45 days of slaughter.

Swine

Doramectin 1% injection (DECTOMAX INJECTABLE SOLUTION) is also approved for use in swine, but the pour-on product is not. For swine the 1% solution is formulated to deliver a dose of 0.3 mg/kg (300 mcg/kg) when injected IM at 1 mL/34 kg (75 lb). The product is effective against gastrointestinal roundworms, lungworms, kidney worms, sucking lice, and mange mites (Arends et al, 1997a; Arends et al, 1997b; Pfizer Animal Health, 2005; Lichtensteiger et al, 1997; Logan et al, 1997; Saeki et al, 1995;

Saeki et al, 1997; Stewart et al, 1996a; Stewart et al, 1996b). It should not be used in swine within 24 days of slaughter.

Dogs and Cats

Although the manufacturer warns against use in species other than pigs and cows, and even states that severe adverse reactions including fatalities in dogs may result (Pfizer Animal Health, 2005), the injectable doramectin product has been used to treat generalized demodicosis in dogs and cats at the dose of 0.6 mg/kg (600 mcg/kg) SC once weekly for 4 weeks after skin scraping is negative (Plumb, 2011b). An oral dose of 0.6 mg/kg has been recommended for canine generalized demodicosis (Merchant, 2009). Such extra-label use should be considered only if labeled products are ineffective, the risk of disease outweighs the risk of treatment, the owner has given informed consent, and testing for *MRD1* status has been recommended in dogs.

Eprinomectin

Eprinomectin is a second-generation macrocyclic lactone synthesized from a fermentation product of *S. avermitilis*. It has extremely broad-spectrum endectocidal activity. The same group that discovered ivermectin synthesized eprinomectin from avermectin B_1. The article that describes the effort to find eprinomectin provides a beautiful description of targeted research and should be read by any scientist interested in understanding the pharmaceutical research process (Shoop et al, 1996a).

Cattle

Eprinomectin was initially approved in a topical formulation and has been marketed more recently as an injectable. Unfortunately the topical formulation has a misleading brand name in that "IVOMEC EPRINEX POUR-ON FOR BEEF AND DAIRY CATTLE" does contain any ivermectin, as do all of the other products with the "IVOMEC" brand name. This is yet another example of a manufacturer focusing on marketing to the detriment of ease of understanding by the end user. At least the active ingredient is in the same drug class and is similar to ivermectin.

Eprinomectin pour-on is an easy-to-use formulation that is applied at 1 mL/10 kg (1 mL/22 lb) and has zero time withdrawal for meat and milk. It is the only macrolide that can be used in lactating dairy cattle because it partitions away from milk (Shoop et al, 1996b). It is effective against the common cattle nematodes, including barber pole worms, *Haemonchus placei;* brown stomach worms, *Ostertagia ostertagi;* small intestinal worms, *Cooperia oncophora, C. punctata,* and *C. surnabada;* small stomach worms, *Trichostrongylus axei* and *T. longispicularis;* bankrupt worms, *T. colubriformis;* thread-necked intestinal worms, *Nematodirus helvetianus;* nodular worms, *O. radiatum;* hookworms, *Bunostomum phlebotomum;* intestinal threadworms, *Strongyloides papillosus;* lungworms, *Dictyocaulus viviparus;* and whipworms, *Trichuris* spp. (Cramer et al, 1997; Gogolewski et al, 1997; Reid et al, 1997; Yazwinski et al, 1997). Efficacy is not affected by coat length or by rain or weather (Gogolewski et al, 1997). It is not surprising that eprinomectin pour-on is also effective against many arthropod ectoparasites, including cattle grubs, *Hypoderma lineatum* and *H. bovis;* sucking lice, *Linognathus vituli, Haematopinus eurysternus,* and *Solenopotes capillatus;* biting lice, *Damalinia (Bovicola) bovis;* mange mites, *Chorioptes bovis* and *Sarcoptes scabiei;* and horn flies, *Haematobia irritans* (Eagleson et al, 1997a; Eagleson et al, 1997b; Thompson et al, 1997).

Eprinomectin injectable (LONGRANGE) is dosed SC at 1 mL/50 kg (110 lb) and is labeled to treat and control many of the same parasites as the pour-on formulation plus *Ostertagia lyrata.* Eprinomectin injectable provides 100-day-persistent effectiveness against small intestinal worms, *Cooperia oncophora* and *C. punctata;* and small stomach worms *Trichostrongylus axei;* 120-day-persistent effectiveness against barber pole worms, *H. placei*; nodular worms, *Oesophagostomum radiatum;* and brown stomach worms, *Ostertagia ostertagi* and *O. lyrata;* and 150-day-persistent effectiveness against lungworms, *Dictyocaulus viviparous.* Like the pour-on, it is labeled to treat *Cooperia surnabada, Trichostrongylus colubriformis,* and grubs *Hypoderma bovis,* and mange mites *Sarcoptes scabiei* var. *bovis.* Unlike the pour-on, the injectable is not labeled to treat *Trichostrongylus longispicularis, Nematodirus helvetianus, Bunostomum phlebotomum,* or *Strongyloides papillosus;* or whipworms, biting lice, sucking lice, or horn flies. Do not slaughter treated cattle within 48 days of injection. The injectable product is not for use in bulls, as reproductive safety testing has not been conducted. Injection site lesions included swelling, hyperemia, or necrosis (Merial Ltd, 2012).

A recent study of eprinomectin injectable in pastured cattle revealed that treated cattle had significantly fewer strongylid eggs than controls, which were treated with vehicle only (Rehbein et al, 2012). Cattle treated with eprinomectin injection had significantly ($P < 0.05$) fewer of the following nematodes postmortem than control cattle: *Dictyocaulus viviparus* (adults and fourth-stage larvae (L4), *Bunostomum phlebotomum, Cooperia curticei, Cooperia oncophora, Cooperia punctata, Cooperia surnabada, Cooperia* spp. inhibited L4, *Haemonchus contortus, Haemonchus placei, Haemonchus* spp. inhibited L4, *Nematodirus helvetianus, Nematodirus* spp. inhibited L4, *Oesophagostomum radiatum, Oesophagostomum* spp. inhibited L4, *Ostertagia leptospicularis, Ostertagia lyrata, Ostertagia ostertagi, Ostertagia* spp. inhibited L4, *Trichostrongylus axei, Trichostrongylus colubriformis, Trichostrongylus* spp. inhibited L4, *Trichuris discolor*, and *Trichuris ovis.* Treated cattle had significant weight gain compared with control cattle. The overall reduction of nematode counts was >92% compared with control cattle.

Rabbits

Eprinomectin treatment can reportedly eliminate rabbit ear mange mites when used extralabel. One study of rabbits infected with *Psoroptes cuniculi* used 2 mg/kg topically, which resulted in complete recovery within 2 weeks (Wen et al, 2010). In another study, rabbits infected with *P. cuniculi* were treated with 0.1, 0.2, or 0.3 mg/kg (100, 200, or 300 mcg/kg) by SC injection. The 0.1 mg/kg group improved but was not cured, whereas *P. cuniculi* was eliminated in both of the other treatment groups (0.2 and 0.3 mg/kg) (Pan et al, 2006).

Donkeys

Extralabel use of 0.5 mg/kg eprinomectin pour-on in 12 donkeys naturally infected with strongyle nematodes revealed 100% efficacy 7 and 14 days after treatment and >99% efficacy until the end of the 56-day study (Gokbulut et al, 2011).

Ivermectin

Ivermectin was the first commercially available macrolide, released for animal use by Merck in 1981, just 6 years after the discovery of avermectins (Holden-Dye and Walker, 2007; Shoop et al, 1995). The avermectins were isolated from the fermentation broth of *Streptomyces avermitilis*. Anthelmintic activity was discovered after actinomycetic broth was administered to mice infected with the nematode *Nematospiroides dubius*. The commercial success of ivermectin inspired other companies to develop analogs including moxidectin, milbemycin oxime, doramectin, selamectin, abamectin, and eprinomectin (Holden-Dye and Walker, 2007). Ivermectin is

effective against many nematodes and arthropods. It is very effective against the immature heartworm, *Dirofilaria immitis,* but it has minimal effect on adult heartworms. The current literature contains reports of use against hundreds of species of parasites in a very long list of hosts, including many exotic and wild animal species.

Administration of ivermectin to pregnant rats, mice, and rabbits produced teratism in fetuses only at or near doses that were maternally toxic. No teratogenesis was noted in cattle, sheep, and dogs when ivermectin was administered to pregnant animals at 4× the recommended dose.

As was previously stated, although ivermectin was originally believed to act by disturbing GABA-mediated neurotransmission, it is now known that it binds with high affinity to glutamate-gated chloride channels, triggering chloride influx, hyperpolarization, paralysis, and death (Arena et al, 1991; Martin, 1993; Martin, 1997b; Shoop et al, 1995; Vercruysse and Rew, 2002; Wolstenholme and Rogers, 2005). In arthropods, ivermectin inhibits transmission of signals at neuromuscular junctions by the same mechanism. Death results from paralysis in both nematodes and arthropods.

Horses

Ivermectin is available as the sole active ingredient in a host of 1.87% paste products (e.g., EQVALAN PASTE 1.87%) designed for easy oral administration and 1% liquid products (e.g., IVERMAX EQUINE ORAL LIQUID) formulated for drench or tubing. Ivermectin is also available in combination with praziquantel, but this formulation is discussed later in the section on broad-spectrum combination products. Ivermectin was previously available for horses as injectable for intramuscular administration, but was withdrawn from the market as the result of adverse reactions such as pain and clostridial infection at the injection site (Barragry, 1987).

Ivermectin has a broad spectrum of activity against nematodes and arthropod parasites of horses when administered PO at 0.2 mg/kg (200 mcg/kg) of body weight. It is used for the treatment and control of large strongyles, small strongyles (including those resistant to some benzimidazoles), pinworms, ascarids, hairworms, large-mouth stomach worms, bots, lungworms, and threadworms. It is also used to treat summer sores caused by *Habronema* and *Draschia* spp. larvae and dermatitis caused by neck threadworm (*Onchocerca* spp.) microfilariae (onchocerciasis).

When used to treat onchocerciasis, a single ivermectin dose often results in clinical remission of signs within 2 to 3 weeks, but sometimes two or three monthly treatments are needed (Rees, 2010). About a quarter of horses treated for onchocerciasis have an adverse reaction, which may occur more frequently in horses with a large burden of neck threadworm microfilariae, presumably as a result of death of a large number of microfilariae and massive release of parasitic antigens. The signs—ventral midline edema and pruritus—occur 1 to 10 days posttreatment and may necessitate therapy with prednisolone or phenylbutazone. If untreated, edema usually resolves in a week to 10 days, and pruritus subsides within about 3 weeks. (Plumb, 2011b). Administering a glucocorticoid just before ivermectin treatment and repeating the steroid 1 to 2 days after treatment reportedly prevents this adverse reaction (Plumb, 2011b).

According to package inserts (RXV; Merial LTD, 2008) ivermectin may be used in horses of all ages, including mares at any stage of pregnancy and breeding stallions, although inserts clarify that foals should be treated initially at 6 to 8 weeks of age. Treating foals that are younger is ill advised because toxicity can occur, presumably as the result of immaturity of the blood-brain barrier (Godber et al, 1995). Disruption of the blood-brain barrier is suspected, but not proven, as the cause of ivermectin toxicity in adult horses given the labeled dose of ivermectin after having access to silver nightshade *(Solanum eleagnifolium)* (Garland et al, 1998; Swor et al, 2009).

Pregnant mares treated orally with 0.6 mg of ivermectin per kilogram throughout the organogenesis period gave birth to normal, healthy foals. Treatment with 0.6 mg of ivermectin per kilogram did not affect the sexual behavior of stallions, and the quality of semen was not affected. Foals are typically treated initially at 6 to 8 weeks of age. Oral administration of 3× the recommended dose of ivermectin was well tolerated by horses. Horses orally dosed at 1.8 mg/kg (9× the recommended dose) did not have signs of toxicity, but when dosed at 2 mg/kg (10× the recommended dose), visual impairment, depression, and ataxia were noted (Plumb, 2011b).

Ivermectin package inserts indicate that the product is for oral use in horses only and caution that the product may cause severe adverse reactions when administered to other species, which may include death in dogs (RXV; Merial LTD, 2008). It is not unusual for avermectin (ivermectin and moxidectin) toxicity to be reported in dogs that were in close proximity to horses during deworming because horses may spit the paste out during administration (Coles and Lynn, 2012). Coprophagic dogs with an ivermectin-sensitive genetic makeup (carrying the multidrug resistance gene mutation) are also at risk for ivermectin toxicity if they eat the feces of a recently treated horse. Ivermectin reaches maximum fecal concentration 2 to 3 days after oral treatment (Perez et al, 2001). By 4 days posttreatment, 90% of the drug has been excreted in the feces. Owners of ivermectin-sensitive, coprophagic dogs should be advised to treat feces from ivermectin-treated horses as toxic waste, disposing of it in a manner that will prevent their dog from eating it (Coles and Lynn, 2012).

Cattle

Ivermectin is available as the sole active ingredient in two formulations for cattle: a 1% (10 mg/mL) liquid for subcutaneous injection (IVOMEC 1% INJECTION FOR CATTLE AND SWINE) and a pour-on 5-mg/mL solution (IVOMEC POUR-ON FOR CATTLE), which are available in products with nearly identical package inserts and are produced by a host of different manufacturers. Ivermectin is also available in a combination injectable product with a flukicide, clorsulon—a product that will be discussed in the combination section.

Ivermectin injection is administered SC at 0.2 mg/kg of body weight to treat and control gastrointestinal roundworms, cattle grubs, lungworms, sucking lice, and mange mites (*Psoroptes ovis* and *Sarcoptes scabiei*). Subcutaneous administration of ivermectin persistently protects cattle from reinfection with *Dictyocaulus viviparus* and *Oesophagostomum radiatum* for 28 days after treatment; *Ostertagia ostertagi, Trichostrongylus axei,* and *Cooperia punctata* for 21 days after treatment; and *Haemonchus placei* and *Cooperia oncophora* for 14 days after treatment (Merial LTD, 2007a). The efficacy of ivermectin against biting lice is erratic. Although not a labeled indication, ivermectin injection may help treat and control adult *Parafilaria bovicola* (Swan et al, 1991), which causes summer bleeding, and has shown good efficacy against the eyeworm, *Thelazia rhodesi* (Soll et al, 1992).

The drug is absorbed, widely distributed in the tissues, and slowly eliminated; and it is excreted in the feces as unaltered ivermectin, which, as previously discussed, probably disturbs the development of coprophilic larvae, although the product insert states

that it is not expected to have an adverse impact on dung-dependent insects. Aquatic insects will certainly be harmed if the water runoff from feedlots freely enters lakes or streams.

Toxic effects may be noted when cattle are dosed at ≥30× the label dose. Ataxia, listlessness, and death may occur after dosing at ≥8 mg/kg (Plumb, 2011b).

As with doramectin, ivermectin products should be used with caution in treating grubs (*Hypoderma* spp. larvae) because if treatment occurs when many larvae are present in the gullet, then bloat or acute esophagitis may result; or if many larvae are present in the vertebral canal, staggering or paralysis may occur as a consequence of spinal cord hemorrhages. These reactions can occur with any drug that kills cattle grub; they are not specific to ivermectin. The result can be deadly for affected cattle, nonetheless. It is best to treat as soon as possible after the end of the heel fly season to avoid such reactions.

The ivermectin concentration in the pour-on formulation for cattle is 5 mg/mL. It is applied at a rate of 1 mL/10 kg to treat and control gastrointestinal roundworms, cattle grubs, lungworms, mange mites, horn flies, and both sucking and biting lice. Pour-on administration of ivermectin persistently protects cattle from reinfection with *Oesophagostomum radiatum* and *Dictyocaulus viviparus* for 28 days after treatment; *Cooperia punctata and Trichostrongylus axei* for 21 days after treatment; *Ostertagia ostertagi, Haemonchus placei, Cooperia oncophora,* and *Cooperia surnabada* for 14 days after treatment; and *Damalinia bovis* for 56 days after treatment. See previous text for comparison of these parasites with those that the injection persistently protects against—a similar but not identical group. Comparison of the injectable and pour-on package inserts reveals that the pour-on, but not the injection, is labeled for biting lice *(Damalinia bovis)* and horn flies. Also, the injectable, but not the pour-on, is labeled for *Psoroptes ovis* (Merial LTD, 2007a; Merial LTD, 2007b).

Do not use ivermectin in female dairy cattle of breeding age, and do not use in lactating dairy cattle, because a withdrawal time in milk has not been established. Do not use in veal calves. The withdrawal time for cattle treated with injectable ivermectin is 35 days, and for cattle treated with pour-on ivermectin is 48 days.

American Bison and Reindeer

The FDA has approved ivermectin injection (IVOMEC 1% INJECTION FOR CATTLE AND SWINE) for the treatment and control of grubs, *Hypoderma bovis,*, in American bison *(Bison bison)* and for treatment and control of warbles, *Oedemagena tarandi,* in reindeer *(Rangifer tarandus)*. The effective dose is 0.2 mg/kg injected subcutaneously. Do not treat these animals within 8 weeks of slaughter.

Sheep

Several manufacturers produce an ivermectin sheep drench (e.g., IVOMEC DRENCH FOR SHEEP) labeled at 0.2 mg/kg for the treatment and control of adults and fourth-stage larvae of barber pole worms, *Haemonchus contortus* and *H. placei* (adults only); brown stomach worms, *Ostertagia circumcincta;* small stomach worms, *Trichostrongylus axei;* bankrupt worms, *Trichostrongylus colubriformis;* Cooper's worms, *Cooperia curticei* and *C. oncophora* (adults only); nodular worms, *Oesophagostomum columbianum* and *O. venulosum* (adults only); thread-necked intestinal worms, *Nematodirus battus* and *N. spathiger;* intestinal threadworms, *S. papillosus* (adults only); large-mouth bowel worms, *Chabertia ovina* (adults only); whipworms, *Trichuris ovis* (adults only); and lungworms, *Dictyocaulus filarial,* and all the larval stages of the nasal bot *Oestrus ovis*. The drench package insert has a residue warning stating that sheep should not be treated within 11 days of slaughter.

Overseas, injectable ivermectin is labeled in sheep for the treatment of infection due to gastrointestinal roundworms, lungworms, and larval stages of the nasal bot (Merial LTD, 2003). Although extralabel in the United States, the parenteral SC dose in sheep is the same as the oral sheep dose and the parenteral cattle dose, 0.2 mg/kg (200 mcg/kg). The overseas label warns against use within 35 days of slaughter in sheep.

Goats

An extralabel dose of 0.2 mg/kg ivermectin by SC injection successfully treated and controlled *Haemonchus, Trichostrongylus, Oesophagostomum, Bunostomum,* and *Strongyloides* spp. parasites in Jamunapari goats (Godara et al, 2011). In another study, the dose ranged from a single SC injection at 0.2 mg/kg to two injections at 0.3 mg/kg, both of which successfully treated goats with lungworm *(Muellerius capillaris)* infection (McCraw and Menzies, 1986), although a more recent study using 0.2 mg/kg SC for treatment of lungworms in goats was unsuccessful, in that results revealed no difference between treated and untreated groups (Lopez et al, 2010). Extralabel ivermectin injection at 0.2 to 0.4 mg/kg SC has been used to treat and control *Sarcoptes scabiei,* although not with 100% efficacy, in the Spanish ibex *(Capra pyrenaica hispanica),* a wild goat (Leon-Vizcaino et al, 2001).

The Food Animal Residue Avoidance Databank (FARAD) estimates that 99% of the drug will be eliminated from goat milk 22 days after a 0.2-mg/kg SC injection of ivermectin but recommends using the cattle withdrawal of 35 days, just to be on the safe side (Baynes et al, 2000). Although not labeled as such, the sheep drench has been used in goats at the sheep dose (0.2 mg/kg PO) or higher. But in goats, it is not uncommon for ivermectin to be dosed PO at 1.5× to 2× the label dose in sheep (Baynes et al, 2000). If goats are dosed PO at 0.2 mg/kg, then the sheep withdrawal interval of 11 days for meat is considered sufficient by FARAD. But if dosed at 0.4 mg/kg PO, FARAD, considering goat ivermectin pharmacokinetics, calculated the meat withdrawal interval to be 14 days. The milk withdrawal interval at 0.2 mg/kg PO is 6 days, and at 0.4 mg/kg PO it is at least 8 days (Baynes et al, 2000).

Swine

Ivermectin 1% injection (again, many manufacturers; e.g., IVOMEC 1% INJECTION FOR CATTLE AND SWINE) is administered subcutaneously in the neck area at a dose level of 0.3 mg/kg. It is indicated for the treatment and control of adults and fourth-stage larvae of large roundworms, *Ascaris suum;* red stomach worms, *Hyostrongylus rubidus;* nodular worms, *Oesophagostomum* species; threadworms, adults and somatic larvae of *Strongyloides ransomi;* adult lungworms *Metastrongylus* spp.; sucking lice, *Haematopinus suis;* and mange mites, *Sarcoptes scabiei* var. *suis.* Injecting ivermectin into sows 7 to 14 days before farrowing prevents colostral transmission of *Strongyloides ransomi*. Although extralabel, ivermectin is active against the swine kidney worm, *Stephanurus dentatus* (Becker, 1986). In short-term studies, ivermectin was injected into swine at up to 30 mg/kg (100× the label dose) without fatal sequelae, but lethargy, ataxia, mydriasis, tremors, lateral recumbency, labored breathing, and other toxicity signs were noted (Barragry, 1987; Plumb, 2011b). Swine should not be treated within 18 days of slaughter (Merial LTD, 2007a).

Extralabel use of ivermectin in potbellied pigs has been advised at 0.3 mg/kg SC or IM given once for susceptible internal parasites and repeated in 10 to 14 days in treating external parasites (Braun, 1995).

Dogs

Ivermectin tablets and chewable tablets (HEARTGARD TABLETS FOR DOGS, HEARTGARD CHEWABLES FOR DOGS) are administered orally at 0.006 mg (6 mcg) per kilogram monthly to prevent the establishment of heartworms, *Dirofilaria immitis*. These products are recommended for dogs 6 weeks of age and older. The initial dose should be given within a month after the first exposure to mosquitoes and throughout the year when mosquitoes are active. The last treatment must be given to dogs within a month after the last exposure to mosquitoes. In the short term, ivermectin has minimal activity against the adult heartworm. It is active only against third- and fourth-stage larvae and circulating microfilariae.

A single oral dose of ivermectin administered within 2 months after infection prevents the establishment of worms in the heart. A single dose of 0.05 mg/kg is adequate to clear the circulating microfilariae when given to dogs 4 weeks after administration of an adulticide. Ivermectin is not approved as a microfilaricide (Hribernik, 1989). Review of the original reference is suggested for more complete information. When ivermectin (0.006 mg/kg) is given to heartworm-positive dogs over several months, the circulating microfilariae are eliminated, resulting in an occult infection. Thus dogs receiving monthly ivermectin should be tested annually with an occult heartworm test (American Heartworm Society, 2012; Bowman et al, 1992; Courtney et al, 1998; Lok and Knight, 1995).

Knight provides an excellent review of heartworm testing and suggested chemoprophylaxis timing for various regions in the United States (Knight, 2000). The American Heartworm Society guidelines for diagnosis, prevention, and management of heartworm infection in dogs should also be consulted (American Heartworm Society, 2012). Although no FDA-approved microfilaricide is available, macrocyclic lactones are safe and effective microfilaricidal drugs for use in heartworm-positive dogs; however, they may cause rapid microfilarial death and should be used with caution in dogs with high counts of microfilaria. Pretreatment with antihistamines and glucocorticosteroids will minimize potential reactions (American Heartworm Society, 2012).

Short-term use of ivermectin alone has minimal effect on adult heartworms, but when given continuously over a prolonged period of 1 to 2 years, or when combined with doxycycline, it may have some utility in treating dogs with adult heartworm infection. The older the adult heartworms are when first exposed to ivermectin, the longer it takes for them to die; because they continue to cause damage during this time, long-term ivermectin therapy generally is not a substitute for melarsomine (IMMITICIDE) therapy (American Heartworm Society, 2005). In addition, a mild hypersensitivity reaction has been observed in dogs with circulating microfilariae that are treated with ivermectin. Many products that contain ivermectin have precautions suggesting removal of adult heartworms and microfilariae before initiation of ivermectin heartworm prophylaxis.

Regarding the combination of ivermectin and doxycycline as a heartworm adulticide, it has been found that *Wolbachia* spp. bacteria are filarial species endosymbionts, that is, their presence is necessary for filial worm survival, and that eliminating these bacteria from heartworm-positive dogs and cats will decrease the host antigenic response (Bazzocchi et al, 2000; McCall et al, 2008). In fact, one study of heartworm-positive dogs compared groups that were treated with three drugs (i.e., melarsomine, doxycycline, and ivermectin), two drugs (i.e., doxycycline and ivermectin), doxycycline alone, ivermectin alone, and melarsomine alone; the authors concluded that the combination of doxycycline and ivermectin was synergistic and could eliminate adult heartworms with less potential for severe thromboembolism than melarsomine alone (McCall et al, 2008). This is discussed in greater depth in the melarsomine section.

Ivermectin as a single subcutaneous injection at 0.2 mg/kg demonstrated high efficacy against the immature and adult roundworms, *Toxocara canis;* hookworms, *Ancylostoma caninum, Ancylostoma braziliense,* and *Uncinaria stenocephala;* and parasitic threadworms, *Strongyloides stercoralis*. Its activity against roundworms, *Toxascaris leonina,* and whipworms, *Trichuris vulpis,* is erratic (USP, 2006).

Ivermectin is safe in Collies and in all breeds, even dogs with mutant *MDR1,* at the approved dose of 0.006 mg (6 mcg) per kilogram. When ivermectin is given at a dose of 0.2 mg/kg (32× the label dose), dogs with mutant *MDR1* exhibit severe adverse reactions, such as mydriasis, ataxia, tremors, drooling, paresis, recumbency, excitability, stupor, and coma. A single oral dose of 2 mg/kg and repeated oral doses of 0.5 mg/kg/day for 14 weeks were well tolerated by dogs of other breeds. Mydriasis, depression, tremors, ataxia, coma, and death have been observed after doses in excess of 20 mg/kg in laboratory dogs (Pulliam et al, 1985). No teratism was observed in fetuses when pregnant bitches received repeated oral doses of ivermectin at 0.5 mg/kg. It appears to be safe for use in pregnant bitches (Wiebe and Howard, 2009).

Ivermectin has been used in the treatment of mange, *Demodex canis,* at 0.4 to 0.6 mg/kg orally daily for 2 to 4 months (Mueller, 2004), but these uses are not approved and should be applied with caution. Current advice is to check for *ABCB1-1Δ* mutation and to not use ivermectin at these doses unless the results are "normal/normal." For demodicosis that is not generalized, start at a trial dose of 0.05 mg (50 mcg) per kg PO daily for a week, and then increase to 0.12 mg/kg/day for a week, the latter of which is the dose *MDR1* mutants often react to. If no reactions occur, increase the dose to 0.2 mg/kg daily for 3 days, then increase the dose by 0.1 mg/kg weekly until the target dose of 0.6 mg/kg/day is reached. If reactions (e.g., mydriasis, ataxia, lethargy) occur at a dose >0.3 mg/kg, consider using an every-other-day schedule at the highest nonreactive dose. Treatment typically takes 3 to 7 months and should continue for 2 months after the last negative skin scrape (Waisglass, 2009).

For generalized demodicosis, the recommendation is to start at 0.1 mg (100 mcg) per kg PO daily and increase to 0.2 mg/kg on day 4 and to 0.3 mg/kg on day 7, and to continue to increase by 0.1 mg/kg every third day until the target dose of 0.6 mg/kg is reached, at which time treatment is continued for 1 to 2 months after two negative skin scrapes (usually 10 to 33 weeks) (Hillier, 2006). Occasionally normal (regarding *MDR1* mutation) dogs on such high doses of systemic macrolides develop a potentially fatal neurotoxicity, the cause of which has not been fully explained (Bissonnette et al, 2009). In these cases, signs typically develop later in the course of treatment and may respond to ivermectin dose reduction.

Other extralabel uses for ivermectin include treatment for a couple of zoonotic diseases, first cheyletiellosis, at a dose of 0.3 mg/kg SC or PO, administered twice, 2 weeks apart (USP, 2006), or 0.3 mg/kg SC twice, 2 to 3 weeks apart (Foil, 2003). Ivermectin has been used to treat sarcoptic mange, which is also zoonotic, at 0.3 to 0.4 mg/kg SC or PO weekly for 4 weeks (Foil, 2003). And ivermectin has been used to treat *Capillaria* spp. and *Eucoleus boehmi* at 0.2 mg/kg once PO, *Pneumonyssoides caninum* at 0.2 mg/kg once SC, and *Oslerus osleri* at 0.4 mg/kg once SC (Plumb, 2011b).

It is important to use care if ivermectin is given condurrently with other drugs. Concurrent use with drugs that interfere with

P-glycoprotein should be avoided. One expert recommends never treating with ketoconazole and ivermectin concurrently (Waisglass, 2009), and Plumb recommends using caution when dispensing ivermectin for dogs on amiodarone, carvedilol, clarithromycin, cyclosporine, diltiazem, erythromycin, itraconazole, ketoconazole, quinidine, spironolactone, tamoxifen, or verapamil (Plumb, 2011b). See the "*MDR1* Mutants" section of this chapter for more on drugs to use cautiously in ivermectin-treated dogs.

Several combination products containing ivermectin are available. For more information, see the section on combination products.

Cats

Ivermectin is approved for cats as an ear mite treatment (ACAREXX OTIC SUSPENSION) and as a heartworm preventive (HEARTGARD CHEWABLES FOR CATS). Monthly doses of 0.024 mg (24 mcg) per kilogram are effective in preventing the development of heartworms, *Dirofilaria immitis* (McTier et al, 1992; Paul et al, 1992). It is also approved for use against hookworms, *Ancylostoma braziliense* and *A. tubaeforme* (Nolan et al, 1992; Roberson et al, 1992). A higher than label dose of 0.3 mg/kg is required to eliminate roundworms, *Toxocara cati* (Blagburn et al, 1987; Kirkpatrick and Megella, 1987). Ivermectin is apparently safe to use in pregnant queens (Wiebe and Howard, 2009).

Ivermectin is also available as a 1% otic suspension (ACAREXX OTIC SUSPENSION). It is approved for the treatment of ear mites, *Otodectes cynotis,* in cats and kittens 4 weeks of age and older. The package insert states efficacy against eggs and immature stages; such efficacy has been confirmed (Bowman et al, 2001).

Ivermectin injection has been used extralabel in cats to treat the lungworm, *Aelurostrongylus abstrusus,* at a one-time dose of 0.4 mg/kg SC (Reinemeyer, 2000).

Milbemycin Oxime

Milbemycin oxime was the second macrocyclic lactone to achieve approval by the FDA. It is a fermentation product of *Streptomyces hygroscopicus* subsp. *aureolacrimosis*. The drug has structural similarities to ivermectin and works by the same mechanism of action.

Dogs

Milbemycin oxime tablets (INTERCEPTOR FLAVOR TABS FOR DOGS & CATS) are formulated to deliver 0.5 mg/kg to dogs. When given every 30 days, they are effective in preventing heartworms *(Dirofilaria immitis)* (Bater, 1989; Grieve et al, 1991). The American Heartworm Society guidelines for diagnosis, prevention, and management of heartworm infection in dogs should be consulted (American Heartworm Society, 2012). Milbemycin oxime, like ivermectin, is known to kill heartworm microfilariae and inhibit the release of new microfilariae, so all dogs on routine monthly heartworm prophylaxis should be tested with adult antigen tests (American Heartworm Society, 2012; Bowman et al, 1992; Courtney et al, 1998; Lok and Knight, 1995). The product also kills hookworms *(Ancylostoma caninum)* and removes and controls roundworms (*Toxocara canis* and *Toxscaris leonina*) and whipworms *(Trichuris vulpis)* (Blagburn et al, 1992; Bowman et al, 1988; Bowman et al, 1990; Bowman et al, 1991; USP, 2006).

Milbemycin oxime has been extensively tested with regard to safety. It is nontoxic to rough-coated Collies at up to 20× the recommended dose (Blagburn et al, 1989) and can safely be given to pregnant and nursing animals. Although an LD_{50} was never determined in dogs, the drug was well tolerated when given to Beagles at 200 mg/kg in a single oral dose. Nursing puppies given 19× the label dose had tremors, vocalizations, and ataxia (Novartis Animal Health, 2010). When given 2.5 mg/kg PO, 8-week-old pups had no signs the first day, but had trembling and ataxia on the second and third days (Plumb, 2011b).

Milbemycin oxime is effective extralabel in treating dogs with amitraz-resistant mange mites *(Demodex canis)* when given at a dosage of 1 to 2 mg/kg daily for 60 to 90 days (Mueller, 2004; Mueller et al, 2012). It is best get a microfilaria test before treatment, to start at a low dose, and work toward a higher dose as needed. Merchant starts at a dose of 1 mg/kg/day with a skin scraping after 30 days, and increases the dosage to 2 mg/kg if minimal or no improvement is noted at that time. After another 30 days, the scrape is repeated, and if not improving, the dose is increased to 3 mg/kg/day, or alternative therapy is discussed (Merchant, 2009). This may be safer than ivermectin in sensitive dogs, but most clients will not be able to afford it if sold at the monthly preventive price (Plumb, 2011b). Conflicting reports have described an increased incidence of neurologic toxicity in *MDR1(–/–)* dogs compared with normal dogs with the wild-type allele (Barbet et al, 2009; Bissonnette et al, 2009). Barbet and Snook found two *MDR1(–/–)* dogs that had neurologic side effects when treated at 1.5 mg/kg/day, but tolerated the drug when the dose was reduced to 0.6 mg/kg/day (Barbet et al, 2009; Geyer and Janko, 2012).

Other extralabel uses include its use in dogs with sarcoptic mange *(Sarcoptes scabiei).* Milbemycin oxime is safe and highly effective against mange mites when given orally at 2 mg/kg twice weekly for 3 to 4 weeks (Mueller, 2007). Milbemycin oxime is also effective extralabel against the nasal mite *(Pneumonyssoides caninum)* when given at 1 mg/kg PO once every 10 days for 3 treatments (Plumb, 2011b).

In humans concurrent milbemycin oxime and benzodiazepines are contraindicated because the effect of the benzodiazepine may be potentiated. Caution is advised when milbemycin oxime is used concurrently with any drug that inhibits P-glycoprotein, especially if the patient is a dog with the *MDR1(–/–)* allele. P-glycoprotein inhibitors include amiodarone, azole antifungals (e.g., ketoconazole), carvedilol, cyclosporine, diltiazem, erythromycin, clarithromycin, quinidine, spironolactone, tamoxifen, and verapamil (Plumb, 2011b). For more see the "*MDR1* Mutants" section of this chapter.

Cats

Milbemycin oxime formulations for cats include a tablet for parasite prevention and an otic solution to treat ear mites. Milbemycin oxime tablets (INTERCEPTOR FLAVOR TABS FOR DOGS & CATS) are formulated to deliver 2 mg/kg in cats and are indicated for prevention of heartworm disease *(Dirofilaria immitis)* and for removal of hookworms *(Ancylostoma tubaeforme)* and roundworms *(Toxocara cati)* (Novartis Animal Health, 2010).

An 0.1% solution of milbemycin oxime (MILBEMITE OTIC SOLUTION) is approved for the treatment of ear mite infestation *(Otodectes cynotis)* in cats and kittens 8 weeks of age or older. It is effective against all ear mite life stages (Novartis Animal Health, 2009).

See concurrent drug cautions in the preceding milbemycin oxime dog section.

Turtles

It is interesting to note that milbemycin oxime is apparently nontoxic in turtles and was somewhat effective in a small study conducted on red-eared sliders *(Chrysemys scripta elegans)* and Gulf Coast box turtles *(Terrapene carolina major)* (Bodri et al, 1993). It is, of course, not approved for this use.

Moxidectin

Moxidectin is a chemically altered product of *Streptomyces cyaneogriseus noncyanogenus*. It has a similar range of activity and safety margin as ivermectin, with FDA-approved products for horses, sheep, cattle, dogs, and cats.

Horses

Moxidectin is available in a 20-mg/mL formulation (QUEST 2% EQUINE ORAL GEL) designed to deliver 0.4 mg/kg to horses and ponies for the treatment and control of large strongyles (*Strongylus vulgaris:* adult and L4/L5 arterial stages; *S. edentates:* adult and tissue stages; *Tridontophorus brevicauda:* adults; *T. serratus:* adults); small strongyle adults (*Cyathostomum* spp., *Cylicostephanus* spp., *Cylicocyclus* spp., *Coronocyclus* spp., *Gyalocephalus capitatus, Petrovinema poculatus*); small strongyle undifferentiated lumenal larvae; ascarids (*Parascaris equorum:* adults and L4 larval stages); pinworms (*Oxyuris equi:* adults and L4 larval stages); hairworms (*Trichostrongylus axei:* adults); stomach worms *(Habronema muscae);* and botfly larvae (*Gasterophilus intestinalis* and *G. nasalis*) (Bello and Laningham, 1994; Fort Dodge Animal Health, 2006; Slocombe and Lake, 1997). The moxidectin product is particularly effective against encysted small strongyles and is labeled to suppress strongyle egg production through 84 days. Because it is fat soluble and very effective against a broad range of parasites, it should not be the first choice product for heavily parasitized thin horses. Signs of overdose noted in horses given ≈1 to 5 mg/kg (2.5× to 12.5× over label dose) moxidectin include weakness, depression, dyspnea, ataxia, tremors, seizures, and coma (Khan et al, 2002). Although moxidectin is labeled as safe for use in mares during breeding, gestation, and lactation, and for foals older than 6 months, dosing should be done carefully, especially in foals.

Cattle

Two moxidectin products are available for cattle: injectable and pour-on. The FDA approved an injectable solution with 1% (10 mg/mL) moxidectin (CYDECTIN INJECTABLE SOLUTION) for use in beef and nonlactating dairy cattle. It is injected SC at 0.2 mg/kg for treatment and control of gastrointestinal roundworms (*Ostertagia ostertagi:* adults and L4 including inhibited larvae; *Haemonchus placei:* adults; *Trichostrongylus axei:* adults and L4; *T. colubriformis:* adults and L4; *Cooperia oncophora:* adults; *C. pectinata:* adults; *C. punctate:* adults and L4; *C. spatulata:* adults; *C. surnabada:* adults and L4; *Nematodirus helvetianus:* adults; *Oesophagostomum radiatum:* adults and L4; and *Trichuris* spp.: adults); lungworms (*Dictyocaulus viviparous:* adults and L4); cattle grubs (*Hypoderma bovis* and *H. lineatum*); mange mites *(Psoroptes ovis);* and sucking lice (*Linognathus vituli* and *Solenopotes capillatus*) (Boehringer Ingelheim Vetmedica, 2005; Eysker and Boersema, 1992; Ranjan et al, 1992; Scholl et al, 1992; Williams et al, 1992). Moxidectin injectable has persistent activity to prevent reinfection from *Dictyocaulus viviparus* and *Oesophagostomum radiatum* for 42 days after treatment, *Haemonchus placei* for 35 days after treatment, and *Ostertagia ostertagi* and *Trichostrongylus axei* for 14 days after treatment. Do not treat cattle younger than 8 weeks of age. Do not treat veal calves or lactating dairy cattle, and do not treat within 21 days of slaughter.

A 0.5% pour-on formulation of moxidectin (CYDECTIN) has 5 mg moxidectin per mL and is approved at 0.5 mg/kg to control all parasites previously mentioned for the injection formulation, along with these additional species or life stages: gastrointestinal roundworms (*Bunostomum phlebotomum:* adult; *Nematodirus helvetianus:* L4); mange mites *(Chorioptes bovis);* sucking lice *(Haematopinus eurysternus);* biting lice *(Bovicola [Damalinia] bovis);* and horn flies *(Haematobia irritans)* (Boehringer Ingelheim Vetmedica, 2005; Morin et al, 1996; Vercruysse et al, 1997). Moxidectin pour-on is approved for use in beef and dairy cattle; neither a preslaughter withdrawal period nor a milk discard time is required. Meat and milk may be used at any time after treatment. Do not use in veal calves or preruminating calves.

Both injectable and pour-on products should be used with caution in treating grubs (*Hypoderma* spp. larvae), because if treatment occurs when many larvae are present in the esophagus, bloat may result, or when present in the vertebral canal, staggering or paralysis may occur. These reactions can occur with any drug that kills cattle grubs. They are not specific to moxidectin, but the result can be deadly for affected cattle. It is best to treat as soon as possible after the end of the heel fly season to avoid such reactions.

Sheep

Moxidectin is approved as an oral drench (CYDECTIN ORAL DRENCH FOR SHEEP) formulated as a 0.1% moxidectin (1 mg/mL) solution for use in sheep. When given PO at a dose of 0.2 mg/kg, it is effective in the treatment and removal of adult and L4 stages of *Cooperia curticei, C. oncophora, Haemonchus contortus, Nematodirus battus, N. filicollis, N. spathiger, Oesophagostomum columbianum, O. venulosum, O. trifurcata, Teladorsagia (Ostertagia) circumcincta, Trichostrongylus axei, T. colubriformis,* and *T. vitrinus* (Boehringer Ingelheim Vetmedica, 2010; Craig et al, 1992).

Regarding extralabel use, moxidectin treatment by injection or PO at 0.2 mg/kg has been useful in treating sheep with lungworm infection (Papadopoulos et al, 2004).

Sheep treated with moxidectin oral solution should not be slaughtered within 7 days of treatment. Moxidectin should not be used in sheep that are producing milk for human consumption because a withholding time has not been established. Once saved as a drug of last resort, it has been used increasingly because of resistance to other products, and now parasites are increasingly becoming resistant to moxidectin, too (Roussel, 2012).

Goats

Sheep and goats share many of the same parasites. Although the oral drench approved for use in sheep is not approved or labeled for goats, it has been used in goats at twice the sheep dose (Snyder, 2009).

Dogs

Moxidectin is known to be very active against heartworms and gastrointestinal nematodes. PROHEART 6 is a sustained-release formulation distributed in two vials containing 10% moxidectin microspheres and sterile vehicle for constitution. The constituted product has 3.4 mg/mL of moxidectin and is given by SC injection at 0.05 mL/kg to provide a dose of 0.17 mg moxidectin/kg body weight. Product mixing or constitution should be performed at least 30 minutes before injection. This product is approved for prevention of heartworms *(Dirofilaria immitis)* and for treatment of existing larvae and adult hookworms (*Ancylostoma caninum* and *Uncinaria stenocephala*). The injection should be given within 1 month of the dog's first mosquito exposure and is repeated every 6 months to provide continuous prevention against and control of heartworms (Fort Dodge Animal Health, 2011).

Moxidectin is the only drug with 100% efficacy in preventing *Ancylostoma caninum* in dams and pups when given at 1 mg/kg SC on pregnancy day 55 (Wiebe and Howard, 2009). In addition, two SC injections of moxidectin given at 1 mg/kg on days 40 and 55 of pregnancy (5 to 13 days before parturition) completely prevented

prenatal and lactogenic *Toxocara canis* infection in puppies (Kramer et al, 2006).

In 2001, PROHEART 6 was approved in the United States for prevention of heartworm disease and for treatment of existing larvae and adult hookworm infection (Blagburn et al, 2001; Lok et al, 2001a; Lok et al, 2001b; McCall et al, 2001). The FDA had concerns about safety as a result of adverse event reports that it received. Extensive studies conducted by the manufacturer showed that a mixture of residual solvents in the moxidectin technical material might be allergenic. In 2002 manufacturing changes were made to address this issue. The manufacturer voluntarily recalled the product from the U.S. market in 2004 to address ongoing FDA safety concerns (Glickman et al, 2005). During this time, the product remained on the market in Australia, Japan, and parts of Europe. In 2005 the manufacturer changed the supplier of one of the vehicle excipients. Product marketed with low levels of residual solvent outside the United States demonstrated improved safety. In the following years, the adverse event rate declined in international markets and the manufacturer continued post-marketing safety studies, which led to restricted return of PROHEART 6 to the U.S. market.

In 2008 the product was reintroduced to the U.S. market with a new label under a postmarketing surveillance initiative based on human drug programs and known as a Risk Minimization Action Plan (RiskMAP), which included several provisions such as veterinarian training and use of a pet owner consent form (Fort Dodge Animal Health, 2008). RiskMAP is to be reviewed periodically and updated as needed. This was the first veterinary drug to be marketed under RiskMAP, a strengthened risk minimization and restricted distribution program. The initial RiskMAP required practicing veterinarians to complete web-based training before using the product. Key components of the training were to make veterinarians aware of which patients are suitable candidates for treatment, to require pretreatment bloodwork, to maintain complete records, and to make a commitment to report adverse reactions promptly. The RiskMAP also required that pet owners sign a consent form before injection of the product. The RiskMAP program is similar to other programs applied in human medicine for important life-saving drugs. The new label advised not to administer the drug to sick, debilitated, or underweight dogs or those with a history of weight loss, and stated that the product should be used with caution in dogs with preexisting allergic disease, including food allergy, atopy, and flea allergy dermatitis. The label in 2008 also warned not to administer moxidectin injection within 1 month of vaccination (Fort Dodge Animal Health, 2008). In 2010 additional changes were made to the label and to the RiskMAP.

The 2010 RiskMAP has been in effect for over two years and still requires veterinarians to complete web-based training and maintain signed owner consent records for each treated dog. Concurrent vaccination can be performed now with caution. If dogs started on PROHEART before 7 years of age remain healthy, their treatment can continue as they grow older. Pretreatment blood work may be indicated but is no longer mandatory (Pfizer Animal Health, 2011). Other provisions apply. Because the RiskMAP is reviewed and updated periodically, the practicing veterinarian is advised to keep abreast of label and RiskMAP changes.

The sustained-release injection provides a 6-month window of protection from heartworms (Fort Dodge Animal Health, 2011). However, it does not clear microfilariae or remove adult heartworms (USP, 2006). With each injection, larval and adult hookworms are killed, but reinfection may occur before the next injection is given, making its use as a sole treatment less than ideal when control of recurrent hookworm disease is the goal.

Moxidectin injection of confirmed ivermectin-sensitive Collies revealed a wider margin of safety than either ivermectin or milbemycin at a dose 30× the label and resulted in no adverse reaction (Paul et al, 2000). Although no specific drug interactions for moxidectin have been reported, concurrent use of moxidectin with other drugs should be carefully considered, especially when ivermectin-sensitive dogs such as those carrying *MDR1(–/–)* are treated. Concurrent use with drugs that interfere with P-glycoprotein should be avoided. Plumb recommends using caution when dispensing the following drugs to dogs on moxidectin: amiodarone, carvedilol, clarithromycin, cyclosporine, diltiazem, erythromycin, itraconazole, ketoconazole, quinidine, spironolactone, tamoxifen, and verapamil (Plumb, 2011b). For more see the "*MDR1* Mutants" section of this chapter.

Adverse event reports recorded from 2008 to 2010 revealed an adverse reaction rate of 3.3 cases reported per 10,000 doses, most of which were allergic reactions and/or GI upset and responded to symptomatic therapy. Dogs with weight loss >10% were more likely to have a severe adverse reaction. Allergic reactions typically included clinical signs of facial edema (with or without urticaria), erythema, and pruritus, with signs occurring within 2 hours of injection. Occasionally vomiting and/or diarrhea, lethargy, and, rarely, fever were noted in conjunction with the allergic reaction. Vomiting or diarrhea occurring immediately after administration was often treated with diphenhydramine or dexamethasone and responded immediately to treatment. These cases were most likely allergic in nature. Less frequently, allergic cases presented with clinical signs of anaphylaxis typical of those seen associated with vaccine allergic adverse reactions (Pfizer Animal Health, 2011).

GI upset reactions typically included clinical signs of hypersalivation, anorexia, vomiting, diarrhea, hemorrhagic diarrhea, and lethargy with onset of signs within 1 day of injection. Most cases that were considered a reaction to the drug resolved in 24 hours; a few cases took as long as 4 days to recover. Treatment included supportive care, maropitant, diphenhydramine, metronidazole, sucralfate, antibiotics, dexamethasone, and metoclopramide (Pfizer Animal Health, 2011).

Selamectin

Selamectin is a novel endectocide that is prepared by semisynthetic modification of doramectin (Bishop et al, 2000).

Dogs and Cats

Selamectin topical solution (REVOLUTION) is formulated for topical application in dogs and cats. It is approved for use in dogs that are at least 6 weeks old and cats that are at least 8 weeks old. The stated dose is a minimum of 6 mg/kg every 30 days. Selamectin appears to be safe to use during pregnancy in dogs and cats (Wiebe and Howard, 2009). Selamectin topical solution is approved in dogs and cats for prevention of heartworm disease caused by *Dirofilaria immitis* and for control of fleas *(Ctenocephalides felis)* and ear mites *(Otodectes cynotis)* (Boy et al, 2000; McTier et al, 2000a; McTier et al, 2000b; McTier et al, 2000c; Shanks et al, 2000b; Shanks et al, 2000c; Six et al, 2000). It is not effective in clearing heartworm microfilariae. In dogs it is approved for the treatment and control of sarcoptic mange *(Sarcoptes scabiei)* and the American dog tick *(Dermacentor variabilis)* (Jernigan et al, 2000; Shanks et al, 2000a). In cats it is also indicated for the treatment and control of hookworms *(Ancylostoma tubaeforme)* and roundworms *(Toxocara cati)* (McTier et al, 2000d; Six et al, 2000).

In Europe selamectin is labeled in dogs to treat roundworms *(Toxocara canis)* and biting lice *(Trichodectes canis),* and in cats to treat biting lice *(Felicola subrostratus).* Selamectin has been used extralabel to control feline lungworms *(Aelurostrongylus abstrusus),*

but treatment was effective in only one of three cats. Other extra-label uses include treatment of dogs with nasal mites *(Pneumonyssoides caninum);* dogs, cats, and rabbits with *Cheyletiella* spp.; and cats with harvest mites *(Neotrombicula autumnalis)* (Fisher and Shanks, 2008). Selamectin has not been effective in treating demodectic mange.

The product was shown to be safe in *MDR1* mutant dogs when given at 40 mg/kg topically (≈7× to 13× the label dose) (Geyer and Janko, 2012). At oral doses of >15 mg/kg *MDR1* mutant dogs have only mild signs of neurological toxicity (Geyer and Janko, 2012). Although no specific drug interactions for selamectin have been reported, concurrent use with other drugs should be carefully considered, especially in treating *MDR1(–/–)* dogs, in which case concurrent use with drugs that interfere with P-glycoprotein should be avoided. Plumb recommends not dispensing the following drugs to *MDR1(–/–)* dogs on selamectin: amiodarone, carvedilol, clarithromycin, cyclosporine, diltiazem, erythromycin, itraconazole, ketoconazole, quinidine, spironolactone, tamoxifen, and verapamil (Plumb, 2011b). For more see the "*MDR1* Mutants" section of this chapter.

BENZIMIDAZOLES

The benzimidazoles represent a large family of broad-spectrum agents that have been used widely for many years in a broad array of animal species. Excellent review articles (Campbell, 1990; Lacey, 1990; Loukas and Hotez, 2006; McKellar and Scott, 1990) discuss the history, mode of action, and spectrum of activity of this useful class of anthelmintics.

Thiabendazole was the first benzimidazole discovered, and it represented a major step forward when it became available more than 30 years ago. At the time of its introduction, thiabendazole was a novel, true broad-spectrum product that was very safe for the host animal. Since that time, parasite resistance to the benzimidazoles has been discovered in several species.

Considerable effort has been devoted to determining the mechanism by which the benzimidazoles act on parasites. Conventional wisdom holds that benzimidazoles bind to tubulin molecules; this inhibits the formation of microtubules and disrupts cell division (Frayha et al, 1997; Lanusse et al, 2009a; Reinemeyer and Courtney, 2001a). Benzimidazoles have a much higher affinity for nematode tubulin versus mammalian tubulin, thus providing selective activity against parasites. Evidence also indicates that the benzimidazoles can inhibit fumarate reductase, which blocks mitochondrial function, depriving the parasite of energy and thus resulting in death.

The benzimidazoles are poorly soluble in water and therefore are generally given by mouth. They tend to be more effective in horses and ruminants because of rapid metabolism into active metabolites by gastrointestinal microbes (Lancusse et al, 2009a). The dose is usually more effective when divided, thus prolonging contact time with the parasite. Two members of the benzimidazole group (albendazole and oxfendazole) have been found to be teratogenic, which limits their usefulness in pregnant animals.

For simplicity, the probenzimidazole drug febantel is included in this section. It is a nonbenzimidazole drug that is metabolized to a benzimidazole. It therefore shares a similar efficacy and mechanism of action with the other benzimidazoles.

Albendazole

Albendazole has potent broad-spectrum anthelmintic activity. It offers a wide margin of safety in cattle when used according to label specifications.

Albendazole has demonstrated a broad spectrum of anthelmintic activity against gastrointestinal nematodes; lung nematodes, including inhibited larval forms; cestodes; and lung and liver trematodes in farm animals, companion animals, and humans. Albendazole (Zentel) is used overseas for the treatment of intestinal helminth infections, hydatid disease, and cysticercosis of humans. Albendazole 11.36% suspension (113.6 mg/mL) is available for treatment of cattle, sheep, and nonlactating goats (VALBAZEN SUSPENSION PI). Cattle and goats are dosed at 10 mg/kg and sheep at 7.5 mg/kg. In cattle and sheep, it is useful for removal and control of liver flukes, tapeworms, stomach worms (including fourth-stage inhibited larvae of *Ostertagia ostertagi*), intestinal worms, and lungworms. In nonlactating goats, it is labeled for the treatment of adult liver flukes.

Albendazole has been associated with teratogenic and embryotoxic effects in rats, rabbits, and sheep when given early in pregnancy. It was identified as an oncogen in 1984, but subsequent studies failed to demonstrate any carcinogenic activity. Albendazole may cause GI and hepatic dysfunction and rarely aplastic anemia. Do not use in pigeons, doves, or alpaca crias (Plumb, 2011b).

Cattle

In cattle albendazole is administered orally at a dose level of 10 mg/kg for removal and control of adult and larval stages of internal parasites including barber pole worms (*Haemonchus contortus* and *H. placei*), brown stomach worms, including fourth-stage inhibited larvae *(Ostertagia ostertagi),* small stomach worms *(Trichostrongylus axei),* bankrupt worms *(Trichostrongylus colubriformis),* thread-necked intestinal worms (*Nematodirus spathiger* and *N. helvetianus*), small intestinal worms (*Cooperia punctata* and *C. oncophora*), hookworms *(Bunostomum phlebotomum),* nodular worms *(Oesophagostomum radiatum),* lungworms *(Dictyocaulus viviparus),* tapeworms (*Moniezia benedeni* and *M. expansa*), and adult liver flukes *(Fasciola hepatica)* (Bogan and Armour, 1987; Pfizer Animal Health; Prichard, 1986; Prichard, 1987).

The safety of albendazole in single and repeated treatments was evaluated in healthy and parasitized cattle. A single dose of 75 mg/kg of body weight was well tolerated. Albendazole was embryotoxic when administered to cows at a dosage rate of 25 mg/kg during the first 7 to 17 days of gestation. The conception rate of cows treated after the twenty-first day of gestation was comparable with that in controls, and all cows gave birth to normal calves. Doses of 300 mg/kg (30× label) have caused death in cattle, but doses of 45 mg/kg (4.5× label) caused no adverse effects (Plumb, 2011b).

In the United States, cattle must not be slaughtered within 27 days after treatment. Also, albendazole should not be used in female dairy cattle of breeding age, and the label cautions that the drug should not be given to pregnant cows during the first 45 days of gestation (Pfizer Animal Health).

Goats and Sheep

In goats albendazole 11.36% suspension is administered at 10 mg/kg, same dose as in cattle, for the removal and control of adult liver flukes *(Fasciola hepatica).* In sheep it is administered as an oral drench at a lower dose level of 7.5 mg/kg for removal and control of adult liver flukes (*Fasciola hepatica* and *Fascioloides magna*), common tapeworms *(Moniezia expansa),* fringed tapeworms *(Thysanosoma actinoides),* brown stomach worms (*Ostertagia circumcincta* and *Marshallagia marshalli*), barber pole worms *(Haemonchus contortus),* small stomach worms *(Trichostrongylus axei),* thread-necked intestinal worms (*Nematodirus spathiger* and *N. filicollis*), Cooper's worms *(Cooperia oncophora),* bankrupt worms *(Trichostrongylus colubriformis),* nodular worms *(Oesophagostomum columbianum),* large-mouth bowel worms *(Chabertia ovina),* and lungworms *(Dictyocaulus filaria)* (Pfizer Animal Health).

Albendazole has been used in sheep at 20 mg/kg for the treatment of the small liver fluke *(Dicrocoelium dendriticum)* (Manga-Gonzalez et al, 2010). Doses of 200 mg/kg (20× label) have caused death in sheep (Plumb, 2011b). Albendazole at 5 mg/kg PO was ineffective in treating goats with lungworm *(Muellerius capillaris)* infection (Lopez et al, 2010).

Do not give to lactating does. Do not give to ewes during the first 30 days of pregnancy or for 30 days after removal of rams. Sheep and goats should not be treated within 7 days of slaughter.

Dogs and Cats

Albendazole is not approved for use in dogs and cats. Dogs treated with 50 mg/kg twice daily for 10 to 14 days may have anorexia (Brown and Barsanti, 1989). Cats treated with 100 mg/kg daily for 14 to 21 days showed weight loss, neutropenia, and mental dullness (Plumb, 2011b). Dogs can be treated for lungworm *(Filaroides hirthi)* infection at a dosage of 25 to 50 mg/kg twice daily for 5 days and repeated in 2 to 3 weeks (Plumb, 2011b). The dog can be treated for bladder worm *(Capillaria plica)* infection at a dosage of 50 mg/kg twice daily for 10 to 14 days, but, as stated previously, anorexia may occur (Brown and Barsanti, 1989). Dogs can also be treated for the lung fluke *(Paragonimus kellicotti)* at a dosage of 25 mg/kg twice daily for 14 days (Reinemeyer, 1995). The same dosage is effective for *Paragonimus* organisms in cats (Plumb, 2011b). Although albendazole is effective against these uncommon parasites, ivermectin and praziquantel are more convenient therapies and are likely to be just as effective. More interesting is the use of albendazole against *Giardia* organisms in dogs at 25 mg/kg twice daily for 2 days (Barr et al, 1993) as was discussed in detail in the antiprotozoal section. Recent evidence suggests that this product may cause aplastic anemia in dogs and cats, so it should be used with caution (Plumb, 2011b).

Febantel

Febantel is a prodrug that is metabolized to fenbendazole and oxfendazole, which are undoubtedly the active parasiticide molecules (McKellar and Scott, 1990). The oral acute toxic dose in mice, rats, and dogs is >10,000 mg/kg. At oral doses greater than 150 mg/kg daily for 6 days, transient salivation, diarrhea, vomiting, and anorexia may be seen in dogs and cats.

Febantel is not available in a single-entity formulation, but combination products of febantel with praziquantel and pyrantel are discussed in the section on combination products.

Fenbendazole

Fenbendazole is a commercially successful benzimidazole that is widely used in domestic animals. The oral LD_{50} for rats and mice is >10,000 mg/kg. Fenbendazole does not have embryotoxic or teratogenic effects in rats, sheep, and cattle. In the rabbit, fenbendazole was fetotoxic but not teratogenic. It is generally considered safe to use in pregnancy in all other species, making it the drug of choice for treating *Giardia* spp. in pregnant animals, which was discussed in greater detail previously in the antiprotozoal section. No carcinogenesis was observed in lifetime studies in rats and mice. Fenbendazole is tolerated at as high as 100× the recommended dose (Plumb, 2011b).

Absorbed fenbendazole is metabolized to at least two active metabolites: oxfendazole sulfoxide and oxfendazole sulfone. In ruminants it is known to undergo enterohepatic cycling, which serves to prolong effective blood levels (USP, 1998). After oral administration, fenbendazole is not completely absorbed. Gut absorption is more a function of drug solubility than of dose given. Area under the curve was similar for doses ranging from 25 to 100 mg/kg in dogs. Bioavailability increased when fenbendazole was administered with food in dogs, but the fat content of the food does not alter bioavailability significantly (McKellar et al, 1993). In sheep, cattle, and swine, almost half of absorbed fenbendazole is excreted unchanged in the feces, <1% in the urine (Plumb, 2011b).

Fenbendazole is a broad-spectrum anthelmintic with activity against gastrointestinal nematodes, lung nematodes, and cestodes in cattle, sheep, goats, and horses. Activity against a variety of helminth parasites in dogs, cats, and many zoo animals also has been reported. In the United States, fenbendazole is available from only one manufacturer, but is approved in a wide variety of formulations for control of helminth parasites in horses, cattle (beef and dairy), dogs, goats, and zoo animals. All are marketed under the PANACUR or SAFE-GUARD brand name and are manufactured by Merck Animal Health. Although it is not approved for use in domestic cats in the United States, many other countries have a cat-labeled fenbendazole product. Fenbendazole has been used extra label in the United States to treat *Giardia* spp. in dogs and cats (Plumb, 2011b). In Europe fenbendazole paste is labeled to treat giardiasis in dogs. It is effective at clearing *Giardia* and is safer than metronidazole.

Cattle

Fenbendazole is available in a wide range of formulated products including suspension, premix and top dress pellets, granules, paste, block, and a free-choice mineral supplement. These products are designed to be administered orally or fed to dairy and beef cattle at 5 mg/kg for the removal and control of lungworms *(Dictyocaulus viviparus);* stomach worms (*Haemonchus contortus, Ostertagia ostertagi,* and *Trichostrongylus axei*); and intestinal worms (*Bunostomum phlebotomum, Nematodirus helvetianus, Cooperia punctata, C. oncophora, Trichostrongylus colubriformis,* and *Oesophagostomum radiatum*) (Yazwinski et al, 1985; Yazwinski et al, 1989).

In beef cattle only, the dose can be increased to 10 mg/kg for the removal of tapeworms *(Monezia benedeni)* and the inhibited fourth-stage larvae of brown stomach worms *(Ostertagia ostertagi);* this dose is not approved for use in dairy cattle. In cattle, fenbendazole is not embryotoxic or teratogenic and does not impair the fertility of bulls (Muser and Paul, 1984). Extralabel, fenbendazole has been shown to be effective against *Giardia* organisms, as was discussed in the antiprotozoal section.

Cattle must not be slaughtered within 8 days of medication with fenbendazole; and dairy cattle of breeding age should not be treated with the 10-mg/kg dose. No milk discard is required for dairy cattle treated with the 5-mg/kg dose. Do not use in veal calves.

Horses

Fenbendazole suspension, granules, or paste (PANACUR) is administered orally to horses at 5 mg/kg for the control of large strongyles (*Strongylus edentatus, S. equinus, S. vulgaris,* and *Triodontophorus* spp.), small strongyles (*Cyathostomum* spp., *Cylicocyclus* spp., *Cylicostephanus* spp., and *Cylicodontophorus* spp.), and pinworms *(Oxyuris equi).* For removal of ascarids *(Parascaris equorum),* a dose of 10 mg/kg is recommended. For control of fourth-stage larvae of *Strongylus vulgaris,* the extralabel dosage is 10 mg/kg daily for 5 days (Leneau et al, 1985; Plumb, 2011b). Pregnant mares, stallions, and foals may be treated safely with fenbendazole at recommended dosages. Fenbendazole has been evaluated for safety in pregnant mares during all stages of gestation and in stallions with doses up to 25 mg/kg; no adverse reproductive effects were found. The biggest problem with fenbendazole is cyathostome resistance (Tarigo-Martinie et al, 2001). Fenbendazole can still be used effectively in horses if supported, at a particular locale, by a fecal egg count reduction test and a favorable egg reappearance period.

Swine

Fenbendazole is approved for a total dose of 9 mg/kg, which is divided and then fed over a 3- to 12-day period in pigs. This dosage is labeled to remove and control lungworms (*Metastrongylus apri* and *M. pudendotectus*); gastrointestinal worms (*Ascaris suum, Oesophagostomum dentatum, O. quadrispinulatum, Hyostrongylus rubidus,* and *Trichuris suis*); and kidney worms *(Stephanurus dentatus)* (Biehl, 1986). No withdrawal time restriction is applied when pigs are treated at the approved dose.

Extralabel use of fenbendazole in potbellied pigs for a 3-day regimen (9 mg/kg PO) has been advised for whipworm treatment (Braun, 1995).

Dogs

Fenbendazole granules (PANACUR) at a dose of 50 mg/kg are mixed in the feed and given to dogs for 3 consecutive days for the removal of roundworms (*Toxocara canis* and *T. leonina*), hookworms (*Ancylostoma caninum* and *Uncinaria stenocephala*), whipworms *(Trichuris vulpis),* and tapeworms *(Taenia pisiformis)* (Reinemeyer, 2000). Fenbendazole is approved for use in dogs that are at least 6 weeks of age and in pregnant bitches. Fenbendazole is the only parasiticide proven to prevent vertical transmission of parasites in dogs (Wiebe and Howard, 2009).

Extralabel indications include prolonged therapy at 50 mg/kg for several weeks, which has been demonstrated to provide excellent activity against the lung fluke, *Paragonimus kellicotti* (Dubey et al, 1979). Fenbendazole is also used extralabel to treat giardiasis, as was discussed in detail in the antiprotozoal section.

Cats

Fenbendazole is not currently approved in the United States for use in domestic cats. This is interesting since it is approved for use in many species of large zoo-animal felines. It is also approved for use in domestic cats in many other countries. When given orally at 50 mg/kg for 3 to 5 consecutive days, it is effective against ascarids, hookworms, strongyloides, and *Taenia* spp. tapeworms. A variety of cat dosing regimens ranging from 20 to 50 mg/kg once to twice daily for 3 to 14 days have been proposed for the treatment of *Aelurostrongylus abstrusus, Capillaria aerophilia, C. feliscati, Paragonimus kellicotti,* and *Eurytrema procyonis* infections (Bowman, 1992; Plumb, 2011b; Reinemeyer, 1995; Roberson and Burke, 1980). Fenbendazole is the only parasiticide proven to prevent vertical transmission of parasites in cats (Wiebe and Howard, 2009). In cats (as in dogs), fenbendazole is used extralabel to treat giardiasis, as was discussed in detail in the antiprotozoal section.

Goats

Fenbendazole is available as a 10% suspension (SAFE-GUARD DEWORMER FOR GOATS) approved as a single oral dose of 5 mg/kg to remove and control the barber pole worm *(Haemonchus contortus)* and the brown stomach worm *(Teladorsagia [Ostertagia] circumcincta).* Some *Haemonchus* populations apparently have developed resistance to fenbendazole. Although fenbendazole reportedly has been used to treat goats with lungworm *(Muellerius capillaris)* infection (McCraw and Menzies, 1986), results have been less than desired. Do not use in dairy goats producing milk for human consumption. Do not treat goats within 6 days of slaughter.

Sheep

Fenbendazole is not approved for use in sheep in the United States. Overseas, oral administration of fenbendazole at 5 mg/kg is recommended for removal of cestodes and gastrointestinal and lung nematodes. Some *Haemonchus* populations apparently have developed resistance to fenbendazole (Falzon et al, 2012). As noted in the next section, the FDA has approved the use of fenbendazole for the treatment of lungworms (*Protostrongylus* species) in Rocky Mountain bighorn sheep.

Zoo Animals

Fenbendazole granules (PANACUR GRANULES 22.2% and SAFE-GUARD DEWORMER 20% TYPE A MEDICATED FEED) are among the few commercial products actually approved by the FDA for use in zoo animals, with the PANACUR product labeled for carnivores and the SAFE-GUARD product labeled for pigs, turkeys, and hoofed stock. The PANACUR label describes use in lions *(Panthera leo),* tigers *(Panthera tigris),* cheetahs *(Acinonyx jubatus),* pumas *(Felis concolor),* jaguars *(Panthera onca),* leopards *(Panthera pardus),* panthers (*Panthera* spp.), Grizzly Bears *(Ursus horribilis),* Polar Bears *(Ursus maritimus),* and Black Bears *(Ursus americanus).* The label recommends 10 mg/kg/day PO for 3 consecutive days. It is used to remove ascarids, hookworms, and tapeworms from these species. The actual list of approved parasite indications is rather complex owing to the large number of host species involved and the common parasites found in each. In summary, the following parasites may be controlled in these zoo animals: roundworms (*Toxocara cati, Toxascaris leonina,* and *Baylisascaris transfuga*), hookworms (*Ancylostoma* spp., including *A. caninum*), and tapeworms (*Taenia hydatigena, T. krabbei,* and *T. taeniaeformis*).

Safety trials in zoo animals (*Felidae* and *Ursidae*) dosed at 100 mg/kg (10× label) showed mild signs of anorexia and loose stool, but no effect on reproduction was noted at this dose, even at 2× the label duration (Merck Animal Health, PANACUR GRANULES 22% product insert).

Fenbendazole (SAFE-GUARD) is also approved by the FDA as a Type A medicated article for use in manufacturing feed for large wildlife and game animals, including feral swine *(Sus scrofa)* to treat kidney worms, roundworms, and nodular worms; bighorn sheep *(Ovis canadensis canadensis)* to treat lungworms; and ruminants of the subfamilies Antilopinae, gazelle and impala; Hippotraginae, addax and oryx; and Caprinae, mouflon and saiga, to treat small stomach worms, thread-necked worms, barberpole worms, and whipworms. These animals are treated daily with 2.5 mg/kg (ruminants), 3 mg/kg (swine), or 10 mg/kg (bighorn sheep) for 3 consecutive days. The label requires that the drug not be given to game animals 14 days before or during hunting season (North American Compendiums, 2012).

Oxfendazole

Oxfendazole is a broad-spectrum benzimidazole approved in the United States for use in cattle. Oxfendazole is metabolized in ruminants to oxfendazole sulfone and fenbendazole, but the primary anthelmintic action is caused by the parent drug (Marriner and Bogan, 1981). Its oral LD_{50} is >1600 mg/kg for Beagle dogs and >6400 mg/kg for rats and mice.

Cattle

Oxfendazole is available in both 9.06% and 22.5% suspensions (SYNANTHIC BOVINE DEWORMER SUSPENSION), labeled to be used at 4.5 mg/kg by oral dosing syringe. The drug is approved for use in beef and nonlactating dairy cattle. It is effective for removal and control of lungworms, roundworms (including inhibited forms of *Ostertagia ostertagi*), and tapeworms *(Moniezia benedeni)* (Todd and Mansfield, 1979). Cattle must not be slaughtered within 7 days of treatment. Because no milk withdrawal time has been established, do not use oxfendazole in female dairy cattle of breeding age.

Other Species

Oxfendazole has been used extralabel in dogs at 10 mg/kg daily for 28 days to treat them for lungworm *(Oslerus osleri)* infection (Bowman, 2006) and in horses at 10 mg/kg, goats at 7.5 mg/kg, sheep at 5 mg/kg, and swine at 3 to 4.5 mg/kg to treat them for susceptible parasites (Plumb, 2011b).

Oxibendazole

Oxibendazole, a broad-spectrum benzimidazole, is apparently effective against small strongyles that are resistant to benzimidazole (Drudge et al, 1979). Its acute oral LD_{50} is >10,000 mg/kg in guinea pigs, hamsters, and rabbits, and >32,000 mg/kg in mice. A single dose of 600 mg/kg was well tolerated by cattle, sheep, and ponies; and no adverse reactions were observed in rats and dogs treated with up to 30 mg/kg daily for 3 months. No evidence of teratogenicity or embryotoxicity was observed in rats, mice, sheep, cattle, or horses.

Horses

Oxibendazole paste or suspension (ANTHELCIDE EQ) is administered orally to horses at 10 mg/kg for the removal and control of large strongyles *(Strongylus edentatus, S. equinus, S. vulgaris),* small strongyles (species of the genera *Cylicostephanus, Cylicocyclus, Cyathostomum, Triodontophorus, Cylicodontophorus,* and *Gyalocephalus*), large roundworms *(Parascaris equorum),* and pinworms (*Oxyuris equi,* including various larval stages) (Drudge et al, 1981a; Drudge et al, 1981b; Drudge et al, 1985). The dose must be increased to 15 mg/kg for treatment of threadworms (DiPetro and Todd, 1987). Although oxibendazole is not effective against botfly larvae, historically it has been one of the last of the benzimidazoles to remain effective against helminths and has been used successfully against parasites resistant to fenbendazole. A study of 44 farms in the southern United States revealed that cyathostomins were resistant to fenbendazole at 98% of the farms and were resistant to oxibendazole at 74% of the farms (Kaplan et al, 2004b). A study of horses in Kentucky revealed good oxibendazole efficacy against ascarids, but not strongyles, when used at 10 mg/kg (Lyons et al, 2008). Do not use in severely debilitated horses or in horses suffering from colic, toxemia, or infectious disease.

Other Species

Oxibendazole has been used extralabel PO in swine at 15 mg/kg and in cattle and sheep at 10 to 20 mg/kg for susceptible parasites (Plumb, 2011b).

Thiabendazole

The discovery of thiabendazole in 1961 marked the beginning of truly broad-spectrum anthelmintics. The first of the benzimidazoles, thiabendazole is a very safe compound. Its acute oral LD_{50} for rats is 3100 mg/kg. Thiabendazole was used as an anthelmintic in sheep, goats, cattle, horses, swine, and other animals. It was active against the adults and some immature forms of nematodes, and it inhibited embryonation of nematode eggs. It was also active against fungi and mites. Owing to its wide margin of safety, thiabendazole was used in animals of all ages and in pregnant and debilitated animals. Thiabendazole was available in a variety of pharmaceutical forms (suspension, bolus, paste, feed block, and top-dressing pellets) under various proprietary names. All but one dosage form have left the market in the United States. Thiabendazole is available in only one formulation (TRESADERM), a combination product that is primarily used in dog and cat ears for its activity against ear mites *(Otodectes cynotis).* It is also labeled for treatment of bacterial and fungal dermatoses.

IMIDAZOTHIAZOLES

Tetramisole, discovered in 1966, was the first of the imidazothiazoles. Tetramisole was actually a racemic mixture of two optical isomers. Only the L-isomer, which is levamisole, has anthelmintic activity. Levamisole has twice the safety margin of the racemic mixture because it is equally active against parasites at half the dose (Courtney and Roberson, 1995). Hence, levamisole was subsequently developed, and now it is the only commercially available anthelmintic in this class.

Imidazothiazoles act as nicotinic agonists that disturb the neuromuscular system, thus causing contraction and subsequent tonic paralysis (Coles et al, 1975; Coles, 1977; Courtney and Roberson, 1995; Martin, 1993). Nicotinic acetylcholine receptors of invertebrate parasites are essential for neurofunction, but differ in physiology and distribution in mammals (Londershausen, 1996). The imidazothiazoles are also known to interfere with the fumarate reduction system, which plays a key role in mitochondrial energy production (Arundel et al, 1985; Behm and Bryant, 1979).

Sheep treated PO at 90 mg/kg with tetramisole die. Signs of toxicity occur at 45 mg/kg. These signs, such as lip licking, salivation, lacrimation, head shaking, ataxia, and muscle tremors, may mimic organophosphate toxicity, but are due primarily to muscarinic and nicotinic effects, and are possibly related to cholinesterase inhibition (Lanusse et al, 2009a). Although the injectable cattle product label cautions increased risk with concurrent administration of cholinesterase inhibitors, and Plumb states that adverse effects are more common when levamisole is administered concomitantly with organophosphates (Plumb, 2011b), simultaneous administration of levamisole and the organophosphate dichlorvos did not alter LD_{50}, but when given concurrently with the nicotine-like drug pyrantel tartrate, the LD_{50} of levamisole decreased considerably (Courtney and Roberson, 1995).

Subcutaneous injection is more toxic than oral administration, but cattle are a bit more tolerant regarding parenteral administration. At twice the therapeutic dosage level, two thirds of cattle treated with injection have muzzle foam and licking of the lips. In dogs, oral doses of tetramisole at 20 mg/kg are tolerated and 40 to 80 mg/kg will cause vomiting, but death does not occur. In contrast, SC dosing of tetramisole at 40 mg/kg is fatal to dogs in 10 to 15 minutes (Lanusse et al, 2009a).

Levamisole

Worldwide, levamisole is marketed for use in cattle, sheep, swine, poultry, and dogs. In the United States, levamisole is formulated as a drinking water additive for swine, injectable solution for cattle, and bolus or oral drench for cattle and sheep. It is used to control gastrointestinal and lung nematodes, but has no activity against flukes, protozoa, or tapeworms (Courtney and Roberson, 1995). In addition to its antinematodal activity, levamisole is used as an immunostimulant in dogs and cats.

Cattle

Levamisole hydrochloride is formulated for administration to cattle as a drench (PROHIBIT SOLUBLE DRENCH POWDER), bolus (LEVASOLE CATTLE WORMER BOLUSES), or injectable solution (LEVASOLE INJECTABLE SOLUTION, 13.65%). The dose for cattle is 8 mg/kg PO and 6 mg/kg by subcutaneous injection of the phosphate salt. These formulations are labeled for efficacy against stomach worms (*Haemonchus, Ostertagia,* and *Trichostrongylus*), intestinal worms (*Trichostrongylus, Cooperia, Nematodirus, Bunostomum,* and *Oesophagostomum*), and lungworms *(Dictyocaulus)* (Baker and Fisk, 1972; Lyons et al, 1972; Lyons et al, 1975a; North American Compendiums, 2012). The injectable solution is also labeled

for efficacy against *Chabertia* spp. (North American Compendiums, 2012). Arrested early fourth-stage larvae of *Ostertagia* species are refractory to levamisole.

Muzzle foam may occur but should subside in a few hours. Swelling may occur at the site of levamisole phosphate injection but should subside in 7 to 14 days. The injectable product label cautions that risk is increased when it is used during stressful procedures and with concurrent administration of cholinesterase inhibitors.

In cattle, sheep, and swine, a level of 0.1 ppm has been established for negligible residues in edible tissues (Plumb, 2011b). Cattle should not be slaughtered within 7 days of injection or 2 days of oral medication. Levamisole is not to be used in dairy animals of breeding age to avoid drug residues in milk.

Sheep

Levamisole hydrochloride is formulated for administration to sheep as a drench (PROHIBIT SOLUBLE DRENCH POWDER) or bolus (LEVASOLE SHEEP WORMER BOLUSES). These formulations are labeled for efficacy against stomach worms (*Haemonchus, Ostertagia,* and *Trichostrongylus*), intestinal worms (*Trichostrongylus, Cooperia, Nematodirus, Bunostomum, Oesophagostomum,* and *Chabertia*), and lungworms (*Dictyocaulus*) (Callinan and Barton, 1979; Craig and Shepherd, 1980; North American Compendiums, 2012). Levamisole has an ample therapeutic margin, but occasionally sheep show side effects (e.g., transient excitability, lip licking, salivation, increased alertness, muscle tremors), even at the recommended dose. Debilitated sheep appear to be more susceptible to toxicity. Sheep should not be slaughtered within 72 hours of treatment.

Swine

Levamisole administered to swine in drinking water (e.g., LEVASOLE SOLUBLE PIG WORMER) removes large roundworms *(Ascaris suum),* nodular worms (*Oesophagostomum* spp.), intestinal threadworms *(Strongyloides ransomi),* and lungworms (*Metastrongylus* spp.). Because the peak concentration, rather than the duration of exposure, is more important regarding anthelmintic effects of levamisole, it is important that treated water is consumed fairly rapidly (Lanusse et al, 2009a). Levamisole may cause temporary salivation or muzzle foaming. Swine infected with lungworms may develop coughing or vomiting, which may last for several hours. These reactions may be caused by the expulsion of paralyzed lungworms from the bronchi. At 3× the label dose, pigs occasionally vomit.

Levamisole injection is not labeled for use in pigs. The LD_{50} of levamisole in pigs when given by SC injection is 40 mg/kg (Lanusse et al, 2009a).

Dogs

Levamisole is labeled in some countries to treat roundworms *(Toxocara, Toxascaris)* and hookworms *(Ancylostoma, Uncinaria)* in dogs and cats, providing 95% efficacy when given at 10 mg/kg/day PO for 2 days, although such use is extralabel in the United States (Lanusse et al, 2009a). It has been recommended to control several canine parasites. Levamisole has been used to control the French heartworm *(Angiostrongylus vasorum),* a parasite that is enzootic to western and southern Europe, and its use has been reported in eastern Canada, at 7.5 mg/kg for 2 consecutive days, followed by 2 days at 10 mg/kg (Bowman, 2006). If the infection is not cleared, the regimen is repeated. In addition, levamisole has been recommended to treat (1) *Crenosoma vulpis* at 8 mg/kg once; (2) *Filaroides osleri* at 7 to 12 mg/kg once daily PO for 20 to 45 days, or 7.5 mg/kg PO twice daily, or 25 mg/kg PO every other day for 10 days; and (3) *Capillaria aerophilia* at 10 mg/kg PO once daily for 5 days, repeat in 9 days (Plumb, 2011b; Reinemeyer, 1995). Levamisole is ineffective against whipworms (Lanusse et al, 2009a). Previously it was used as a microfilaricide, but other products are more appropriate for that indication at this time.

Cats

As previously stated, although extralabel in the United States for use in cats, levamisole is labeled in some countries to treat roundworms *(Toxocara, Toxascaris)* and hookworms *(Ancylostoma, Uncinaria)* and has demonstrated 95% efficacy when given at 10 mg/kg/day PO for 2 days (Lanusse et al, 2009a). Levamisole has been used to control *Capillaria aerophilia* in cats at 10 mg/kg PO once daily for 5 days, repeat in 9 days (Reinemeyer, 1995). It has also been used in cats to treat *Aelurostrongylus abstrusus* and *Ollulanus tricuspis* (Plumb, 2011b). Previously levamisole was used as a microfilaricide, but other products are more appropriate for that indication at this time.

Opossum

Levamisole use in opossums *(Didelphis virginiana)* is extralabel, but according to the National Opossum Society, it is the drug of choice for controlling internal parasites. The National Opossum Society website advocates treating opossums over 200 g in body weight with levamisole at a dose of 6 mg/kg by SC injection, and repeating the dose in juvenile and adult animals (not infants) in about 3 weeks as needed on the basis of fecal testing for roundworms, hookworms, and whipworms (National Opossum Society, 2010).

Birds

Levamisole has been recommended extralabel to treat poultry as medicated drinking water at 40 mg/kg. Because the peak concentration, rather than the duration of exposure, is more important regarding anthelmintic effects of levamisole, it is important for treated water to be consumed fairly rapidly (Lanusse et al, 2009a). A variety of dosing rates, schedules, and routes for anthelmintic treatment of parakeets, ratites, and other birds are reviewed by Plumb (Plumb, 2011b).

Reptiles and Amphibians

Levamisole has been recommended extralabel to treat (1) reptiles and amphibians with nematode infection at 5 to 10 mg/kg PO, and (2) aquatic turtles, frogs, and toads with thorny-headed worm (*Acanthocephalan* spp.) infection at 5 to 10 mg/kg PO, SC, or by intracoelomic injection, with the dose repeated again in 2 weeks (de la Navarre, 2003).

Rabbits

Levamisole has been recommended extralabel to treat rabbits with (1) gastric nematodes at 12.5 to 20 mg/kg PO, or (2) nematodes that are not gastric at the same dose, but by the SC route (Plumb, 2011b).

Llamas

Levamisole has been recommended extralabel to treat llamas with susceptible nematodes at 5 to 8 mg/kg PO, IM, or SC (Plumb, 2011b).

TETRAHYDROPYRIMIDINES

The tetrahydropyrimidines include the numerous salts of pyrantel, morantel, and oxantel, the latter of which is available only outside the United States. All act as nicotinic agonists that disrupt the neuromuscular system, causing contraction and subsequent tonic paralysis by their action at synaptic and extrasynaptic nAChRs on

nematode muscle cells (Aubry et al, 1970; Eyre, 1970; Lanusse et al, 2009a; Martin, 1993; Martin, 1997b). In vitro experiments indicate that pyrantel is 100× more potent than acetylcholine. The nAChRs of invertebrate parasites are essential for neurofunction, but different in physiology and distribution than in mammals (Londershausen, 1996).

Pyrantel

Introduced in 1966, pyrantel is the most widely used of all the tetrahydropyrimidine anthelmintics (Lanusse et al, 2009a). Pyrantel is now available under a wide variety of trade names in the form of tablets, chewable tablets, paste, oral suspension, medicated pellets, and medicated feed (North American Compendiums, 2012).

The tartrate salt of pyrantel is a white powder, soluble in water, which is absorbed more readily than the pamoate salt and is used in horses and swine (Lanusse et al, 2009a). Pyrantel tartrate is well absorbed after oral administration in the rat, dog, and pig. Plasma levels peak within 3 to 6 hours (Lanusse et al, 2009a). It is not as well absorbed in ruminants. Pyrantel tartrate is rapidly metabolized. In dogs, and in no other species, it is primarily eliminated by way of the urinary tract.

Pyrantel pamoate, on the other hand, is poorly absorbed from the gastrointestinal tract and is primarily eliminated through the feces, with less than 15% excretion through the urinary tract (USP, 2005). The pamoate salt of pyrantel is a yellow powder, insoluble in water. It is available as a ready-to-use suspension or paste in horses and as a suspension or chewable tablet in dogs. The fact that pyrantel pamoate is poorly absorbed from the intestine adds to its safety in very young or weak animals. Pyrantel salts are stable in solid form but photodegrade when dissolved or suspended in water, resulting in reduction of potency.

Because pyrantel and piperazine appear to be pharmacologic antagonists, they should not be used concurrently. Pyrantel also should not be given concurrently with levamisole or morantel, but in this case, because they have similar mechanisms of action. There is an increased risk of side effects when pyrantel is used concurrently with organophosphates (Plumb, 2011b).

Dogs

Pyrantel pamoate is available as tablet, chewable tablets, and a palatable suspension, marketed as NEMEX and many other trade names, and is indicated for the removal of roundworms *(Toxocara canis, Toxascaris leonina)* and hookworms *(Ancylostoma caninum, Uncinaria stenocephala)* from dogs and puppies (Clark et al, 1991; Jacobs, 1987b; Klein et al, 1978; Linquist, 1975). The recommended dose of 5 mg/kg of pyrantel pamoate suspension is administered orally. For animals weighing 2.25 kg or less, the dose is increased to 10 mg/kg. Tablets may be administered directly or placed in a small portion of food.

This product has also been used to treat Physaloptera stomach worms in dogs, although such use is not approved (Clark, 1990; Lanusse et al, 2009a). It may have some effect on tapeworms as well, but other drugs are commonly used to treat tapeworm infection in small animals. Pyrantel pamoate is safe for nursing and weanling pups, pregnant bitches (Wiebe and Howard, 2009), males used for breeding, and dogs infected with *Dirofilaria immitis*. It does not appear to be teratogenic in either rats or rabbits at oral doses of up to 1000 to 3000 mg/kg (Wiebe and Howard, 2009). The oral LD_{50} is >690 mg/kg in dogs or 138× the label dose (Lanusse et al, 2009a). In chronic toxicity studies, dogs had no adverse effects when given 20 mg/kg/day for 3 months, but did have ill effects at 50 mg/kg/day (Lanusse et al, 2009a).

Horses

Pyrantel is available as the tartrate or pamoate salt in horses.

PYRANTEL TARTRATE IN HORSES. The tartrate salt of pyrantel is a water-soluble white powder, which is used as a medicated feed. Unlike pyrantel pamoate, pyrantel tartrate is well absorbed after oral administration, with plasma levels peaking in 2 to 3 hours, followed by rapid metabolism and elimination in the urine. Pyrantel tartrate (STRONGID C) is fed at a dose of 2.6 mg/kg of body weight daily for prevention of *Strongylus vulgaris* larval infestation and for control of adult large strongyles *(S. vulgaris, S. edentatus),* and adult and fourth-stage larval small strongyles (*Cyathostomum* spp., *Cylicocyclus* spp., *Cylicostephanus* spp., *Cylicodontophorus* spp., *Poteriostomum* spp., *Triodontophorus* spp.), pinworms *(Oxyuris equi),* and ascarids *(Parascaris equorum)* (Cornwell and Jones, 1968; Drudge et al, 1982; Lyons et al, 1975b).

Pyrantel tartrate is safe for use in horses and ponies of all ages, including foals and pregnant mares. Foals may be treated as soon as they take grain. Stallion fertility is not affected by the use of pyrantel tartrate. It can be used concurrently with insecticides, tranquilizers, muscle relaxants, and central nervous system depressants.

One downside of daily treatment with pyrantel is the development of parasite resistance to pyrantel. Another downside of daily pyrantel is that foals raised on daily pyrantel do not acquire resistance to strongyles, as indicated by response to mixed strongyle L_3 challenge (Monahan et al, 1997). So although this medicated feed is safe and easy to administer, indiscriminate use throughout a herd, without focusing use toward particular horses in need, will, as with all anthelmintics, be detrimental to the maintenance of healthy refugia and eventually will lead to increased parasitic resistance.

PYRANTEL PAMOATE IN HORSES. Several manufacturers have pyrantel pamoate products approved for horses. It is available as a paste in concentrations of 171, 180, or 226 mg (base)/mL (i.e., EXODUS PASTE, STRONGID PASTE, and PYRANTICPASTE, respectively). It is also available as a flavored suspension at 50 mg (base)/mL (e.g., STRONGID-T), which is approved for administration at 6.6 mg (base)/kg to remove and control adult populations of large strongyles *(Strongylus vulgaris, S. edentatus, S. equinus),* small strongyles, pinworms *(Oxyuris equi),* and large roundworms *(Parascaris equorum)* in horses and ponies. Ordinarily other drugs are used to eliminate tapeworms, but a single oral pyrantel pamoate dose of 13.2 mg/kg has been shown to be 83% to 98% effective against tapeworms (Craig et al, 2003; Slocombe, 1979). In 2005 the same dose was FDA approved for removal and control of *Anoplocephala perfoliata* in horses and ponies, but currently 13.2 mg/kg for tapeworms is considered extralabel (Food and Drug Administration, 2005).

Swine

The only pyrantel product labeled for pigs is tartrate salt (i.e., Banminth 48), which, when fed at 96 g/ton as the sole ration for 3 days, prevents the migration and establishment of large roundworm *(Ascaris suum)* infection, and when fed continuously also aids in the prevention of nodular worm (*Oesophagostomum* spp.) infection. The package insert lists other feed rates as well. Pyrantel is the only approved anthelmintic that will prevent the appearance of "milk spots" on the liver of pigs when administered continuously. It does so by killing the larvae of *A. suum* in the lumen of the gut (Biehl, 1986).

Pyrantel should not be given to pigs within 24 hours of slaughter. Because the drug is photodegradable, it should be used immediately after the package is opened. Pyrantel tartrate should not be mixed with rations containing bentonite.

Extralabel use of pyrantel has been advised for potbellied pigs at 6.6 mg/kg for ascarids and nodular worms, but the author does not clarify whether this refers to the tartrate versus the pamoate salt (Braun, 1995).

Cattle, Sheep, and Goats

Pyrantel tartrate is not approved by the FDA for use in cattle, sheep, or goats but has been recommended or considered effective at 25 mg/kg PO in treating barber pole worm *(Haemonchus contortus),* brown stomach worm (*Ostertagia ostertagi* and *Teladorsagia [Ostertagia] circumcincta*), small stomach worm *(Trichostrongylus axei),* bankrupt worm *(Trichostrongylus colubriformis),* thread-necked worm (*Nematodirus battus* and *N. spathiger*), small intestinal worm (*Cooperia* spp.), hookworm (*Bunostomum* spp.), large-mouth bowel worm (*Chabertia* spp.), and nodular worm (*Oesophagostomum* spp.) infections (Arundel et al, 1985; Campbell and Rew, 1985; Lanusse et al, 2009a; Reinemeyer and Courtney, 2001a).

Morantel Tartrate

Morantel is the 3-methyl analog of pyrantel. Morantel tartrate is used for control of gastrointestinal nematodes in cattle and goats. Morantel tartrate has an oral LD_{50} of 5g/kg in mice and is a safer drug than pyrantel tartrate, which has an oral LD_{50} of only 170 mg/kg in mice (Lanusse et al, 2009a). Signs of overdose include increased respiratory rate, profuse sweating, ataxia, and other cholinergic effects (Plumb, 2011b). Do not add to feeds containing bentonite. Do not use concurrently with levamisole or pyrantel because they have similar mechanisms of action. Do not use with piperazine because of the antagonistic mechanism of action. Use with care and watch for adverse effects if using with organophosphates (Plumb, 2011b).

Cattle

Morantel tartrate (i.e., RUMATEL 88) is formulated as a 19.4% concentrated medicated feed that is mixed in a complete feed or top dressed to deliver 9.7 mg/kg (0.44 g/100 lb) of body weight for the removal of stomach worms (*Haemonchus* spp., *Ostertagia* spp., and *Trichostrongylus* spp.), worms of the small intestine (*Cooperia* spp., *Trichostrongylus* spp., and *Nematodirus* spp.), and worms of the large intestine *(Oesophagostomum radiatum)* (Anderson and Marais, 1975; Ciordia and McCampbell, 1973; Conway et al, 1973). Activity against larval stages of these nematodes appears to be variable. Morantel may be administered to lactating dairy cows without requiring milk withdrawal. Cattle should not be slaughtered within 14 days after treatment. It may be given simultaneously with vaccines and injectable drugs without concern.

Goats

Morantel tartrate (e.g., GOAT CARE-2X) is mixed in a complete feed or top dressed to deliver 9.7 mg/kg (0.44 g/100 lb) of body weight for the removal of adult barber pole worms *(Haemonchus contortus),* brown stomach worms *(Teladorsagia [Ostertagia] circumcincta),* and small stomach worms *(Trichostrongylus axei)* in goats. Goats should not be slaughtered within 30 days of treatment. Morantel may be administered to lactating dairy goats without requiring milk withdrawal.

CYCLIC DEPSIPEPTIDES

Emodepside

Emodepside is the first cyclic depsipeptide to be approved for use against animal parasites in the United States. It has low to moderate acute toxicity in mammalian species; the oral LD_{50} in rats is >500 mg/kg, and no rat mortality was reported with dermal exposure at >2000 mg/kg (Plumb, 2011b). Emodepside binds to a presynaptic latrophilin-like receptor in the pharynx and body wall muscle of parasitic nematodes, which results in flaccid paralysis and death (Harder et al, 2005; Lanusse et al, 2009a). With this novel mode of action, it is fully effective against benzimidazole-, levamisole-, and ivermectin-resistant nematodes of sheep and cattle (Harder et al, 2005; Kaminsky et al, 2008), although no product with emodepside is labeled for use in these species in the United States. It is not available as the sole ingredient but is available only when combined with praziquantel for use in cats in a product that is discussed in the broad-spectrum combinations section.

PIPERAZINE

Piperazine was used for treating human gout in the early 1900s because it is an excellent uric acid solvent. Its anthelmintic activity was discovered in the 1950s (Courtney and Roberson, 1995). Since then, a wide variety of piperazine salts have been derived for use as anthelmintics in swine, poultry, horses, dogs, and cats. Piperazine produces a neuromuscular blockade through disruption of GABA neurotransmission. Most data suggest that the receptors in nematodes and insects resemble the mammalian GABA subtype but are clearly different from their vertebrate counterparts (Londershausen, 1996; Martin, 1997b). Piperazine is safe to use in all species but has a narrow spectrum of action, limited primarily to roundworms (Papich, 2007; Reinemeyer and Courtney, 2001a).

Currently no piperazine products are available on the U.S. market for horses, cattle, sheep, or goats, probably because of its narrow spectrum. Various salts of piperazine (e.g., adipate, hydrochloride, sulfate, monohydrate, citrate, dihydrochloride) are used as anthelmintics in swine, poultry, dogs, and cats. The amount of piperazine base in each salt, hence the amount of anthelmintic activity, varies widely (e.g., citrate: 35%, adipate: 37%, phosphate: 42%, hexahydrate: 44%, sulfate: 46%, chloride: 48%, and dihydrochloride salts: 50% to 53%) (Courtney and Roberson, 1995; Reinemeyer and Courtney, 2001a). Anthelmintic activity depends on freeing piperazine base in the gastrointestinal tract. Piperazine is rapidly absorbed from the gastrointestinal tract and is quickly cleared by urinary excretion. Elimination is virtually complete within 24 hours. Piperazine should be used with caution, if at all, in animals with hepatic or renal dysfunction. The drug may not be effective in animals with intestinal hypomotility because the paralyzed worms may recover from the effects of the drug before they are passed in the stool. Occasional adverse reactions observed in dogs and cats include ataxia, diarrhea, and vomiting. Horses sometimes have transient softening of the feces after piperazine treatment (Plumb, 2011b).

Piperazine is available as tablets, solution, and soluble powder under many proprietary names (e.g., PIPA-TABS, TASTY PASTE, PIG SWIG). The drug is practically nontoxic but should be used with caution in animals with hepatic or renal dysfunction. Its oral LD_{50} in rats is 4.9 g/kg, in chickens 8 g/kg, and in mice 11.4 g/kg. Piperazine can be administered to animals of all ages.

Dogs and Cats

Several piperazine products are on the market for dogs and cats, including adipate, citrate, and dihydrochloride salts. Piperazine is administered orally at 45 to 65 mg/kg (Riviere and Papich, 2009), although higher doses (100 to 250 mg/kg) have been reported in the literature (English and Sprent, 1965; Jacobs, 1987a; Jacobs, 1987b; Plumb, 2011b; Sharp et al, 1973). It is effective against adult roundworms, *Toxocara canis, T. cati,* and *Toxascaris leonina*. Treatment of nursing pups at 2, 4, 6, and 8 weeks of age removes >90%

of prenatally acquired *Toxocara canis* (Lanusse et al, 2009a). It is ineffective against whipworms.

Swine

Piperazine monohydrochloride (PIG SWIG) and dipiperazine sulfate (WAZINE-17, WAZINE-34) are dosed in drinking water at 110 mg/kg (see product insert for mixing directions) to remove large roundworms *(Ascaris suum)* and nodular worms (*Oesophagostomum* spp.) (Biehl, 1986).

Chickens and Turkeys

The same piperazine products used in swine are used in chickens and turkeys: piperazine monohydrochloride (PIG SWIG) and dipiperazine sulfate (WAZINE-17, WAZINE-34), which are administered to poultry via drinking water for the control of roundworms (*Ascaridis* spp.).

Piperazine administered in water for 2 days at 32 mg of base per kilogram is very effective against roundworms *(Ascaridia galli),* but not against the cecal worm *(Heterakis gallinarum)* (Lanusse et al, 2009a; Reinemeyer and Courtney, 2001a).

ORGANOPHOSPHATES

Dichlorvos

Dichlorvos is an organophosphate that is effective against many internal and external parasites. See the insecticide section of this chapter for an introduction to dichlorvos with a review of its history and mechanism of action. Its mammalian toxicity and treatment of such toxicity are reviewed in the organophosphate and carbamate section. Its use as an organophosphate, taken internally to kill parasites, especially nematodes in swine, is discussed herein.

Swine

Dichlorvos is formulated for pigs in polyvinyl chloride resin pellets (ATGARD SWINE DEWORMER). The dose is 11.2 to 21.6 mg/kg once PO, or it is mixed into a gestation feed to provide 1000 mg/head daily during the last 30 days of gestation (mixed as described in the package insert). It is mixed into a complete meal-type feed (not unground grain or pelleted meal). Dichlorvos is labeled for the removal and control of adult, sexually immature, and/or fourth-stage larvae of the whipworm *(Trichuris suis),* nodular worm (*Oesophagostomum* sp.), large roundworm *(Ascaris suum),* and mature thick stomach worm *(Ascarops stongylina),* occurring in the GI lumen of pigs, boars, weaner, fatteners, and open or bred gilts and sows (Arundel et al, 1985; Biehl, 1986).

For best results, gilts and sows should be medicated shortly before farrowing and again at weaning. It is best to administer the medicated feed to small lots of compatibly sized pigs (e.g., single litters) at one time, so they can be watched while feeding to ensure that all eat their share. Preliminary fasting is unnecessary, but alternative sources of feed should be excluded during the medication period.

When given at labeled dosages to breeding swine, dichlorvos has no adverse effects on production and does not cause abortion or premature birth, impaired fertility, fewer pigs per litter, or decreased litter survival or performance. No preslaughter withdrawal period is required when the drug is used at the recommended dosage level. Dichlorvos should not be used with other cholinesterase-inhibiting chemicals, taeniacides, antifilarials, muscle relaxants, phenothiazine tranquilizers, or central nervous system depressants. As discussed in the organophosphate and carbamate subsection of the insecticide section of this chapter, atropine and pralidoxime (2-PAM) are the recommended antidotes for organophosphate poisoning.

ISOQUINOLONES

The cestocidal isoquinolones are represented by two closely related drugs: praziquantel and epsiprantel. This cestocidal class is the safest and most effective yet approved in the United States. They attack the parasite neuromuscular junction and the tegument. These drugs cause increased cell membrane permeability to calcium and resulting loss of intracellular calcium. This effect causes instantaneous contraction and paralysis of the parasite (Andrews et al, 1983). The second effect is devastating vacuolization and destruction of the protective tegument (Arundel et al, 1985; Frayha et al, 1997). The combined effects of paralysis and tegmental destruction provide excellent activity against cestodes.

Praziquantel

Praziquantel was the first cestocidal isoquinolone approved in the United States. It has marked anthelmintic activity against a wide range of adult and larval cestodes and trematodes of the genus *Schistosoma.* Oral administration results in nearly complete absorption and rapid distribution throughout the body and across the blood-brain barrier. Praziquantel has high oral bioavailability, high protein binding, and a marked first-pass effect, which is especially extensive in sheep (Lanusse et al, 2009b). Although 80% of the drug is eliminated in the urine, the main site of inactivation is the liver, with only trace amounts of unchanged drug excreted in the urine (Roberson and Courtney, 1995). The drug is metabolized to 4′-hydroxy-praziquantel in dogs, both of which are roughly similar in pharmacologic activity. In dogs the oral half-life of the parent compound is 1.3 hours, versus 1.92 hours for the metabolite (Lanusse et al, 2009a).

Praziquantel is a very safe anthelmintic. Vomiting is typically observed at high dosage rates. Hence an oral LD_{50} for dogs has not been established because they vomit at dosages >200 mg/kg (Lanusse et al, 2009a). Rats tolerated daily administration of up to 1000 mg/kg for 4 weeks, and dogs tolerated up to 180 mg/kg/day for 13 weeks. Injected doses of 200 mg/kg were lethal in cats (Plumb, 2011b). It can be used safely in breeding and pregnant animals without restriction. Praziquantel did not induce embryotoxicity, teratogenesis, mutagenesis, or carcinogenesis, nor did it affect the reproductive performance of test animals (Lanusse et al, 2009a). Occasional adverse experiences in clinical use include pain on injection, anorexia, diarrhea, salivation, vomiting, sleepiness, staggering, and weakness. Overdoses have been reported to cause diarrhea, depression, incoordination, tremors, salivation, and vomiting.

Dogs and Cats

Praziquantel (DRONCIT) is administered orally or is injected subcutaneously per dosing charts listed in package inserts. It is labeled for the removal of *Dipylidium caninum, Taenia taeniaeformis, T. pisiformis, T. hydatigena, T. ovis, Mesocestoides corti, Echinococcus granulosus, E. multilocularis, Spirometra* spp., *Diphyllobothrium latum, D. erinacei,* and *Joyeuxiella pasquali* (Andersen et al, 1978; Andersen et al, 1979; Gemmell et al, 1977; Gemmell et al, 1980; Kruckenberg et al, 1981; Lanusse et al, 2009a; Thakur et al, 1978; Thomas and Gonnert, 1978). The single therapeutic dose ranges listed by Lanusse et al are 3.8 to 12.5 mg/kg in dogs and 4.2 to 12.7 mg/kg in cats (Lanusse et al, 2009a). The package inserts have extensive information about using this drug to help control *E. multilocularis,* including the life cycle of the parasite, difficulty

of diagnosis, and other public health considerations (Bayer Healthcare, 2003).

Praziquantel has been used extralabel at a high dose—25 mg/kg PO daily for 3 days (Roberson and Courtney, 1995) or 23 to 25 mg/kg PO every 8 hours for 3 days—to treat lung fluke *(Paragonimus kellicotti)* infection in dogs and cats (Plumb, 2011b; Reinemeyer, 1995). Praziquantal has been recommended extralabel for the treatment of intestinal fluke (*Alaria* spp.) infection of dogs and cats at 20 mg/kg (Ballweber, 2004). It has also been recommended, extralabel, to treat dogs and cats for several other flukes (Plumb, 2011b). Praziquantel injection is not intended for use in puppies or kittens younger than 4 weeks of age. Several combination products contain praziquantel. See the section on combination products for more information.

Sheep, Goats, and Chickens

Although not approved for use in these species, praziquantel may be used for tapeworm infection from *Avitellina* spp., *Stilesia* spp., *Moniezia* spp., *Choanotaenia infundibulum, Davainea proglottina,* and *Raillietina cesticellus*. Sheep and goats may be treated with a dose of 10 to 15 mg/kg, and chickens with a dose of 10 mg/kg (Reinemeyer and Courtney, 2001b).

Horses

Although not approved as a sole ingredient in any equine products, praziquantel may be used for tapeworm infections from *Anoplocephala perfoliata.* Praziquantel has been administered to horses as a single dose of 1.23 mg/kg using the injectable product, but delivered via nasogastric tube (Craig et al, 2003). Praziquantel is approved in combination with the macrocyclic lactone moxidectin or ivermectin for use in horses. See the section on combination products for more information.

Epsiprantel

Epsiprantel (CESTEX) was the second cestocidal isoquinolone approved in the United States. Unlike its cousin praziquantel, epsiprantel is poorly absorbed after oral administration. Less than 0.1% is recovered from the urine; no metabolites are known (Lanusse et al, 2009b; Plumb, 2011b). It is eliminated in the feces unchanged (Lanusse et al, 2009b). Because of its low bioavailability, systemic toxicity and teratogenic effects are very unlikely, but the safety of epsiprantel in pregnant dogs and cats has not been proven. In acute toxicity studies in mice and rats, the oral minimum lethal dose of epsiprantel was shown to be greater than 5000 mg/kg. Doses as high as 36× the label dose were well tolerated in dogs and caused vomiting in some kittens (Plumb, 2011b). Cats given the drug daily at 40× the label dose for 4 days had minimal signs. Dogs given 90× the label dose for 14 days had no significant adverse events (Pfizer Animal Health, 2007).

Epsiprantel treatment as an oral film-coated tablet, at 2.75 mg/kg for cats or 5.5 mg/kg for dogs, effectively removes tapeworms in the cat (*Dipylidium caninum* and *Taenia taeniaeformis*) and dog (*Dipylidium caninum, Taenia pisiformis,* and *T. hydatigena*) (Corwin et al, 1989; Manger and Brewer, 1989). Using it to remove *T. hydatigena* is extralabel. Evidence suggests that the drug is effective against *Echinococcus granulosa* and *E. multilocularis,* but the data are insufficient to recommend a dosage that will completely clear the infection from those treated (Arru et al, 1990; Thompson et al, 1991). Epsiprantel was given concurrently with anti-inflammatory drugs, insecticides, and nematocides with no incompatibilities observed (Pfizer Animal Health, 2007). It should not be used in puppies and kittens younger than 7 weeks of age.

ARSENICALS

Heavy metals such as arsenic and antimony are well represented in the history of anthelmintics. To date, safer and more effective drugs for the most common parasites have largely replaced arsenicals. Their use is now limited to removal of adult heartworms *(Dirofilaria immitis).* Thiacetarsemide (CAPARSOLATE) is no longer available commercially in the United States and thus will not be covered in this chapter (see previous editions of this text if necessary). Arsenical therapeutic effect depends on a reaction between the arsenic salt and sulfhydryl-containing enzymes (Gilman et al, 1990). Inactivation of parasite enzyme systems results in death. Arsenic is widely known as a toxin in humans and animals. Due caution is certainly required when arsenicals are used.

Melarsomine

Melarsomine dihydrochloride (IMMITICIDE) is the only arsenical anthelmintic commercially available on the U.S. veterinary market. It provides 92% to 98% efficacy against adult heartworms *(Dirofilaria immitis)* in dogs (Dzimianski et al, 1992; Keister et al, 1992; Keister et al, 1995; Miller et al, 1995), but its use is contraindicated in cats because of toxicity problems. The arsenic content of the product is less than that of thiacetarsemide, making melarsomine less toxic to the patient. Unlike thiacetarsemide, which binds to blood cells, melarsomine and its metabolites remain free in the plasma, resulting in higher, longer-lasting plasma levels. It is rapidly absorbed; maximal concentration (C_{max}) is noted 8 minutes after injection (Lanusse et al, 2009a). The parent drug and the arsenoxide metabolite are rapidly eliminated in the feces, probably by biliary excretion. The arsenic acid metabolite is rapidly eliminated in the urine, so no significant bioaccumulation occurs (Keister et al, 1995).

Melarsomine is labeled to be administered intramuscularly at a dose of 2.5 mg/kg for two injections given 24 hours apart to dogs at low risk of thromboembolic complications, but this regimen kills only 90% of the adult worms (American Heartworm Society, 2012). Dogs that have moderate risk of thromboembolism may be treated with an alternative three-injection regimen of a single injection followed by a rest period of 1 to 2 months, after which two standard injections are given. This latter three-injection regimen is reportedly less hazardous for the patient and is more efficacious, killing 98% of the worms, and is therefore the preferred regimen recommended by the American Heartworm Society in all stages, except severe heartworm disease complicated by caval syndrome, for which melarsomine is contraindicated (American Heartworm Society, 2012). Injections should be made deep into the lumbar epaxial muscles along L3 to L5. Peak blood level is achieved about 8 minutes after injection (Lanusse et al, 2009a), and the half-life is 3 hours (Plumb, 2011b).

About one third of dogs treated will have injection site reactions, most of which resolve within a week, but firm nodules at the injection site can persist indefinitely (Plumb, 2011b). Additional adverse reactions include elevated hepatic enzymes, coughing, gagging, depression, lethargy, anorexia, fever, pulmonary congestion, and vomiting (Papich, 2007; Plumb, 2011b). This drug exemplifies the problem of parasite removal by poisoning the patient just enough to kill the parasite, hopefully without damaging the patient too much. It has a narrow therapeutic range. The toxic dose is only 2.5 to 3× the recommended dose and can result in panting, pulmonary inflammation, salivation, vomiting, edema, and death. Dimercaprol (also known as BAL in oil) is antidotal in dogs overdosed with melarsomine (Lanusse et al, 2009a; Plumb, 2011b).

Safety has not been determined in breeding, pregnant, or lactating dogs. That said, clinical studies indicate that treatment is well tolerated even in dogs that have clinical signs of heartworm disease

(Case et al, 1995; Miller et al, 1995; Vezzoni et al, 1992). As was previously mentioned in the ivermectin section of this chapter, the American Heartworm Society guidelines for diagnosis, prevention, and management of heartworm infection in dogs should be consulted before treating a heartworm-infected dog with melarsomine (American Heartworm Society, 2012). Treatment with a macrocytic lactone before administration of melarsomine should be considered along with other methods to reduce the potential for melarsomine adverse reactions. For example, as was mentioned in the ivermectin section, one study of heartworm-positive dogs comparing groups that were treated with three drugs (i.e., melarsomine, doxycycline, and ivermectin), two drugs (i.e., doxycycline and ivermectin), doxycycline alone, ivermectin alone, or melarsomine alone led the authors to conclude that the combination of doxycycline and ivermectin was synergistic (McCall et al, 2008). All dogs treated with ivermectin plus doxycycline (with or without melarsomine) were free of microfilariae in 9 weeks. This may be related to the elimination of *Wolbachia* spp. bacteria, which are filarial endosymbionts. Doxycycline PO at 10 mg/kg twice daily (BID) for 4 weeks has been shown to eliminate more than 95% of *Wolbachia* organisms in the filarial nematode *Wuchereria bancrofti*, resulting in amicrofilaremia for 12 months (American Heartworm Society, 2012). McCall et al found that administration of doxycycline plus ivermectin for several months before (or without) melarsomine resulted in elimination of adult heartworms with less severe thromboembolism than did treatment with melarsomine alone (McCall et al, 2008).

Warning: Do not give melarsomine by SC or IV injection.

MISCELLANEOUS ANTHELMINTICS

Clorsulon

Clorsulon, a benzene sulfonamide compound, is not available as a sole ingredient in any products labeled for use in animals in the United States. Clorsulon is very effective in cattle against the immature and mature liver fluke *(Fasciola hepatica)*. The drug is not given to lactating cattle because milk withdrawal has not been established. Clorsulon is not effective against the rumen fluke *(Paramphistomum)* (Plumb, 2011b).

Previously, before November 2008, clorsulon was available as a drench and was given to cattle and sheep at a dose of 7 mg/kg. A single dose was effective in removing *F. hepatica* (Kilgore et al, 1985).

Currently clorsulon is available only in combination with ivermectin. For more information, see the section on combination products.

Hygromycin B

Hygromycin B is an antibiotic produced by *Streptomyces hygroscopicus* that has anthelmintic properties. It is added to swine and poultry feed (HYGROMIX 8 PI). In chickens it aids in the control of large roundworms *(Ascaris galli)*, cecal worms *(Heterakis gallinae)*, and capillary worms *(Capillaria obsignata)*. In pigs it aids in the control of large roundworms *(Ascaris suis)*, nodular worms *(Oesophagostomum dentatum)*, and whipworms *(Trichuris suis)*. Hygromycin B should be used for ≤8 weeks during gestation and lactation. In breeding pig stock, it should be used with caution if at all because of hearing and vision impairment side effects. If sows are affected, they are less responsive to squeals, which may result in crushed baby pigs (Elanco Animal Health).

BROAD-SPECTRUM COMBINATIONS

The veterinary practitioner is always looking for anthelmintic products that cover ever-increasing spectra of parasites. Broad-spectrum products provide two important advantages. First, they obviate dosing with several different products at once when a patient has a mixed parasite infection, making administration easier. Second, they provide peace of mind that a treated animal will be cleared of possibly undiagnosed parasites. For instance, a puppy from the animal shelter will be better served by use of a product that is effective in removing both roundworms and hookworms than a product that is effective against roundworms only. The spectrum of anthelmintics can be increased in one of two ways: by tackling the arduous task of discovering a single broad-spectrum chemical, or by combining several compatible active ingredients to build the desired spectrum of activity.

In this section the combination products are discussed. In many cases combination product formulation and dosing regimens are different from those of the single-entity drug ingredients. The toxicity and mechanism of action of the individual ingredients were covered earlier in this chapter.

Ivermectin and Clorsulon

A handful of products contain 10% w/v clorsulon and 1% w/v ivermectin (e.g., IVOMEC PLUS) and are approved for use in cattle. The product is injected subcutaneously behind the shoulder at a dose of 1 mL/50 kg of body weight. This dose volume delivers 0.2 mg ivermectin and 2 mg clorsulon per kilogram of body weight. It is effective against the liver fluke *(Fasciola hepatica)* plus all the parasites that ivermectin treats and controls: gastrointestinal roundworms, cattle grubs, lungworms, sucking lice, and mange mites (*Psoroptes ovis* and *Sarcoptes scabiei*). It is not effective against the rumen fluke *(Paramphistomum)* (Plumb, 2011b). Do not treat cattle within 49 days before slaughter. Do not use the product in female dairy cattle of breeding age, because no milk withholding time has been established. Do not use in veal calves.

Ivermectin and Pyrantel Pamoate

Ivermectin combined with pyrantel pamoate is available in flavored chewables or tablets (e.g., TRI-HEART PLUS, HEARTGARD PLUS) for dogs. Because the heartworm preventive dose of ivermectin is not effective against gastrointestinal parasites, pyrantel pamoate is added to provide action against these important parasite species. The product is formulated to deliver a target dose of 0.006 mg (6 mcg) of ivermectin and 5 mg of pyrantel pamoate per kilogram of body weight. It is given PO to dogs every 30 days to prevent heartworms, *Dirofilaria immitis*, and to remove roundworms (*Toxocara canis* and *Toxascaris leonina*), and hookworms (*Ancylostoma caninum* and *Uncinaria stenocephala*) (Clark et al, 1991). The product should be given at monthly intervals during the heartworm season. Recent studies have shown that adult heartworms are not able to maintain detectable levels of microfilariae when exposed to ivermectin, so an antigen test should be used to reveal the presence of adult heartworms (Bowman et al, 1992). Safety tests indicate the ivermectin-pyrantel combination is well tolerated (Clark et al, 1992). Do not give this medication to dogs younger than 6 weeks of age or to those with existing heartworm infection.

Ivermectin and Praziquantel

Two oral paste products (EQUIMAX, ZIMECTRIN GOLD) containing ivermectin and praziquantel are approved for use in horses. The addition of praziquantel extends the parasitic spectrum of ivermectin to include the tapeworm *(Anoplocephala perfoliata)*. The formulation of the active ingredients and dosing are different for these products. Equimax paste (ivermectin 1.87%/praziquantel 14.03%) is given orally at a dose of 0.2 mg/kg for ivermectin and 1.5 mg/kg body weight for praziquantel. ZIMECTRIN GOLD (ivermectin

1.55%/praziquantel 7.75%) is given orally at a dose of 0.2 mg/kg for ivermectin and 1 mg/kg body weight for praziquantel.

Both combination products are approved for the treatment and control of *Anoplocephala perfoliata,* large strongyles, small strongyles (including those resistant to some benzimidazoles), pinworms, ascarids, hairworms, large-mouth stomach worms, bots, lungworms, and threadworms. They are also used to treat summer sores caused by *Habronema* and *Draschia* spp. larvae and dermatitis caused by neck threadworm (*Onchocerca* spp.) microfilariae (onchocerciasis).

When used to treat onchocerciasis, a single dose often results in clinical remission of signs within 2 to 3 weeks, but sometimes two to three monthly ivermectin treatments are needed (Rees, 2010). See the ivermectin section for information about an adverse reaction (pruritus and ventral edema) that occurs in about a quarter of horses treated for onchocerciasis.

Oral administration of 10× the recommended dose of ZIMECTRIN GOLD was well tolerated in 5-month-old foals. The package insert states that ZIMECTRIN GOLD has not been tested in pregnant mares, in breeding stallions, or in foals younger than 5 months of age, but also reports that it was found safe when given at 3× the recommended dose in 2-month-old foals. On the other hand, the EQUIMAX paste package insert safety section indicates that it can be used in horses as young as 4 weeks of age, in breeding stallions, and in breeding, pregnant, or lactating mares. Do not use either product in horses intended for food.

Pyrantel and Praziquantel

Two-way combinations of praziquantel and pyrantel are approved for use in dogs (VIRBANTEL FLAVORED CHEWABLES) and in cats and kittens (DRONTAL TABLETS).

Dogs

The canine product is formulated to deliver 5 mg of praziquantel and 5 mg of pyrantel pamoate per kilogram of body weight. A single dose is given to dogs to remove tapeworms (*Dipylidium caninum* and *Taenia pisiformis*), hookworms (*Ancylostoma caninum, A. braziliense,* and *Uncinaria stenocephala*), and roundworms (*Toxocara canis* and *Toxascaris leonina*).

Cats

The feline product is formulated to deliver at least 5 mg of praziquantel and 20 mg of pyrantel pamoate per kilogram. A single dose is given to cats and kittens to remove tapeworms (*Dipylidium caninum* and *Taenia taeniaeformis*), hookworms *(Ancylostoma tubaeforme),* and roundworms *(Toxocara cati).* The product is 98% effective and is well tolerated. Cats maintained in conditions of heavy or constant parasite exposure should be reevaluated in 2 to 4 weeks. This combination product should not be used in kittens weighing less than 1.5 pounds or in those younger than 4 weeks of age.

Ivermectin, Pyrantel Pamoate, and Praziquantel

Ivermectin combined with pyrantel pamoate and praziquantel is available in flavored tablets (IVERHART MAX CHEWABLE TABLETS) for dogs. Adding praziquantel to the two-way combination product of ivermectin and pyrantel pamoate mentioned earlier extends the parasite spectrum to include the tapeworms. The product is formulated to deliver a target dose of 0.006 mg (6 mcg) of ivermectin, 5 mg of pyrantel pamoate, and 5 mg of praziquantel per kilogram of body weight. It is given PO to dogs every 30 days to prevent heartworms *(Dirofilaria immitis)* and to treat and control roundworms (*Toxocara canis* and *Toxascaris leonina*), hookworms (*Ancylostoma caninum, A. braziliense,* and *Uncinaria stenocephala*), and tapeworms (*Dipylidium caninum* and *Taenia pisiformis*). Studies have shown that adult heartworms are not able to maintain detectable levels of microfilariae when exposed to ivermectin, so an antigen test should be used on treated dogs to reveal the presence of adult heartworms (Bowman et al, 1992). Use caution with sick or underweight animals and with dogs weighing <10 lb. Do not give this medication to dogs younger than 8 weeks of age or to those with existing heartworm infection. (See the ivermectin section of this chapter for a discussion of administration of ivermectin to dogs harboring adult heartworms—a procedure that carries some risk and is not a labeled indication for use.)

Pyrantel, Praziquantel, and Febantel

A three-way combination of febantel, praziquantel, and pyrantel is available in the United States for use in dogs as a tablet or chewable product (DRONTAL PLUS TABLETS) formulated to deliver at least 25 mg febantel, 5 mg praziquantel, and 5 mg pyrantel pamoate per kilogram. A single dose is given to dogs to remove tapeworms (*Dipylidium caninum*, *Taenia pisiformis,* and *Echinococcus granulosus*), hookworms (*Ancylostoma caninum* and *Uncinaria stenocephala*), ascarids (*Toxocara canis* and *Toxascaris leonina*), and whipworms *(Trichuris vulpis),* and to remove and control *Echinococcus multilocularis* (Bowman and Arthur, 1993; Cruthers et al, 1993; Bayer Animal Health, 2007). This combination is effective against the nematodes when given in a single oral dose. (Febantel alone requires three daily doses to be effective in monogastric animals.) This combination should not be used in pregnant dogs, in dogs weighing less than 2 lb, or in puppies younger than 3 weeks of age.

Moxidectin and Praziquantel

An oral paste containing moxidectin and praziquantel (QUEST PLUS) is approved for use in horses and ponies. The product has 20 mg/mL of moxidectin and 125 mg/mL of praziquantel formulated to provide 0.4 mg/kg of moxidectin and 2.5 mg/kg of praziquantel when given as directed. The addition of praziquantel extends the parasitic spectrum of moxidectin to include the tapeworm *(Anaplocephala perfoliata)* plus the parasites that moxidectin treats and controls: large strongyles (*Strongylus vulgaris:* adult and L4/L5 arterial stages; *S. edentates:* adult and tissue stages; *Tridontophorus brevicauda:* adults; *T. serratus:* adults); small strongyles: adults (*Cyathostomum* spp., *Cylicostephanus* spp., *Cylicocyclus* spp., *Coronocyclus* spp., *Gyalocephalus capitatus, Petrovinema poculatus*), small strongyle undifferentiated luminal larvae; ascarids (*Parascaris equorum:* adults and L4 larval stages); pinworms (*Oxyuris equi:* adults and L4 larval stages); hairworms (*Trichostrongylus axei:* adults); stomach worms *(Habronema muscae);* and botfly larvae (*Gasterophilus intestinalis* and *G. nasalis*).

This moxidectin combination product is particularly effective against encysted small strongyles and is labeled to suppress strongyle egg production for 84 days. Because moxidectin is fat soluble and very effective against a broad range of parasites, this product should not be the first choice for heavily parasitized thin horses. Although moxidectin is safe for use in mares during breeding, gestation, and lactation, and for foals older than 6 months, this combination product has not been tested in mares during breeding, gestation, and lactation, or in breeding stallions.

Moxidectin and Imidacloprid

One of the more recent topical combination products to hit the market contains imidacloprid for external parasites and moxidectin for internal parasites (ADVANTAGE MULTI). The canine product provides a minimum of 10 mg/kg of imidacloprid and 2.5 mg/kg of moxidectin, whereas the feline product provides the same dose

of imidacloprid and only 1 mg/kg of moxidectin. It is important not to use the canine product on cats because cats are more sensitive than dogs to moxidectin.

Dogs

The canine product is approved topically to kill adult fleas, to treat flea *(Ctenocephalides felis)* infestations, to prevent heartworms *(Dirofilaria immitis),* and to treat and control adult and larval hookworms (*Ancylostoma caninum* and *Uncinaria stenocephala*), roundworms (*Toxocara canis:* adult and larval; *Toxascaris leonine:* adult), and whipworms (*Trichuris vulpis:* adult) (Arther et al, 2005b). The canine product has not been tested in dogs that weigh less than 1.36 kg (3 lb) or that are younger than 7 weeks of age. Nor has it been tested in breeding, pregnant, or lactating dogs. Dogs should be tested for the presence of heartworms before administration. The canine product is not effective against adult heartworms, nor should it be used for clearing microfilariae. It was well tolerated at 5× the label dose. Oral ingestion of the product by dogs may cause serious reactions, including depression, salivation, dilated pupils, lack of coordination, panting, and generalized tremors. Thus it is important to prevent dogs from licking the product from the application site, especially avermectin-sensitive dogs, in which signs may include coma and death. Field studies revealed that nearly 15% of treated dogs were pruritic. Owners also complained of residue left at the application site and of a medicinal odor during application.

Warning: Do not use the dog product on cats.

Cats

The feline product is approved topically to kill adult fleas, to treat flea *(Ctenocephalides felis)* infestations, to treat and control ear mite *(Otodectes cynotis)* infestations, to prevent heartworms *(Dirofilaria immitis),* and to treat and control adult and larval hookworms *(Ancylostoma caninum)* and roundworms *(Toxocara cati)* (Arther et al, 2005a). It should not be used on cats that weigh less than 0.9 kg (2 lb) or on cats younger than 9 weeks of age. This product was well tolerated when 5× the label dose was given to 9-week-old kittens. Cats dosed with a single dose at 10× the label exhibited mild transient hypersalivation. In a field study, adverse reactions included, in order of frequency, a host of behavioral changes (e.g., agitated excessive grooming, hiding, pacing, spinning), discomfort (e.g., scratching, rubbing, head shaking), lethargy, hypersalivation, polydipsia, and coughing and gagging. Foreign market experience includes reports of application site reactions and irritation as well. Oral ingestion of the product may cause hypersalivation, tremors, vomiting, and decreased appetite.

Warning: Do not use the dog product on cats.

Emodepside and Praziquantel

This product is formulated for use in cats (PROFENDER) as a topical spot-on that contains 1.98% emodepside and 7.94% praziquantel. The prefilled applicators deliver a minimum dose of 3 mg/kg emodepside and 12 mg/kg praziquantel when applied to the skin. The active ingredients are readily absorbed through the skin, enter systemic circulation, and act on target parasites in the gastrointestinal tract. It is labeled for use in cats and kittens that are at least 8 weeks of age and is considered safe to use in heartworm-positive cats (Bayer Animal Health, 2010). The product is safe and effective when used to treat and control hookworm infection caused by *Ancylostoma tubaeforme* (adults, immature adults, and fourth-stage larvae), roundworm infection caused by *Toxocara cati* (adults and fourth-stage larvae), and tapeworm infection caused by *Dipylidium caninum* (adults) and *Taenia taeniaeformis* (adults) in cats (Altreuther et al, 2005a; Charles et al, 2005; Reinemeyer et al, 2005). The product was very effective and safe when used in a large-scale clinical study comparing it with topical selamectin–oral epsiprantel (Altreuther et al, 2005b). Studies in rats and rabbits suggest that emodepside may interfere with fetal development (Bayer Animal Health, 2010). Women who are pregnant or who may become pregnant should avoid direct contact with this product and should wear disposable gloves if handling the product is necessary.

Although not approved in the United States, there is an emodepside and praziquantel combination tablet for oral administration to dogs available in Europe. It treats dogs suffering from or at risk for roundworms, hookworms, whipworms, and tapeworms (European Medicines Agency, 2013).

Milbemycin Oxime and Lufenuron

A two-way combination of milbemycin oxime and lufenuron (SENTINEL) is approved for use in dogs. It is formulated to deliver a minimum dose of 0.5 mg of milbemycin oxime and 10 mg of lufenuron per kilogram of body weight. When given every 30 days, it is effective in preventing heartworms *(Dirofilaria immitis)* and in controlling flea populations. The product also kills hookworms *(Ancylostoma caninum)* and removes and controls roundworms (*Toxocara canis* and *Toxascaris leonina*) and whipworms *(Trichuris vulpis).* It should not be used in puppies younger than 4 weeks of age or in those that weigh less than 2 pounds. This product is approved for concurrent administration with nitenpyram (CAPSTAR) for quick knockdown of preexisting flea populations.

RESISTANCE

This section reviews some parasite resistance topics in general and discusses specific resistance issues in several animal species of importance.

Resistance has been heralded when a greater proportion of parasitic organisms within a population are still alive after exposure to a compound than were alive when the general susceptible population was initially exposed to the compound. It would be more precise to define resistance as a drug exposure–induced genetic change in a population of a certain parasitic species, which causes the minimal effective dose that previously killed a defined proportion of the population to be less effective (Shoop et al, 1995). Heritability is the most important feature of resistance (Lanusse et al, 2009a). The fact that a population of parasites, not specific individuals, becomes resistant is of paramount significance. Knowledge of the history (Kaplan, 2004) and mechanisms of action (Lanusse et al, 2009a) of nematode resistance is important to gain a full understanding of the problem, but herein summarization of these aspects will be followed by practical strategies to maintain drug effectiveness for as long as possible.

Helminths first developed resistance to phenothiazine in the late 1950s. The barber pole worm *(Haemonchus contortus)* of sheep was the first to develop resistance, followed shortly thereafter by small strongyles in horses (Kaplan, 2004). These parasites were also the first to develop resistance to thiabendazole. Then several sheep nematodes developed resistance to benzimidazole with equine helminths following suit. So far, once resistance develops, reversion to susceptibility does not occur (Lanusse et al, 2009a; Love and Christley, 2004). As new anthelmintics were introduced, it was found that resistance was documented within 10 years for all major classes, including the avermectin-milbemycin class, just as Sangster predicted back in 1999 when recommending surveillance of the egg reappearance period (ERP) as a tool to reduce anthelmintic usage (Kaplan et al, 2004b; Kaplan et al, 2007; Sangster, 1999).

For many years ivermectin was used without the development of resistant parasites, but in 1993 Shoop reported that a limited number of sheep and goat nematodes had developed ivermectin resistance (Shoop, 1993). Ivermectin-moxidectin–resistant parasites have passed from goats to sheep farmed on the same pasture (USP, 2006). Thoughtfully designed anthelmintic protocols and animal management systems are important to limit resistance to macrocyclic lactones (USP, 2006).

HORSES

Only 11 new endoparasiticides have been developed for horses since 1917 (Love and Christley, 2004). For various reasons, including toxicity and resistance, many have become obsolete, such as febantel, levamisole, trichlorfon, dichlorvos, phenothiazine, and carbon disulfide (Love and Christley, 2004). Before broad-spectrum anthelmintics became available, rotation between drug classes was used to achieve control of various equine gastrointestinal parasites. Rotation programs were set up for a valid purpose at that time—to provide good efficacy against large and small strongyles, ascarids, and pinworms. But with the introduction of broad-spectrum anthelmintics, rotation was no longer necessary for that purpose. Rotation was still recommended, but with a different purpose—prevention of resistance, a purpose that may have been based on a logical hypothesis, but was without scientific proof (Kaplan and Nielsen, 2010).

Many years ago, widespread implementation of rotational interval dosing of anthelmintics resulted in the reduction of *Strongylus vulgaris* while small strongyle (cyathostome) populations grew. Wide availability of macrocyclic lactones led to routine anthelmintic treatment of every horse in the herd every 4 to 8 weeks, regardless of need; this became standard practice and hastened the emergence and escalation of anthelmintic resistance (Kaplan, 2002). We now find that resistance to all classes of equine anthelmintics is emerging in the United States. Small strongyles and ascarids are resistant to pyrantel (Brazik et al, 2006); small strongyles are resistant to ivermectin (Lyons et al, 2008), fenbendazole (Authier, 2000), and oxibendazole (Kaplan et al, 2004b); and ascarids are becoming resistant to ivermectin and moxidectin (Reinemeyer, 2009). A recently published text on equine parasite control provides the following overview of current levels of resistance to three classes of anthelmintics (Reinemeyer and Nielsen, 2013):

- Benzimidazole resistance is widespread in cyathostomins, but nonexistent in ascarids
- Pyrimidine resistance is common in cyathostomins and just starting in ascarids
- Macrolide lactone resistance is widespread in ascarids and just starting in cyathostomins

As has been stated, once resistance develops, reversion to susceptibility does not occur (Love and Christley, 2004). There are controversial studies that suggest otherwise (Blanek et al, 2006; Blanek et al, 2008; Brady et al, 2008). Nonetheless, it is not logical to continue to use a drug to which helminths are resistant; in fact it may be dangerous (Kaplan and Nielsen, 2010).

An important principle for evidence-based management of parasite populations is maintenance of healthy herd refugia. Refugia are parasites that are not exposed to anthelmintics and therefore are not subjected to selection for resistance. Experts suggest that improving refugia will delay the emergence of anthelmintic resistance (Kaplan and Nielsen, 2010; Nielsen et al, 2007). Parasite burdens are not uniformly distributed among herd members, and it is common to find that a relatively small percentage of the herd bears most of the internal parasites. Parasite load is heaviest in foals and young adult horses. About 20% to 30% of horses carry about 80% of the worms. An individual horse's shedding level—high or low—tends to be consistent from one year to the next (Nielsen et al, 2006).

A McMaster slide can be used to perform a quantitative fecal egg count (FEC), the result of which will determine which horses should be treated. Horses with an FEC of fewer than 200 eggs per gram (EPG) should not be treated because (1) the risk of clinical signs due to parasitic disease is low, and (2) the parasites that low-shedding horses harbor will maintain refugia on the farm, which is healthier for the entire herd.

A second evidence-based principle for managing parasites involves determination of the efficacy of currently used anthelmintics on each and every farm. Because tremendous differences in efficacy of a specific anthelmintic may be noted from farm to farm, even in the same region, it is important to perform a fecal egg count reduction test (FECRT) to determine anthelmintic efficacy for each and every herd. To accomplish this, the EPG should be determined for each horse before treatment, and again 10 to 14 days after treatment; if anthelmintic treatment does not reduce the fecal egg count by ≥90% for benzimidazoles or pyrantel, or by ≥95% for macrolides, then parasites on that farm are resistant to that anthelmintic (Kaplan and Nielsen, 2010). Because a reduction in the time it takes for eggs to reappear after anthelmintic treatment precedes development of resistance (Sangster, 1999), monitoring the ERP will be helpful in detecting resistance to ivermectin and moxidectin.

If an anthelmintic is effective on the farm, then it is important to stay with that anthelmintic as long as it continues to be effective. There is nothing to be gained by "rotating" to a less effective anthelmintic. However, that said, "rotating" from a macrolide to a drug that is efficacious for tapeworms could be important because macrolides are not efficacious against cestodes.

Tapeworms may not be causing a resistance problem, but may be causing more pathology in horses than was previously suspected, in part because of difficulty in diagnosis (Jordan et al, 1999; Proudman et al, 1998). The fact that tapeworms are ubiquitous and challenging to diagnose contributes to ongoing discussion among parasitologists regarding their pathogenicity (Elsener and Villeneuve, 2011; Matthews et al, 2004). Horses harboring large numbers of tapeworms may be difficult to identify because the parasites shed eggs sporadically, and clinical signs associated with tapeworm infection, such as colic, are erratically manifested, confounding efforts to accurately estimate parasite load and the effect that the load has on clinical disease. Although serologic and molecular methods of cestode detection have been evaluated (Traversa et al, 2008), fecal egg counts from samples collected just before treatment, 24 to 48 hours after treatment, and 16 to 21 days after treatment provide cost-effective and valuable information about the prevalence of tapeworms and the efficacy of anticestodal treatments (Elsener and Villeneuve, 2011). Many tapeworm-infected horses are negative on fecal exam before treatment but are positive 24 to 48 hours after treatment, revealing a higher prevalence than was previously suspected (Elsener and Villeneuve, 2011; Slocombe, 1979). The use of centrifugal fecal flotation increases the likelihood of finding tapeworm eggs compared with gravitational fecal flotation.

An effective equine parasite management program consists of several other key components. First, proper dosing is important for maximizing efficacy. Veterinarians and horse owners are notoriously inaccurate in estimating body weight, which can lead to under-dosing, decreased efficacy, and increased resistance.

Therefore use of a weigh tape or a scale to determine body weight is highly recommended. In addition, fresh manure should not be spread on pastures, because the practice seeds the pastures with additional parasites. Instead, equine manure should be composted before spreading. Removing feces from pastures twice a week will also help decrease the spread of parasites. Other recommended management practices include making efforts to do the following:

- Avoid high stocking densities
- Refrain from feeding hay or concentrates directly from the ground
- Rotate non-equid species through the pasture to graze, if possible, which will allow host-specific horse parasites to die off
- Refrain from dragging currently occupied pastures, which will hamper natural fecal avoidance tendencies

These simple management techniques can help prolong the efficacy of currently available anthelmintics. Culling the high shedders will make parasite control easier, as will selection of low shedders for breeding stock, which should eventually result in a more genetically vigorous and parasite-resistant herd. For more on the use of evidence-based medicine and realistic herd management techniques to develop individualized and practical equine parasite control programs for clients see Reinemeyer and Nielsen's instructive recently published text, *Handbook of Equine Parasite Control* (Reinemeyer and Nielsen, 2013).

CATTLE

For a long time, ivermectin was reliable regarding lack of parasite resistance, but in 2002, evidence of *Cooperia oncophora* resistance to ivermectin was discovered (Mejia et al, 2003). Ivermectin-resistant *Cooperia* spp. continue to be an ongoing issue (Almeida et al, 2013). One problem is that current anthelmintic treatment programs are based on the assumption that pastured animals contain a variety of parasite species, with the most pathogenic being one of the gastrointestinal roundworms—the brown stomach worm, *Ostertagia ostertagi*. So treatment times are based on that which is optimal concerning *O. ostertagi* transmission. But the appearance of resistant species has changed this archetype because current treatment programs result in cattle with monospecific infection of *Cooperia* or *Nematodirus,* often in high numbers. Although the precise reason for this phenomenon has yet to be proven, it is likely due to several factors, including the removal of parasites that directly compete for preferred gut sites, differences in immune response effectiveness elicited by monospecific versus multiple-species infections, and differences in optimal application for different species (Gasbarre, 2011).

Another factor to consider is the dearth of information regarding the true pathogenicity of either *Cooperia punctata* or *Nematodirus helvetianus*. Although numerous studies have explored *Ostertagia* pathogenicity and some have examined *Cooperia oncophora,* few studies have focused on the pathogenicity of *Cooperia punctata*. One study performed on cattle in an experimental feedlot demonstrated drug-resistant *C. punctata* in high numbers associated with significantly reduced average daily gain.

The nearly exclusive use of macrolides to control cattle nematodes has likely changed parasites genetically. The end result is that cattle parasites are less susceptible to macrolides. Basing cattle parasite control programs on the results of increased fecal testing, especially fecal egg count reduction testing (FECRT) combined with more limited and specific, targeted anthelmintic administration in the appropriate season, is necessary at this time.

SHEEP AND GOATS

As has been mentioned, *Haemonchus contortus* was the first parasite to develop resistance (Kaplan, 2004). More recently, nematode development of multidrug resistance has threatened the viability of the small ruminant business—an industry that has been growing rapidly in the United States. A recent study of sheep and goat farm anthelmintic resistance in eight southern states, Puerto Rico, and St. Croix revealed that *H. contortus* was the most common parasite, and that its population was resistant to moxidectin on 24% of farms, levamisole on 54%, ivermectin on 76%, and benzimidazole on 98% (Howell et al, 2008). On 48% of the farms, *H. contortus* populations were resistant to all three classes of anthelmintics, and on 17%, they were resistant to all three classes as well as moxidectin. As has been discussed, ongoing routine (especially whole herd) use of anthelmintics with disregard for the parasite burden of specific individuals hastens the development of resistance. As an example, the USP ivermectin monograph cautions that to delay the onset of ivermectin parasite resistance, routine use of ivermectin oral solution in goats should be stopped (USP, 2006).

One way that use of anthelmintics in sheep and goats is decreased is by using FAMCHA, an acronym derived from the name of the originator of the system, Dr. Francois "Faffa" Malan (FAffa MAlan CHArt) (van Wyk and Bath, 2002). This system, first used in Africa, is based on chart classifying animals into categories according to their level of anemia and treating only those at risk. The FAMACHA eye color chart follows:

1 = red, nonanemic
2 = red-pink, nonanemic
3 = pink, mildly anemic
4 = pink-white, anemic
5 = white, severely anemic

FAMCHA scores are assigned at least every 2 weeks for all weaned and mature sheep and goats. They are also helpful in monitoring for other changes such as body condition, weight, and, in dairy goats, milk yield. Excellent reviews of the FAMCHA system are available for interested practitioners (Kaplan et al, 2004a; van Wyk and Bath, 2002). The system was effective when small ruminant producers were trained in its use in the southern United States and Puerto Rico (Burke et al, 2007) and should help maintain a nonresistant parasitic refugia population.

Practitioners should strongly promote this system to small ruminant producers and should keep abreast of other alternatives to routine anthelmintic treatment, such as use of copper oxide wire particles, condensed-tannin–containing forages, and nematode-trapping fungi (Howell et al, 2008).

DOGS

There is concern that selection for *Dirofilaria immitis* macrolide resistance is occurring. Reports of prophylactic lack of efficacy (LOE), especially in the Mississippi delta, surfaced in 2005 (Hampshire, 2005). Although the most popular explanations focused on missed doses or altered pharmacokinetics of an individual, mutterings of possible resistance were not completely drowned out. Selection for resistance could be taking place because 10% to 20% of heartworm-infected dogs begin a monthly preventive without adulticide therapy and with persistent circulating microfilaria, exposing those microfilaria to macrolides (Bowman and Mannella, 2011). Although *D. immitis* resistance to ivermectin has been reported and use of a 7-day microfilaria suppression test has been described to confirm such resistance (information that could be used to document geographic distribution of resistant heartworm strains) (Geary et al, 2011), more research is needed.

Current reviews on the topic will provide the interested practitioner with additional information (Bowman and Mannella, 2011; Geary et al, 2011; Bowman, 2012).

SUMMARY

The arc of antiparasite products for veterinary medicine is now past its zenith. The early editions of this textbook chronicled a vast array of narrow-spectrum products with small margins of safety. Subsequent editions heralded the rise of broad-spectrum products like ivermectin and fipronil that provided enormous safety margins and complete lack of resistance. These broad-spectrum products placed the older products into the therapeutic dustbin because they could no longer compete in the commercial marketplace. Now that the new-generation products have secured their hold on the world market we begin to notice the emergence of resistance or the more palatable term "tolerance." First we try to deny the existence, next we try marginalizing it to a few geographic regions or a few problem species, but nevertheless the problem seems to be on the rise. In retrospect, this is the expected outcome of products applied on a global scale to trillions of parasitic species. We should be expecting it when it arrives.

Now that we have realized the emergence of tolerance (i.e., resistance), the veterinary practitioner is forced to take a more rational approach to the use of antiparasite products; we must use products that are known to be effective for that species on that farm in that location; we must use products only on the animals that need them rather than treating the entire flock or herd; and we must use products in combination to help extend their useful lifespan.

We have no way of knowing if or when the next breakthrough molecules will be available for use in veterinary medicine. It is in our collective self-interest, and in the interest of the animals that are entrusted to our care, that we understand the parasites that threaten them and understand the best choices for antiparasite therapy. It is only when we are equipped with this knowledge that we are fully capable of fulfilling our duties as veterinarians.

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CHAPTER 7

Diagnostic Parasitology

For busy veterinarians it is necessary to achieve fairly accurate identification of parasites with a reasonable expenditure of effort. The conventional system is to take advantage of the site and host specificities of parasites and list them according to their customary locations in or on their customary hosts. With the use of this system, it is good to recognize that it must fail in abnormal cases. Whenever doubt arises or the exact identity of a parasite is essential (e.g., as for publication), recourse must be had to detailed morphologic study, preferably by a recognized expert.

The diagnostic categories used in the following discussion do not adhere consistently to any particular level of taxonomic nomenclature. This is because the goals of typologic taxonomy differ from those of applied parasitology. Taxonomists strive to arrange living organisms into ranks and files in a way that, to their tastes at least, best displays the phylogenetic relationships among them. However, the needs of clinicians and clinical parasitologists are best served by diagnostic categories that do not happen to coincide consistently with any particular level of the taxonomist's classification scheme. Therefore we identify an egg from one of several dozens of species of canine tapeworms as a taeniid egg rather than as a *Taenia pisiformis* egg because it is practically impossible to carry the identification of such eggs below the family level. Fortunately, all members of this particular family except *Echinococcus* respond in about the same way to anthelmintic therapy, and the infective larvae of all develop in vertebrate intermediate hosts. Therefore the diagnostic category "taeniid" is adequate to the needs of effective treatment and control. In another instance, the recognition of a worm as a member of its particular phylum may suffice. For example, an acanthocephalan from a pig is almost certain to be *Macracanthorhynchus hirudinaceus*. In still other instances, however, species identification is necessary. For example, the distinction between *Toxocara canis* and *Toxascaris leonina* is important from the standpoints of both animal parasite control and public health related to human larval toxocariasis. Of course, the larvae of *T. leonina* will also persist in the musculature of murine paratenic hosts, so it is a bit uncertain as to whether this is also a zoonotic agent.

Unfortunately many important practical distinctions transcend even the lowest levels of conventional systematics. There exist infraspecific races of many nematodes that may differ remarkably in pathogenicity, antigenicity, and response to pharmacologic agents and yet on morphologic grounds fall into the same species. Here we must make our way with whatever criterion proves helpful.

FECAL EXAMINATION

QUALITATIVE FECAL EXAMINATION

Direct Smear

The direct smear made by breaking up a very small particle of feces in a drop of saline is a simple, quick method. When examining outpatients, many small animal practitioners routinely smear the feces adhering to the rectal thermometer directly on a microscope slide. Use of a coverslip improves the optics, subdues eddy currents, and helps prevent soiling of the objective lens of the microscope. The use of saline rather than water prevents the lysis of fragile trophozoites of protista and amoebic organisms that are subject to distortion by osmotic changes. Because the resulting suspension must be thin enough to read through, only a small particle of feces can be examined, but limited efficiency is the only shortcoming of this technique. Negative findings are inconclusive, but positive results are just as valid as those obtained with the more efficient concentration techniques. In fact, the smear presents advantages over concentration techniques in dealing with delicate forms such as nematode larvae and protistan trophozoites, which may be distorted or destroyed by concentration media, and with particularly heavy eggs that fail to float in them. Direct smears of fresh fecal material also allow us to observe the motility of amoebae, flagellates, nematode larvae, and the like. As a rule, the concentration techniques should supplement rather than supplant the smear, but in practice one or the other technique is adopted as a matter of routine.

Detecting Parasite Antigens in Feces

The detection of antigens in feces **(coproantigens)** by various antigen-capture immunoassays is becoming more and more routine. Methods have been known for some time for the laboratory detection of parasite antigens in feces, particularly those of *Giardia* and *Cryptosporidium*. Now the method is available for routine in-house detection of the cyst wall antigen of *Giardia* in the feces of dogs and cats using the IDEXX SNAP *Giardia* test (IDEXX Laboratories, Westbrook, Maine), and many labs now run similar plate tests for submitted veterinary samples.

The need to be able to distinguish the eggs of taeniid tapeworms in dogs for the purpose of distinguishing the dangerous eggs of *Echinococcus granulosus* and *Echinococcus multilocularis* from the eggs of *T. pisiformis* and other *Taenia* species has led to the development of coproantigen detection enzyme-linked immunosorbent assays

(ELISAs) for these parasites. Thus it is possible to detect the antigens of this parasite in certain laboratories and to distinguish those of *E. granulosus* and *E. multilocularis,* as well as to distinguish them from those of *Taenia* species and other intestinal parasites and pathogens (Deplazes et al, 1999). It has been shown that these types of antigen assays can be used to follow experimental infections with echinococcosis in dogs and to monitor treatment efficacy (Jenkins et al, 2000). It appears that these assays are now good enough to begin to be used routinely in surveys of canine populations to detect the presence of these parasites and perhaps to monitor the success of control programs.

Coproantigens are being examined as to their usefulness in the diagnosis of bovine trichostrongylids. This could prove highly useful for prescreening cattle and other ruminants for various drug-testing protocols. In cattle experimentally infected with *Ostertagia ostertagi,* a coproantigen capture ELISA gave very good results in experimentally infected cattle, showing a rise over the course of the infection (Agneessens, Claerebout, and Vercruysse, 2001); unfortunately, in this early stage, ELISA values were not very well correlated with worm numbers at necropsy, but some correlation was evident. More recently promise has also been found in using an ELISA to look for *Teladorsagia circumcincta* in sheep in which cross-reactivity could be minimized by heat treating of the fecal sample (Johnson, Behnke, and Coles, 2004). There is every reason to believe that such tests are going to become more and more common, and perhaps routine, as they have become for *Giardia*.

Polymerase Chain Reaction

Various genetic markers for different parasites found in feces are now being routinely detected in the case of several protista. The most commonly used are various assays for *Cryptosporidium* and *Giardia* (e.g., O'Handley et al, 2000; Xiao et al, 2001), and this is being driven mainly by the desire to determine the source of parasites that may have caused zoonotic infections in different waterborne outbreaks. More recently, work has begun on the detection of different trichostrongylid species (Schnieder, Heise, and Epe, 1999; Zarlenga et al, 2001). Once such work has been incorporated into a quantitative assay, it may be possible to determine, with DNA extracted from feces, the relative abundance of different worms within the ruminant host. The reverse line blot hybridization method recently used for the identification of horse strongyles, if applied to feces of hosts and their respective helminths would be a very powerful tool to aid in the diagnosis of infection in most domestic animals (Traversa et al, 2007).

Flotation Concentrations of Eggs and Cysts

All flotation techniques take advantage of a difference in the buoyancy of parasites relative to food residues. If some feces are suspended in water, the eggs and solid fecal particles will settle out, allowing the supernatant fats and dissolved pigments to be decanted. If the sediment is then resuspended in a solution intermediate in density between the eggs and fecal debris, the former will float, whereas the latter will sink. In general, techniques based on the flotation principle work well for nematode and cestode eggs and for some protistan cysts but fail to float some trematode eggs, and distort protozoan trophozoites and certain nematode larvae and protistan cysts beyond recognition. Zinc sulfate (specific gravity 1.18) is superior to sucrose of equal density for floating protozoan cysts and nematode larvae because it is slower to shrink and distort them.

Feces puddling is by no means an exact science. The actual procedure followed is less important than a show of respect for the basic principles involved. A workable procedure is outlined as follows:

1. Mix a teaspoonful or so of feces with enough water to make a semisolid suspension. Use a tongue depressor and a paper cup.
2. Place two layers of single-sheet gauze over a second paper cup, and empty the fecal suspension into it. Return the gauze with the solid waste to the first cup and discard.
3. Pinch the rim of the second paper cup to form a pouring spout, and transfer contents to 15-mL centrifuge tubes.
4. Centrifuge for 3 minutes, and decant the supernatant containing fats and dissolved pigments.
5. Add concentrated sucrose solution (specific gravity 1.33) to 1 cm from the top of the tube, and resuspend the sediment with an applicator stick. Insert stopper, and mix by four or more inversions. The viscosity of the sugar solution impedes mixing, but the solution must nevertheless be thoroughly mixed with the sediment.
6. Centrifuge for 5 minutes. Without removing the tube from the centrifuge, pick up the surface film containing eggs and cysts by touching it gently with a "glass nail" or a wire loop. Transfer the surface film to a microscope slide, and add a coverslip. *Variant:* Alternatively, after step 5 has been completed, the centrifuge tube may be filled to the brim with saturated sucrose solution and a coverslip applied to the top. After centrifuging, remove the coverslip by lifting it straight up, and place it and its adherent film of sugar solution on a glass slide. This variant will not work with fixed angle-head centrifuges.
7. Scan the slide under ×100 magnification. To avoid omission or overlap of fields, start by scanning along one edge of the coverslip from one corner to the other. Then shift one field width and continue scanning. The shift can be executed precisely by concentrating attention on any object that happens to lie at or near the edge of the field and moving that object to the other edge with the mechanical stage adjustment. As skill in identification is acquired, the scanning may be done under ×50 magnification with considerable saving of time. Very small objects such as *Giardia* cysts and *Cryptosporidium* oocysts must, of course, be hunted with the high dry lens and perhaps studied further under oil immersion.

Gravitational force may be used in lieu of centrifugal force, but it is weaker and therefore takes longer. Several commercially available, disposable fecal analysis kits that work by gravity afford satisfactory results. If sodium nitrate solution (specific gravity 1.20) is used as flotation medium, the preparation is ready for microscopic examination in 10 minutes. Saturated sucrose solution, because of its greater viscosity, requires 15 to 20 minutes to yield equivalent results. A disadvantage of sodium nitrate is that the slide must be examined promptly. Otherwise, osmotic distortion may have rendered the parasites difficult to identify, or crystallization of the medium may have totally obscured the microscopic field.

Fecal Sedimentation Techniques

Sedimentation techniques, like direct fecal smears, demonstrate objects that are too heavy or too delicate to concentrate by the techniques just described. Sedimentation is more sensitive than the direct smear in terms of the number of organisms demonstrated, and the slide is easier to read because much of the fecal debris has been removed. Sedimentation is particularly appropriate for trematode and acanthocephalan eggs, amoebae, ciliates, and formalin-fixed *Giardia* cysts. However, sedimentation is far less sensitive than flotation in concentrated sucrose for most nematode eggs and coccidian oocysts including *Cryptosporidium,* less sensitive than flotation in zinc sulfate (specific gravity 1.18) for fresh *Giardia* cysts and *Filaroides* larvae, and less sensitive than the Baermann technique described later for larvae of *Strongyloides, Aelurostrongylus,*

and *Dictyocaulus* and other active nematode larvae. It is unfortunate that there is not one best technique that serves all purposes equally well. However, considering the extreme diversity of the organisms with which we deal, our techniques are remarkably few and simple.

The formalin-ether method should be avoided at all costs because ethyl ether has blown up enough people already. The formalin-ethyl acetate method is safer and is probably just as good. Formalin preserves the feces, stops or slows development of most parasites, and reduces the odor of the sample. Ethyl acetate removes fats, pigments, and other substances that interfere with microscopic study. The following outline is freely adapted from Faler and Faler (1984):

1. Mix a teaspoonful or so of feces with 10 mL of water or 10% neutral buffered formalin.
2. Strain the mixture through a tea strainer or two layers of cheesecloth.
3. Transfer the strained mixture to a 15-mL centrifuge tube.
4. Centrifuge for 1 to 2 minutes at 1500 to 2000 rpm.
5. Discard the supernatant.
6. Resuspend the sediment in 10 mL of water or 10% formalin, and repeat steps 4 and 5 until the supernatant is clear.
7. Resuspend the sediment in 10 mL of water or 19% formalin, and add 3 mL reagent grade ethyl acetate.
8. Insert the stopper and shake the preparation vigorously for 30 seconds.
9. Remove stopper and centrifuge for 1 minute at 2000 rpm.
10. Decant supernatant, transfer a portion of the sediment to a microscope slide, and examine.

Note: To duplicate the sensitivity of flotation techniques in detecting most nematode eggs and coccidian oocysts, examine at least half of the sediment microscopically.

Concentration of Nematode Larvae by the Baermann Technique

With the Baermann technique, advantage is taken of the inability of most nematode larvae to swim against gravity. The vertical migrations of nematode larvae on vegetation occur in moisture films, where surface tension translates their sinusoidal body movements into effective locomotion. By contrast, nematode larvae tend to sink gradually in an appreciable body of water within which there is no surface tension. A typical Baermann apparatus is illustrated in Figure 7-1. Break up a fairly large fecal specimen (5 to 15 g); place it in a tea strainer or wrap it in cheesecloth; and place it in lukewarm water in the funnel. The warmth stimulates larval motility, and many larvae will come to the surface of the fecal mass, fall off, and descend to the pinch clamp. In heavy infections, larvae can be drawn off in a drop of water after an hour or so, but when few larvae are present, it may be necessary to leave the "Baermann" set up overnight. If more than a single drop of water is drawn for examination, it will be necessary to centrifuge, decant, and pipette a drop of sediment. Many refinements and modifications of this technique have been performed, but the same simple principle underlies them all.

The infective first-stage larvae of *Filaroides osleri* and *Filaroides hirthi* are lethargic and do not migrate out of the fecal mass. The Baermann technique is therefore an utter failure with respect to *Filaroides* larvae, and it is necessary to resort to the flotation concentration technique with zinc sulfate (specific gravity 1.18) as flotation medium.

Culture of Nematode Larvae

Generic identification of strongylid eggs usually requires rearing infective-stage larvae. Well-formed horse and sheep feces contain just the right amount of water and can usually be successfully cultured merely by placing a few pellets in a covered jar that has been rinsed with 0.1% sodium carbonate solution to inhibit mold growth, and by storing the jar in a drawer or on a dark shelf at room temperature for a week to 10 days. The walls of the jar should always be covered with droplets of condensed moisture. If the culture appears to be drying out, add a few drops of water or sodium carbonate solution. When the jar is returned to the light after incubation, larvae will soon be found squirming about in the condensation droplets on the walls of the jar.

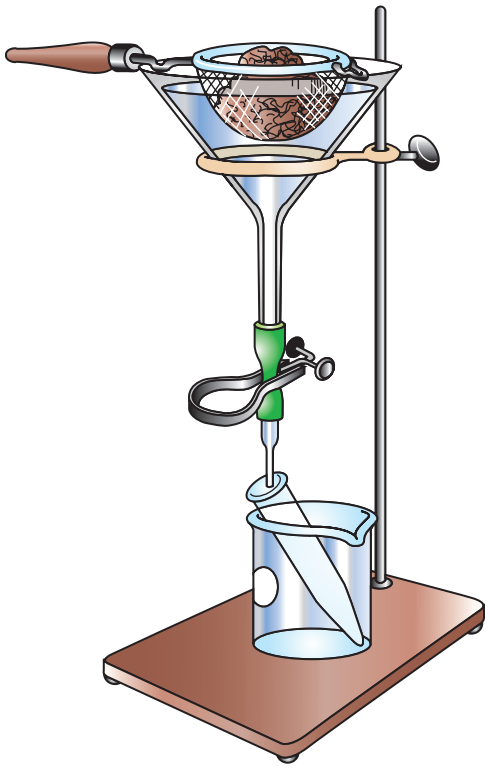


FIGURE 7-1. Baermann apparatus for separating and concentrating nematode larvae from feces, minced tissues, and soil samples. The specimen is placed in the basket of a tea strainer or is wrapped in cheesecloth and immersed in lukewarm water in the funnel. Nematode larvae unable to swim against gravity descend to the pinch clamp and may then be recovered in a small volume of water. A few minutes to several hours may be required, depending on the kind of larvae and the degree of infection.

Cattle feces of similar consistency can also be cultured without further preparation, but usually cattle feces are more fluid and require the addition of vermiculite or sand to produce a damp but not wet culture.

All fecal cultural techniques are essentially qualitative because various species of nematodes have differing optimum conditions for hatching, development, and survival. As a result, the relative abundance of species of third-stage larvae harvested from cultures is not a simple function of the relative abundances of species of strongylid eggs that were present at the start. *Haemonchus contortus* or *Strongyloides papillosus* larvae tend to predominate in culture whenever eggs of either of these species are present in the feces, and the possible clinical importance of *Trichostrongylus* or *Cooperia* should not be discounted because they are represented by only a small number of larvae.

Culture of dog feces for the demonstration of *Strongyloides stercoralis* filariform larvae consists of merely storing the specimen in a jar at room temperature. Filariform larvae of the homogonic

generation appear by 24 to 48 hours, but if the isolate under study is principally or entirely heterogonic, substantial numbers of filariform larvae will not appear in less than 96 hours.

When larvae can be seen swimming in droplets of condensed moisture on the walls of the culture jar, rinse the walls of the jar with a small volume of water, collect the rinsings, and concentrate the larvae by centrifugation. Few larvae will be lost with the supernatant if the decanting is done by simply inverting the centrifuge tube in a single motion. Sediment containing the larvae can then be taken up in the small volume of water retained by cohesion and transferred with a bulb pipette to a microscope slide.

Nutrient agar plates provide excellent growing conditions for certain nematode eggs or larvae that have been separated from feces and concentrated by the techniques already described. For example, rhabditiform larvae that have been concentrated from dog feces by the Baermann technique are deposited on the surface of the agar in a small volume of water and incubated at room temperature. If these are *Strongyloides* larvae, the culture will be found teeming with infective filariform larvae and/or rhabditiform adult worms in less than 2 days.

Identification of larvae often requires that they be killed in an extended posture. This is easily accomplished by judiciously warming the droplet of water before applying the coverslip. Hold a lighted match below the slide and view the cessation of motion and extension of larvae from above. "Relaxation" is the customary euphemism applied to the thermal death of nematodes. Because *Strongyloides* tend to revive, it may be necessary to heat them up again. Avoid overheating the larvae because this distorts them. As an alternative to heating, a drop of Lugol's solution may be added at the edge of the coverslip. This both relaxes and stains the larvae.

Whenever measurements are critical, the coverslip must be supported, or it will press on the larvae and distort them. Ring the coverslip with petroleum jelly to avoid this effect and to retard evaporation. The coverslip may be ringed quickly and conveniently as follows: Spread some petroleum jelly in a thin film on the heel of the left hand. Then, while holding a coverslip edgeways between the thumb and the forefinger of the right hand, draw each edge of the coverslip in turn through the film to obtain a uniform dam of petroleum jelly all around the perimeter.

Culture of Coccidian Oocysts for Sporulation

Mix a small amount of feces or concentrated suspension of oocysts with 1% potassium dichromate solution, and make a shallow pool of this mixture in a Petri dish. Sporulating oocysts need a lot of air, so the pool must be shallow to favor diffusion of oxygen, but do not let the culture dry out; add more dichromate solution if necessary. Sporulation is usually complete after 2 to 4 days' incubation at room temperature, but some species require weeks.

Micrometry

Measuring the lengths of parasites with a microscope equipped with a calibrated eyepiece micrometer sometimes provides the most efficient means of reaching a diagnosis. An object micrometer is a glass microscope slide etched with a linear scale 1 or 2 mm long and subdivided into units of 10 µm (0.01 mm). An eyepiece micrometer is a glass disc etched with a scale of arbitrary units. The disc is inserted into the microscope eyepiece, and the scale may be used to compare linear dimensions of objects in the microscopic field. For example, the ratio of length to width of a particular kind of egg may be determined. To measure absolute lengths, however, one must first calibrate the eyepiece micrometer for each objective magnification against the scale of the object micrometer.

1. Focus the ×10 objective on the scale of the object micrometer.
2. Rotate the eyepiece until the eyepiece scale and the objective scale are parallel.
3. Align their zero marks by adjusting the mechanical stage (Figure 7-2).
4. Locate any point past the halfway mark at which the two scales are in perfect register. The ratio of the object length to the number of eyepiece scale divisions up to this point provides a factor for converting all subsequent eyepiece micrometer measurements made with the ×10 objective to absolute units. In Figure 7-2, 40 eyepiece scale divisions correspond exactly to 170 mm of the object micrometer scale, yielding a ratio of 4.25 mm per scale division.
5. Repeat the calibration procedure for all objective magnifications.

Note: Microscopes with variable tube lengths and other sources of variation in secondary magnification must be brought into the same state of adjustment each time measurements are taken, or else

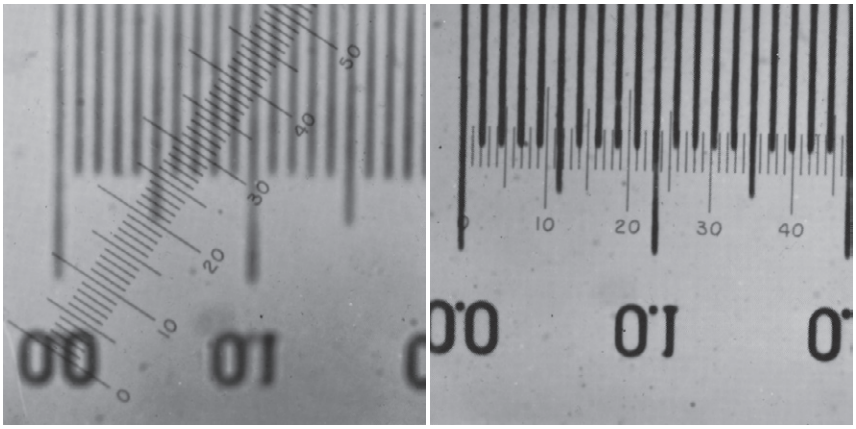


FIGURE 7-2. Eyepiece micrometer calibration. *Left,* The object micrometer scale is out of focus, and the eyepiece micrometer scale is about one-eighth turn out of alignment. *Right,* The scales have been made parallel by rotating the eyepiece; the object scale has been brought into focus, and the zero line (0.0) of the object scale has been aligned with the zero (0) line of the eyepiece scale by adjustment of the mechanical stage. Notice that 0.17 mm (170 mm) equals 40 eyepiece divisions (measuring consistently from the right edges of the rather thick object scale lines), so that at this magnification each ocular division equals 4.25 mm. An oocyst measuring 9 by 5.5 divisions would therefore be 38.2 mm long by 23.4 mm wide.

they must be recalibrated anew. Any variation of the interpupillary distance of certain binocular microscopes alters the tube length and is easily overlooked as a source of error.

QUANTITATIVE FECAL EXAMINATION

Dilution Egg Counts

The Cornell-McMaster dilution egg counting technique as described in the following paragraphs is based on the work of Stoll (1923 and 1930), Gordon and Whitlock (1939), Whitlock (1941), and Kauzal and Gordon (1941).

Briefly, a sample of feces is weighed and is vigorously mixed with water in the proportion of 1 g/15 mL. Aliquots of 0.3 mL are drawn from this suspension and are mixed with equal parts of saturated sucrose solution in a counting chamber. The parasite eggs float in this medium and come to rest at the undersurface of the chamber cover. In this way, all the eggs in a 0.02-g subsample are brought into the same focal plane of a microscopic field that is relatively free of fecal debris. The number of eggs counted in this aliquot is multiplied by 50 to yield an estimate of the number of eggs per gram of feces.

Materials Required

1. Balance sensitive enough to indicate a change of as little as 0.1 g in sample weight.
2. Mixing apparatus (Figure 7-3) consisting of a 250- to 300-mL graduated cylinder with a height-to-diameter ratio of about 2 to 1 (the cylinder in Figure 7-3 was made by sawing off a 500-mL plastic cylinder at the 300-mL mark) and an electric hand drill with a special beater. The beater may be easily fabricated with a brass rod for the shank and a strip of old inner tube for the beater. The beater shank should glide freely through a hole in a rubber stopper that fits the graduated cylinder.
3. Counting chamber (Figure 7-4). Two microscope slides separated by two thicknesses of slide cut into narrow strips and cemented together with aquarium cement. The upper and lower slides should be offset slightly to facilitate filling the chamber. To clean the chamber, rinse under a stream of cold water.
4. Avian tuberculin syringe, 1 mL. The needle hub may be ground off to avoid plugging by coarse debris.
5. Saturated sucrose solution. Add granulated table sugar to boiling water, stirring continuously until no more will dissolve. Cool. Add a few phenol crystals to inhibit mold growth. The specific gravity at room temperature should be at least 1.31.
6. Paper cups, tongue depressors, and dissecting needles.

Procedure

1. Weigh out 10 g of feces in a paper cup (correct for tare) and add to 150 mL water in the graduated cylinder. If less than 10 g of feces is available, reduce the volume of water to preserve the 1:15 proportion.
2. Mix feces and water thoroughly. With the hand drill mixer, only a few seconds is required.
3. (Optional) The suspension may be passed through a tea strainer to remove coarse debris that might interfere with microscopic examination. This is often necessary when horse manure is examined but should be avoided if possible because it may yield lower counts.
4. Place 0.3 mL saturated sucrose solution in each half of the counting chamber (see Figure 7-4).
5. Stir the fecal suspension, withdraw two 0.3-mL aliquots, and add one to each pool of sucrose solution in the counting chamber.

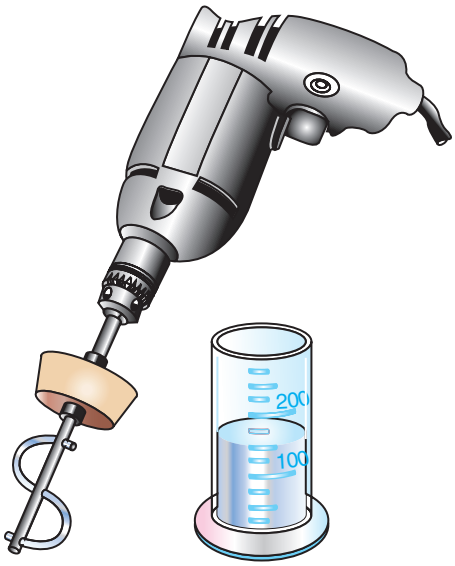


FIGURE 7-3. Mixing apparatus for preparing fecal suspensions.

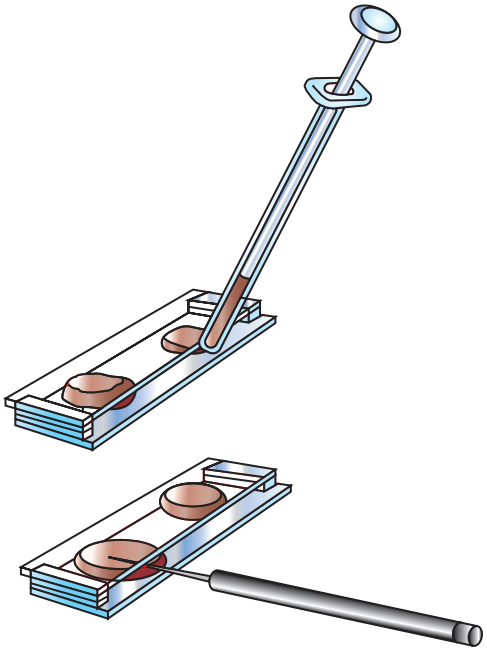


FIGURE 7-4. Loading the counting chamber. Two 0.3-mL volumes of saturated sucrose solution are placed in the counting chamber. Then a 0.3-mL aliquot of fecal suspension is added to each volume of sucrose solution and is thoroughly mixed with a dissecting needle.

6. Mix each aliquot-sucrose pool thoroughly with a dissecting needle, and allow the preparation to stand for about 15 minutes.
7. Count all the eggs in each pool while scanning with the low power of the microscope. The focal plane containing the eggs may be quickly located by the presence of air bubbles. Take care to include eggs lying in the optically darkened borders of the pools.

Variations of this technique with the use of calibrated chambers overcome the difficulty of counting eggs in the optically darkened borders of the pools. Unfortunately such chambers often prove difficult to obtain commercially.

An alternative method with an electric stir plate, a magnetic stir bar, a 100-mL beaker, and magnesium sulfate (Epsom salts) of a

specific gravity of 1.2 as the flotation medium is described in the following procedure with a precalibrated counting chamber (Chalex Corporation, Box 187 Wallowa, OR, 97885, chalexcorp@att.net).

1. Place beaker on balance, tare it, and weigh out 4 g of feces into the beaker.
2. Add approximately 10 mL of the magnesium sulfate solution, and mix well using applicator sticks or a tongue depressor to break up the fecal matter as much as possible.
3. Bring the volume to 60 mL with additional flotation medium, and add a stir bar. Stir for 5 minutes at moderate speed.
4. Using a glass slide to make a score mark, score a pasture pipette halfway between the tip and the barrel and break off the tip to produce a wider bore. (**Caution:** Pasteur pipettes have caused numerous laboratory accidents; use with care.)
5. Load the pipette with the fecal material from the stirring beaker, and fill both chambers on the precalibrated counting chamber.
6. Let the preparation stand 5 minutes to allow the eggs to float to the surface, and then count all eggs within the grids of both chambers using the ×10 objective.
7. Calculate eggs per gram of feces by multiplying the total number of eggs counted in the two chambers by 50.

Concentration Egg Counts

Dilution egg count procedures are less reliable than concentration egg counts for quantifying low levels of parasitic infection (see Statistical Considerations, later). Of course, there is a limit to the number of eggs that can be counted conveniently, so one must choose the procedure best suited to the level of infection. A practical solution is proposed as follows:

1. Weigh out 10 g of feces in a paper cup (correct for tare), and add to 150 mL water in the graduated cylinder. If less than 10 g of feces is available, reduce the volume of water to preserve the 1:15 proportion.
2. Mix feces and water thoroughly. With the hand drill mixer, only a few seconds is required.
3. (Optional) The suspension may be passed through a tea strainer to remove coarse debris that might interfere with microscopic examination. This step is often necessary when horse manure is examined but should be avoided if possible because it may yield lower counts. *Note:* So far, the procedure is identical to the dilution egg count procedure described before.
4. Draw a 15-mL (1-g solids equivalent) aliquot of well-mixed fecal suspension, and transfer it to a 15-mL centrifuge tube.
5. Centrifuge for 3 minutes, and decant the supernatant containing fats and dissolved pigments.
6. Add concentrated sucrose solution (specific gravity 1.3) to 1 cm from the top of the tube, and resuspend the sediment with an applicator stick. Insert stopper and mix by four or more inversions.
7. Add concentrated sucrose solution to the brim, and place a coverslip on top.
8. Centrifuge for 10 minutes. Do not use a fixed-angle centrifuge. The cups must be horizontal during centrifugation.
9. After centrifuging, remove the coverslip by lifting it straight up, and place it and its adherent film of sugar solution on a glass slide.
10. Scan the slide under ×50 to ×100 magnification, counting eggs as you go. To avoid omission or overlap of fields, start by scanning along one edge of the coverslip from one corner to the other. Then shift one field width and continue scanning. The shift can be executed precisely by concentrating attention on any object that happens to lie at or near the edge of the field and moving that object to the other edge with the mechanical stage adjustment.

The number of eggs counted by this procedure provides a minimum estimate of the number of eggs per gram of feces. The estimate can be improved by adding another drop of concentrated sucrose solution to the centrifuge tube, placing a second coverslip on top, and repeating steps 7 through 10. If too many eggs are present on the first coverslip to be counted conveniently, repeat the procedure with a smaller aliquot or resort to dilution egg counting. Perhaps because the sucrose solution used is twice as concentrated, the concentration procedure is more efficient than the dilution procedure in detecting *Eimeria* oocysts.

Interpretation of Egg Count Data

Statistical Considerations

If it were possible to obtain a uniform distribution of parasite eggs in the fecal suspension, we could expect to find the same number of eggs in all aliquots. However, as we mix the suspension, the distribution of eggs does not become uniform but instead becomes a random distribution and stays random as long as we continue mixing. Aliquots from a thoroughly mixed suspension thus represent fair samples drawn from a random distribution, and the numbers of eggs counted in replicate aliquots vary in a predictable fashion.

When relatively rare objects are distributed at random in space (or relatively infrequent events are distributed at random in time), the number of objects to be found in each sample volume (or the number of occurrences in each sample time interval) follows a Poisson distribution. In a 150-mL fecal suspension there is room for well over a billion eggs, yet even in acute haemonchosis, there rarely will be more than a half million present. This means that for every 2000 volumes the size of one *Haemonchus* egg, no more than one volume will actually contain an egg. Therefore eggs counted in aliquots drawn from a well-mixed fecal suspension meet the specifications for "relatively rare objects distributed at random in space," and we can expect the number counted in each sample volume to follow a Poisson distribution.

Applications

Egg-counting techniques may be applied, in principle, to any patent parasitic infection of any host. For practical purposes, however, they find their greatest utility in estimating levels of strongyle infections in ruminants and horses. Under conditions of ordinary husbandry, these species of domestic animals always shed strongyle eggs in their feces, except when they have recently been treated with an effective anthelmintic drug. Therefore the question is not whether these animals are infected with strongyles but, instead, what level of infection is present.

Determining Rates of Environmental Contamination

Most contemporary methods used to control strongyles in grazing livestock depend heavily on periodic medication with anthelmintic drugs to suppress the production of eggs and thereby curtail contamination of the pastures. Unfortunately, when populations of parasites are repeatedly exposed to anthelmintics for several years, they develop resistance to these anthelmintics and their chemical congeners. The more frequently anthelmintic medications are applied, the more rapidly does resistance to them develop. To slow or stop the development of resistance, one should administer anthelmintics only when they are actually needed to reduce a significant rate of pasture contamination. This can be accomplished by performing periodic fecal egg counts on a representative sample

of the herd. When egg output is low, treatment may be delayed until it has reached a point deemed significant in relationship to the extent and productivity of pasture, the stocking rate, the species and susceptibility of hosts, and the objectives of the husbandry operation. The critical number of eggs per gram at which the herd ought to be treated cannot be specified without taking all of these factors into consideration. For example, 1000 eggs per gram might supposedly be an appropriate critical number for clinically normal sheep grazing at low stocking rate under weather conditions favorable to *H. contortus*. However, it would be best not to exceed 100 eggs per gram for brood mares with foals at their sides grazing a small paddock. In both of these cases, the critical number would be subject to revision according to the results achieved and any significant modifications of management practices.

Diagnosing Clinical Illness

High egg counts (e.g., more than 5000 eggs/g for sheep and goats or more than 500 eggs/g for cattle) are easy to interpret. They indicate that these animals are infected with many reproductively active parasites. However, high counts do not necessarily indicate that the host is suffering from clinical parasitic disease, because healthy, well-nourished hosts can often support and compensate for very impressive populations of parasites. Negative egg counts indicate that the host is uninfected or is infected with nonreproductive worms (e.g., developing or arrested larvae, infertile adults). Negative egg counts are typical of the early stages of winter ostertagiosis in cattle and peracute hookworm disease in newborn pups. Such facts tend to discredit quantitative fecal analysis in the minds of those who require short lists of simple, plausible rules. However, when interpreted by minds familiar with the biology of both host and parasite, egg counts provide one valuable insight into the interactions taking place between them.

GENERAL IDENTIFICATION OF EGGS, CYSTS, AND LARVAE

PARASITE VERSUS PSEUDOPARASITE

One must first learn to distinguish between parasites and superficially similar but unrelated objects such as air bubbles, pollen grains, hair, plant fibers, fat droplets, and corn smut spores. Identification of **pseudoparasites** may occasionally shed light on the host's recent dietary adventures. Suppose, for example, that we find *Moniezia expansa* eggs in a specimen of dog feces. We know then that the dog has recently eaten sheep feces because *M. expansa* is a parasite of sheep and never of dogs. Actually, because *M. expansa* is a true parasite when it is in a sheep, its egg should be called a **spurious parasite** rather than a pseudoparasite when it is found in dog feces, but perhaps that distinction is a bit too pedantic. For practical purposes, if a dog or a cat is passing an unidentifiable object in its feces, give the animal an enema, confine it for 24 to 36 hours, and do another fecal examination. If the unidentifiable object is still there, chances are it is a parasite, whereas if it is gone, it was probably a pseudoparasite. It is probably more efficient to learn to identify the bona fide parasites and to ignore the irrelevant rubbish scattered about them rather than trying to identify all objects in the microscopic field. However, some commonly observed objects have regular shapes. Examples of these more common pseudoparasites are shown in Figure 7-5.

Fecal specimens for parasitologic examination should be fresh and not contaminated with soil or bedding. If feces are allowed to stand, single cells develop into morulae, larvae hatch, and oocysts begin to sporulate. Identification of developmental stages other than those usually encountered is possible but requires greater skill. Contamination with soil or bedding will likely lead to confusion because the specimen may be invaded by free-living nematodes and arthropods. Starting, however, with a fresh, uncontaminated specimen may frequently allow a more specific identification by observation of the subsequent development in fecal culture.

NEMATODE EGGS

Nematodes have eggs. An egg contains a fertilized zygote, with fertilization of the ovum by the amoeboid sperm having occurred within the oviduct and the seminal receptacle before the eggs enter the uterus. The shell proper of the nematode egg is a smooth, homogeneous, transparent capsule of chitin. An internal lipid layer (vitelline membrane) and a narrow fluid-filled space separate the capsule from its contained embryo. Depending on the parasite, the egg may be passed with the zygote in a single-cell stage, having undergone a number of divisions, or already developed to contain a fully formed first-stage larva. In some cases, first-stage larvae hatch from the eggs within the host and are passed in the feces.

Nematode eggs representative of the different orders and superfamilies of these parasites have characteristics that typify the group. Thus an egg can usually be identified as that of an oxyurid, ascaridid, spirurid, rhabditoid, strongylid, or trichinelloid. In general, nematode eggs vary in size from 30 µm to 100 µm in greatest diameter, although a few examples such as *Nematodirus* may be up to 200 µm in length.

The Oxyurid Egg

The eggs of the oxyurid parasites of ruminants, horses, and primates tend to have a rather thick, colorless shell and to contain a larva when observed. Most of the eggs also appear flattened on one side. The large pinworm of the horse, *Oxyuris equi,* has an egg that appears to have an operculum on one end. Dogs and cats are not hosts to pinworms, so the presence of these eggs in their feces should be considered a spurious finding unless proven otherwise (Figure 7-6).

The Ascaridoid Egg

The eggs of the ascaridoid parasites of domestic animals are typically thick shelled and oblong to spheric in appearance. In some ascaridoid eggs is an apparent operculum, as in some eggs such as those of *Porrocaecum* from hawks (Figure 7-7). When passed in the feces, these eggs tend to contain a single cell. Some eggs, such as those of *Toxocara, Parascaris,* and *Ascaris,* are covered with an albuminous coat applied by the female over the chitinous eggshell; this layer of protein may be smooth as in *Toxascaris* (Figure 7-8), rough as in *Parascaris* (see Figure 7-77), or uniformly and distinctively patterned as in *Toxocara* (Figure 7-9). The material may be tanned in the fecal stream, giving it a dark-brown color as in *Ascaris* and *Parascaris*. This material may sometimes break off from the eggshell, and the egg will then appear with a clear smooth shell. The eggs of infertile ascaridoids are sometimes found in the feces, and their shape is often less regular than that of the fertilized egg. The eggs of ascaridoids tend to be large overall, around 80 to 100 µm in diameter.

The Spirurid Egg

The eggs of the spirurid nematodes found in feces are of at least two basic types. One type of egg, represented by those of *Physaloptera* and *Spirocerca,* is about 30 µm long, is covered by a thick colorless shell, and contains an embryo. These eggs are typical of spirurids transmitted by terrestrial coprophagous insects (Figure 7-10). The other spirurids, such as *Habronema* and *Draschia,* have very thin

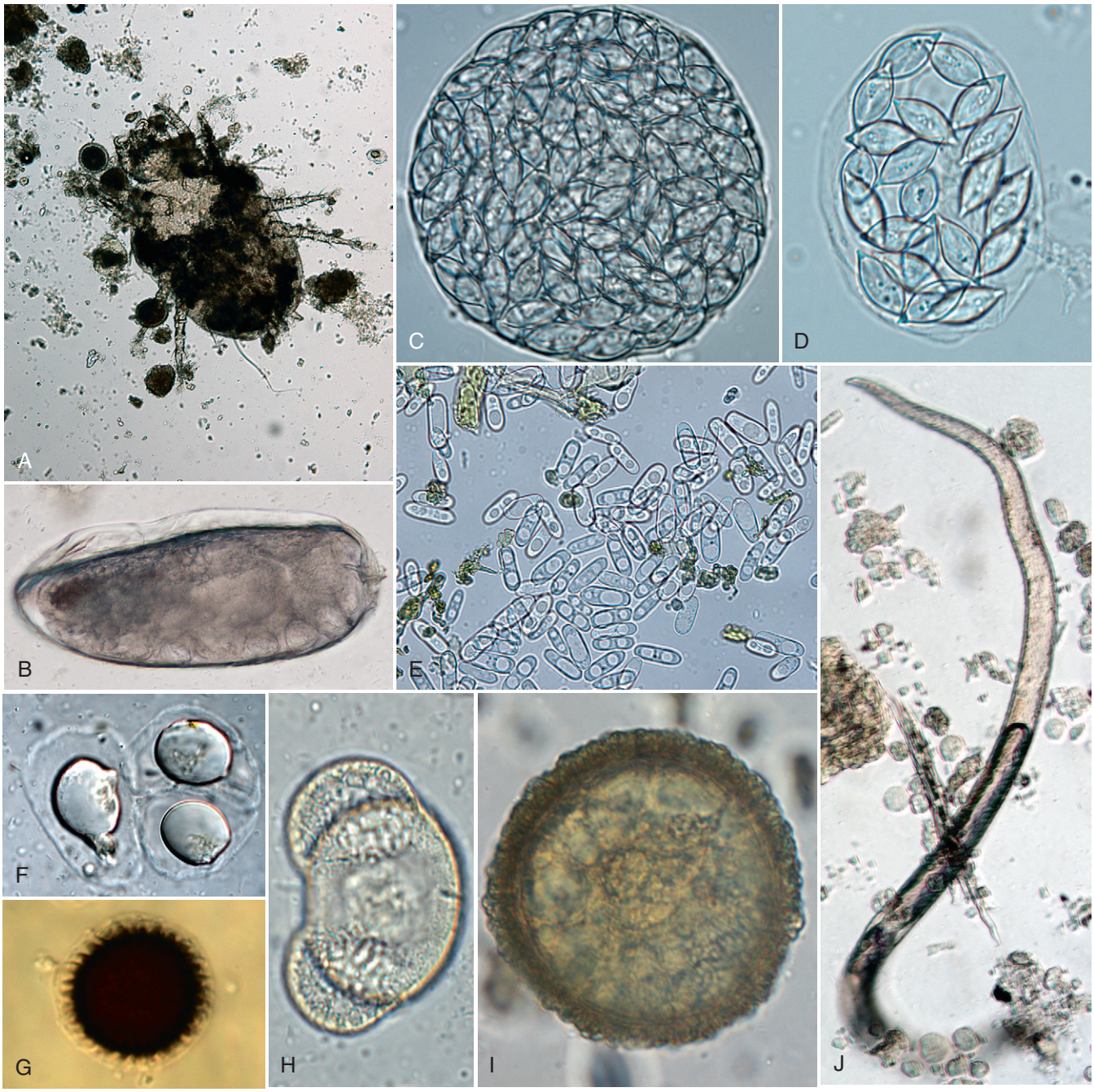


FIGURE 7-5. Pseudoparasites. **A,** *Cheyletiella blakei* (≈500 µm), an arachnid parasite of the cat, and *Toxocara cati* eggs. **B,** Egg of *C. blakei* (≈240 µm). **C,** *Monocystis,* a gregarine parasite of the earthworm (≈55 µm). **D,** *Monocystis* sporulated (each sporulated oocyst ≈15 µm long). **E,** *Cyniclomyces guttulatus (Saccharomycopsis guttulata),* yeast (each about 10 to 20 µm long) found in the feces of dogs and rabbits with some believed perhaps pathogenic. **F,** Plant cells (≈50 µm in diameter). **G,** Corn smut spore (≈20 µm). **H,** Pine pollen (≈60 to 70 µm long). **I,** Hemlock pollen (≈70 µm). **J,** Plant hair (≈500 µm long).

eggshells that may be distorted by the contained larva (see Figure 7-77). These eggs and the contained larva are typical of those spirurids transmitted by flying insects that obtain their infection through the feeding of maggots on fecal material. Filariids are an ovoviviparous form of spirurid that produces microfilariae rather than eggs.

The Rhabditoid Egg

The rhabditoid eggs found in the feces of domestic animals are of two types. One type represents the spurious eggs of soil nematodes that have been ingested by a host or even laid by free-living coprophagous nematodes that have invaded a fecal pat. The second type of rhabditoid egg represents the eggs of those parthenogenetic females of the *Strongyloides* species that produce eggs (Figure 7-11). In domestic animals in North America, only *S. stercoralis* of the dog and human typically produces larvae. Other *Strongyloides* species, such as *Strongyloides felis* of cats in Australia and Southeast Asia and various species in wildlife hosts, also shed larval stages in the feces. The eggs of the *Strongyloides* species shed by horses, swine, and ruminants are typically small, with a thin colorless shell, and

contain a larva. In feces that are not fresh, the small size of these eggs, less than 50 µm, will be one of the best criteria for separating these eggs from those of developed strongylid eggs.

The Strongyle Egg

Females of the superfamilies Strongyloidea, Trichostrongyloidea, and Ancylostomatoidea lay rather thin-walled ellipsoidal eggs containing an embryo in the morula stage of development, and this same stage is found in the host's feces (Figures 7-12, 7-13, and 7-14). In this text, such eggs are collectively referred to as "strongyle" eggs because that is what most clinicians and diagnostic parasitologists call them. The eggs of Metastrongyloidea are also thin-walled and ellipsoidal, but the developmental stage deposited in the host's tissues by different species of female metastrongyloids varies from a single cell (e.g., *Muellerius*) to a first-stage larva that is ready to hatch (e.g., *Filaroides*). Even those laid in the single-cell stage develop to the first larval stage and may have hatched by the time they appear in the feces. Therefore either larvated eggs (e.g., *Metastrongylus*) or first-stage larvae are found in the feces of hosts with patent metastrongyloid infection.

A Diagnostic Dilemma

With few exceptions, the generic identity of individual strongyle eggs cannot be established reliably by microscopic inspection or micrometry (see Figure 7-61). *Nematodirus* eggs stand out because of their large size, and *Bunostomum phlebotomum* eggs have sticky surfaces that accumulate debris, but the rest look very much alike. Necropsy of a few animals to establish an accurate diagnosis is justifiable if the unit value of the animal is sufficiently low or the herd sufficiently large. Owners of valuable animals are understandably reluctant to sacrifice them, however, and recourse must be had

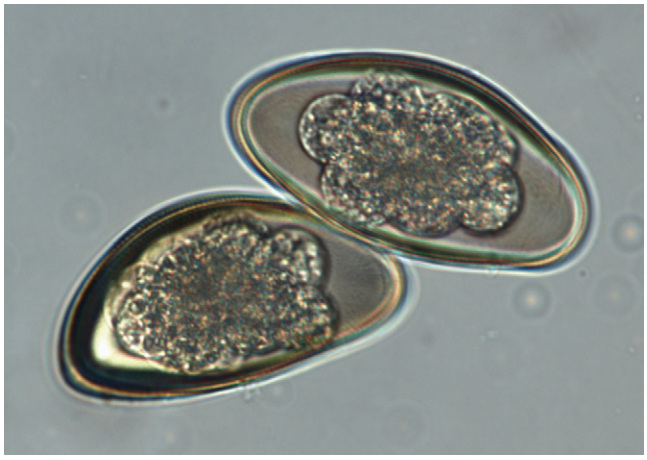


FIGURE 7-6. Oxyurid (pinworm) eggs from a bearded dragon, *Pogona vitticeps*.

FIGURE 7-7. Ascaridoid, *Porrocaecum,* egg from a red-tailed hawk, *Buteo jamaicensis*.

FIGURE 7-8. Development of *Toxascaris leonina* eggs. **A,** One-cell stage typically found in fresh fecal specimens, with shell layers indicated by opposed arrowheads. **B,** Two-celled stage. **C,** Morula stage. **D,** Infective larva in eggshell. **E,** Infective larva artificially hatched in vitro. Hatching of ascarid eggs does not normally occur until they have been ingested by a host (×425).

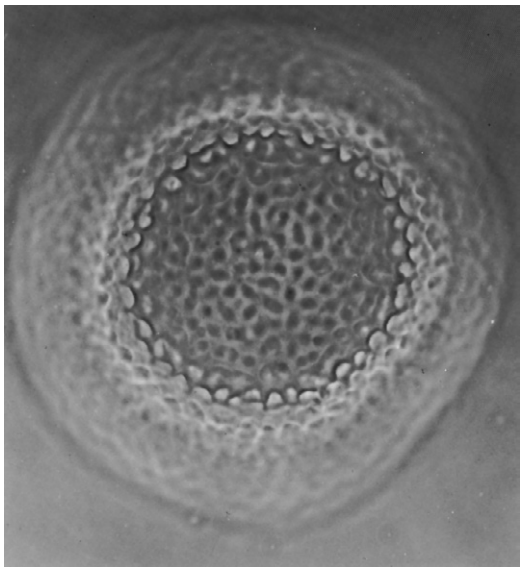


FIGURE 7-9. Surface of a *Toxocara canis* egg cleared in Berlese solution to show the distinctive dimpled pattern of the protein layer (phase contrast ×660).

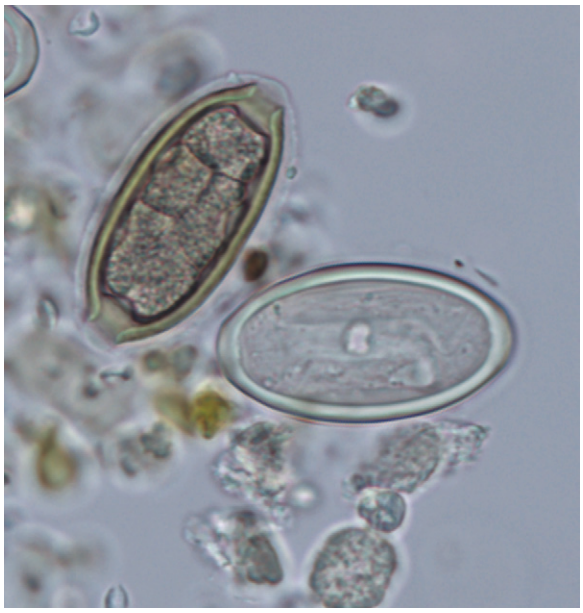


FIGURE 7-10. Eggs of a spirurid *(Tetrameres)* and a trichinelloid (capillarid) from a duck.

FIGURE 7-11. Rhabditoid egg *(Strongyloides papillosus)* from a goat.

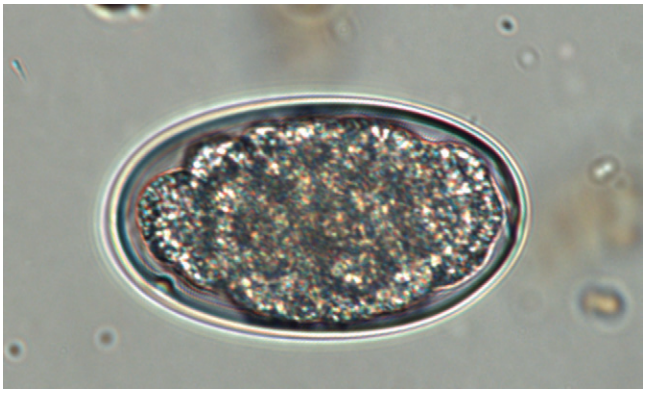


FIGURE 7-12. Strongylid egg (*Obeliscoides cuniculi*, Trichostrongyloidea) from a rabbit.

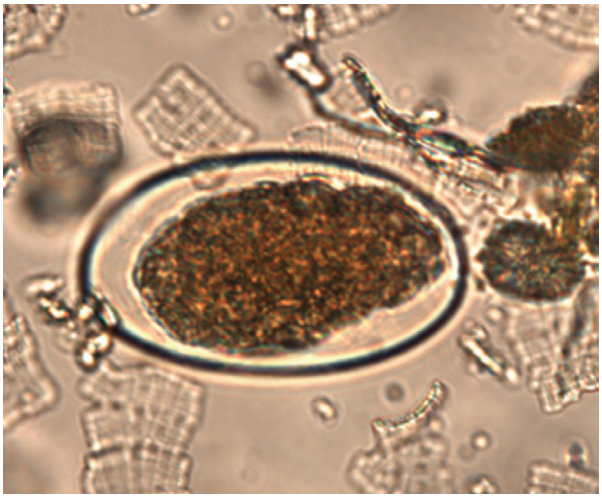


FIGURE 7-13. Strongylid egg (*Oesophagostomum* sp., Strongyloidea) from a gorilla; this egg was fixed with formalin, and the morula appears somewhat contracted.

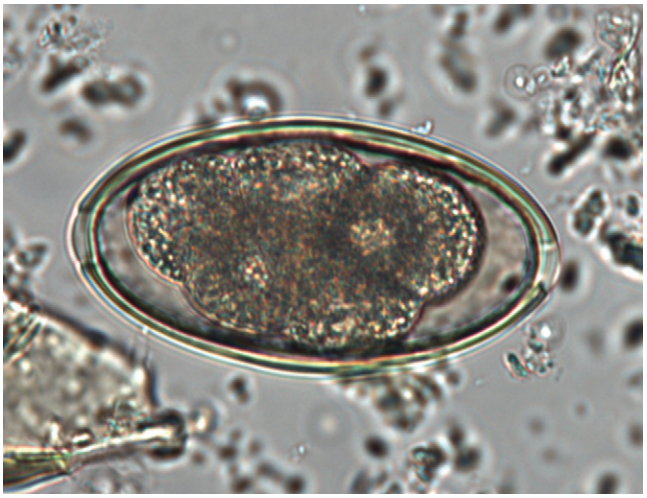


FIGURE 7-14. Strongylid egg (*Syngamus* sp., Strongyloidea) from a crow, *Corvus brachyrhynchos*.

to larval identification (see discussion of identification of strongyle infective larvae). Whenever the situation is too urgent to afford the necessary delay of culturing, the clinical signs should be clear enough to suggest a reasonably accurate diagnosis.

The Trichinelloid Egg

The eggs of *Trichuris* and capillarids are typically brown-shelled with polar plugs and tend to be elongate or barrel-shaped. *Trichuris* is confined to mammalian hosts. Therefore when these eggs are found in other vertebrates, the first thought should be that they are capillarid eggs. The eggs of the *Trichuris* species tend to have smooth shells, whereas those of capillarids tend to have various forms of delicate surface ornamentation (e.g., pits, roughened areas, small wavy lines). Capillarid eggs, unlike those of *Trichuris* species, may have polar plugs that are not on the same straight-line axis (Figure 7-15). However, the eggs of *Trichuris* may become highly distorted after drug therapy that has not removed all female worms. Both *Trichuris* and capillarids tend to have eggs that are single-celled or in the early stages of division when passed in the feces. The eggs of *Anatrichosoma* and *Trichosomoides* are different in that they contain a fully formed larva. In the dog, the eggs of the capillarids are smaller than the egg of *Trichuris vulpis,* which is about 80 µm long. Unfortunately, this is not necessarily true for other mammalian hosts.

NEMATODE LARVAE

The larvae of nematodes shed in the feces are most readily identified with reference to the host parasitized and therefore are discussed for each host as appropriate. The initial goal must be to identify them for what they are and not confuse them with hairs, threads, or plant fibers. The more common problem is finding an artifact and thinking it a nematode larva. Most individuals will recognize a larva when they see one (Figure 7-16). The important thing is to not forget to look for them. The nematode larvae found in the feces of domestic animals all tend to be around 300 µm in length. Special attention must be paid to the relative lengths of the buccal capsule and esophagus, the structure of the tail, and the size and position of the genital primordium. If feces are old or are collected from the soil, many nematode larvae may be present that have hatched from eggs of developed parasitic forms or from soil or coprophagous nematodes that have invaded the fecal matter. The process of identification is much more difficult in these situations.

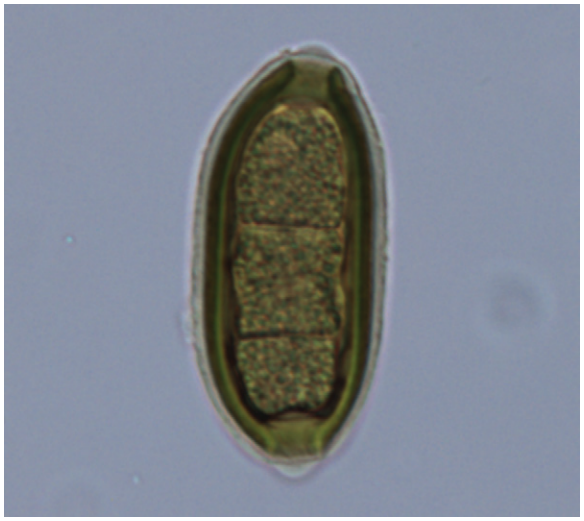


FIGURE 7-15. Trichinelloid egg (capillarid) from a duck.

TREMATODE EGGS

The eggs of trematode parasites of vertebrates tend to have a golden to dark-brown color and to have an operculum on one end (Figure 7-17). The eggs can vary in size from 20 to 200 µm in maximum length. Some of these eggs contain a fully formed miracidium when passed in the feces, whereas others contain a number of developing cells. In the identification of trematode eggs, attention must be given to the size and shape of the eggs, as well as to whether they contain an embryo, whether the operculum appears as a simple cap or a cap in an indented seat or rim on the eggshell, and whether there are any structures on the shell such as bumps or spines opposite the operculum. The eggs of the schistosomes are not operculate, contain fully developed miracidia when passed with the feces, and often have different types of spines on one end of the eggshell

FIGURE 7-16. *Didelphostrongylus* larva (Metastrongyloidea) from the feces of an opossum, *Didelphis virginiana*.

FIGURE 7-17. Trematode egg (Strigeidae) from a great horned owl, *Bubo virginianus*.

depending on the species involved. Trematode eggs tend to be denser and not to float as well as those of nematodes in many of the lighter flotation media, and in sugar the eggs often rupture and appear as empty brown shells that may be collapsed on one side. When dealing with the schistosomes, one must take care to wash the feces with saline rather than water because water induces the miracidium to hatch, making the eggs harder to find and identify.

CESTODE EGGS

Some tapeworms commonly shed eggs into the fecal stream (e.g., *Diphyllobothrium*), whereas others more typically shed segments (e.g., *Taenia*). However, it is not uncommon to find eggs or egg capsules of the latter type in fecal matter from which the segments may have crawled away before collection. The larvae that develop in these eggs will have six hooklets (three pairs) (Figures 7-18 and 7-19), but larvae in the eggs of the pseudophyllidean tapeworms *Diphyllobothrium* and *Spirometra* will not have developed by the time the eggs are passed in the feces. The eggs of these two species are also operculate and may initially be confused with the egg of a trematode. Confusion may persist even after a good deal of study of the actual eggs and pictures of them. The eggs of the cyclophyllidean tapeworms contain six hooklets when passed, which will help to identify the eggs as tapeworm eggs (see Figure 7-19). The "shells" of cyclophyllidean tapeworms can vary markedly (e.g., the thick, brown surface of a taeniid egg, the thin shells on the individual eggs of *Dipylidium,* and the odd-shaped square to round eggs of the various anoplocephalid genera; see Figures 7-18 and 7-76). Tapeworm eggs appear to behave erratically in different flotation media and can be hard to demonstrate even when present. Sugar solution works well for taeniid eggs, but not for many of the other egg types that may be encountered.

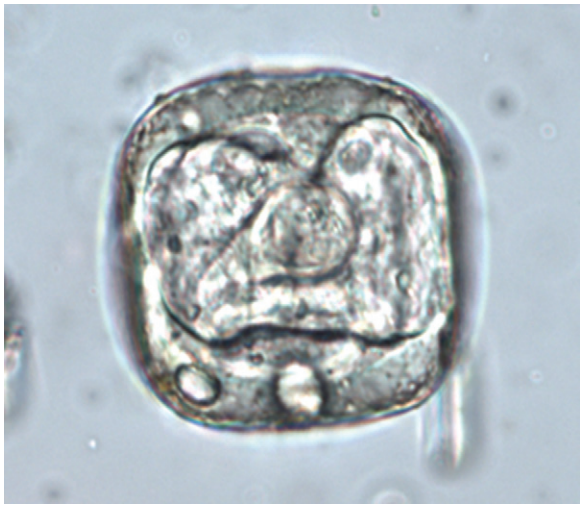


FIGURE 7-18. Tapeworm egg (Anoplocephalidae) from a gorilla, *Gorilla gorilla berengi*.

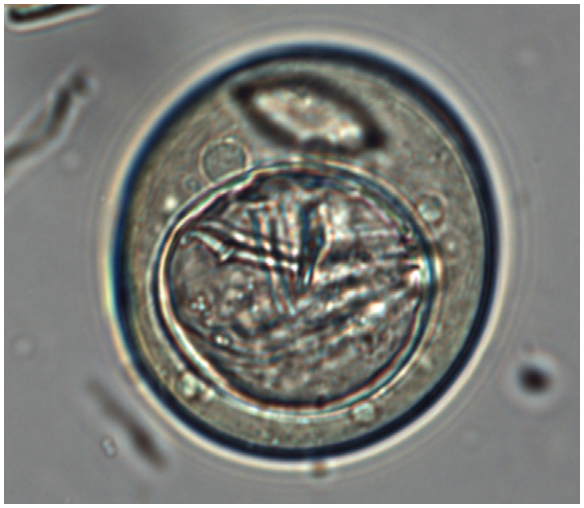


FIGURE 7-19. Tapeworm egg (Cyclophyllidean) from a chicken. Note the hooklets in the embryo (oncosphere) within the egg. Underneath the egg is a *Monocystis* sporocyst that was probably ingested in an earthworm.

ACANTHOCEPHALA EGGS

The eggs of Acanthocephala tend to be elongated and have shells composed of three layers (Figure 7-20). If the larva inside can be seen, the spines present on one end of the larva can often be identified, which clinches the diagnosis. The eggs of some Acanthocephala often appear dark brown in the feces (e.g., *Macracanthorhynchus* species) and are probably tanned in a manner similar to ascaridoid eggs, because the eggshells are clear when the eggs leave the female worm. Not all acanthocephalan eggs are brown, and the very clear ones may be difficult to observe, particularly if one is not expecting to find them. Numerous Acanthocephala are present in wildlife hosts, and it is here that skill has to be developed in diagnosing infection with different species.

PENTASTOMID EGGS

In the United States the eggs of pentastomids will most typically be observed in the feces of snakes or gulls. Elsewhere in the world they can be observed in the feces of dogs and other hosts. Pentastomid eggs are typically quite large, 100 to 200 µm in diameter, with a thin external shell that surrounds what looks like a developing mite. The developing larva is often separated from the eggshell by a rather large area of empty space. The difficulty lies in determining whether what is observed is the egg of a pentastomid or the egg of a mite that has been ingested. It is not uncommon to find in feces the eggs of free-living mites and parasitic mites ingested while an animal is grooming. The pentastomid developing within the egg will typically have four or six small claws, which might help distinguish the mite from the pentastomid (see Figures 2-125 and 8-10).

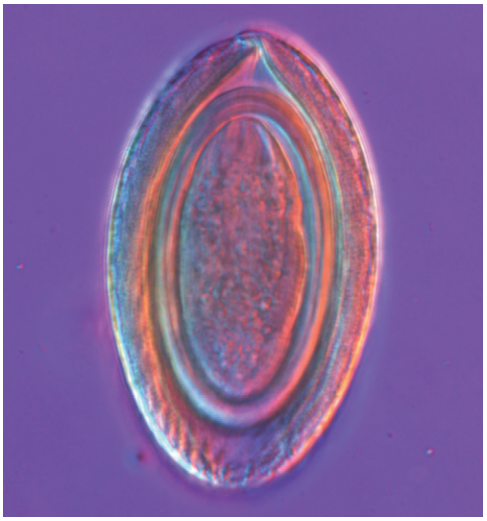


FIGURE 7-20. Acanthocephalan egg *(Macracanthorhynchus ingens)* from a raccoon; note the several layers to the eggshell and the contained acanthor larva.

PROTISTAN CYSTS AND OOCYSTS

The cysts and oocysts of the protista will range from 4 to 30 µm in greatest diameter, with the odd large cysts of *Balantidium* and *Buxtonella* (Figure 7-21 and see Figures 7-98 and 7-116) reaching sizes of 40 to 60 µm and the thick-walled oocysts of *Eimeria leuckarti* and *Eimeria macusaniensis* (Figure 7-22) reaching 80 µm in length. The cysts of *Giardia* appear rather clear in both zinc-sulfate and sugar preparations, and their overall appearance is similar to that of amoebae that are more round in outline. In many flotation media, cysts of *Giardia* will appear collapsed internally with the ovoid cyst wall remaining intact, whereas collapsed cysts of amoebae may appear much like ping-pong balls that have been indented to varying degrees on one side. The oocysts of *Cryptosporidium* are very small and can be found near the surface of the coverslip. They are much easier to see in a sugar flotation, where they will appear as a hyaline pink body, than in zinc-sulfate, where they appear to be clearer. The oocysts of *Isospora* and *Eimeria* do very well in sugar flotation media and present a crisp, clear image of a shell and a central sporoblast. On many species of *Eimeria,* the micropyle and micropylar cap, when present, can be easily discerned. On some *Eimeria* species, it may be difficult to make out the micropyle on all specimens. The oocysts of *Toxoplasma* are similar in size to the cysts of *Giardia*. If the aperture on the condenser diaphragm is not closed and the light coming through the scope is too bright, many of the smaller protista will disappear into the background.

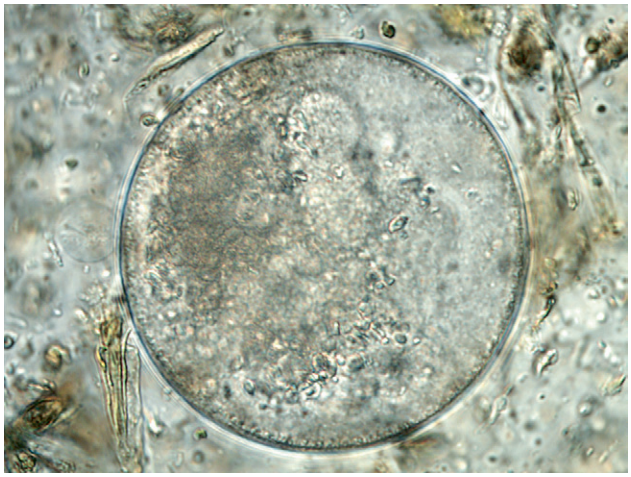


FIGURE 7-21. Ciliate cyst *(Buxtonella sulcata)* from the feces of a cow.

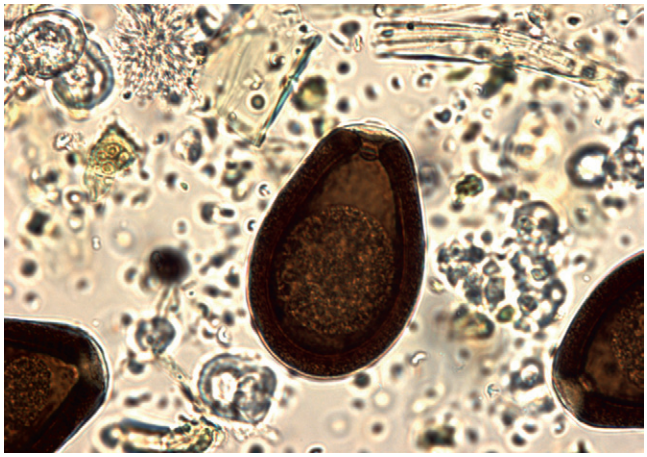


FIGURE 7-22. Coccidian oocyst *(Eimeria macusaniensis)* from a llama.

SKIN SCRAPINGS FOR MANGE DIAGNOSIS

Skin scrapings for mange diagnosis must be obtained in a manner that takes into account both the nature of the lesion and the location of the mite in question; also, it must be remembered that not all mites live in or on the skin (Figure 7-23, and see Figures 2-98 to 2-100).

For lesions with minimal epidermal hyperplasia and lesions caused by deeply burrowing mites (e.g., *Sarcoptes, Demodex*), dip a scalpel blade into mineral oil, pinch a fold of skin firmly between the thumb and forefinger, and, while holding the blade at right angles to the skin, scrape until blood begins to seep from the abrasion. Most animals do not object to deep scraping, although local anesthesia may occasionally be required. Much of the detritus will adhere to the layer of mineral oil on the scalpel blade and may be transferred to a microscope slide and searched for mites.

For lesions with marked epidermal hyperplasia and exfoliation and lesions caused by lice and superficially dwelling mites (e.g., *Chorioptes*), scrape the detritus into an ointment tin using the cover as a scraper. Examine scrapings under a stereoscopic microscope or with a hand lens to find the lice and mites crawling about. Dip fine-tipped thumb forceps or a dissecting needle into Berlese solution, and use this sticky mounting medium to pick up mites and transfer them to a slide for closer study under the compound microscope. Berlese solution is made by mixing 200 g chloral hydrate, 30 g gum arabic, 20 g glycerin, and 50 mL distilled water; boiling this mixture for 5 to 15 minutes; and filtering it through cheesecloth. Berlese solution clears the specimen and hardens to produce a permanent preparation. Unfortunately, chloral hydrate is now regulated as a narcotic, and different lots of gum arabic vary considerably in quality, so that good Berlese solution is getting hard to come by. Glycerin is a reasonably satisfactory temporary mounting medium. Five percent sodium or potassium hydroxide solution also may be used as a temporary mounting medium that digests epidermis and hair, thus helping to clear the microscopic field of debris.

If the scraping contains much debris, and no lice or mites have been found by inspection with the stereoscopic microscope or the hand lens, proceed as follows:

1. Add 10 volumes of 5% KOH to 1 volume of skin scrapings in a large (500- to 1000-volume capacity) beaker, cover with a watch glass or funnel to return condensate, and heat until hair and epidermal scales dissolve. It may be necessary to boil the mixture, but do not allow it to boil dry. Beware of spattering lye!

FIGURE 7-23. *Orthohalarachne attenuata* (mite, Halarachnidae) from the nasopharynx of a northern fur seal, *Callorhinus ursinus*.

2. Allow to cool.
3. Transfer to a centrifuge tube, centrifuge, decant supernatant, resuspend sediment in water, and centrifuge again. These steps dispose of interfering soaps. Decant the supernatant.
4. Transfer sediment to a Petri dish, and search for mites and eggs with a stereomicroscope or a ×10 pocket lens, or proceed with step 5.
5. Add saturated sucrose solution to the tube, and centrifuge again. Pick mites off the top of the sucrose solution with a wire loop or a glass nail, and transfer them to a microscope slide for study under the compound microscope.

Ear mites may be removed from the external ear canal with a cotton swab. If the swab is placed on a dark background in sunlight or near an incandescent lamp, white *Otodectes* mites may be seen crawling about within a few minutes.

NECROPSY PROCEDURES

Occasionally, severe or fatal parasitosis may escape antemortem diagnosis. For example, pups with peracute hookworm disease may bleed to death before shedding an egg. When disease breaks out in a flock of sheep, postmortem examination of a few sick animals often provides the most efficient and economical means of arriving at a diagnosis. In strongylid infections of sheep, various combinations of primary and secondary pathogens often yield a confusing array of clinical signs that may be resolved by identification and enumeration of the worms.

Necropsy findings must be correlated with the case history and clinical signs to arrive at a definitive diagnosis. This is especially true of parasitic diseases. For example, a diagnosis of acute haemonchosis must rest not only on the demonstration of a sufficient number of *H. contortus* worms in the abomasum, but also on the existence of clinical anemia. If there is no anemia, then there is no haemonchosis. In fact, *H. contortus* worms sometimes desert a moribund host so that, on necropsy examination, pallor and edema of the tissues is found, but no worms. The correct diagnosis is still haemonchosis.

OPENING THE CADAVER

Arrange a ruminant cadaver on its left side to get the rumen out of the way. Cadavers of other species are about equally accessible from either side, but you should adopt a consistent approach to develop a mental image of the normal appearance and location of the various organs, so that any abnormal relationship will be quickly noticed. Incise the skin along the midline from the submaxillary space to the perineum. Reflect the skin from one side, including the superficial thoracic muscles and the pectoral limb with it, so as to lay bare the rib cage. Cut the ribs close to the axial muscles and the costal cartilages close to the sternum. Lift away the rib cage, severing attachments to the diaphragm in the process. Incise the abdominal wall along the midline, taking pains to avoid puncturing the viscera. Carry the incision across the brim of the pubis, and reflect the abdominal wall. Split the pubic symphysis or incise the ligaments of the hip joint and reflect the pelvic limb.

THORACIC VISCERA

Incise the intermandibular muscles, hyoid apparatus, and other attachments, and dissect the tongue, larynx, trachea, and esophagus. Removal of the heart and lungs is facilitated by traction on the trachea and esophagus. The points of attachment (aorta, cavae, azygos vein, various ligaments) are easily found and severed. Remove the thoracic viscera from the carcass. Lay open the tracheobronchial tree, heart chambers, cavae, aortic trunk, and ramifications of the pulmonary arteries, and inspect the contents and linings for macroscopic parasites. Very small metastrongylid nematodes (e.g., *Muellerius capillaris, Aelurostrongylus abstrusus, Filaroides hirthi*) are practically invisible grossly. These may be demonstrated in squash preparations of their grayish subpleural nodules. The Baermann technique is useful for demonstrating larvae of lung nematodes (e.g., *Muellerius, Aelurostrongylus*) but usually fails in the case of *F. hirthi* because the larvae of this parasite are too lethargic to migrate out of the lung tissues.

ABDOMINAL VISCERA

Examine the peritoneum for cysticerci, tetrathyridia, encysted pentastomids, and acanthocephalan nymphs. *Strongylus edentatus* larvae may often be observed immediately beneath the parietal peritoneum of horses. Examine the surface of the liver for migration racks of ascarid, taeniid, and *Fasciola* larvae, and the kidneys for encysted *Toxocara* larvae. The equine pancreas is a favorite location for *Strongylus equinus* larvae. Place double ligatures around the cardia (or omasoabomasal junction), pylorus, and ileocecal junction, thus isolating the stomach, small intestine, and large intestine. These regions provide differing environments for distinct sets of parasites, and valuable diagnostic information is lost by pooling the collection from the entire gut. Open one region at a time, carefully poking through the ingesta and scanning the mucosa for the smaller forms. Many parasites of dogs, cats, horses, and pigs are large enough to see with the unaided eye, but a few very small ones are important (e.g., *Strongyloides, Trichinella*). Scrape the mucosa of the small intestine and examine the scrapings for small nematodes, coccidia, and the like.

Most of the important nematode parasites of ruminants are very small, and great care must be taken not to overlook them. The population of nematodes sufficient to kill a heifer may pass the notice of a careless prosector completely. The following technique accomplishes the concentration and separation of these worms from much of the ingesta and mucosal debris and, with a bit of extra effort, provides an estimate of the number of worms present.

1. Transfer all ingesta from a particular organ (the abomasum is an easy one with which to begin) to a bucket; scrub or lightly scrape the mucosal surface to ensure complete transfer of worms.
2. Add several quarts of tepid water, mix, and allow to stand for about 5 minutes, so that the worms and heavy debris can settle to the bottom; then decant the supernatant. Repeat this process until the sediment consists principally of worms and coarse ingesta.
3. Transfer a small amount of sediment to a Petri dish and examine with transillumination, preferably under a magnifying glass or a stereoscopic microscope. If the worms have been taken from the cadaver of a recently dead animal, they will become very active in the tepid water and can be easily detected and fished out with forceps for closer examination.

The small intestine is long, and life is short. Most of the important nematode parasites of the ruminant small intestine can be collected by flushing a liter of water through its first 6 meters. Insert a funnel into the pyloric end of 6 meters of unopened small intestine and pour a beaker of water into it. Massage the water along the length of gut and collect it at the other end; then proceed with steps 2 and 3 above.

A popular alternative to step 2 is to rinse sediment vigorously over a sieve with openings small enough to retain the parasites but large enough to pass water and fine debris. The sieve then may be inverted and back-rinsed to transfer the parasites and coarse debris

to a collecting vessel. If time or facilities to examine sediment for parasites are lacking, the sediment can be preserved in 10% formalin and attended to later. Be sure to sieve preserved sediments once again to remove the formalin before attempting to isolate and study the parasites; this may save you a big headache.

Because we are almost certain to find parasites in sheep, young cattle, and horses, it follows that evaluation of necropsy findings must rest on the abundance of the parasites, as well as on their identity. To obtain an estimate of worm numbers, substitute step 3a for step 3, and proceed as follows:

3a. Transfer the washed sediment to a graduated cylinder, and fill with water to 1 liter. We now have all the worms from some particular organ suspended in 1 liter.
4. Stir the suspension thoroughly, and withdraw a 50-mL aliquot.
5. Pour a small portion of the 50-mL aliquot into a Petri dish, and count all of the worms. Continue until the 50 mL is used up. The number of worms counted times 20 provides an estimate of the total number of worms in the particular organ.

The worm count must be interpreted in the light of other necropsy findings, especially the nutritional status of the cadaver and lesions specifically related to the parasites found. Etiologic significance should be attached to *Trichostrongylus* or *Cooperia* only if it is apparent that the animal has suffered severe and protracted diarrhea. The presence of even 10,000 *Trichostrongylus* worms in a well-nourished lamb carcass with formed fecal pellets in the rectum suggests only that we should search further for the cause of death. Etiologic significance should be attached to *Haemonchus* only if the carcass shows signs of anemia. Cattle with ostertagiosis can become emaciated on full feed. These animals do not even lose their appetite but develop malabsorption that causes them to starve to death in the midst of plenty. It is just as well not to accuse the farmer of starving the animal to death when in fact *Ostertagia* is the culprit.

PARASITES OF DOGS

STAGES IN FECES

The common internal parasitisms of dogs can usually be diagnosed on the basis of the microscopic appearance of eggs, cysts, or larvae found in the feces. Micrometry or fecal culture may be necessary when more specific identification is required than can be accomplished on the basis of microscopic appearance alone.

Nematode Eggs

Eggs of some nematode parasites of dogs are shown in Figures 7-24 and 7-25.

The stage of embryonic development of eggs found in fresh fecal specimens varies among nematode species and thus provides us with diagnostic criteria. In fresh fecal specimens, *Toxocara, Toxascaris, Trichuris,* and the capillarid eggs of *Eucoleus aerophilus* and *Aonchotheca putorii* contain a single cell. The *Ancylostoma* or *Uncinaria* embryo has already segmented to produce a morula, as has the capillarid egg of *Eucoleus boehmi*. Many spirurid eggs contain first-stage larvae, and *Strongyloides* and *Filaroides* have already hatched and appear in the feces as first-stage larvae. The development of a typical nematode egg is portrayed in Figure 7-8.

Recovery of *E. aerophilus* eggs from respiratory mucus by tracheal swab requires general anesthesia. The presence of *Pearsonema plica* eggs in fresh fecal specimens represents contamination with urine. Urine specimens may also contain eggs of *Dioctophyme renale,* but these have much larger and rougher shells than do the eggs of *P. plica,* and the eggs of *D. renale* are typically in a two-cell stage when passed. *Ancylostoma* and *Uncinaria* eggs have a smooth, clear, colorless, ellipsoidal shell and contain an embryo in the morula stage of development. *Ancylostoma caninum* eggs average less than 65 µm, whereas *Uncinaria stenocephala* eggs average more than 70 µm in length. Examination of *Ancylostoma braziliense* eggs from cats and dogs in Florida revealed the average length of the eggs of this hookworm to be 53 µm (Lucio-Forster et al, 2012). (**Caution:** The eggs of strongyle parasites of domestic herbivores frequently find their way into dog feces through coprophagy and may be confused with hookworm eggs.) Eggs of the order Spirurida are usually smooth-walled and contain a larva. The most important of these, *Spirocerca lupi,* produces very small (30 by 12 µm), cylindrical eggs with rounded ends.

Nematode Larvae

If the canine fecal sample is fresh and is not contaminated with soil or extraneous organic material, larvae found swimming about the microscopic field may be *Strongyloides stercoralis* or one of the following metastrongyloids: *Filaroides osleri, Filaroides hirthi, Crenosoma* sp., or *Angiostrongylus vasorum*. The esophagus of metastrongyloid larvae is longer than the rhabditiform esophagus of the first-stage *Strongyloides* larva, and the tail may have a slight kink as in *Filaroides* or a dorsal spine as in *Angiostrongylus,* whereas the tail of the *Strongyloides* and *Crenosoma* first-stage larva tapers smoothly to a point (Figure 7-26).

If the sample is stale, hookworm larvae may have developed and hatched. These somewhat resemble *Strongyloides* rhabditiform larvae but have a longer buccal capsule and smaller genital rudiment (see Figure 7-26). Should doubt remain, culture the feces for the development of infective stages. The infective sheathed third-stage larvae of hookworms do not begin to appear until after 5 to 7 days of incubation at room temperature, whereas homogonic *Strongyloides* filariform larvae appear as early as 24 to 36 hours, and heterogonic filariform larvae appear in about 4 days. *Strongyloides* filariform larvae are slender, with a very long esophagus, and the tip of their tail appears notched or truncated (Figure 7-27). If the specimen is contaminated with soil or extraneous organic material, free-living nematodes and their larvae may confuse the issue. Under such circumstances, the best course is to obtain a fresh sample directly from the dog's rectum.

Tapeworm Segments

Detached segments of cyclophyllidean tapeworms are often found crawling about on the perineum or fresh feces of infected dogs (and cats). Hand lens inspection permits identification for practical purposes. Owners sometimes submit for identification shriveled objects that are actually dehydrated tapeworm segments (Figure 7-28, *A*). If these are soaked in water, they will usually regain their familiar appearance (Figure 7-28, *B*). Should doubt remain, the "reconstituted" segment may be squashed between two microscope slides bound together with adhesive tape. The segment may then be identified by the microscopic appearance of its eggs and such organs (e.g., genital pore, uterine diverticula or capsules, parauterine organ) as may persist in gravid segments of various species (Figures 7-29, 7-30, and 7-31). Taeniid segments are roughly rectangular with a single, lateral genital pore and contain taeniid eggs (see Figure 7-30). *Dipylidium* segments are shaped somewhat like cucumber seeds, have a genital pore on each lateral margin, and contain eggs clustered in packets (uterine capsules) (see Figure 7-30). *Mesocestoides* segments have a dorsal genital pore and eggs massed in a central, thick-walled parauterine organ (see Figure 7-30), and fresh segments are said by some to resemble sesame seeds.

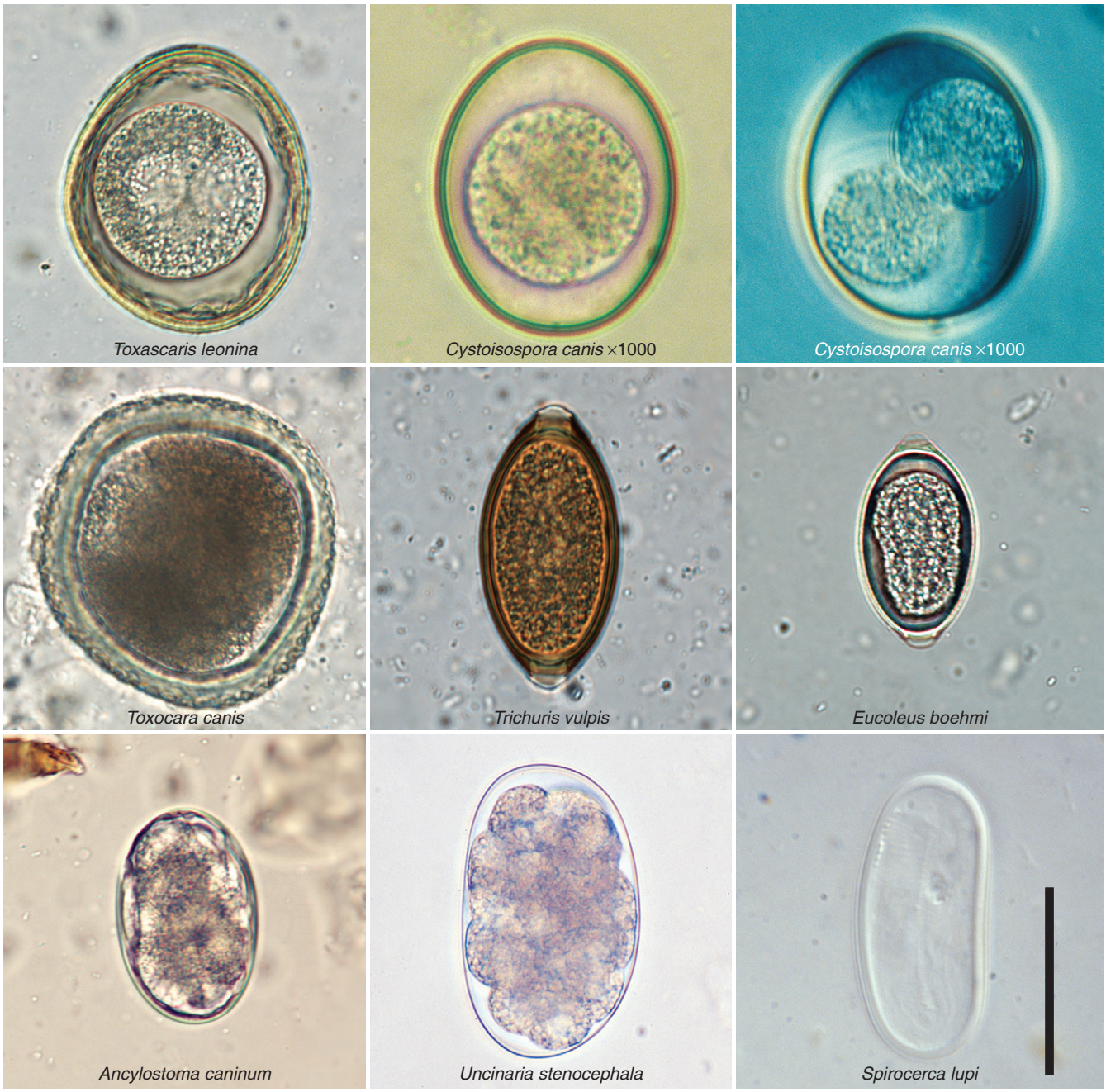


FIGURE 7-24. Eggs of some nematode parasites of dogs (×400, except for *Cystoisospora canis* and *Spirocerca lupi;* bar represents 50 µm). *Toxascaris leonina* produces a colorless, subspheric to ellipsoidal egg shell with a smooth shell surface and a prominent lipid layer containing one or sometimes two cells in fresh specimens. *Cystoisospora canis,* a coccidian oocyst and not a nematode egg, is portrayed here ×1000 to illustrate how easily it might be confused with *T. leonina,* unless the difference in size or the absence of a lipid layer is noticed. *Toxocara canis* produces a yellowish brown, subspheric egg with a uniformly pitted shell surrounding a single cell in fresh specimens. *Trichuris vulpis* and capillarid eggs are lemon-shaped and have bipolar plugs. *T. vulpis* eggs average more than 75 µm, whereas those of capillarids average less than 75 µm, in length.

Tapeworm Eggs

In the majority of tapeworms, segmentation, gastrulation, and embryogeny of the egg take place within the uterus of the adult worm. This is the case in the common cyclophyllidean tapeworm eggs. In the case of the diphyllobthridian eggs, the egg, surrounded by yolk cells, does not begin to embryonate until it leaves the uterus and enters the environment.

Cyclophyllidean Eggs

Cyclophyllidean eggs are all characterized by the six-hooked larva within (see Figure 7-31). Taeniid eggs are spheric or subglobular with a radially striated embryophore (a shell-like covering), and contain an embryo (oncosphere or hexacanth embryo) with three pairs of hooks (see Figures 4-36 and 7-31, *A*). If the hooks are not clearly visible, they may sometimes be demonstrated by pressing a

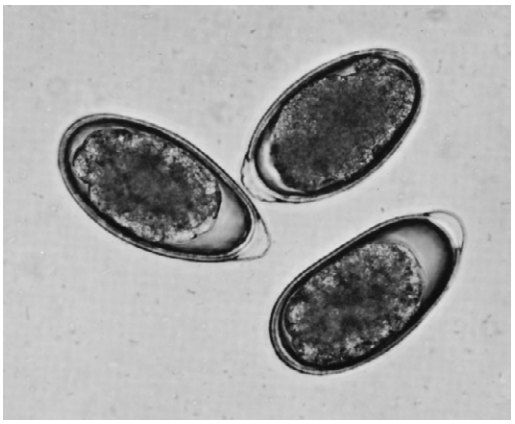


FIGURE 7-25. *Gnathostoma spinigerum* from a dog (×400). This dog belonged to a pet shop owner who occasionally fed it defunct tropical fish. Infection with this exotic parasite probably was acquired by eating one of these fishes.

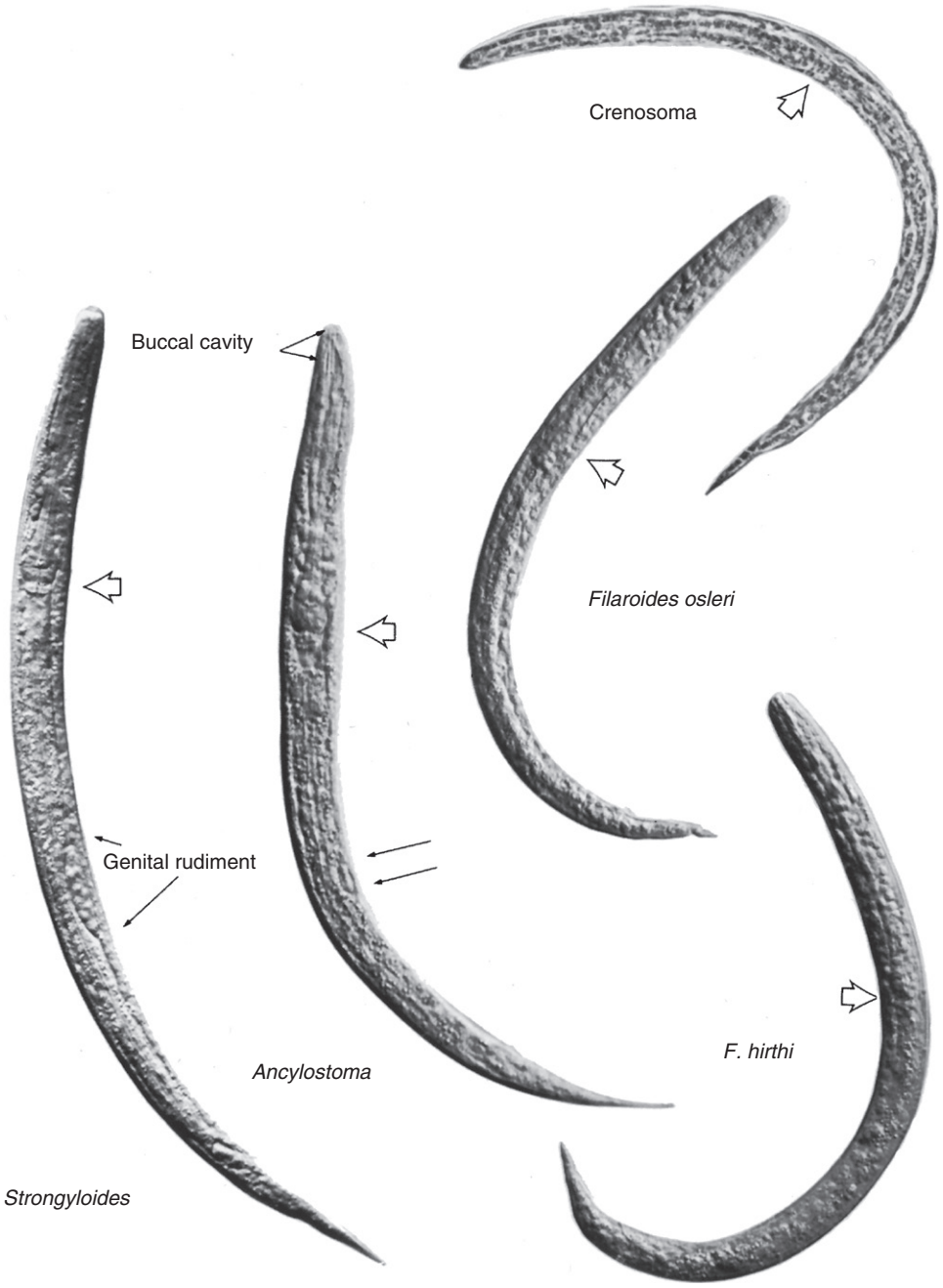


FIGURE 7-26. First-stage larvae of some nematode parasites of dogs. *Crenosoma* and *Filaroides* species are metastrongyloid lungworms and usually undergo no development in fecal cultures. *Strongyloides* and *Ancylostoma* first-stage larvae can be distinguished by differences in the relative sizes of their genital rudiments and relative lengths of their buccal cavities. In fecal cultures, both *Strongyloides* and *Ancylostoma* develop to the infective stage (see Figure 7-28).

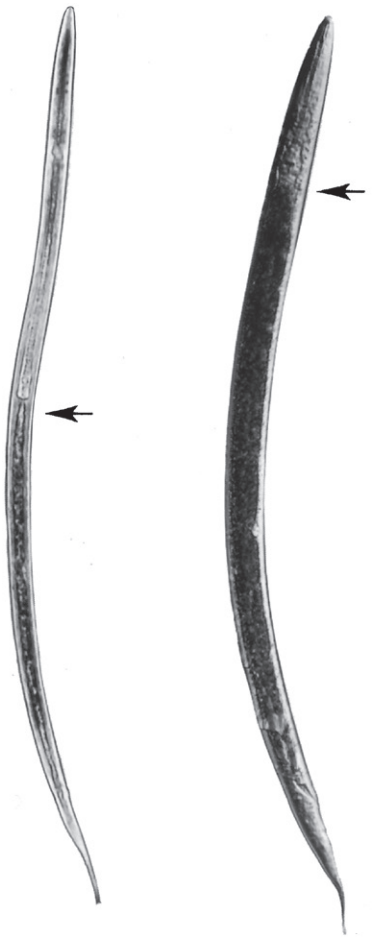


FIGURE 7-27. Third-stage infective larvae of *Strongyloides* and *Ancylostoma*. *Strongyloides* infective larvae have a very long esophagus, and the tip of the tail appears to be notched. (Actually, it is composed of four small projections of the double lateral alae.) *Ancylostoma* infective larvae are usually enclosed in the uncast cuticle (sheath) of the second stage, here seen extending slightly beyond the tail of the third stage. The arrows point to the esophageal-intestinal junctions.

needlepoint on the coverslip to break the embryophore (see Figure 7-31, *B*). The eggs of *Echinococcus* are a serious menace to human health and cannot be distinguished from those of *Taenia*. In *Echinococcus* endemic areas, therefore, the discovery of taeniid eggs in canine fecal samples demands prompt anthelmintic therapy and caution in the handling and disposal of feces. The eggs of Dipylidiidae are spheric or subspheric with an unstriated embryophore, contain an oncosphere, and are enveloped in a uterine capsule. In *Dipylidium* there may be up to 29 eggs per capsule (see Figure 7-31, *D*). In *Joyeuxiella* and *Diplopylidium,* only one egg is present per uterine capsule. The eggs of *Mesocestoides* are oval and thin-shelled and contain an oncosphere (see Figure 7-31, *C*).

Diphyllobothriid Eggs

Diphyllobothriid eggs are discharged continuously through the uterine pores of many segments along the body of the worm and hence are passed independently of any detached segment. *Diphyllobothrium* and *Spirometra* eggs are oval with an operculum at one pole and a small button at the other (Figure 7-32, *A*), which often makes them difficult to distinguish from certain trematode eggs (Figure 7-32).

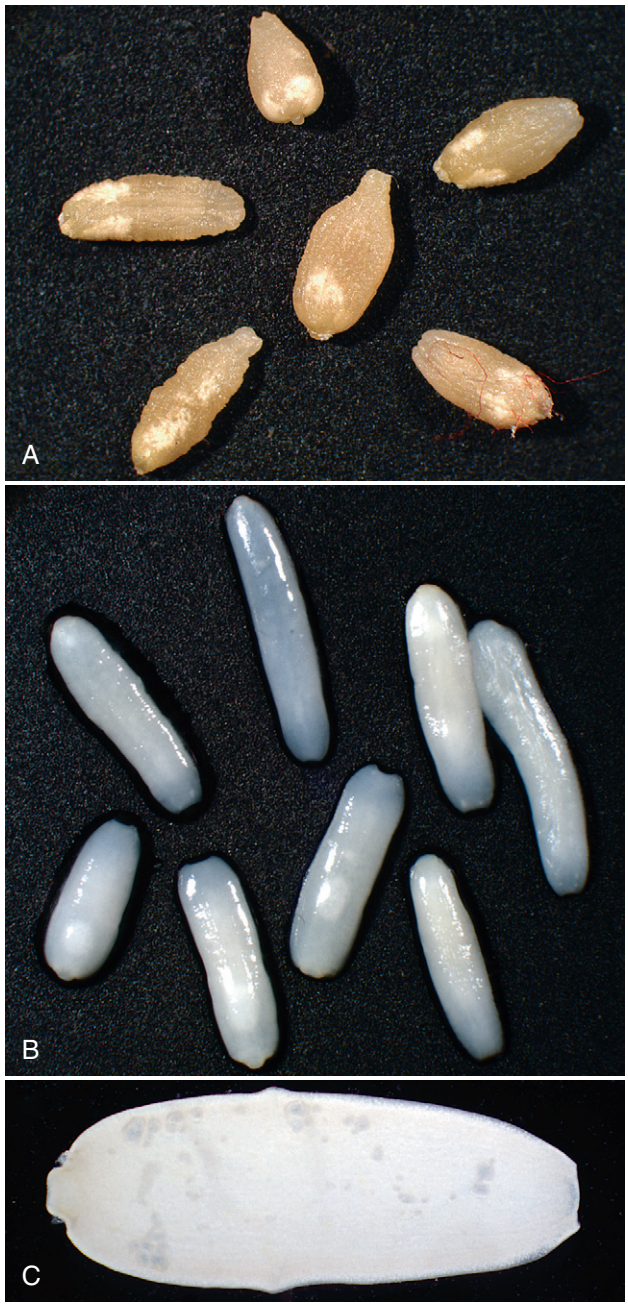


FIGURE 7-28. A, Dehydrated *Dipylidium* segments dried for at least 1 month. **B,** Same segments after soaking in water for about 1 hour. **C,** Rehydrated *Dipylidium* segment showing the two genital pores after compression under a coverslip on a slide.

Acanthocephalan Eggs

Acanthocephalan eggs have a thick outer and thinner inner shell enclosing an embryo called an *acanthor*. The external surface of the egg of *Macracanthorhynchus* is elegantly patterned (Figure 7-33).

Trematode Eggs

Eggs of most digenetic trematodes have an operculum at one pole and contain an embryo whose stage of development varies with the species in question (Figure 7-34). Schistosome eggs, on the other hand, lack an operculum and contain a fully developed miracidium that hatches shortly after the egg comes in contact with water. Many, but not all, schistosome eggs have a sharp spine. If a dog has

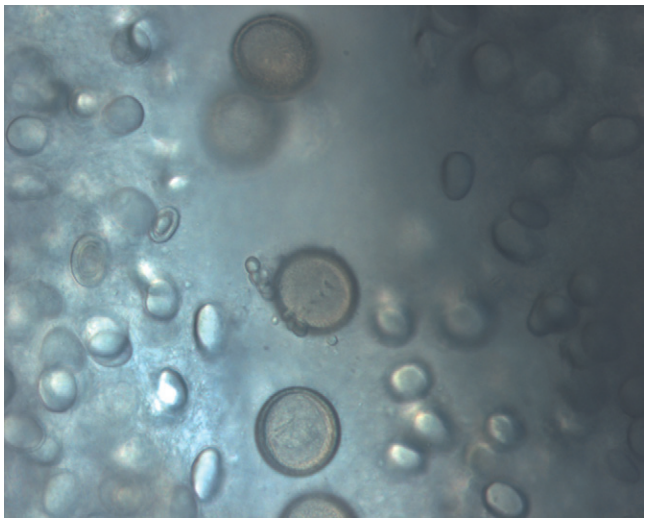


FIGURE 7-29. Taeniid segment in squash preparation.

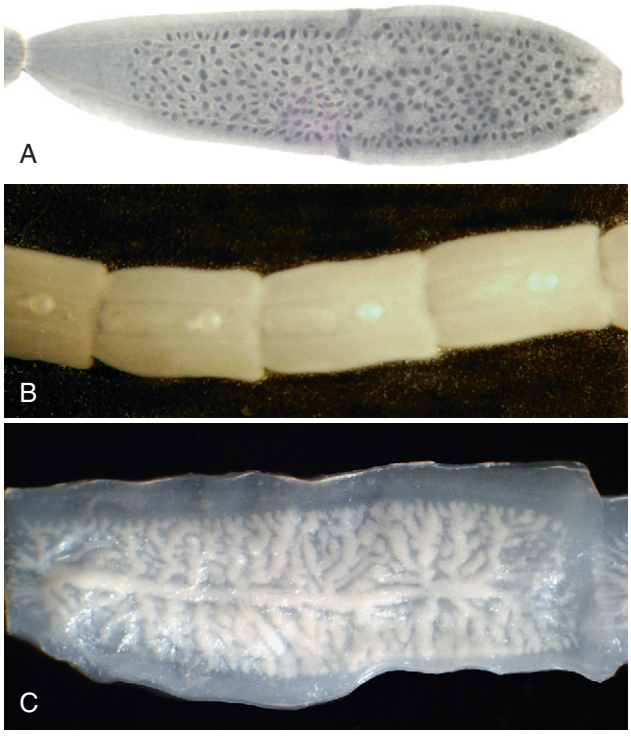


FIGURE 7-30. **A,** Single segment of *Dipylidium caninum*. **B,** Three segments of *Mesocestoides* sp. with the characteristic central parauterine organ. **C,** Segment of *Taenia pisiformis*.

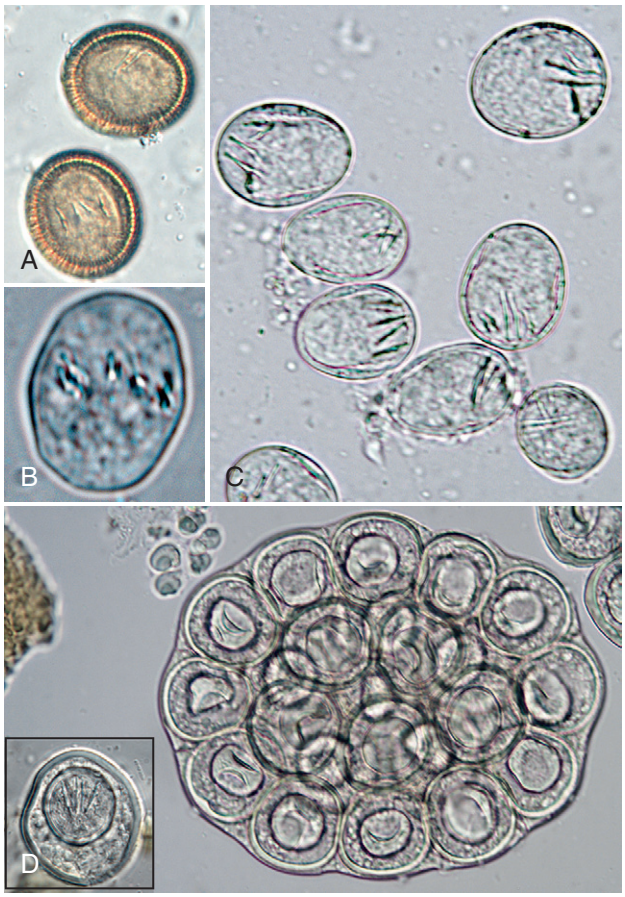


FIGURE 7-31. Tapeworm eggs. **A,** Two taeniid eggs. **B,** Hatched oncosphere of *Taenia pisiformis*. **C,** Eggs of a *Mesocestoides* sp. removed from the parauterine organ by pressure on the eggshell. **D,** *Dipylidium caninum* egg capsule (inset is a single egg showing the six hooklets).

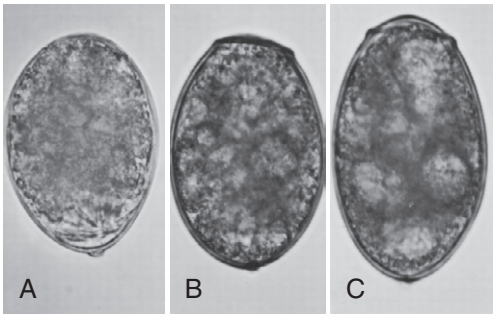


FIGURE 7-32. Operculate eggs (×400). **A,** *Diphyllobothrium* egg. **B** and **C,** Unidentified eggs; their prominent opercula suggest that, except for their small size, these might be *Paragonimus* eggs (see Figure 7-36, *B*). This figure illustrates the difficulty of distinguishing *Diphyllobothrium* eggs from those of certain trematodes.

fed recently on trematode-infected tissues such as sheep liver infected with *Dicrocoelium* or *Fasciola,* or rabbit entrails infected with *Hasstilesia,* the presence of myriad trematode eggs in its fecal specimen may lead to misdiagnosis.

Coccidian Oocysts and Sporocysts

Cystoisospora

Cystoisospora, Hammondia, and *Neospora* oocysts have colorless, ovoid or ellipsoidal, smooth-surfaced walls without micropyle or polar cap and contain a single sporont when passed in the feces of the host (see Figure 7-24). Sporulation occurs in 2 to 4 days at room temperature. The fully sporulated *Cystoisospora* oocyst then contains two sporocysts, each of which contains four sporozoites (Figure 7-35, *A*). Because dogs tend to be coprophagic, oocysts of various other coccidia, especially *Eimeria* species of herbivores, are very common pseudoparasites in dog feces. If the *Eimeria* species in question have micropyles, polar caps, or other distinguishing features, they present no diagnostic problem (Figure 7-35, *B*), but many species are difficult to differentiate from *Cystoisospora* species. Differentiation of *Eimeria* and *Cystoisospora* may be accomplished by fecal culture for oocyst sporulation. Sporulated *Eimeria* oocysts

contain four sporocysts, each of which contains two sporozoites (Figure 7-35, *C*).

Identification of species of *Cystoisospora, Hammondia,* and *Neospora* requires micrometry. Oocyst dimensions in micrometers for species infecting dogs are as follows: *Cystoisospora canis,* 32 to 42 × 27 to 33; *Cystoisospora ohioensis,* 19 to 27 × 18 to 23; *Cystoisospora burrowsi,* 17 to 22 × 16 to 19; *Hammondia heydorni,* 10 to 13 × 10 to 13 (Trayser and Todd, 1978); and *Neospora caninum,* 11.7 × 11.3 (Lindsay, Upton, and Dubey, 1999). Although minor differences are observed sometimes between the oocyst sizes of *C. ohioensis* and *C. burrowsi* and those of *H. heydorni* and *N. caninum,* morphologic differentiation is not possible, thus specific identification requires either animal inoculation or molecular methods.

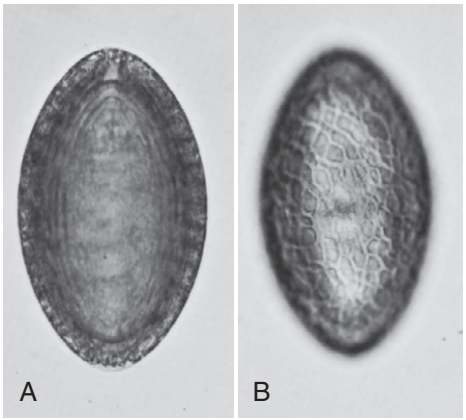


FIGURE 7-33. *Macracanthorhynchus ingens* (Acanthocephala) egg (×400). **A,** Acanthor in focus. **B,** Surface of shell in focus.

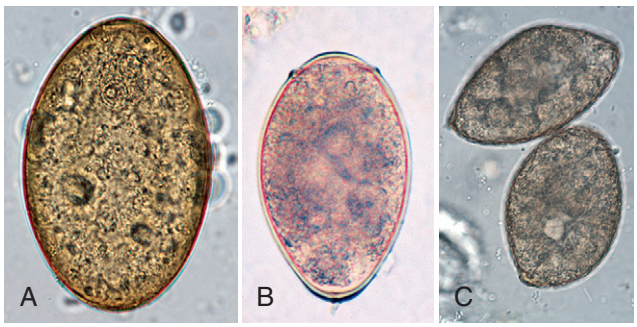


FIGURE 7-34. Trematode eggs (×400). **A,** *Alaria* sp. **B,** *Paragonimus kellicotti.* **C,** *Nanophyetus salmincola.* (Specimen courtesy Dr. Jay Stewart, Aumsville Animal Clinic, Aumsville, Oregon.)

Sarcocystis

Sarcocystis species sporulate within the host, and the fragile oocyst wall often breaks so that the sporocyst containing four sporozoites is the form usually found in the feces (see Figure 7-54, *D*). Sporocysts measure 11 to 28 × 7 to 13 µm, but it is not possible to distinguish species of *Sarcocystis* by micrometry of sporocysts (Dubey, 1976). The host relationships of common species of *Sarcocystis* are presented in Table 3-1.

Amoebae

Entamoeba histolytica, a serious human pathogen, may appear in canine fecal specimens as either trophozoite or cyst. The trophozoites are more likely to be encountered in diarrheal feces and the cysts in formed fecal specimens. Trophozoites of *E. histolytica* are 10 to 30 µm across, and their nuclei have marginated chromatin and a small central endosome. *E. histolytica* trophozoites display amoeboid movement and often ingest erythrocytes. The mature cysts are 10 to 20 µm in diameter and contain four nuclei.

Entamoeba coli trophozoites are 20 to 30 µm in diameter. Their nuclei have a relatively large eccentric endosome. Erythrocytes are not found in *E. coli* trophozoites. As many as eight nuclei may be counted in *E. coli* cysts (see Figure 7-120).

Entamoeba gingivalis, a parasite of the oral cavity, infects both man and dog. Only trophozoites ranging in size from 5 to 35 µm are found in oral scrapings.

Flagellates

Giardia trophozoites are less than 21 µm long and are bilaterally symmetric and pear-shaped. Two nuclei with large central endosomes look like a pair of eyes (see Figure 7-104). *Giardia* cysts are less than 12 µm long, are ellipsoidal, and contain four nuclei.

Trichomonas and related genera do not form cysts and occur in feces (usually diarrheal) only as mononucleated trophozoites.

Ciliates

Balantidium coli trophozoites are ovoid with a cytostome at one end; measure 25 to 150 µm in diameter; contain one macronucleus and one micronucleus, two contractile vacuoles, and inclusions; and are covered with rows of cilia (see Figure 3-7). Cysts are spheric or

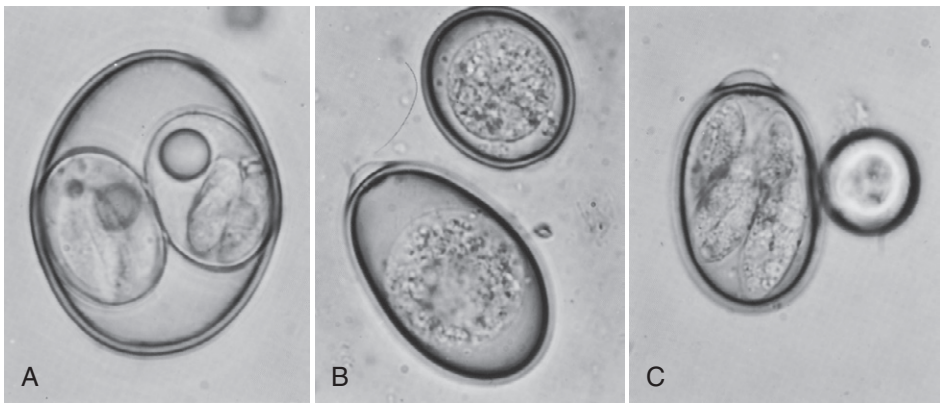


FIGURE 7-35. Coccidian oocysts (×1000). **A,** *Cystoisospora canis,* sporulated. **B,** *Eimeria* spp., one-cell stage. **C,** *Eimeria* sp., sporulated. *Cystoisospora* species sporulated oocysts contain two sporocysts, each of which contains four sporozoites. *Eimeria* species sporulated oocysts contain four sporocysts, each of which contains two sporozoites. See Figure 7-24 for *Cystoisospora canis* one-cell stage.

ovoid, measure 40 to 60 µm in diameter, and have a wall that appears to consist of two membranes (see Figure 3-7).

FIXATION AND IDENTIFICATION OF MICROFILARIAE IN BLOOD

The simplest procedure for diagnosing the presence of microfilariae in the blood of dogs is to place a drop of heparinized venous blood on a slide, add a coverslip, and examine the preparation under low and high dry magnification. Microfilariae reveal their presence by agitating the erythrocytes in their immediate vicinity. In general, if more than 5 or 10 microfilariae are observed per drop of blood, they are probably *Dirofilaria immitis*. If fewer than that are observed, they may represent either heartworm or another filariid parasite infection. In North America the only other filariid recognized in the blood of dogs is *Acanthocheilonema reconditum* (Newton and Wright, 1956, 1957), but in certain other parts of the world, other species need to be dealt with. The following procedure is about 15 times as sensitive as the direct smear and permits more accurate differentiation of microfilariae of *D. immitis* and *A. reconditum*.

Technique of Knott (1939) Modified

1. Draw a sample of venous blood into a syringe containing a suitable anticoagulant such as ethylenediaminetetraacetic acid (EDTA) or heparin.
2. Draw 1 to 2 mL of air into the syringe, and mix the blood and the anticoagulant by rocking the syringe so as to run the air bubble back and forth along the length of the barrel. Prolonged delay and thermal extremes are to be avoided. Remix blood immediately before proceeding with step 3.
3. Place 1 mL of blood in a 15-mL centrifuge tube. Add 10 mL of 2% formalin, stopper, and mix by inverting and shaking. *Note:* When submitting blood samples to a laboratory for identification of microfilariae, complete only steps 1, 2, and 3 to prepare them for shipment.
4. Wait 2 or 3 minutes.
5. Centrifuge for about 5 minutes, and pour off the supernatant by inverting the centrifuge tube only once. Remove with absorbent paper the drop that clings to the rim of the tube.
6. Add one drop of 0.1% methylene blue to the sediment, mix, and transfer some stained sediment to a slide for microscopic examination.

Other microfilarial concentration techniques may be used, but the Knott test is preferred because it is standard, it is inexpensive, and it includes the best preparative technique for specimens submitted to the laboratory. The quality and concentration of the formalin solution are critical. Two percent formalin is 2 mL of stock 37% formaldehyde solution (i.e., formalin) and 98 mL of distilled water. This reagent tends to deteriorate in storage and should be made up fresh periodically.

Differentiation of Microfilariae

Microfilariae of *Dirofilaria immitis* are 6.0 to 7.0 µm wide, whereas those of *Acanthocheilonema reconditum* are less than 5.6 µm wide. When compared with a red blood cell ghost, those of *D. immitis* are wider at midbody than the ghosts, and those of *A. reconditum* are narrower than the RBC is wide (Figure 7-36). Length measurement is a more tedious and less reliable differential criterion. When fixed by the preceding technique, the tails of *A. reconditum* microfilariae tend to be curved like an ovariectomy hook. The anterior end of the *D. immitis* microfilaria tapers gently, whereas that of *A. reconditum* maintains about the same diameter throughout. The cephalic hook of *A. reconditum* (Figure 7-37) is demonstrable with the ×40 objective of any modern, standard, compound microscope

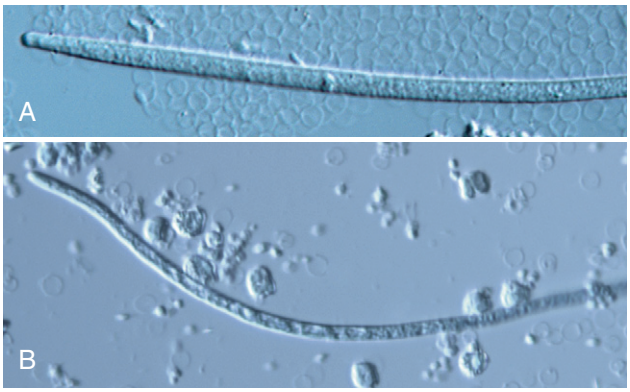


FIGURE 7-36. Anterior to midbody images of microfilariae of *Dirofilaria immitis* **(A)** and *Acanthocheilonema reconditum* **(B)**. The microfilaria of *A. reconditum* is thinner than that of *D. immitis,* and thinner than the red blood cell (RBC) ghosts at midbody, while *D. immitis* is wider than the RBC ghosts.

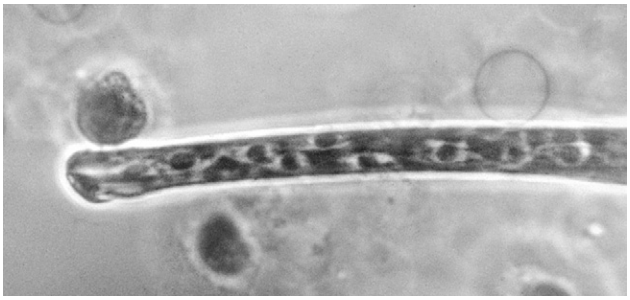


FIGURE 7-37. Image of the cephalic hook of *Acanthocheilonema reconditum;* it can be hard to get the microfilaria rolled just so to make the hook visible.

in samples prepared by the Knott technique described earlier. It is not necessary to resort to thick smears or special stains to demonstrate the cephalic hook. Patience is required at first, but with practice the cephalic hook provides the quickest, easiest, and most reliable differential criterion.

Microfilariae of *D. immitis* can be distinguished from those of the exotic subcutaneous dwelling *Dirofilaria repens* by antigen tests, molecular methods, and comparison of the anterior ends of Giemsa-stained blood films (Figure 7-38).

ANNOTATED HOST-ORGAN LISTING OF PARASITES OF DOGS

Toxoplasma gondii may occur in any tissue of any host as extracellular or intracellular tachyzoites or as bradyzoites in cysts (see Figures 3-20 and 8-35). *Neospora caninum* may occur in similar locations (see Figures 3-21 and 8-36).

Alimentary System

Mouth

PROTISTA. *Trichomonas canistomae* (Trichomonadida). Found around gum margins; nonpathogenic.

Esophagus and Stomach

NEMATODES. *Spirocerca lupi* (Spirurida). Found in fibrous nodules in the wall of the esophagus and sometimes in the stomach (see Figures 8-103 to 105). Larvae migrate through the adventitia of the arteries and aorta to the walls of the stomach or esophagus. Adults encyst in nodules that communicate with the lumen of these two organs. Cysts may be found in other locations as well. Chronic

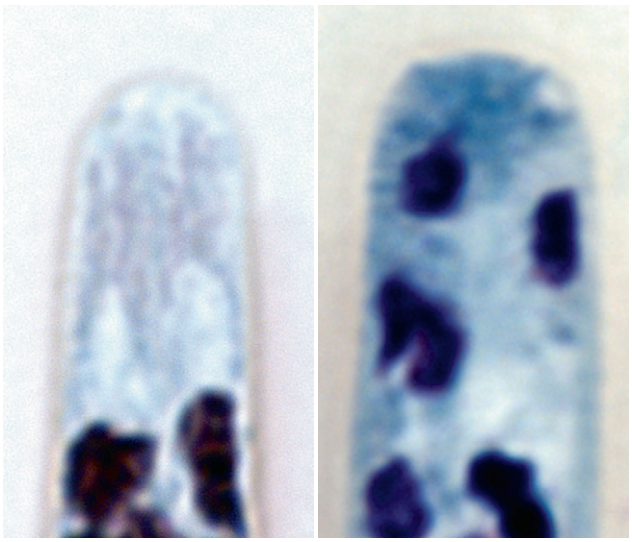


FIGURE 7-38. In Giemsa-stained blood films of *Dirofilaria immitis* and *Dirofilaria repens,* the latter of which is typically seen in the United States only in dogs from other countries, the anterior cephalic space in *D. immitis* is free of nuclei, while typically a pair of anterior nuclei are present in the microfilaria.

infection is associated with dysphagia, vomiting, esophageal osteosarcoma, aortic aneurysm (rupture rare), and pulmonary osteoarthropathy.

Physaloptera rara and *Physaloptera praeputialis* (Spirurida). Adult worms (see Figures 4-142 and 4-143) are found with their anterior end embedded into the gastric mucosa. Infection can be asymptomatic or may be associated with vomiting and anorexia.

Gnathostoma spinigerum (Spirurida). Relatively rare in North America (see Figures 4-140 and 4-141). Adults encyst in nodules in the stomach wall. Larval migration through the liver and other organs is destructive. Rupture of nodules containing adult worms into the peritoneal cavity can cause a medical emergency.

Small Intestine

NEMATODES. *Toxocara canis* and *T. leonina* (Ascaridoidea) are large cream-colored worms (see Figures 4-128 and 4-129). *Toxocara* has a ventriculus intercalated between the esophagus and the intestine (Figure 7-39, *A*), whereas *Toxascaris* has none (Figure 7-39, *C*). The ventriculus is visible in transilluminated fresh specimens under the stereoscopic microscope and in fixed, cleared specimens under the compound microscope. Large, fixed specimens may be dissected to determine the presence or absence of a ventriculus. The tail of male *Toxocara* is finger-like (Figure 7-39, *B*), whereas the tail of male *Toxascaris* tapers to a point (Figure 7-39, *D*). Female *Toxocara* and *Toxascaris* may be distinguished by comparing their eggs (see Figure 7-24). In acquiring diagnostic skill, one must not be content with merely comparing general impressions of the microscopic image with a set of pictures in a book. Persons basing their diagnoses on superficial appearances often confuse *Toxascaris* eggs with *Cystoisospora canis* oocysts less than half as large. In Figure 7-24, a *T. leonina* egg ×425 and a *Cystoisospora canis* oocyst ×1000 have been placed side by side to show how easily this mistake could be made. The matter may be resolved with an ocular micrometer or, more simply, through observation of whether a distinct lipid layer is present *(Toxascaris)* or absent *(Cystoisospora)*. Ascarids in the small intestine may cause bloating and can interfere with intestinal motility and digestion

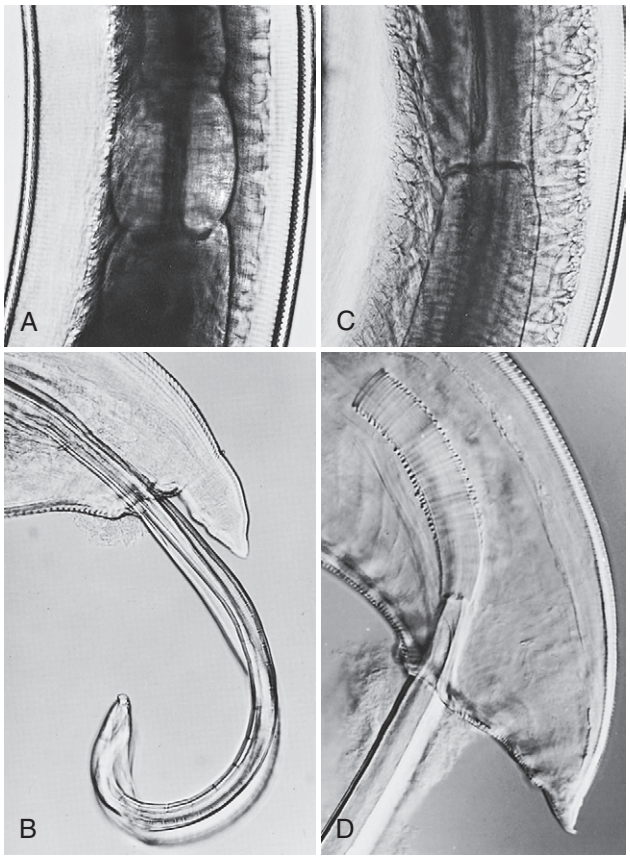


FIGURE 7-39. **A** and **B,** In *Toxocara,* a ventriculus is intercalated between the esophagus and the intestine **(A),** and the tail **(B)** of the male is finger-like. **C** and **D,** In *Toxascaris,* there is no ventriculus between the esophagus and the intestine **(A),** and the tail of the male tapers gradually **(B).**

FIGURE 7-40. *Toxocara canis* worms in the intestine of a dog at necropsy.

(Figure 7-40). Mucoid diarrhea, vomiting, abdominal distention, emaciation, and a failure to thrive may all be noted as clinical signs. Infection with *T. leonina* is less pathogenic and usually results in diarrhea and vomiting in only the worst cases.

Baylisascaris procyonis, the raccoon roundworm, is showing up in dogs as adult worms. This is a dangerous parasite because the zoonotic disease produced by the ingestion of embryonated eggs can be devastating and life threatening. Although a rather rare condition, cases are regularly occurring. When fully mature, the worms tend to be larger than *Toxocara canis* or *T. leonina,* and the eggs can be differentiated by the facts that they are smaller, have a

FIGURE 7-41. *Ancylostoma caninum* female attached to the intestinal mucosa at a feeding site.

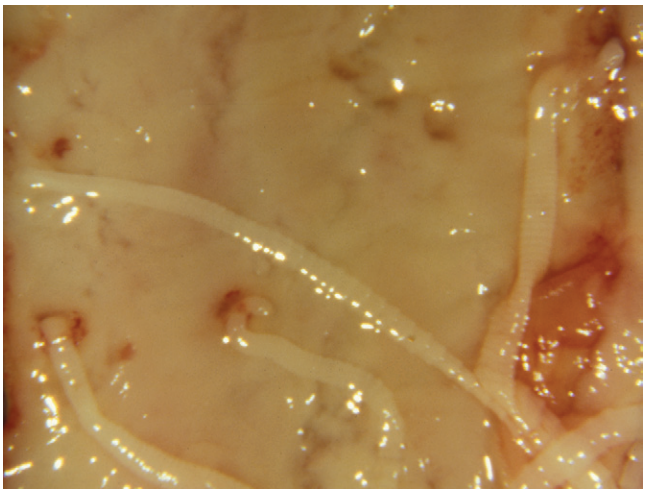


FIGURE 7-42. Anterior ends of *Taenia pisiformis* with the attachment sites from three scolices.

rough external shell (see Figure 4-136), and appear browner than the eggs of the two common dog ascaridoids. Infected dogs typically have no clinical signs.

Ancylostoma caninum, A. braziliense, and *Uncinaria stenocephala* (Ancylostomatoidea). Mature hookworms are found anchored to the mucosa by their buccal capsules unless the cadaver has cooled out or the host has died of an overdose of barbiturate, in which case many specimens will be found unattached. Preadult *A. caninum* burrow deeply and destructively in the mucosa (Figure 7-41), and the mesenteric lymph nodes may be hemorrhagic as a result during the prepatent phase of severe infection. An adult *A. caninum* is colored red, whereas *A. braziliense* and *U. stenocephala* are grayish white. The red color of *A. caninum* quickly fades on fixation, however. Specimens may be differentiated by microscopic examination of their buccal structures: *A. caninum* has three pairs of pointed teeth on the ventral border of the buccal capsule; *A. braziliense* has one pair of pointed teeth; and *U. stenocephala* has a pair of rounded plates instead of teeth (see Figure 4-98). *A. caninum* sucks much more blood than either of the other hookworm species affecting dogs. Suckling pups experience peracute infection owing to transmammary transmission of larvae; infection may be fatal. Affected pups will have pale mucous membranes, and they can pass soft liquid stools containing partially digested blood.

S. stercoralis (Rhabditoidea). The tiny (2.2 mm) parthenogenetic parasitic female worms (see Figure 4-114) may be found in scrapings of the mucous membrane. Clinical signs vary from none to watery diarrhea.

Trichinella spiralis (Trichinelloidea). The small adults are found threaded through the mucosa of the duodenum and produce "prelarvae" that enter the intestinal mucosa (see Figure 4-161). Vomiting or mild diarrhea may result.

CESTODES. *T. pisiformis, Taenia hydatigena, Taenia ovis, Taenia multiceps,* and *Taenia serialis* (Taeniidae). Adult tapeworms typically (Figure 7-42; see also Figures 4-33 to 4-35 and 4-37) cause no significant signs.

E. granulosus and *E. multilocularis* (Taeniidae). Adult tapeworms typically (see Figure 4-43) cause no significant signs.

Dipylidium caninum, Diplopylidium, and *Joyeuxiella* (Dipylidiidae). Typically without clinical signs (Figure 7-43; see also Figures 4-53, 4-55, and 7-28 to 7-30), infection can result in impaction in young puppies.

Mesocestoides species (Mesocestoididae). Typically, infection is without clinical signs (see Figures 4-58 and 7-30).

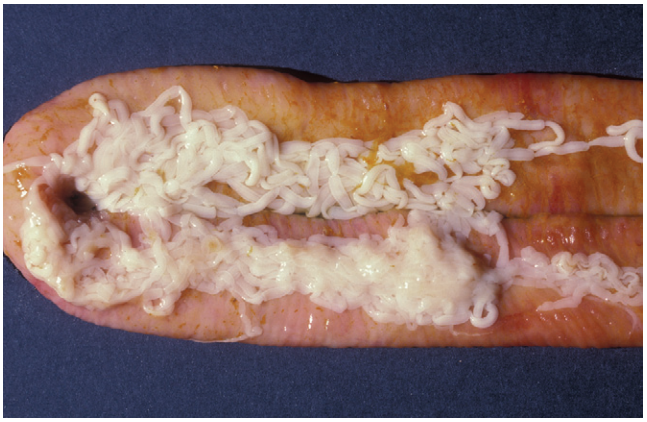


FIGURE 7-43. *Dipylidium caninum* in the intestine of a dog at necropsy.

Diphyllobothrium latum (Diphyllobothriidae). Typically, infection is without any signs (see Figures 4-25, 4-26, and 7-32, *A*).

TREMATODES. *Alaria americana* (5 mm), *Alaria arisaemoides* (10 mm), *Alaria canis* (3.2 mm), and *Alaria michiganensis* (1.9 mm) (Diplostomatidae) (see Figures 4-21 and 4-22).

Mesostephanus appendiculatum (1.8 mm) and *Mesostephanus longisaccus* (1 mm) (Cyathocotylidae). These cyathocotylids resemble *Alaria* in having a bulbous tribocytic organ but differ in not being divided into distinct forebody and hindbody regions.

Echinochasmus schwartzi (2.1 mm) (Echinostomatidae) is a slender echinostomatid with a collar of spines surrounding the oral sucker.

Apophallus venustus (1.4 mm), *Cryptocotyle lingua* (2.2 mm), and *Phagicola longa* (1.2 mm) (Heterophyidae). Dogs ingesting fish and acquiring *C. lingua* can have severe enteritis.

Plagiorchis species. This small (1.2 mm) plagiorchiid has a spindle-shaped, spinous body with well-developed suckers; the genital pore is anterior to the ventral sucker.

Nanophyetus salmincola (1.1 mm; see Figure 4-13) and *Sellacotyle mustelae* (0.4 mm) (Troglotrematidae) are ovoid and pear-shaped, respectively, and have spinous bodies and well-developed suckers. *N. salmincola* is host to *Neorickettsia helminthoeca,* which causes salmon poisoning in dogs. Signs include hemorrhagic enteritis and lymphadenopathy.

ACANTHOCEPHALA. *Oncicola canis* is small (14 mm) and spindle-shaped (see Figure 4-176). *Macracanthorhynchus ingens* is very large (see Figures 4-172 and 7-33); dogs acquire infection by ingesting millipedes, with diarrhea as the main clinical sign.

PROTISTA

FLAGELLATES. *Giardia* (see Figure 7-104) trophozoites on mucosa of the small intestine can be visualized in scrapings examined by microscopy. Diarrhea and vomiting may occur, typically in younger animals. Other infected dogs may or may not have signs but may have periodically soft feces with a foul odor. Cysts are often excreted without clinical signs.

COCCIDIA. *Cystoisospora canis, C. ohioensis, C. burrowsi, Hammondia heydorni,* and *Neospora caninum* (Apicomplexa) oocysts contain a single sporont when shed in the feces (see Figure 7-24). Schizonts, gamonts, and oocysts may also be found in histologic sections or mucosal scrapings. These coccidia cause damage to host enterocytes. Young animals and immunocompromised animals are most often affected. The main clinical sign is diarrhea, which usually is watery but may also contain mucus or blood.

Sarcocystis cruzi, Sarcocystis ovicanis, Sarcocystis miescheriana, Sarcocystis bertrami, Sarcocystis fayeri, and *Sarcocystis hemionilatrantis* (see Table 2-1 and Figure 7-54) (Apicomplexa) have sexual stages in the mucosa, usually with no clinical signs.

Cryptosporidium canis (Apicomplexa) has minute stages on the apical margins of the enterocytes that would be difficult to see without histologic sections. Most infections occur in dogs younger than 6 months and in dogs that are immunocompromised.

Cecum and Colon

NEMATODES. *T. vulpis* (Trichuroidea) (Figure 7-44; see also Figures 4-167, 7-24, 8-113, and 8-114). In small numbers, worms are found in the cecum; in heavier infections, worms also are found with their anterior end embedded in the mucosa of the colon and rectum. Most dogs are without clinical signs. Dogs can have large bowel diarrhea characterized by hematochezia; mucus and straining are the main clinical signs. Diarrhea may lead to dehydration or pseudohypoadrenocorticism in middle-aged and older dogs as a result of isotonic fluid loss causing hyponatremia, metabolic acidosis, and hyperkalemia.

PROTISTA. *E. histolytica* and *E. coli* are cyst-forming amoebae. Trophozoites of *E. histolytica* may contain phagocytosed erythrocytes. Infection with these organisms seems very rare in dogs in the United States.

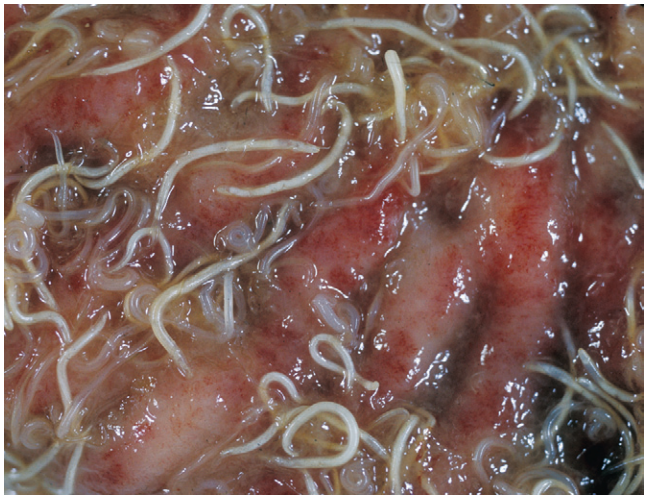


FIGURE 7-44. *Trichuris vulpis* posterior ends of worms on the mucosa of the cecum; the anterior portions of the worms are embedded in the mucosa.

Trichomonas species and *Pentatrichomonas hominis* are non–cyst-forming mucosoflagellates. They can be found by examination of mucus and will lyse in water, so saline preparations are required.

B. coli (ciliate) (see Figure 3-7) has caused colitis in dogs on very rare occasions.

Liver and Pancreas

NEMATODES. *Toxocara canis* and *T. leonina* (Ascaridoidea) sometimes erratically invade the common bile duct or pancreatic duct, causing obstruction or rupture.

Calodium (Capillaria) hepaticum (Trichinelloidea) (see Figure 8-117) is found in the liver of dogs, usually as an incidental necropsy finding.

NEMATODE LARVAE. *Toxocara canis* (Ascaridoidea) can have encapsulated larvae widely distributed in adult animals, especially in skeletal muscle and in the kidneys, but also in the liver.

TREMATODES. *Opisthorchis tenuicollis, Opisthorchis viverrini, Clonorchis sinensis, Metorchis albidus,* and *Metorchis conjunctus* (Opisthorchiidae) in bile ducts (see Figures 4-10 and 4-17); infections are usually asymptomatic unless a large worm burden is present, in which case severe hepatic dysfunction can result.

Eggs of *Heterobilharzia americana* (Schistosomatidae) in tissues are surrounded by a granulomatous reaction; granulomatous lesions in the liver may be associated with elevation of hepatic enzymes. Flushing the vascular system of the liver with saline may produce large numbers of paired flukes. Clinical signs are nonspecific and may include anorexia, lethargy, weight loss, and diarrhea.

Peritoneum and Peritoneal Cavity

CESTODE LARVAE. *Mesocestoides* tetrathyridia (see Figures 8-65 to 8-67) can be associated with massive infection owing to asexual multiplication that may be associated with diarrhea, abdominal distention, pain, and weakness.

NEMATODE. *D. renale* is a giant red worm (up to 1 m, Trichinelloidea) in the peritoneal cavity or renal pelvis (see Figure 4-159). Besides the occasional free adult in the peritoneal cavity, third-stage larvae cross through the peritoneal cavity on their way to the liver, where they molt to the fourth stage. Fourth-stage larvae again traverse the peritoneal cavity before entering the renal capsule. A serofibrinous to chronic fibrinous peritonitis can result.

Respiratory System

Nasal Passages

NEMATODES. *Eucoleus (Capillaria) boehmi* (Trichinelloidea) may cause sneezing.

ARTHROPODS. *Pneumonyssoides caninum* (Mesostigmata) (see Figures 2-99, 7-45, and 8-8). Clinical signs include reverse sneezing, chronic nasal discharge, nasal irritation, and epistaxis. Inflammation of the nasal cavity may result in loss of the sense of smell.

Linguatula serrata (130 mm, Pentastomida). These organisms are bloodsucking, wormlike parasites of the nasal cavity and paranasal sinuses. They can cause epistaxis, inflammation, and respiratory distress.

Trachea and Bronchi

NEMATODES. *Filaroides osleri* (Metastrongyloidea) (see Figures 4-111 and 7-26). *F. osleri* occurs in nodules near the bifurcation of the trachea with clinical signs of respiratory distress.

Crenosoma vulpis (Metastrongyloidea) (see Figures 4-107 and 7-26) are small worms (16 mm) found on bronchial and bronchiolar mucosa, causing most typically signs of chronic cough, dyspnea, and exercise intolerance.

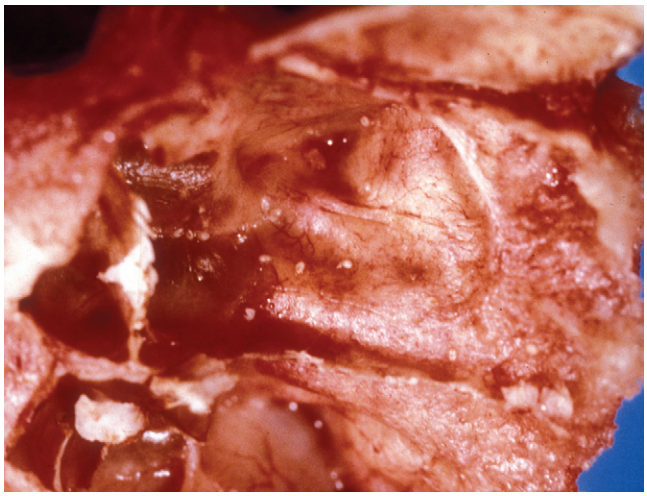


FIGURE 7-45. *Pneumonyssoides caninum* mites (Mesostigmata, Halarachnidae) in the nasal sinuses of a dog at necropsy. (Courtesy Dr. John M. King.)

Eucoleus (Capillaria) aerophilus (Trichinelloidea) is associated with signs of coughing.

Lung Parenchyma

NEMATODES. *Filaroides hirthi* and *Filaroides milksi (Andersonstrongylus milksi)* (Metastrongyloidea) (Georgi, 1975; and see Figures 4-71, 7-26, 8-89, and 8-90). Most dogs are asymptomatic, but immunocompromised dogs may show signs of severe pneumonia that can be fatal.

D. immitis (Filarioidea) (see Figure 4-150) organisms are large (30 cm) worms that occur in pulmonary arteries.

NEMATODE LARVAE. Petechial hemorrhages, areas of focal necrosis, and nodular inflammation of lung tissue may be caused by migrating nematode larvae. Such lesions should be investigated by preparing squashes and by performing the Baermann technique. Identification of nematode larvae in histologic preparations is considered in Chapter 8.

Angiostrongylus vasorum (Metastrongyloidea) eggs and larvae cause respiratory lesions, and clinical signs are varied. Dogs may experience exercise intolerance, weight loss, subcutaneous edema due to congestive heart failure and lung damage, or coagulation abnormalities.

Strongyloides stercoralis (Rhabditoidea) filariform larvae (see Figure 7-28) are migrating larvae that can also cause areas of ecchymotic and petechial hemorrhage throughout the lung parenchyma.

A. caninum, A. braziliense, and *U. stenocephala* (Ancylostomatoidea) (see Figure 7-27).

Toxocara canis (Ascaridoidea) (see Figure 7-115) migrating larvae can cause pneumonia.

Microfilariae of *Dirofilaria immitis* (Onchocercidae).

TREMATODES. *Paragonimus kellicotti* (Troglotrematidae) (see Figures 4-14, 4-15, and 7-34, *B*) live in fluke-filled cysts that are surrounded by large areas of granulation tissue around the escaping eggs. These organisms can cause severe loss of lung function.

Vascular System

Pulmonary Artery, Right Side of the Heart, and Venae Cavae

PROTISTA. *Toxoplasma gondii,* cardiac muscle (Apicomplexa).

Trypanosoma cruzi (hemoflagellate) amastigotes in heart muscle cause acute myocarditis by myocardial invasion and cycles of multiplication and cell rupture. Weakness, exercise intolerance, syncope, lymphadenopathy, pale mucous membranes, neurologic signs, and signs of right- or left-sided heart failure manifested on the electrocardiogram (ECG) as decreased QRS complexes and heart block can be seen clinically. Chronic infection can progress to dilated cardiomyopathy, and dogs can show signs of weakness, exercise intolerance, syncope, ventricular tachycardia, and sudden death.

NEMATODES. *Dirofilaria immitis* (300 mm, Filarioidea) occurs in right ventricle, right atrium, pulmonary arteries, and, rarely, venae cavae (see Figure 4-150). Adult worms live in the pulmonary arteries and cause clinical signs indicative of cardiac, pulmonary, hepatic, and renal involvement. In heavy infection, worms can invade the right side of the heart and cause congestive heart failure and ascites. Clinical signs can include coughing, exercise intolerance, dyspnea, syncope, hepatomegaly, and abnormal heart and lung sounds on auscultation. Vena cava syndrome can also occur as the result of obstruction by adult worms.

Angiostrongylus vasorum (25 mm, Metastrongyloidea) is much smaller than *D. immitis* and is located in the pulmonary arterial branches. First-stage larvae resembling those of *Aelurostrongylus* (see Figure 7-50) are shed in the host's feces. Dogs may experience exercise intolerance, weight loss, subcutaneous edema due to congestive heart failure and lung damage, or coagulation abnormalities.

Toxocara canis (Ascaridoidea) larvae in cardiac muscle.

Mesenteric and Portal Veins

TREMATODES. *Heterobilharzia americana* (Schistosomatidae) (see Figures 4-24 and 8-50) produces disease through the eggs that erode their way through the intestinal mucosa, causing granulomatous reactions in the liver.

Blood

NEMATODE MICROFILARIAE. *D. immitis* and *A. reconditum* (Filarioidea) (see Figure 7-36).

PROTISTA. *Babesia canis* (Apicomplexa) (see Figure 3-27) will be seen only at necropsy if blood films are made. Clinical signs of canine babesiosis include pale mucous membranes, icterus, hemoglobinemia and hemoglobinuria, depression, weakness, fever, anorexia, and splenomegaly.

T. cruzi (hemoflagellate) trypomastigotes may be scarce in blood films. Examine heart muscle histologically for amastigotes (see Figure 8-15).

Skeletal Muscles

Protista

N. caninum (see Figure 3-21, Apicomplexa) causes disease mainly in dogs younger than 6 months, which will show signs of paralysis. The pelvic limbs are more severely affected than the thoracic area, and signs of progressive muscle atrophy are present.

Nematode Larvae

T. spiralis (Trichinelloidea) (see Figures 4-163, 7-100, and 8-116) usually does not cause clinical signs in dogs.

A. caninum (Ancylostomatoidea) larvae are present in vacuoles in muscle fibers with little or no evidence of host reaction (see Figure 8-86).

Connective Tissues

Protista

Hepatozoon americanum (Apicomplexa) can cause myositis and periosteal bone proliferation with changes that might be evident

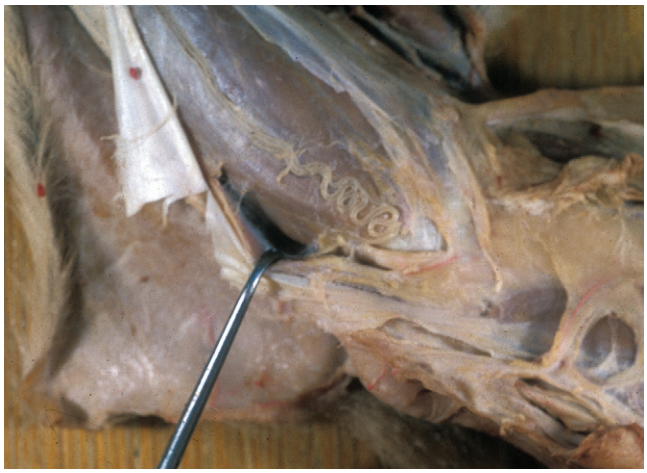


FIGURE 7-46. *Dracunculus insignis* discovered in canine dissection as part of anatomy class in 1966.

on radiographs. The organisms can form large cysts in the muscles, and muscle atrophy, hyperesthesia, and reluctance to move can result.

Nematodes

Acanthocheilonema reconditum (32 mm, Filarioidea) without clinical signs.

Dirofilaria immitis (300 mm, Filarioidea) (see Figure 4-149) migratory stages and ectopically migrating adults.

Dracunculus insignis (360 mm, Spirurida) (Figure 7-46, and see Figures 4-138, 4-139, and 8-108) causes subcutaneous nodules with associated pyoderma and focal erythema. Larvae with very long tails may be expressed from the nodules.

Insect Larvae

Cuterebra (30 mm, Cuterebridae) (see Figures 2-31, 2-32, 8-1, and 8-2) larvae migrate to the skin to create a warble. The skin can be sensitive in this area, and a draining tract may be present. The spiracles of the larvae may be seen protruding from the fistula, and the larvae can be extracted from the opening.

Cochliomyia hominivorax (17 mm, Calliphoridae) (see Figures 2-12 and 2-19).

Phaenicia sericata, Phormia regina, and *Protophormia terraenovae* (17 mm, Calliphoridae) (see Figures 2-12 and 2-19).

Wohlfahrtia vigil and *Wohlfahrtia opaca* (Sarcophagidae) (see Figure 2-19).

Urogenital System

Kidney

NEMATODES. *D. renale* (up to 1 meter, Dioctophymatoidea). A giant red worm in the renal pelvis or peritoneal cavity (see Figure 4-159). The right kidney is most often affected. Clinical signs include enlargement of the right kidney, hematuria, urinary tract infection, and, rarely, renal failure if both kidneys are affected. Eggs of *D. renale* are typically passed in the urine of infected animals (Figure 7-47).

NEMATODE LARVAE. *Toxocara canis* (Ascaridoidea) (Figure 7-48) larvae can cause nodular lesions to form in the kidneys but typically do not cause clinical signs.

Urinary Bladder

NEMATODES. *Pearsonema (Capillaria) plica* (60 mm, Trichinelloidea) organisms can be found in the epithelium of the urinary bladder and usually do not cause signs. If large numbers are present, dogs can have pollakiuria, dysuria, hematuria, and stranguria.

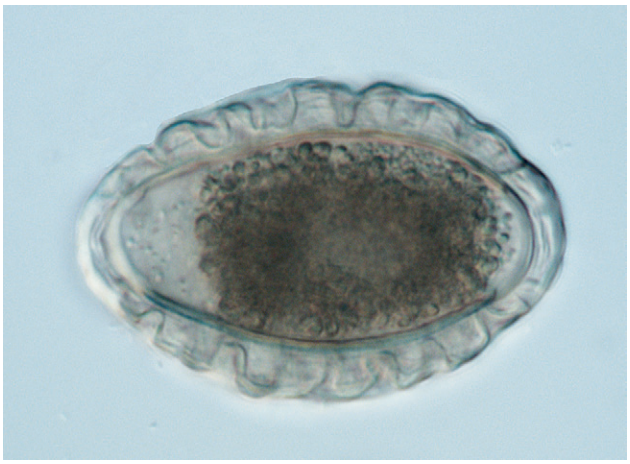


FIGURE 7-47. Egg of *Dioctophyma renale* from the urine of a dog in Trinidad. Typically, such eggs contain two cells, but the eggs examined in this sample were all similar in that they did not contain two cells. The eggs are about 80 µm long. (Specimen courtesy Dr. Miguella Mark-Carew.)

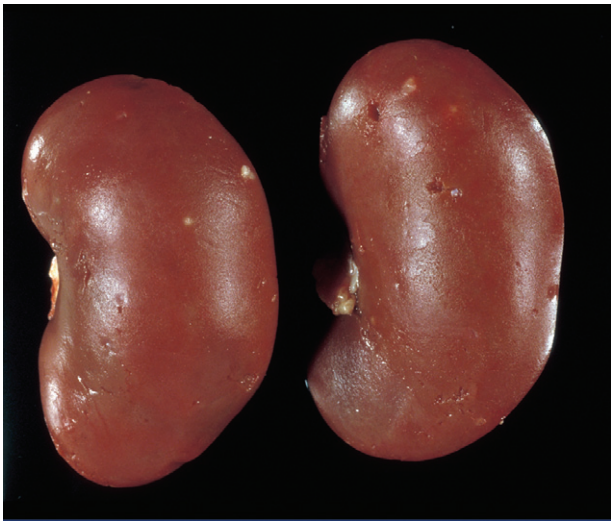


FIGURE 7-48. *Toxocara canis* lesions in canine kidneys.

Nervous System

Brain and Spinal Cord

PROTISTA. *N. caninum* (Apicomplexa) (see Figure 3-21) can appear in older dogs, causing signs of central nervous system involvement that include seizures and tremors, whereas cerebellar involvement will result in postural deficits.

NEMATODES. *Baylisascaris* species (Ascaridoidea) larvae have on rare occasions caused neurologic disease in dogs (Thomas, 1988).

Angiostrongylus cantonensis causes severe neurologic disease in dogs in Australia. The presence of the worm in the southeastern United States and in Hawaii would suggest that sooner or later, unfortunately, cases will be reported in the United States.

Eye

Nematodes

Toxocara canis (Ascaridoidea) has on rare occasions been found in the retina (Hughes, Dubielzig, and Kazacos, 1987).

D. immitis (Filarioidea) (see Figure 4-150) can occur erratically in the anterior chamber of the eye or the epidural space.

Thelazia californiensis (19 mm, Spirurida) (see Figure 4-144) can on occasion be found in the conjunctival sac and ducts of the lacrimal gland.

Onchocerca lupi (Filarioidea) has been fourd in pea-sized nodules on the screa in the southeastern United States.

Skin and Hair

Insects

Adult dipterans.

L. setosus (Anoplura) (see Figure 2-51).

Trichodectes canis (Mallophaga) (Figure 7-49, and see Figure 2-59).

Heterodoxus spiniger (Mallophaga) has club-shaped antennae that lie in cephalic grooves, and the anterior margin of the head is pointed; the organism is restricted to warm climates.

Ctenocephalides canis, Ctenocephalides felis, Pulex irritans, and *Echidnophaga gallinacea* (Siphonaptera) (see Figures 2-35, 2-36, 2-38, 2-42, and 2-44).

Arachnids

Rhipicephalus sanguineus, Dermacentor variabilis, Dermacentor andersoni, Amblyomma americanum, Amblyomma maculatum, Ixodes species, and others (Ixodidae) (see Figures 2-70, 2-71, 2-75 to 2-83, and 2-87 to 2-91).

Sarcoptes scabiei (Sarcoptidae) (see Figures 2-103 and 8-3) mites cause alopecia that usually spares the dorsum. Skin lesions are reddish and are covered with yellowish crusty material. Severe self-trauma to the skin may result from intense pruritus.

Otodectes cynotis (Psoroptidae) (see Figure 2-112) causes otitis with predisposition to secondary infection.

Demodex canis (Demodicidae) (see Figures 2-116 and 8-6) can normally be found on dogs in low numbers, and usually they do not cause disease. Demodicosis can be a localized problem, usually affecting the face and presenting as alopecia and scales surrounding the eyes and the mouth. Generalized demodicosis causes large reddened scaly alopecic coalescing patches on the head, legs, and trunk.

FIGURE 7-49. *Trichodectes canis* in the hair of a dog.

Folliculitis and furunculosis can be present, and generalized lymphadenopathy is typical. Secondary bacterial infections cause inflammation and exudation.

Cheyletiella yasguri (Cheyletidae) (see Figure 2-117) is not generally associated with signs; may cause mild dermatitis.

Nematode Larvae

Rhabditis strongyloides (Rhabditida) (see Figures 4-113 and 8-72) larvae cause a pruritic hyperemic dermatitis. The larvae are usually free living on decaying organic matter, and therefore the lesions are typically distributed on areas of the body that come in contact with the ground, such as the feet and the ventral thorax and abdomen.

PARASITES OF CATS

STAGES IN FECES AND URINE

Cats share a few parasites (e.g., *Toxascaris leonina*, *Eucoleus* [*Capillaria*] *aerophilus*, *Dipylidium caninum*, *Paragonimus kellicotti*) with dogs, and cross-infection with others may occur on rare occasions. In other parts of the world, cats and dogs may share numerous trematodes acquired through ingestion of fish. However, the most common cat parasites (Figures 7-50 to 7-56) are different species of the genera found in dogs (e.g., *Toxocara cati, Ancylostoma tubaeforme, Cystoisospora felis*). *Ancylostoma braziliense* (see Figure 7-57) is shared between dogs and cats. The capillarid found in the urinary bladder of the cat is considered to be a species separate from that of the dog and goes by the name *Personema feliscati* (see Figure 7-52).

Nematode Eggs and Larvae

The most common nematodes of the cat are *Toxocara cati* and *Ancylostoma tubaeforme;* in the southeastern United States, a good percentage of the hookworm eggs might also be those of *Ancylostoma braziliense*. The eggs of *A. braziliense* can be distinguished from those of *A. tubaeforme* with careful microscopy; the eggs of *A. braziliense* are smaller (see Figure 7-51). Cats can harbor *T. leonina,* but this seems to be less common now than it once was. Pseudoparasitism in cats is usually the result of predation rather than coprophagy. For example, the eggs of *Calodium (Capillaria) hepaticum* accumulate in infected rodent livers and may be found in the feces of a cat that has eaten such a rodent (see Figure 8-117). Feline *Trichuris* infection often excites lively debate because its rare appearances in cats in North America violate a time-honored belief that it does not exist at all. In any case, it is certainly of little practical importance, aside from its tendency to complicate the differential diagnosis of pulmonary and gastrointestinal capillariasis. Cats are host to capillarids, typically *Eucoleus aerophilus* from the respiratory system and *Aonchotheca putorii* from the stomach and small intestine.

Cestode Eggs and Segments

Cats most commonly are host to one of four tapeworms, although they can be infected with *Diphyllobothrium latum* and a few other unusual species. The four tapeworms most commonly found in cats in North America are *Spirometra mansonoides, Taenia taeniaeformis, Dipylidium caninum,* and *Mesocestoides* species. The eggs of *Spirometra* species are brown, fairly elongate, and operculate. The eggs of *T. taeniaeformis* are nearly spheroid. In the case of cats that use litter pans, people are often aware of the segments passed by cats: Rectangular segments are taeniid segments, cucumber seed–shaped segments are those of *D. caninum*, and the small sesame seed–shaped segments are those of *Mesocestoides* species. Cats can be

FIGURE 7-50. Stages of nematode parasites found in the feces of cats. *Toxocara cati* eggs are smaller and more delicate than *Toxocara canis* eggs (see Figure 7-24). *Toxascaris leonina* is a parasite of both cats and dogs. *Aelurostrongylus abstrusus* larvae may be identified by their curiously shaped tail. *Mammomonogamus* is a parasite of the respiratory tract and eustachian tubes of cats in the Caribbean and Asia. *Eucoleus aerophilus* is the lung capillarid of cats. *Aonchotheca putorii* is the gastrointestinal capillarid of cats. *A. tubaeforme* is the common hookworm of the cat. *Physaloptera praeputialis* is the stomach spirurid of the cat with a thick-shelled larvated egg that is passed in the feces.

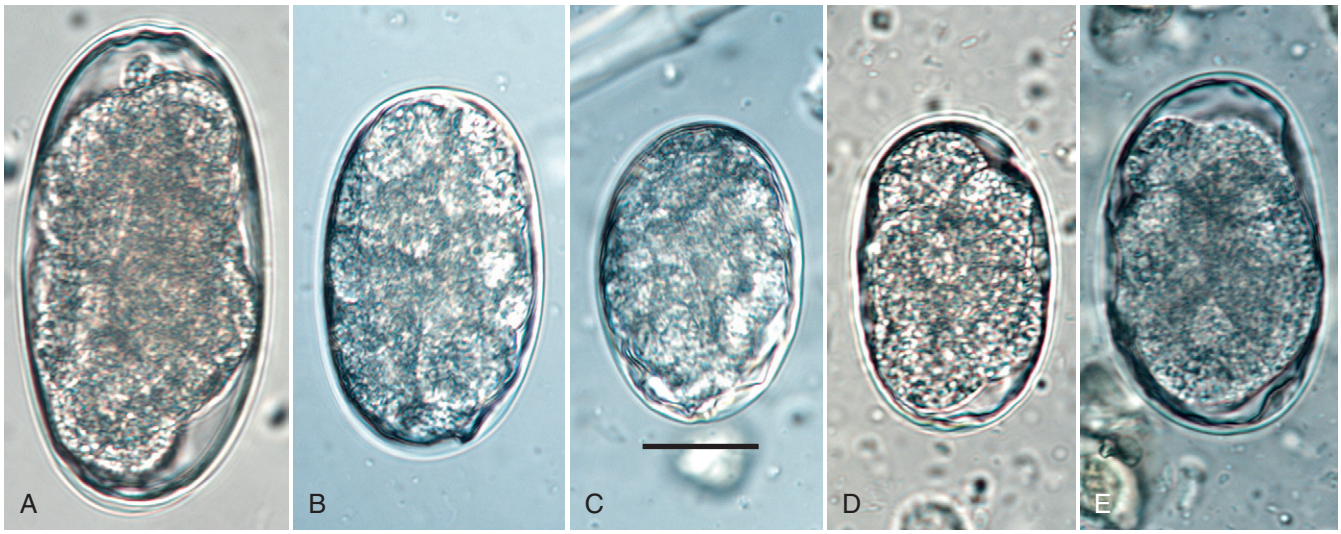


FIGURE 7-51. Hookworm eggs of dogs and cats in the United States. In this figure, the eggs are all at the same magnification (Bar = 20 µm): *Uncinaria stenocephala* from a dog **(A),** *Ancylostoma canium* **(B),** *Ancylostoma braziliense* from a dog **(C),** *Ancylostoma braziliense* from a cat **(D),** and *Ancylostoma tubaeforme* from a cat **(E)**. Occasionally in the United States, one might run across other species (e.g., *Ancylostoma pluridentatum* in a cat), and in other parts of the world, the eggs of *Ancylostoma ceylanicum* might be present in dogs or cats.

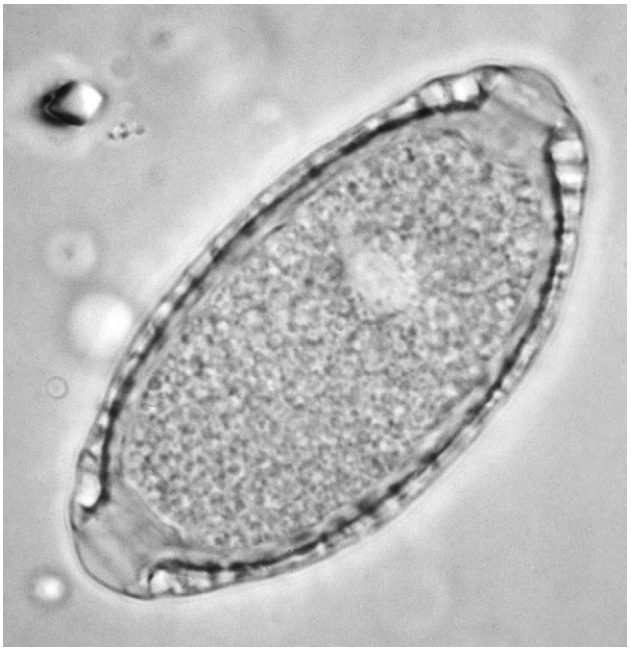


FIGURE 7-52. The egg of a *Pearsonema* species. The species *Pearsonema plica* and *Pearsonema feliscati* are considered to be the capillarids of the bladder of dogs and cats, respectively.

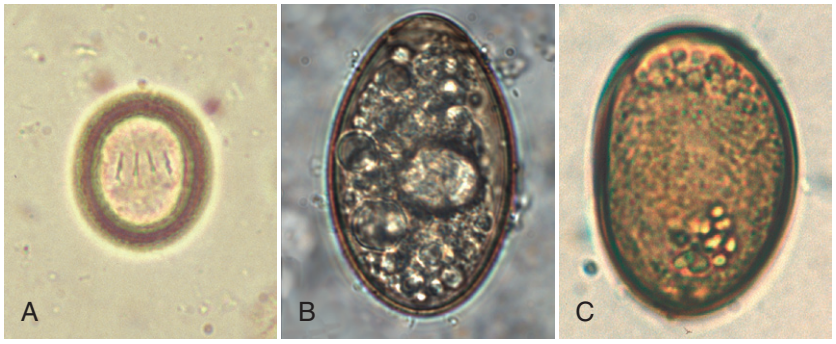


FIGURE 7-53. Eggs of cat platyhelminths. **A,** *Taenia taeniaeformis*. This taeniid cestode egg has a radially striated embryophore and contains a fully developed oncosphere. **B,** *Spirometra mansonoides*. This diphyllobothriid cestode egg has an operculate capsule and contains an undeveloped embryo. **C,** *Platynosomum fastosum*. This dicrocoeliid trematode egg also has an operculate capsule but contains a fully developed miracidium.

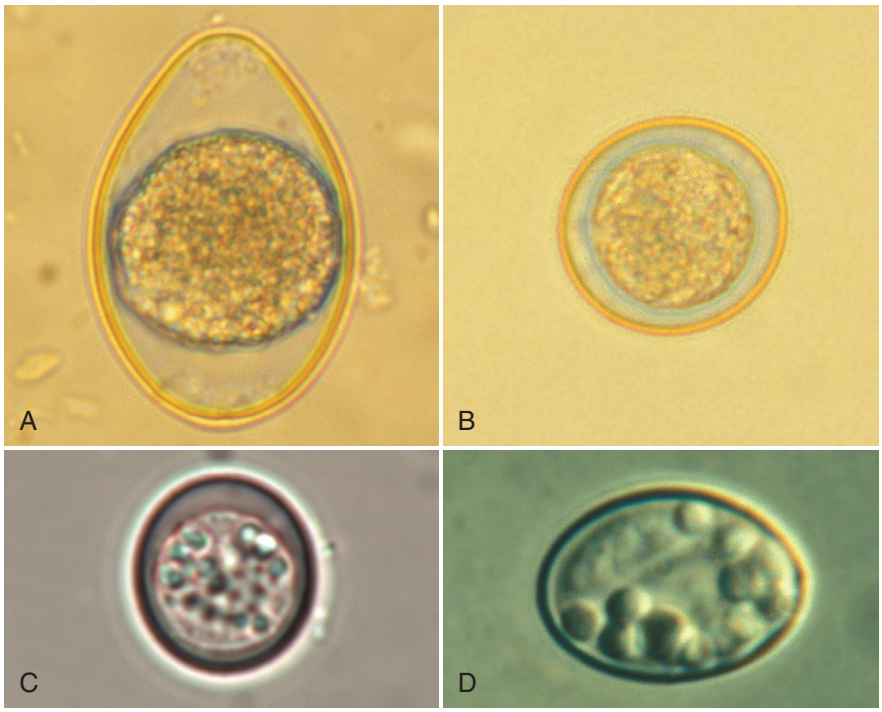


FIGURE 7-54. Coccidian cysts of cats. **A,** *Cystoisospora felis* (×1000). **B,** *Cystoisospora rivolta* (×2000). **C,** *Toxoplasma gondii* (×2000). **D,** *Sarcocystis* sp. (×2000). *Sarcocystis* sporocysts released by rupture of the oocyst wall are only slightly larger than *T. gondii* but are ovoid rather than subspheric and contain four sporozoites.

infected with species of *Echinococcus* but are less likely to be infected than dogs.

Trematode Eggs

Cats around the world are host to nearly 100, or perhaps more, species of trematodes (Bowman et al, 2002). The trematodes live in the mouth, intestinal tract, pancreatic and bile ducts, nasal fossae, lungs, and blood vessels. In all cases the eggs make their way into the feces. The eggs of most of these trematodes are operculate, but the eggs of the Schistosomatidae are not. Some of the eggs are embryonated when passed (e.g., those of *Platynosomum fastosum*), whereas others (e.g., *Paragonimus kellicotti*) contain the zygote surrounded by yolk. Some eggs can be quite large, such as those of *Alaria* species, whereas others are very small, such as those of *Metagonimus* species.

Cystoisospora, Hammondia, Besnoitia, and *Toxoplasma*

The species of *Cystoisospora* infecting cats are entirely distinct from those infecting dogs. The largest oocyst is that of *Cystoisospora felis*. A midsize oocyst is *Cystoisospora rivolta*. Several species and genera produce smaller oocysts, including *Besnoitia darling, Besnoitia wallacei,* and *Besnoitia jellisoni,* along with *Toxoplasma gondii* and *Hammondia hammondi*. Careful micrometry affords differentiation of the larger species of oocysts, but, unfortunately, the most important species, *Toxoplasma,* remains confounded with *Hammondia*. Until this dilemma is resolved, oocysts smaller than 14 µm should be regarded as *Toxoplasma*, just to be on the safe side (see Figure 7-54 and Table 7-1).

Sarcocystis

Sarcocystis sporulates within the host, and the fragile oocyst wall often breaks. Therefore the sporocyst measuring 9 to 12 × 7 to 12 µm and containing four sporozoites is the form usually found in the feces (see Figure 7-54). It is not possible to easily distinguish species of *Sarcocystis* by micrometry.

TABLE 7-1 Oocyst Dimensions in Cat Parasites

Species	Oocyst Dimensions (µm)
Cystoisospora felis	38-51 × 27-39
Cystoisospora rivolta	21-28 × 18-23
Besnoitia darling	11-13 × 11-13
Besnoitia wallacei	16-19 × 10-13
Toxoplasma gondii	11-13 × 9-11
Hammondia hammondi	11-13 × 10-12

Cryptosporidium

The oocysts of *Cryptosporidium felis* are best floated in saturated sucrose solution. Because the oocysts are a mere 5 µm in diameter, slides must be scanned at high dry magnification. *Cryptosporidium* oocysts tend to lie in the focal plane immediately below the coverslip (i.e., at the top of the air bubbles) (see Figure 3-16).

ANNOTATED HOST-ORGAN LISTING OF PARASITES OF CATS

T. gondii may occur in any tissue of any host as extracellular or intracellular tachyzoites or as bradyzoites in cysts (see Figures 3-20 and 8-35). Sexual reproduction with formation of oocysts (see Figure 7-54) occurs only in the intestinal mucosae of members of the cat family (Felidae).

Alimentary System

Mouth

PROTISTA. *Trichomonas felistomae* (Trichomonadida) is found around the gum margins and is mainly observed in cats infected with feline immunodeficiency virus (FIV), feline leukemia virus (FeLV), or feline infectious peritonitis (FIP) or in cats suffering from gingivitis; it is nonpathogenic.

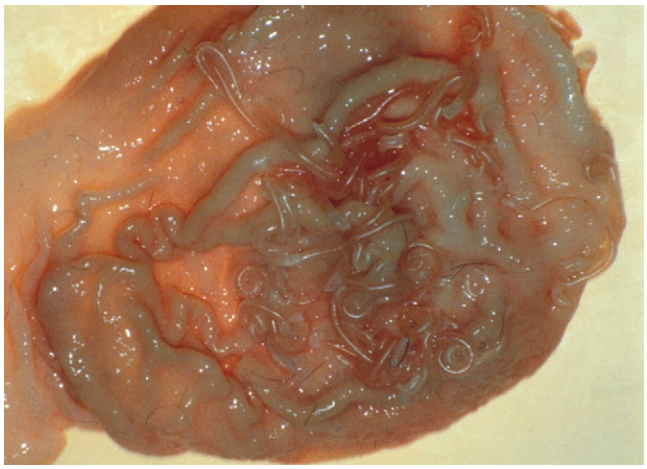


FIGURE 7-55. *Physaloptera praeputialis* in the stomach of a cat.

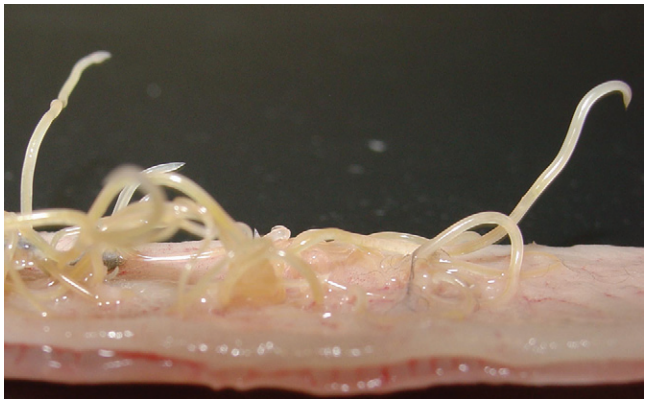


FIGURE 7-56. *Toxocara cati* in the intestine of a cat at necropsy.

Stomach and Esophagus

NEMATODES. *Gnathostoma spinigerum* (Spirurida) (see Figures 4-140 and 4-141), with head attached to stomach mucosa, may cause gastric wall perforation.

Physaloptera praeputialis and *P. rara* (Spirurida) (Figure 7-55; see also Figures 4-142 and 4-143), with the anterior end attached to the gastric mucosa, may be diagnosed endoscopically and may cause vomiting.

Ollulanus tricuspis (1 mm, Trichostrongyloidea) (see Figure 4-82) living in the stomach wall of infected cats causes chronic gastritis that results in vomiting, anorexia, weight loss, and possibly death.

Aonchotheca (Capillaria) putorii (Trichinelloidea) (see Figure 7-50) usually causes no clinical signs; it has been reported to cause perforation at the caudal aspect of the pylorus.

Small Intestine

NEMATODES. *Toxocara cati* (Ascaridoidea) (Figure 7-56; see also Figures 7-39 and 7-50) infection is usually without clinical signs except in very heavy infections.

Toxascaris leonina (Ascaridoidea) (see Figures 7-39 and 7-51) infection is typically without signs.

A. tubaeforme (Ancylostomatoidea) (see Figures 4-99, 7-50, and 7-51) usually causes no clinical signs, but cats may have weight loss, regenerative anemia, and loose, tarry stools, and infection has resulted in death due to significant blood loss from the intestinal mucosa.

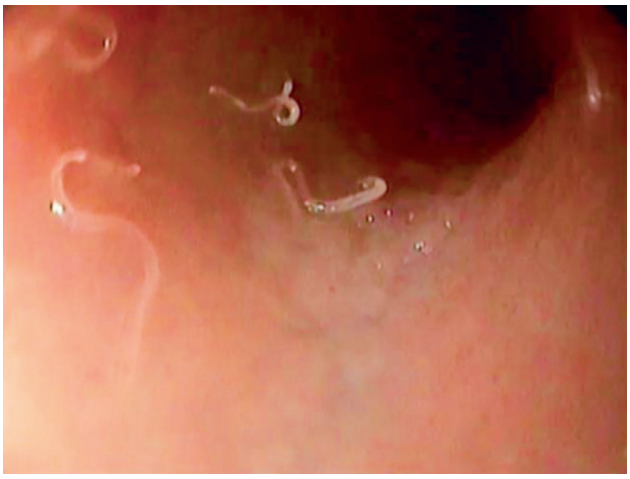


FIGURE 7-57. Adults of *Ancylostoma braziliense* as viewed by endoscopy in the duodenum of a cat.

A. braziliense (Ancylostomatoidea) (see Figures 4-99 and 7-57) causes less blood loss than *A. tubaeforme*, and experimentally infected kittens have maintained unaffected red blood cell parameters.

U. stenocephala (Ancylostomatoidea) (see Figures 4-98 and 7-51) infections in cats in the United States are very rare.

Strongyloides felis (common in Australia; Speare and Tinsley, 1987) (5 mm, Rhabditida) (see Figure 4-114).

T. spiralis (Trichinelloidea) (see Figure 4-161) causes signs referable to mild gastrointestinal upset, such as vomiting and diarrhea, maybe bloody diarrhea.

Aonchotheca (Capillaria) putorii (Trichinelloidea) (see Figure 7-50) is present in the small intestine as well as in the stomach.

CESTODES. *Taenia taeniaeformis* (Taeniidae) (see Figures 4-34 and 4-36) occurs typically without signs. In rare cases impactions have been reported.

Echinococcus multilocularis (Taeniidae) (see Figure 4-43) occurs typically without signs.

Dipylidium caninum (Dipylidiidae) (see Figures 4-53 to 4-55, 7-28, 7-30, and 7-31) occurs typically without signs.

Mesocestoides lineatus (Mesocestoididae) (see Figures 4-58 and 7-30) occurs typically without signs.

Spirometra mansonoides (Diphyllobothriidae) (see Figures 4-27 and 4-29) may be associated with diarrhea, emaciation, or vomiting.

TREMATODES. *Alaria marcianae* (5 mm, Diplostomatidae) (see Figures 4-20 to 4-22) occurs typically without signs.

Apophallus venustus (1.4 mm, Heterophyidae) occurs typically without signs.

Phagicola longa (1.2 mm, Heterophyidae) occurs typically without signs.

Mesostephanus milvi (1.8 mm, Cyathocotylidae) occurs typically without signs.

ACANTHOCEPHALA. *Oncicola* species (see Figure 4-161) occur typically without signs.

PROTISTA. *Cystoisospora felis, C. rivolta, Besnoitia* species, *Hammondia hammondi,* and *Toxoplasma gondii* (Coccidia) (see Figure 7-54) stages occur in the intestinal epithelium, where they might cause enteritis and perhaps mild diarrhea.

Sarcocystis hirsute, Sarcocystis tenella, Sarcocystis porcifelis, and *Sarcocystis leporum* (Coccidia) (see Table 2-1 and Figure 7-54) occur with sexual stages in the intestinal epithelium.

Giardia sp. (see Figure 3-6) trophozoites present on intestinal epithelium may be detected in mucosal scrapings. *Giardia* infections in cats usually occur without signs, but diarrhea may occur.

Cryptosporidium felis (see Figure 3-10; Apicomplexa) asexual and sexual stages occur in the apical portion of the epithelial cells; they are probably visible only via histologic sections. Infection is usually without signs, although occasionally it is accompanied by severe diarrhea.

Large Intestine

NEMATODES. *Strongyloides tumefaciens* (5 mm, Rhabditida) forms large tumor-like nodules in the large intestine, which are detected on abdominal palpation as a firm, fibrotic colon.

Trichuris campanula and *Trichuris serrata* (exotic, South America: Trichinelloidea) (see Figures 4-165 and 7-50).

Liver, Bile Ducts, and Gallbladder; Pancreatic Duct

NEMATODES. *Calodium (Capillaria) hepaticum* (Trichinelloidea) (see Figure 8-117).

Toxocara canis (Ascaridoidea) larvae, granulomas (see Figure 8-99) (Parsons et al, 1988).

TREMATODES. *Opisthorchis tenuicollis* and *Opisthorchis felineus* (30 mm, Opisthorchiidae) in gallbladder and bile ducts is likely to induce cirrhosis, cholecystitis, and the development of edema and ascites owing to continuing periportal fibrosis.

Metorchis albidus (4.6 mm) and *Metorchis conjunctus* (6.6 mm) (Opisthorchiidae) in bile ducts are associated with icterus and cholangiohepatitis, ascites, jaundice, and emaciation.

Amphimerus pseudofelineus (22 mm, Opisthorchiidae) occurs in gallbladder and bile ducts with anorexia, weight loss, diarrhea, vomiting, and icterus.

Parametorchis complexus (10 mm, Opisthorchiidae) occurs in bile ducts (see Figure 4-17).

Clonorchis sinensis (Asia) (Opisthorchiidae) (see Figure 4-10) occurs in gallbladder and bile ducts with occasional pancreatic duct involvement causing progressive liver cirrhosis.

Platynosomum fastosum (Platynosomum concinnum) (8 mm, Dicrocoeliidae) (see Figures 4-19 and 7-53) occurs in tropical climates in gallbladder and bile ducts, causing anorexia, weight loss, vomiting, depression, mucoid diarrhea, jaundice, and hepatomegaly.

Eurytrema procyonis (3.3 mm) (Dicrocoeliidae) occurs in the pancreatic duct, bile ducts, and gallbladder, causing cirrhosis and pancreatic atrophy and fibrosis (Figure 7-58).

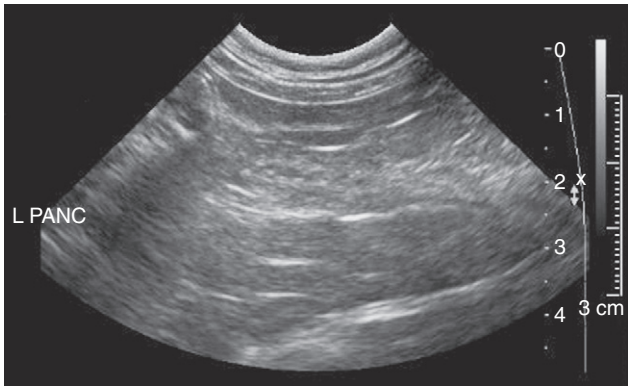


FIGURE 7-58. Long-axis ultrasonic image of the left limb of the pancreas of a cat with *Eurytrema procyonis*. The pancreas is mildly enlarged and the thickened walls of the mildly distended pancreatic duct have a beaded appearance.

Respiratory System

Nasal Cavity, Trachea, and Bronchi

NEMATODES. *Eucoleus (Capillaria) aerophilus* (Trichinelloidea) (see Figure 7-50).

Mammomonogamus species (Syngamidae) (Figure 7-59) occurs in nares and nasopharynx; species seen in the middle ear.

Lung Parenchyma

NEMATODES. *Aelurostrongylus abstrusus* (9 mm, Metastrongyloidea) (see Figures 7-52 and 8-85) occurs in terminal respiratory bronchioles and alveolar ducts, with most signs related to developing eggs lodged in the tissues; cats harboring large worm burdens may experience bronchopneumonia and show signs of open-mouthed abdominal breathing.

TREMATODES. *Paragonimus kellicotti* and other *Paragonimus* species outside the United States (Troglotrematidae) (see Figures 4-14, 4-15, and 7-34, *B*) occur in nodules, typically in pairs or in greater numbers within the cysts; animals are generally without signs, but respiratory distress or even death may be associated with the infection.

INSECTS. *Cuterebra* species (Diptera) can migrate through the lung tissue of cats (see Figure 8-1).

Vascular System

Heart

NEMATODES. *Dirofilaria immitis* (Filarioidea) (see Figures 4-149, 4-150, and 8-109) occurs in arteries; cats very typically have few worms and signs of infection from migrating developmental forms.

Toxocara canis (Ascaridoidea) larvae, granulomas (Parsons et al, 1988) (see Figure 7-106).

Mesenteric Veins

TREMATODES. *Schistosoma japonicum* (Schistosomatidae) in cats in southeast Asian countries.

Blood

PROTISTA. *Cytauxzoon felis* (piroplasm) (see Figure 3-28) occurs with merozoites in erythrocytes and schizonts in macrophages in the lumen of vessels in most organs. Cats may show signs of anemia, depression, anorexia, dehydration, fever, icterus, and enlargement of the liver and spleen.

NEMATODE MICROFILARIAE. *Dirofilaria immitis* (Filarioidea) (see Figure 7-36) rarely produces microfilariae in cats; other filarids infect cats in other parts of the world.

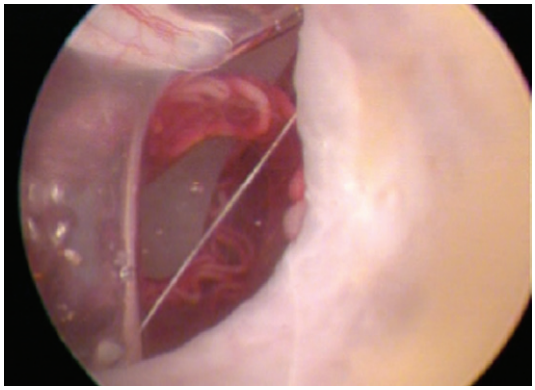


FIGURE 7-59. *Mammomonogamus auris* in the middle ear of a cat as viewed through an otoscope. (Courtesy Dr. Edgar Tudor, Paradise Animal Hospital, Saipan, United States.)

Skeletal Muscles

Nematode Larvae

Trichinella spiralis (Trichinelloidea) (see Figures 4-163, 7-100, and 8-116).

Connective Tissues

Insect Larvae

Cuterebra species (Diptera) (30 mm) (see Figures 2-32, 8-1, and 8-2) occur as migrating forms.

Urogenital System

Kidneys

NEMATODES. *Toxocara canis* (Ascaridoidea) larvae, granulomas (Parsons et al, 1988) (see Figure 7-48).

Urinary Bladder

NEMATODES. *Pearsonema (Capillaria) plica* (60 mm) and *Pearsonema feliscati* (32 mm) (Trichinelloidea) (see Figure 7-52).

Nervous System

Nematodes

D. immitis (Filarioidea) adults migrate erratically in meninges and ventricles (see Figures 4-149 and 4-150).

Insect Larvae

Cuterebra species (Diptera) (30 mm) (see Figures 2-32, 8-1, and 8-2) larvae can migrate through spinal cord and brain with clinical signs largely dependent on the path taken; seizures, vestibular signs, blindness, dementia, circling, disorientation, and death have all been noted.

Eye

Protista

Toxoplasma gondii (Coccidia) can cause iritis, uveitis, detached retina, iridocyclitis, keratic precipitates, mydriasis, anisocoria, and delayed pupillary reflex.

Skin and Hair

Insects

Adult dipterans.

Felicola subrostratus (Mallophaga) live clinging to the hairs of cats to which they attach their eggs (Figure 7-60, and see Figure 2-47).

Ctenocephalides felis, Ctenocephalides canis, and *E. gallinacea* (Siphonaptera) (see Figures 2-35, 2-36, and 2-44).

Insect Larvae

Cuterebra species (Diptera) (30 mm) (see Figures 2-32, 8-1, and 8-2) larvae penetrate the skin of the cat after internal migrations and form a subcutaneous warble. The third-stage larvae and its spiracles can often be seen through the pore of the warble.

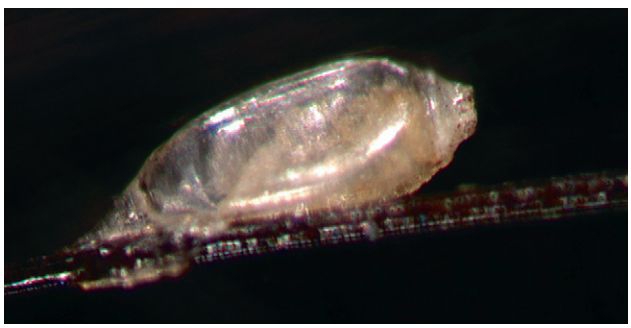


FIGURE 7-60. Egg of *Felicola subrostratus* glued to a cat's hair.

Arachnids

Dermacentor species, *Haemaphysalis leporispalustris, Ixodes* species, and *Amblyomma americanum* (Ixodidae) (see Figures 2-70, 2-75, 2-79, and 2-86 to 2-90).

Notoedres cati and *Sarcoptes scabiei* (Sarcoptidae) (see Figures 2-101, *A,* and 2-103 to 2-106).

O. cynotis (Psoroptidae) (see Figure 2-102, *A,* and 2-112).

Lynxacarus radovskyi (Listrophoroidea) (see Figure 2-115).

Cheyletiella blakei (Cheyletidae) (see Figure 2-117).

Demodex cati (Demodicidae) (see Figure 2-116).

Neotrombicula whartoni and *Walchia americana* (Trombiculidae) (see Figures 2-119 to 2-121). *N. whartoni,* a bright red chigger, has been found in the external ear canal of cats. *W. americana,* normally a parasite of the gray squirrel *Sciurus carolinensis,* is capable of causing a severe and generalized dermatitis in cats (Lowenstine, Carpenter, and O'Connor, 1979).

PARASITES OF RUMINANTS

STAGES IN FECES

Nematode Eggs

Other than the numerous eggs of various strongylid parasites that will be present in the feces, one commonly finds the eggs of *Strongyloides, Trichuris,* and capillarids (Figure 7-61). The strongylid eggs present in ruminant feces cannot be readily identified to genus or species with the exception of certain types (e.g., *Nematodirus battus*). When a more specific diagnosis is required, it is necessary to culture the stages present in the feces to the infective stage.

Eggs of the following ruminant nematodes are not illustrated in Figure 7-61. *Toxocara vitulorum* (parasite of cattle) eggs look a lot like *Toxocara canis* eggs, are subglobular with a uniformly pitted surface, and contain a single cell when passed. *Note:* Patent *Ascaris suum* infections are occasionally reported from sheep and cattle. *A. suum* eggs (see Figure 7-98) are easy to distinguish from those of *T. vitulorum. Gongylonema* eggs are thick walled, have bipolar opercula, and contain vermiform embryos (Figure 7-62). *Skrjabinema ovis* eggs are typical pinworm eggs, with one side slightly flattened (see Figure 7-71).

Identification of Strongyle Infective Larvae

Identification of third-stage infective larvae in cultured ruminant feces is challenging but not formidable. Usually two or more genera are present, and one can best determine just how many there are by scanning the slide at low power and mentally grouping those of similar appearance. Certain species stand out from the crowd. For example, *Strongyloides* larvae are more slender than any of the others, lack a sheath, and have a long cylindric esophagus and truncated tail. Two sizes, of which the larger is "standard," are portrayed in Figure 7-63. Dr. Georgi encountered both sizes in a single culture. Similarly, *Bunostomum* species are distinguished from other sheathed strongyle larvae by their smaller size. Other genera of sheathed larvae may be grouped according to the length of their caudal sheath extension (the extension of the sheath beyond the tip of the larva's tail): short, *Trichostrongylus* and *Ostertagia;* medium, *Haemonchus* and *Cooperia;* and long, *Oesophagostomum* and *Chabertia,* as illustrated in Figures 7-63 and 7-64. Within these groupings, further identification depends on micrometry and observation of such morphologic details as the caudal tubercles of *Trichostrongylus,* the "oval bodies" of *Cooperia,* and the number and

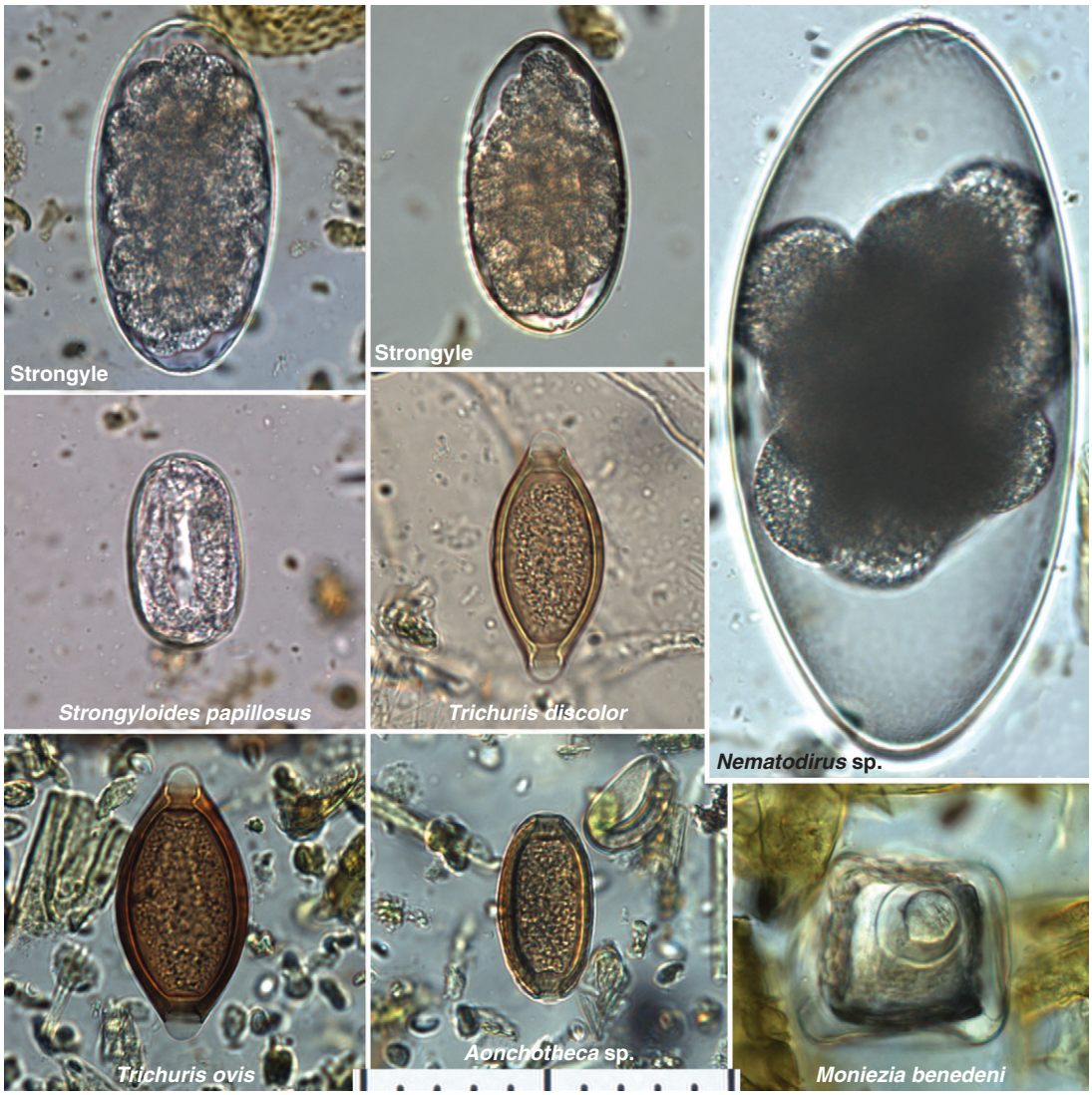


FIGURE 7-61. Eggs of common ruminant parasites. Strongyle eggs are ellipsoidal, are smooth-walled, and contain a morula. Although *Nematodirus* species eggs are very large, some species are considerably smaller than the one shown here. *Marshallagia marshalli* eggs *(not shown)* are also very large but differ from *Nematodirus* eggs in having more parallel sides and less pointed poles. *S. papillosus* eggs are slightly smaller than strongyle eggs and contain a rhabditiform larva in fresh fecal specimens. On incubation, the larvae soon hatch and develop into infective filariform larvae (see Figure 4-115) or free-living adult males and females, predominantly the latter. *Trichuris* eggs of ruminants are more than 60 µm long; those of *Capillaria* are less than 60 µm long. *Moniezia* eggs contain a pear-shaped embryophore containing an oncosphere. *Thysanosoma* eggs *(not shown here)* are grouped in uterine capsules.

FIGURE 7-62. Egg of *Gongylonema pulchrum* from a worm removed from the esophagus of a cow.

shape of the intestinal cells of *Oesophagostomum* and *Chabertia*. The odd larva may defy identification, but accurate diagnosis of the predominating genera in a culture is not a difficult task. Proceed as follows.

Place a drop of the larval suspension on a microscope slide. Relax the larvae by gentle warming or by adding a drop of Lugol's solution (5 g iodine crystals and 10 g potassium iodide in 100 mL distilled water). Ring the coverslip with petroleum jelly for support and thus prevent distortion of the larvae. Avoid higher magnifications at first but instead scan the slide under low power to get an impression of how many different kinds of larvae are present. Then seek representatives of each kind; examine these under higher power; and take whatever measurements may be necessary for generic or specific diagnosis. The data in Table 7-2 are taken from the works of Dikmans and Andrews (1933, sheep) and Keith (1953, cattle). The number of intestinal cells is 16, except as otherwise noted. Taxa grouped with braces are similar in appearance and require more care for their differentiation than is required for comparisons among other groups.

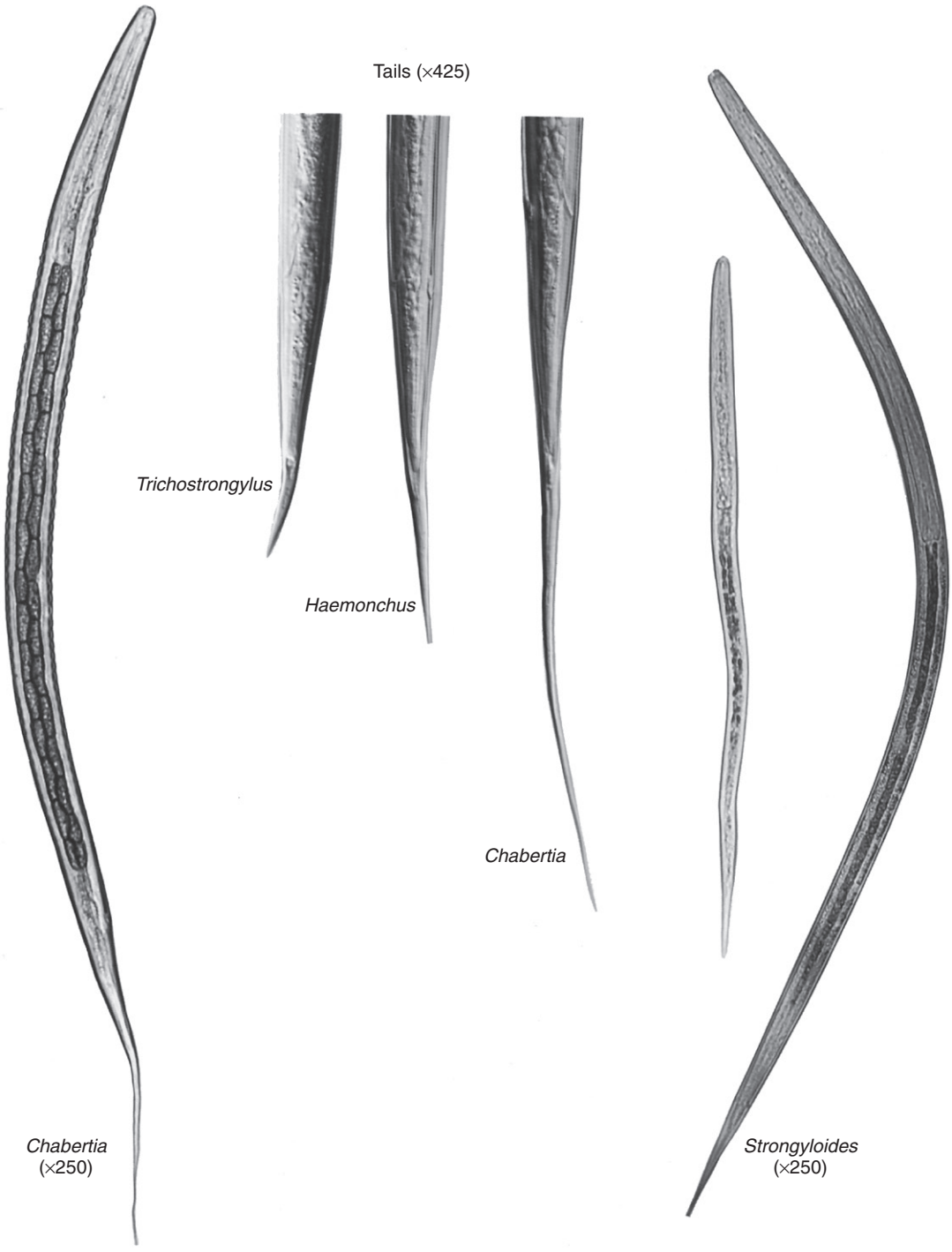


FIGURE 7-63. Infective third-stage larvae of nematode parasites of sheep. Both large and small *Strongyloides* infective larvae are represented at the same magnification.

Lungworm Larvae

D. viviparus is the only lung nematode of cattle. *Dictyocaulus filaria, Protostrongylus rufescens,* and *Muellerius capillaris* are common lung nematodes of sheep and goats in North America. Differential diagnosis is based on morphologic features of the first-stage larvae found in the host's feces (Figure 7-65). *Dictyocaulus* species larvae are tough enough to be countable by the Cornell-McMaster egg-counting technique, but counting should be done promptly to avoid osmotic shriveling of the larvae. For sensitive qualitative diagnosis of lungworm infection, Baermann is the technique of choice.

Cestode Eggs

The eggs found in the feces of cattle are all from tapeworms within the family Anoplocephalidae (see Figures 4-50 and 7-61). The egg of *Moniezia benedeni* has a fairly thick shell and seems to be cuboidal in shape. The other species of anoplocephalid tapeworms for the most part seem to have fairly thin shells that distort in various flotation media (and also clear to some extent). In almost all cases, careful inspection of the egg will allow visualization of the piriform apparatus containing the larva with its six hooklets.

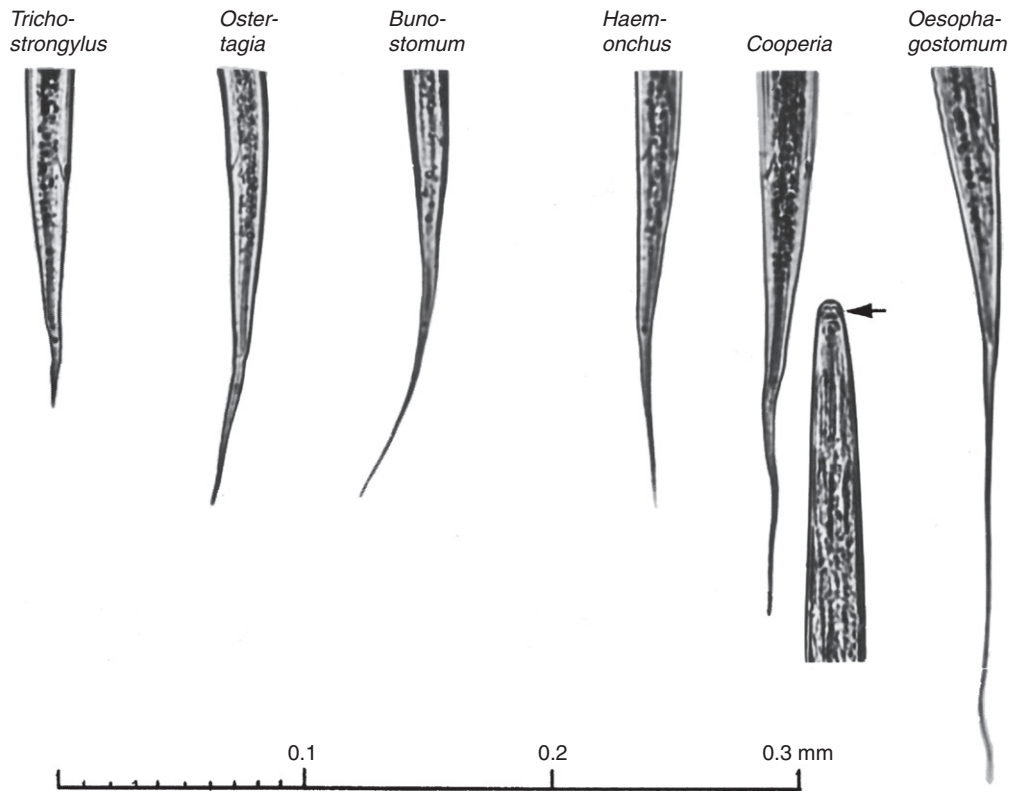


FIGURE 7-64. Tails of infective third-stage larvae of nematode parasites of cattle and the anterior end of a *Cooperia* larva showing the conspicuous oval bodies *(arrow),* which represent optical cross sections of a bundle of fibers surrounding the buccal capsule (×350). (From Whitlock JH: *The diagnosis of veterinary parasitisms,* Philadelphia, 1960, Lea & Febiger.)

Trematode Eggs

Trematode eggs may fail to float in the concentrations of sugar solutions ordinarily used. They are best concentrated by washing feces through sieves to remove coarse debris, then centrifuging the washings. The eggs will be found in the sediment. The formalin–ethyl acetate sedimentation technique is also appropriate. The operculum of trematode eggs is sometimes difficult to see. When in doubt, press the coverslip with a pencil point. Usually, the type of operculum found on trematode eggs will pop open under such pressure (Figure 7-66, *B*).

Fasciola hepatica eggs are large (up to 150 µm) and operculate and contain a cluster of yolk cells (Figure 7-66, *A*). *Fasciola gigantica* (Africa, Hawaii, Philippines, and India) eggs are like those of *F. hepatica* but larger (more than 150 µm). Eggs of *Fascioloides magna,* normally a parasite of deer, resemble those of *F. hepatica* but are infrequently found in the feces of infected domestic ruminants because the eggs are trapped in the hepatic cysts containing the adult worms in cattle, and because the flukes fail to mature in sheep and goats. Paramphistomatid (rumen fluke) eggs are large and easily confused with those of *Fasciola* species (see Figure 7-66, *B*). *Dicrocoelium dendriticum* eggs are small (50 µm), lopsided, and yellowish brown and contain a miracidium (Figure 7-66, *C*). *Eurytrema pancreaticum* (Far East) eggs resemble those of *D. dendriticum*. Schistosome eggs lack an operculum, contain a fully developed miracidium, and are armed with a spine.

Coccidia of Ruminants

Oocysts of *Eimeria* species are often found in considerable numbers in the feces of healthy ruminants. Even experimental lambs raised on wire become infected with coccidia. Despite their frequent occurrence in healthy animals, coccidia are quite capable of causing serious disease in cattle, sheep, and goats. At times, severe disease signs appear before oocysts are shed in the feces. Diagnosis of clinical coccidiosis must be based not only on identification of the oocysts in the feces (Figures 7-67 and 7-68), but also on consideration of the case history and clinical signs.

Figure 7-67 presents the unsporulated and sporulated oocysts of nine species of *Eimeria* from sheep. Goats have a closely similar set, which do not, however, cross-infect and are probably all distinct species. Corresponding species of *Eimeria* of sheep and goats are listed in Table 7-3. The species listed for sheep are those illustrated in Figure 7-67. *Eimeria ahsata, Eimeria bakuensis,* and *Eimeria crandallis* differ mainly in size, and the ranges overlap, so differentiating the three species is problematic. Therefore these three species are listed in Table 7-3 under "*Ahsata* group," and their counterpart goat parasites are listed under "*Arloingi* group." Oocysts of *Eimeria caprovina, Eimeria absheronae,* and *Eimeria caprina* resemble those of *Eimeria faurei* very closely, so we have also assigned these species to an unofficial composite group. (In the table, asterisks indicate those species most likely to be responsible for clinical signs of coccidiosis.)

Cryptosporidium

The oocysts are best concentrated by flotation in saturated sucrose solution. Because the oocysts of *Cryptosporidium parvum* and *Cryptosporidium bovis* are a mere 5 µm in diameter, the slide must be scanned under high dry magnification. *Cryptosporidium* oocysts tend to lie in the focal plane immediately below the coverslip (i.e., at the top of the air bubbles) (see Figure 3-10). Cattle are hosts to several species of *Cryptosporidium: C. parvum* and *C. bovis* of the

TABLE 7-2 Table of Measurements of Infective Third-Stage Larvae of Strongyles Infecting Sheep and Cattle

Genus (Sensu Latu)*	MEASUREMENTS (MICRONS) Overall	Tail of Sheath†	Extension of Sheath‡	Special Morphologic Features
Strongyloides				
Sheep	574-710	Sheath		Length of esophagus at least 1/3 length of body cauda
Cattle	524-678	Absent		extremity of larva truncated
Trichostrongylus				
Sheep	622-796	76-118	21-40	Tiny tubercles on tip of tail
Cattle	619-762	83-107	25-39	
Ostertagia				
Sheep	797-910	92-130	30-60	
Cattle	784-928	126-170	55-75	
Haemonchus				
Sheep	650-751	119-146	65-78	Sheath kinked at tip of tail; anterior end tapers
Cattle	749-866	158-193	87-119	
Cooperia oncophora				
Sheep	804-924	124-150	62-82	Two conspicuous oval bodies at anterior end of esophagus
Cattle	809-976	146-190	79-111	
Cooperia spp.				
Sheep	711-850	97-122	35-52	Two conspicuous oval bodies at anterior end of esophagus
Cattle	666-866	109-142	47-71	
Nematodirus				
Sheep	922-1118	310-350	250-290	Unlikely to be encountered in cultures less than 2 weeks old;
Cattle	1095-1142	296-347	207-266	forked tail with rodlike process; intestine has eight cells
Bunostomum				
Sheep	514-678	153-183	85-115	Small size, long tail sheath
Cattle	500-583	129-158	59-83	
Oesophagotomum				
Sheep	771-923	193-235	125-160	16-24 triangular intestinal cells
Cattle	726-857	209-257	134-182	
Chaberia				
Sheep	710-789	175-220	110-150	24-32 rectangular intestinal cells

*Taxa grouped with braces are similar in appearance and require more care for differentiation than other groups.
†Anus to tip of sheath.
‡Tip of larva to tip of sheath.

small intestine and *Cryptosporidium andersoni* of the abomasum are probably the most common, but cattle can also be infected with *Cryptosporidium ryanae* and *Cryptosporidium ubiquitum*. The oocysts of *C. andersoni* are larger than those of *C. parvum*, being about 7 µm in diameter, and are ellipsoidal (see Figure 3-11). Sheep and goats are infected as neonates with *C. parvum*, but they can also be infected with *Cryptosporidium xiaoi*.

Other Protista

Cattle, sheep, and other ruminants can also be host to other protista. Among the most common groups of protozoa seen in the feces of cattle, sheep, and other ruminants are amoeba cysts, considered in these hosts to be commensals. Also, *Giardia* can sometimes be found in the feces of ruminants, some with signs of infection and some without. Cattle are also host to a commensal protistan, *Buxtonella sulcata*, which has cysts in the feces that look very similar to those of *B. coli* of pigs.

ANNOTATED HOST-ORGAN LISTING OF PARASITES OF RUMINANTS

Neospora caninum may occur as extracellular or intracellular tachyzoites or as bradyzoites in cysts (see Figure 8-35). *T. gondii* may occur in any tissue of any host as extracellular or intracellular tachyzoites or as bradyzoites in cysts (see Figure 8-35).

Alimentary System

Mouth, Esophagus, and Forestomachs

PROTISTA. *Sarcocystis* (Apicomplexa) sarcocysts occur in muscles of tongue and esophagus (see Figures 8-32 and 8-33).

CESTODE LARVAE. *Taenia* species (Taeniidae) cysticerci occur in muscles of tongue (see Figures 4-38 and 8-60).

INSECT LARVAE. *Hypoderma lineatum* (Diptera: Hypodermatidae) in wall of esophagus.

NEMATODES. *Gongylonema pulchrum* (150 mm) and *Gongylonema verrucosum* (100 mm) (Spirurida) (see Figures 4-145, 4-146,

Dictyocaulus viviparus (×250) *Dictyocaulus filaria* (×250)

Protostrongylus sp. (×425) *Muellerius* sp. (×425)

FIGURE 7-65. First-stage larvae of ruminant lungworms. *Dictyocaulus viviparus* is the only lungworm of cattle, and *D. viviparus* first-stage larvae are the only larvae of parasitic nematodes found in fresh cattle dung. Notice the prominent granules. *Dictyocaulus filaria* first-stage larvae from sheep are large and have bluntly rounded tails and a "button" at the mouth, and likewise have prominent granules. *Protostrongylus rufescens* larvae are rather stout and have conically tapering tails without spines. *Muellerius capillaris* larvae have a curiously shaped tail with a dorsal spine *(inset)*.

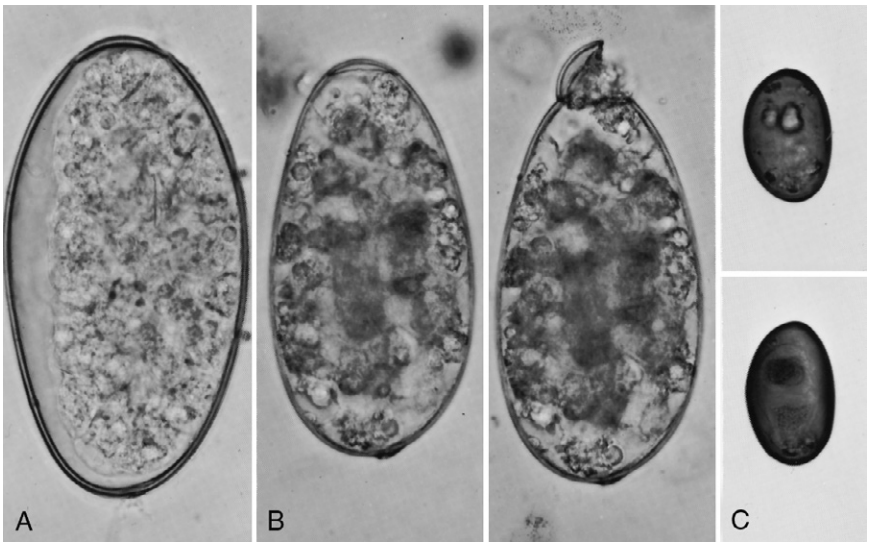


FIGURE 7-66. Eggs of some trematode parasites of ruminants (×425). **A,** *Fasciola hepatica.* **B,** *(right and left)* Paramphistominae. **C,** *(top and bottom), Dicrocoelium dendriticum.*

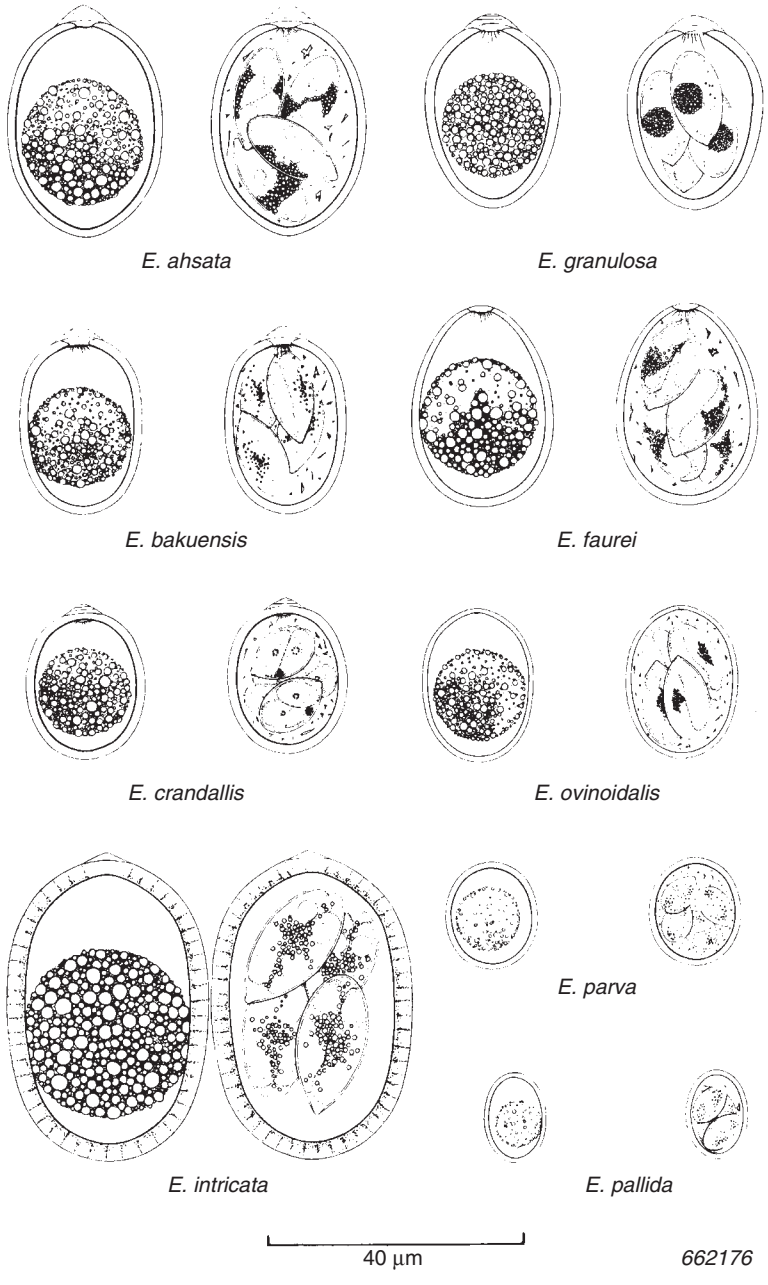


FIGURE 7-67. Unsporulated and sporulated oocysts of nine species of *Eimeria* of sheep (×1000). (From Joyner LP, Norton CC, Davies SFM, Watkins CV: The species of coccidia occurring in cattle and sheep in the southwest of England, *Parasitology* 56:533, 1966. Crown copyright. Reproduced with permission from the Controller of Her Britannic Majesty's Stationery Office.)

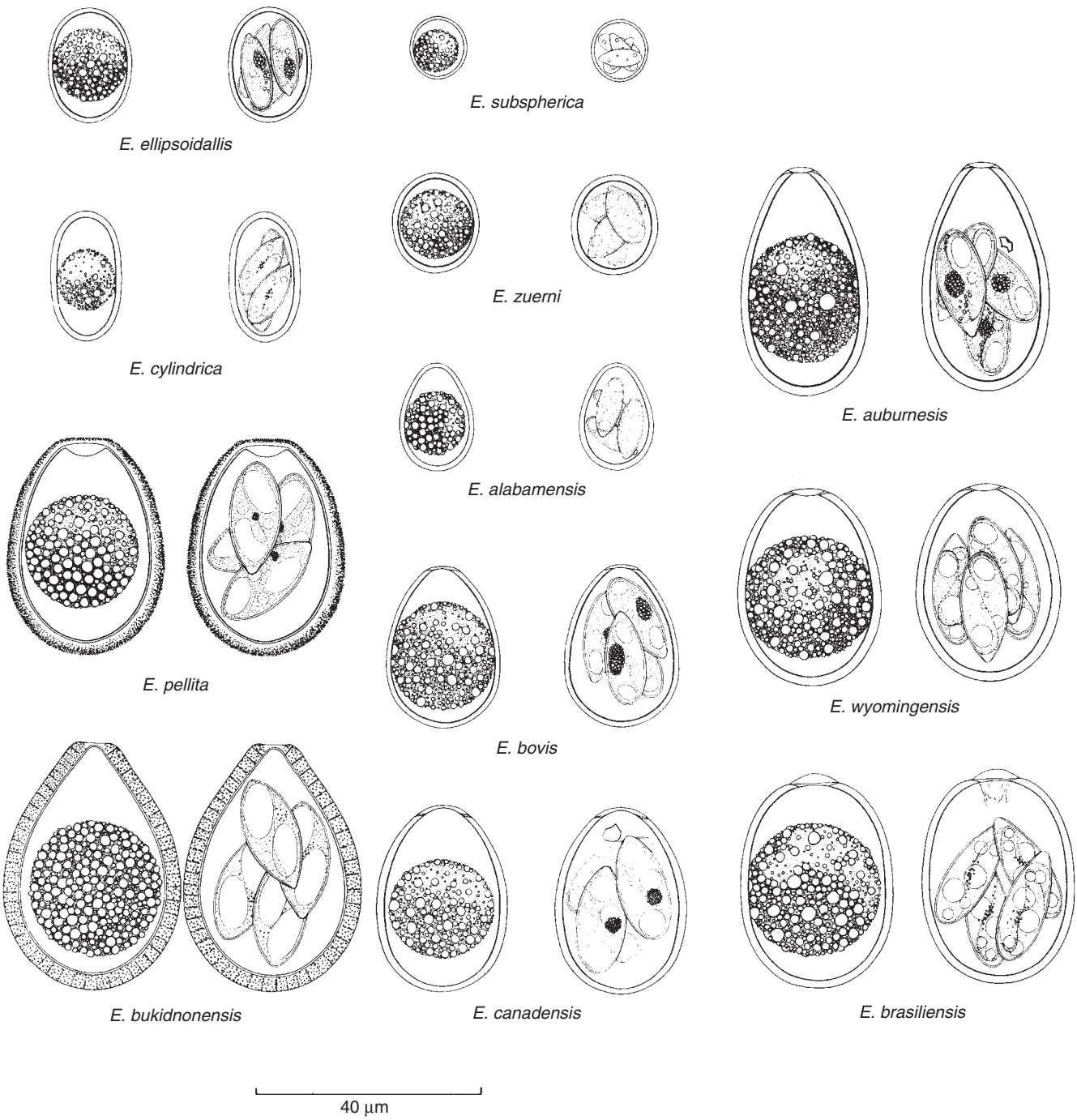


FIGURE 7-68. Unsporulated and sporulated oocysts of 12 species of *Eimeria* of cattle (×1000). (From Joyner LP, Norton CC, Davies SFM, Watkins CV: The species of coccidia occurring in cattle and sheep in the southwest of England, *Parasitology* 56:536, 1966. Crown copyright. Reproduced with permission from the Controller of Her Britannic Majesty's Stationery Office.)

TABLE 7-3 Corresponding Species of *Eimeria* of Sheep and of Goats

SHEEP	GOATS	SHEEP	GOATS
Ahsata Group	**Arloingi Group**	**Faurei Group**	**Absheronae Group**
Eimeria ahsata, Eimeria bakuensis,** and *Eimeria crandallis*	*Eimeria arloingi,* Eimeria hirci,* and *Eimeria christenseni**	*Eimeria faurei* and *Eimeria caprovina*	*Eimeria absheronae,* Eimeria caprina,* and *Eimeria caprovina*
Eimeria intricata	*Eimeria kocharii**	*Eimeria ovinoidalis*	*Eimeria ninakohlyakimovae*
Eimeria granulosa	*Eimeria jolchijevi*	*Eimeria parva*	*Eimeria alijevi**
		Eimeria pallida	*Eimeria pallida*

*Species most likely to be responsible for clinical signs of coccidiosis.

and 7-62 and 7-118) occur woven in a neat, sinusoidal pattern in the esophageal *(G. pulchrum)* or ruminal *(G. verrucosum)* mucosa.

TREMATODES. *Cotylophoron cotylophoron, Paramphistomum cervi, Paramphistomum liorchis,* and *Paramphistomum microbothroides* (Paramphistomatidae) (see Figure 4-12).

Abomasum

PROTISTA. *Eimeria gilruthi* (Coccidia) megaschizonts (see Figure 8-27).

Cryptosporidium andersoni (Apicomplexa) occurs usually without clinical signs (see Figure 3-111).

NEMATODES. *Haemonchus contortus, Haemonchus placei, Haemonchus similis, Mecistocirrus digitatus, Ostertagia ostertagi, Ostertagia bisonis, Ostertagia (Teladorsagia) circumcincta, Ostertagia orloffi, Ostertagia trifurcata, Ostertagia (Grosspiculagia) lyrata, Ostertagia (Grosspiculagia) occidentalis, Ostertagia (Telodorsagia) davtiani, Ostertagia (Pseudostertagia) bullosa, M. marshalli,* and *Trichostrongylus axei* (Strongylida: Trichostrongyloidea) (see Figures 7-63 and 7-64, Table 7-4). These parasites, depending on the species, cause anemia, diarrhea, abomasitis, and so on (Figure 7-69).

Small Intestine

NEMATODES. *T. vitulorum* (30 cm, Ascaridoidea) is only rarely seen in the United States (see Figure 4-135), although it can be common in the developing world. It has an esophageal ventriculus and produces subspheric eggs with a pitted *T. canis*–like shell surface. *A. suum,* a very occasional parasite of ruminants, lacks a ventriculus and produces ellipsoidal eggs with a mammillated shell surface.

Cooperia curticei, Cooperia bisonis, Cooperia oncophora, Cooperia pectinata, Cooperia punctata, Cooperia spatulata, Cooperia occidentalis, Trichostrongylus colubriformis, Trichostrongylus longispicularis, Trichostrongylus capricola, Trichostrongylus vitrinus, Nematodirus helvetianus, Nematodirus spathiger, Nematodirus filicollis, Nematodirus abnormalis, Nematodirus lanceolatus, and *N. battus* (Strongylida: Trichostrongyloidea) occur, with the typical associated sign in heavy infection being diarrhea (see Table 7-4).

Bunostomum phlebotomum (cattle) and *Bunostomum trigonocephalum* (sheep) (25 mm, Ancylostomatoidea) (see Figure 4-96) are capable of causing anemia in younger animals with heavy infection.

Strongyloides papillosus (6 mm, Rhabditida) (see Figure 4-115) can cause diarrhea and anemia when present in large numbers.

Aonchotheca (Capillaria) bovis and *Aonchotheca (Capillaria) brevipes* (Trichinelloidea) (see Figure 7-61).

Oesophagostomum species third- and fourth-stage larvae (Strongyloidea) (see Figure 4-90).

CESTODES. *Moneizia expansa* and *Moneizia benedeni* (Anoplocephalidae) (Figure 7-70; see also Figures 4-49, 4-50, and 7-61) typically occur with no clinical signs.

Thysanosoma actinoides and *Wyominia tetoni* (Anoplocephalidae) typically occur with no clinical signs.

Thysaniezia, Stilesia, and *Avitellina* (Anoplocephalidae) are exotic anoplocephalids of ruminants.

PROTISTA. *Eimeria* species (Coccidia) (see Figures 7-67, 7-68, and 8-20 to 8-24), depending on the species involved, can cause severe enteritis with bloody diarrhea; stages may be visible in wet mount scrapings of the intestinal mucosa.

C. parvum, C. bovis, and *C. andersoni* (Apicomplexa) can occur, with *C. parvum* being a cause of diarrhea in calves younger than 30 days of age (see Figures 3-10 and 3-11).

Giardia species (flagellate) (see Figure 7-104) can cause diarrhea in young animals and sometimes in adults.

Cecum and Colon

NEMATODES. *Oesophagostomum radiatum* (cattle), *Oesophagostomum columbianum* (sheep and goats), *Oesophagostomum venulosum* (sheep and goats), and *Chabertia ovina* (sheep and goats) (18 to 22 mm, Strongyloidea) (see Figures 4-88 to 4-92). The fourth-stage larvae of *O. radiatum* in cattle and *O. columbianum* in sheep may be found in abscesses in the gut wall (see Figure 4-92).

TABLE 7-4 Nematodes in the Abomasum and Small Intestine

Genus	Length (mm)	Figure(s)
ABOMASUM		
Haemonchus	14-30	4-72, 4-75
Mecistocirrus	43	4-77
Ostertagia	7-9	4-66, 4-72
Trichostrongylus axei	7	4-70, 4-72
SMALL INTESTINE		
Cooperia	6-16	4-72, 4-78
Trichostrongylus	6-7	4-70, 4-72
Nematodirus	20-25	4-72, 4-76

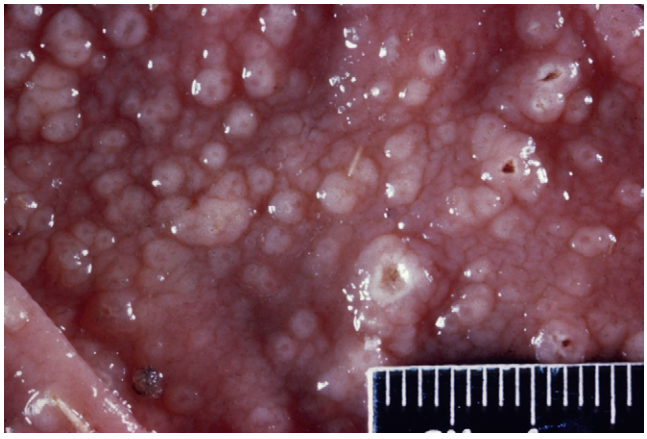


FIGURE 7-69. Lesions in the abomasum of a cow caused by larvae of *Ostertagia ostertagi*.

FIGURE 7-70. *Moniezia benedeni* in the intestine of a cow at necropsy.

FIGURE 7-71. *Skrjabinema caprae* egg and an *Eimeria* oocyst in the feces of a goat (×400).

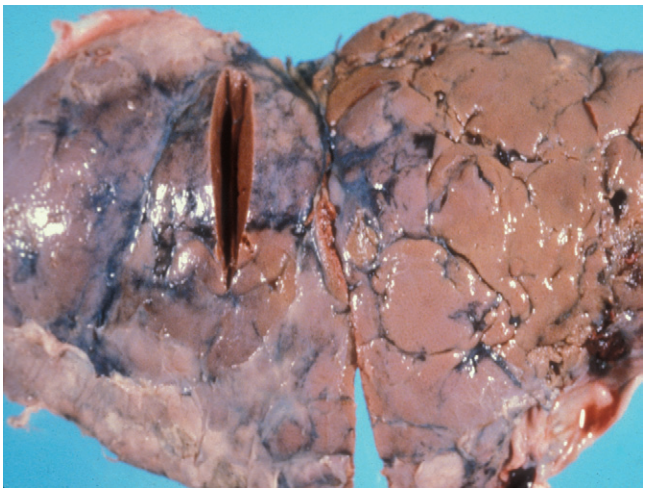


FIGURE 7-72. Liver of a sheep that has been fatally infected with *Fascioloides magna* showing the typical lesions and deposits of black "fluke pigment."

Trichuris discolor (52 mm, cattle) and *Trichuris ovis* (70 mm, sheep and goats) (Trichinelloidea) (see Figures 4-166 and 7-61) can be associated with clinical diarrhea.

Skrjabinema ovis and *Skrjabinema caprae* (8 to 10 mm, Oxyurida) infections are usually without clinical signs (Figure 7-71).

PROTISTA. *Eimeria* species (Coccidia) (see Figures 7-67, 7-68, and 8-20 to 8-24).

Entamoeba bovis and other species of amoebae are considered nonpathogenic parasites or commensals of the large bowel of ruminants.

Buxtonella sulcata (ciliate) is a commensal of the large bowel of cattle (see Figure 7-21).

Liver

NEMATODES. *Ascaris suum* (Ascaridida) of swine will on rare occasions sometimes appear in the bile ducts of sheep and cattle.

Stephanurus dentatus (Strongyloidea) (see Figure 4-93) has immature larvae that can migrate through the bovine liver and cause severe trauma.

NEMATODE LARVAE. The larvae of *Toxocara vitulorum* undergo a liver–lung migration on their way back to the intestine after the infective eggs are ingested.

CESTODES. *Thysanosoma actinoides* and *Wyominia tetoni* (Anoplocephalidae) can sometimes be found in the bile ducts of ruminants into which they have migrated soon after the animal's death; with rapid ligation of the duct, the worms will be found in the small intestine.

CESTODE LARVAE. *Echinococcus granulosus* and *E. multilocularis* hydatids (Taeniidae) (see Figures 4-44 to 4-48, 8-57, 8-58, and 8-64) cause signs that may be severe, depending on the location of the cysts produced.

Taenia hydatigena cysticerci (Taeniidae) (see Figure 4-38).

TREMATODES. *Fasciola hepatica, F. gigantica,* and *F. magna* (Fasciolidae) (Figure 7-72; see also Figures 4-1 to 4-9 and 7-66, *A*). *F. hepatica* (30 mm) is endemic in western and Gulf states of the United States and in Hawaii, Puerto Rico, British Columbia, and eastern provinces of Canada. *F. gigantica* (75 mm) is endemic in Hawaii and Africa. *F. magna* (100 mm) occurs in foci throughout North America; remember that the final host is typically the white-tailed deer. The migratory tracts and lesions produced by *F. magna* can be marked in ruminants with large deposits of black "fluke pigment," often fatal in smaller ruminants.

Dicrocoelium dendriticum (Europe, Asia, Africa, South America) has been introduced into North America, and it occurs in central New York State and the Pacific Northwest; it causes chronic hepatic fibrosis.

Eurytrema pancreaticum (Asia and Brazil) (Dicrocoeliidae) (see Figures 4-18 and 7-66, *C*).

Peritoneum and Peritoneal Cavity

NEMATODE. *Setaria labiatopapillosa* (Filarioidea) (see Figure 4-156) are large white filarids that are sometimes noted as incidental findings in the abdominal cavity of cattle.

CESTODE LARVAE. *Taenia hydatigena* larvae (Taeniidae) (see Figure 4-38) are cysticerci that often have a long "neck" anterior to the scolex.

PENTASTOMID NYMPHS. *Linguatula serrata* (Pentastomida) (see Figure 2-123) larvae can be found in the abdominal cavity of viscera of ruminants, most commonly in Africa.

Respiratory System

Nasal Cavity and Paranasal Sinuses

INSECT LARVAE. *Oestrus ovis* larvae in sheep and goats (Oestridae) (see Figure 2-23) are found in the nasal sinuses; they may be rather small or quite large (10 to 20 mm), depending on their developmental age.

Trachea and Bronchi

NEMATODES. *Dictyocaulus viviparus* (80 mm, cattle) (Trichostrongyloidea) is the only lung-dwelling nematode found in cattle; these can cause severe respiratory distress when present in large numbers.

Dictyocaulus filaria (100 mm, sheep and goats) (Trichostrongyloidea) (see Figures 4-83 and 7-65) can cause respiratory distress in the infected host (see Figure 4-83).

Protostrongylus rufescens (50 mm; sheep) (Metastrongyloidea) (see Figures 4-70 and 7-65).

Mammomonogamus laryngeus (Syngamidae) (see Figure 7-59). Male and female worms are fused in copula; they are endemic in Puerto Rico and various Caribbean islands. These worms have a large strongylid buccal capsule.

Lung Parenchyma

NEMATODES. *Muellerius capillaris* (Metastrongyloidea) (see Figure 4-105).

Oesophagostomum columbianum larvae (erratic migration) (see Figure 4-92).

LARVAL NEMATODES. Both *Toxocara vitulorum* and *Ascaris suum* are capable of causing and have caused respiratory signs in cattle during larval migration.

CESTODE LARVAE. *Echinococcus granulosus* (Taeniidae) (see Figures 4-44, 4-45, and 8-64) have cysts that can become quite large when present in lung tissue.

Vascular System

Heart

CESTODE LARVAE. *Taenia saginata* (Taeniidae) cysticerci are found in the muscles of cattle in the United States.

Taenia ovis (Taeniidae) cysticerci are found in various muscles of sheep and have recently appeared among sheep in Canada (Figure 7-73).

Arteries

NEMATODES. *Elaeophora schneideri* (sheep; Filarioidea) occurs in the western United States.

Elaeophora poeli (cattle; Filarioidea) is an exotic infection in Africa and Asia.

Onchocerca armillata (cattle; Filarioidea) is an exotic infection in Africa and Asia.

Veins

TREMATODES. All *Schistosoma* species (Schistosomatidae) (see Figure 4-24) are exotic. *S. japonicum* is found in Asia with a wide mammalian host range. Species in cattle, sheep, and goats include *Schistosoma bovis* (Africa, Asia, southern Europe), *Schistosoma nasalis, Schistosoma matthei, Schistosoma indicum, Schistosoma spindale,* and *Schistosoma turkestanica* (Asia).

Lymph Nodes

PENTASTOMIDS. *Linguatula serrata.*

Blood

NEMATODE MICROFILARIAE. *Setaria labiatopapillosa* (cattle; Filarioidea).

Elaeophora schneideri (sheep; Filarioidea).

PROTISTA. *Babesia bigemina, Babesia bovis, Babesia divergens, Babesia argentina, Theileria parva, Theileria annulata,* and *Theileria mutans* (piroplasms) (see Figure 3-27) are all basically exotic at this time in the United States.

Trypanosoma theileri (cattle) and *Trypanosoma melophagium* (sheep) (hemoflagellates) (see Figure 3-2). Rarely seen in blood films, these organisms are readily demonstrable by blood culture.

Skeletal Muscles and Connective Tissues

Cestode Larvae

T. saginata (Taeniidae) cysticerci are found most frequently in muscles of mastication, tongue, heart, and muscular portion of the diaphragm of cattle; scolex with four suckers but no hooks.

T. hydatigena (Taeniidae) (see Figure 4-38) cysticerci are sometimes found in skeletal muscles but more commonly in liver or on peritoneal membranes.

Taenia ovis (Taeniidae) cysticerci are pea-sized vesicles found in the heart and esophagus and beneath the epicardium and diaphragmatic pleura of sheep and goats (see Figure 7-73).

Insect Larvae

Hypoderma bovis and *H. lineatum* (Hypodermatidae) (see Figure 2-25) larvae overwinter in the northern climates within cattle, with *H. bovis* in the spinal canal and *H. lineatum* in tissues around the esophagus.

Nematodes

Onchocerca gutturosa, Onchocerca lienalis, Onchocerca bovis, and *Onchocerca gibsoni* (Filarioidea). Adult *Onchocerca* worms are found in deep connective tissues, microfilariae in the dermis. In Australian cattle, *O. gibsoni* produce nodules in the brisket that require extensive trimming. We have seen *O. gibsoni* in corned beef purchased at a local supermarket.

Protista

Sarcocystis species (Apicomplexa) sarcocysts in muscles (Figure 7-74; also see Table 2-1 and Figure 8-32).

Urogenital System

Protista

Tritrichomonas foetus (Trichomonadida) (see Figures 3-4 and 3-5).

Toxoplasma gondii (Apicomplexa) placentas of aborting sheep.

Neospora caninum (Apicomplexa) placentas of aborting cattle.

FIGURE 7-73. Muscle of a sheep from Canada with three evident cysticerci of *Taenia ovis.* (Photograph courtesy Dr. Andrew Peregrine, Ontario Veterinary College, University of Guelph, Ontario, Canada.)

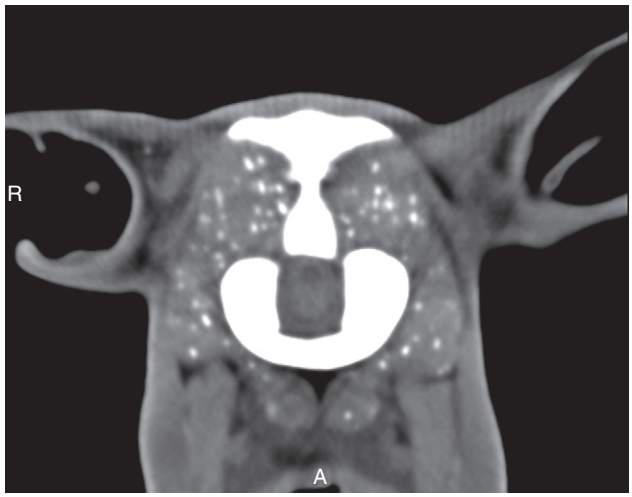


FIGURE 7-74. Computed tomographic image of the cranial portion of the head of a llama taken in the Cornell Hospital for Animals showing calcified lesions that were identified histologically as sarcocysts.

Nervous System

Brain, Spinal Cord, and Meninges

PROTISTA. *Sarcocystis*-like organism (Apicomplexa) in brain of cattle (Dubey, Perry, and Kennedy, 1987).

NEMATODE. *Parelaphostrongylus tenuis* (Metastrongylidae) (see Figures 4-106, 8-93, and 8-94). Adults are typically found in white-tailed deer. Larvae and young adults that find their way into sheep and goats migrate through the spinal cord and brain, causing paralysis. Infections in cattle are rare but are reported.

CESTODE LARVAE. *Taenia multiceps* (Taeniidae) occur in the brain of sheep and goats, causing "gid" (see Figures 4-42 and 8-62); the organism is exotic and supposedly is no longer found in North America.

INSECT LARVA. *Hypoderma bovis* (Hypodermatidae) larvae in the spinal canal of cattle.

Eye

Nematodes

Thelazia californiensis (sheep), *Thelazia gulosa* (cattle), and *Thelazia skrjabini* (cattle) (Spirurida), in conjunctival sac and lacrimal duct (see Figure 4-144), may be associated with conjunctivitis and development of granulation tissue.

Skin and Hair

Insects

DIPTERAN ADULTS. *Musca autumnalis, Stomoxys calcitrans,* and *Haematobia irritans* (Muscidae) (see Figures 2-13, 2-14, and 2-15) adults spend a good deal of time on cattle; *S. calcitrans* is more likely to rest off of cattle when not feeding.

Glossina species (Africa) (see Figure 2-16).

Melophagus ovinus (Hippoboscidae) (see Figure 2-17) pupae and adults of the ked are found on the fleece.

Hypoderma bovis and *H. lineatum* (Hypodermatidae), the gadflies, are rarely seen as they hover about cattle gluing their eggs to hairs on the animals.

Tabanidae (see Figures 2-10 and 2-11) land on cattle typically only long enough to feed.

DIPTERAN LARVAE. *Hypoderma bovis* and *H. lineatum* (30 mm, Hypodermatidae) (see Figures 2-22 and 2-25) bots mature in warbles in the skin of cattle, typically along the back of the animal.

Calliphoridae and Sarcophagidae (see Figures 2-12, 2-18, and 2-19) maggots can be serious pests of ruminant, newborns, and animals that are wounded or down and soiled for an extended period.

ANOPLURANS. *Haematopinus eurysternus, Haematopinus quadripertusus, Haematopinus tuberculatus, Linognathus vituli, Solenopotes capillatus* (cattle), *Linognathus ovillus, Linognathus pedalis, Linognathus oviformis* (sheep), *Linognathus oviformis,* and *Linognathus stenopsis* (goat) (see Figures 2-46, 2-48, 2-50, and 2-52).

MALLOPHAGANS. *Damalinia (Bovicola) bovis* (cattle), *Damalinia ovis* (sheep), *Damalinia caprae, Damalinia limbatus, Damalinia (Holokartikos) crassipes* (goats) (see Figure 2-57).

SIPHONAPTERA. *Echidnophaga gallinacea* (see Figures 2-36 and 2-44).

Ctenocephalides felis can cause severe distress in calves and has even been reported to cause the death of calves, lambs, and sheep, although mainly in tropical settings overseas (see Figure 2-35).

Arachnids

METASTIGMATA: IXODIDAE. *Amblyomma americanum, A. cajennense, A. maculatum, A. inornatum* (Mexico), *A. oblongoguttatum* (Central and South America), and *A. variegatum* (imported to Caribbean from Africa, eradication from area in process) (see Figures 2-74, 2-89, and 2-90).

Rhipicephalus annulatus and *Rhipicephalus microplus* (see Figures 2-84 and 2-85); *B. annulatus* is considered exotic and should be reported if found on cattle.

Dermacentor andersoni, Dermacentor albipictus, Dermacentor occidentalis, Dermacentor nigrolineatus, Dermacentor variabilis, and *Dermacentor (Otocentor) nitens* (see Figures 2-86 to 2-88).

Ixodes cookie, Ixodes pacificus, Ixodes scapularis (see Figures 2-71 and 2-78).

METASTIGMATA: ARGASIDAE. *Otobius megnini* (spinose ear tick) (see Figure 2-74), with larvae and nymphs in the ears.

Ornithodoros coriaceus and *Ornithodoros turicata* (see Figure 2-73) will get on the hosts only long enough to feed.

ASTIGMATA. *S. scabiei* (see Figures 2-101 and 2-103) can cause severe dermatitis, especially in cattle.

Chorioptes bovis (see Figures 2-102, 2-103, and 2-111).

Psoroptes ovis (see Figures 2-100, 2-107, and 2-108) is considered eradicated for the most part from the United States, but this and other very similar mites turn up in the ears of llamas and other American camelids, various wild sheep, and cattle in the southern and western parts of the United States.

PROSTIGMATA. *Demodex bovis, Demodex ovis,* and *Demodex caprae* (see Figures 2-116 and 8-7) can cause very large lesions in the skin of goats and cattle, each containing thousands of mites.

Psorobia bos (cattle) and *Psorergates ovis* (sheep and goats) (Psorergatidae) are the ruminant itch mites.

Trombiculidae (see Figures 2-119 to 2-121), chiggers, are larvae of free-living adult mites and can cause severe pruritus often localizing within the ears.

MESOSTIGMATA. *Raillietia auris* (cattle) and *Raillietia caprae* (goats). Ear mites (see Figure 2-96).

Protista

Besnoitia besnoiti (Coccidia), exotic.

Nematodes

Stephanofilaria stilesi (6 mm, Filarioidea). Very small adult filariids in skin of ventral abdomen, exotic.

Parafilaria bovicola (Filarioidea). Adults in subcutaneous tissues cause "summer bleeding" in cattle, exotic.

Onchocerca gutturosa, O. lienalis, and *O. bovis* (Filarioidea). Microfilariae found in dermis of cattle.

Elaeophora schneideri (Filarioidea) microfilariae can be found in the skin, usually in the head region.

Rhabditis strongyloides (Rhabditida) (see Figures 4-107 and 8-72) larvae will enter the hair follicles of animals on occasion if they are resting on damp hay or other bedding.

PARASITES OF HORSES

STAGES IN FECES

The intestinal parasites of horses form a unique group. Horses host only two coccidian species, *C. parvum* and *E. leuckarti* (Apicomplexa) (Figure 7-75), and only three species of tapeworms (*Anoplocephala magna, Anoplocephala perfoliata,* and *Paranoplocephala mamillana*), all of which belong to the family Anoplocephalidae (Figure 7-76). Nematodes form the largest group (Figure 7-77), which includes one ascarid *(Parascaris equorum)*, two pinworms (*O. equi* and *Probstmayria vivipara*), one rhabditoid nematode *(Strongyloides westeri),* three habronematid spirurids (*Habronema muscae, Habronema microstoma,* and *Draschia megastoma*), and many

strongylids that are all members of the Strongyloidea except one, *T. axei* in the Trichostrongyloidea. Although the horse hosts no hookworm or whipworm, 54 species of strongylids more than make up for these deficiencies. The strongylids are cosmopolitan in distribution, and naturally infected horses tend to harbor a dozen or more species simultaneously. The diagnostic dilemma associated with strongylid eggs is thus accentuated in the case of the horse. However, the major diagnostic categories can be identified by fecal culture (Figure 7-78).

Most parasite stages that float in a sugar flotation of equine feces are relatively easy to recognize. *P. equorum* eggs are yellowish brown with thick, subspheric, rough-surfaced shell walls and contain one cell. Eggs are often found with their external protein layer partially or completely detached. The exposed portions of such shells are smooth and clear. Strongyle eggs present the usual differential diagnostic problem. Recourse may be had to fecal culture and identification of infective third-stage larvae (see Figure 7-78). *S. westeri* eggs are smaller than strongyle eggs and contain a rhabditiform larva in fresh specimens. *O. equi* eggs are more likely to be recovered from anal scrapings than from fecal specimens. The egg shown here was collected by momentarily pressing the adhesive side of a piece of cellophane tape against a horse's anus and then was mounted by sticking the tape to a microscope slide (see Figure 7-77).

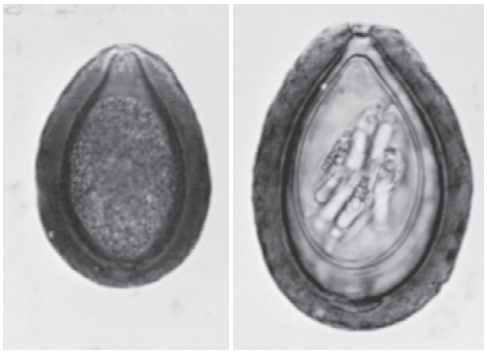


FIGURE 7-75. *Eimeria leuckarti* unsporulated *(left)* and sporulated *(right)* oocysts (×425).

The eggs of *Draschia, Habronema,* and the equine tapeworms tend not to float very well in various solutions because they are fairly fragile and hard to float in common flotation media. They can actually even be very hard to find when it seems that they should be present in large numbers, such as when the adults are present in the same animal on the necropsy table. *Draschia* and *Habronema* eggs are cigar-shaped and contain a vermiform embryo. Such eggs are difficult to demonstrate in feces. If a technique for antemortem diagnosis of gastric habronemiasis is essential, resort to xenodiagnosis using *Musca domestica* larvae for *D. megastoma* and *H. muscae,* and *S. calcitrans* larvae for *H. microstoma*.

IDENTIFICATION OF EQUINE MICROFILARIAE

Equine microfilariae as drawn by Dr. Jay Georgi are shown diagrammatically in Figure 7-79. The reality is that after the approval

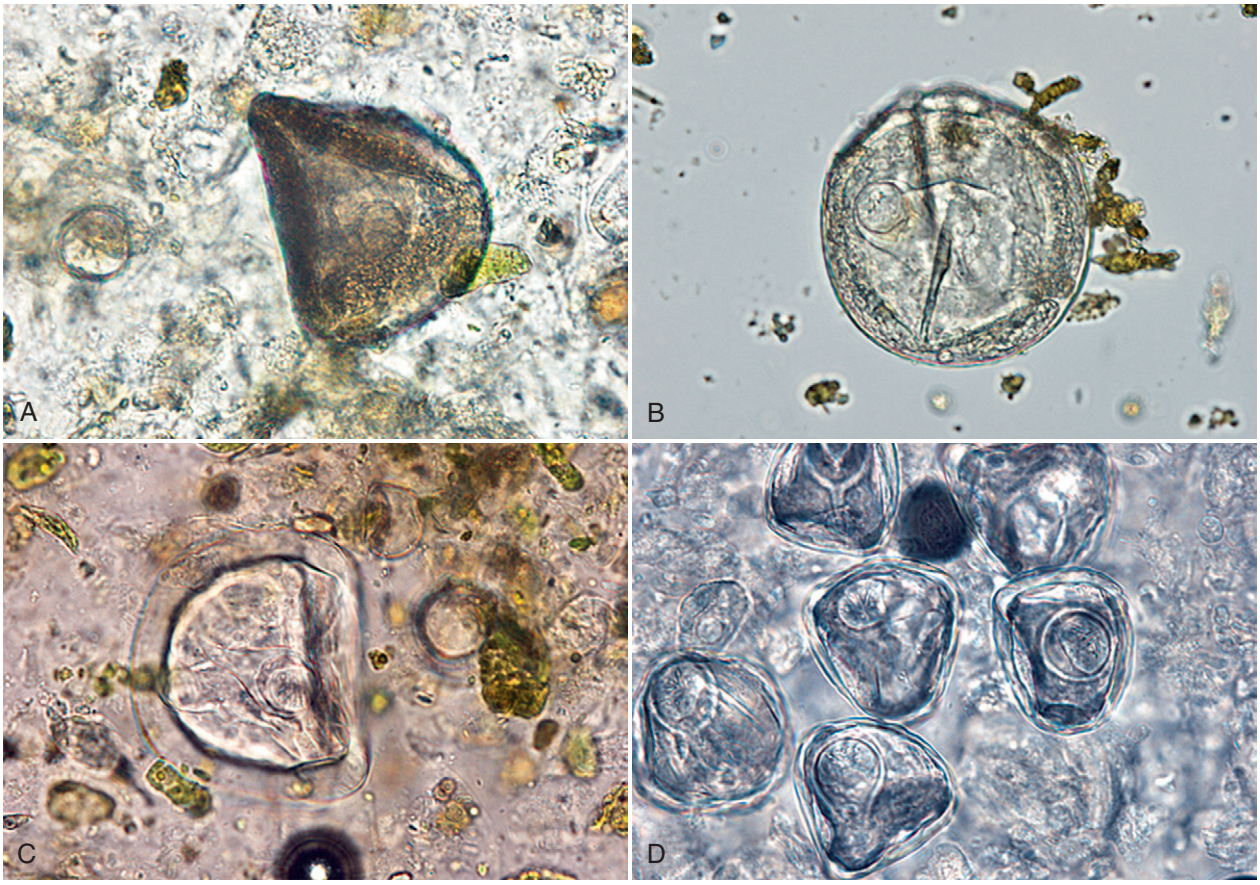


FIGURE 7-76. Eggs of *Anoplocephala perfoliata* in three different preparations. **A,** Egg in water. **B,** Egg in a zinc sulfate centrifugal flotation. **C** and **D,** Eggs in a centrifugal sugar flotation, viewed with brightfield **(C)** and Nomarski **(B)** optics. The eggs of *Anoplocephala magna* and *Paranoplocephala mamillana* are similar, but the eggs of *P. mamillana* are only three fourths as large as these.

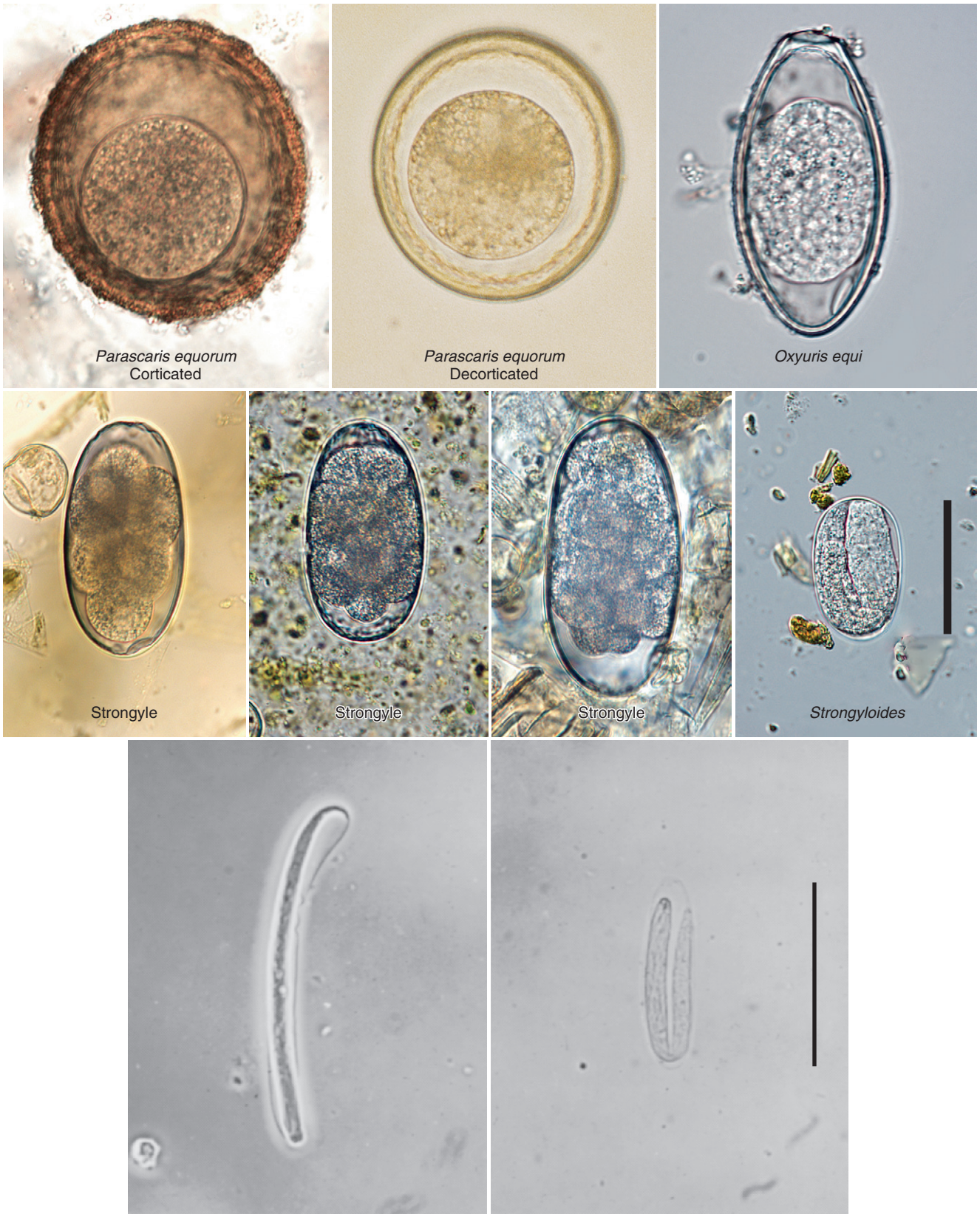


FIGURE 7-77. Eggs of some nematode parasites of horses.

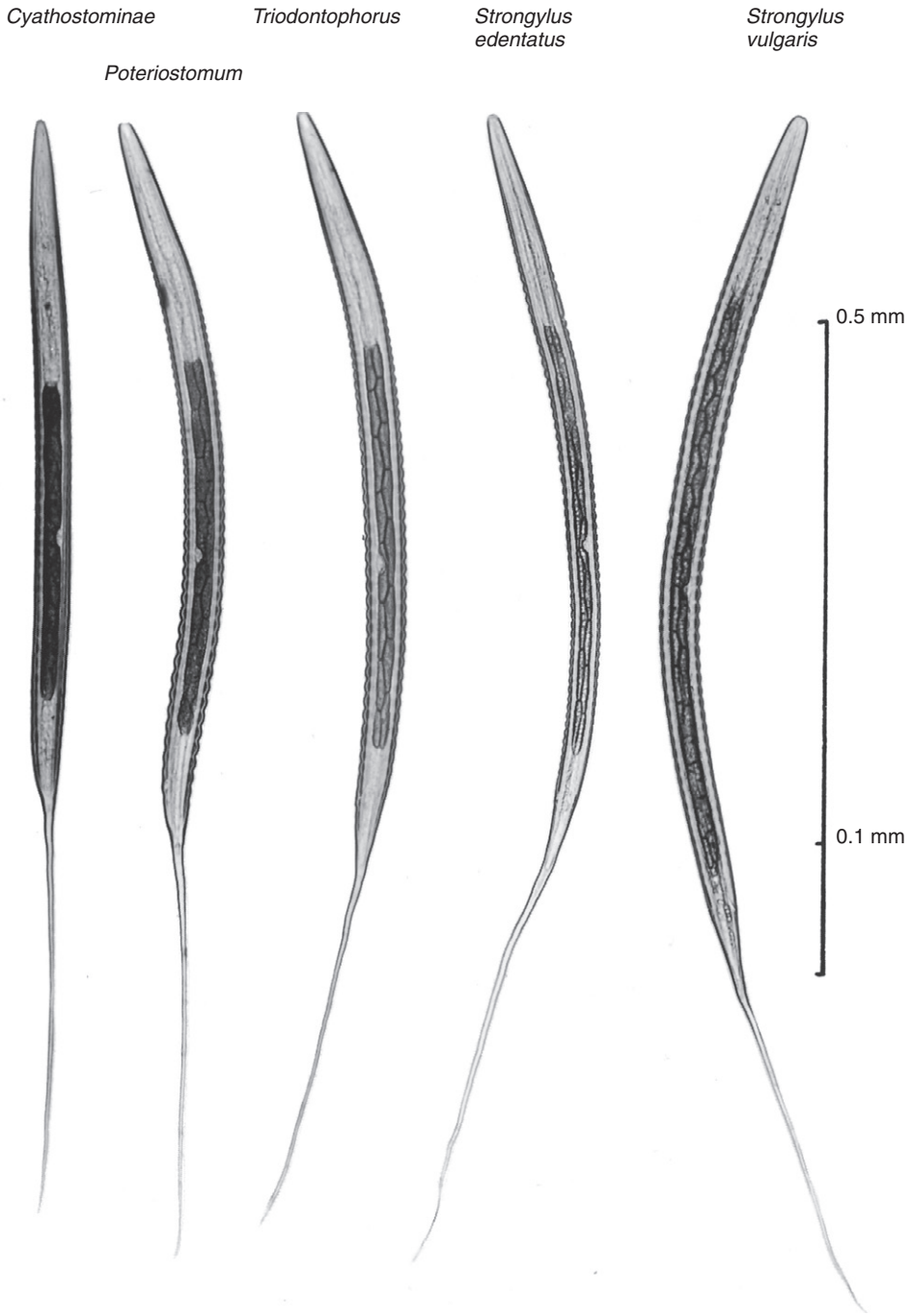


FIGURE 7-78. Infective third-stage larvae of some horse strongylids. Larvae of the subfamily Cyathostominae, represented here by *Cyathostomum catinatum,* have eight intestinal cells. *Gyalocephalus capitatus (not shown)* has 12, *Poteriostomum* has 16, *Triodontophorus* has 18 (but the *Triodontophorus serratus* larvae shown here have only 16), *Strongylus edentatus* has 18 to 20, and *Strongylus vulgaris* has 32 intestinal cells. *S. vulgaris* is easily distinguished from all the rest by its large size and long column of intestinal cells.

of ivermectin and the other avermectins for use in horses, it is hard to find microfilariae commonly in horses any more; routine ivermectin administration to horses may be reducing transmission or suppressing microfilariae.

The sheathed microfilariae of *Setaria equina* may be demonstrated in blood samples by the techniques described for detecting the microfilariae of the canine heartworm.

Parafilaria multipapillosa microfilariae may be found in blood discharged from "summer bleeding" nodules caused by the adult female worms. They are less than 200 µm long, are unsheathed, and have a rounded posterior extremity (Supperer, 1953).

Microfilariae of *Onchocerca cervicalis, Onchocerca reticulate,* and *Elaeophora böhmi* may be demonstrated by excising a small piece of skin from near the linea alba and placing it in physiologic saline solution. The microfilariae of these three species will soon be observed migrating out of the dermis into the saline solution. Leave the preparation set up overnight to detect low levels of microfiladerma.

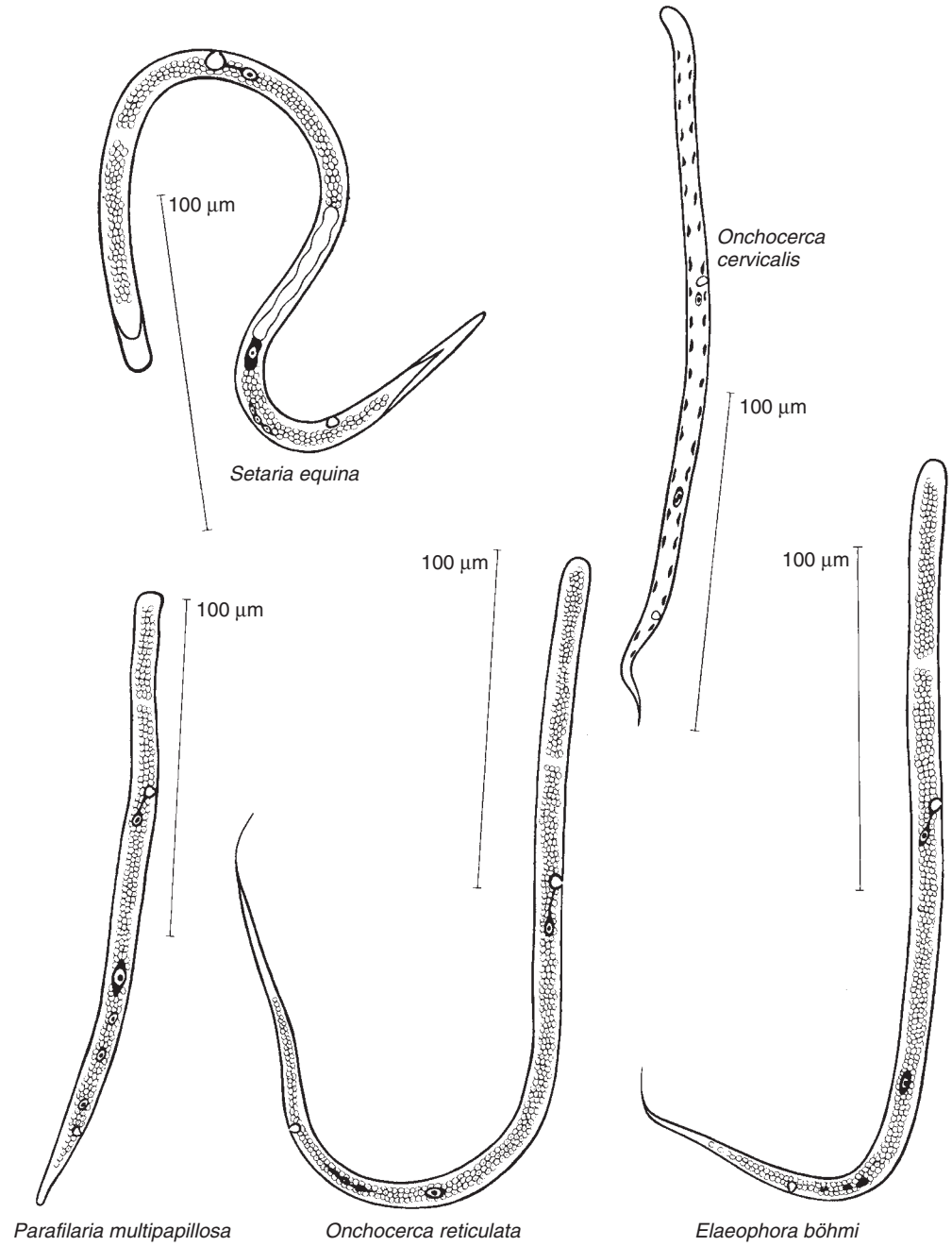


FIGURE 7-79. Microfilariae of filariid parasites of horses. (Redrawn from Supperer T: Filarosen der Pferde in Osterreich, *Wiener Tieraztliche Monatschr* 40(4):193–220, 1953.)

Onchocerca cervicalis microfilariae are slender, delicate, and 207 to 240 µm long.

Onchocerca reticulata microfilariae are 330 to 370 µm long and have a long, whiplike tail ending in a fine point.

Elaeophora böhmi microfilariae are 300 to 330 µm long and may be distinguished from *O. reticulata* by a difference in the distance from the genital cell to the tip of the tail, which is greater than 140 µm for *O. reticulata* and less than 120 µm for *E. böhmi*.

ANNOTATED HOST-ORGAN LISTING OF PARASITES OF HORSES

Neospora species may occur as extracellular or intracellular tachyzoites or as bradyzoites in cysts (see Figure 3-21). *T. gondii* may occur in any tissue of any host as extracellular or intracellular tachyzoites or as bradyzoites in cysts (see Figure 3-20).

Alimentary System

Mouth

INSECT LARVAE. *Gasterophilus intestinalis, Gasterophilus nasalis,* and *Gasterophilus haemorrhoidalis* (Diptera: Gasterophilidae) (Figure 7-80; see also Figures 2-22 and 2-26 to 2-30) larvae can be found in the tongue, in the interdental pockets, or at the base of the tongue.

PROTISTA. *Trichomonas equibuccalis* (Trichomonadida) is found around gum margins of cheek teeth.

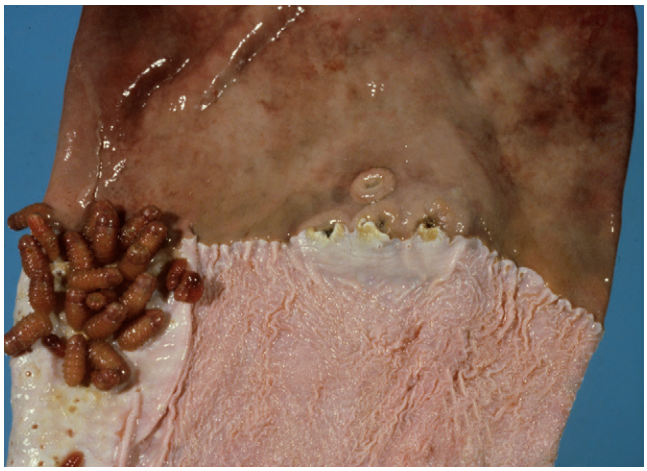


FIGURE 7-80. Stomach of a horse showing the attachment of bots, *Gasterophilus intestinalis,* and a lesion produced at the margo plicatus by infection with the spirurid *Draschia megastoma*.

Stomach

NEMATODES. *Draschia megastoma, Habronema muscae,* and *H. microstoma* (Spirurida) (see Figure 4-148) are found in the stomach, with *H. muscae* and *H. microstoma* on the mucosa and *D. megastoma* in nodules at the margo plicatus (see Figure 7-80).

Trichostrongylus axei (Trichostrongyloidea) (see Figures 4-72 and 4-74) infections may cause hypertrophic gastritis with mucosal proliferations and are often associated with shared pasturage with cattle.

INSECT LARVAE. *Gasterophilus intestinalis* (Diptera: Gasterophilidae) (see Figures 2-22, 2-26 to 2-30, and 7-80), even with their specific designation, are found in the stomach.

Small Intestine

NEMATODES. *Parascaris equorum* (Ascaridoidea) (Figure 7-81, and see Figure 7-77) can be anywhere in length from 1 inch to 2 feet; often with regular deworming, only small worms are found at necropsy.

Strongyloides westeri (Rhabditida) (see Figures 4-112, 4-114, 7-77, and 8-74) is very small and is threaded through the intestinal mucosa.

CESTODES. *Anoplocephala magna, Paranoplocephala mamillana* (Anoplocephalidae) (see Figures 4-51, 4-52, and 7-76).

PROTISTA. *Cryptosporidium* species (Apicomplexa) can cause serious diarrhea in neonatal foals (see Figure 3-10).

Eimeria leuckarti (Coccidia) (see Figures 7-75 and 8-25) has large schizonts and oocysts that may be demonstrated in mucosal scrapings.

Giardia species (flagellate) (see Figure 7-104) can be found as trophozoites in light scrapings of the mucosal surface of the anterior small intestine.

INSECTS. *Gasterophilus nasalis* and *G. haemorrhoidalis* (Diptera: Gasterophilidae) larvae are found in the duodenum.

Large Intestine

NEMATODES. *Oxyuris equi* (150 mm) and *Probmayria vivipara* (3 mm) (Oxyurida) (see Figures 4-117 to 4-119 and 7-77). *O. equi* (Figure 7-82) is commonly seen because the females crawl out of the anus, causing tail rubbing; *P. vivipara* is almost never seen (see Figure 7-120).

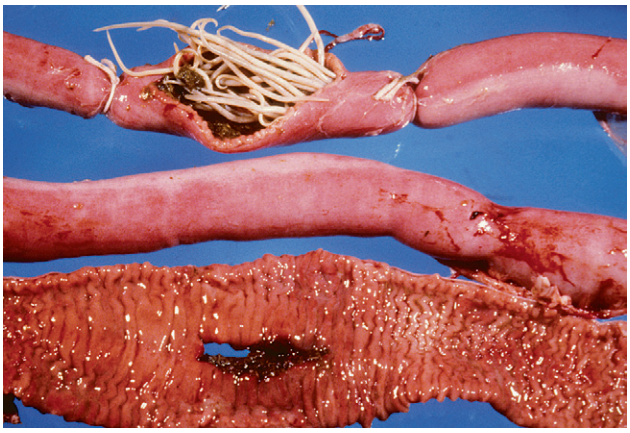


FIGURE 7-81. Linear ulcer in a horse associated with a very heavy infection with *Parascaris equorum* seen at Cornell University in 1984. (Courtesy Dr. John M. King.)

FIGURE 7-82. *Oxyuris equi* adults recovered from a horse at necropsy.

FAMILY STRONGYLIDAE. The horse is host to about 60 species belonging to the family Strongylidae, and as many as 20 different species are often found in the same horse.

SUBFAMILY STRONGYLINAE. *Strongylus vulgaris, S. edentatus, S. equinus, Triodontophorus serratus, Triodontophorus brevicauda, Triodontophorus tenuicollis, Triodontophorus nipponicus, Oesophagodontus robustus,* and *Craterostomum acuticaudatum* (Figure 7-83; see also Figures 4-63 and 7-86 [bottom row]).

SUBFAMILY CYATHOSTOMINAE. Genera: *Cyathostomum, Cylicocyclus, Cylicostephanus, Cylicodontophorus, Poteriostomum, Paraposteriostomum, Petrovinema, Coronocyclus,* and *Gyalocephalus* (Figures 7-84 to 7-94).

Each series can be identified by careful study of the stomal region alone. With fresh specimens, detail sufficient for identification can be seen without recourse to clearing agents. Simply mount the specimen under a coverslip in a drop of water. With this simple preparation, it is usually possible to roll the specimen so that both dorsal and lateral aspects may be studied. Even preserved specimens may be studied in this manner but tend to be considerably less transparent than fresh specimens. For comparisons to be made easily, illustrations of the species that bear the greatest resemblance to one another have been grouped together. The nomenclature of J. Ralph Lichtenfels' excellent monograph *Helminths of Domestic Equids* (*Proc Helminthol Soc Wash*, 42, 1975) along with an update on the taxonomy of the group (Lichtenfels et al, 1998) is the system that has been applied in the following pictorial key.

Text continued on p. 384

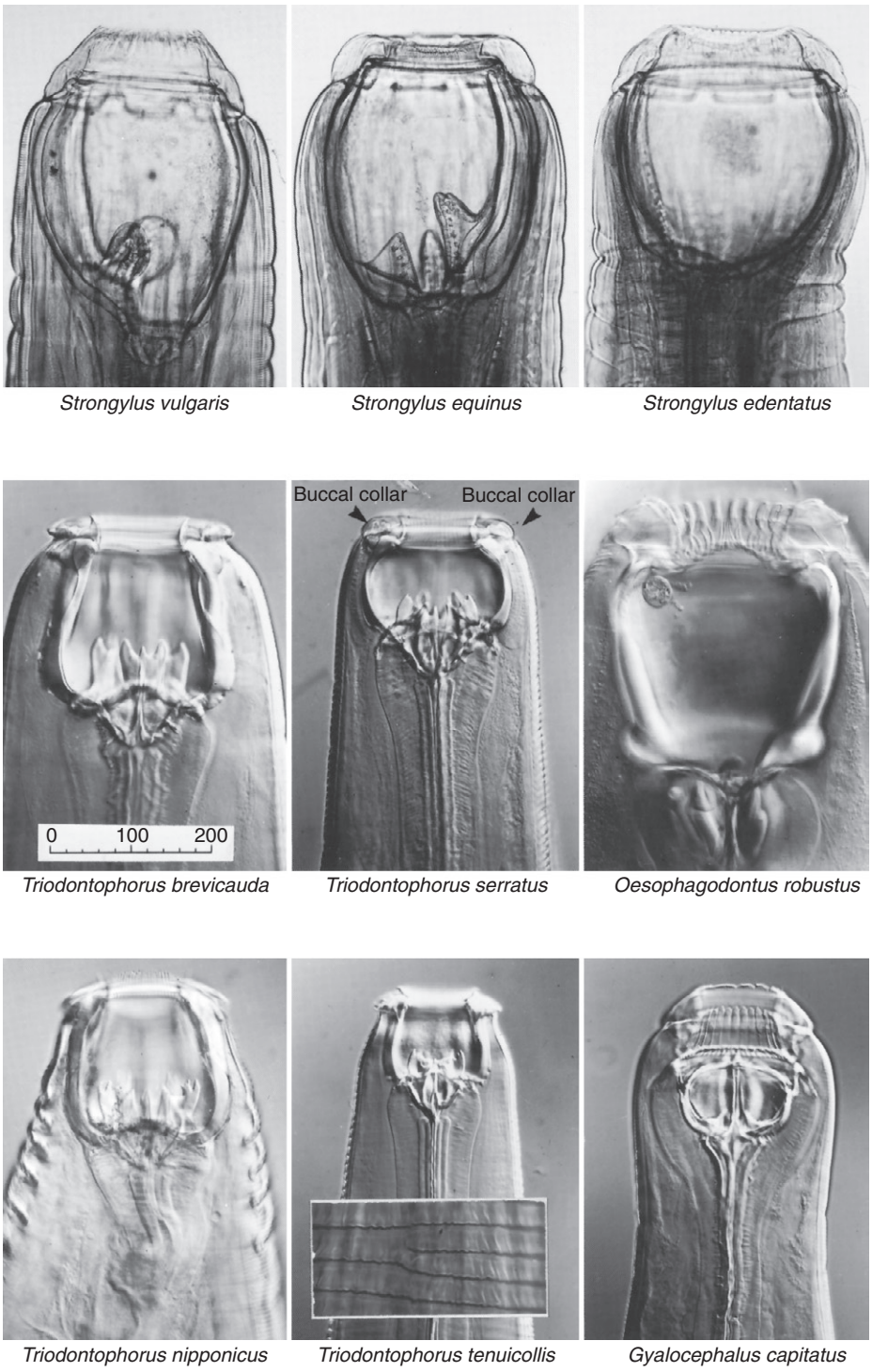
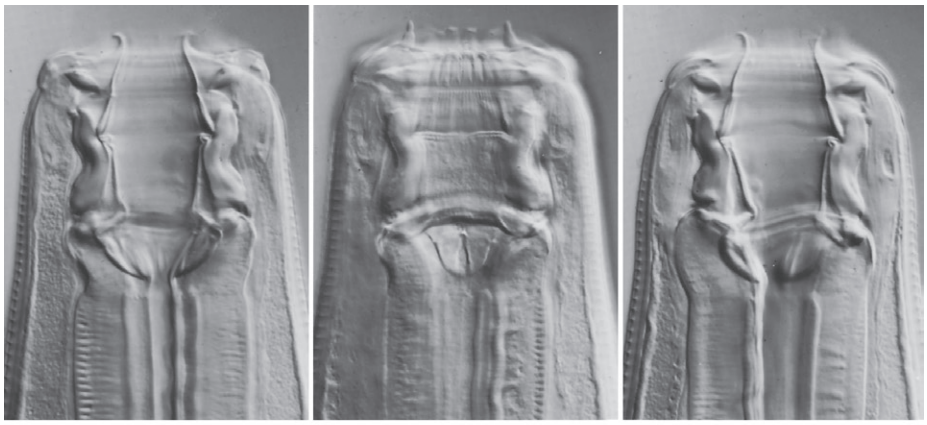
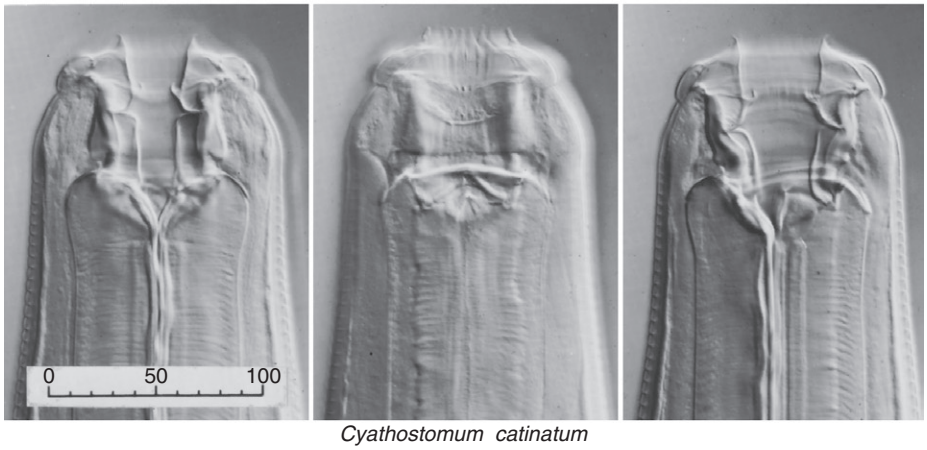


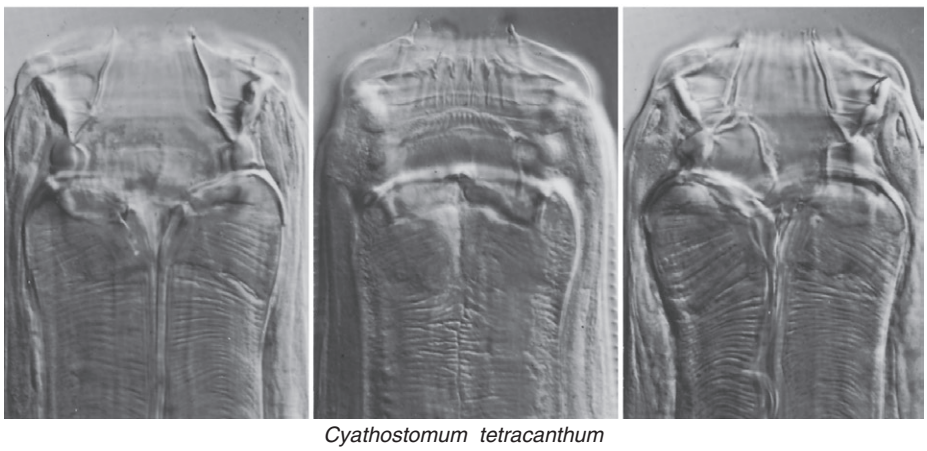
FIGURE 7-83. Members of the subfamily Strongylinae (large strongyles) and *Gyalocephalus capitatus* (subfamily Cyathostominae). *Strongylus vulgaris* and *Oesophagodontus robustus* (×72); *Strongylus equinus* (×40); *Strongylus edentatus* (×33); *Triodontophorus* spp. and *Gyalocephalus capitatus* (×112). (*Strongylus* species cleared and mounted by the glycol methacrylate method of Pijanowski et al: *Cornell Vet* 62:333, 1972.)



Coronocyclus coronatum

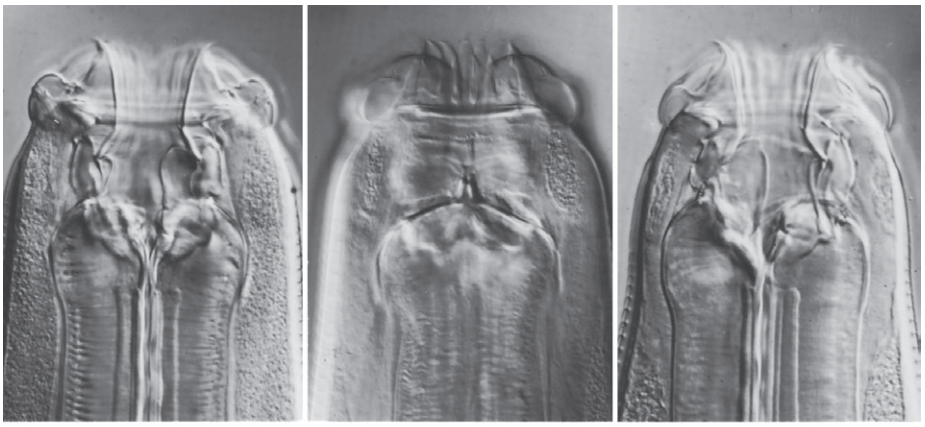


Cyathostomum catinatum

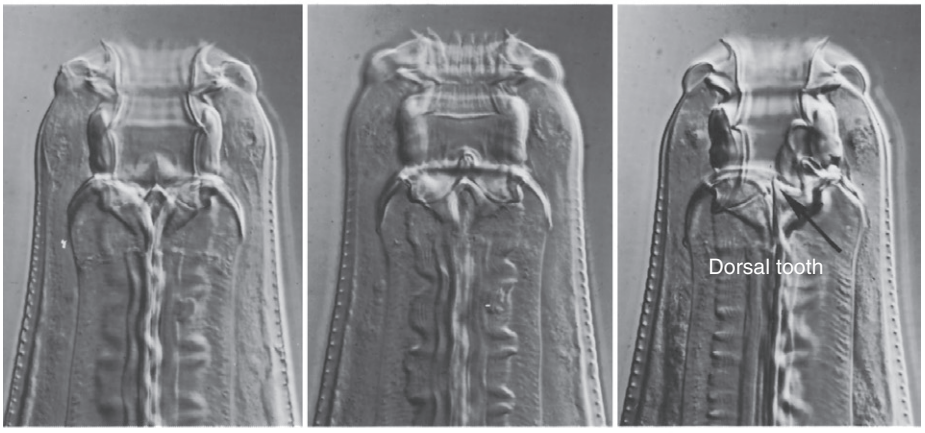


Cyathostomum tetracanthum

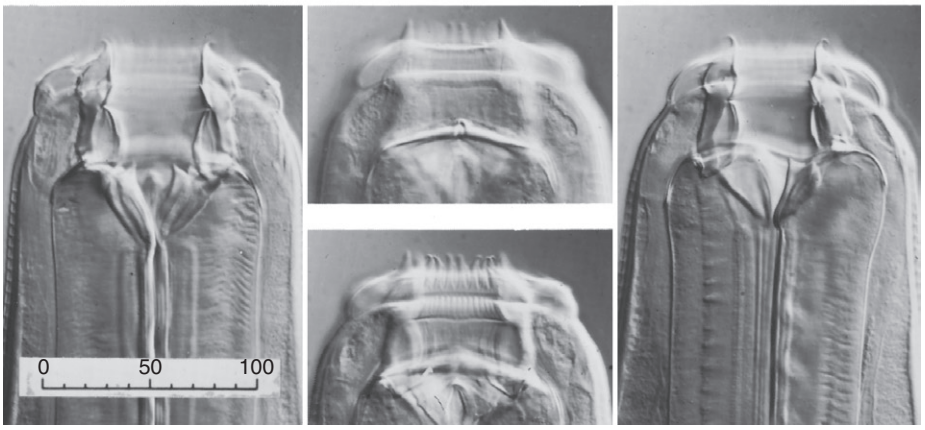
FIGURE 7-84. Members of the subfamily Cyathostominae. Dorsoventral *(left)*, dorsal surface *(center)*, and lateral *(right)* views of the heads of *Coronocyclus coronatus (top row)*, *Coronocyclus catinatum (middle row)*, and *Cyathostomum tetracanthum (bottom row)*. (All ×283.)



Coronocyclus labiatum

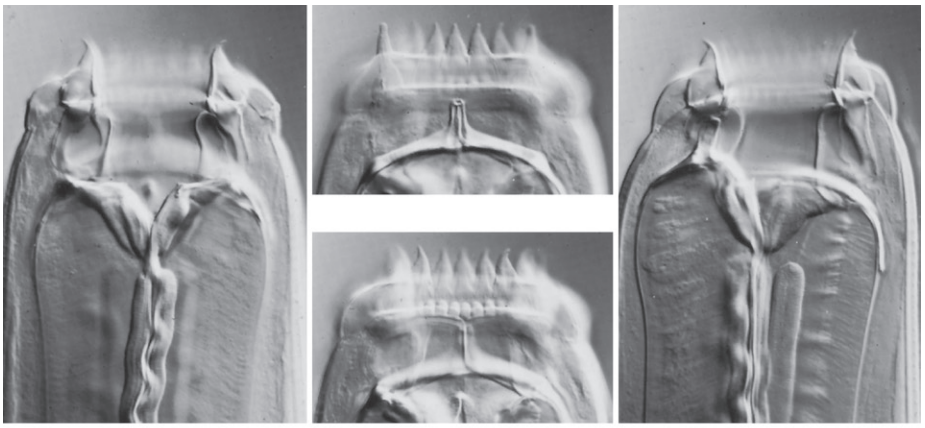


Coronocyclus labratus

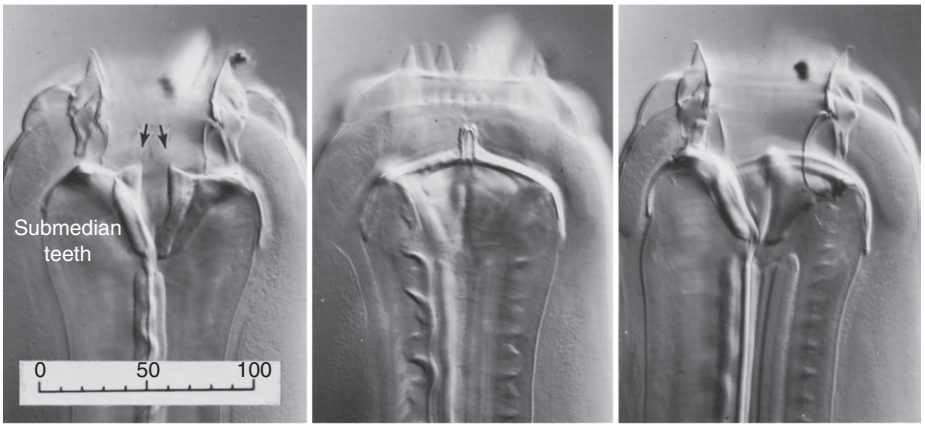


Cylicostephanus goldi

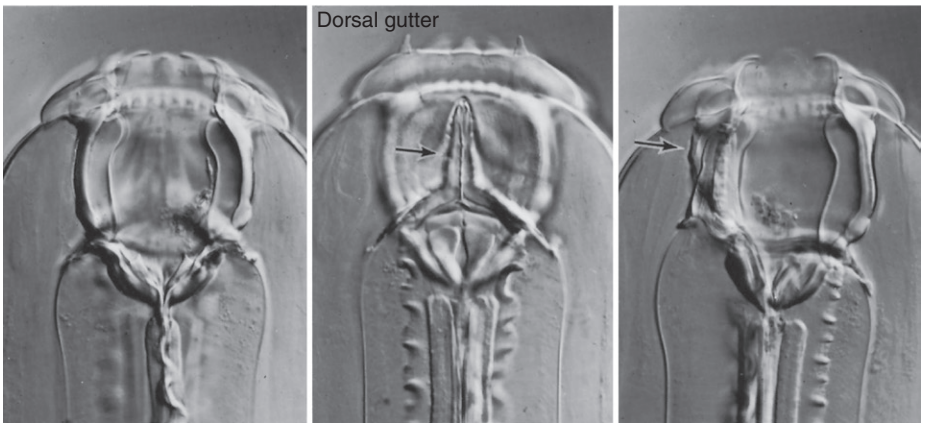
FIGURE 7-85. Members of the subfamily Cyathostominae. Dorsoventral *(left)*, dorsal surface *(center)*, and lateral *(right)* views of the heads of *Coronocyclus labiatus (top row)*, *Coronocyclus labratus (middle row)*, and *Cylicostephanus goldi (bottom row)*. (All ×283.)



Cylicostephanus asymetricus

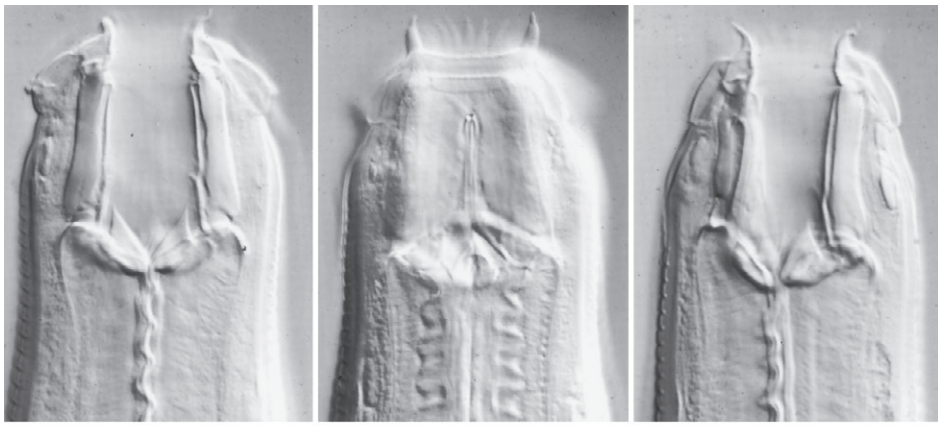


Cylicostephanus bidentatus

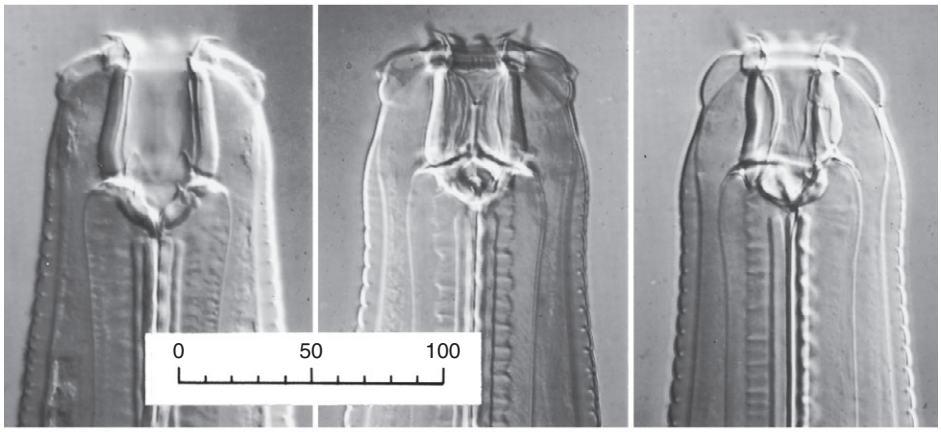


Craterostomum acuticaudatum

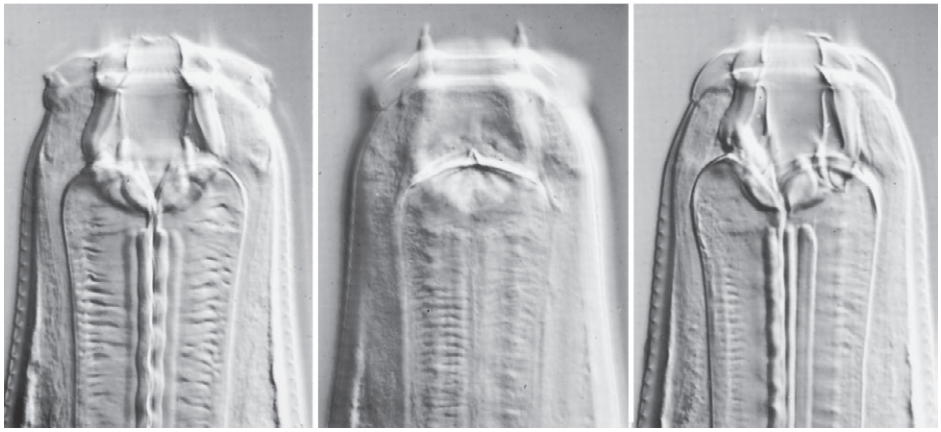
FIGURE 7-86. Members of the subfamily Cyathostominae and *Craterostomum acuticaudatum* (subfamily Strongylinae). Dorsoventral *(left)*, dorsal surface *(center)*, and lateral *(right)* views of the heads of *Cylicostephanus asymetricus (top row)*, *Cylicostephanus bidentatus (middle row)*, and *Craterostomum acuticaudatum (bottom row)*. (All ×283.)



Cylicostephanus calicatus

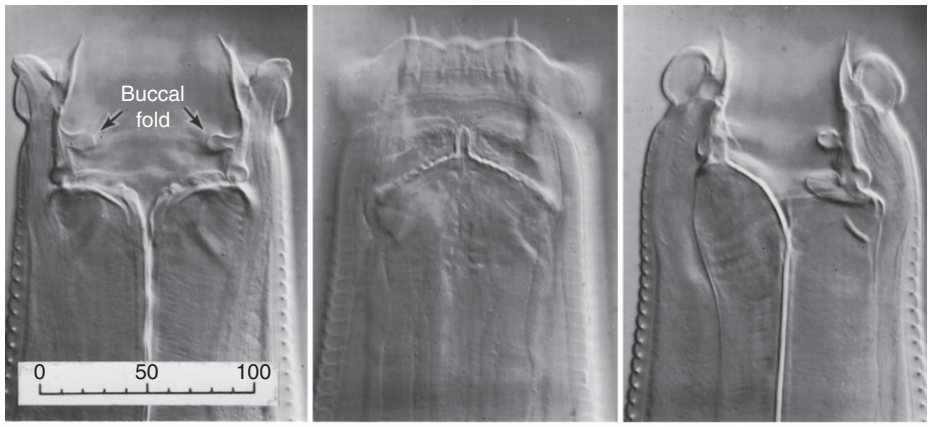


Cylicostephanus minutus

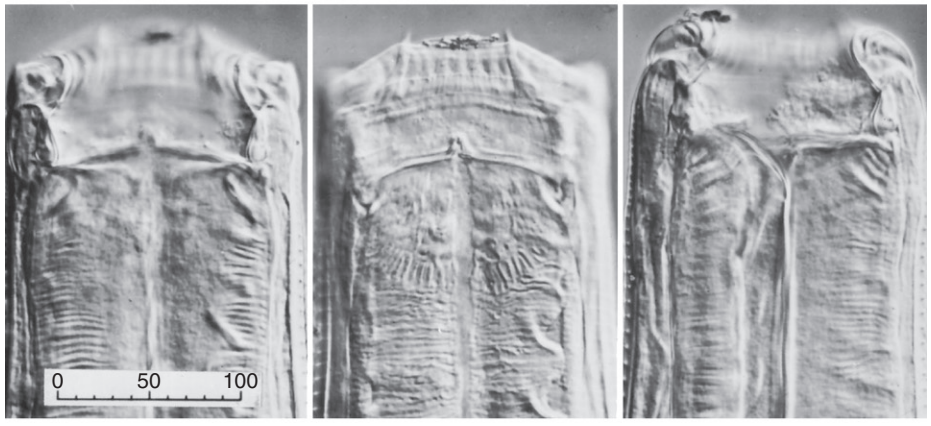


Cylicostephanus longibursatus

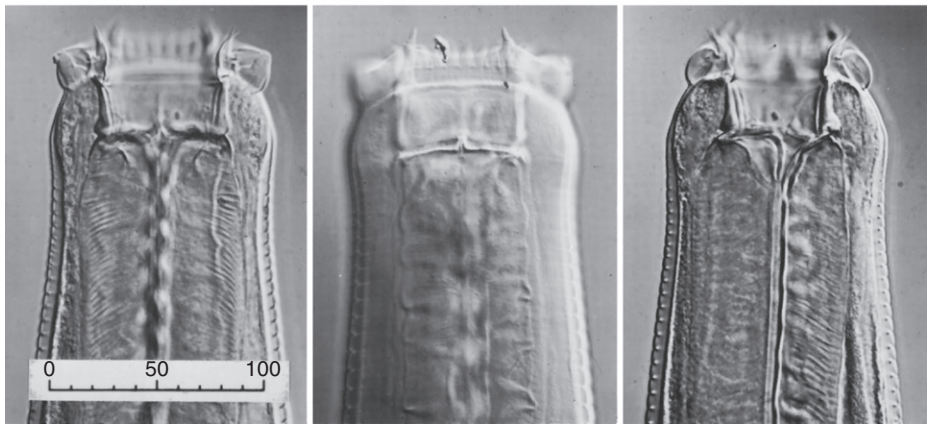
FIGURE 7-87. Members of the subfamily Cyathostominae. Dorsoventral *(left)*, dorsal surface *(center)*, and lateral *(right)* views of the heads of *Cylicostephanus calicatus (top row)*, *Cylicostephanus minutus (middle row)*, and *C. longibursatus (bottom row)*. (All ×425.)



Cylicocyclus nassatus

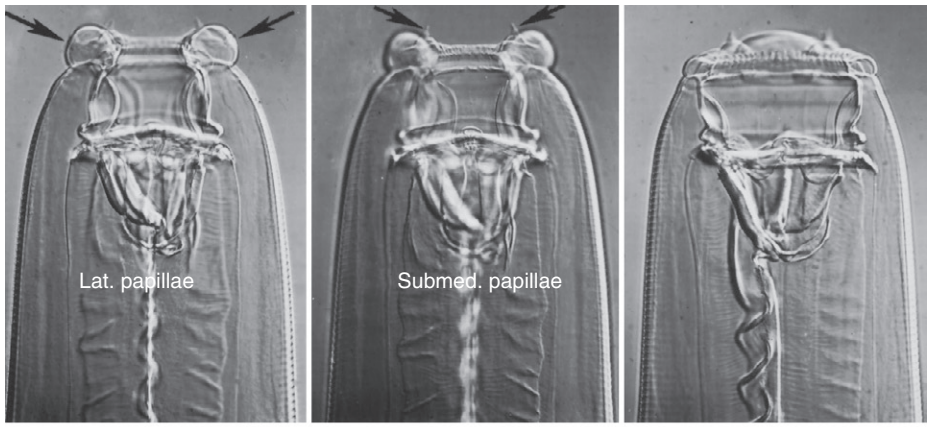


Cylicocyclus ashworthi

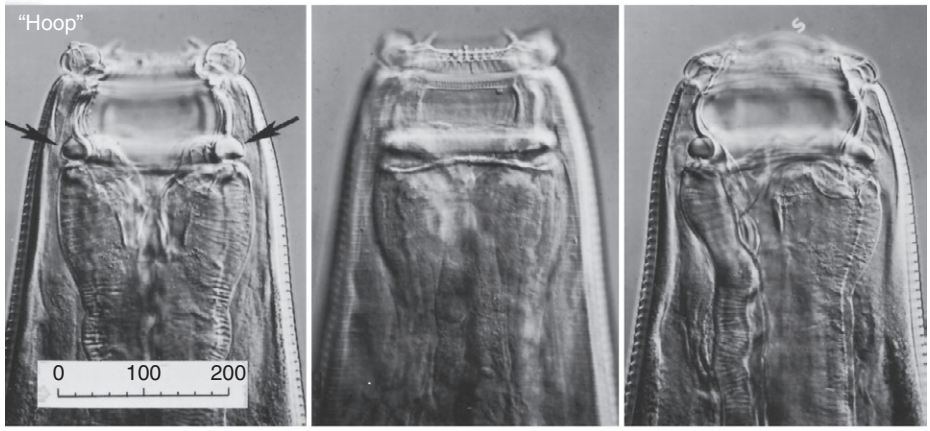


Cylicocyclus leptostomus

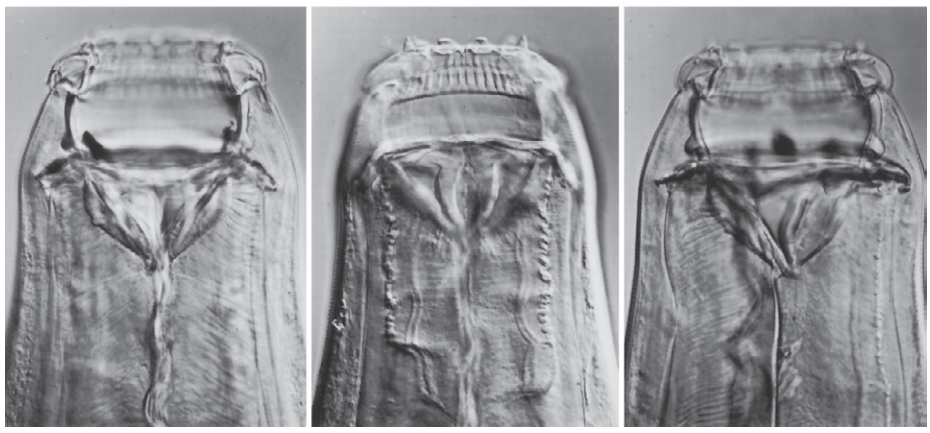
FIGURE 7-88. Members of the subfamily Cyathostominae. Dorsoventral *(left)*, dorsal surface *(center)*, and lateral *(right)* views of the heads of *Cylicocyclus nassatus (top row)*, *Cylicocyclus ashworthi (middle row)*, and *Cylicocyclus leptostomus (bottom row)*. (*C. nassatus* and *C. leptostomus* ×283. *C. ashworthi* ×242.)



Cylicocyclus elongatus

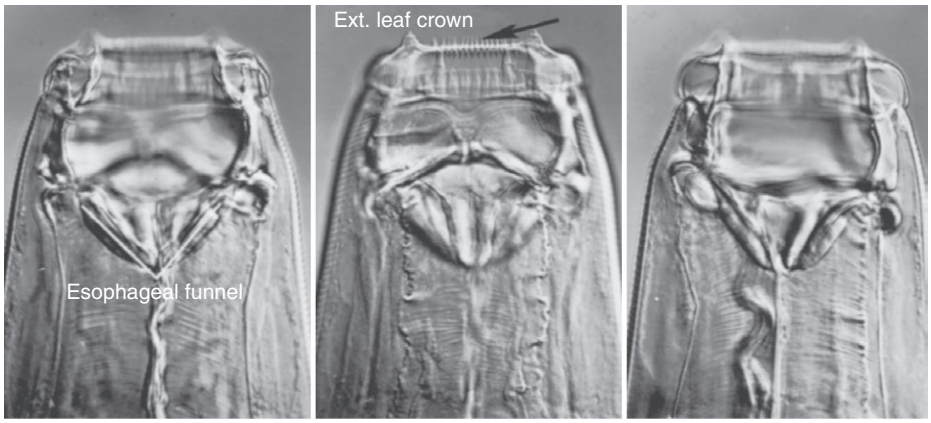


Cylicocyclus insigne

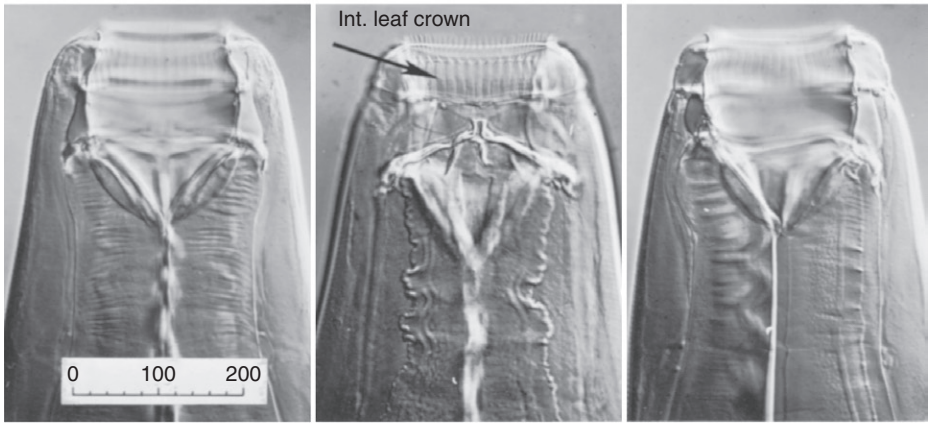


Cylicocyclus ultrajectinus

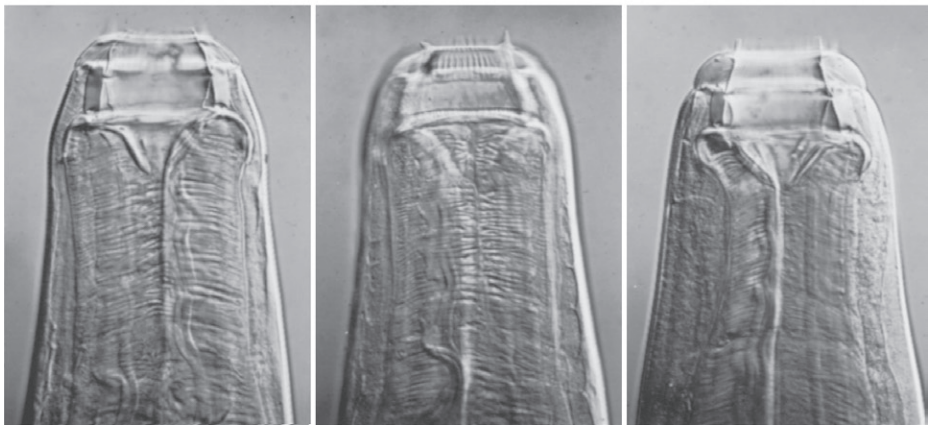
FIGURE 7-89. Members of the subfamily Cyathostominae. Dorsoventral *(left)*, dorsal surface *(center)*, and lateral *(right)* views of the heads of *Cylicocyclus elongatus (top row)*, *Cylicocyclus insigne (middle row)*, and *Cylicocyclus ultrajectinus (bottom row)*. (All ×112.)



Poteriostomum imparidentatum

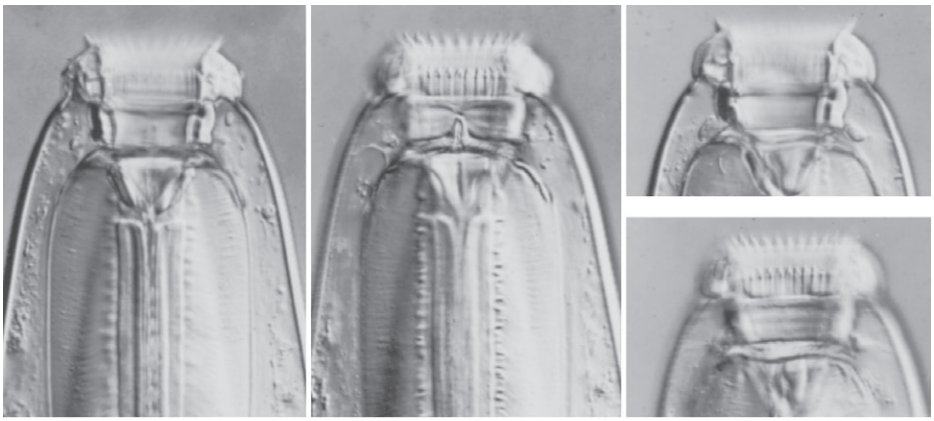


Poteriostomum ratzii

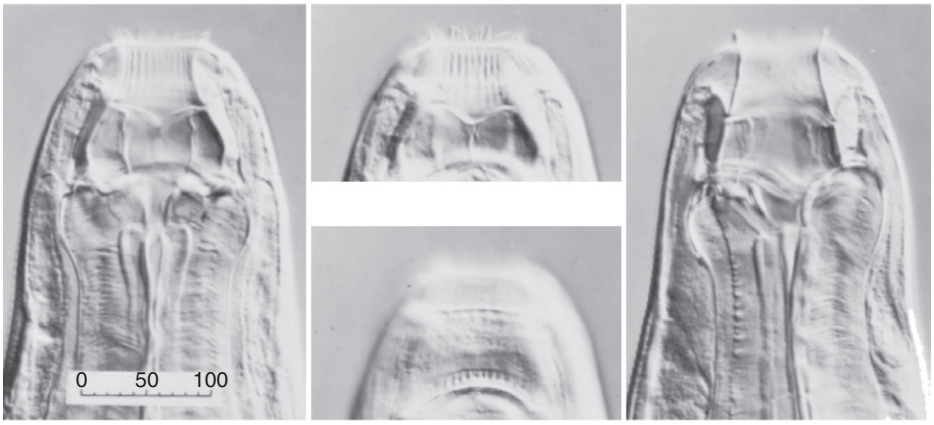


Paraposteriostomum mettami

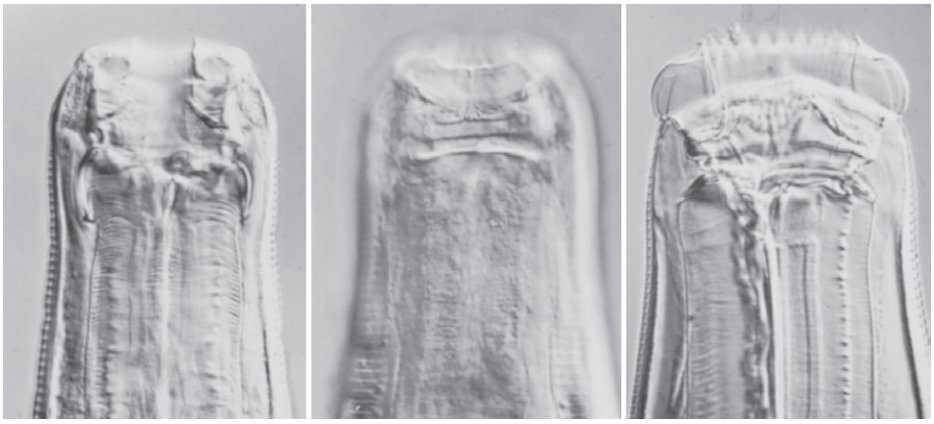
FIGURE 7-90. Members of the subfamily Cyathostominae. Dorsoventral *(left)*, dorsal surface *(center)*, and lateral *(right)* views of the heads of *Poteriostomum imparidentatum (top row)*, *Poteriostomum ratzii (middle row)*, and *Paraposteriostomum mettami (bottom row)*. (All ×112.)



Cylicodontophorus bicoronatus



Paraposteriostomum euproctus



Cyathostomum pateratum

FIGURE 7-91. Members of the subfamily Cyathostominae. Dorsoventral *(left)*, dorsal surface *(center)*, and lateral *(right)* views of the heads of *Cylicodontophorus bicoronatus (top row)*, *Paraposteriostomum euproctus (middle row)*, and *Cyathostomum pateratum (bottom row)*. (All ×170.)

CESTODE. *Anoplocephala perfoliata* (Anoplocephalidae) (see Figures 4-52 and 7-76) is found mainly in the cecum; this tapeworm tends also to cluster in the ileum near the ileocecal valve, where it is associated with ulceration and chronic inflammation of the ileal wall.

INSECT. *Gasterophilus haemorrhoidalis* (Diptera: Gasterophilidae) larvae sometimes attach briefly as they make their way out of the intestinal tract into the environment.

Liver

NEMATODE LARVAE. *Parascaris equorum* (Ascaridoidea) passes through the liver on its way to the lung after the infective-stage eggs are ingested.

Strongylus edentatus and *S. equinus* (see Figures 8-79 to 8-82) will wander through the liver for a time before patency.

CESTODE LARVAE. *Echinococcus granulosus* (Taeniidae) (see Figures 4-44 to 4-46 and 8-64) hydatid cysts are very rare in horses in most of the world, especially so in the United States.

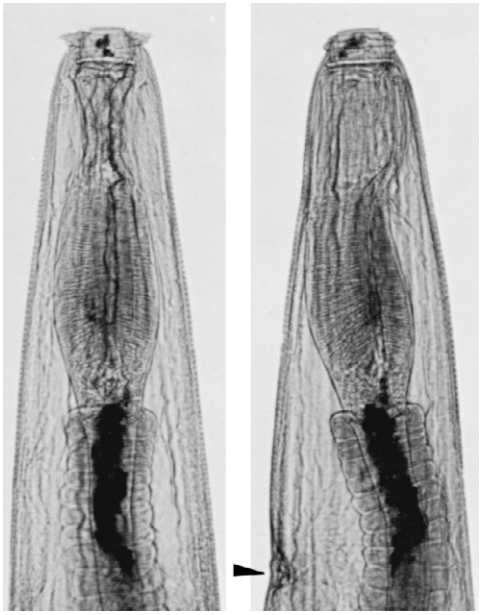


FIGURE 7-92. *Cylicocyclus auriculatus* (subfamily Cyathostominae) (×50). Note prominent lateral head papillae. *Arrowhead* indicates position of excretory pore.

Pancreas

NEMATODE. *Strongylus equinus* (Strongylinae) (see Figure 8-82) larvae migrate sometimes into the pancreas before patency.

Peritoneum and Peritoneal Cavity

NEMATODES. *Setaria equina* (150 mm; Filarioidea) (see Figures 4-157 and 7-73) adults live in the peritoneal cavity.

Strongylus edentatus (44 mm; Strongylinae) (see Figures 8-78 to 8-81) larvae migrate.

Respiratory System

Paranasal Sinuses

INSECT LARVA. *Rhinoestrus purpureus* (Oestridae) is an exotic nasal bot.

Bronchi and Bronchioles

NEMATODE. *Dictyocaulus arnfieldi* (65 mm; Trichostrongyloidea) (see Figure 4-83) is found in horses; donkeys are thought to help maintain the infection among equines.

Lung Parenchyma

NEMATODE. *Strongylus edentatus* (aberrant migration) (see Figures 8-78 and 8-80).

Parascaris equorum (Ascaridoidea) has larvae that routinely make a liver-to-lung migration in the horse before returning to the intestinal tract. There is reason to believe that many of the larvae that do not develop to the adult stage in horses still make it to the lungs and cause eosinophil-associated pathology.

Vascular System

Arteries

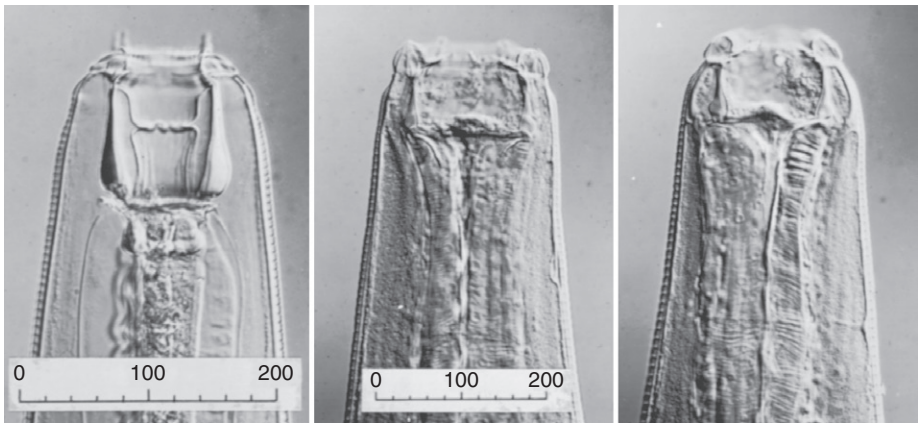
NEMATODES. *Strongylus vulgaris* (Figures 7-95 and 7-96) larvae migrating through the walls of the mesenteric arteries produce remarkably severe lesions in the walls of these vessels.

Elaeophora böhmi (Filarioidea) (see Figure 7-79) is found in intimal nodules of the wall of the aorta and other vessels. It is exotic.

Blood

NEMATODE MICROFILARIA. *Setaria equina* (Filarioidea) (see Figure 7-79).

PROTISTA. *Babesia caballi* (piroplasm) (see Figure 3-27) could be seen in fixed red blood cells.



Petrovinema poculatum *Cylicocyclus radiatus*

FIGURE 7-93. Members of the subfamily Cyathostominae.

FIGURE 7-94. *Cylicocyclus brevicapsulatus*, the only homely member of the subfamily Cyathostominae (×168).

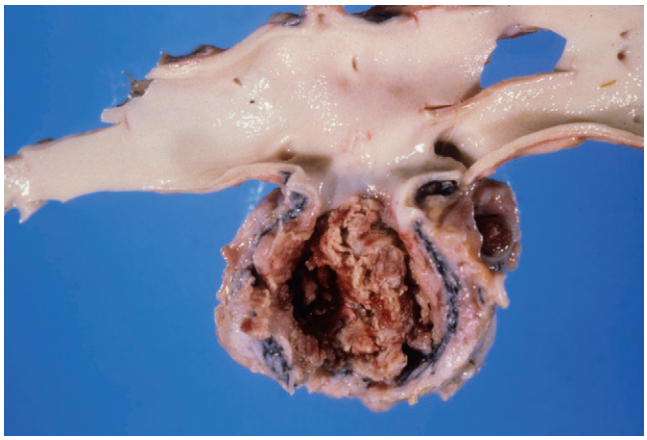


FIGURE 7-95. *Strongylus vulgaris* verminous arteritis and aneurysm in a pony aorta discovered during junior surgery.

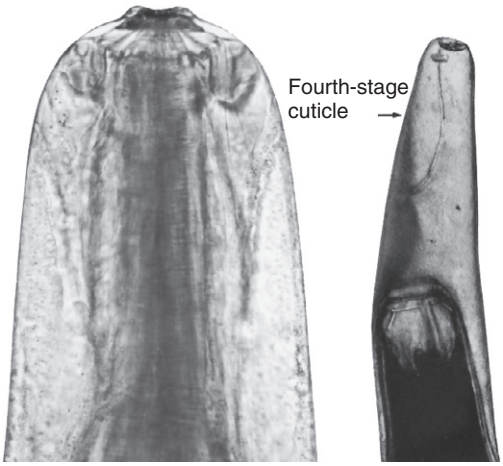


FIGURE 7-96. *Strongylus vulgaris* fourth stage (*left*, ×108) and immature fifth stage (*right*, ×38) from a mural thrombus of the cranial mesenteric artery of a horse.

Skeletal Muscles and Connective Tissues

Nematodes

Trichinella spiralis (Trichinelloidea) first-stage larvae have been found in Europe in horses fattened for human consumption (see Figure 7-100).

Onchocerca cervicalis (Filarioidea) adults are found in the nuchal ligament.

Protista

Sarcocystis bertrami and *Sarcocystis fayeri* (coccidians) (see Table 2-1 and Figures 8-32 and 8-33) occur as sarcocysts within muscle fibers.

Insect Larvae

Hypoderma bovis and *H. lineatum* (Diptera: Hypodermatidae) (see Figure 2-25) will on occasion migrate erratically into the subcutaneous dorsal tissues of horses.

Nematode Microfilariae

Onchocerca cervicalis and *O. reticulata* (Filarioidea) (see Figures 7-79, 8-111, and 8-112) microfilariae are found in the dermis.

Urogenital System

Kidneys

NEMATODE. *Halicephalobus gingivalis* (Rhabditida) can be found in various viscera of the horse as adult females and larvae, with one site of infection often being the kidney.

PROTISTA. *Klossiella equi* (coccidian) (see Figure 8-30).

Testes

NEMATODE. *Strongylus edentatus* (Strongylinae) (see Figures 8-78 to 8-81) sometimes have immature adults in vaginal tunics.

Nervous System

Brain and Spinal Cord

NEMATODES. *S. vulgaris* (Strongylinae) (see Figure 7-96) may have fourth-stage larvae or young adults migrating erratically; even one worm can cause fatal neurologic disease.

Setaria species (Filarioidea) (see Figures 4-33, 4-157, 4-158, and 7-73) can undergo erratic migration with neurologic disease; this seems to happen most often in Asia.

Halicephalobus gingivalis (Rhabditoidea) causes neurologic disease that can be fatal (see Figure 8-73).

Draschia megastoma (Spirurida) (Mayhew et al, 1983).

Parelaphostrogylus tenuis has also been reported now to cause neurologic disease in horses.

INSECTS. *Hypoderma bovis* and *H. lineatum* (Diptera: Hypodermatidae) may have larvae undergoing erratic migration in the atypical equine host; one larva can cause fatal neurologic disease.

PROTISTA. Equine protozoan myelitis (EPM) organism (Apicomplexa) *Sarcocystis neurona* (see Figures 3-25 and 3-26).

Eye

Nematodes

Thelazia lacrymalis (Spirurida) (see Figure 4-144) is found in the conjunctival sac and lacrimal ducts.

Draschia megastoma and *Habronema* species (Spirurida) larvae may cause habronemic conjunctivitis.

Onchocerca species microfilariae (see Figure 7-79).

Skin and Hair

Insects

Musca autumnalis and *Stomoxys calcitrans* (Diptera: Muscidae) (see Figures 2-13 and 2-14).

Hippobosca equina and *Lipoptena cervi* (Diptera: Hippoboscidae) (see Figure 2-17) are the keds of horses. *H. equina* tends to be rare in the United States; *L. cervi* of deer is common but fortunately only rarely gets on horses.

Gasterophilus intestinalis, G. nasalis, and *G. haemorrhoidalis* (Diptera: Gasterophilidae) females will hover around horses while they lay their eggs glued to hairs.

Tabanus and *Chrysops* species (Diptera: Tabanidae) (see Figures 2-10 and 2-11) will attack in bright sun long enough to inflict a painful bite.

Haematopinus asini (Anoplura).

Damalinia equi (Mallophaga: Ischnocera).

Echinophaga gallinacea (Siphonaptera) (see Figure 2-36).

Triatoma sanguisuga (Hemiptera: Triatominae) (see Figure 2-64).

Insect Larvae

Hypoderma bovis and *H. lineatum* (Diptera) (see Figure 2-25) larvae are found in the subcutis of the saddle area.

Arachnids

Amblyomma, Anocentor, Rhipicephalus, Dermacentor, Haemaphysalis, Hyalommaa, and *Ixodes* (Metastigmata: Ixodidae) (see Figures 2-74 and 2-91).

Sarcoptes scabiei (Sarcoptidae; Astigmata) (see Figures 2-100 and 2-102).

Psoroptes ovis and *Chorioptes bovis* (Psoroptidae; Astigmata) (see Figures 2-100, 2-101, and 2-107 to 2-110).

Trombiculidae (Prostigmata) (see Figures 2-119 to 2-122).

Demodex equi (Prostigmata) (see Figure 2-115).

Nematode Microfilariae and Larvae

Parafilaria multipapillosa (Filarioidea) (see Figure 7-79) has microfilariae in serosanguineous discharge from ulcerated nodules.

Onchocerca cervicalis and *O. reticulata* (Filarioidea) (see Figures 8-111 and 8-112) have microfilariae almost universally present in the dermis of horses, especially the dermis of the ventrum, if they have not been on routine avermectin therapy.

Rhabditis strongyloides (Rhabditida) (see Figure 4-113) can cause dermatitis in horses if they are down, as, for example, on straw for a day or two after surgery.

Draschia megastoma, H. muscae, and *H. microstoma* (Spirurida) have larvae that excite exuberant granulomatous reactions in skin wounds, areas of skin subject to frequent wetting, and ocular conjunctiva (see Figure 7-80).

PARASITES OF SWINE

STAGES IN FECES

Intestinal Protista include eight species of *Eimeria* and *Cystoisospora suis* (Figure 7-97), *Cryptosporidium suis, Entamoeba polecki, Iodamoeba buetschlii, Endolimax nana, Giardia* species, other flagellates, and the very common ciliate *B. coli* (Figure 7-98). Other than the species of *Eimeria, Cystoisospora,* and *Cryptosporidium,* most of these parasites will not be seen in sugar flotations owing to distortion.

A number of common eggs are found in pig feces that include nematodes and an acanthocephalan (Figure 7-99). The fertile eggs of the ascaridoid *A. suum* have a rough, bile-stained, external protein layer. Infertile *A. suum* eggs can be common and appear a little longer and thinner than the fertilized eggs; the middle wall of the shell tends to be thinner, and the central portion looks disorganized. The

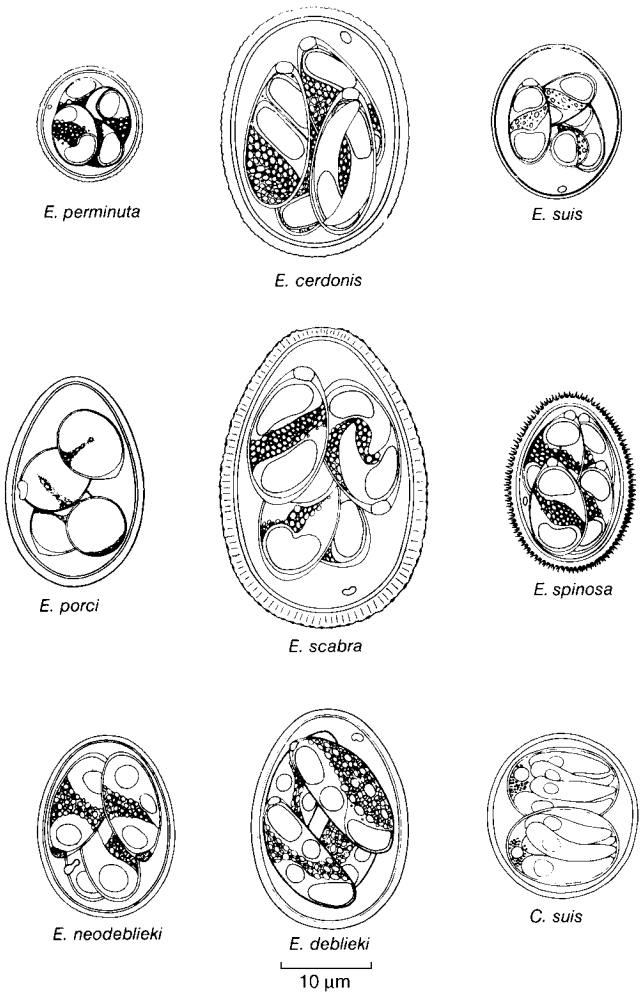


FIGURE 7-97. Sporulated oocysts of eight species of *Eimeria* and one species of *Cystoisospora* from swine. (From Vetterling JM: *J Parasitol* 51:909, 1965.)

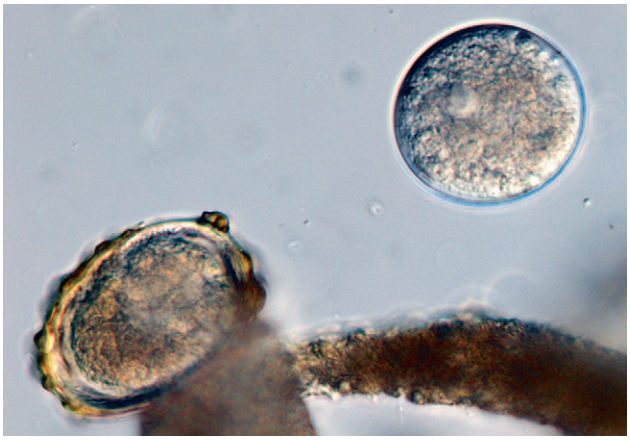


FIGURE 7-98. Cyst of *Balantidium coli* and the egg of *Ascaris suum.* The cyst is about the same size as the *A. suum* egg (≈60 µm).

spirurids *Ascarops* and *Physocephalus* produce thick-walled, larvated eggs. *Strongyloides ransomi* (Rhabditida) eggs resemble those of *S. papillosus* (Rhabditida) and are thin-shelled and larvated (see Figure 7-61). Strongyle eggs in pig feces may represent infection with *Hyostrongylus rubidus* (Trichostrongyloidea), *Oesophagostomum*

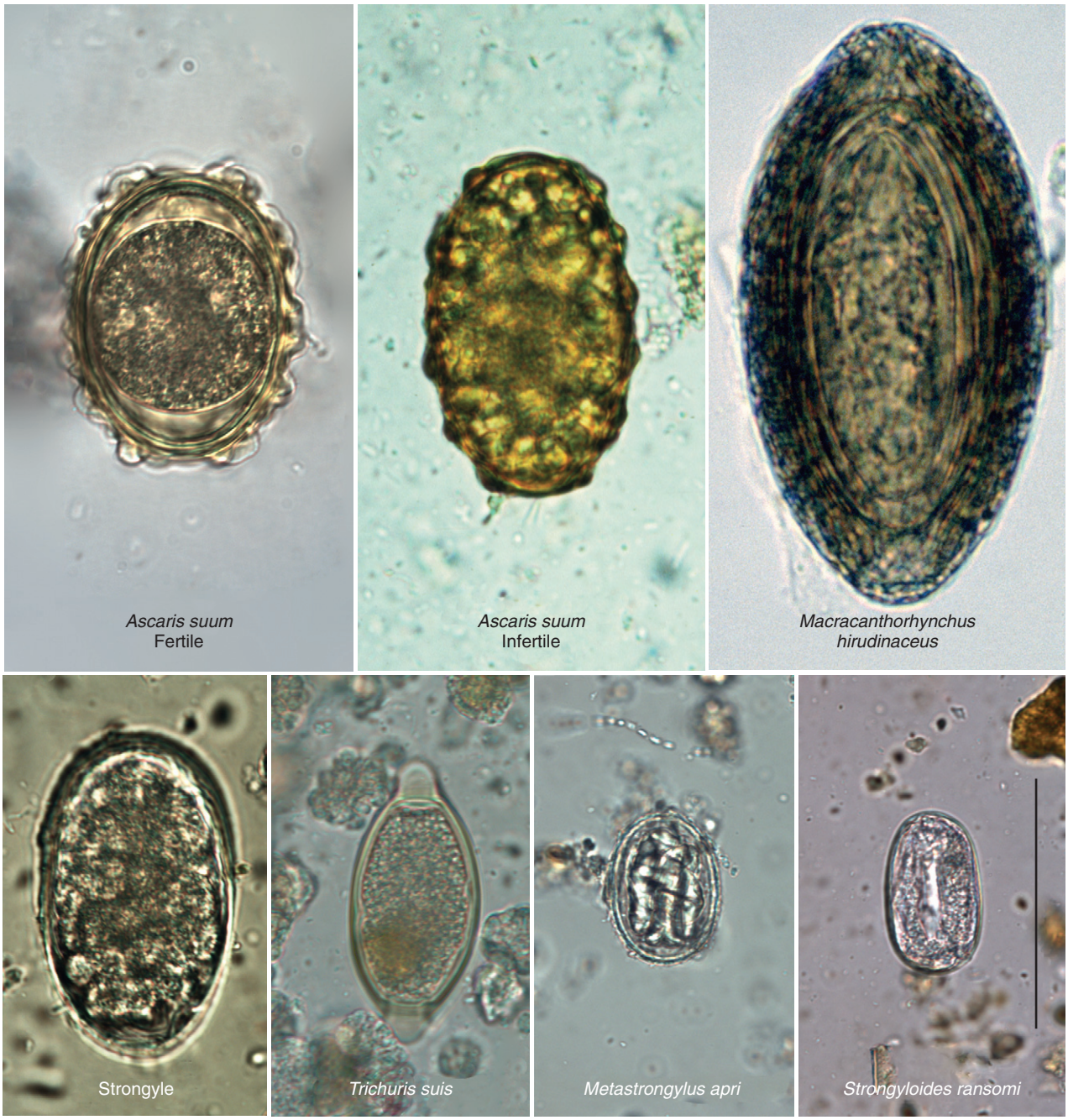


FIGURE 7-99. Eggs of some parasites found in the feces of pigs. (Image of infertile *Ascaris* egg courtesy Dr. M. Dale Little.)

species (Strongyloidea), or *Globocephalus urosubulatus* or *Necator americanus* (Ancylostomatoidea), but most commonly with only the first two. The Metastrongyloidea parasitic organism in swine is unusual compared with many in that it has an earthworm rather than a molluscan intermediate host, and unlike most metastrongyloids of domestic animals, *Metastrongylus apri, Metastrongylus salmi,* and *Metastrongylus pudendotectus* have eggs passed in feces rather than larvae. The eggs are small and subglobular and contain a larva. *Trichuris suis* (Trichinelloidea) living in the mucosa of the cecum and colon are typical of the genus, are almost identical to the *Trichuris trichiura* of humans, and are smaller than the eggs of the dog whipworm, *T. vulpis*. *M. hirudinaceus* (Acanthocephala) eggs have three concentric, ellipsoidal shells surrounding the acanthor embryo.

STAGES IN URINE

The eggs of *Stephanurus dentatus* (Strongyloidea) are large and morulated and are found in urine specimens from infected swine. The last urine voided contains the highest concentration of eggs.

EXAMINATION FOR TRICHINAE

Squash Preparation

Moderate to heavy *T. spiralis* infections can be diagnosed by simply squashing bits of muscle tissue between two glass slides and scanning under low power. The diaphragm and masseter muscles are especially likely to yield positive findings.

1. Detach a small scrap of meat and place it on a microscope slide.

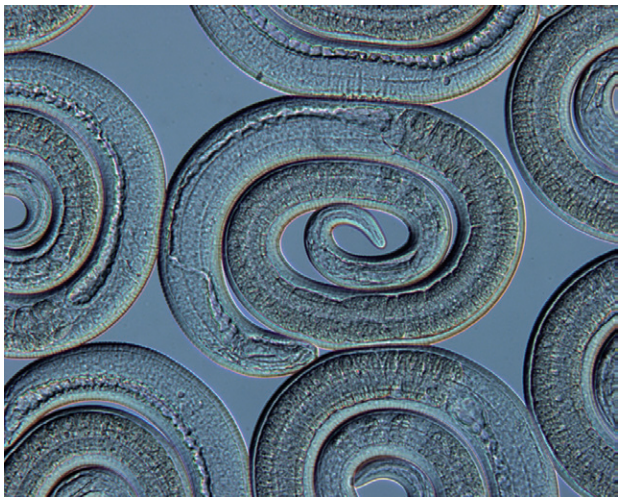


FIGURE 7-100. *Trichinella spiralis* larvae in a fresh digest preparation of rat muscle cysts.

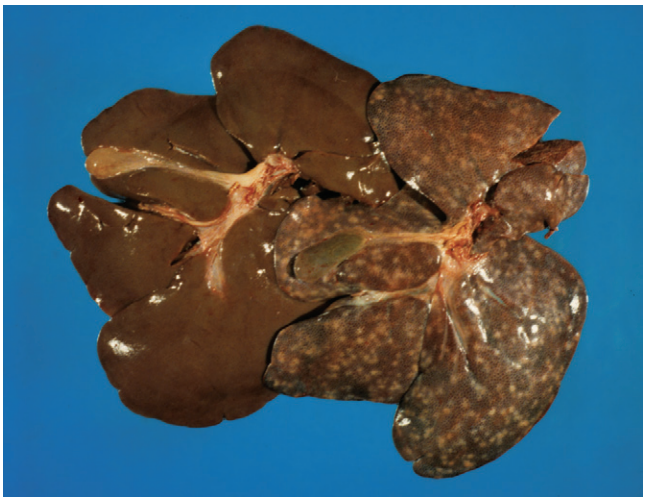


FIGURE 7-101. Lesions induced in the liver of a pig exposed to the infective eggs of *Ascaris suum (right);* normal liver on left.

2. Cover with a second microscope slide, and press the two slides together with the thumb and forefinger, thus squashing the scrap of meat.
3. While maintaining pressure, bind the slides firmly together by wrapping each end with adhesive tape.
4. Trim off any meat protruding from between the slides to avoid contaminating the microscope stage.
5. Scan the entire field under low power. Larvae, if present, are easily visible (Figure 7-100). *Note:* This procedure is also applicable to other tissue-dwelling parasites such as the smaller lungworms of sheep and carnivorans, encysted *Toxocara* larvae, and the like.

Tissue Digestion

Peptic digestion is used to detect light infection with *T. spiralis* and other nematodes in tissues. Gastric juice digests the muscle tissue but not the larvae of *T. spiralis*. Pepsin-acid solution consists of 0.2 g granular pepsin and 1.0 mL concentrated hydrochloric acid in 100 mL distilled water.

1. Weigh out 4 g of tissue and mince it with a scalpel.
2. Add 100 mL of pepsin-acid solution, and allow it to stand for about 1 to 6 hours at 37° C.
3. Decant excess supernatant carefully, suspend sediment, and transfer to a Petri plate.
4. Count larvae under a dissecting microscope. Larvae may be retrieved with a Pasteur pipette for closer study under the compound microscope.

ANNOTATED HOST-ORGAN LIST OF PARASITES OF SWINE

Toxoplasma gondii may occur in any tissue of any host as extracellular or intracellular tachyzoites or as bradyzoites in cysts (see Figure 8-35).

Alimentary System

Mouth and Esophagus

NEMATODES. *Gongylonema pulchrum* (150 mm; Spirurida) (see Figures 4-145, 4-146, 7-62, and 7-118).

Eucoleus (Capillaria) garfiai (Trichinelloidea) is found in the epithelia of the tongue of wild pigs.

Stomach

NEMATODES. *Physocephalus sexalatus* (see Figure 4-147), *Ascarops strongylina, Gnathostoma hispidum* (see Figure 4-142 and 4-143), and *Simondsia paradoxa* (Spirurida).

Hyostrongylus rubidus (9 mm) and *Ollulanus tricuspis* (1 mm) (Trichostrongyloidea) (see Figures 4-72 and 4-80).

Aonchotheca (Capillaria) gastrosuis (Trichinelloidea).

Small Intestine

NEMATODES. *Ascaris suum* (410 mm; Ascaridoidea) (Figure 7-101; see also Figures 4-120 to 4-121 and 7-99).

Globocephalus urosubulatus (6 mm; Ancylostomatoidea) (see Figure 4-97).

Strongyloides ransomi (5 mm; Rhabditida) (see Figures 4-114 and 4-115).

Trichinella spiralis (4 mm; Trichinelloidea) (see Figures 4-161 to 4-163 and 7-100).

ACANTHOCEPHALA. *Macracanthorhyncus hirudinaceus* (470 mm) (see Figure 4-155).

PROTISTA. *Eimeria debliecki* and about 10 other species of *Eimeria* (coccidians); usually infection is without clinical signs.

Cystoisospora suis (coccidian) causes enteritis in the small intestine of young animals.

Cryptosporidium suis (Apicomplexa).

Giardia species (mucosoflagellate) (see Figure 7-104; see also Figure 3-6); infection is usually without signs.

Cecum and Colon

NEMATODES. *Oesophagostomum dentatum, Oesophagostomum brevicaudum, Oesophagostomum georgianum,* and *Oesophagostomum quadrispinulatum* (Strongyloidea) (see Figures 4-86 and 4-87).

Trichuris suis (Trichinelloidea) (Figure 7-102; see Figures 4-167 and 7-99).

PROTISTA. *Entamoeba polecki, Endolimax nana, Iodamoeba buetschlii,* and others (amoebas) are considered for the most part to be commensals.

Chilomastix mesnili, Tetratrichomonas buttreyi, Trichomitus rotunda, and *Tritrichomonas suis* (mucosoflagellates) are considered for the most part to be commensals.

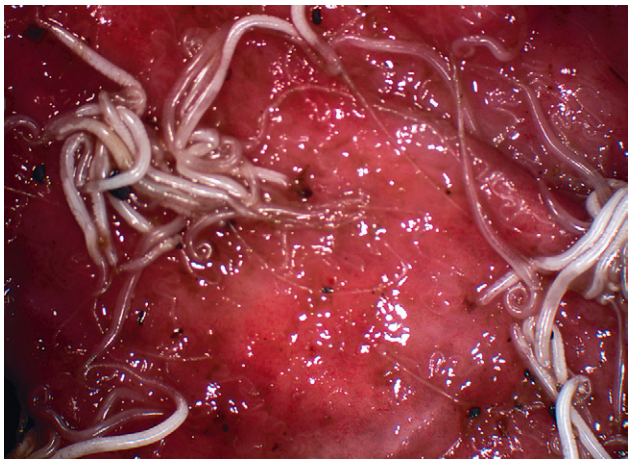


FIGURE 7-102. Rectum of pig with attached *Trichuris suis.* (Specimen courtesy Dr. Mary C. Smith.)

Balantidium coli (ciliate) (see Figure 3-7) is a commensal organism that can on occasion cause colitis.

Liver, Pancreas, and Peritoneal Cavity

NEMATODE LARVAE. *Ascaris suum* (Ascaridoidea) (see Figures 7-101 and 4-116) has migrating larvae that cause "milk spot" lesions on the liver surface.

Stephanurus dentatus (Strongyloidea) migrating larvae in liver and pancreas (see Figure 4-93).

TREMATODES. *Fasciola hepatica* and *F. gigantica* (Fasciolidae) (see Figures 4-2 and 4-11).

CESTODE LARVAE. *Echinococcus granulosus* (Taeniidae) (see Figures 4-44 to 4-46 and 8-64) hydatids are very rare in the United States.

Taenia hydatigena (Taeniidae) (see Figure 4-48) cysticerci can be found on rare occasions, mainly in wild pigs.

Respiratory System

Bronchi and Bronchioles

NEMATODES. *Metastrongylus apri, M. salmi,* and *M. pudendotectus* (Metastrongyloidea) (see Figures 4-104 and 7-99) can cause signs of respiratory distress in pigs.

Lung Parenchyma

NEMATODE LARVA. *Ascaris suum* (Ascaridoidea) larvae migrate through after the liver and cause disease in reaction to their passage.

CESTODE LARVA. *Echinococcus granulosus* (Taeniidae) (see Figures 4-44 to 4-46 and 8-64) hydatids in pigs in the United States seem to be very rare.

TREMATODE. *Paragonimus kellicotti* (Troglotrematidae) (see Figures 4-14, 4-15, and 7-34, *B*) would be an excellent parasite of wild pigs and is liable to do very well in pigs fed crayfish.

Skeletal Muscles and Connective Tissues

Nematode Larva

Trichinella spiralis and *Trichinella murrelli* (Trichinelloidea) (see Figures 7-100 and 8-116) larvae can be present in very large numbers per gram of pig muscle without the pig showing signs of disease.

Cestode Larvae

Taenia solium (Taeniidae) (see Figure 8-60) cysticerci in muscle are a potential problem in areas where humans who might be infected with adults, especially those from certain developing countries, are working around pigs as animal handlers; cysts cause carcass condemnations.

FIGURE 7-103. *Haematopinus suis* from pig.

Spirometra mansonoides (Diphyllobothriidae) (see Figures 4-31 and 8-68) spargana can occur in pigs, which serve as paratenic hosts.

Trematode Larvae

Alaria (mesocercariae, Diplostomatidae).

Protista

Sarcocystis miescheriana, S. porcifelis, and *Sarcocystis suihominis* (coccidians) (see Table 2-1 and Figures 8-32 and 8-33) sarcocysts occur in the muscles of pigs.

Urogenital System

Nematode

Stephanurus dentatus (45 mm; Strongylida) (see Figure 4-93). Stout, white worms occur in the kidneys, ureters, urinary bladder, perirenal fat, pork chops, spinal canal, and elsewhere as a result of erratic migrations.

Skin and Hair

Insects

Musca and *Stomoxys* (Diptera) (see Figures 2-13 and 2-14).

Haematopinus suis (Anoplura) (Figure 7-103; see Figure 2-49).

Pulex irritans, Echidnophaga gallinacea, and *Tunga penetrans* (Siphonaptera) (see Figures 2-34, 2-36, 2-38, and and 2-44).

Arachnids

Metastigmata (ticks) (see Figures 2-75 and 2-90).

Sarcoptes scabiei (Astigmata) (see Figures 2-101 and 2-103) continues to be a problem in pigs.

Demodex phylloides (Prostigmata) (see Figure 2-116) causes pimples on pigs full of huge numbers of mites.

PARASITES OF LABORATORY RABBITS AND RODENTS

Many parasites lose all opportunity to complete their life history the day their host becomes a member of a laboratory animal colony. Although they may limit the usefulness of their immediate hosts as experimental subjects, such parasites present no continuing problem of control. Heartworm infection, for example, renders a dog unfit for experiments involving the circulatory or respiratory system but, in the absence of mosquitoes, must remain confined to the host in which it arrived. On the other hand, a surprising variety

of arthropod, Protistan, and helminth parasites do succeed in maintaining impressive populations even in reasonably hygienic laboratory animal colonies. Hair-clasping mites, mucosoflagellates, coccidians, *Hymenolepis* tapeworms, and pinworms are particularly common. The following incomplete outline includes only the common parasites of laboratory rabbits, rats, mice, guinea pigs, monkeys, and apes.

A few of the more common parasites of rodents and rabbits are represented in Figure 7-104.

ANNOTATED HOST-ORGAN LISTING OF COMMON PARASITES OF RABBITS

Toxoplasma gondii may occur in any tissue of any host as extracellular or intracellular tachyzoites or as bradyzoites in cysts (see Figure 8-35). *N. caninum* may occur in similar locations (see Figures 3-21 and 8-36).

Alimentary System

Stomach

NEMATODES. *Obeliscoides cuniculi* and *Graphidium strigosum* (18 to 20 mm; Trichostrongyloidea) (Figure 7-105). Spicules of *O. cuniculi* are 0.54 mm; of *G. strigosum*, 2.4 mm.

Intestine

NEMATODES. *Trichostrongylus retortaeformis* and *Nematodirus leporis* (Trichostrongyloidea) (see Figures 4-72 and 4-74).

S. papillosus (6 mm; Rhabditida).

Passalurus ambiguus (11 mm; Oxyurida) (see Figure 4-116).

Trichuris leporis (Trichinelloidea).

CESTODE. *Cittotaenia ctenoides* (Anoplocephalidae) (see Figure 7-104).

PROTISTA. *Eimeria* species (coccidian) (see Figure 7-94). Ten species of *Eimeria* parasitize the intestinal epithelium and cause diarrhea and emaciation.

Cryptosporidium cuniculus—a potential zoonotic agent.

Entamoeba cuniculi (amoeba). Nonpathogenic.

Liver and Peritoneal Cavity

PROTISTA. *Eimeria stiedae* (Coccidia) causes biliary coccidiosis (see Figure 8-28).

LARVAL NEMATODES. *Toxocara canis* migrates through the liver of rabbits on the way to the lungs and musculature (Figure 7-106).

CESTODE LARVAE. *Taenia pisiformis* (Taeniidae) (Figure 7-107) cysticerci initially migrate through the liver but ultimately settle down to mature in the peritoneal cavity.

Skin and Hair

Arachnids

Psoroptes cuniculi (Astigmata) (Figures 7-108 and 7-109; see also Figures 2-100, 2-107, and 2-108) can cause severe ear canker in rabbits.

Sarcoptes and *Chorioptes* (Astigmata) (see Figures 2-101 to 2-103 and 2-109).

Leporacarus gibbus (Listrophoridae) (Figure 7-110).

Cheyletiella parasitovorax (Prostigmata) (see Figure 2-117).

ANNOTATED HOST-ORGAN LISTING OF COMMON PARASITES OF RATS

Toxoplasma gondii may occur in any tissue of any host as extracellular or intracellular tachyzoites or as bradyzoites in cysts (see Figure 8-35). *N. caninum* may occur in similar locations (see Figures 3-21 and 8-36).

Alimentary System

Stomach and Intestines

NEMATODES. *Nippostrongylus brasiliensis* (6 mm; Trichostrongyloidea) (Figure 7-111).

Strongyloides ratti (Rhabditida) (see Figures 4-114 and 4-115).

Gongylonema neoplasticum (Spirurida) (see Figure 7-118).

Syphacia muris and *Aspiculuris ratti* (Oxyurida).

Heterakis spumosa (16 mm; Ascaridida).

Trichinella spiralis and *T. murrelli* (Trichinelloidea) (see Figure 4-148).

Trichuris muris (Trichinelloidea).

CESTODE. *Hymenolepis diminuta* (Hymenolepididae) (see Figure 7-104). Scolex without hooks.

PROTISTA. *Eimeria nieschultzi* and other species (coccidians) (see Figure 7-104).

Giardia (mucosoflagellate) (see Figure 7-104).

Liver

NEMATODE. *Calodium* (*Capillaria*) *hepaticum* (Trichinelloidea) (see Figure 8-117).

CESTODE LARVAE. Strobilocercus of *Taenia taeniaeformis* (Taeniidae) (see Figure 8-61).

Hydatid cysts of *Echinococcus multilocularis* (Figure 7-112).

PROTISTA. *Hepatozoon muris* (plasmodium) has schizogony occurring in the hepatic cells; gamonts are found in the monocytes of the circulating blood. The vector is a mesostigmatid mite, *Echinolaelaps echidninus*.

Urogenital System

Nematodes

Trichosomoides crassicauda (Trichinelloidea) (see Figures 4-169 and 8-120) live threaded through the bladder epithelium; the male lives in the reproductive system of the female worm.

Skin and Hair

Insects

Polyplax spinulosa (Anoplura) (Figure 7-113).

Xenopsylla cheopis (Siphonaptera) (see Figure 2-37).

Arachnids

Ornithonyssus bacoti (Mesostigmata).

Radfordia ensifera (Prostigmata).

Notoedres muris (Astigmata) (see Figures 2-104 and 2-105).

ANNOTATED HOST-ORGAN LISTING OF COMMON PARASITES OF MICE

Toxoplasma gondii may occur in any tissue of any host as extracellular or intracellular tachyzoites or as bradyzoites in cysts (see Figure 8-35). *N. caninum* may occur in similar locations (see Figures 3-21 and 8-36).

Alimentary System

Stomach and Intestines

PROTISTA. *Cryptosporidium muris* (stomach) and *C. parvum* (small intestine) (see Figures 3-11, *C. andersoni*, and 3-10, *C. parvum*).

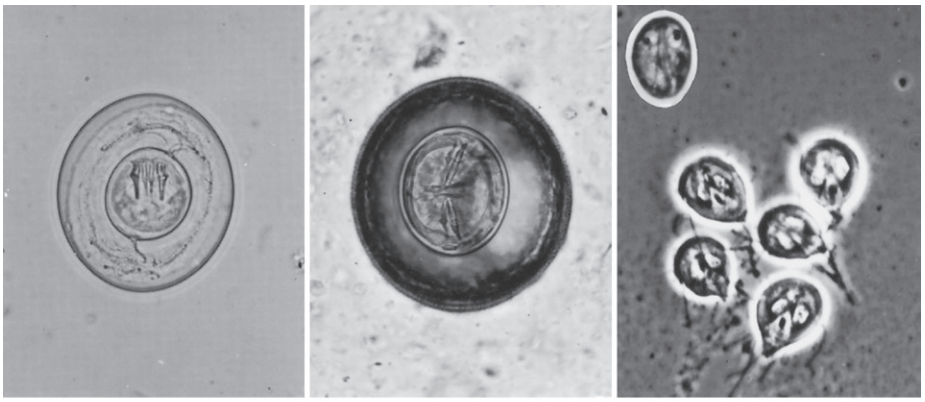
NEMATODES. *Heligmosomoides polygyrus* (syn. *Nematospiroides dubius;* Trichostrongyloidea) organisms are reddish and tightly coiled.

N. brasiliensis (6 mm; Trichostrongyloidea) (see Figure 7-111).

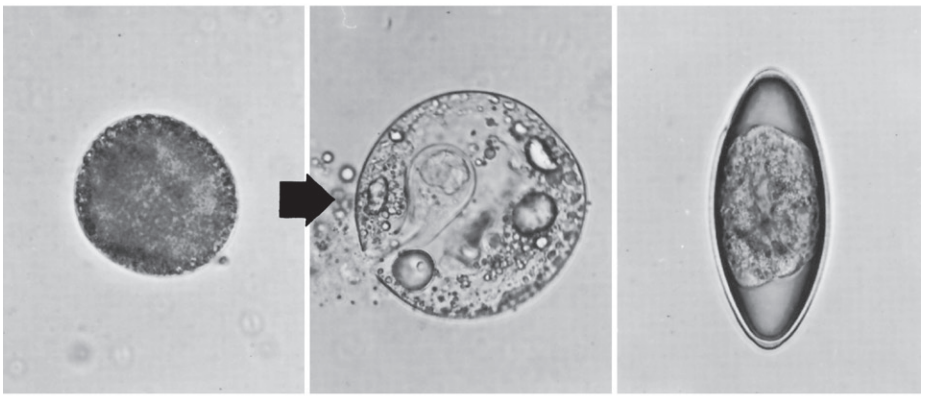
Syphacia obvelata and *Aspiculuris tetraptera* (Oxyuroidea) (Figure 7-114).

Heterakis spumosa (Ascaridida).

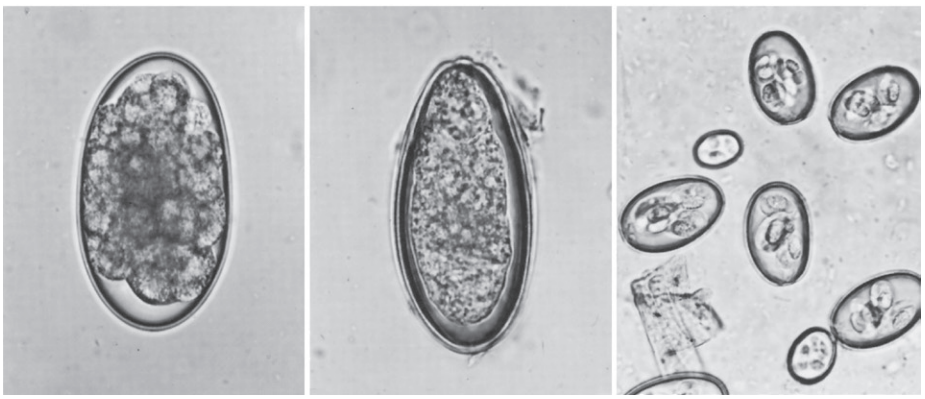
Trichuris muris (Trichinelloidea).



Hymenolepis nana *H. diminuta* *Giardia*



Cittotaenia ctenoides *Aspiculuris*



Obeliscodes cuniculi *Passalurus ambiguus* *Eimeria*

FIGURE 7-104. Common parasites of laboratory mice, rats, and rabbits. For a more comprehensive listing of laboratory animal parasites by host and organ, see text. *Mouse and rat: Hymenolepis nana* and *Hymenolepis diminuta* (Hymenolepididae) are also parasites of humans. *H. nana* infection in rodent colonies is directly infective to human beings; no intermediate host is required by this tapeworm. Various beetles and cockroaches serve as intermediate hosts for *H. diminuta* and, facultatively, for *H. nana*. *Giardia* (Mastigophora) trophozoites *(group of five, center)* and cysts *(inset, upper left)* are common parasites of mice. *Rabbit: Cittotaenia ctenoides* (Anoplocephalidae) eggs appear as amorphous spheres *(left of arrow)* until crushed by pressure on the coverslip *(right of arrow)*, whereupon the oncosphere and pear-shaped embryophore become visible. *Obeliscoides cuniculi* eggs are typical strongyle eggs. *Passalurus ambiguus* (Oxyuridae) are somewhat asymmetric and have a cap at one end. *Eimeria*, sporulated oocysts. Avoid mistaking *Saccharomycopsis guttulatus* (see Figure 7-6) for a bona fide parasite of the rabbit. All ×425 except *Giardia* (×1000).

FIGURE 7-105. *Obeliscoides cuniculi*, stomal end *(left)* and bursa and spicules of male *(right)* (×120).

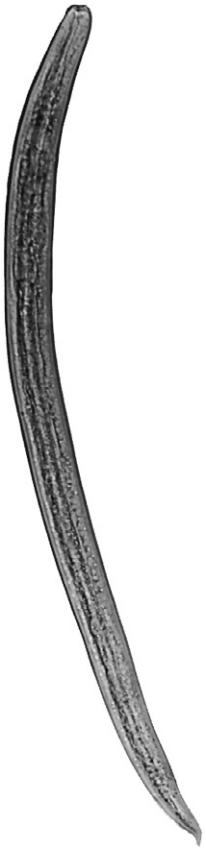


FIGURE 7-106. *Toxocara* larva from a rabbit's liver (×250).

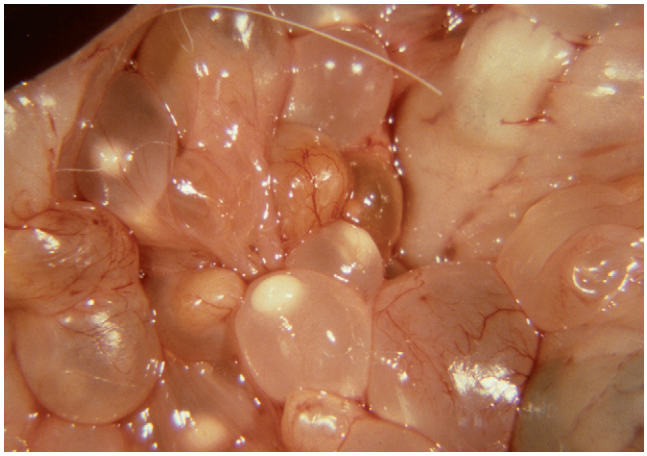


FIGURE 7-107. Cysticerci of *Taenia pisiformis* in the abdominal cavity of an experimentally infected domestic rabbit.

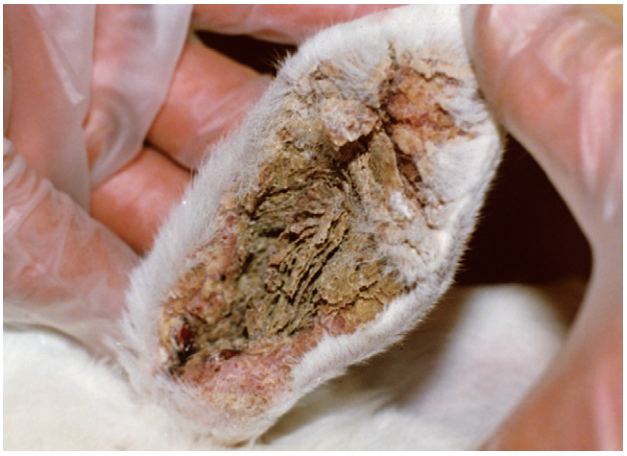


FIGURE 7-108. Ear of a rabbit infested with *Psoroptes cuniculi*.

FIGURE 7-109. *Psoroptes cuniculi* from a rabbit; *inset* shows the segmented pretarsi.

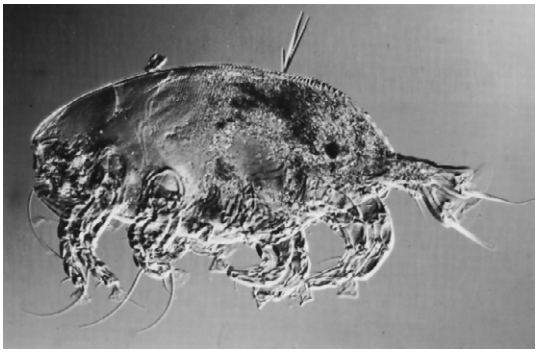


FIGURE 7-110. *Leporacarus gibbus*, a hair-clasping mite of rabbits (×100). (Courtesy Dr. Stephen Weisbroth.)

FIGURE 7-111. *Nippostrongylus brasiliensis.* **A,** Bursa and spicules of male (×125). **B,** Caudal end of female (×150). **C,** Esophageal region (×150).

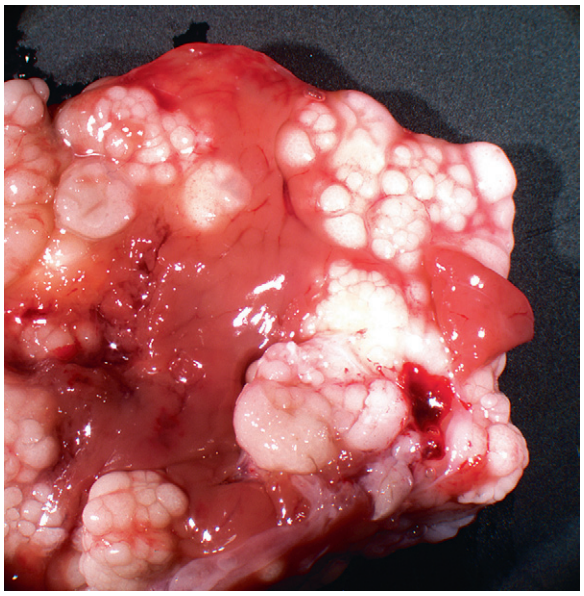


FIGURE 7-112. Liver of cotton rat experimentally infected with *Echinococcus multilocularis* producing the alveolar hydatid cysts that have replaced most of the liver tissue.

CESTODES. *Hymenolepis nana* and *H. diminuta* (Hymenolepididae) (see Figure 7-103). The scolex of *H. nana* is armed with hooks; that of *H. diminuta* is unarmed.

LARVAL NEMATODES. Migrating *Toxocara canis* can cause disease in small mammals as it migrates (Figure 7-115).

Urogenital System

Kidneys

PROTISTA. *Klossiella muris* (coccidian) usually seen on histosections (see Figure 8-30).

Skin and Hair

INSECTS. *Polyplax serrata* (Anoplura) (see Figures 2-53, 2-54, and 7-113).

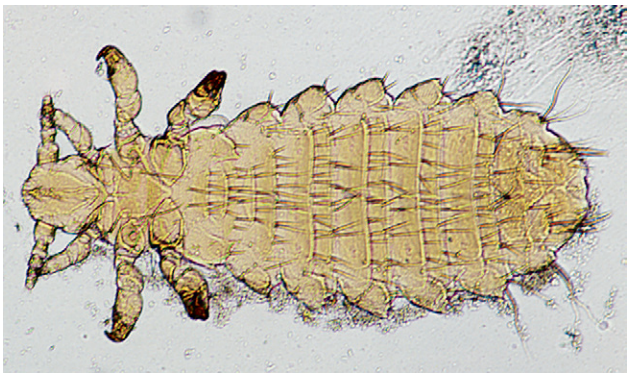


FIGURE 7-113. *Polyplax spinulosa* male (×108).

FIGURE 7-114. Pinworms of mice: *Syphacia obvelata* male *(left)* and *Aspiculuris tetraptera* anterior end *(right)* (×80).

ARACHNIDS. *Myobia musculi* and *Radfordia affinis* (Prostigmata) (see Figure 2-118). Myobiids do not migrate away from a dead host; the carcass must be scanned carefully with a stereoscopic microscope to find them.

Myocoptes musculinus (Astigmata) (see Figure 2-114).

Ornithonyssus bacoti and *Allodermanyssus sanguineus* (Mesostigmata) (see Figure 2-93, *Ornithonyssus sylviarum*).

ANNOTATED HOST-ORGAN LISTING OF COMMON PARASITES OF GUINEA PIGS

Toxoplasma gondii may occur in any tissue of any host as extracellular or intracellular tachyzoites or as bradyzoites in cysts (see Figure 8-35).

Alimentary System

Nematode

Paraspidodera uncinata (Oxyurida).

Cestode

Hymenolepis nana (see Figure 7-104).

Protista

Eimeria caviae (coccidian).

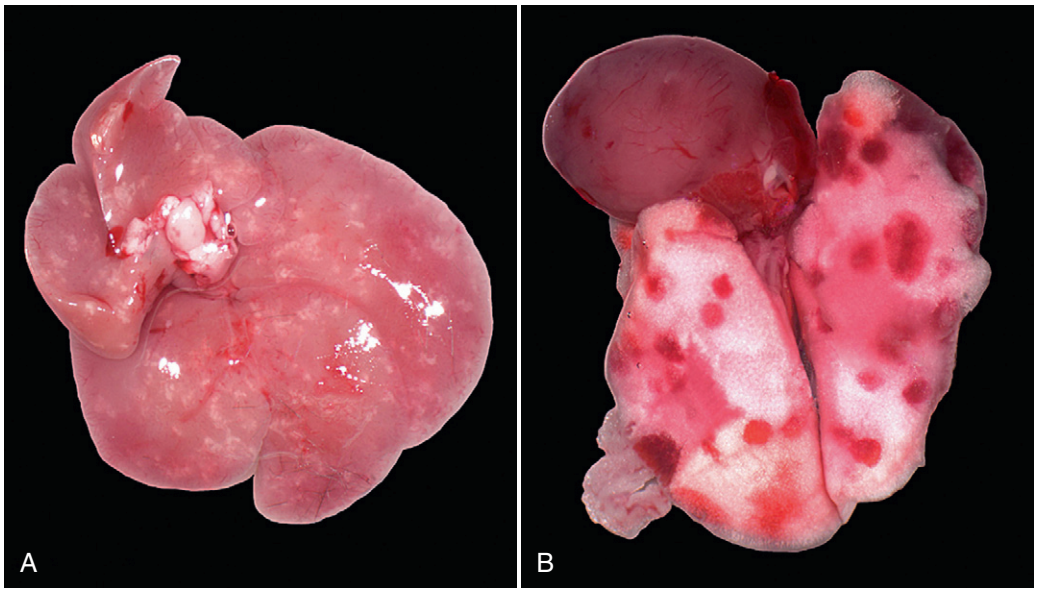


FIGURE 7-115. Liver and lungs of mice experimentally infected with the infective eggs of *Toxocara canis*. **A,** Liver removed from a mouse 2 weeks after a challenge infection imposed on a month-old infection. **B,** Lungs of a mouse 3 days after being infected with 125 eggs of *Toxocara canis.*

Balantidium species (ciliate) (Figure 7-116, and see Figure 3-7).

Cryptosporidium wrairi (see Figure 3-10, *C. parvum*).

Skin and Hair

Insects

Gliricola porcelli, Gyropus ovalis, and *Trimenopon hispidum* (Mallophaga) (Figure 7-117, and see Figure 2-62).

Arachnids

Chirodiscoides caviae (Astigmata) (see Figure 2-113).

Trixacarus caviae (Astigmata) can cause severe mange in guinea pigs that can be fatal.

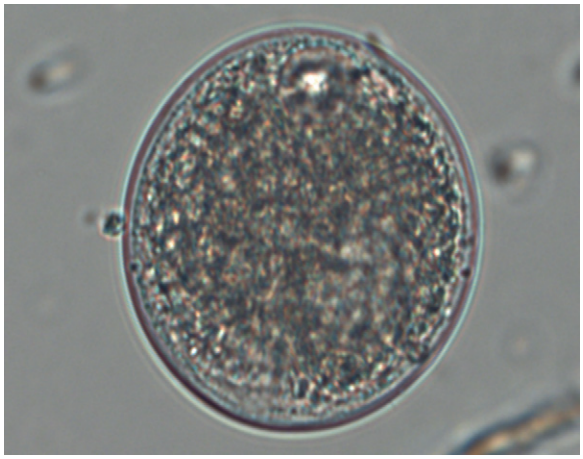


FIGURE 7-116. *Balantidium coli* cyst in the feces of a guinea pig.

PARASITES OF MONKEYS AND APES

The kinds of parasites to be found depend on the species and the geographic origin of the monkey and on the duration and environmental conditions of its captivity. Certain parasites (e.g., *Strongyloides, Oesophagostomum*) flourish in captive monkeys. Others, especially those whose natural intermediate hosts are no longer available, tend to fade away. In mixed colonies, parasites that are not discriminating in their selection of hosts may spread to species of monkeys that, for geographic or ecologic reasons, rarely or never infect in the wild. Such cross-infections are more likely to cause disease because of the lack of mutual adaptation of host and parasite. The following therefore represents a composite listing of the more common parasites of monkeys and apes without particular regard for natural host species' preferences or geographic origins.

STAGES IN FECES

Many parasites are shared with humans, and a text such as *Atlas of Human Parasitology* (Ash and Orihel, 2007) can be consulted for identification of the many shared parasites.

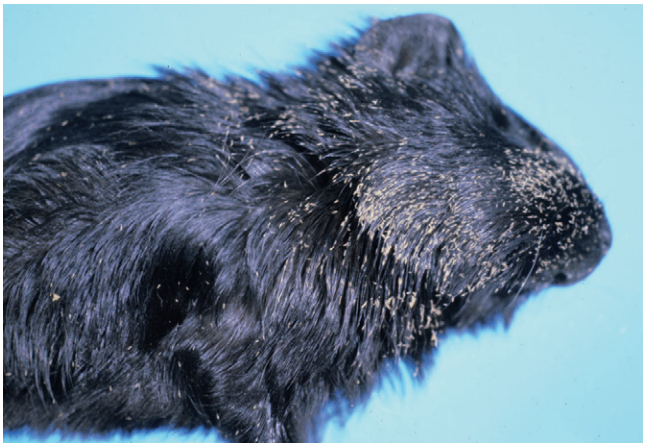


FIGURE 7-117. Guinea pig infested with the louse *Gliricola porcelli.*

Alimentary System

Nematodes

Cephalobus parasiticus (Rhabditida). These harmless parasites of the stomach and intestines of *Macaca iris mordax* (and probably others) resemble the free-living generation of *Strongyloides*. Their rhabditiform larvae may be confused with those of *Strongyloides* on fecal examination. They do not, however, develop into filariform larvae, so the dilemma may be resolved by culturing the fecal specimen.

Strongyloides fuelleborni and *S. stercoralis* (Rhabditida) (see Figure 4-109). Simian strongyloidosis is a human health hazard.

Nochtia nochti (Trichostrongyloidea). Bright red worms lie within or protrude from gastric papillomata in the prepyloric region of the stomach. Cross sections of *N. nochti* in histologic preparations display 16 distinct longitudinal cuticular ridges and channeled lateral alae.

Trichostrongylus, Molineus, and *Nematodirus* (Trichostrongyloidea) (see Figure 4-72).

Oesophagostomum (*Conoweberia*) *apiostomum, Oesophagostomum stephanostomum,* and *Ternidens deminutus* (Strongyloidea) (Figure 7-118, and see Figure 4-88). Stout-bodied "nodular worms" with leaf crowns and transverse ventral cervical groove.

Necator, Ancylostoma, and *Globocephalus* (Ancylostomatoidea) (see Figures 4-97 and 4-98).

Ascaris lumbricoides (Ascaridoidea) (see Figures 4-120 and 4-121).

Trichuris species (Trichinelloidea) (Figure 7-119, and see Figure 4-151).

Enterobius species (Oxyurida) (see Figure 7-118). Pinworms are quite host specific. Generally speaking, a species of pinworm infects a genus of monkeys. *Enterobius vermicularis* and *Enterobius anthropopitheci* occur in chimpanzees. *Enterobius* species are usually considered nonpathogenic, but sometimes they invade the wall of the intestine and produce serious or even fatal disease.

Probstmayria species occur in various primates and sometimes are found in fecal specimens (Figure 7-120).

Streptopharagus, Gongylonema, Protospirura, Physocephalus, Rictularia, Physaloptera (Spirurida) (see Figures 4-130, 4-131, 4-133, 4-134, and 7-118). *Protospirura muricola,* a parasite of rodents that uses the cockroach *Leucophaea maderae* as intermediate host, has been observed to cause perforation of the stomach in captive monkeys (Foster and Johnson, 1939).

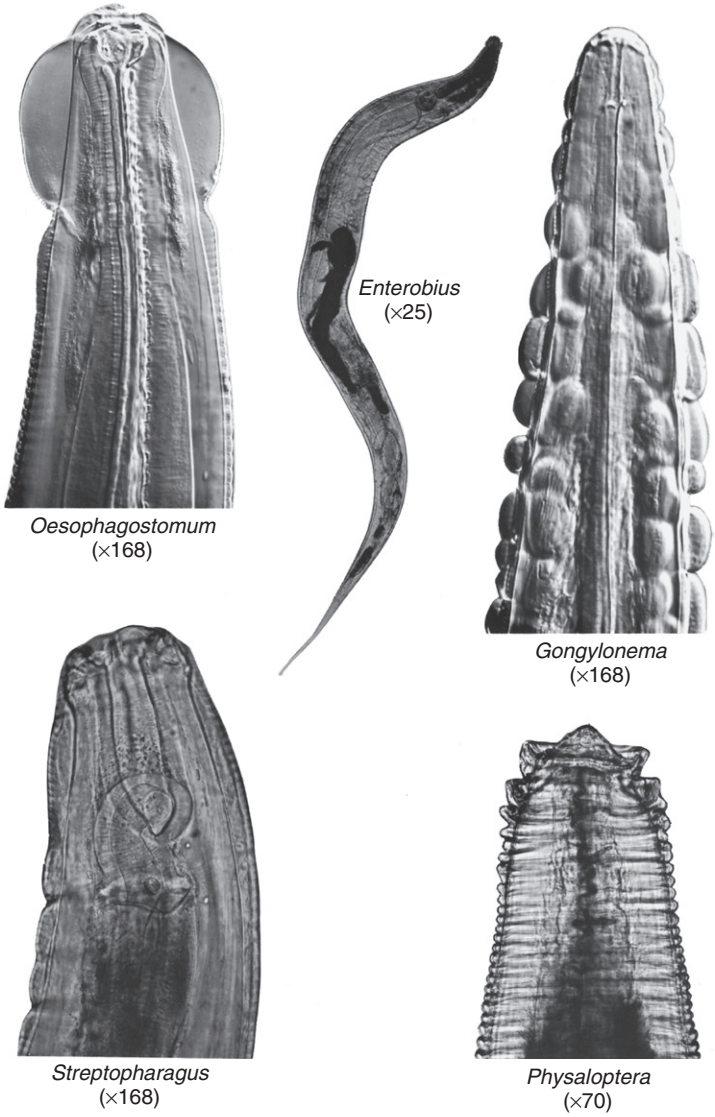


FIGURE 7-118. Some nematode parasites of monkeys and apes. (Courtesy Dr. M. M. Rabstein.)

Cestodes

Bertiella studeri (Anoplocephalidae) is large and has four suckers and no hooks (Figure 7-121).

Hymenolepis nana (Hymenolepididae) (see Figure 7-103) is very small, has four suckers, and has hooks.

Acanthocephalans

Prosthenorchis and *Moniliformis* (see Figure 7-121).

Trematode

Gastrodiscoides hominis (Paramphistomatidae).

Protista

B. coli (ciliate) (see Figure 3-7) causes acute enteritis (Teare and Loomis, 1982).

Entamoeba coli or *Entamoeba coli,* like amoebae, are common in primates (Figure 7-122), but they can also occasionally be infected with the pathogenic *Entamoeba histolytica*.

Giardia lamblia (flagellate) (see Figure 7-104).

Liver and Pancreas

Protista

Hepatocystis kochi schizonts.

Entamoeba histolytica (amoeba) can cause hepatic abscess.

Nematodes

Calodium (Capillaria) hepaticum (Trichinelloidea) (see Figure 8-117) occurs with worms and eggs in hepatic parenchyma.

Trichospirura leptostoma (Spirurida) is a 10- to 20-mm worm with a long capillary pharynx; associated with varying degrees of fibrosing pancreatitis. Found in pancreatic duct of American primates.

Respiratory System

Nose and Throat

NEMATODE. *Anatrichosoma* (Trichinelloidea) (see Figure 7-121, and see Figures 8-118 and 8-119).

ANNELIDS. The leeches that attack the pharyngeal mucosa of monkeys are large, black annelids with a large cup-shaped caudal sucker. The presence of this bloodsucking parasite is suggested by chronic epistaxis in a recently captured monkey. When the host drinks infested water, the young leeches enter the mouth, nose, pharynx, or larynx and attach to the mucous membrane. They remain in these locations for several weeks unless removed.

ARACHNID. *Rhinophaga* species.

Lungs

NEMATODES. *Filaroides* (Metastrongyloidea).
Metathelazia (Spirurida).

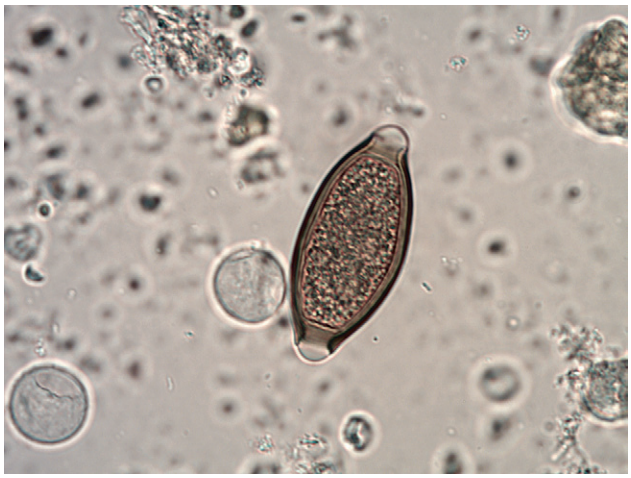


FIGURE 7-119. *Trichuris* species from the feces of a patas monkey *(Erythrocebus patas).* Also, two unstained amoeba cysts are present.

FIGURE 7-120. *Probstmayria* species from the feces of a gorilla *(Gorilla beringei beringei)* identified in an ethyl acetate–formalin sediment examination. (Specimen provided by Dr. Jessica M. Rothman, Hunter College, New York.)

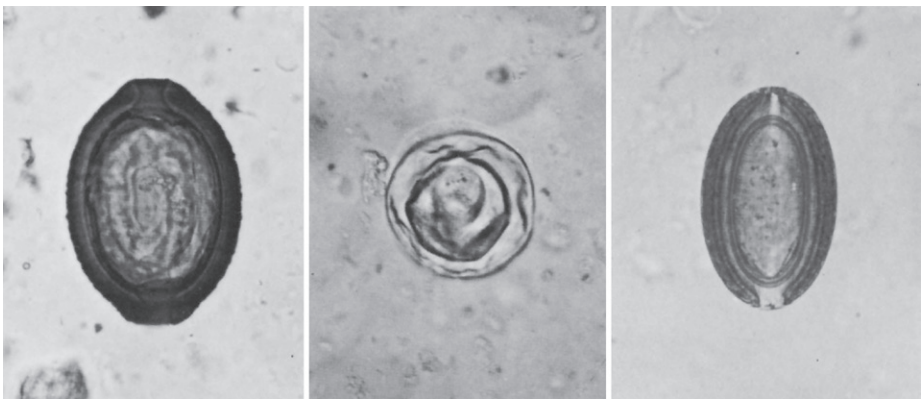


FIGURE 7-121. Three parasites of primates. For a more complete listing of simian parasites by host and organ, see text. *Anatrichosoma cynomolgi* adult worms tunnel in the nasal mucosa. Anoplocephalid eggs have a pear-shaped embryophore surrounding the oncosphere. *Prosthenorchis elegans* (Acanthocephala) eggs have a thick outer shell and thin inner shells enclosing the embryo (acanthor).

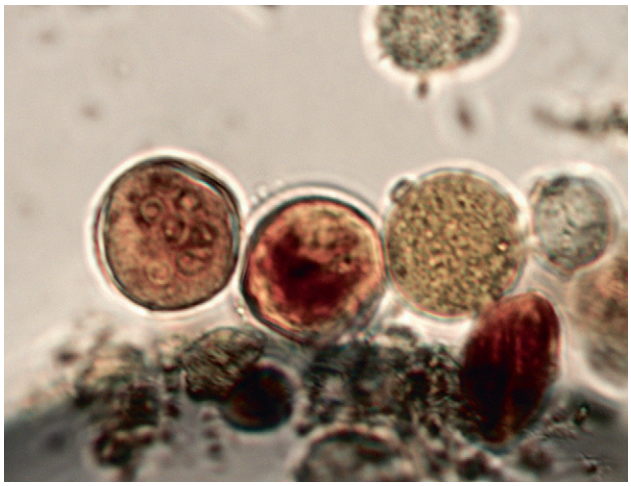


FIGURE 7-122. *Entamoeba coli*–like cyst in the feces of a patas monkey *(Erythrocebus patas)*; one cyst has eight visible nuclei.

CESTODE LARVA. *Echinococcus granulosus* (Taeniidae) (see Figures 4-44 to 4-46 and 8-64).

ARACHNID. *Pneumonyssus simicola* (Mesostigmata) (see Figure 8-8).

Serous Cavities

NEMATODE. *Dipetalonema* species (Filarioidea) (see Figure 4-145).

CESTODE LARVAE. *Taenia hydatigena* (cysticercus) (see Figure 4-48).

Mesocestoides (tetrathyridium) (see Figures 8-65 to 8-67).

Spirometra mansonoides (plerocercoid) (see Figures 4-31 and 8-68).

PENTASTOMID NYMPHS. *Porocephalus, Armillifer,* and *Linguatula*.

ACANTHOCEPHALANS. *Prosthenorchis* species (see Figure 7-121).

Blood

Nematode Microfilariae

Dirofilaria, Dipetalonema, Tetrapetalonema, Loa, and *Brugia* (Filarioidea). Differentiation of the many kinds of microfilariae found in monkeys from all parts of the tropics is a task for the specialist. Many species remain to be described.

Protista

Simian malaria organisms, *Plasmodium* and *Hepatocystis*.

Muscles and Connective Tissues

Nematodes

Onchocerca, Dirofilaria, Dipetalonema, Tetrapetalonema, Loa, and *Brugia* (Filarioidea) (see Figure 4-155). *Onchocerca* microfilariae are found in the dermis.

Cestode Larvae

Taenia (cysticercus).

Mesocestoides (tetrathyridium) (see Figures 8-65 to 8-67).

Spirometra (plerocercoid) (see Figures 4-31 and 8-68).

Skin and Hair

Insects

Pedicinus and *Pthirus* (Anoplura) (see Figure 2-55).

Nematodes

Anatrichosoma cutaneum (Trichinelloidea). Very slender (25 by 0.2 mm) worms give rise to subcutaneous nodules, edema about the joints, and elongated, serpiginous blisters of the palms and soles. Adult females burrow in the epidermis of the palms and soles (see Figure 7-121).

Onchocerca microfilariae.

Dracunculus (Spirurida) (see Figures 4-138 and 4-139).

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CHAPTER 8

Histopathologic Diagnosis

Mark L. Eberhard

The microscopic identification of parasites in tissue sections is an interesting challenge. Often a diagnostician is provided with a single slide that shows only pieces of the parasite. In an attempt to identify an object believed to be a parasite, one should gather as much information about the patient as possible, including life history and clinical signs. It is also important to be familiar with the kinds of parasites most likely to be found in the particular host and tissue under study, as well as in the specific geographic area. The host-organ listing of parasites in the preceding chapter should be considered as a checklist of possibilities. The main objective of this section is to emphasize some of the major microscopic anatomic features of parasites that can be helpful in their identification in histologic sections. For arthropods and metazoan parasites, several defining characteristics can be listed for each group of parasites, but the presence or absence of a body cavity and digestive tract and the type and distribution of muscle fibers are important criteria to be considered in making an initial placement into a major group.

For further reading and assistance with diagnosis of parasites in tissues, the following sources are helpful. A report dealing with the present subject is "Identification of Parasitic Metazoa in Tissue Sections" by MayBelle Chitwood and J. Ralph Lichtenfels, first published in *Experimental Parasitology*, volume 32, pages 407 to 519, 1972, and later reprinted as a monograph by the U.S. Department of Agriculture. Texts dealing with the subject include *Pathology of Tropical and Extraordinary Diseases*, volumes 1 and 2, edited by C.H. Binford and D.H. Connor, Washington, DC, 1976, Armed Forces Institute of Pathology (AFIP); *Pathology of Infectious Diseases*, volumes 1 and 2, by D.H. Connor, F.W. Chandler, D.A. Schwartz, H.J. Manz, and E.E. Lack, Stamford, Connecticut, 1997, Appleton & Lange; *An Atlas of Protozoan Parasites in Animal Tissues*, by C.H. Gardiner, R. Fayer, and J.P. Dubey, USDA Agriculture Handbook No. 651, U.S. Government Printing Office, Washington, DC, 1988, and edition 2, published by AFIP, American Registry of Pathology, Washington, DC; *Diagnostic Pathology of Parasitic Infections With Clinical Correlations*, edition 2, by Y. Gutierrez, Philadelphia, 1990, Lea & Febiger; *Parasites in Human Tissues* by T.C. Orihel and L.R. Ash, Chicago, 1995, American Society of Clinical Pathology (ASCP) Press; *Parasitic Diseases in Nonhuman Primates,* volume 2, by K. Strait, J.G. Else, and M.L. Eberhard, in *Nonhuman Primates in Biomedical Research: Diseases,* edited by C.R. Abee, K. Mansfield, S.D. Tardif, and T. Morris, San Diego, 2012, Academic Press; and *Pathology of Infectious Diseases,* volume 1, *Helminthiases,* by W.M. Meyers, R.C. Neafie, A.M. Marty, and D.J. Wear, AFIP, 2000, American Registry of Pathology, Washington, DC.

ARTHROPODS

Arthropods, composed of hundreds of thousands of species, have such diverse features that describing them succinctly is nearly impossible. Arthropods do have some shared features—for example, they have a segmented body, a chitinous exoskeleton, a coelom, and jointed appendages. The chitinous exoskeleton, the cuticle, in histologic sections usually appears thick and dark, but usually the exoskeleton itself does not take up stain. Over some parts of the body, especially in areas between segments or joints in an appendage, the cuticle can be very thin. The striated muscle of arthropods is diagnostic for this group of pathogens if they can be found in the sections. The larger arthropods also have a respiratory system that is composed of a racemose tracheal system, which in section appears as variously sized tubes coursing throughout the body. The larger of the tracheal branches have chitinous reinforcing rings. Arthropods also can contain fat bodies that often appear darkly stained in sections. Smaller parasitic arthropods often have rounded to elongate bodies that are apparent in tissue sections, and sometimes one is fortunate enough to observe sections through paired, jointed legs. All together, these features are fairly complete in defining an arthropod in section.

Three major groups of arthropods are likely to appear in histologic section. The insects (subphylum Mandibulata, class Insecta) contain the maggots of various myiasis-producing flies, and these commonly appear in histologic sections. The mites are in the class Arachnida of the subphylum Chelicerata, and these creatures, because of their small size and ability to colonize various mainly superficial body surfaces, such as skin and respiratory mucosae, also appear in sections of lesions. Ticks tend to remain superficial to the host, attaching only long enough to feed, so typically, unless there is a strange clinical presentation or an interested researcher, they do not appear in histologic sections. The Pentastomids are a group of parasitic crustaceans that have larval stages that parasitize vertebrates.

MAGGOTS

Maggots in tissue are the larvae of dipteran flies and may represent species that require a living host or species, causing secondary myiasis such as that caused by various *Calliphora* and *Sarcophaga*.

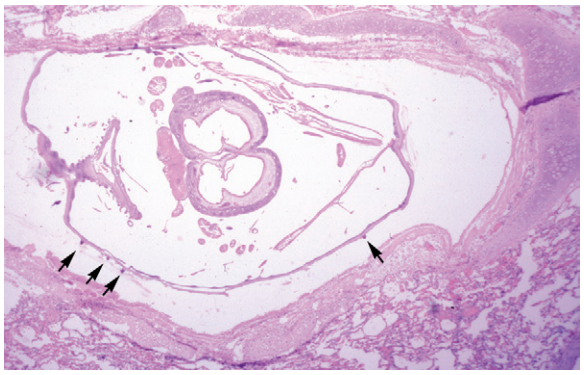

FIGURE 8-1. *Cuterebra* in the lung of a rabbit (×5). The internal organs lie in a body cavity rather than in a parenchymatous matrix; *arrows* indicate spines on the cuticle.

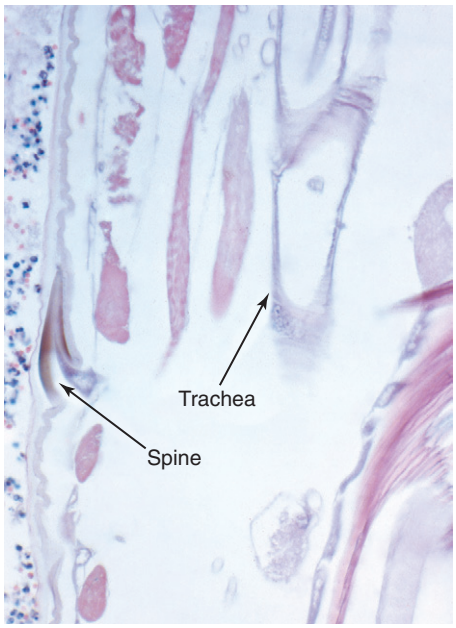

FIGURE 8-2. *Cuterebra* larva (bot) in the brain of a cat (×220).

Both types of maggots display similar features, and the difficulty lies in making a generic diagnosis based strictly on morphology. The spiracular plate is important in identification of fly larvae and may need to be retrieved from the wet tissues or paraffin block (see Figure 2-22).

Sections of maggots will display the typical features of an arthropod (e.g., body cavity; Figure 8-1), segmentation, striated muscles attached at various points to the chitinous exoskeleton, and tracheae, often with cuticular rings (Figure 8-2). Some species have prominent spines (see Figure 8-2). *Cuterebra* larvae are obligate endoparasites of rodents and lagomorphs; these larvae may invade dogs, cats, and occasionally humans. Typically, they are found in cervical subcutaneous tissues, but in dogs and cats they migrate into the central nervous system with disastrous results (see Figures 8-1 and 8-2). First-stage *Hypoderma* larvae migrate extensively in cattle, and erratic migration through the brain of horses has been reported.

MITES

Mites tend to be rather small—millimeters or smaller in size. With many species, eggs, larvae (six legs), nymphs (eight legs), and adults (eight legs) are all found in section, and in a section of an adult can be found all the component parts of a typical arthropod—segmented

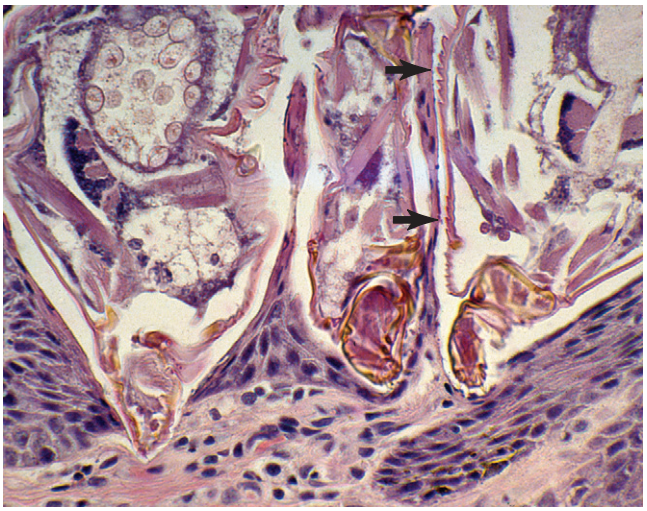

FIGURE 8-3. *Sarcoptes* mites in the skin of a dog (×230); *arrows* indicate spines on the cuticle.

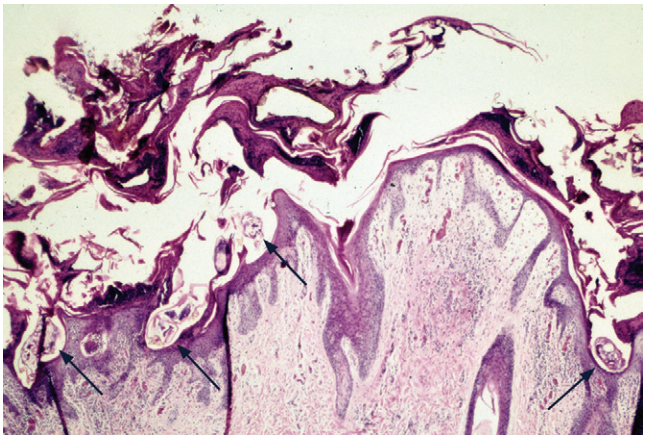

FIGURE 8-4. Hyperkeratosis caused by *Sarcoptes scabiei* in the pig (×22). The mites *(arrows)* are found in the deeper layers of the greatly thickened epidermis.

legs, spines, and hairs externally, and striated muscles, reproductive organs, intestine, yolk glands, and developing eggs internally, may be seen in section. Mites that live in the skin—*Sarcoptes, Notoedres, Knemidocoptes,* and *Trixacarus*—are very small and round, feed at the stratum germinativum and dermis (Figure 8-3), and have spines on their dorsum (Figure 8-4). In some hosts such as the red fox, *Vulpes vulpes,* and pigs, sarcoptic mange is characterized by extraordinary hyperkeratosis (see Figure 8-4), and similar hyperkeratosis is seen in cats with *Notoedres* infection (Figure 8-5). Hyperkeratosis is typical of mange caused by *Chorioptes* and *Cheyletiella* in certain hosts, but the mites lie more superficially in the stratum corneum.

Demodex organisms are cigar-shaped mites found in hair follicles or with associated sebaceous glands (Figure 8-6), although some such as *Demodex gatoi, Demodex criceti,* and *Demodex injai* tend to be superficial. In dogs with severe demodectic mange, *Demodex canis* may be found in the lymph nodes. Very large nodular lesions can be found in the skin in goats and can be seen occasionally with demodectic mange in cattle and swine (Figure 8-7).

Mites of the respiratory tract (e.g., *Pneumonyssus, Sternostoma*) have more delicate exoskeletons than their ectoparasitic relatives. *Pneumonyssus simicola* and *Pneumonyssoides caninum* of the primate lung and canine nasal passages look very much superficially like any other mesostigmatid mite (Figure 8-8).

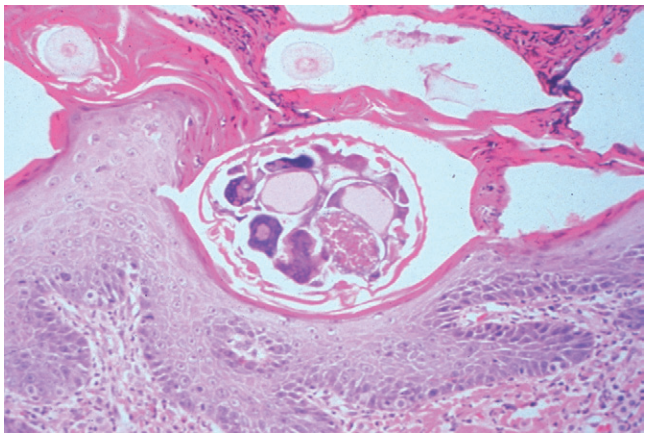

FIGURE 8-5. *Notoedres cati* in the skin of a cat (×150). These mites lie in the stratum corneum.

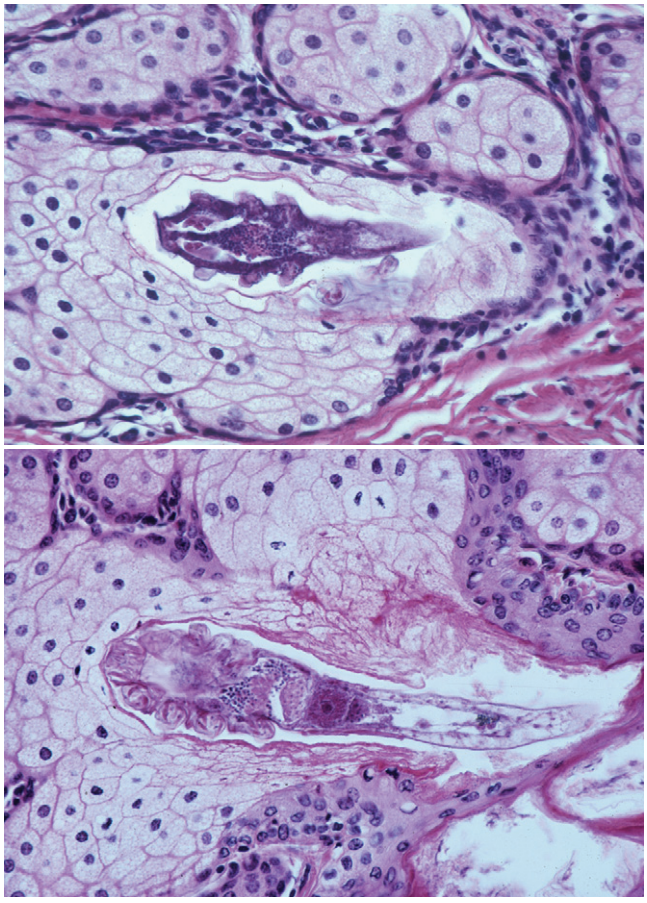

FIGURE 8-6. *Demodex canis* in the hair follicle of the vulva of a ewe. *Top*, Larva. *Bottom*, Adult (×430).

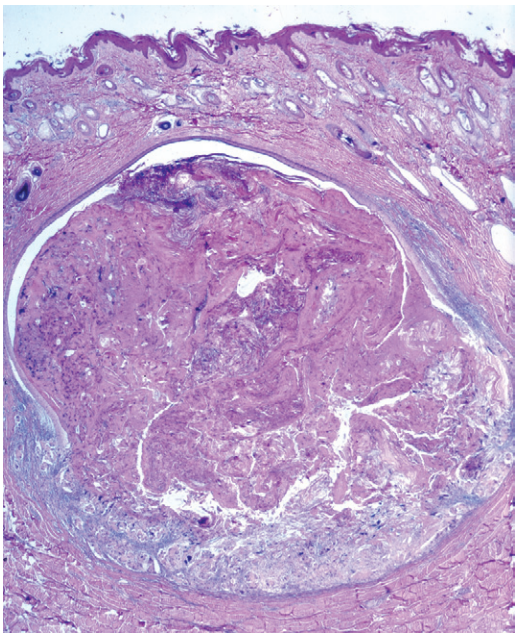

FIGURE 8-7. Demodectic mange in a bull (×16). Demodectic mange in cattle takes the form of nodular accumulations of myriad mites and cellular debris in proportions depending on the age of the lesion.

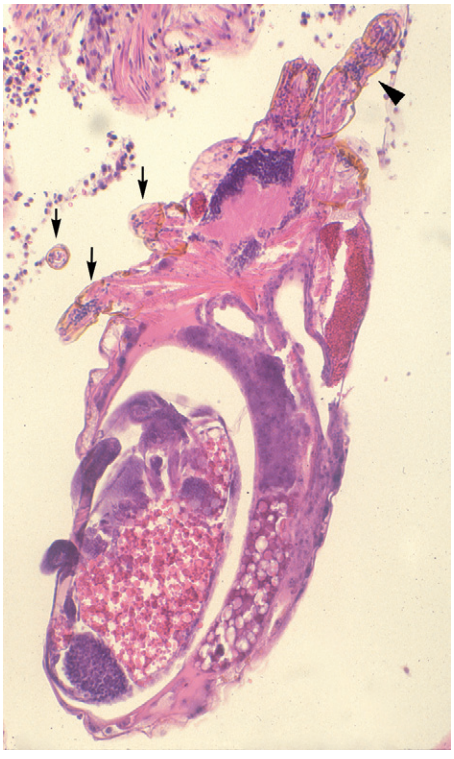

FIGURE 8-8. *Pneumonyssus simicola* in the lung of a rhesus monkey (×92). The mite contains a developing larva. The *arrows* indicate the legs, and the *arrowhead* indicates a palp. (Courtesy Dr. Castleman.)

Trombiculid larvae (chiggers) feed through a stylostome or feeding tube extending into the dermis (Figure 8-9); very typically, the mites become dislodged, and all that remains is the very pruritic lesion.

PENTASTOMIDS

Pentastomids are so called because of the early belief that they had five mouths; in reality, they possess one mouth surrounded by four hooks (see Figure 2-100). The adults of these bizarre crustaceans are wormlike parasites in the respiratory passages of predaceous reptiles, birds, and mammals that for the most part become infected when they ingest nymphs encysted in the tissue of their prey. It is in the vertebrate prey that the nymphs appear in tissue sections after the host has ingested an egg (Figure 8-10) containing a larva with four or six appendages. The pseudosegmented body of the nymph has a spheric to oval shape and is covered by a thick cuticle with sclerotized openings—stomata (Figures 8-11, 8-12, and 8-13).

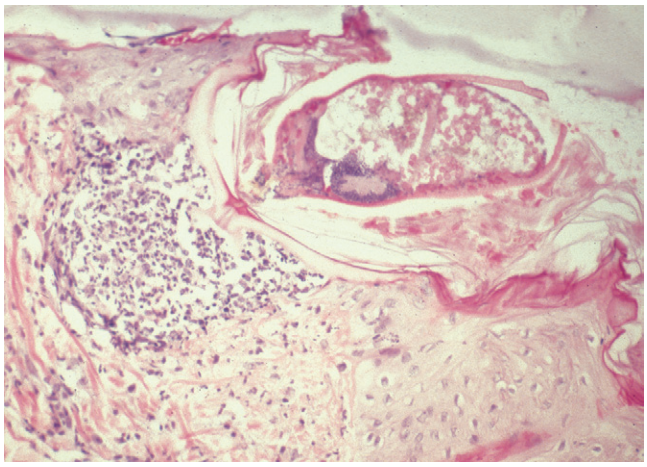

FIGURE 8-9. *Walchia americana* in the skin of a cat (×225). The stylostome or feeding tube extends to an area of dermis infiltrated with inflammatory cells.

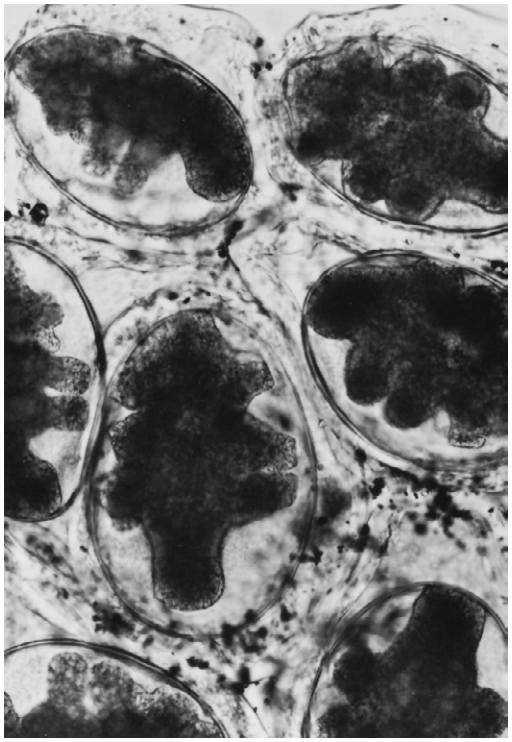

FIGURE 8-10. Pentastomid eggs with developing embryos (×160).

Pentastomids have a complete digestive system with a mouth and an anus, and in section the intestine is often surrounded by large acidophilic glands (see Figures 8-11 and 8-12). These acidophilic glands are a good distinguishing characteristic for this group of organisms; they stain bright pink with prominent blue nuclei in hematoxylin and eosin (H&E)-stained sections. The musculature is striated and is located within the subcuticular region.

PROTOZOA

Protozoa that are found in sections tend to be highly specialized individual cells with distinctive nuclei and other structures that may occur singly or in "nests" either within or external to the cells of the host. At the light microscope level, it is often difficult to ascertain many details of the individual cells, and often electron microscopy of material will provide the added detail required for a diagnosis. Also, immunohistochemical or in situ hybridization methods can often be used for some infections (e.g., for *Toxoplasma gondii, Neospora caninum,* and *Sarcocystis neurona*) to make a definitive diagnosis as to a genus or species of parasite in a particular case.

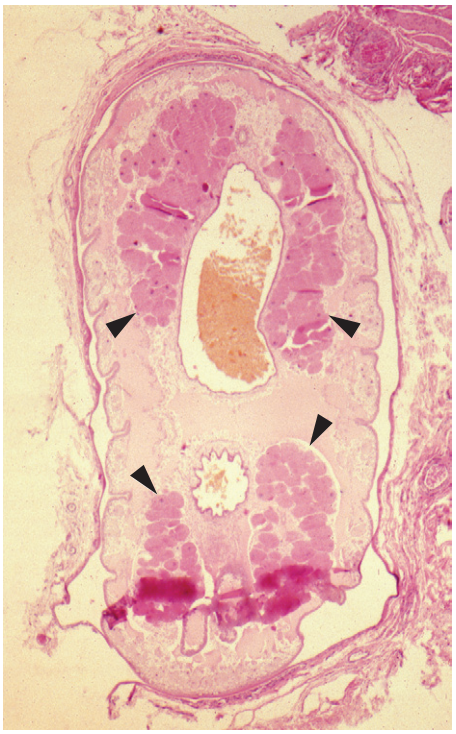

FIGURE 8-11. Pentastomid nymph from near the bladder of a cynomolgus monkey (×94). The cuticle is marked by deep annulations, and the nymph contains large acidophilic glands *(arrowheads)*.

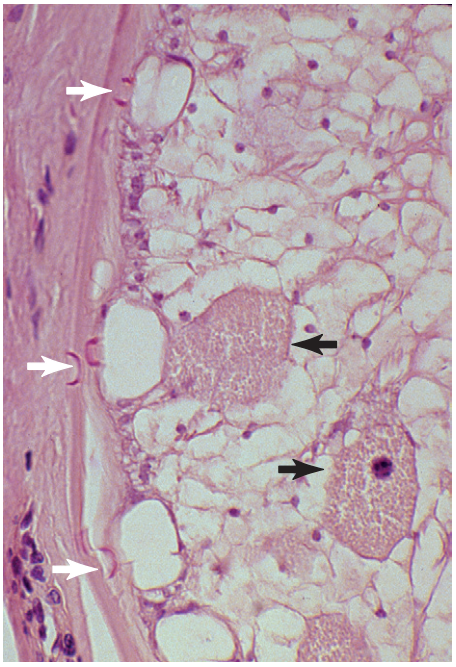

FIGURE 8-12. Pentastomid tissue showing pores *(white arrows)* and acidophilic glands *(black arrows)* (×290).

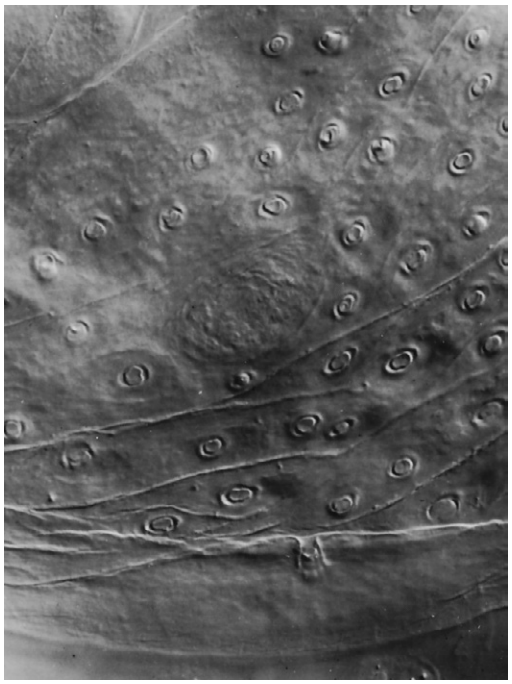

FIGURE 8-13. Surface view of the cuticle of a pentastomid showing the pores (×440).

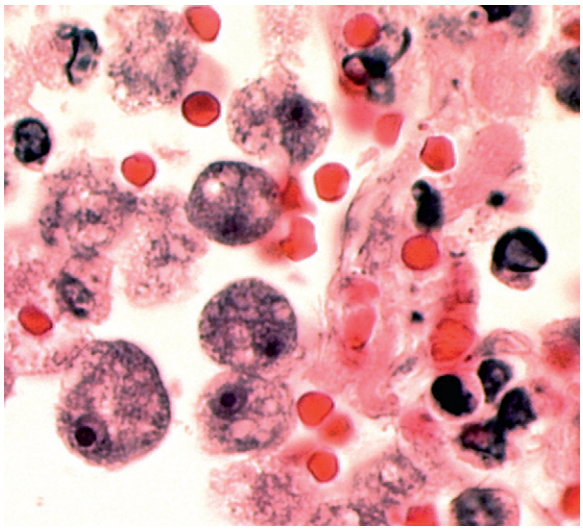

FIGURE 8-14. *Acanthamoeba* in the brain of a horse succumbing to the infection (×1200); note the large nucleolus in the nuclei of each of the amoeba parasites. (Courtesy Dr. Govinda Visvesvara.)

With a great many of the protozoa, it is often difficult to distinguish even distantly related organisms purely on the basis of the structures seen in sections because of preparation, because of the way they are fixed and stained, and because one is near the working resolution of the light microscope. Therefore a group of amastigotes of *Trypanosoma cruzi* may look very similar to a pseudocyst of rounded zoites of *T. gondii*. This should be fairly straightforward because the amastigotes should be seen to contain the identifying kinetoplast, but they may be visible in only a portion of the organisms, and the typically elongate zoites of *T. gondii* may appear simply as round small nucleated cells in some sections. Often it is very helpful to also take into account the history, clinical signs, and overall pathologic changes seen in a case when making a diagnosis.

AMOEBAE

Amoebae are extracellular parasites that feed through the process of engulfing bacteria, cell debris, or other cells as food material. A vast majority of these organisms are nonpathogenic parasites or commensals living typically in the large intestine of animals. However, two forms do cause disease. Primates are host to *Entamoeba histolytica,* which can colonize the bowel wall and move to ectopic sites where the organisms establish cysts that most often include the liver, but they can also be found in lungs or brain tissue; reptiles have a similar pathogen, *Entamoeba invadens,* which can cause serious disease in these hosts with extraintestinal lesions. These amoebae tend to have nuclei typical of the genus with a central dot of chromatin, the **karyosome, endosome,** or nucleolus, and chromatin is also clumped around the inner surface of the nuclear membrane. These parasites can be found to contain erythrocytes, sometimes several, in various states of digestion. The other main group of disease-causing amoebae are facultative parasites that include the genera *Naegleria, Acanthamoeba,* and *Balamuthia,* which have infected dogs, sheep, cattle, primates, and horses (Daft et al, 2005). These forms live in the environment but can invade the tissue if they gain access through the nose or through wounds with lesions typically occurring in the brain or skin, but they can be found elsewhere. In sections these amoebae tend to appear in clear spaces from artifactual contraction of surrounding tissues during fixation and specimen preparation, to have foamy cytoplasm, and to have characteristic nuclei that contain a very dense endosome surrounded by a clear halo internal to the nuclear wall (Figure 8-14).

FLAGELLATES

Typical flagellates that occur in the tissues of vertebrates include two that are known as amastigote stages living within host cells. Amastigotes are small, round to oval bodies measuring 1.5 to 4 µm in diameter (often smaller after tissue processing); they contain a nucleus and a rod-shaped **kinetoplast.** They do not store period acid–Schiff (PAS)-positive material. The two groups of organisms that have these stages are *Trypanosoma cruzi* and various species within the genus *Leishmania*.

Both trypomastigote and amastigote stages of *T. cruzi* occur in the vertebrate host, but generally only the amastigotes are seen in tissue sections; the trypomastigote stage is found almost exclusively in the blood. *T. cruzi* amastigotes are generally found in muscle cells of the esophagus, colon, and heart, where they may be responsible for megaesophagus, megacolon, and myocarditis (Figure 8-15), respectively.

The amastigotes of *Leishmania* parasitize only one cell type in the vertebrate host, the macrophage—typically histiocytes. Therefore they can be found in skin, bone marrow, and visceral organs such as the spleen and Kupffer cells of the liver. Again, the diagnostic organelle within the parasite is the kinetoplast, but diagnosing the infection in tissue sections can be somewhat difficult because of shrinkage of cells during fixation, which can make visualization of the nucleus and kinetoplast challenging. One of the major differentiations to be considered is whether one is dealing with leishmaniasis or an infection with *Histoplasma* organisms. Needle biopsies or impression touch preps from cutaneous lesions or lymph node and bone marrow aspirates may be prepared and stained with Wright-Giemsa solution, and in these preparations, the full structure of the organism, including both the nucleus and the kinetoplast, is generally more clearly visible (Figure 8-16).

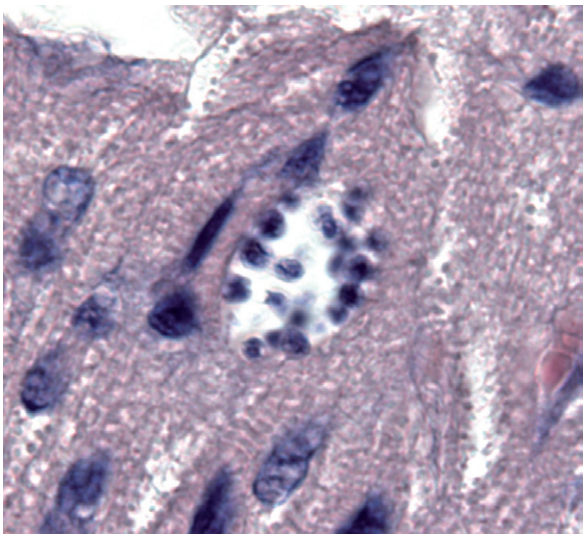

FIGURE 8-15. *Trypanosoma cruzi* amastigotes in cardiac muscle of a dog (×1300). Both nucleus and kinetoplast can be seen in individual organisms. (Courtesy Dr. Stephen C. Barr.)

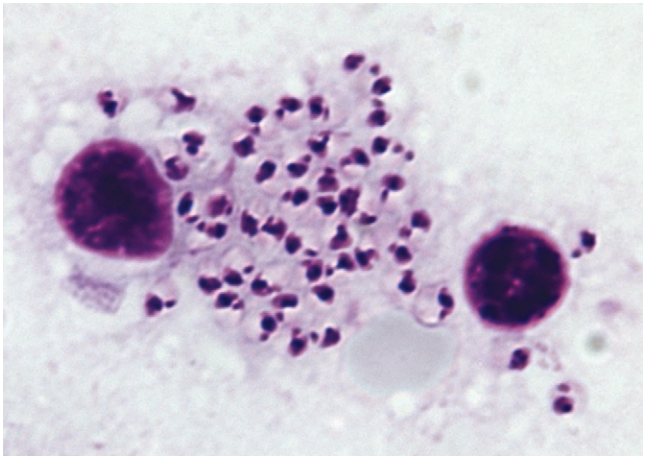

FIGURE 8-16. *Leishmania* amastigotes in a touch prep of an axillary lymph node of a dog (×690). The nucleus and kinetoplast are clearly evident in several of the organisms.

CILIATES

Balantidium coli trophozoites live within the contents of the cecum and colon of pigs but may secondarily invade the wall of the large intestine of swine that have various forms of enteritis. Trophozoites are characterized by their large size and the presence of a **macronucleus** and a **micronucleus** and cilia (Figures 8-17 and 8-18). Rumen ciliates may be found in the lung as a result of terminal inhalation of ruminal contents, in which case no evidence of an inflammatory reaction is found. Rumen ciliates may also be found in hepatic vessels in cases of very severe enteritis (Figure 8-19). In horses with severe enteritis, the extravagantly shaped ciliates normally present in the large intestine may secondarily penetrate the submucosa. These ciliates have large, often polymorphic macronuclei, and some have tufts of long cilia.

APICOMPLEXA

Coccidia

The coccidia are members of the phylum Apicomplexa. Included in this discussion are members of the genera *Eimeria, Klossiella,*

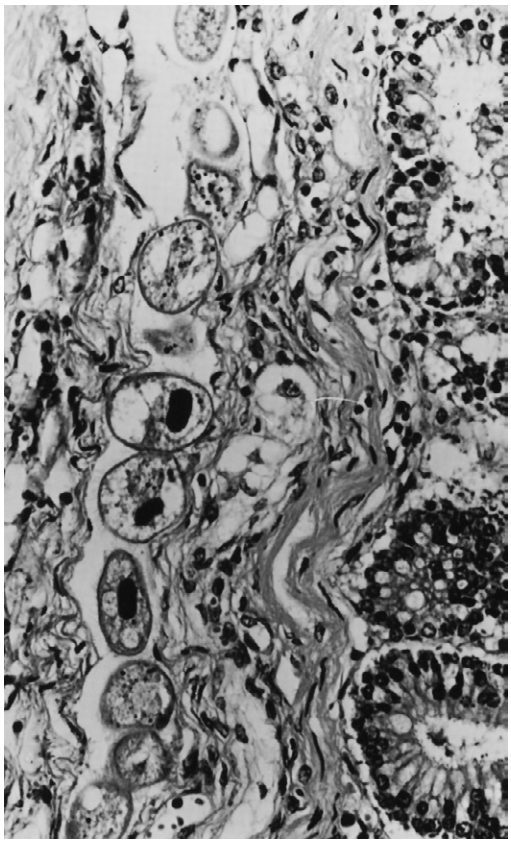

FIGURE 8-17. *Balantidium coli* in the submucosa of the large intestine of a pig (×280).

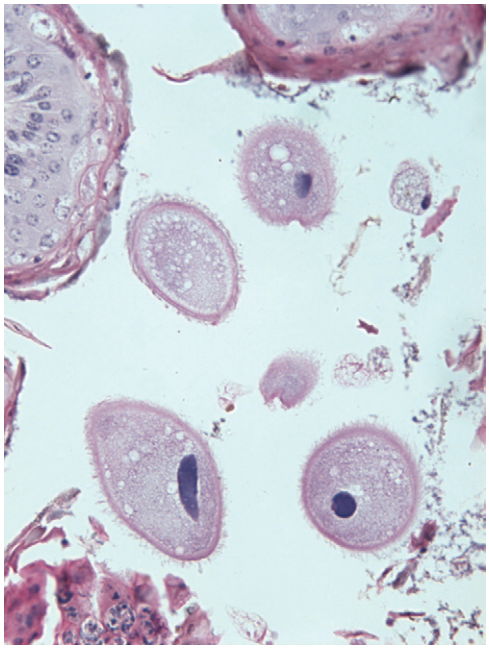

FIGURE 8-18. Rumen ciliates (×360).

Cystoisospora, Hammondia, Besnoitia, Sarcocystis, Neospora, and *Toxoplasma*. The life history and development of the major genera of coccidians are described in Chapter 3. There seems to be a good deal of consensus around placement of the genus *Cryptosporidium* within the gregarines rather than with the coccidia, but for convenience, these species are still included in this section. The genera

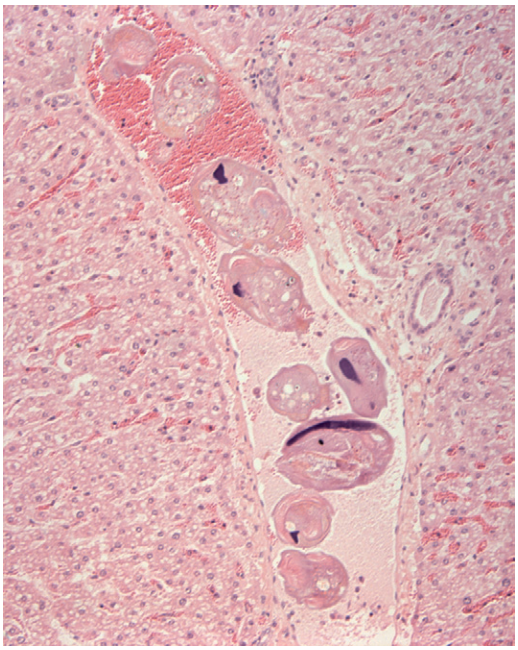

FIGURE 8-19. Ciliate in vein in the liver of a goat with severe suppurative lymphangitis (×250).

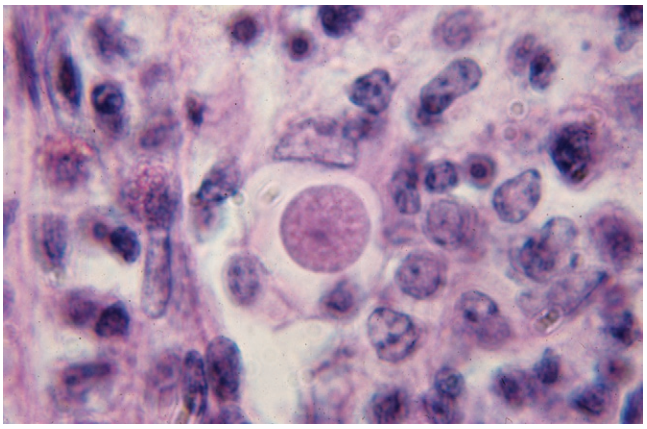

FIGURE 8-20. *Eimeria bovis* trophozoite in an intestinal epithelial cell of a cow (×1300).

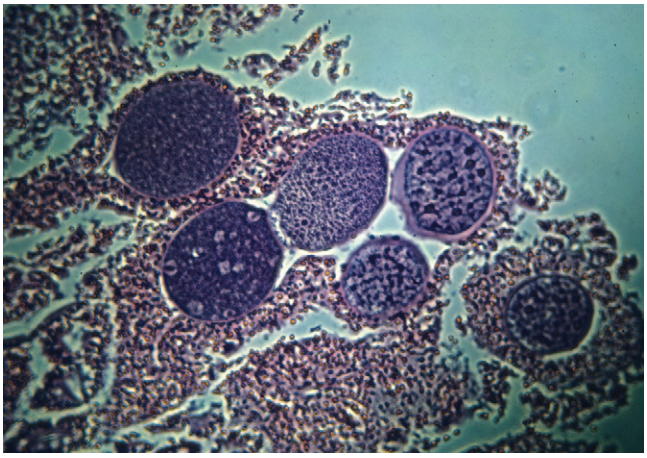

FIGURE 8-21. *Eimeria bovis* schizonts in several stages of development in intestinal epithelial cells of a calf (×250).

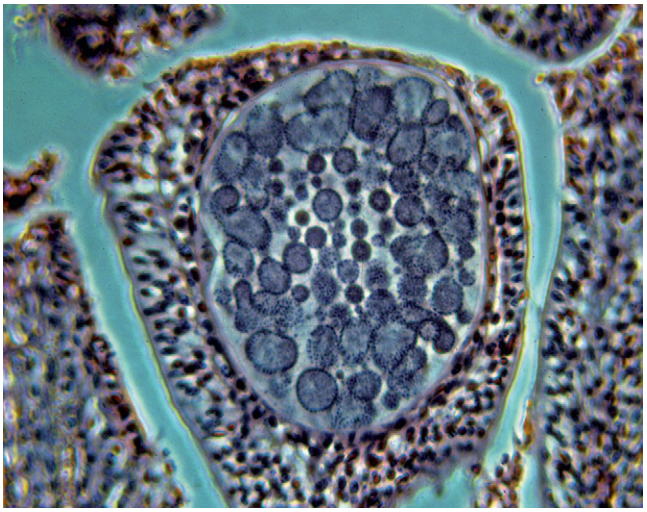

FIGURE 8-22. Another young *Eimeria bovis* schizont in an intestinal epithelial cell of a calf (×400).

Eimeria and *Cryptosporidium* seem to be completely monoxenous (i.e., with transmission always between members of one type of host with no paratenic or intermediate hosts), and almost all of the stages seen in section occur within the epithelium of the gastrointestinal tract or rarely the gallbladder. *Klossiella* is also apparently monoxenous, with direct transmission between hosts and almost all stages found in the epithelium of the renal system. The other coccidia are facultatively (*Cystoisospora* and *Toxoplasma*) or obligatorily (*Sarcocystis, Hammondia, Neospora,* and *Besnoitia*) heteroxenous, that is, they have a paratenic or intermediate host. For heteroxenous species of coccidia, the stages often seen in tissue are the stages causing disease in the prey animal that is serving as the paratenic or intermediate host. A description of the histologic appearance of the various stages follows, but host specificity, site specificity, life cycle, and details of development characteristic of the genera and species of coccidia must be taken into consideration in arriving at a diagnosis.

Eimeria and *Cystoisospora*

ASEXUAL STAGES. The infective stage contained in the oocyst is the **sporozoite,** which is a product of a reduction division that occurs in the oocyst (Apicomplexa are haploid except immediately after fusion of the gametes). When a sporozoite enters a cell, it rounds up as a **trophozoite** in a membrane-lined **parasitophorous vacuole** (Figure 8-20). Not every species of coccidian stays within a parasitophorous vacuole, and this fact can be a useful adjunct in generic and specific diagnoses.

Trophozoites multiply asexually within cells by several processes. In the case of *Eimeria* they typically undergo a special type of cellular division called **schizogony** (other terms that describe this form of division with various nuances are **merogony** and **endopolygeny**). In this type of division the apical complex divides into numerous copies around the periphery of the cell, the nucleus lobulates with portions associated with each apical complex, and finally the cell membrane contracts and divides to form a few to thousands of individual organisms (Figures 8-21 and 8-22). Depending on the species, schizonts may be found in enterocytes, biliary epithelial cells, endothelial cells, renal epithelial cells, or even uterine epithelial cells. Ordinary meronts contain from less than ten to hundreds of merozoites; some meronts (megaschizonts) (see Figure 8-21) may contain more than 100,000 merozoites.

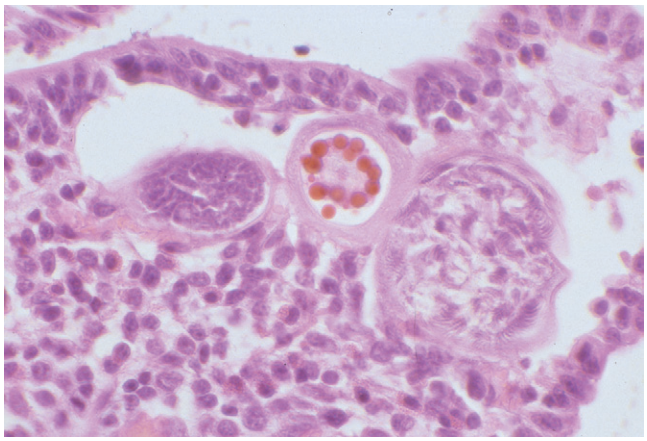

FIGURE 8-23. *Eimeria auburnensis* male gamonts surrounding a developing oocyst in the intestinal epithelial cells of a calf (×1050).

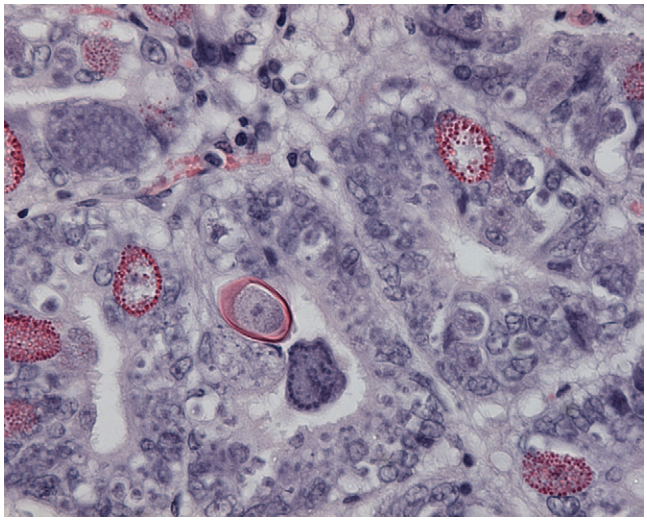

FIGURE 8-24. *Eimeria* oocysts developing in the intestinal epithelium of a goat (×900).

SEXUAL STAGES. A merozoite produced by the final schizogonic generation enters a fresh host cell and develops into a male or a female gametocyte. The female gametocyte enlarges, stores food materials, and induces a hypertrophy of both the cytoplasm and the nucleus of its host cell. When mature the female gametocyte is called a **macrogamete** (Figure 8-23). The male gametocyte also induces hypertrophy of the cytoplasm and the nucleus of its host cell (see Figure 8-23) as it undergoes repeated nuclear division and becomes multinucleate. Each nucleus is finally incorporated into a flagellated **microgamete.** (The microgametes of *Cryptosporidium* species are without flagella.) When a macrogamete is penetrated and fertilized by a microgamete, it becomes a **zygote.** Wall-forming bodies, already present in the macrogamete, then become clearly visible as large, spheric, eosinophilic granules in the cytoplasm of the zygote (see Figure 8-23). These later coalesce to form the oocyst wall (Figure 8-24).

EXAMPLES. In the horse, *Eimeria leuckarti* forms large schizonts and very thick-walled and obvious oocysts (Figure 8-25). The oocyst of *Cystoisospora canis* seems to develop within the lamina propria rather than in the epithelial cells (Figure 8-26). *Eimeria gilruthi* is atypical in that it forms megaloschizonts in the abomasum that are visible to the naked eye (Figure 8-27). *Eimeria stiedae* of the rabbit lives in and causes proliferation of the biliary epithelium and can produce a lethal hepatitis (Figure 8-28).

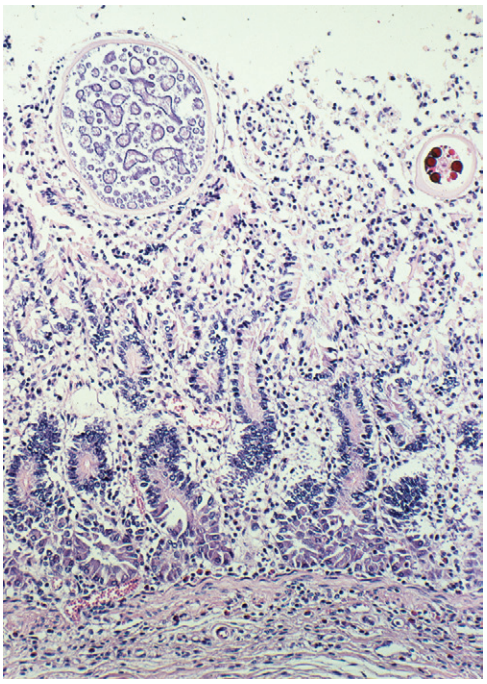

FIGURE 8-25. *Eimeria leuckarti* schizont and developing oocyst in the intestinal mucosa of a foal from Switzerland (×250). (Courtesy Dr. Maja Suter.)

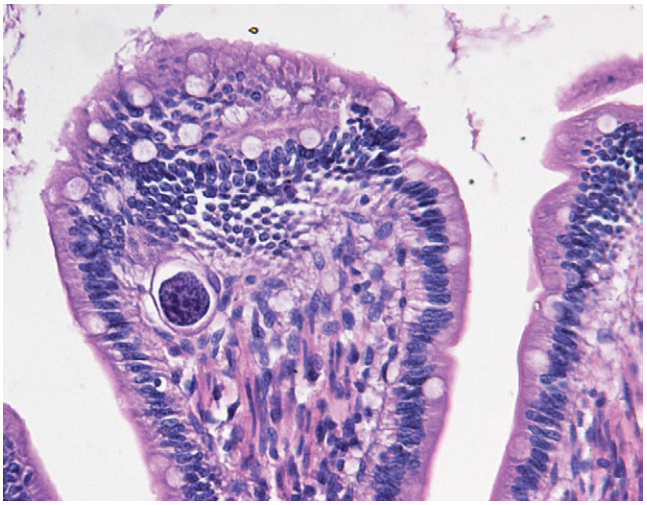

FIGURE 8-26. Oocyst of *Cystoisospora canis* developing in the lamina propria of the colonic mucosa of a dog (×900).

Cryptosporidium

The minute (5 to 7 µm) stages appear as basophilic spheres on the luminal surface of epithelial cells of the gastrointestinal tract of vertebrates (Figure 8-29); on rare occasions, typically in the immunocompromised host, infection of respiratory or gallbladder epithelia may also occur. The infection is very superficial and appears to protrude from the surface of the cell, but these are intracellular parasites, and all stages—schizonts, gametes, oocysts, and so on—form underneath the host cell membrane.

Klossiella

A parasite of the equine kidney, *Klossiella equi* is usually an accidental histopathologic finding. Schizogony occurs in the

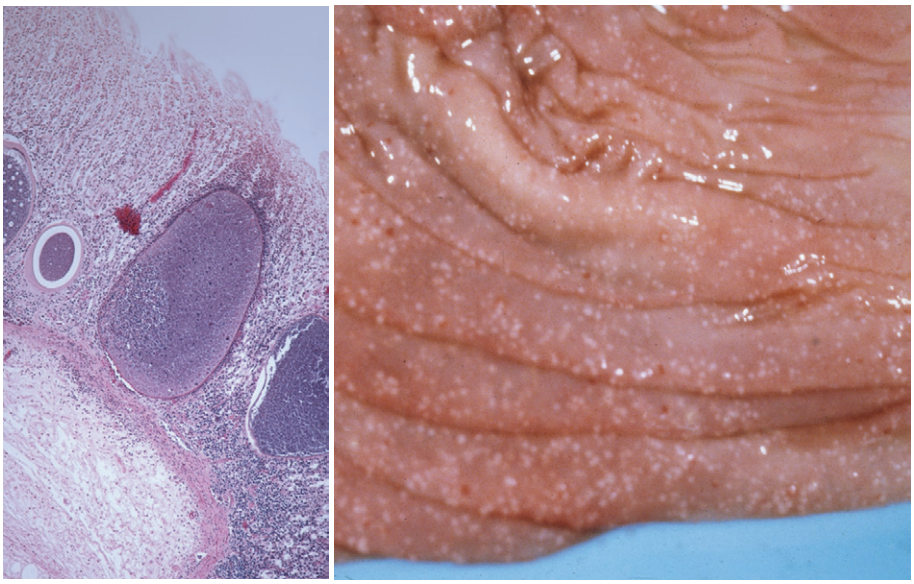

FIGURE 8-27. *Eimeria gilruthi* megaloschizonts in the abomasum of a sheep (histosection on left, ×100; gross on right, ×5).

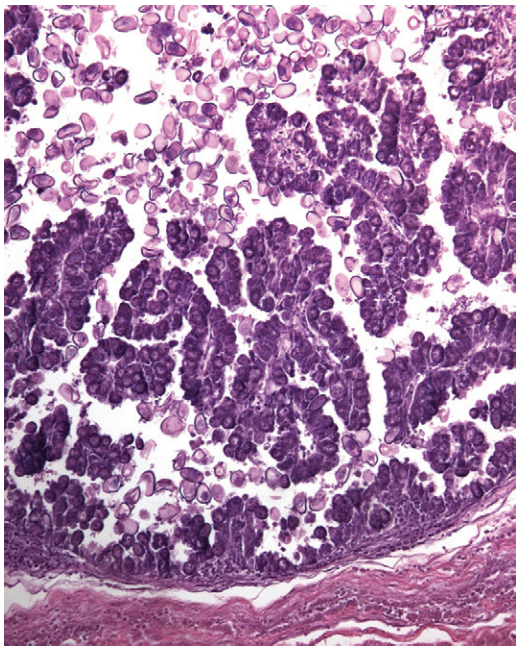

FIGURE 8-28. *Eimeria stiedae* developing in the bile duct epithelium of a rabbit (×100).

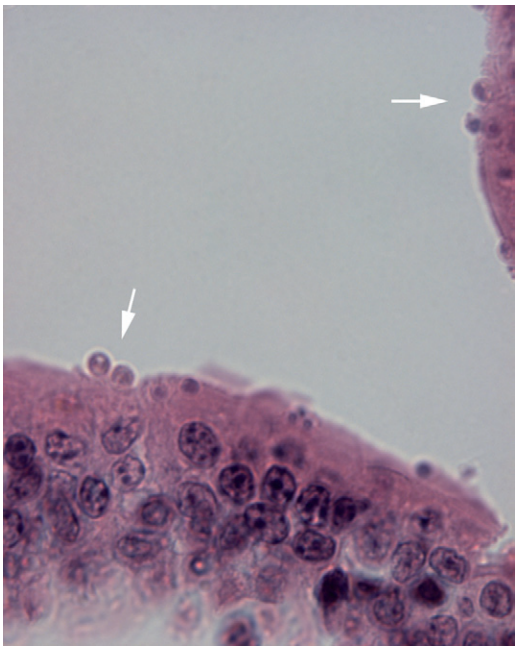

FIGURE 8-29. *Cryptosporidium parvum*. Developing stages *(arrows)* of *Cryptosporidium parvum* on the mucosa of a naturally infected calf (×500).

glomerular endothelium and in the proximal convoluted tubules of the kidneys. The distinctive sporonts (Figure 8-30) in the renal tubular epithelium produce as many as 40 sporoblasts, which develop into sporocysts, each of which may contain eight to 15 sporozoites. A similar species, *Klossiella muris,* will show up in histologic sections of murine kidneys.

Sarcocystis

The early schizonts of *Sarcocystis* occur in various endothelial cells of different organs (Figure 8-31). **Sarcocysts,** the stages found in the intermediate host, are found in skeletal and cardiac muscle fibers (Figures 8-32 and 8-33); they vary in size from a few micrometers in diameter to macroscopically visible objects, stain intensely with hematoxylin, and are packed full of bradyzoites that are larger than those of *Toxoplasma*. Septa subdivide the interior of the sarcocyst but may escape notice because they stain poorly or not at all with H&E. Often the cyst wall is described as hirsute (hairy) because of the many prolongations that give the cyst its apparent striated border. The hirsute wall and the septa dividing the zoites within the sarcocysts are often diagnostic.

Hammondia

Hammondia appears very similar morphologically to *Toxoplasma gondii;* the distinctions are biologic and molecular more than structural. The life cycle is obligatorily heteroxenous, but stages very similar to those described for *Toxoplasma* in the next paragraph are found in the tissues of many warm-blooded vertebrate animals that serve as prey to dogs and cats. These parasites have not been found

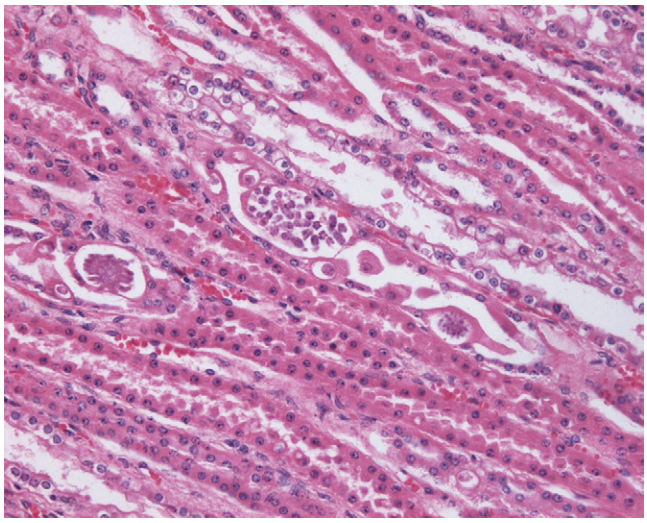

FIGURE 8-30. Sporonts of *Klossiella equi* in the renal tubular epithelium of a horse (×250).

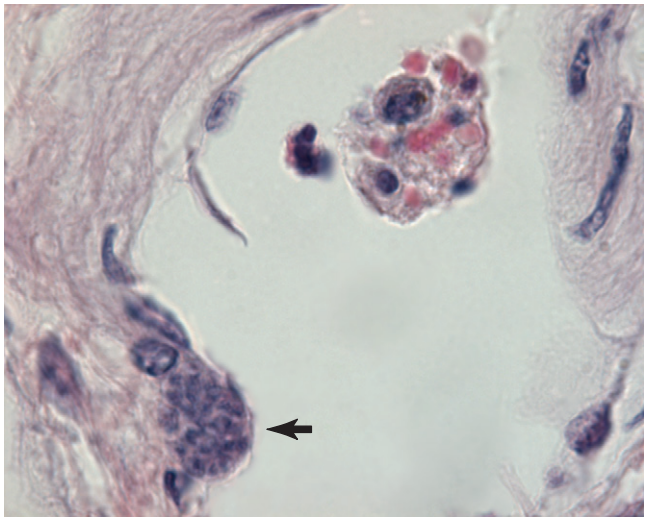

FIGURE 8-31. *Sarcocystis cruzi* schizont *(arrow)* in endothelium of a small artery of a calf with a fatal, naturally acquired infection (×800). (Courtesy Dr. Paul Frelier.)

to cause disseminated disease in immunosuppressed or immunocompromised hosts.

Toxoplasma

The stages that occur within the epithelial cells of the cat are to a great extent comparable with what occurs with *Eimeria* and *Cystoisospora* (Figure 8-34). It is within the genus that the names **tachyzoite** and **bradyzoite** were first used to describe the different life stages that occur in the paratenic hosts. Within these hosts the only form of division that occurs is **endodyogeny,** which is similar to schizogony, but only two daughter cells are formed in each dividing organism. The only schizonts seen with *T. gondii* occur in the intestinal epithelial cells of felids (see Figure 8-34). Tachyzoites divide rapidly and for the most part cannot withstand pepsin digestion for any length of time. Bradyzoites divide slowly, are resistant to pepsin digestion, and form cysts in tissue that are most easily observed in histologic sections of brain stained with PAS, because the slowly dividing forms store PAS-positive material. Cats can have cysts of bradyzoites throughout their bodies just as other hosts

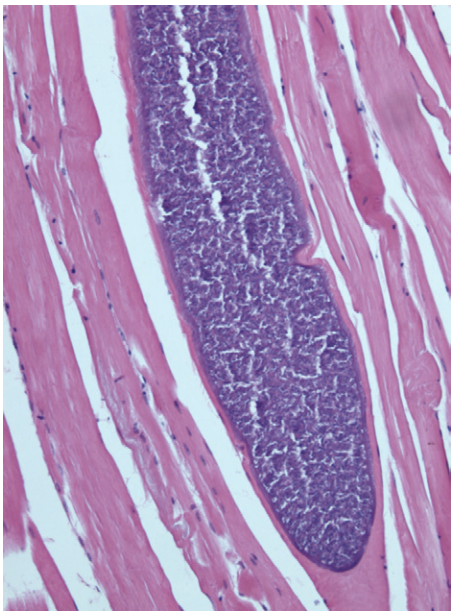

FIGURE 8-32. Sarcocyst of *Sarcocystis muris* in skeletal muscle of a mouse (×200). (Courtesy Dr. Marguerite Frongillo.)

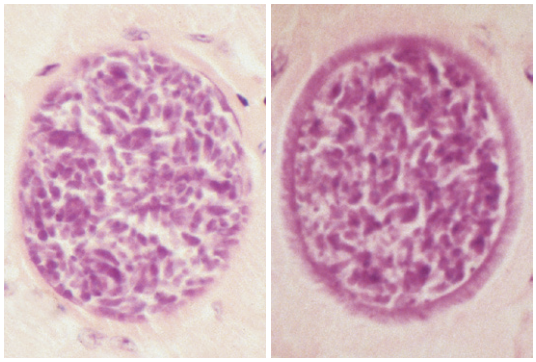

FIGURE 8-33. Sarcocysts of *Sarcocystis cruzi (left)* and *Sarcocystis bovifelis (right)* in skeletal muscle of a cow (×300). The cyst wall of *S. bovifelis* is thicker and appears striated.

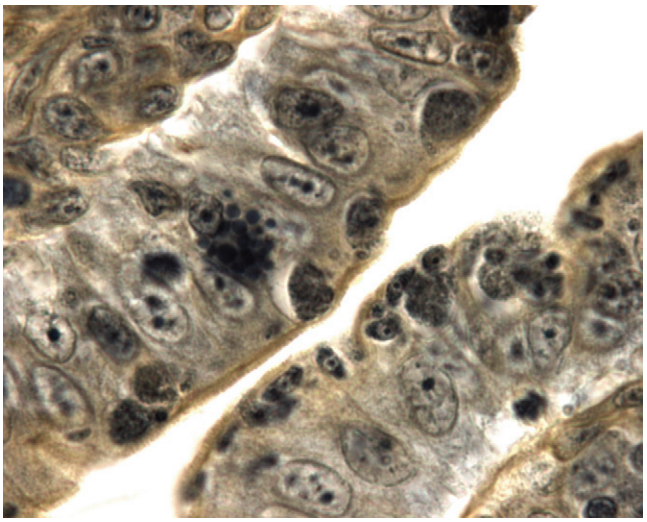

FIGURE 8-34. *Toxoplasma gondii* development stages in the intestinal epithelia of an experimentally infected cat (×800).

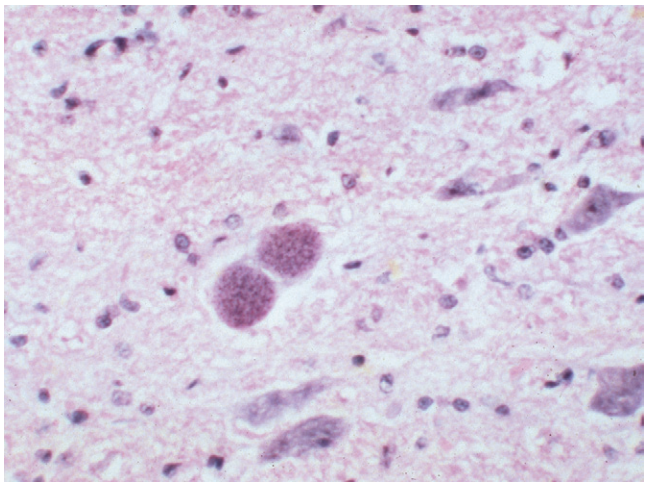

FIGURE 8-35. *Toxoplasma gondii* bradyzoites in a cyst in the brain of a cat (×800).

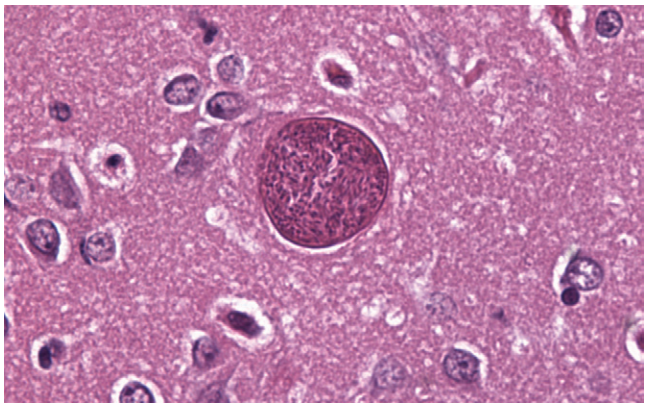

FIGURE 8-36. *Neospora caninum* bradyzoites in a cyst in the brain of a dog (×1200).

(Figure 8-35). Tachyzoites accumulate as "groups" intracellularly; bradyzoites become tightly packed in "intracellular cysts." The latter, when found in striated muscle fibers, might be confused with sarcocysts or accumulations of *T. cruzi* amastigotes.

Neospora

Under the light microscope, the cysts of *N. caninum* are almost indistinguishable from those of *T. gondii*. The major distinction that was recognized in the early description of this species was the thicker "cyst wall" that occurred around bradyzoites (Figure 8-36).

Besnoitia

Besnoitia is mainly considered to be exotic to domestic animals in the United States, although wildlife such as opossums can be infected. These organisms described as *Besnoitia bennetti* have been described from donkeys in the United States (Dubey et al, 2005). The typical presentation is very large cysts without septa that are often found in the skin, although viscera may also be affected (Figure 8-37).

Hemosporidians

A number of the Apicomplexa genera have heteroxenous life cycles with the sexual stages occurring in invertebrates and the asexual stages occurring in vertebrates (e.g., *Plasmodium, Theileria,*

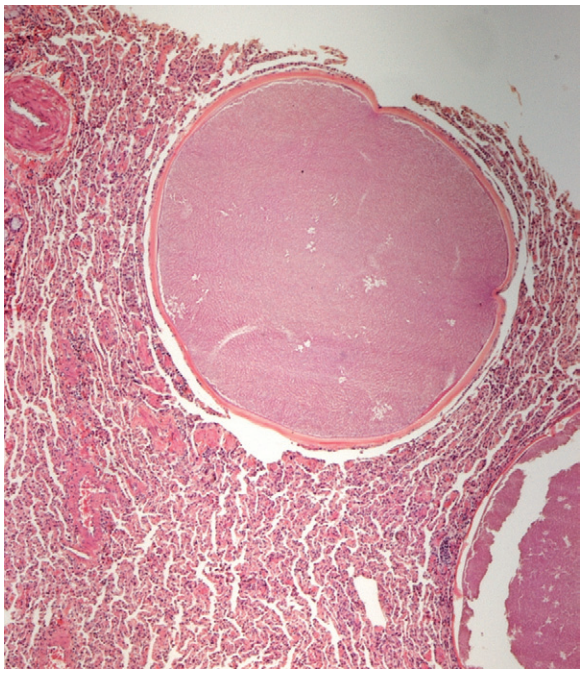

FIGURE 8-37. *Besnoitia* cyst in the lung of an opossum (×40).

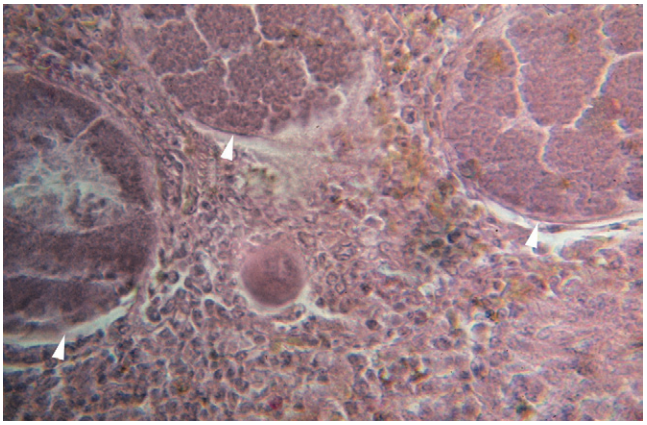

FIGURE 8-38. *Leucocytozoon simondi* megaloschizonts in the liver of a Canada goose (×100).

Hepatocystis, Leucocytozoon). With most of these parasites there is a good deal of description relative to the stages found in the blood of the host, whereas very little time is spent describing the various stages, typically schizogonous stages, that may occur in the viscera of hosts that can be seen in sections. *Babesia* infects only red blood cells, whereas *Theileria* infects erythrocytes and lymphocytes; because these are two of the most important hemosporidians of domestic animals, there is little need to focus much attention on the schizont stages that occur in tissues. However, some other species do cause pathology and have stages in the tissues—schizonts that can be quite large and damaging.

Leucocytozoon

These species in chickens—*Leucocytozoon caulleryi* and *Leucocytozoon simondi*—produce megaloschizonts in chickens and geese (Figure 8-38), respectively, that can be highly pathogenic. These schizonts can be very large and detrimental to the host.

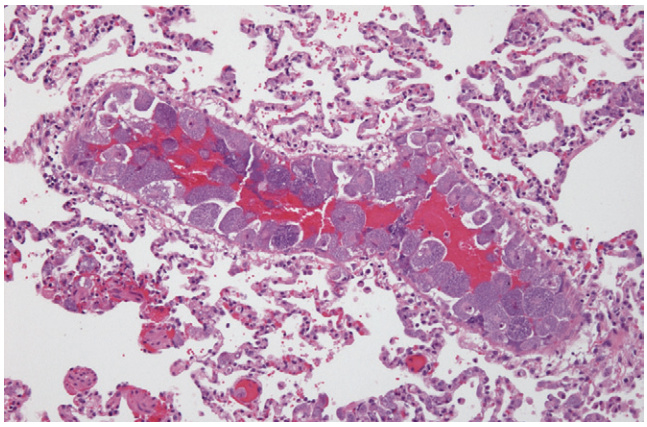

FIGURE 8-39. *Cytauxzoon felis*. A pulmonary vein of a cat filled with multiple, enlarged, mononuclear cells containing schizonts (×100).

Hepatozoon

Hepatozoonosis in the United States is associated with *Hepatozoon americanum,* which has cystic stages in the muscle of the dog host that are associated with chronic muscle pain in these animals. These stages are often used to assist diagnosis, which is still often made as a result of muscle biopsy.

Cytauxzoon

Cytauxzoon is a parasite that kills cats, often very acutely. Large schizonts can occur within the macrophages and cause them to become enormous. This is the reason for the name of the genus and it is what makes the infection so deadly for cats. Sections throughout the body will have vessels plugged with these giant cells (Figure 8-39).

HELMINTHS

In examining helminths in section, basically two types can be seen: solid bodied (the acoelomates) and bodies in which tubes are suspended within a tube (the pseudocoelomates). Trematodes and cestodes are of the solid body type; nematodes and acanthocephalans represent the "hollow" body types. The problem can be that a trematode or a cestode may have all sorts of cavities in various organs that give them the appearance of having a pseudocoele, and nematodes may be so packed with organs and eggs or larvae that one starts to doubt whether one is looking at a nematode or not. The trematodes and cestodes are covered with a syncytial tegument, whereas the nematodes and acanthocephalans are covered with a secreted cuticle.

TREMATODES

Most trematodes are parasites of the digestive tract, but they only rarely show up in tissue sections. Trematodes in tissues are typically those in which the adults live in other tissues. Trematodes can be found throughout the bodies of vertebrates, in bile ducts, pancreatic ducts, body cavities, lungs, ureters, blood vessels, and so on. In a few cases larval stages can be found in domestic animals, where they may or may not be causing disease.

The characteristics of trematodes in sections form a composite group of useful features, but because often the goal is to differentiate trematodes from cestodes, part of the characterization includes how they differ from cestodes. Of course, for almost every characteristic, there is one group that composes an exception. Trematodes have a solid but spongy body that usually contains no large cavities

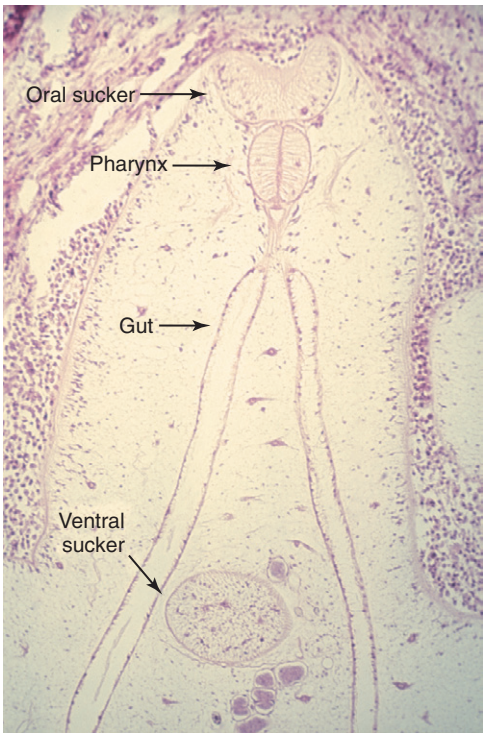

FIGURE 8-40. *Amphimerus pseudofelineus* in the small intestine of an ocelot (×40). (Courtesy Dr. M. Dale Little.)

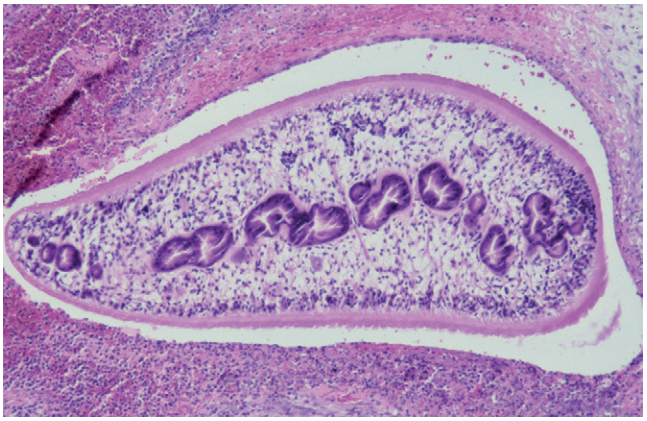

FIGURE 8-41. Migrating larva (marita) of *Fasciola hepatica* in the liver of a cow (×40).

and is not divided into cortical and medullary layers as the body of cestodes is. Trematodes have an intestine that is usually bifurcate ending in a blind cecum. (An example of an exception is the Cyclocoelidae, in which fusion of the posterior gut forms a continuous loop [Figure 8-40]). Unlike tapeworms, trematodes do not contain calcareous corpuscles. The body is covered with the syncytial integument that often has spines. Muscles are present below the integument, usually in an outer circular layer, a middle longitudinal layer, and an inner diagonal layer (which may also be external to the longitudinal layer or absent) (Figure 8-41). Sex organs in the adult flukes are monoecious hermaphrodites, except for Schistosomatidae, which have separate males and females. The eggs have typical shapes, and the shells are often brown to golden in sections. There are typically two suckers—one around the mouth and one ventral (often anterior to midbody) (Figures 8-42 and 8-43; see also Figure 8-40)—and an excretory system that is difficult to see and empties through a pore at the posterior end of the body.

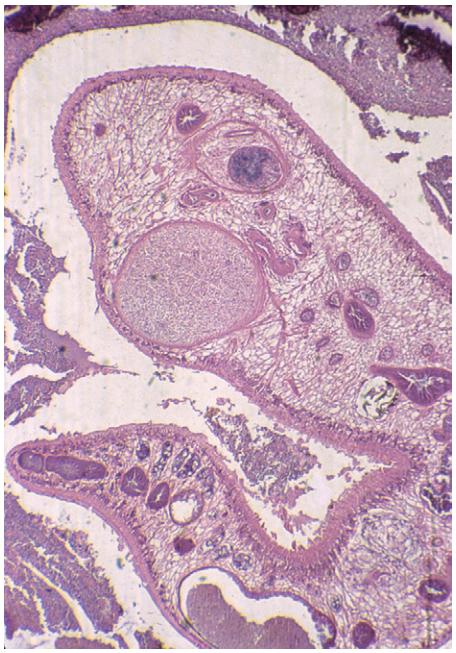

FIGURE 8-42. *Fasciola hepatica* in the bile duct of a cow (×20).

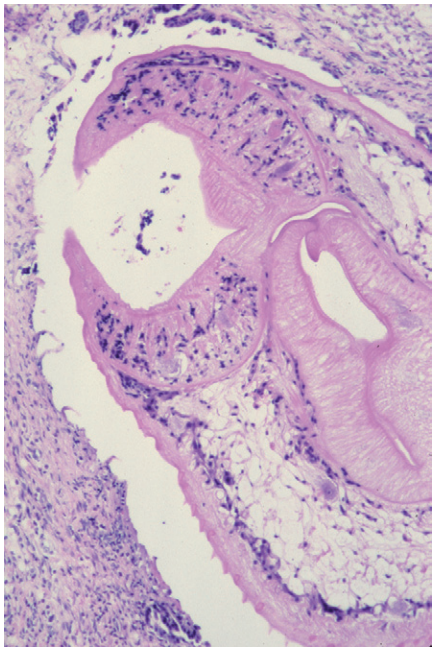

FIGURE 8-43. *Fasciola hepatica* in the bile duct of a rat (×20). (Courtesy Dr. Helen Han Hsu.)

Once a trematode is identified as such, the next step is to try to determine the family or genus. This involves calculating or guessing at the overall size and looking at the arrangement of the sex organs, the types of sucker if they are sectioned, and the extent and branching of the intestine and excretory system (Figure 8-44; see also Figure 8-40). If there are eggs, they can be very helpful once the size, shape, type of operculum, and state of development (with or without a miracidium) (Figure 8-45) have been noted. The spines on the surface of the body can also be very helpful in diagnosis; they will have to be examined to determine their number, size, and location on the body of the fluke (Figure 8-46).

Although both trematodes and cestodes have suckers, the oral sucker of trematodes is connected to a gut (see Figure 8-40),

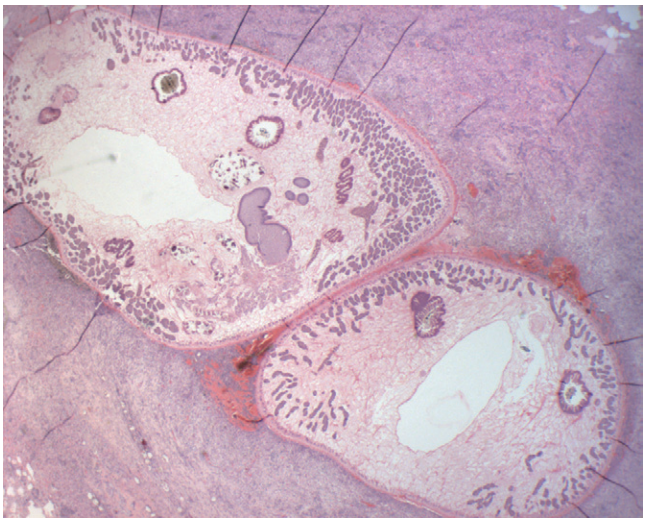

FIGURE 8-44. *Paragonimus kellicotti* in the lung of a cat (×5).

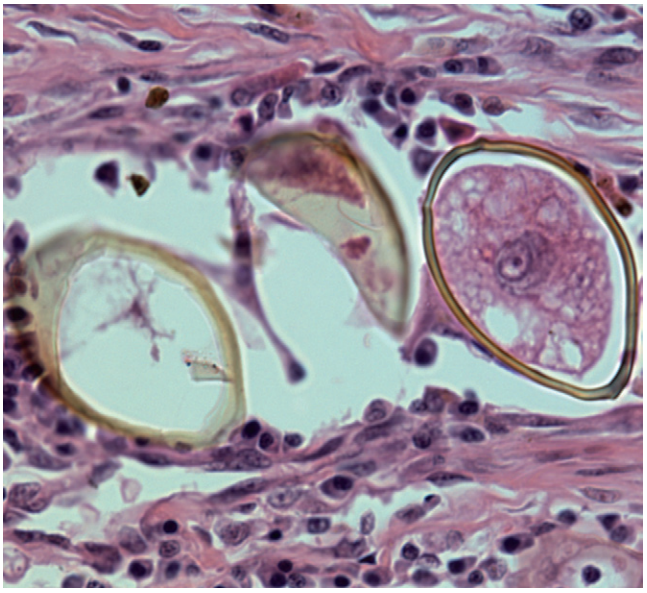

FIGURE 8-45. *Paragonimus kellicotti* eggs in the lung of a cat (×800); note the seated operculum of the shell and the central zygote in the egg on the right.

whereas a gut is lacking in cestodes. The ventral sucker of trematodes is not connected to a gut. Sections through the uterus may contain eggs, which by their size, shape, and state of embryonic development may provide clues to the identity of the specimen (Figure 8-47). The arrangement of the sex organs and the distribution of vitelline glands in the trematode body are a much-used taxonomic characteristic (see Figures 8-44 and 8-46). For example, these glands lie both dorsal and ventral to the gut in *Fasciola,* but all lie ventral to the gut in *Fascioloides*. The body form of some trematodes is quite distinctive. For example, heterophyids have small bodies with distinct spines and tend to be inserted in intestinal crypts (Figure 8-48), whereas diplostomatids are divided into a flattened forebody and a cylindric hindbody (Figure 8-49). In the dioecious schistosomatids, the slender female is enclosed in the gynecophoral groove of her stouter male partner (Figure 8-50). Adult trematodes lay eggs that can persist in the tissues for a long time, causing granulomatous inflammatory reactions in tissues (Figures 8-51 and 8-52).

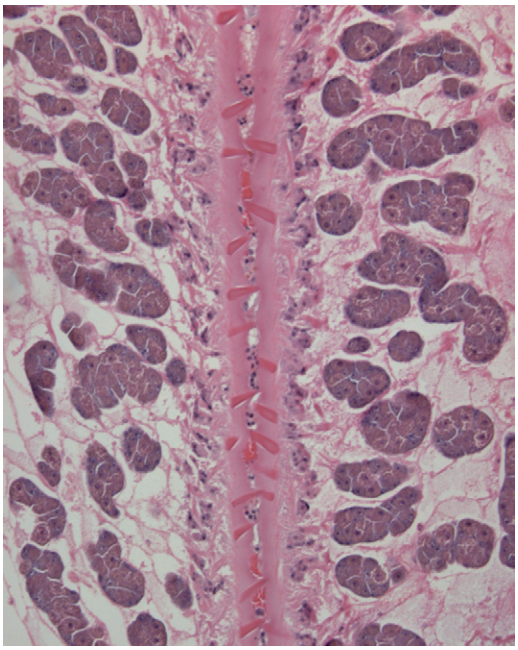

FIGURE 8-46. *Paragonimus kellicotti,* a pair of worms showing the spines on the cuticle and the vitelline glands (×800).

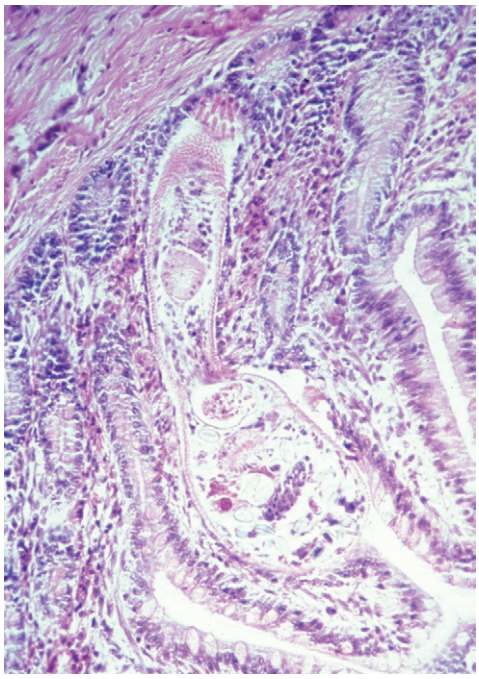

FIGURE 8-48. Heterophyid fluke in the intestine of a raccoon (×40); the spines on the anterior end are quite obvious, as is the relationship of the fluke to the intestinal mucosa.

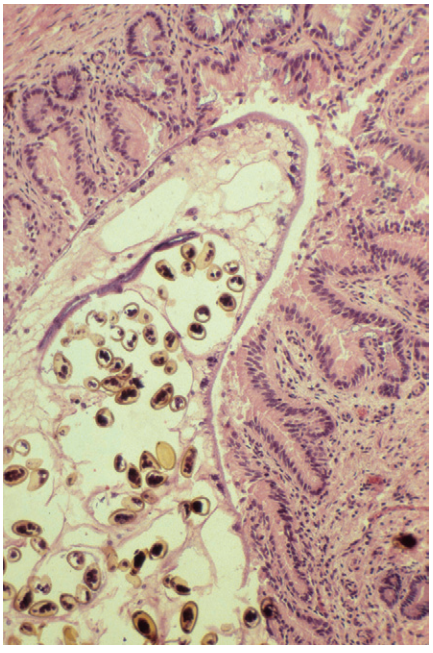

FIGURE 8-47. *Dicrocoelium dendriticum* in the bile duct of a sheep (×40). The typical eggs of this parasite can be seen within the uterus of the fluke.

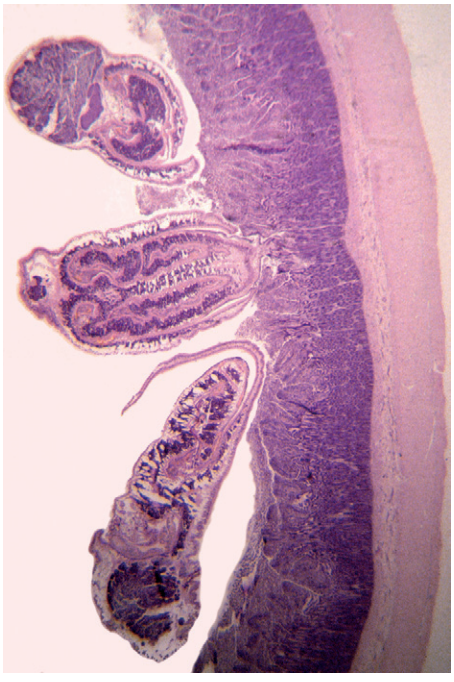

FIGURE 8-49. *Alaria* organisms in the small intestine of a dog (×10). *Alaria,* typical of the family Diplostomatidae, is divided into forebody and hindbody.

Larval trematodes, specifically mesocercariae and metacercariae, are not uncommonly seen in tissue sections. They are often rather small; sometimes they are encountered singly, and other times numerous organisms are present. They are, like adult trematodes, composed of a solid parenchymal body with an outer tegument, but often little other internal structure is seen (Figure 8-53). Because they represent immature stages, no reproductive structures are evident. No calcareous corpuscles are present, and this helps to distinguish them from larval tapeworms.

CESTODES

Tapeworms seen in sections are most likely to be larval forms, although there is always a chance that a section of a proglottid may be seen in an unusual location. Tapeworms, unlike trematodes, have no intestine in any stage of the larvae or adults. Like trematodes, the internal organs of cestodes are embedded in a parenchymatous matrix; there is no body cavity. There are two principal zones of

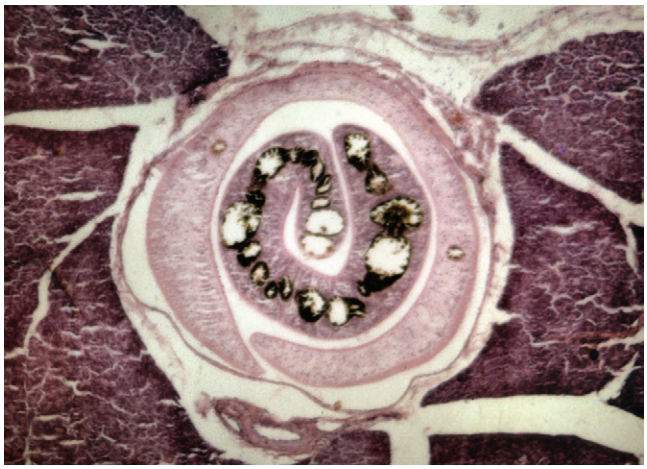

FIGURE 8-50. *Heterobilharzia americana* in a pancreatic vein of a beagle (×80). The smaller female is seen being held in the gynecophorous canal of the male.

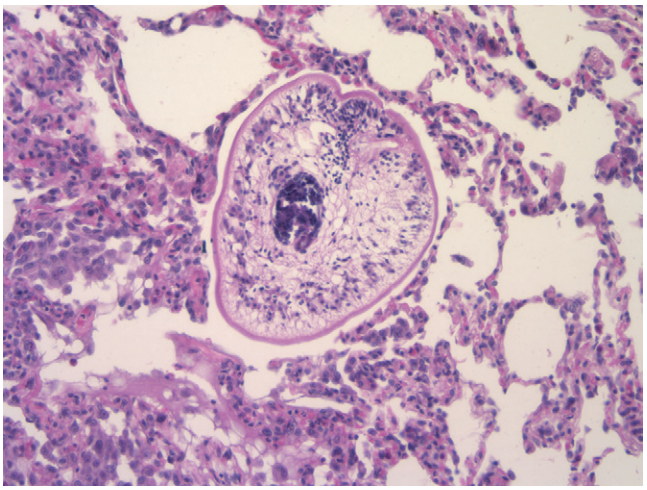

FIGURE 8-53. Mesocercariae of an unidentified trematode in the lung of a raccoon (×125).

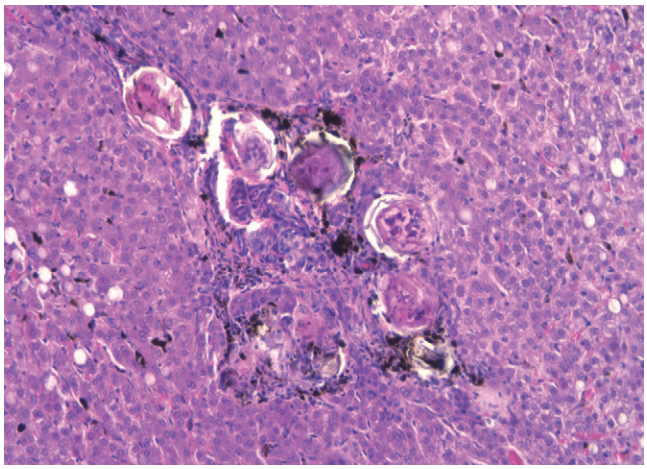

FIGURE 8-51. *Heterobilharzia americana* eggs in the liver of a naturally infected raccoon (×140).

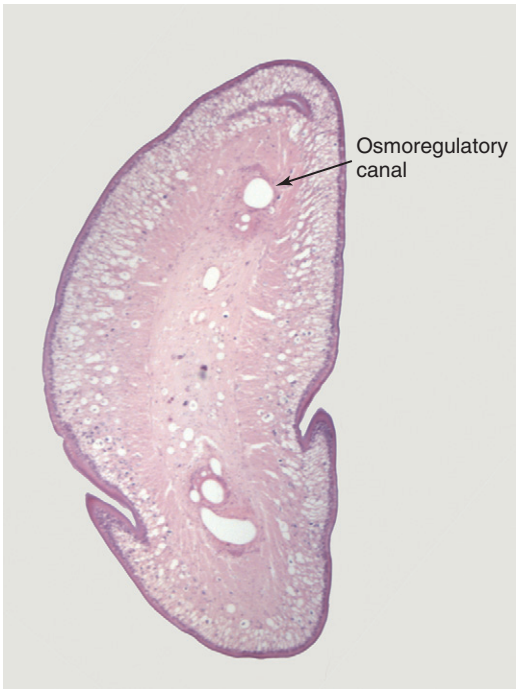

FIGURE 8-54. *Taenia taeniaeformis* strobilocercus from a vole (×20). Cestodes have a solid spongy body with no body cavity and no digestive system. The internal organs of cestodes are embedded in a loose matrix, a parenchymal meshwork of loosely arranged cells divided into distinct outer and inner portions by a system of longitudinal subtegumental and transverse parenchymal muscle fibers.

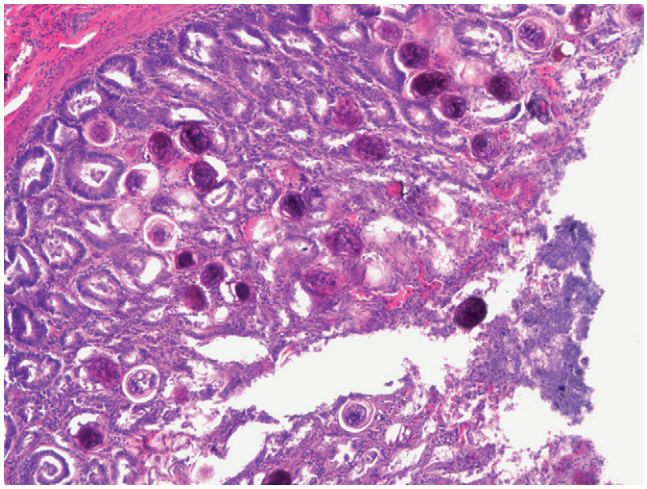

FIGURE 8-52. *Heterobilharzia americana* eggs in the intestine of a naturally infected raccoon (×58).

nonstriated muscle fibers—**subtegumental** and **parenchymal** (Figure 8-54). It is through the tegument that tapeworms absorb their nutrients from the host, and the syncytial surface, especially in adult forms, is thrown into numerous villus-like projections for this purpose. Within the tapeworm, the parenchymal zone divides the parenchyma into a **cortex** lying outside a longitudinal layer of fibers and a **medulla** lying within a transverse layer of muscle fibers; the medulla contains the osmoregulatory ducts and reproductive organs if these are present. **Calcareous corpuscles** are typical of cestode tissues and, especially in larvae, may provide the only evidence that the specimen is a tapeworm (Figures 8-55 and 8-56). Tapeworms are covered by a tegument formed by the cytoplasmic

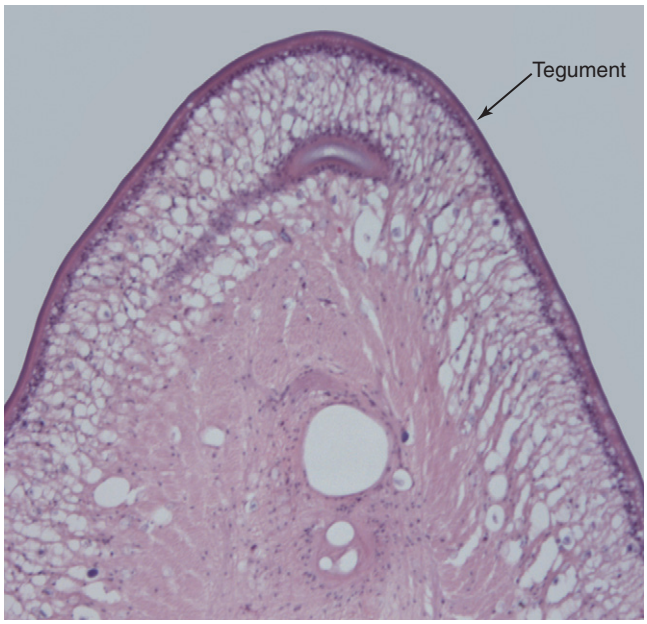

FIGURE 8-55. *Taenia taeniaeformis* strobilocercus at higher power showing the subtegumental and parenchymal muscle layers (×100).

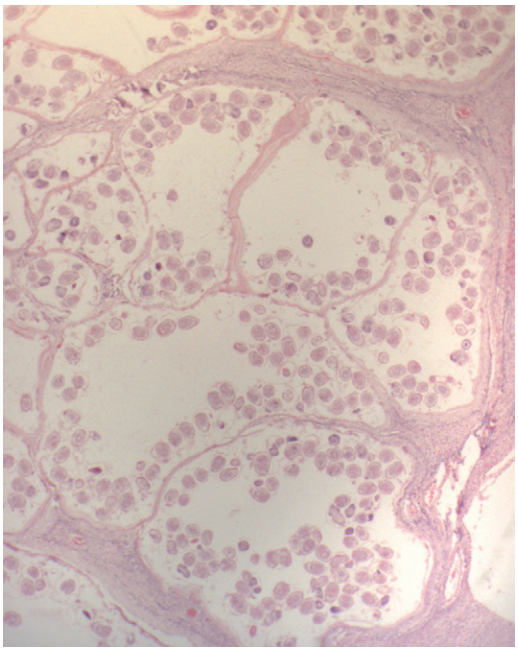

FIGURE 8-57. *Echinococcus multilocularis* alveolar hydatid (×10) showing multiple germinal areas within the cyst.

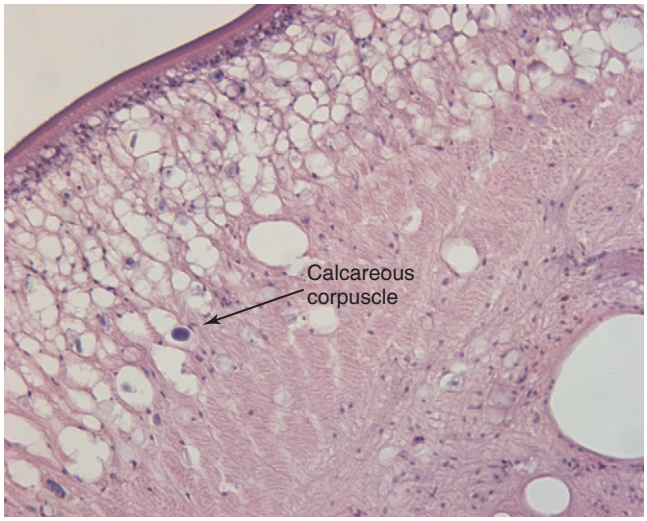

FIGURE 8-56. *Taenia taeniaeformis* strobilocercus at higher power showing calcareous corpuscles (×100).

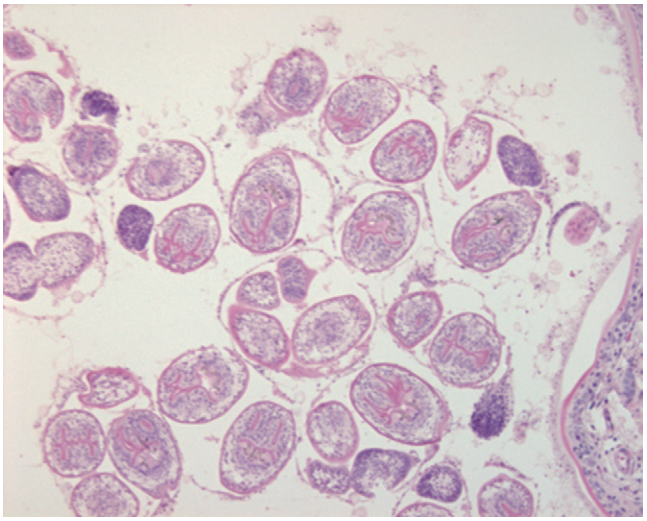

FIGURE 8-58. *Echinococcus multilocularis* alveolar hydatid (×100) showing a number of protoscolices.

projections of epidermal cells, which appears in histologic sections as a thick, homogeneous noncellular external layer supported by a basal membrane.

The larva of a tapeworm that is found in a vertebrate host represents the precursor to the adult form and typically bears the holdfast or scolex of the adult in some rudimentary or embryonic form (Figures 8-57 to 8-68). After the host is ingested, much of the larva will be digested away and the small holdfast will attach to the intestinal mucosa and grow the adult strobili, which contains all the varied sexual organs and associated structures. In veterinary medicine, although it often seems as though we are dealing with a huge number of types and forms of bothria, scolices, suckers, and hook shapes, the reality is that compared with the large numbers of tapeworm families with different forms of holdfasts occurring in a wide range of vertebrates (e.g., the Trypanorhyncha, the Tetraphyllidea), we are really dealing only with the few forms that occur in terrestrial mammals. If one is lucky, the sections are through the head of the larva, which aids greatly in identifying the parasite beyond the simple designation of larval tapeworm based on body structure and the presence of calcareous corpuscles, but unfortunately much of the time, one has only sections through the body of the larva, and then the diagnosis, based simply on morphology, almost always remains somewhat obscure.

Because veterinary medicine until very recently focused almost exclusively on the common domestic mammals used as food and human companions, the most common tapeworm larvae seen are those of the taeniid tapeworms that have a mammalian final host and a mammalian intermediate host. The typical taeniid metacestode larvae are the **cysticercus** (see Figure 8-60), the **coenurus** (see Figure 8-62), the **strobilocercus** (see Figures 8-54 to 8-56 and 8-61), the **unilocular hydatid** (see Figures 8-59 and 8-64), and the **alveolar hydatid** (see Figures 8-57 and 8-58). For information on

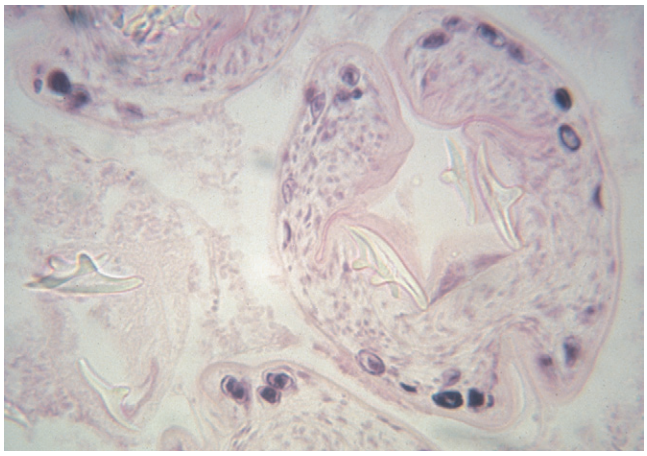

FIGURE 8-59. *Echinococcus vogeli* from an agouti rat *(Dasyprocta leporina)* in Brazil showing the typical claw-hammer–shaped taeniid hooks on the protoscolex (×400). (Courtesy Dr. M. Dale Little.)

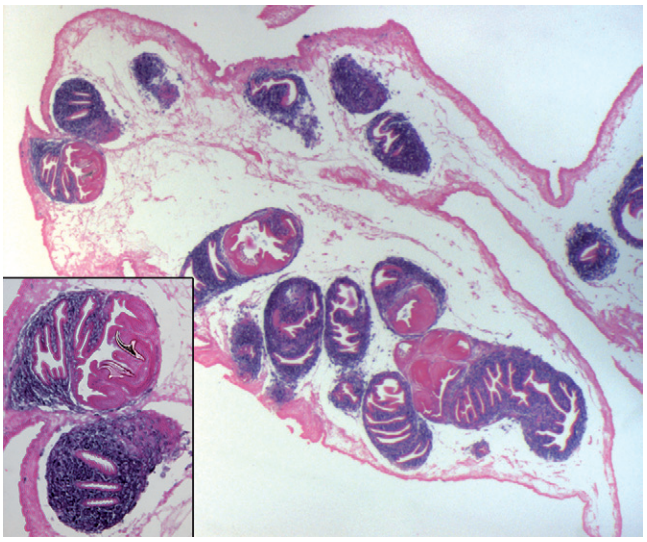

FIGURE 8-62. *Taenia multiceps* coenurus showing several scolices on a thin bladder wall (×45); enlarged view shows the hooks on one of the scolices (×250). (Courtesy Ward's Biological Supply.)

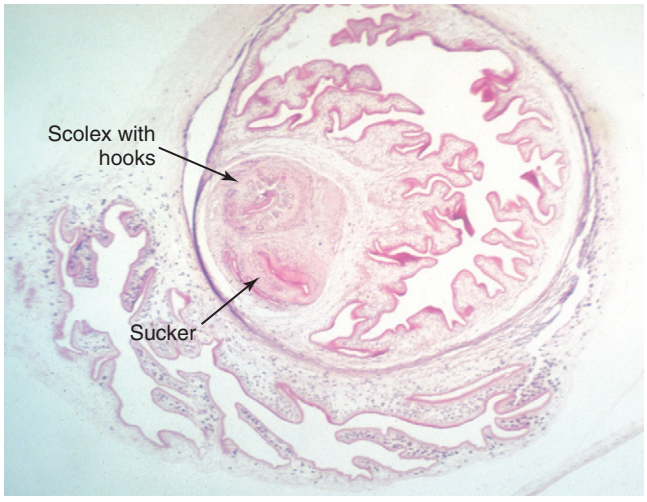

FIGURE 8-60. *Taenia solium* cysticercus in the brain of a dog that can be identified by the shapes and the measurement of the hooks on the scolex (×5). (Courtesy Dr. M. Dale Little.)

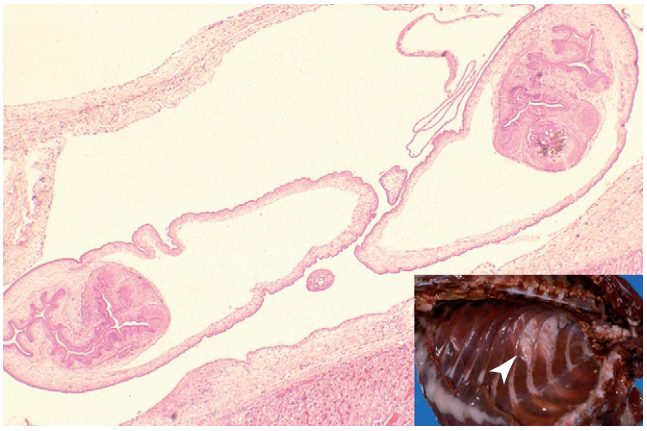

FIGURE 8-63. *Taenia crassiceps*. Cysticerci shown in a gross lesion in a naturally infected woodchuck. Sections through two cysticerci showing each with an inverted scolex (×250). This is an unusual cysticercus in that it proliferates by budding and may be found widely disseminated in various tissues of rodents. *Inset,* Gross specimen of cysticerci *(arrowhead)* in woodchuck at necropsy.

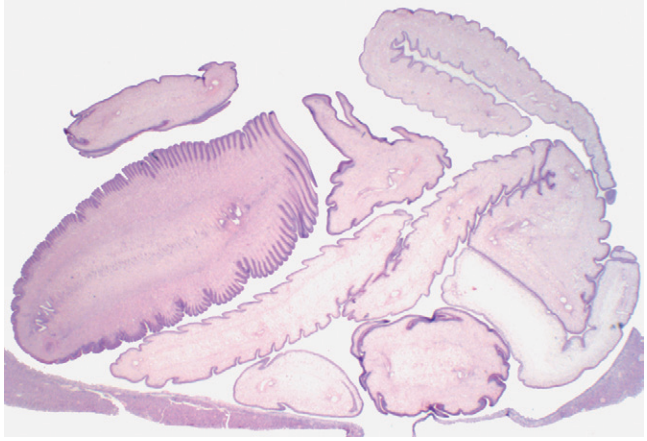

FIGURE 8-61. *Taenia taeniaeformis* strobilocercus encysted in the liver of a meadow vole (×5).

hosts and site specificity, refer to the appropriate host-organ list and details in the previous chapters. If the histologic section includes only the bladder wall of the larvae, there will be little more than calcareous corpuscles to identify it as cestode tissue. A section through the scolex of the larvae that includes the typical claw-hammer–shaped hooks of this group (see Figure 8-59) identifies the specimen as a taeniid. *Taenia saginata,* the "beef tapeworm" of man, forms an exception in not having hooks in the larval nor adult stages. Often the scolex of the tapeworm is inverted in the body and will not evert until the larva is ingested by the final host.

Tentative identification of species of taeniid larvae may be based on their host and site specificity. For example, a cysticercus attached to the peritoneal membranes of a cottontail rabbit is very probably the larva of *Taenia pisiformis,* whereas a cysticercus on the peritoneal membranes of a ruminant of pig is most likely the larva of *Taenia hydatigena*. Further evidence is provided by hook length measurements if both long and short hooks happen to lie in the

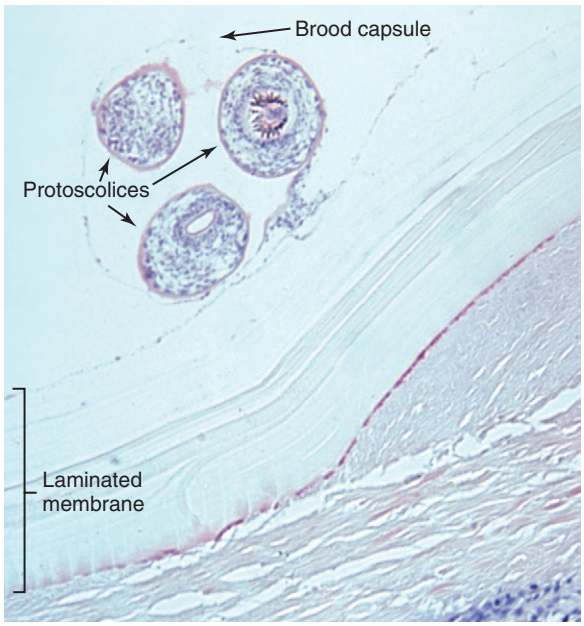

FIGURE 8-64. Echinococcus granulosus hydatid cyst (×200).

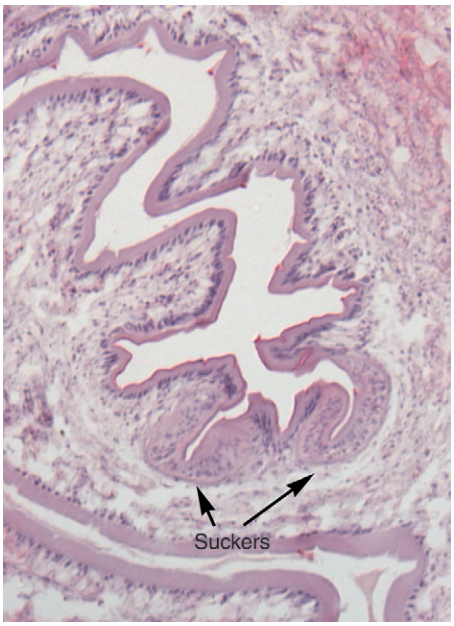

FIGURE 8-66. *Mesocestoides* tetrathyridium from the peritoneal cavity of a baboon (*Papio* sp.); region of scolex shows two suckers *(arrows)* (×200).

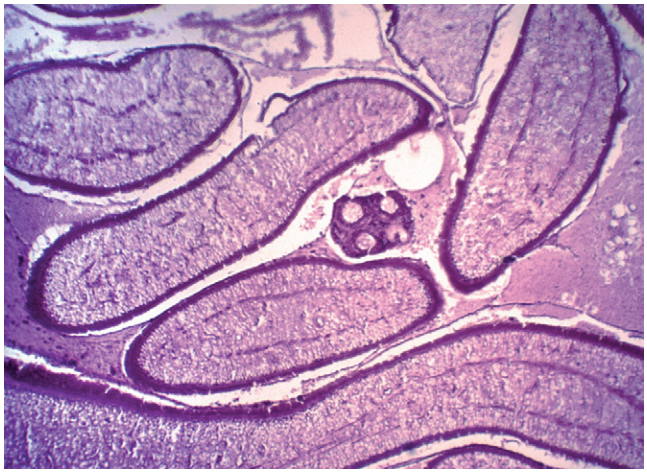

FIGURE 8-65. *Mesocestoides* tetrathyridium from the heart of a rice rat (*Oryzomys* sp.) from Colombia (×40); note the scolex without hooks in the middle of the image.

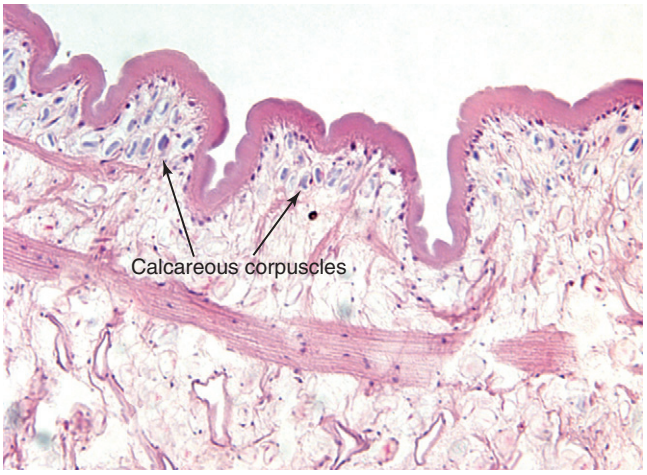

FIGURE 8-67. *Mesocestoides* tetrathyridium of Figure 8-66 parenchyma with large calcareous corpuscles *(arrows)* (×250).

plane of section, or if they can be isolated from the wet tissues. Verster (1969) may be consulted for hook-length data. In the coenurus more than one scolex is connected to the same bladder wall. *Taenia crassiceps* presents a source of confusion in this regard by forming many cysticerci by budding. These all lie within the same host cysts but are not attached to a common bladder wall (see Figure 8-63). Strobilocerci of *Taenia taeniaeformis* are cysticerci that have precociously begun to elongate and segment as larvae and are found in the liver of rodents (see Figures 8-54 to 8-56 and 8-61).

Hydatid cysts manifest expansive growth and have thick, laminated membranes separating the germinative layer, which bears sessile small scolices (termed *protoscolices*) or brood capsules, from the surrounding host connective tissue capsule. In "sterile hydatid cysts" (cysts without protoscolices), the laminated membrane is the only diagnostic characteristic available. Alveolar hydatids have much thinner laminated membranes, and their manner of growth is invasive instead of expansive.

The remaining tapeworm larvae that are typically found in the tissues in sections are solid-bodied wormlike threads or ribbons (that can be very long) that course through the tissues or peritoneal cavities of the host. These two larvae are the **tetrathyridium** of the Mesocestoididae (see Figures 8-65 to 8-67) and the **plerocercoid** (or sparganum) of the pseudophyllidean tapeworms (see Figure 8-68). Plerocercoids of *Spirometra* organisms (see Figure 8-67) are ribbon-like larvae that are unsegmented and undifferentiated. They have no bladder, and the scolex is not always developed, so no sections through bothria may be evident no matter how many serial sections are prepared. Calcareous corpuscles observed in a parenchymatous matrix without evidence of other structures may be the only feature by which to identify a plerocercoid. Tetrathyridia of *Mesocestoides* organisms differ from plerocercoids in that they possess four suckers with no hooks (see Figure 8-66), and their calcareous corpuscles tend to be large but not as dense as those of other larvae (see Figure 8-67). Tetrathyridia can undergo massive asexual proliferation in the intermediate host (often seen in dogs),

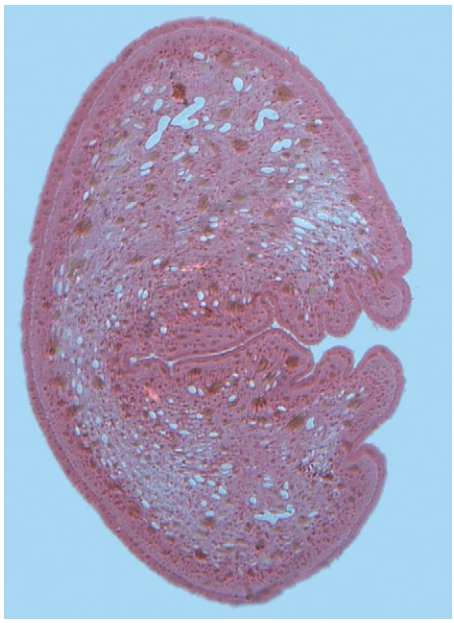

FIGURE 8-68. *Spirometra mansonoides* plerocercoid from the subcutaneous tissues of a mouse (×108).

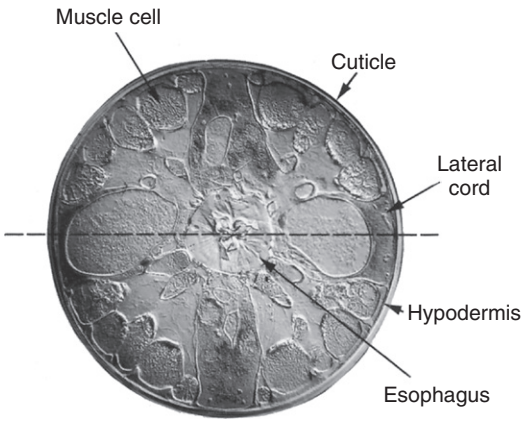

FIGURE 8-69. *Strongylus vulgaris*. Cross-section through the esophageal region of *Strongylus vulgaris* showing the division of somatic musculature into dorsal and ventral fields by the lateral cords. In this particular body region of *S. vulgaris,* the dorsal and ventral cords are exceptionally well developed, and these anatomically separate their respective muscle fields into halves. However, functional separation, expressed in terms of coordinated muscular activity, remains predominantly dorsoventral (×62).

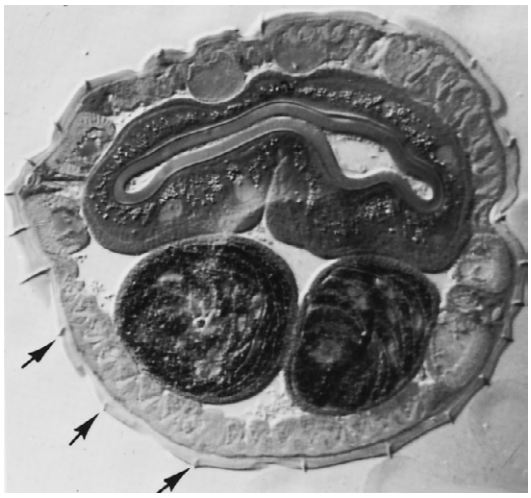

FIGURE 8-70. *Haemonchus contortus* in cross-section at the level of the intestine (×140). The entire circumference of the cuticle is marked by longitudinal ridges *(arrows)*, and the prominent brush border on the luminal surface of the gut is evident.

forming thousands of organisms that are atypical perhaps owing to the rapid multiplication, and are very difficult to identify as anything other than tapeworm tissue.

NEMATODES

Living nematodes are pseudocoelomic animals that have a fluid-filled body. The body externally is covered with a cuticle composed of collagen, and movement occurs by muscle cells in quadrants along the body wall working in apposition to the cuticle, which allows the worms to move sinusoidally. Nematodes typically have a mouth and an anus connected by a digestive tract. In sections the worms typically appear round and do have the internal organs floating within the pseudocoelomic cavity. The genital primordium appears in larvae, but it tends not to grow to any extent until the worms reach the fourth larval stage. One feature of worms becoming adults after the fourth and final molt of their development is that the vulva of the female finally opens through the cuticle (becomes patent).

In sections (Figures 8-69 and 8-70) the nematode in most cases is divided into two dorsolateral and two ventrolateral **quadrants** by the **hypodermis.** A syncytial layer below the cuticle secretes the cuticle. The hypodermis tends to extend into the body of the worm in dorsal, ventral, and lateral cords, which is what divides the body into its four apparent sections. The **nervous system** of a nematode consists of a major nervous ganglion that typically encircles the esophagus and that has major sets of fibers running through the ventral and dorsal hypodermal cords. Nematodes also have an **excretory-secretory system** that usually empties through an **excretory pore,** which is located on the ventral side of the worm near the level of the nerve ring or more anteriorly. The excretory system may have columns that extend posteriorly in the form of arms that extend into each of the lateral cords. In some adenophorean nematodes, cords may be found in addition to the typical four. Also, in the Trichinelloidea the hypodermis tends to be organized into **bacillary bands** rather than lateral cords, with one in *Trichuris,* two in *Trichinella,* and three or four typical in the various capillarids. From the bacillary bands, pores can be seen extending from the band through the cuticle.

The **cuticle** covers the external surface of the worm and, to varying degrees in different groups, the lining of the esophagus, the posterior portion of the digestive tract, the vagina, and the opening of the excretory system. The cuticle may appear layered in histologic sections, especially in forms with thicker cuticles. The cuticle may have major modifications above the lateral hypodermal cord where it forms large wings of cuticle called *cephalic alae* (when only on the head), which may or may not be continuous with **lateral alae** running the length of the worm. Some forms, mainly the Trichostrongyloidea, have numerous additional longitudinal ridges running the length of the body. Adult worms are liable to have all sorts of modifications on the anterior end that are usually less apparent in the larvae; these consist of lips, large buccal capsules, teeth, and so on. The cuticle may also have spines, striations, bosses (thickened bumps), pores (in some Adenophorea), and so on. In adult male nematodes with spicules, the spicules are composed of sclerotized cuticle and have a myriad of forms and shapes with some being spined.

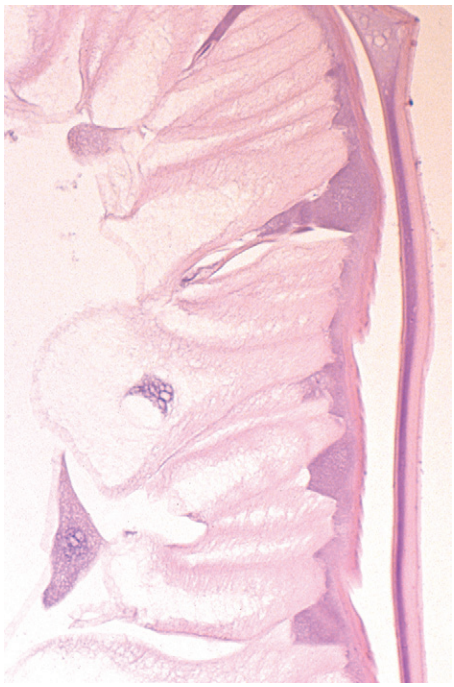

FIGURE 8-71. *Eustrongyloides* sp. (×170) from a great blue heron. Each somatic muscle cell is composed of a basement membrane adjacent to the hypodermis, contractile muscle fibers, and a delicate sarcoplasmic portion containing the nucleus. The coelomyarian muscle cells have a darkly staining contractile portion extending up the lateral sides of the muscle cell, which gives the cell a cylindric appearance, and an abundant, noncontractile cytoplasmic portion that appears to be empty with most stains.

The **muscle cells** of the body that provide locomotion lie along the body underneath the hypodermal layer (Figure 8-71). These muscle cells have their long axis oriented along the length of the worm and vary in number and shape; the muscle cells of nematodes are unlike those of many other organisms because the muscles send processes to the dorsal and ventral nerve cords rather than having the nerves extending to the muscle cells. When a section has only a few muscle cells (about three to five) per quadrant, this is termed **meromyarian,** and when there are more cells, the worms are described as **polymyarian.** Muscle cells are also described as to their appearance. If the cells have their contractile elements all oppressed to the hypodermis with an empty cell body above them, they are termed **platymyarian.** If the cells have contractile portions that extend up the side of the cell body, they are called **coelomyarian.** Typically, cells that are platymyarian are few in number per quadrant, hence *meromyarian,* whereas coelomyarian muscle cells tend to be numerous per quadrant and polymyarian. The Ascaridida and Spirurida tend to have polymyarian, coelomyarian muscles; the Rhabditida, Oxyurida, and Strongylida tend to have meromyarian, platymyarian muscles. The Adenophorea are varied.

The digestive tract of the nematodes consists of an esophagus, an intestine, and a rectum (in male nematodes this is actually a cloaca, but the distinction is almost never used). Many of the characteristics of the digestive tract of adult nematodes are also present in their respective larvae stages. This can be a very useful asset in diagnosis using histologic sections.

The **esophagus** tends to be divided into a dorsal and two subventral portions by a triradiate lumen that is typically lined with cuticle. There are muscles within the esophagus that pull on the lumen to open the esophagus for feeding. Within the different sections there may also be various glandular elements. The esophagus may be muscular throughout its length or may have an anterior portion that is muscular and a posterior portion that is glandular. The Rhabditida have a muscular esophagus typically divided into a distinct corpus, isthmus, and bulbus. The Oxyurida have a muscular esophagus with a large valved bulb before the junction with the intestine. The Strongylida for the most part have a simple muscular esophagus. The Ascaridida have an esophagus that may have a large glandular area, the ventriculus, at its base, and they may also have ventricular ceca. The Spirurida typically have an esophagus that is muscular anteriorly and glandular posteriorly. The Trichinelloidea tend to have a stichosome esophagus (described later), whereas the Dioctophymatoidea have a muscular esophagus with many large branching glands.

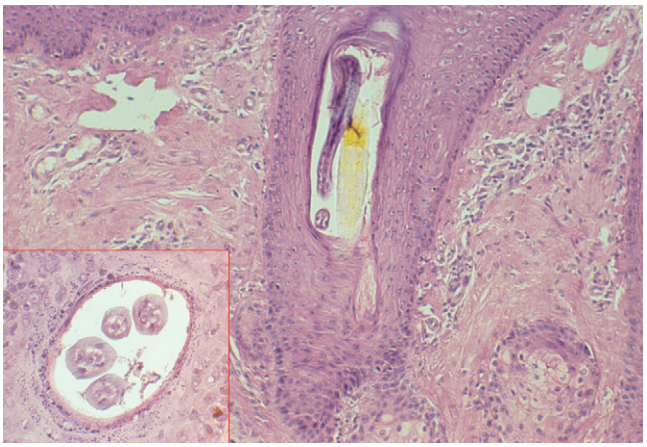

FIGURE 8-72. *Rhabditis (Pelodera) strongyloides* in a hair follicle of a dog (×130). The inset is an enlarged view that shows the double lateral alae (×400).

The **intestine** of nematodes is fairly simple in all nematodes; it is composed of a single layer of columnar cells that have a microvillous border. In the Strongylida, the intestine is lined with a very few cells **(oligocytous)** that are syncytial and polynucleate, and it will often appear that only two such cells line the lumen at any given section. In the Rhabditida, the intestine appears lined by only two cells at each level. The Oxyurida, Ascaridida, and Spirurida have many cells **(polycytous)** to myriad cells **(myriocytous)** lining the intestinal lumen; these cells tend to be uninucleate for the most part but can vary markedly in height around the lumen, especially within the Spirurida. In the Adenophorea, those we are concerned with typically have a polycytous intestine with uninucleate cells. In most of the nematodes that we will see in section, the anus is subterminal (i.e., there is a tail beyond the anus). The only group for which this is not the case is the Adenophorea, in which the anus is terminal.

Rhabditida

Pelodera strongyloides larvae are found in the hair follicles of dogs, swine, and cattle (Figure 8-72). They have double lateral alae.

Halicephalobus gingivalis is another normal saprophytic nematode that has been reported to invade mammalian tissue and disseminate to various sites, most notably the brain (Figure 8-73), with fatal outcome. The infection has been reported widely in horses. These worms are small—adult females are 250 to 450 µm in length by no more than about 25 µm in diameter—and only females and larvae have been reported in tissues, suggesting that they are parthenogenetic. Distinctive features in section, in addition to the small size and location, include the presence of a rhabditoid esophagus, a single genital tube, and a thin body wall in which

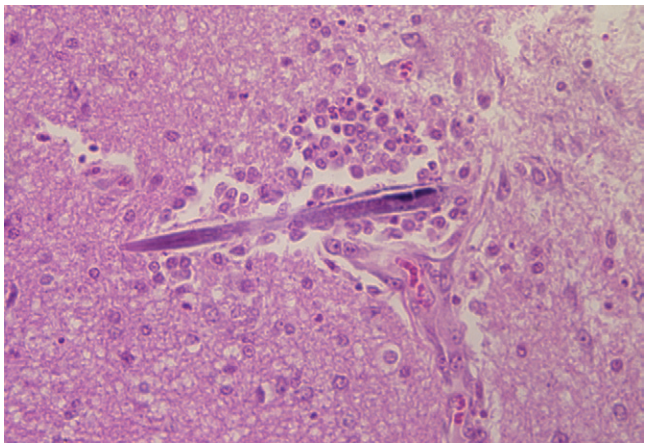

FIGURE 8-73. *Halicephalobus (Micronema)* gingivalis in brain of horse (×200).

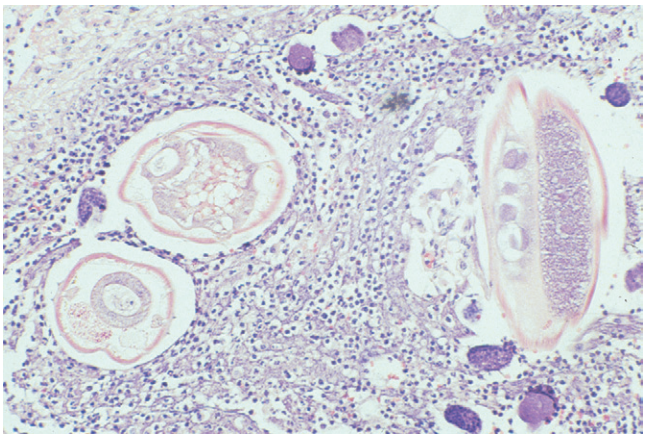

FIGURE 8-75. *Molineus barbatus* in the small intestine of a *Cebus* monkey (×200).

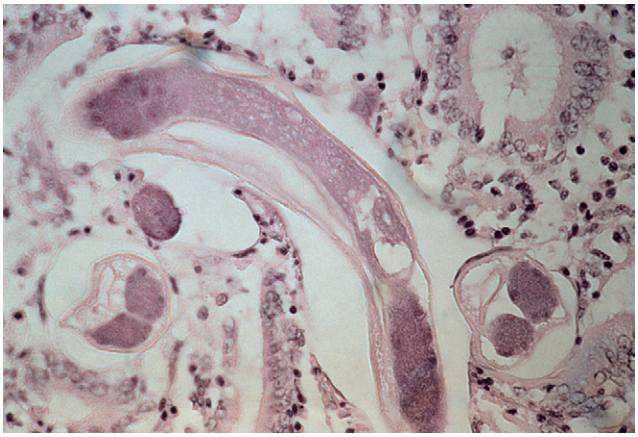

FIGURE 8-74. *Strongyloides westeri* in the mucosa of the small intestine of a horse (×250).

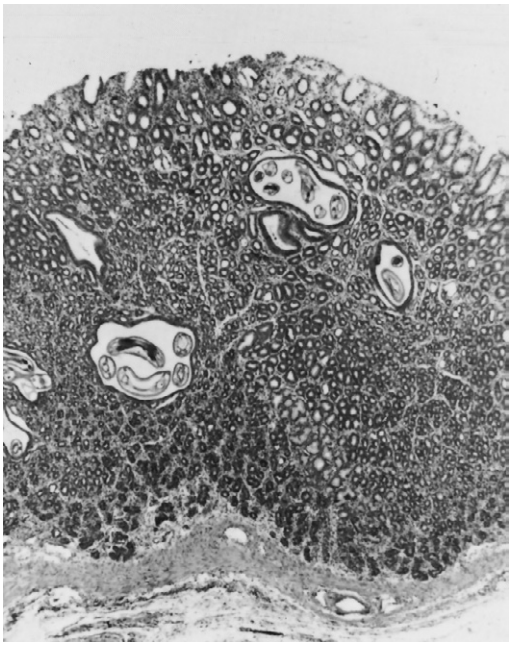

FIGURE 8-76. *Ostertagia ostertagi* in the abomasal mucosa of a heifer (×25). (Courtesy Dr. Lois Roth.)

the cuticle, hypodermis, and muscle layers cannot be distinctly separated.

Strongyloides is a group of parthenogenetic species, and only female worms and larvae are found in the tissues. The adult parasitic female worms of this species are found deep in the mucous membrane of the small intestine (Figure 8-74) and are characterized by meromyarian and platymyarian muscles, a simple intestine composed of only two cells, and the eggs in utero, which are few in number, lined up in single rows and often with developing larvae. *Strongyloides* larvae (see Figure 7-27) have double lateral alae.

Strongylida

There are four superfamilies: Trichostrongyloidea, Strongyloidea, Ancylostomatoidea, and Metastrongyloidea.

Trichostrongyloidea

The adults of this group tend to be small worms that typically inhabit the stomach or small intestine. In cross-section they are characterized by a small number of platymyarian muscle cells and an intestine composed of few cells, often with prominent nuclei and a microvillous border. Most trichostrongyles, with the exception of *Trichostrongylus* specimens, have marked longitudinal ridges on the surface of the cuticle (Figure 8-75). Fourth-stage larvae are found throughout the mucosa of the stomach and intestine of ruminants and a wide range of other hosts. *Trichostrongylus axei* fourth-stage larvae and juvenile adults are found between the basement membrane and epithelial cells of the abomasal mucosa. *Ostertagia* fourth-stage larvae and juvenile adults are found in dilated gastric glands of the abomasum (Figures 8-76 and 8-77).

Strongyloidea

Most adult strongyles inhabit the intestinal tract and are larger than the trichostrongyles. In section they exhibit characteristic features, including platymyarian muscles and the typical strongyle intestine. The cuticle is not adorned with ridges. In the strongyles the presence of a large buccal capsule and specialized mouthparts is of great taxonomic value, but these features are often not seen in tissue sections.

Some of the larval stages of strongyles are spent in tissues other than the gut, whereas some form nodules in the intestinal wall. *Strongylus vulgaris, Strongylus edentates,* and *Strongylus equinus* migrate extensively and sometimes erratically in the horse. *S. edentatus* tends to migrate retroperitoneally, and it is characterized by a thick, multilayered cuticle (Figure 8-78). *S. equinus* immature adults are frequently found in the pancreas; sections

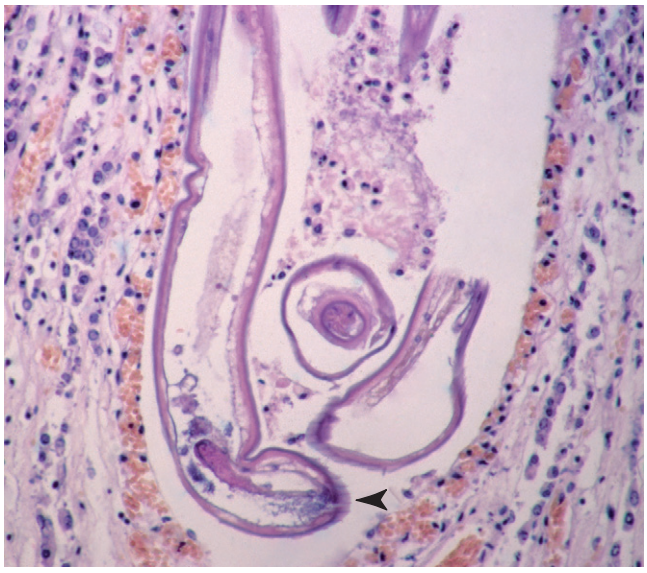

FIGURE 8-77. *Ostertagia ostertagi* in the abomasal mucosa (×200) showing the longitudinal cuticular ridges typical *(arrowhead)* of the superfamily Trichostrongyloidea.

FIGURE 8-79. *Strongylus edentatus* immature male in the lung of a horse (×15). Two sections of worm are visible. The upper is a cross-section near the caudal end of the worm (see also Figure 8-77), and the lower is an oblique section through the buccal capsule (see also Figure 8-78).

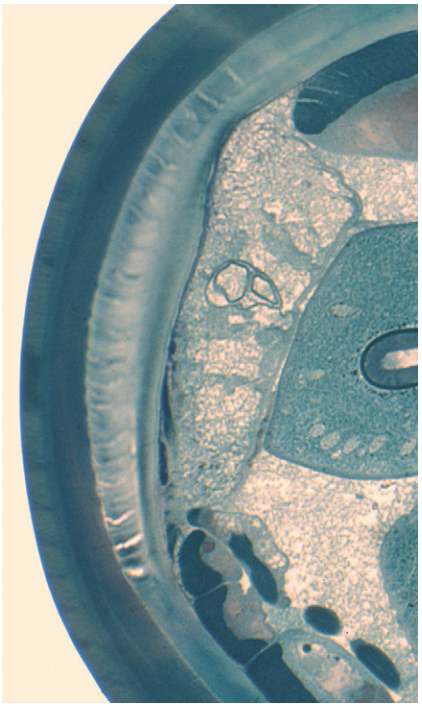

FIGURE 8-78. *Strongylus edentatus*. Cross-section showing the thick, multilayered cuticle of this species (×220).

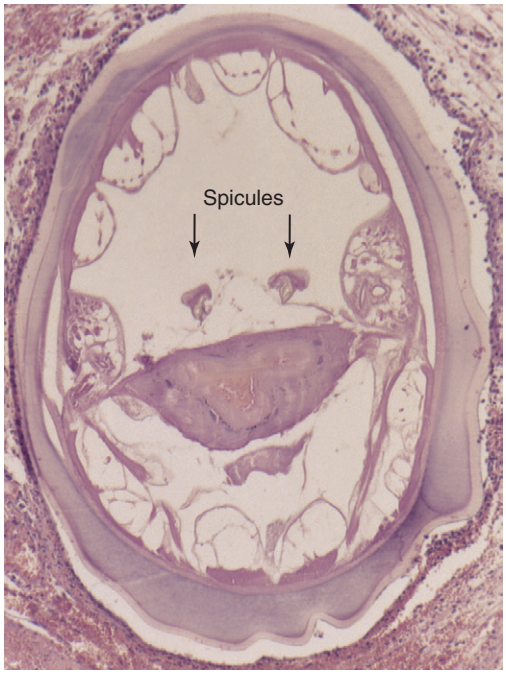

FIGURE 8-80. *Strongylus edentatus*. Higher magnification of Figure 8-79, showing a section through the caudal end of the worm (×100). Note the thick, multilayered cuticle, spicules, and prominent lateral cords. The cytoplasm of the meromyarian-platymyarian muscle cells was lost in histologic processing (see also Figure 8-75).

through the buccal capsule reveal the presence of teeth at their base (Figures 8-79 to 8-82).

Oesophagostomum and related worms are common parasites of livestock and monkeys and have worldwide distribution. They are often referred to as *nodular worms* because developing larvae produce remarkable nodular abscesses in the intestinal wall of the vertebrate host during development, leading to the adult stage. Most often seen in section as developing worms inside these nodules (Figures 8-83 and 8-84), the larvae have a relatively thick but smooth cuticle, prominent lateral cords, and muscle cells that are platymyarian and meromyarian, typically with only a small number of muscle cells per quadrant. The gut is composed of a small number of multinucleate cells with a conspicuous microvillous (brush) border.

Ancylostomatoidea

The Ancylostomatoidea, typically referred to as *hookworms,* inhabits the gut as adults and have typical strongyle features in section (Figure 8-85). The larvae of hookworms are relatively small, usually

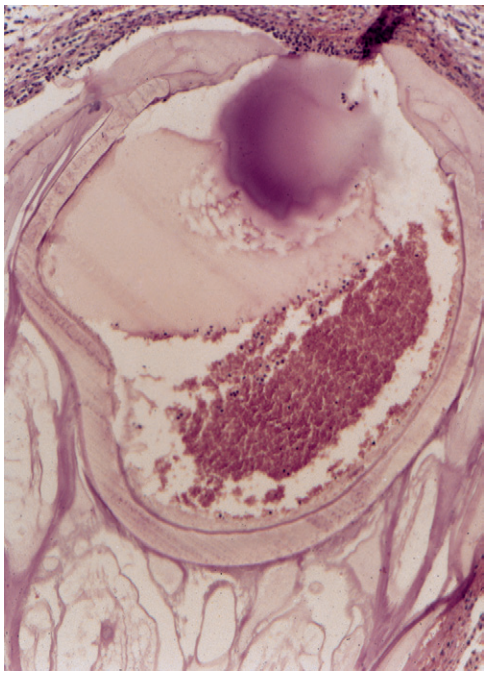

FIGURE 8-81. *Strongylus edentatus*. Higher magnification of Figure 8-79, showing the buccal capsule (×100).

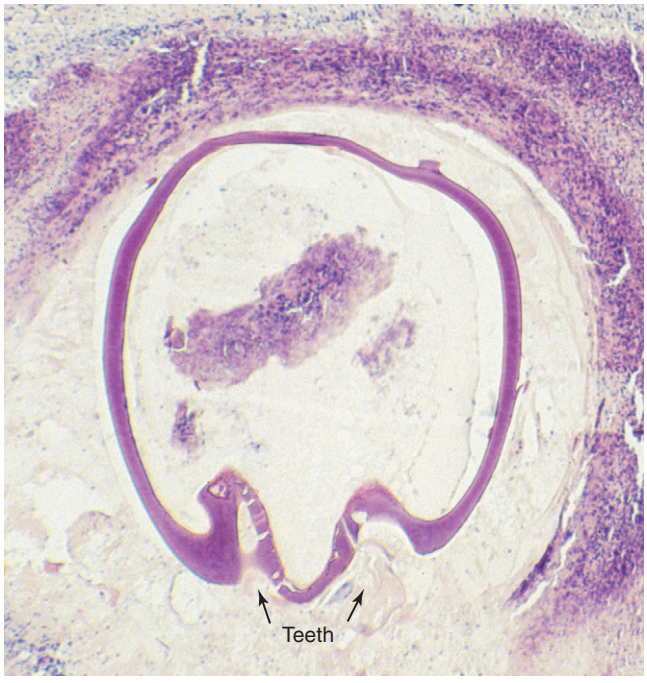

FIGURE 8-82. *Strongylus equinus* immature adult worm in the pancreas of a horse (×100). Although moribund, the teeth *(arrows)* in the base of the buccal capsule are still readily visible and distinguish this species from *S. edentatus*.

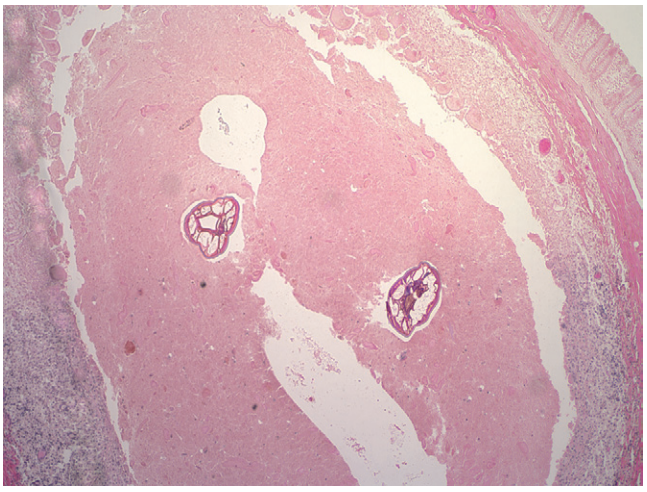

FIGURE 8-83. *Oesophagostomum* sp. Section through a nodule in wall of the large intestine of cynomolgus monkey containing two sections of *Oesophagostomum* larva (×25).

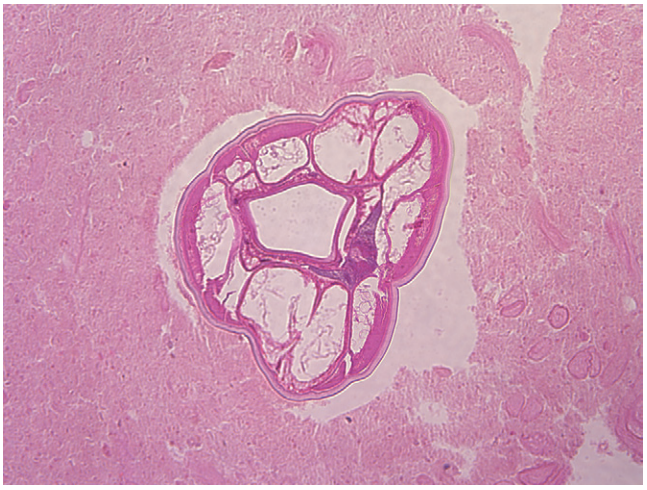

FIGURE 8-84. *Oesophagostomum* sp. Higher magnification of Figure 8-83 showing a section through *Oesophagostomum* larva (×120). Note the small number of platymyarian muscle cells and a prominent brush border on the epithelial cells of the gut.

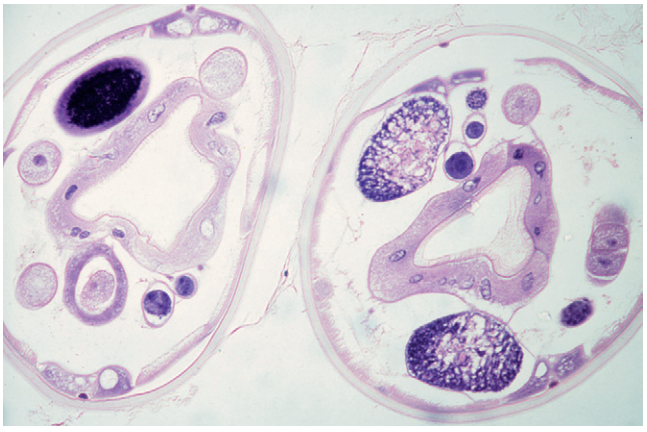

FIGURE 8-85. *Ancylostoma caninum* adult females from the intestine of a dog show the platymyarian muscles and the small number of syncytial intestinal cells (×80). (Courtesy Dr. M. Dale Little.)

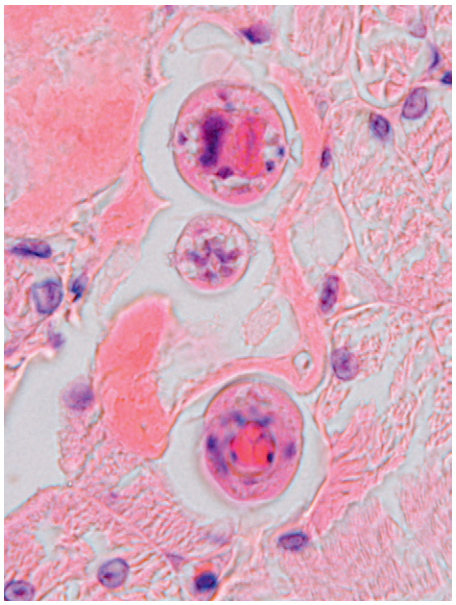

FIGURE 8-86. *Ancylostoma caninum* third-stage larvae within skeletal muscle fibers (×1250). Note the double lateral alae.

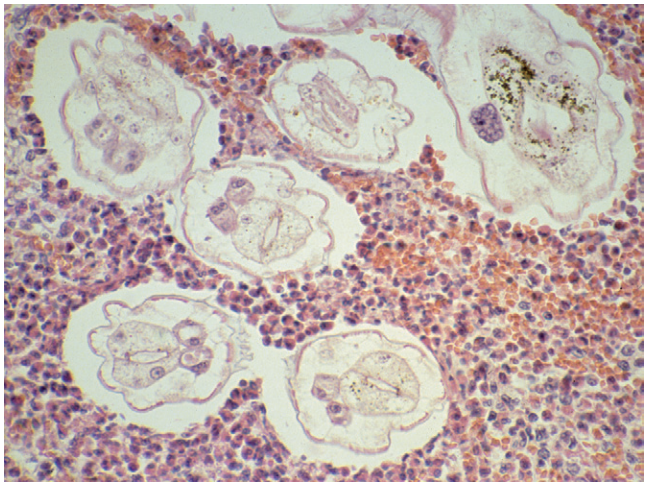

FIGURE 8-87. *Aelurostrongylus abstrusus* adults in section of a nodule in the lung of a cat (×250).

only 14 to 16 µm in diameter, and have double lateral alae (Figure 8-86).

Metastrongyloidea

Adult metastrongyles, often referred to as *lungworms,* typically parasitize the lungs or airways, but some may invade blood vessels or the central nervous system. In section the body wall tends to be thin, the musculature is often polymyarian and coelomyarian in nature, and the gut is the typical strongyle type, although the microvilli are less prominent than in other strongyles. Many metastrongyles contain embryonated eggs or larvae in utero and shed these stages into the surrounding tissues.

Cats are typically host to only a single lungworm, *Aelurostrongylus abstrusus*. Adults, eggs in varying stages of development, and larvae are found in nests in the lung parenchyma (Figure 8-87). Diagnosis is usually fairly easy because domestic cats have few other worms causing similar lesions; however, wild felids may be host to related forms.

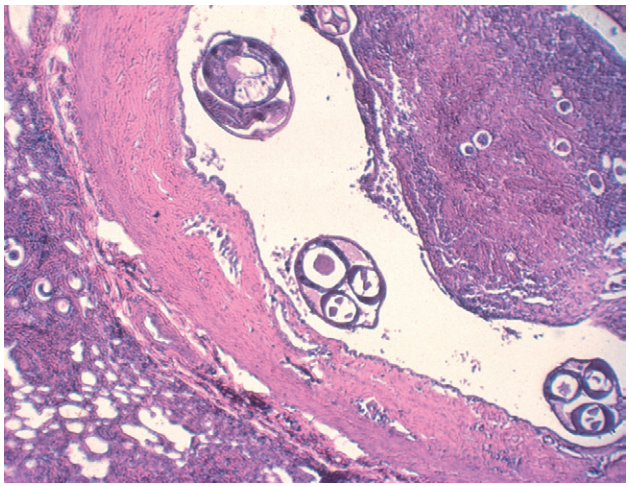

FIGURE 8-88. *Angiostrongylus vasorum* in the pulmonary artery of a dog (×100). (Courtesy Dr. M. Dale Little.)

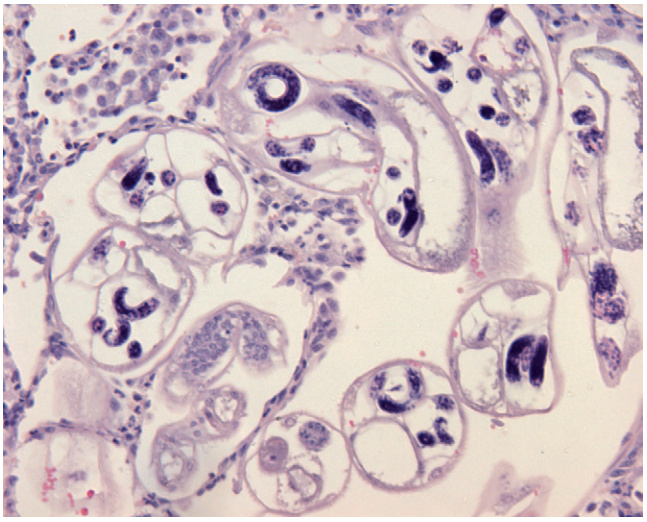

FIGURE 8-89. *Filaroides hirthi* in canine lung tissue (×100). The dark objects are eggs and larvae in the uterus of female worms.

Dogs can be infected with several lungworms, but they tend to live in markedly different locations, making diagnosis easier than it would be otherwise. *Angiostrongylus vasorum* adults may be found in the right side of the heart and in pulmonary vessels of dogs, whereas the eggs and larvae are found in the lung parenchyma. This infection was exotic to North America but has now appeared in the far east of Canada (Figure 8-88). *Filaroides hirthi* adults are found in the lung parenchyma of the dog (Figures 8-89 and 8-90). Eggs contain first-stage larvae when laid, and the eggs do not accumulate in lung tissue. Autoinfection by *F. hirthi* may lead to a state of hyperinfection in which lung tissue is almost completely replaced by adult worms and larvae may be found widely scattered in lymph nodes, pancreas, intestinal tract, liver, and brain. *Filaroides osleri* adults are found in fibrous nodules projecting into the lumen of the trachea and principal bronchi (Figures 8-91 and 8-92).

Sheep and goats can be host to several species of lungworms. *Muellerius capillaris* is found in nodules in the lung parenchyma. These nodules contain adult worms, eggs in varying stages of development, and larvae. If the tails of larvae can be located in the tissue section, *Muellerius* organisms can be distinguished from *Protostrongylus* organisms (see Figure 7-65). *Protostrongylus* species adults

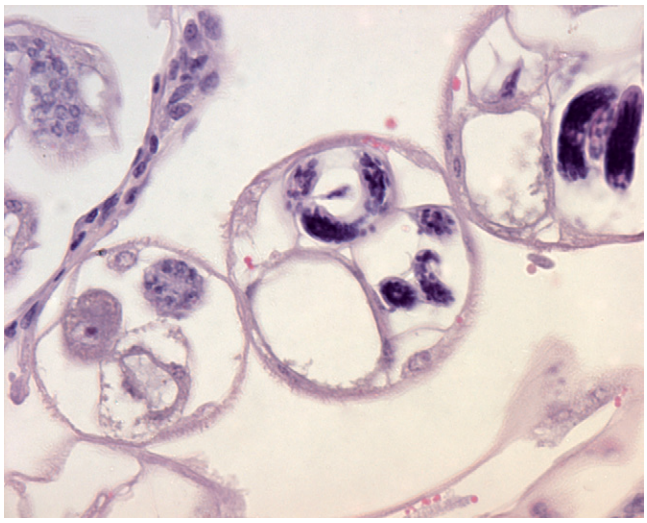

FIGURE 8-90. *Filaroides hirthi,* enlarged view, showing the nature of the intestine, composed of a very few cells (×200).

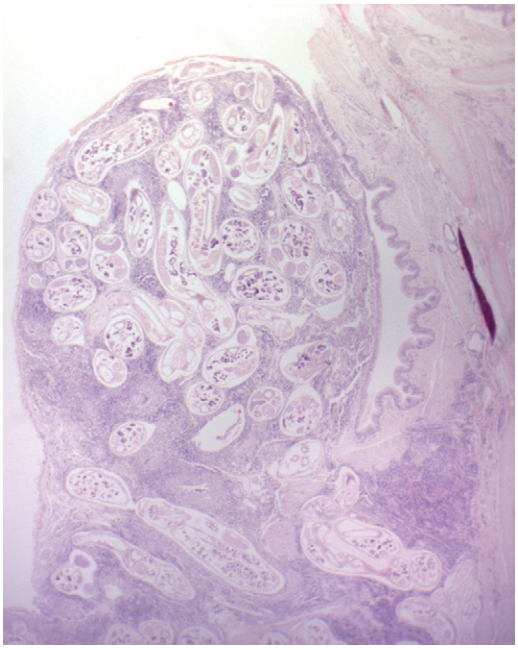

FIGURE 8-91. *Filaroides osleri* in fibrous nodules in the trachea of a dog (×26).

may be found in either parenchymal nodules or airways. *Dictyocaulus* species (Trichostrongyloidea) adults are found in airways. *Parelaphostrongylus tenuis* adults are found in the meninges and nervous tissue of the spinal cord and brain of sheep and goats (Figures 8-93 and 8-94), but their eggs and larvae, which are indistinguishable from those of *Muellerius* organisms, are found widely scattered in the lung parenchyma rather than concentrated in nests.

Oxyurida

The oxyurids are generally smallish worms that as adults typically inhabit the large intestine or cecum. In section most species have prominent lateral alae. The esophagus has characteristic sections consisting of corpus, isthmus, and terminal bulb, which can occasionally be seen in sections. The musculature is platymyarian and meromyarian, and generally only two or three muscle cells are located in each quadrant (Figure 8-95). The intestine is variable but

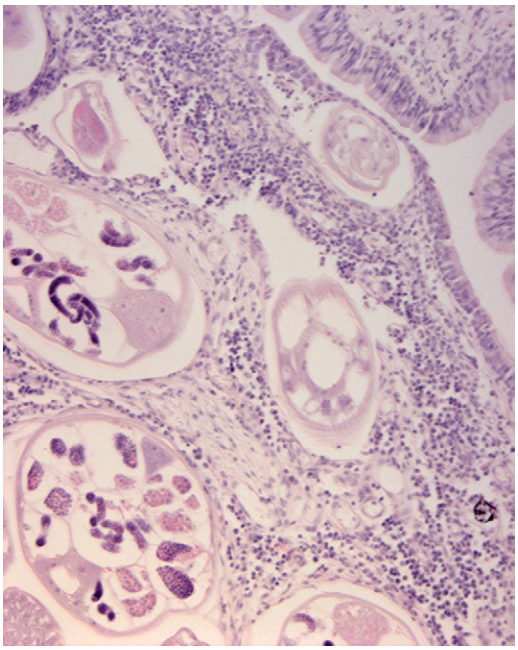

FIGURE 8-92. *Filaroides osleri*, enlarged view, showing the nature of the intestine and the very thin body wall (×180).

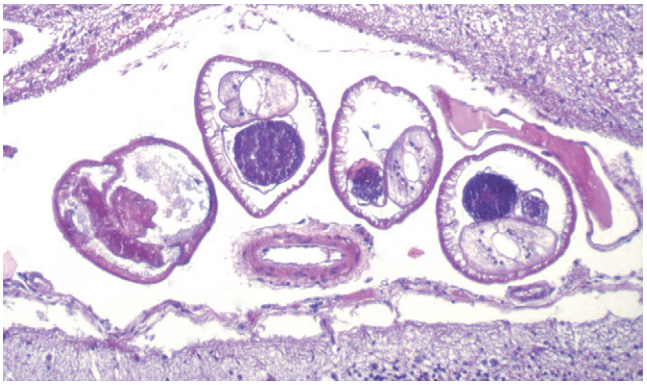

FIGURE 8-93. *Parelaphostrongylus tenuis* in the meninges of a goat (×25).

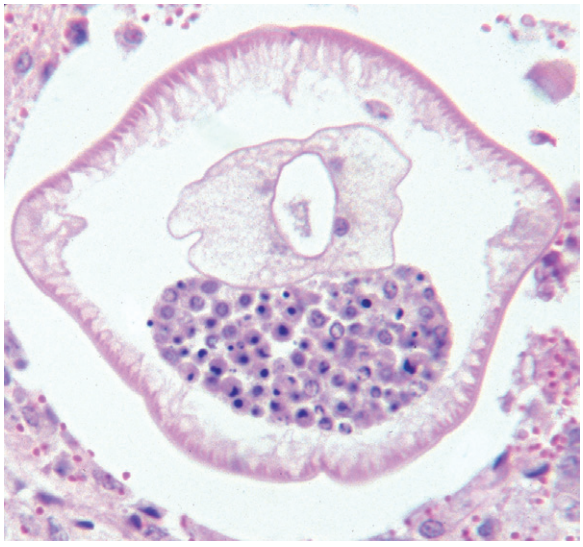

FIGURE 8-94. *Parelaphostrongylus tenuis* (×290) illustrating the nature of the intestine, with very few cells.

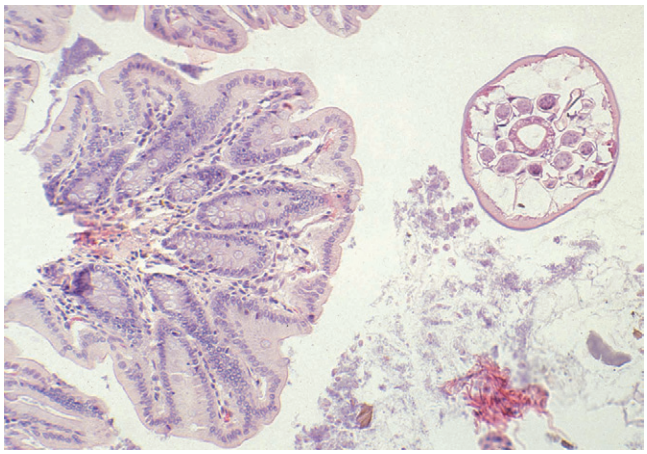

FIGURE 8-95. *Aspiculuris* sp. in the intestine of a rat. This oxyurid (pinworm) has platymyarian muscle cells and at this level of the body lacks lateral alae.

is cuboidal to columnar with a single nucleus per cell. The presence of typical eggs in utero often assists with identification.

Ascaridida

The ascarids comprise a diverse group of worms, and as adults, some, such as *Ascaris* and *Parascaris* organisms, are the largest of the intestinal nematodes. In tissue section, in addition to their large size, the ascarids characteristically have a thick, multilayered cuticle, polymyarian-coelomyarian muscles (often with cytoplasmic processes that extend into the body cavity), an intestine with numerous columnar epithelial cells and short microvilli, and large lateral cords (Figures 8-96 to 8-98). The Ascaridida are often divided into two large groups or superfamilies. One, the Ascaridoidea, parasitize land-dwelling vertebrates, whereas the second group, the Heterocheiloidea, parasitize birds, fish, and marine mammals. Members of the Ascaridoidea, including the genera *Ascaris, Parascaris, Toxocara, Toxascaris,* and *Baylisascaris,* have three simple lips on the anterior end; a thick, multilayered cuticle; a club-shaped esophagus; columnar epithelial gut cells with a single nucleus near the base of each cell; prominent coelomyarian-polymyarian muscle; and typical eggs in the uterus that have a thick shell, often wrinkled or sculptured on the surface. Genera in Heterocheiloidea, such as *Anisakis, Terranova, Contracaecum,* and *Porrocaecum,* have much the same features in section, except that all in this group also have a cecum (anteriorly directed), a ventricular appendix (posteriorly directed), or both. These may be obvious if sections are cut through the level of the esophageal-intestinal junction.

Those ascarids that parasitize mammals often have larvae that are capable of tissue migration, and larvae of genera such as *Toxocara* (Figure 8-99), *Baylisascaris* (Figure 8-100), and *Lagochilascaris* (Figure 8-101) cause "larval migrans" syndrome. Ascarid larvae have single lateral cuticular alae. They also have a single excretory cell with H-shaped anterior and posterior projections called *excretory columns*. The presence of single lateral alae and paired excretory columns makes ascarid larvae relatively easy to distinguish in tissue sections (see Figures 8-99 to 8-101). *Toxocara* larvae migrating or arrested in somatic tissues tend not to exceed 21 µm in diameter, but *Baylisascaris* larvae continue to grow as they migrate and may reach 55 to 70 µm in diameter.

Spirurida

The order Spirurida consists of the superfamilies Gnathostomatoidea, Physalopteroidea, Rictularioidea, Thelazioidea, Spiruroidea,

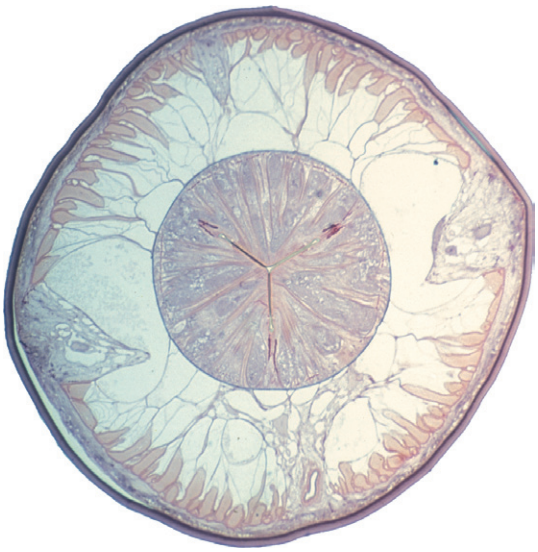

FIGURE 8-96. Cross-section of *Ascaris suum* at the level of the muscular esophagus (×25). The esophagus has a dorsal and two sublateral portions divided by the Y-shaped cuticular lining. The muscles are polymyarian. The lateral cords are prominent, and also visible are the dorsal and ventral nerve cords and the lumen of the excretory duct on the ventral cord. (Courtesy Dr. M. Dale Little.)

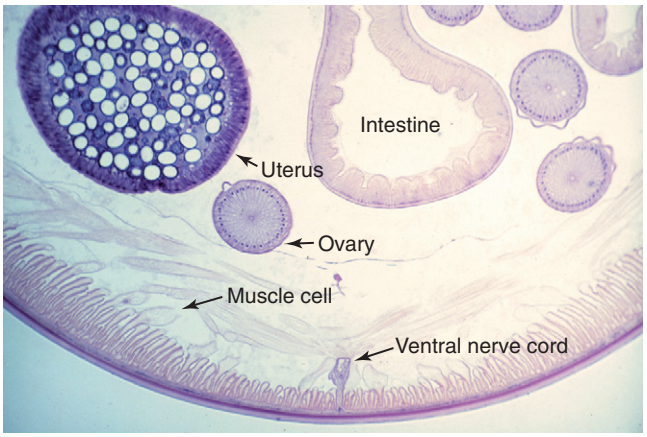

FIGURE 8-97. *Ascaris suum* female (×10) section through midbody showing the many-celled intestine, the ovary with a central rhachis, the uterus full of developing eggs, the ventral nerve cord, and the cytoplasmic portions of the muscle cells extending to the ventral nerve cord. (Courtesy Dr. M. Dale Little.)

Dracunculoidea, and Filarioidea. The spirurids represent an extremely diverse group of nematodes that parasitize a wide range of hosts and anatomic locations in those hosts. As adults, spirurids range in size from thin and threadlike in the case of *Gongylonema,* to stout, robust worms such as *Gnathostoma,* to incredibly long worms in the case of *Dracunculus*. Some species localize in the lumen of the gut, others are associated with the wall of the gut, and others have moved away from the gut entirely. Despite this variability, a number of similarities in both biologic and morphologic aspects have been noted. As a group the spirurids use insects as intermediate hosts. In many species, small, thick-shelled eggs containing a well-developed larva are passed in the feces and are ingested by an insect intermediate host. In the Dracunculoidea, female worms migrate to the surface and release first-stage larvae into water, where they are ingested by copepods. In the Filarioidea, not only have the adult worms moved away from the gut, but the

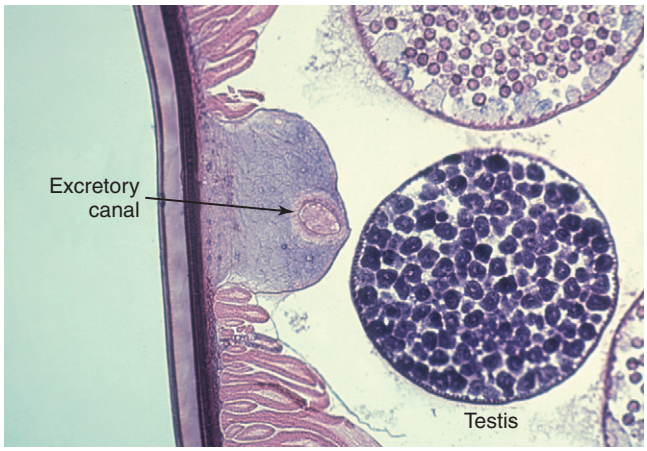

FIGURE 8-98. *Ascaris suum* male (×20) showing a section through the lateral cord with the prominent excretory canal and a section through several loops of the testis. (Courtesy Dr. M. Dale Little.)

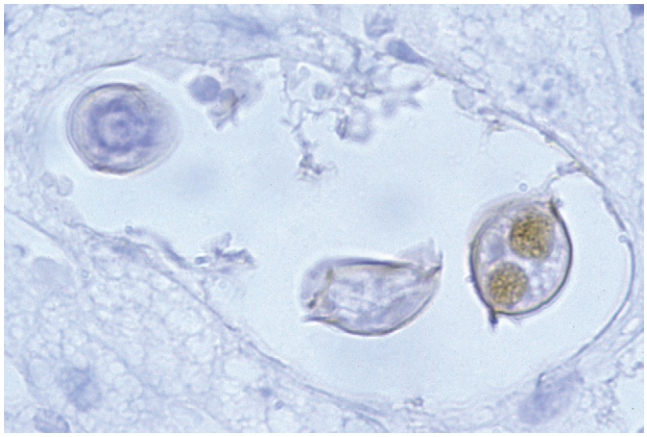

FIGURE 8-99. *Toxocara canis* larva (×650) in an experimentally infected mouse, stained with a monoclonal antibody using immunoperoxidase to show the location of the large branches of the excretory cell that extend posteriad from the single excretory cell along each of the two lateral cords *(right section)*. The *section on the left* is through the esophagus and is anterior to the branching, and the *central section* is posteriad to the termination of the excretory columns.

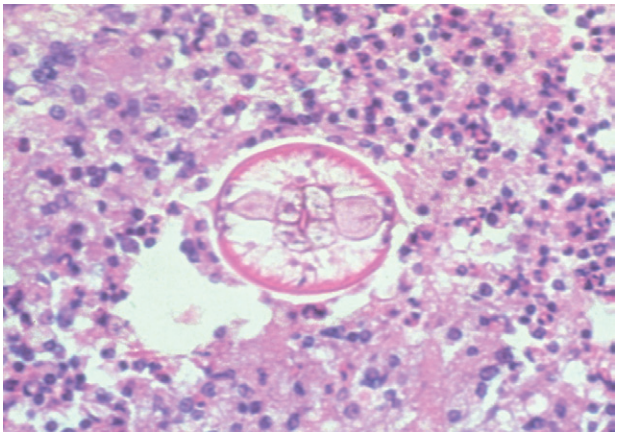

FIGURE 8-100. *Baylisascaris procyonis* (×400) in the brain of a porcupine showing the large excretory columns, the intestine with a patent lumen, and lateral alae.

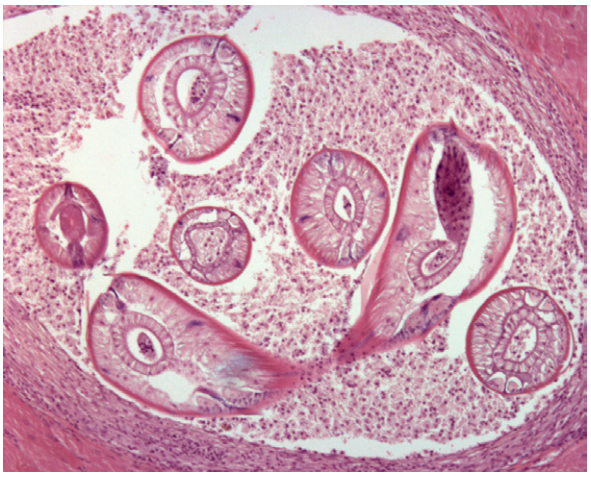

FIGURE 8-101. *Lagochilascaris sprenti* (×100) larvae in an experimentally infected mouse. These ascarid larvae grow quite large, and sections can be seen through the esophagus and through numerous levels of the many-celled intestine.

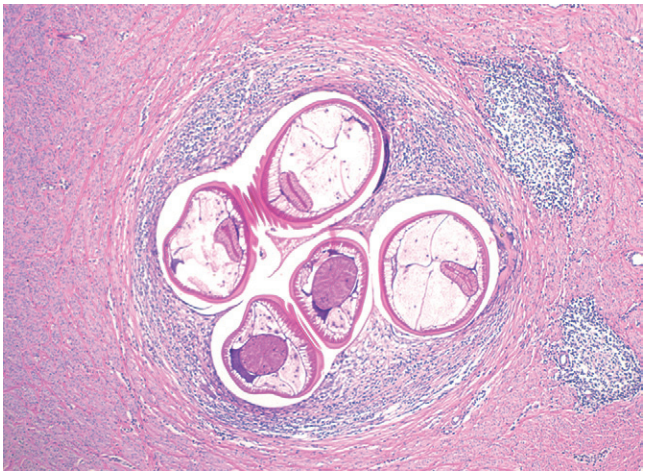

FIGURE 8-102. Spirurid larva in a granuloma in the uterine wall of a rhesus monkey (×36).

female worms release motile larvae called **microfilariae** that may circulate in the blood or reside in the skin and are picked up by blood-sucking insects that serve as intermediate hosts. Features of spirurids in tissues include a cuticle that often has some ornamentation, including spines, bosses, transverse striations, or longitudinal ridges. The esophagus tends to be long and divided into anterior muscular and posterior glandular portions; the glandular portion is very cellular and stains much more intensely. The general spirurid intestine is often large and folded on itself and is composed of many cells, often with the nuclei arranged in a row, a prominent brush border, but a rather weak basement membrane. The lateral cords are prominent, and the musculature is polymyarian-coelomyarian in nature. In most spirurids, female worms contain small, thick-shelled eggs containing a larva. In the case of the Dracunculoidea and Filarioidea, large numbers of larvae or microfilariae, respectively, are contained in utero. This combination of features makes the spirurids relatively distinctive in sections.

Spirurid larvae are, on occasion, seen in tissue sections and have some of the same morphologic features seen in adult worms, including polymyarian-coelomyarian muscle cells, prominent lateral cords, and a distinctive intestine composed of many tall columnar cells (Figure 8-102).

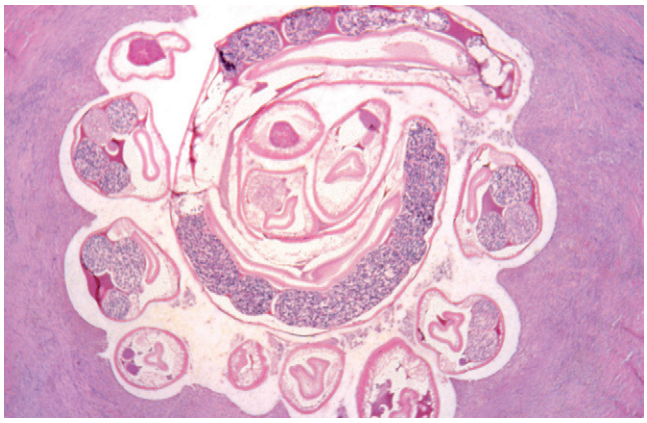

FIGURE 8-103. *Spirocerca lupi* (×22) in a nodule in a dog. (Case described in Georgi ME, Han H, Hartrick DW: *Spirocerca lupi* [Rudolphi, 1809] nodule in the rectum of a dog from Connecticut, *Cornell Vet* 70:43, 1980.)

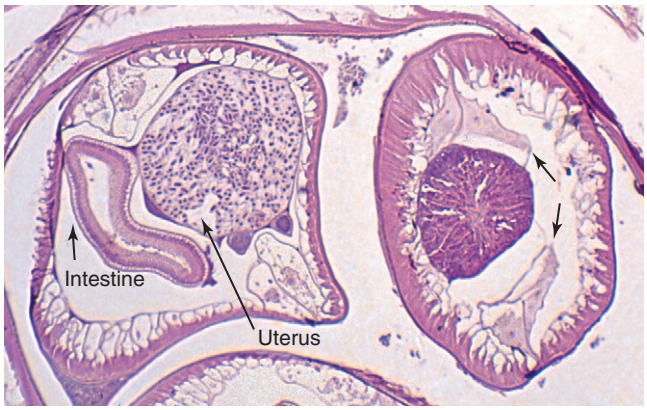

FIGURE 8-104. *Spirocerca lupi* (×50) sections through the region of the glandular esophagus showing the lateral cords *(arrows)* projecting into the pseudocoelom, and showing the nature of the intestine, with a prominent brush border and many cells with nuclei lined up in a row and uterus filled with tiny eggs.

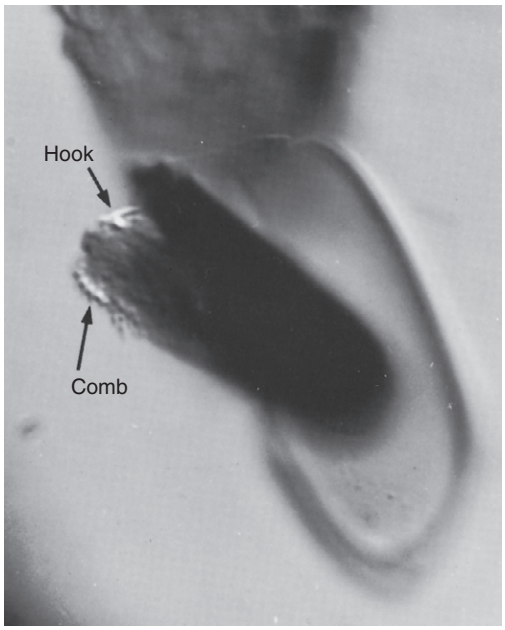

FIGURE 8-105. *Spirocerca lupi* egg with broken shell from which the larva projects (×1800).

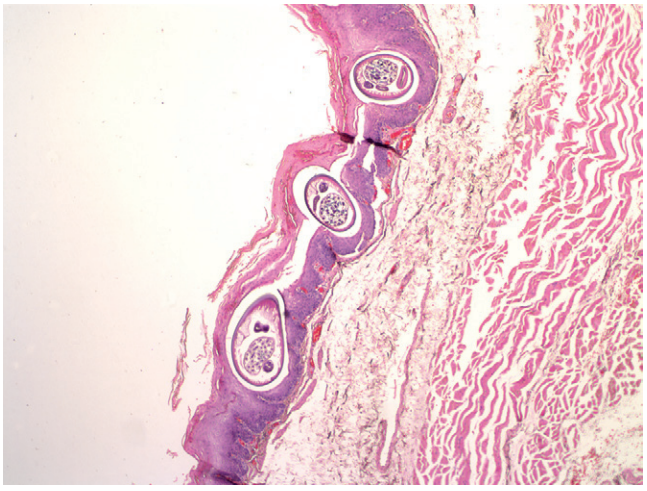

FIGURE 8-106. *Gongylonema* (×22), cross-section through gravid female embedded in esophagus of stumptail macaque monkey.

Spirocerca lupi (Figures 8-103 to 8-105) provides an example of the superfamily Spiruroidea. The adults typically are found in nodules in the wall of the esophagus and stomach, and sometimes in the wall of the aorta or rectum. In cross-section they are characterized by large lateral cords that project into the body cavity; an intensely stained glandular esophagus (see Figure 8-104); an intestine with a prominent brush border and many cells with the nuclei lined up in a row, which gives the appearance of three layers; a uterus filled with small eggs containing intensely stained larva; and coelomyarian-polymyarian muscle cells (see Figures 8-103 and 8-104). The larvae have hooks and combs associated with the stoma, although these structures require oil immersion microscopy to be seen properly (see Figure 8-105).

The genus *Gongylonema,* another member of the Spiruroidea group, is encountered in the tissues of animals with some frequency and has several distinctive morphologic features. Typically found threaded in the mucosa of the mouth, esophagus (Figure 8-106), or stomach, the members of *Gongylonema* have characteristic spirurid features in section, including a divided esophagus, a polymyarian-coelomyarian musculature, and the presence of small, thick-shelled, embryonated eggs (Figure 8-107). *Gongylonema* organisms are distinctive, however, in that the anterior end has large cervical alae and is covered with cuticular plaques or bosses on the anterior end, and the lateral cords are asymmetric (see Figure 8-107).

Dracunculus insignis, of the superfamily Dracunculoidea, is characterized by flat lateral cords separating semilunar dorsal and ventral muscle fields composed of coelomyarian-polymyarian muscles, a very reduced intestine, and a large uterus filled with larvae (Figure 8-108).

Members of the superfamily Filarioidea, although they have many typical spirurid features in section, are relatively distinct. Most distinctive is their location, as adults, in virtually all tissues except the gut. Filarids range greatly in size, from some that are only 1 or 2 cm in length to others such as *Dirofilaria immitis,* in which the female worm may reach 30 cm in length by 1 mm in diameter; however, all tend to be slender. The cuticle may be thin or thick and in some groups contains distinctive ridges or striations. The musculature is coelomyarian-polymyarian, the esophagus may be divided but is generally not as prominent as in other spirurids,

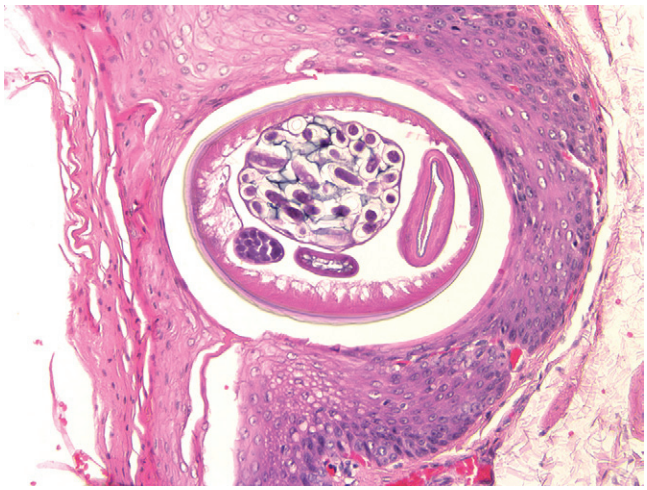

FIGURE 8-107. *Gongylonema* (×125) at a higher magnification showing the presence of unequal lateral cords and embryonated eggs, many containing larvae.

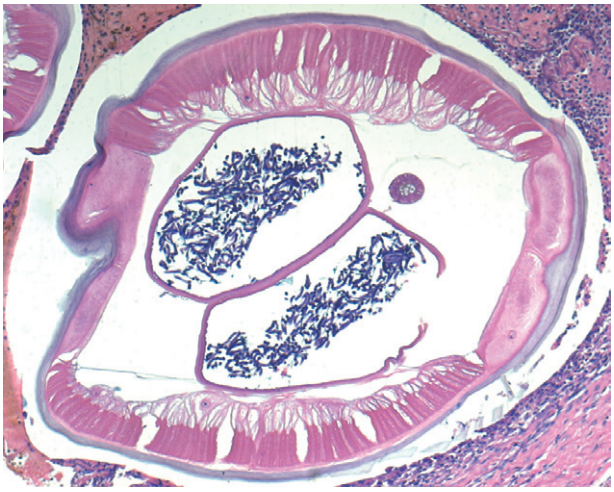

FIGURE 8-109. *Dirofilaria immitis* (×65) in the pulmonary artery of a dog. The thick, smooth cuticle, large coelomyarian-polymyarian muscles, small intestine, and paired uteri are evident.

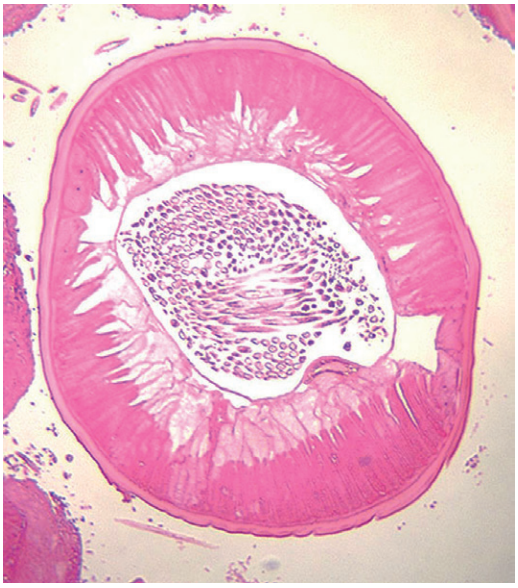

FIGURE 8-108. *Dracunculus insignis* (×60). Cross-section of *Dracunculus insignis* in the subcutaneous tissue of a raccoon. The two lateral cords on either side of the body and heavy dorsal and ventral muscle bands surrounding the uterine tube packed with larvae are evident.

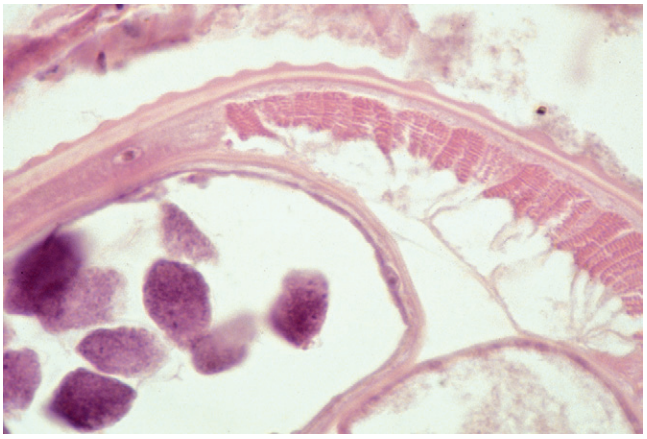

FIGURE 8-110. *Dirofilaria tenuis* (×220), high-power cross-section through a portion of the subcutaneous tissues of a raccoon. The longitudinal ridges on the surface of the cuticle are evident.

and the intestine is typically a simple tube. One of the most characteristic features of filarids is the presence of microfilariae filling the uterus. Many species of filaria infect animals, and several examples will serve to illustrate the group.

D. immitis, the dog heartworm, is well recognized for the disease it produces in canines, felines, and humans. The adult worms live in the circulatory system, typically in the chambers and great vessels of the heart. The worms, as just stated, are large; have a thick, multilayered but smooth cuticle; have prominent coelomyarian-polymyarian muscles; have broad lateral cords; have a weak intestine; and, in the female, have paired uteri filled with microfilariae (Figure 8-109). Many other *Dirofilaria,* such as *Dirofilaria repens* of the dog and *Dirofilaria tenuis* of the raccoon, live in subcutaneous locations and are distinctive in that the cuticle has prominent longitudinal ridges marked with transverse striations, giving the external surface a beaded or corn-row appearance (Figure 8-110).

The genus *Onchocerca,* another common filarial infection of domestic animals, provides a good example of specific filarial anatomy in section. Adult female *Onchocerca* organisms are thin and extremely long and have distinctive cuticular structures. These worms possess external circular ridges and striae in the inner layer of the cuticle (Figure 8-111). Not only are these ridges and striae specific to the genus *Onchocerca,* but the number of striae per ridge has been shown to have great value in distinguishing various species within the genus. Also distinctive of adult female *Onchocerca* organisms are the muscle cells, which often appear to be weak and poorly developed, and a prominent amount of hypodermal tissue, even underlying the muscle cells (Figure 8-112). As far as it is known, adult *Onchocerca* organisms inhabit dense connective tissue, are tightly coiled, and, in some species, form distinct fibrous nodules.

Enoplida

Trichinelloidea

This group contains the trichinelloids, the trichuroids, the capillarids, and the trichosomoids. In this group the most characteristic feature, both grossly and in section, is the **stichosome esophagus,** a small cylindric tube surrounded by individual **stichocytes** that compose the stichosome. The other distinctive feature of these

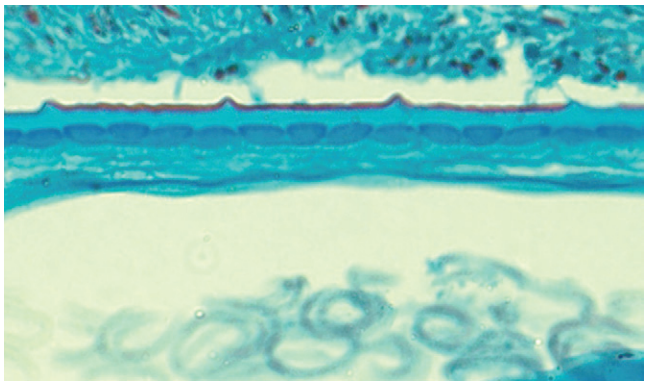

FIGURE 8-111. *Onchocerca cervicalis* (×560) female in the nuchal ligament of a horse. The outer circular cuticular ridges and striae in the inner layer of the cuticle are evident. In *O. cervicalis* there are four striae per ridge—one directly under and three between each ridge.

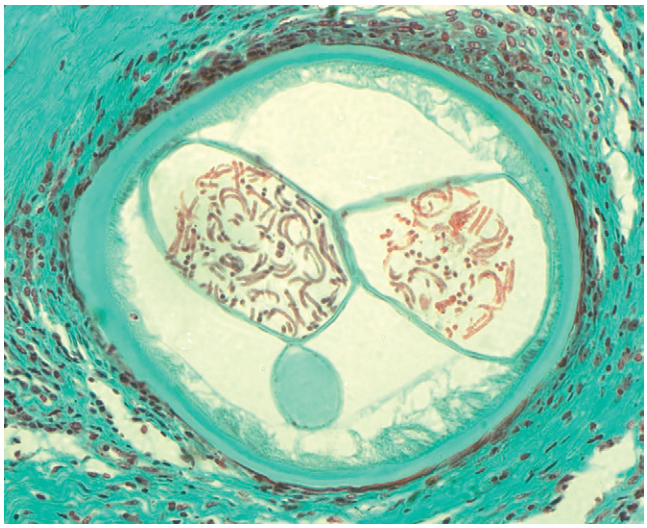

FIGURE 8-112. *Onchocerca cervicalis* (×340) female in cross-section in the nuchal ligament of a horse. The thick cuticle, prominent hypodermal tissue between the cuticle and muscle layers, and wispy muscle cells are all prominent, as are the paired uteri and small intestine.

worms in section is the presence of a bacillary band(s). The **bacillary band** is a specialized section of the cuticle and hypodermis that includes specialized hypodermal gland cells. In *Trichuris* there is a single bacillary band in the esophageal region (Figure 8-113), whereas in *Trichinella* and capillarids, two bacillary bands run the length of the esophagus. In addition, the female reproductive tract is a single tube, the anus is usually terminal, the muscles are coelomyarian-polymyarian, and the eggs typically have bipolar prominences (plugs) and are frequently in an unembryonated state when passed or seen in tissues. Occasionally, eggs may develop and hatch in utero, as in the case of *Trichinella*. The first-stage larva is typically the infective stage for the definitive host. Most worms in this group display a high order of site specificity and, except for *Trichinella,* a high order of host specificity as well. The host-organ listings should prove helpful in dealing with this group of parasites.

Adult *Trichuris,* as their common name *whipworm* suggests, have a whip-shaped body. The thin "whiplash" anterior portion is threaded through the epithelium of the large intestine, whereas the stout "handle" portion normally lies free in the lumen (Figure 8-114). Immature *Trichuris* lie entirely within the mucosa and are of uniform diameter.

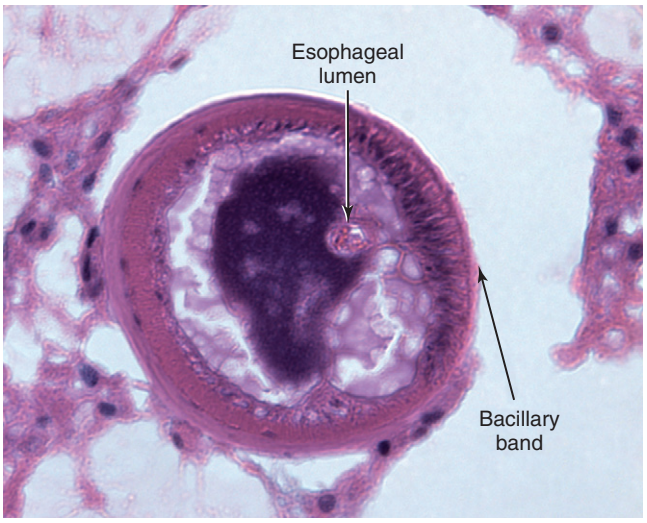

FIGURE 8-113. *Trichuris vulpis* (×500) in the cecum of a dog showing a cross-section of the esophageal region.

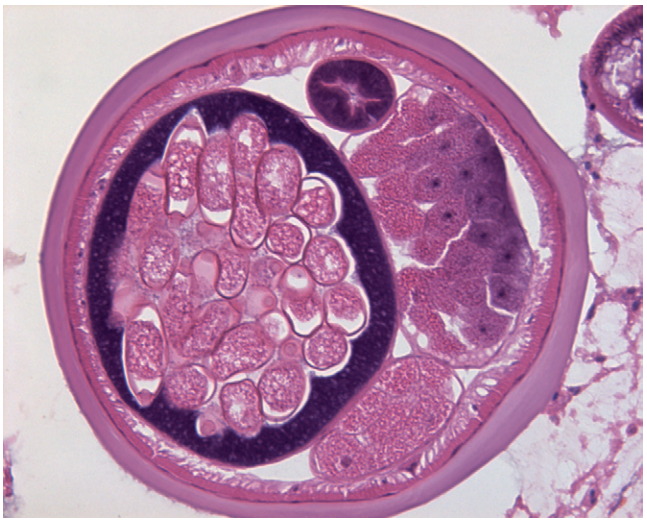

FIGURE 8-114. *Trichuris vulpis* (×250) in the cecum of a dog showing sections through the very small intestine and the thick-walled uterus filled with typical *Trichuris vulpis* eggs.

Adult *Trichinella* are found threaded in the mucosa of the small intestine (Figure 8-115), and in tissue section the adults resemble *Strongyloides,* except that they have a tubular esophagus embedded in the stichosome, male worms exist, and in female worms the uterus contains prelarvae instead of segmenting eggs. *Trichinella* larvae are found characteristically coiled in a "nurse cell" (Figure 8-116) in striated muscle, and they are characterized by stichocytes surrounding the esophagus. Capillarids infecting the intestinal mucosa are somewhat larger than *Trichinella* and have eggs with bipolar plugs in their uteri.

The presence of single-celled eggs with bipolar plugs in the uterus is the best criterion for identifying capillarids in tissue sections (Figure 8-117). *Trichuris* species have larger eggs and are found only in the large intestine of mammals, practically the only epithelium in which capillarids will not be found.

Other common but less frequently seen members of this group include *Anatrichosoma* in the nasal mucosa or palate of primates and marsupials (Figures 8-118 and 8-119) and *Trichosomoides* in the bladder of rats (Figure 8-120). Both have larvated eggs with bipolar plugs, and two or one bacillary band, respectively.

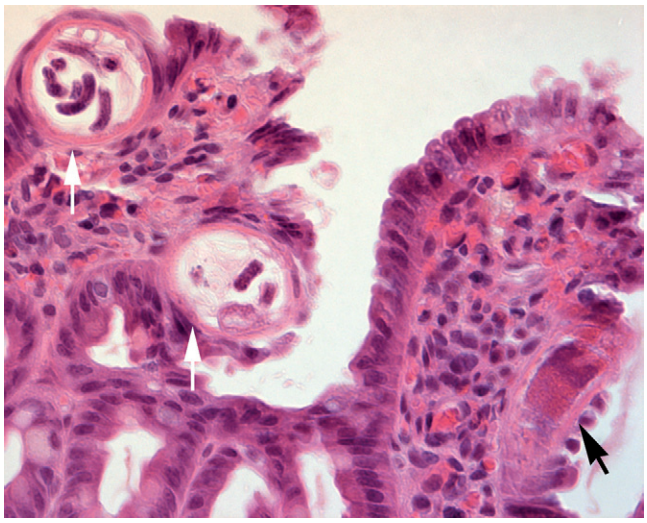

FIGURE 8-115. *Trichinella spiralis* adult in the mucosa of the small intestine of a rat (×480). Two cross-sections through a female *(white arrows)* contain prelarvae and a longitudinal section through the stichosome esophagus *(black arrow)*.

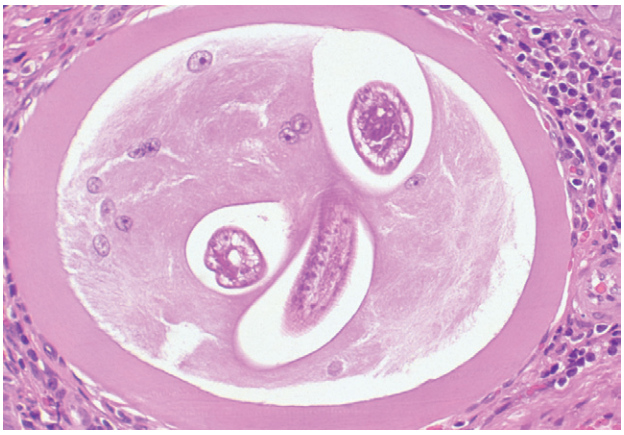

FIGURE 8-116. *Trichinella spiralis* first-stage larvae in a skeletal muscle fiber of a cat (×425).

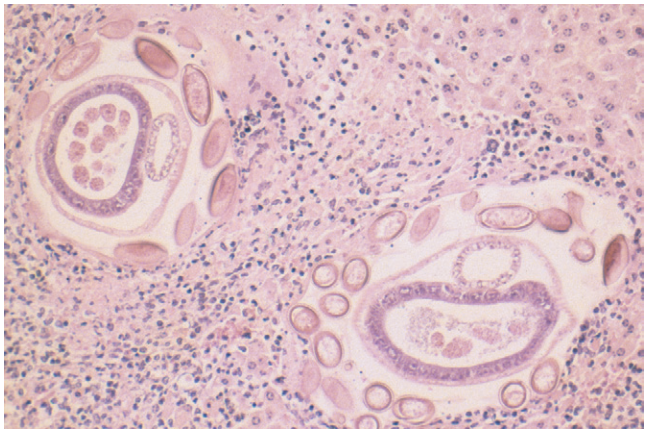

FIGURE 8-117. *Calodium (Capillaria) hepaticum* in the liver of a rat (×360). Eggs with bipolar plugs are visible in the tissue surrounding the worm.

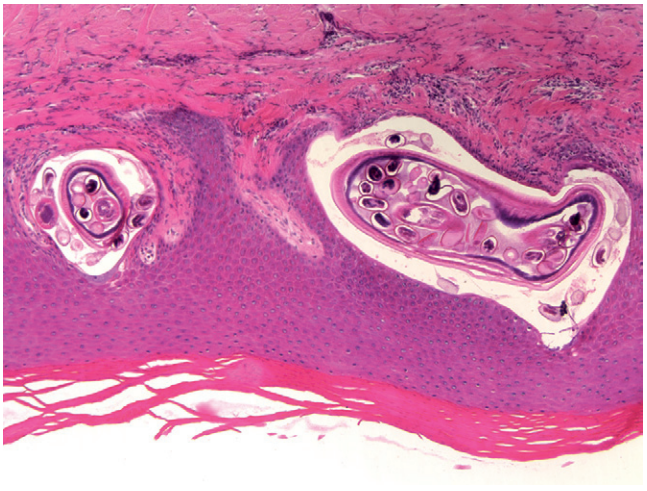

FIGURE 8-118. *Anatrichosoma buccalis.* Cross-section through gravid female *Anatrichosoma buccalis* embedded in palate of opossum (×60).

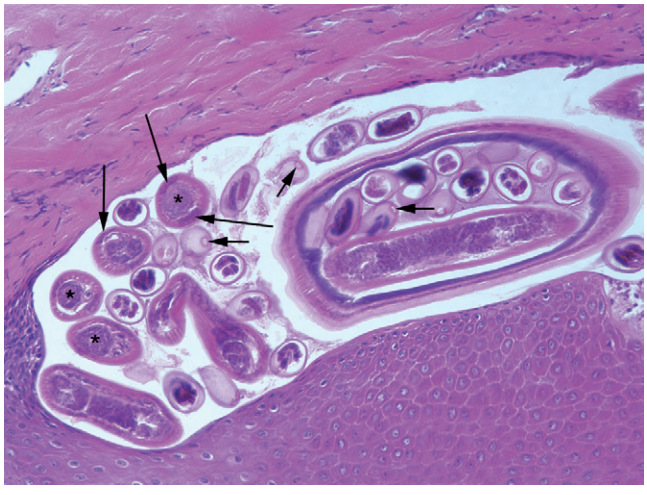

FIGURE 8-119. *Anatrichosoma buccalis*. Higher magnification of worm in Figure 8-114, illustrating stichocytes *(asterisks)*, bacillary bands *(long arrows)*, and polar plugs *(short arrows)* in the eggs (×125).

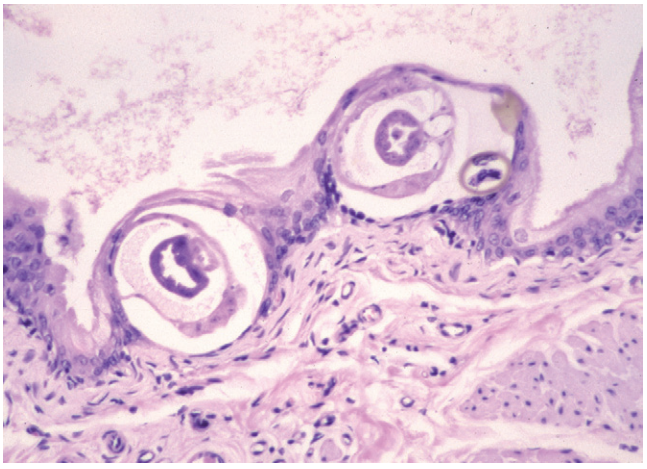

FIGURE 8-120. *Trichosomoides crassicauda* in the urinary bladder mucosa of a rat (×480).

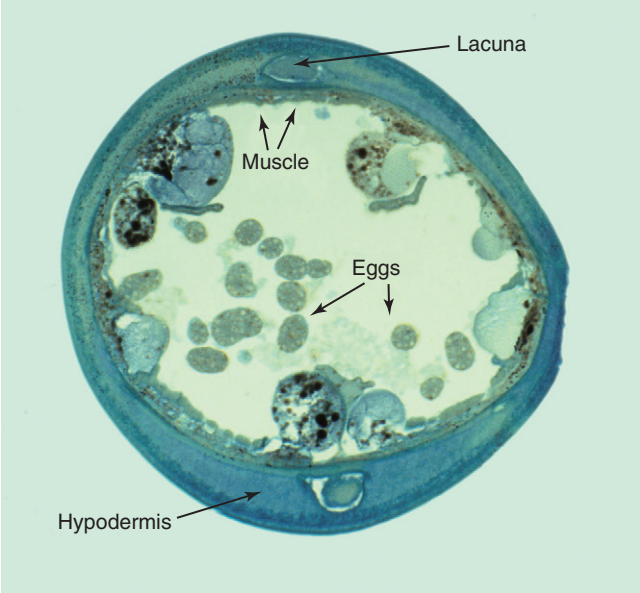

FIGURE 8-121. Cross-section of a female acanthocephalan, *Neoechinorhynchus* (×150). "Eggs" are actually clusters of oogonia called *ovarian balls* that float free in the body cavity.

Anatrichosoma, although occurring in the same general location (i.e., mouth and throat) as *Gongylonema,* can easily be distinguished on morphologic features, including smaller diameter, presence of stichosomes and bacillary bands in the anterior end, and polar plugs in the eggs (see Figure 8-119).

ACANTHOCEPHALANS

Adult male and female Acanthocephala are pseudocoelomates that live in the intestine of vertebrates, where they gain nutrients through their external covering (i.e., they have no intestinal tract; Figure 8-121). Hosts include all vertebrate classes, fish, amphibians, reptiles, birds, and mammals. Eggs passed in feces are ingested by the intermediate host, an arthropod; and infection is acquired through ingestion of the intermediate host. The wormlike adults possess a spiny proboscis that is used for attachment to the intestinal mucosa and can be retracted inside the body; this why they are often called *thorny-* or *spiny-headed worms* (Figure 8-122). The fluid-filled pseudocoelom contains cells of the reproductive system, testes, and cement glands in males. Females have a reproductive system wherein balls of ovarian tissue float about and sperm migrate into the pseudocoelom to fertilize the eggs. A "uterine bell" sorts the eggs according to their developmental stage, and mature eggs, containing a larva called an *acanthor,* pass into the uterus, out of the body, and into the feces. The intermediate host is typically an arthropod in which a stage called a cystacanth develops; the cystacanth can sometimes use vertebrate paratenic hosts, and this is typically the stage that will be seen in histologic sections (Figure 8-123).

The body wall is thick and multilayered and is very distinctive in histologic sections. There is an outer tegument (outer plasma membrane and three fibrous layers that contain lacunae [channels] that may serve as a means of moving nutrients around the body), a thin "dermis" layer, and a layer of circular and longitudinal muscle tubules that are highly distinctive. In the cystacanth there are no reproductive organs, but there are two lemnisci—muscular and glandular structures that serve to evert and retract the thorny proboscis. The thick hypodermis lying external to the muscle layer provides the major clue as to the identity of a cystacanth.

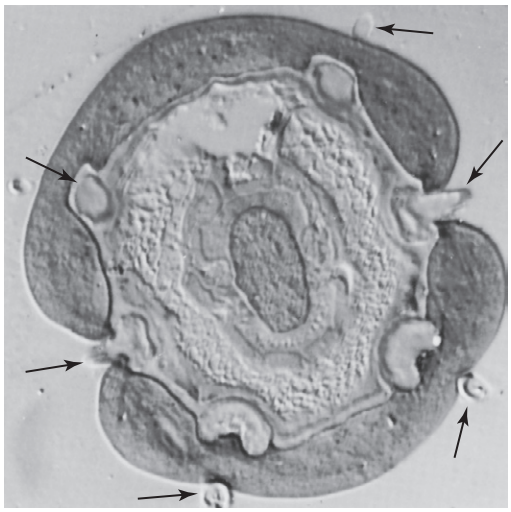

FIGURE 8-122. *Neoechinorhynchus*. Cross-section through the proboscis showing hooks *(arrows)* (×320).

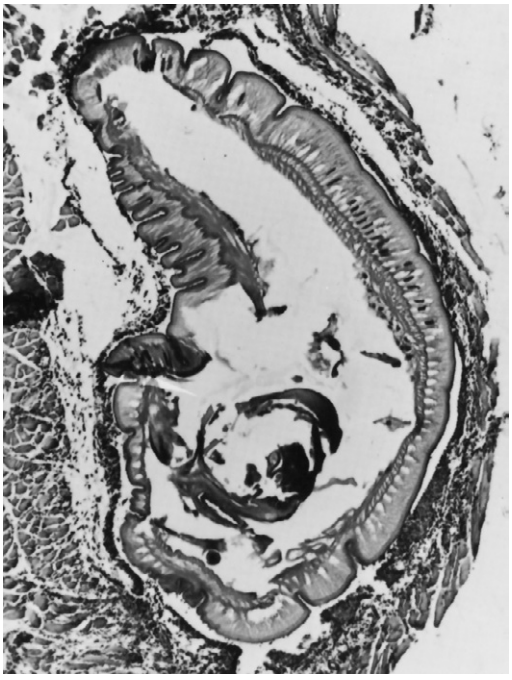

FIGURE 8-123. *Macracanthorhynchus ingens*. Cystacanth in skeletal muscle of a golden hamster *(Mesocricetus auratus)* (×66). (Courtesy Dr. G.R. Fahnestock.)

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CHAPTER 9

Vaccinations

Marshall W. Lightowlers

Vaccines are biologic preparations that improve immunity to disease. The terms *vaccination* and *immunization* are used interchangeably. Most vaccines incorporate a source of antigen that is specific to a particular pathogen. Before the discoveries of Edward Jenner and Louis Pasteur, it had been recognized that for some diseases, those that recovered were immune to reinfection. This led to practices whereby individuals were deliberately exposed to virulent pathogens so as to induce subsequent immunity—at least in those that survived the vaccination! Examples include variolation for small pox and leishmanization for cutaneous leishmaniasis in humans. Later the practice was used to prevent tick fever in cattle and is used even now, either as a carefully controlled exposure to virulent parasites for prevention of coccidiosis in chickens, or together with chemotherapy for poultry coccidiosis and also for East Coast fever in cattle in parts of Africa. The term *vaccine* was coined by Louis Pasteur in honor of Edward Jenner's discovery of the protective efficacy of cowpox infection against smallpox (Levine and Lagos, 1997). The potential for vaccines to transform man's ability to overcome some diseases was recognized from the earliest times and was embodied in Thomas Jefferson's 1806 letter to Edward Jenner, in which he wrote, "Future nations will know by history only that the loathsome smallpox has existed" (Hopkins, 1983). Vaccination has also transformed disease control for many animal diseases, playing a pivotal role in eradication of the first veterinary disease, rinderpest, declared in June 2011.

An advance on the use of virulent organisms as vaccines has been the use of attenuated pathogens; notable examples include the Sabin vaccine for polio in humans. Several vaccines of this type are in current commercial use for diseases of veterinary importance, including some parasitic diseases. Live vaccines provide advantages in that the immunity they stimulate is generally strong; however, disadvantages are associated with aspects such as the vaccine strain reverting to virulence, possibility for inadvertent transmission of contaminant microorganisms, particularly viruses, and the need to maintain a cold chain vaccine so as to maintain the vaccine's infectivity. For safety and quality control reasons, there is a preference for vaccines to contain nonliving antigens, preferably defined antigens.

As a general rule, eukaryotic parasites do not stimulate a high level of immunity, and persistent infections and reinfections are the norm. In comparison with bacterial and viral pathogens, vaccination has proved ineffective against most parasitic diseases. Nevertheless, there are notable exceptions whereby the existence of naturally acquired immunity has favored successful vaccine development. The vaccines described here are currently registered commercial vaccines. A number of commercial antiparasite vaccines have been produced but are no longer marketed, such as Jensen-Salsbery Laboratories' Canine Hookworm Vaccine, the Fort Dodge Sarcocystis Nerona Vaccine, and Intervet's Neospora Caninum Vaccine. These vaccines are not considered here. Brief mention is made of vaccines for which commercial application appears to be imminent. A summary of commercial antiparasite vaccines is provided in Table 9-1. There has never been a commercial antiparasite vaccine for use in humans.

PROTOZOAL INFECTIONS

COCCIDIOSIS VACCINES

The first commercial antiparasite vaccines were developed for use against coccidiosis in chickens. Development of these vaccines was made possible following the realization that initial exposures of birds to the parasites stimulated a high level of resistance to challenge infections (Beach and Corl, 1925; Tyzzer, 1929), and that this could be achieved without causing serious pathology or death through the initial administration of low numbers of oocysts (Dickinson, 1941; Johnson, 1927). In practice, one of the most challenging aspects of applying this knowledge to the development of commercial vaccines involved establishing procedures by which to reliably deliver oocysts to the birds en masse without a high risk of inducing clinical disease (see reviews by Shirley et al, 2005; Williams, 2002b).

The earliest commercial use of vaccines based on live wild-type parasites incorporated the concomitant application of chemotherapeutic agents to prevent clinical disease in some birds, and the practice continues in some circumstances to the present day. Safer live vaccines incorporating attenuated parasites followed, and the first commercial coccidiosis vaccine comprising nonliving antigens has become available. Effective vaccines have been developed for coccidiosis in both chickens and turkeys. Commercially available vaccines for coccidiosis are summarized in Table 9-2. Anticoccidial vaccination is a complex topic, wherein different vaccine formulations are available comprising different combinations of *Eimeria* species for application in particular target birds (breeders, layers, broilers) and for different target species (chickens and turkeys). More extensive discussions concerning the various vaccine formulations can be found in Shirley et al (2005) and Williams (2002a, 2002b).

TABLE 9-1 Commercial Antiparasite Vaccines*

Parasite	Vaccine Recipient	Registered Name	Company†	Antigen Type
ANTIPROTOZOAL				
Babesia bovis, B. bigemina	Cattle	Numerous‡	Local§	Live attenuated
Babesia canis	Dog	Pirodog	Merial	Subunit
Babesia canis, B. rossi	Dog	Nobivac Piro	Intervet International bv	Subunit
Eimeria sp.‖	Chicken	Coccivac, Immunocox, Nobilis COX ATM, others	Merck Animal Health, Vetech Laboratories, Intervet International, others	Live nonattenuated
Eimeria sp.‖	Chicken	Paracox, Livacox, Eimeriavax, others	Biopharm, Schering-Plough Animal Health, Bioproperties, others	Live attenuated
Eimeria sp.‖	Chicken	CoxAbic	Phibro Animal Health Corporation	Subunit
Eimeria sp.‖	Turkey	Coccivac, Immunocox	Merck Animal Health, Vetech Laboratories	Live nonattenuated
Leishmania donovani	Dog	Leishmune	Pfizer	Subunit
Leishmania donovani	Dog	Leish-Tec	Hertape Calier	Recombinant subunit
Theileria annulata	Cattle	Numerous	Local	Live attenuated
Theileria parva	Cattle	Several	Local	Live nonattenuated
Toxoplasma gondii	Sheep	Toxovax	Intervet	Live attenuated
Trichomonas foetus	Cattle	TrichGuard	Boehringer Ingelheim	Inactivated parasites
ANTHELMINTH				
Dictylcaulus viviparus	Cattle	Bovilis, Huskvac	Intervet	Live attenuated
ANTITICK				
Boophilus microplus	Cattle	Gavac	Heber Biotec S.A.	Recombinant subunit

*Marketing and availability of these vaccines are subject to commercial decisions at any time, hence the list cannot be considered to be accurate or comprehensive on an ongoing basis. These vaccines were being commercially marketed to some extent around the time of writing or in the recent past.
†Marketing arrangements may lead to the sale of vaccines under license in particular regions, hence the company specified may not be responsible for marketing the indicated vaccine in some areas.
‡Vaccines prepared using similar methods in a number of countries.
§A number of different institutes or companies involved with manufacture and marketing.
‖Up to eight different species of *Eimeria* may be included in a combined vaccine. Vaccines may be marketed in a number of different variants containing different combinations of oocysts of various species.

A variety of technologies have been developed for delivery of live coccidiosis vaccines, including administration in drinking water, spray on feed or within an edible gel, and administration via intraocular, intra-yolk sac, and in ovo routes. Consideration of various methods can be found in an article by Williams (2002a).

Live Coccidiosis Vaccines Containing Wild-Type Strains

The first anticoccidial vaccines were based on controlled exposure of birds to live wild-type parasites. Much of the following information has been derived from a comprehensive review of the history of the development of coccidial vaccines (Williams, 2002b). The first commercially successful coccidiosis vaccine was developed by S.A. Edgar of Auburn University, in Auburn, Alabama, apparently with the original vaccine batches prepared in the basement of Edgar's home! This univalent vaccine containing *E. tenella* oocysts was marketed by Dorn and Mitchell, Inc., from 1952 as DM Cecal Coccidiosis Vaccine. It was soon superseded by a multivalent vaccine containing *E. acervulina, E. hagani*, *E. necatrix,* and *E. tenella,* marketed as Coxine. Importation of the vaccine into The Netherlands by the company Nobilis saw it marketed under the trade name Nobilis, which is still used for some products today. A new formulation in which *E. hagani* was replaced by *E. maxima* was subsequently marketed in Europe as CocciVac, except in Belgium and The Netherlands, where it was marketed as NObiCOX. Later the trade names CocciVac and Coccivac came to be used in many markets. (The species named *E. hagani* and *E. mivati* are regarded by many as invalid [Shirley et al, 1983]).

Following the release of the first multivalent vaccine, Coxine, now Coccivac, similar vaccines have come onto the market. The first of these was Immucox, marketed from 1985 by Vetech Laboratories, initially in Canada and now registered and sold in 29 countries. The method of application of these vaccines is different. Coccivac is applied via a spray in the hatchery or is sprayed onto food, and Immucox is applied in water together with thickening agents so as to reduce sedimentation, or in an edible gel. At the present time, ADVENT, Coccivac-B (Figure 9-1), Coccivac®-D, Immucox (Figure 9-2), Immucox EM1, and Nobilis COX ATM vaccines all comprise nonattenuated oocysts from a variety of *Eimeria* species. The Coccivac-D formulation, for use in birds grown beyond 8 weeks of age, such as layer or broiler-breeder replacements, contains oocysts from eight separate species of *Eimeria.* As well as containing oocysts from several different species, the Immucox and Nobilis COX ATM vaccines include oocysts from antigenically different strains of *E. maxima*. Use of anticoccidials is recommended as a matter of course in some vaccination programs; for example, with Nobilis COX ATM, ionophorous anticoccidials such as monensin, narasin, or salinomycin

TABLE 9-2 Coccidiosis Vaccines for Chickens

Vaccine (Manufacturer)	Parasite Species[†]	Vaccine Type[‡]	Bird Type	Administration	First Registered
ADVENT (Novus International)	*E. ac, E. ma, E. te*	WT	Broilers	Hatchery spray, water or feed	2002 (USA)
Coccivac-B (Merck Animal Health)	*E. ac, E. ma, E. miv, E. te*	WT	Broilers	Hatchery spray or feed	1952 (USA)
Coccivac-D (Merck Animal Health)	*E. ac, E. br, E. ha, E. ma, E. miv, E. ne, E. pr, E. te*	WT	Breeders/layers	Ocular, hatchery spray or feed	1989 (USA)
CoxAbic (Phibro Animal Health Corporation)	*E. ma*	NL	Breeders	Intramuscular	2002 (Israel)
Eimerivac Plus (Guangdong Academy of Agricultural Sciences)	*E. ac, E. ma, E. mi, E. ne, E. te*	A (P)	Breeders/layers	Feed spray or water	2013? (China)[‖]
	E. ac, E. ma, E. mi, E. te	A (P)	Broilers	Feed spray or water	2013? (China)[‖]
Eimeriavax 4m (Bioproperties Pty)	*E. ac, E. ma, E. ne, E. te*	A (P)	Breeders/layers	Ocular, spray	2003 (Australia)
Eimeriavax 3m (Bioproperties Pty)	*Eace, Emax, Eten*	A (P)	Broilers	Ocular, spray	2010 (Australia)
Hipracox Broilers (Laboratorios Hipra, S.A.)	*E. ac, E. ma, E. mi, E. pr, E. te*	A (P)	Broilers	Water	2006 (UK)
Immucox I (Vetech Laboratories)	*E. ac, E. ma, E. ne, E. te*	WT	Broilers	Water or oral gel	1985 (Canada)
Immucox II (Vetech Laboratories)	*E. ac, E. br, E. ma, E. ne, E. te*	WT	Breeders/layers	Water or oral gel	1985 (Canada)
Inmuner GEL-Coc (Vacunas Inmuner)	*E. ac, E. br, E. ma, E. te*	A (P)	Broilers, breeders/ layers	Oral	2005 (Argentina)
Inovocox, Inovocox EM1,[§] (Pfizer)	*E. ac, E. ma* ×2, *E. te*	WT	Broilers	In ovo via the Embrex Inovoject system	2006 (USA)
Livacox Q (Biopharm)	*E. ac, E. ma, E. ne, E. te*	A (P, EA)	Breeders/layers	Hatchery spray, water or feed	1992 (Czech Republic)
Livacox T (Biopharm)	*E. ac, E. ma, E. te*	A (P, EA)	Broilers	Hatchery spray, water or feed	1992 (Czech Republic)
Nobilis COX ATM (Intervet International bv)	*E.ac, E. ma* ×2, *E. te*	WT	Broilers	Water or feed	2001 (The Netherlands)
Paracox-8 (Schering Plough Animal Health)	*E. ac, E. br, E. ma* ×2, *E. mi, E. ne, E. pr, E. te*	A (P)	Breeders/layers	Water or feed	1989 (UK)
Paracox-5 (Schering Plough Animal Health)	*E. ac, E. ma* ×2, *E. mi, E. te*	A (P)	Broilers	Hatchery spray, water or feed	1989 (UK)
Supercox (Qilu Animal Pharmaceutical Company)	*E. ac, E. ma, E. te*	WT, A (P)	Broilers	Water	1996 (China)

*Information sourced from Williams, 2002a, 2002b; Shirley et al, 2005; and company-related sources.
[†]*E. ac, E. acervulina; E. br, E. brunette; E. ha, E. hagani; E. ma, E. maxima; E. mi, E. mitis; E. miv, E. mivati; E. ne, E. necatrix; E. pr, E. praecox; E. te, E. tenella.*
[‡]A, attenuated; EA, egg adapted; NL, nonliving; P, precocious; WT, wild-type.
[§]Inovocox is formulated with two strains of *E. maxima,* whereas Inovocox EM1 contains one strain.
[‖]Application for regulatory approval under consideration since 2010.

are recommended to be used in the feed during a period of 3 to 4 weeks after vaccination. In the case of the ADVENT vaccine, which is given during the first day of a chick's life, one of the options suggested by the manufacturer is that administration of the vaccine may be followed by the use of an anticoccidial drug in the grower diet, beginning after 10 days of age. With the Coccivac vaccines, it is recommended that postvaccination reactions are monitored closely and, if necessary, treatment initiated at 10 to 14 days' post vaccination with amprolium.

An in ovo vaccine delivery system has been developed by Pfizer for injection of oocysts into 18- to 19-day-old embryonated eggs using the Embrex Inovoject system. Two vaccines are licensed for use in the United States: Inovococ, containing oocysts of *E. acervulina, E. tenella,* and two strains of *E. maxima,* and a similar vaccine, Inovococ EM1, containing one strain of *E. maxima*. This delivery method provides potential advantages in consistency of delivery and early exposure of birds to the parasite.

Live, wild-type vaccines have also been produced for turkeys, such as Coccivac-T, which contains oocysts of *E. adenoeides, E. dispersa, E. gallopavonis,* and *E. meleagrimitis,* and Immunocox T, which contains oocysts of *E. adenoeides* and *E. meleagrimitis*.

One interesting outcome of the use of live vaccines such as Coccivac, which contain parasites highly sensitive to anticoccidial chemicals, is that a poultry production facility that has been using

FIGURE 9-1. One of the Coccivac range of coccidiosis vaccines for chickens. These vaccines are the current multivalent incarnations of the original *Eimeria tenella* vaccine developed in Auburn, Alabama, and first marketed in 1952. The vaccine incorporates virulent parasites, and chickens are protected following exposure to a closely controlled dose of sporocysts.

FIGURE 9-2. One of the several coccidiosis vaccines that have been developed using virulent wild-type parasites, Immucox was first marketed in 1985 by Vetech Laboratories in Canada and now is registered and sold in 29 countries.

anticoccidials and therefore is populated with drug-resistant parasites can be repopulated with drug-sensitive parasites through use of the vaccine (Chapman, 1994). This basic principle had been established by Jeffers (1976). Use of drug-sensitive vaccine isolates in turkeys can also restore drug sensitivity in cases where resistance has been a problem (Mathis and McDougald, 1989).

Live Coccidiosis Vaccines Incorporating Attenuated Parasites

A logical extension of the development of effective live vaccines for coccidiosis in poultry would be the development of nonliving vaccines, including recombinant vaccines. Notwithstanding substantial effort, no commercial recombinant vaccines have been introduced, and only relatively recently (2002), the first and only nonliving coccidiosis vaccine, CoxAbic, was registered. However, efforts to develop live vaccines comprising attenuated parasites have been remarkably successful. Two methods have been effective in achieving attenuation: selection for precocious development, and adaptation through growth in chicken embryos; the former was by far the most successful.

FIGURE 9-3. The Paracox vaccines for chickens contain viable sporocysts of a number of species of *Eimeria*. Unlike the vaccines containing wild-type parasites, Paracox contains parasites that have been attenuated by selection for precocious development. The Paracox vaccine was the first coccidiosis vaccine to be developed with the use of attenuated parasites.

While working with Hess & Clark, Inc., in Ashland, Ohio, Thomas K. Jeffers derived a line of *E. tenella* of reduced virulence by selecting for the earliest oocysts to appear in the feces during serial passage in chickens (Jeffers, 1975). The virulence of parasites derived from a single 10th-generation oocyst of a line selected for precocious development was significantly attenuated and stable through at least 25 generations of relaxed selection. The preciously developing parasites nevertheless stimulated immunity, which protected birds against wild-type strains, and Jeffers concluded his 1975 paper by describing the discovery with the prophetic statement, "Such attenuated strains have potential use in live coccidia vaccines." Remarkably the discovery was not patented (Williams, 2002b), and it was not until 1989 that a commercial vaccine based on precocious parasite lines, Paracox, became available (Shirley, 1989). Since that time, several different vaccines have been developed using similar principles, and these vaccines are now used extensively in many countries. Precocious lines of *Eimeria* have been developed for every commercially important species infecting chickens; Paracox-8 (Figure 9-3) contains oocysts of precocious parasites of *E. acervulina, E. brunetti, E. mitis, E. necatrix, E. praecox,* and *E. tenella,* plus oocysts of two antigenically different strains of *E. maxima*. In 2002 Paracox was recorded as registered in 33 countries (Williams, 2002b). Several other manufacturers produce vaccines incorporating precocious lines, as summarized in Table 9-2. Bioproperties Pty Ltd is in the process of seeking regulatory approval to market products similar to their Australian Eimeriavax vaccines (Figure 9-4) in Europe and other countries.

An alternative method by which to attenuate *Eimeria* has been through embryo adaptation. In 1965 Peter L. Long demonstrated that sporozoites of *E. tenella* could complete their life cycle in the chorioallantoic membrane of a chicken embryo (Long, 1965); he subsequently showed that parasites that had been repeatedly passaged in this way were attenuated (Long, 1972). Commercialization of a vaccine incorporating such an attenuated strain of *E. tenella* has been achieved by Biopharm in the Czech Republic with its development of trivalent and quadrivalent Livacox T and Livacox Q vaccines, containing a combination of *E. tenella* oocysts from an embryo-adapted line and oocysts of other species obtained by selection for precocious development. Livacox T has been marketed since 1992 in more than 42 countries. Embryo adaptation has not been used more extensively in coccidiosis vaccine

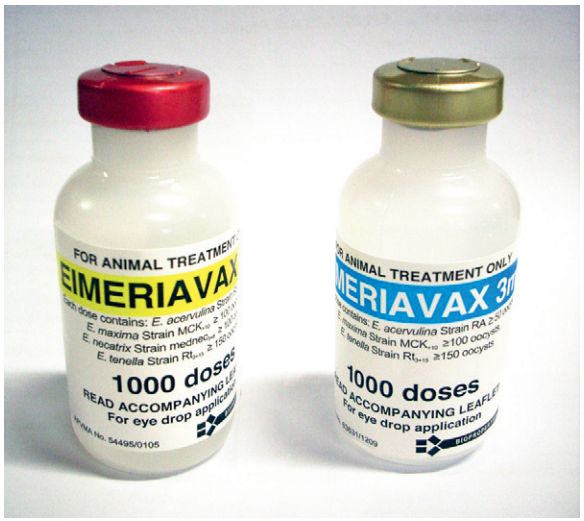

FIGURE 9-4. One of several examples of coccidiosis vaccines for chickens and turkeys that contain attenuated lines of *Eimeria*, which have been selected for precocious development. This vaccine example is manufactured and marketed in Australia. (Courtesy Bioproperties.)

development because important species such as *E. acervulina* and *E. maxima* are unable to complete their life cycle in eggs (Shirley and Long, 1990), and the immunogenicity of embryo-passaged *E. necatrix,* for example, is poor.

Nonliving Coccidiosis Vaccines

CoxAbic is the only nonliving vaccine against coccidiosis and was the first effective subunit vaccine against any protozoan parasite when it was first marketed by ABIC, Israel, in 2002. CoxAbic is unique in its approach to vaccination, because the vaccine is used in laying hens with immunity being transferred to the chick via IgY in the yolk, and in its ability as a monospecies product to protect against challenge with all *Eimeria* species of the chicken. One particular advantage of the vaccine is that it is necessary to vaccinate only one hen to effectively vaccinate many chicks. Balancing this advantage is the complex and expensive nature of the production process necessary to obtain commercial quantities of parasite gametocytes. Development of CoxAbic has been reviewed by Sharman et al (2010).

Studies of antibody responses to *E. maxima* and passive transfer of immunity were undertaken after M. Elaine Rose and Peter L. Long, at the Houghton Poultry Research Station in the UK, confirmed immunity in chickens after initial exposure of birds to the parasite (Rose and Long, 1962). Serum taken from birds 14 days after a primary infection was found to be capable of passively transferring protection (Rose, 1971). Protection was also shown to be passed from an immune hen to chicks via maternal antibodies transferred in the egg yolk (Rose, 1972; Rose and Long, 1971). Subsequently, Michael Wallach and colleagues at the Hebrew University Hadassah Medical School in Israel identified gametocyte antigens as being immunogenic (Pugatsch et al, 1989) and implicated macrogametocyte antigens in particular as possibly being the protective antigens associated with immunity that could be transferred with antibody (Wallach et al, 1989). Wallach and colleagues produced a monoclonal antibody that bound these antigens, and they showed that polyclonal antibodies raised against the affinity purified native antigens were capable of passively transferring protection against *E. maxima* challenge infection (Wallach et al, 1990). Subsequently, direct immunization of hens was shown to protect chicks from developing a pathologic infection with *E. maxima* and greatly reduced oocyst production following a challenge infection (Wallach et al, 1992). This served as the basis for a commercial vaccine. Floor pen trials confirmed protection in chicks from vaccinated hens and showed that, unlike protection derived from viable vaccines, there was substantial cross-protection between birds receiving antibodies directed against *E. maxima* antigens and challenge infections with other *Eimeria* species (Wallach, 1997). An average of 60% to 70% reduction in oocyst output was demonstrated in birds challenged with *E. acervulina, E. maxima,* and *E. tenella* over four trials (Wallach, 1997, 2003). Chickens produced from hens immunized with CoxAbic have been found to perform equally well as those in which coccidiosis was managed by other means (by use of a live vaccine or through coccidiostats in feed) (Wallach et al, 2008). Cross-reactivity between gametocyte antigens in different species of *Eimeria* enables CoxAbic to provide broad protection against coccidiosis. Natural immunity develops through exposure to *Eimeria* infection early in life in chicks from vaccinated hens, such that the birds remain protected after maternal antibody wanes (Wallach et al, 2008).

CoxAbic was developed by Abic Biological Laboratories Teva Ltd, which was acquired by Phibro Animal Health Corporation in 2009. The CoxAbic vaccine is marketed in Argentina, Brazil, Columbia, India, Israel, Mexico, Romania, South Africa, Thailand, Turkey, Venezuela, and Vietnam.

BOVINE BABESIOSIS

One of the earliest uses of vaccination in the field of parasitology involved the practice whereby blood from adult cattle that had survived a bout of babesiosis was injected into young, relatively insusceptible calves, leading in most cases to a mild fever and subsequent immunity to what otherwise may have been a lethal natural challenge arising when animals were exposed to tick-infested pastures (Connaway and Francis, 1899; Nuttall, 1913; Pound, 1897). Connaway and Francis (1899) refer to earlier work undertaken by C.J. Pound in Australia (Pound, 1897) in their description of investigations undertaken at the University of the State of Missouri's Experiment Station, the Missouri State Board of Agriculture, and the Texas Agricultural Experiment Station, where more than 400 cattle that were to be transported to Texas were inoculated with blood from animals in Texas that had survived and recovered from Texas fever. The procedure was effective, inducing immunity in the recipients, with losses from both the inoculation and subsequent exposure of the cattle to a tick-infested environment described as being less than 8%. Key to the effectiveness of this practice was the reduced virulence of parasites in the blood of an animal that had recovered from a bout of babesiosis when transferred to a naive recipient, compared with parasites in the blood of a clinical case (Callow and Tammemagi, 1967). This antiparasite vaccination procedure was undertaken on a commercial basis in Australia for some 80 years (Callow, 1977), and similar practices were later followed in Europe for *Babesia divergens* infections (Taylor, 1989). Callow (1977) suggests that the practice was most efficient when the vaccine was prepared by government laboratories, as was the case in examples in Algeria (Sergent et al, 1945a) and Australia (Callow, 1977).

A breakthrough in the development of a more reliable and commercial vaccine came with the serendipitous discovery by Bill Callow at the Tick Fever Research Centre near Brisbane, Australia, that *Babesia bovis,* which had been serially passaged through splenectomized calves at 4- to 7-day intervals for eight or more passages, showed reduced virulence but nevertheless was able to induce immunity in recipients against subsequent exposure to virulent

FIGURE 9-5. Current Australian commercial vaccine for control of "tick fevers" in cattle caused by *Babesia bovis, B. bigemina,* and *Anaplasma marginale*. Although originally developed in Australia, similar vaccines are manufactured locally and used in several countries.

FIGURE 9-6. One of two vaccines for canine babesiosis marketed in Europe, Nobivac Piro combines soluble parasite antigens from *B. canis* and *Babesia rossi*. Antigens are prepared from parasite cultures maintained in vitro. Although the vaccine is registered in several countries, it is used principally in France. (Courtesy Intervet International B.V.)

parasites (Callow et al, 1979, 1997). This discovery arose after splenectomized animals were used as vaccine blood donors so as to increase parasitemia and enable Callow and colleagues to cope with what was at the time (mid-1960s) an increasing demand for a blood-based vaccine (Callow et al, 1997). The same procedure was not successful in lowering the virulence of *B. bigemina;* however, it was found that repeated, slow passage of *B. bigemina* through intact calves did lower virulence, and this has formed the basis for a successful commercial vaccine (Dalgliesh et al, 1981). Vaccines based on this technology and incorporating also the rickettsia *Anaplasma centrale* (which provides cross-protection against *Anaplasma marginale*) continue to be produced in Australia, where they are very effective. Callow (1977) calculated a cost-benefit ratio of 43:1 in relation to use of bovine babesiosis vaccines in Australia over a 32-year period. The technology has been adopted in several other countries, with live vaccine produced locally in Argentina and possibly in other South American countries, South Africa, and Israel.

Currently, the Tick Fever Centre in Brisbane manufactures and markets two trivalent tick fever vaccines. Both comprise viable organisms of *B. bovis, B. bigemina,* and *A centrale*. The Combavac 3 in 1 vaccine must be stored at –196° C under liquid nitrogen and transported under liquid nitrogen. The concentrate has a shelf life of 5 years when kept under liquid nitrogen but must be used within 8 hours after the frozen vaccine is thawed and reconstituted, provided it is maintained at <20° C. The Trivalent Tick Fever Chilled Vaccine (Figure 9-5) is distributed refrigerated and has a refrigerated shelf life of 4 days.

Improved quality control procedures have increased the safety of the live babesiosis vaccines for cattle (Callow et al, 1997); nevertheless, concerns remain about the potential for transmission of adventitious agents or the virulence of vaccine parasites in some circumstances. The long-held desire to transfer the bovine babesiosis vaccines to nonliving or, preferably, recombinant antigen vaccines remains. However, despite substantial research effort, no commercial nonliving babesiosis vaccine has been developed for cattle.

CANINE BABESIOSIS

Two commercial vaccines are marketed in France for canine babesiosis: Pirodog and Nobivac Piro. Pirodog was the first canine babesiosis vaccine to be marketed—in 1987. The vaccine was the result of collaboration between scientists at the University of Illinois and Rhône Mérieux in France. The vaccine contains so-called exoantigens that are secreted by the parasite. These antigens can be found in the blood of infected animals (Mahoney, 1966; Ristic et al, 1971) and can be used as a vaccine to induce immunity (Sibinovic et al, 1967). Studies by Nocard and Motas (1902) on canine babesiosis due to *B. canis* indicate the clear potential for development of a nonliving vaccine and the importance of a specific antibody in protection against the parasite. Development of a microaerophilous stationary phase cultivation method for *Babesia,* first for *B. bovis* (Levy and Ristic, 1980) and later for *B. canis* (Ristic, 1984), paved the way for the development of commercial vaccines. Practical use of the Pirodog vaccine shows that the vaccine does offer significant protection against clinical babesiosis in dogs (Moreau and Laurent, 1984; Moreau et al, 1989); however, some vaccinated animals do succumb to clinical disease. In an overview of data from some 150,000 vaccinated animals, Moreau et al (1989) surmise that failure of the vaccine in some animals may be seen when animals have already been infected with *B. canis,* with prior exposure affecting responses to the vaccine, and they lament the tendency of clients to present animals for vaccination only after they are likely to have been exposed to infection. However several other possible explanations have been put forth for the apparent failure of the vaccine in some circumstances, and an integrated approach to control of the disease is recommended by Bourdoiseau (2006). The vaccine is registered and sold by Merial in France (the main market), The Netherlands, Spain, Portugal, Italy, Switzerland, Croatia, and Russia. Advice concerning use of the vaccine indicates that dogs must be in "perfect condition" when vaccinated, and vaccination must be undertaken only at least 8 weeks after clinical babesiosis. The vaccine is recommended to be given to puppies as two immunizations, 3 to 4 weeks apart, with the first injection given at the 5th month of age, followed by annual or bi-annual boosters. The lyophilized vaccine comprises culture antigens of *B. canis* plus Quil A adjuvant, and after rehydration, the vaccine is delivered subcutaneously.

Schetters et al (1995) observed that strain variation in *B. canis* limited the effectiveness of vaccination using antigens from a single strain type, and this encouraged the development of a new commercial vaccine, Nobivac Piro, marketed by Intervet International BV (Figure 9-6). The vaccine combines soluble parasite antigens

(exoantigens) from both *B. canis* and *Babesia rossi,* the rationale being that the combination of cross-protective antigens from the two species may provide broader protection against antigenic variants. Experimental trials supported this hypothesis (Schetters et al, 2001). The process involved in development of the vaccine has been described by Schetters et al (2007a). An isolate of *B. canis* was obtained from a clinical case in France, and *B. rossi* was obtained from a dog in South Africa. Master seed and working seed lots were established from parasites that were passaged through a splenectomized dog. Production of the vaccine in cultures was initiated from these working seeds. Production of the commercial vaccine involves concentration and γ-irradiation of culture supernatants to ensure the absence of viable parasites; then aliquots of antigen from the two species are combined and the vaccine freeze-dried. The vaccine diluent contains 250 μg Quil A adjuvant. The recommended vaccination schedule and vaccine delivery method for Nobivac Piro are similar to those noted earlier for Pirodog, with annual boosters suggested at the beginning of the tick season.

Inclusion of *B. rossi* antigens broadened the effectiveness Nobivac Piro for different antigenic variants of *B. canis* and provided protection against infection with *B. rossi* itself (Schetters et al, 2007b). The vaccine appears to impact the two species differently. In the case of *B. canis,* it is considered to be an anti-disease vaccine rather than an anti-infection vaccine, with antibodies induced by vaccination neutralizing putative toxic components in the material released by the parasites in vivo (Schetters et al, 2009). In the case of *B. rossi,* the vaccine has a more dramatic effect on parasite proliferation and can be considered to be an antiparasite vaccine (Schetters et al, 2007b, 2009). The vaccine was registered in Europe in 2004 and also in Switzerland, Iceland, Norway, South Africa, and Brazil; most sales of the vaccine take place in France.

A potentially important recent development has been the publication, in patent form, of details concerning the cloning of a protective antigen contained within Nobivac Piro (Schetters et al, 2012). Sera from immune dogs were used to identify an antigen of approximately 41 kDa in supernatants of *B. canis* cultures. The antigen was cloned and was found to be associated with a protein with predicted size of 33 kDa. Protein was expressed in *Escherichia coli* as a 6xHis fusion, and the affinity purified product was used in vaccination trials in dogs against a challenge infection with *B. canis,* with saponin used as adjuvant. Vaccinated animals were protected against clinical disease and developed a substantially reduced parasite load compared with unvaccinated controls. Clearly a wealth of additional studies would be needed to determine effectiveness in various circumstances; however, the antigen appears to have potential for development as a commercial, recombinant antigen vaccine for canine babesiosis.

THEILERIOSIS VACCINES

Theileria parva

Theileria parva is a genetically diverse species complex, members of which cause diseases known as East Coast fever, Corridor disease, and Rhodesian theileriosis or January disease in cattle in parts of Africa. East Coast fever is believed to have become a serious problem for cattle in southern Africa after the importation in 1901 of animals from West Africa following the 1895 rinderpest panzootic (Lawrence, 1992). Infection with *T. parva* cannot be transferred using the blood from clinical cases; however, Theiler (1911) found that infection could be transferred to naive animals using cells from tissues of infected animals, and that animals that recovered from an infection induced in this way were immune to a subsequent challenge infection from infected ticks. This observation served as the basis for the rationale whereby James Spruell and colleagues (1914) in the Transkeian Territories of South Africa gave 283,000 head of cattle intravenous injections with material derived from the lymph nodes and spleen of animals with well-advanced *T. parva* infection, following processing of these tissues through a meat mincer. Although some 25% of treated animals died following the procedure, most of the remainder were found to be immune, and that immunity was boosted after subsequent natural exposure of the vaccinees to infected ticks. Nevertheless, unreliability of the method saw it abandoned. Attempts to develop a nonliving vaccine were unsuccessful. Neitz (1953) showed that if infected ticks were fed on cattle that were treated with drugs belonging to the tetracycline group, the animals survived and were immune. This observation formed the basis for the development of an infection and treatment vaccination method that continues to be used. Attempts to use tissue-derived schizonts or cultured parasites as the source of infective material were frustrated by the requirement for use of a large number of parasites to effect immunization, and led to unreliability of the "take" of the immunizing infection in recipients (Brown et al, 1978; Dolan et al, 1982; Pirie et al, 1970). Mat Cunningham and colleagues at the East African Research Organization station at Muguga, just outside Nairobi in Kenya, established procedures for preparing a stabilate of infective sporozoites from ticks fed on infected cattle (Cunningham, 1973, 1977). A titrated dose of sporozoites could then be inoculated, with concurrent and repeated use of tetracyclines, to immunize cattle. Use of long-acting formulations of tetracyclines reduced the number of injections required in such a way that the procedure could be applied practically (Radley, 1981).

Various factors have significantly complicated the use of the infection and treatment vaccination procedure for *T. parva,* one of which is antigenic variability in the parasite. The isolate used in initial experiments at the Muguga research station (Muguga isolate) was found to protect against some parasite isolates but not others. Subsequently, a combination of isolates that has come be known as the *Muguga cocktail* was found to be more reliable (Radley et al, 1975) but not universally effective (Morrison and McKeever, 2006; Uilenberg, 1999). Another, related factor influencing the isolates used in vaccines in different areas of Africa arises from the carrier state that develops in animals that have recovered from an initial *T. parva* infection (natural or vaccine induced). Concerns about the introduction of vaccine-derived parasite strains into areas where those strains were not present encouraged the use of locally derived strains for vaccine production (Morrison and McKeever, 2006; Uilenberg, 1999). This has limited the market potential for a commercially produced vaccine where costs of production and distribution are major factors affecting vaccine availability. Batches of sporozoites must be extracted from ticks fed on infected cattle; they must then be titrated in cattle so the optimal infective dose can be determined. Cryopreserved stabilates are required to be stored in liquid nitrogen and distributed under liquid nitrogen because the parasites quickly lose viability at ambient temperatures and must be inoculated into cattle within a few hours after thawing. The production cycle of each batch of vaccine takes about 18 months. Each of these issues contributes to the cost of the vaccine. Despite these difficulties and deficiencies, the infection and treatment vaccination practice for *T. parva* continues to be used in Kenya, Tanzania, Malawi, Uganda, Zambia, and Zimbabwe, and indeed has been the subject of renewed interest and investment. The Muguga cocktail vaccine, comprising three different stabilates, is currently registered and used in Tanzania, Malawi, and Kenya, and other parasite stabilates are used in Zambia and Zimbabwe. With support from the Department for International Development (DFID; UK) and the Bill & Melinda Gates Foundation, the

Global Alliance for Livestock Veterinary Medicines is partnering with organizations in Eastern, Central, and Southern Africa to improve production and commercialization of vaccine based on the Muguga cocktail.

Theileria annulata

Unlike *T. parva,* infection with *Theileria annulata* can be passaged to cattle by injection of blood from an infected animal. Vaccination of cattle against *T. annulata* using blood transfer was initiated by Edmond Sergent and collaborators in Algeria (Sergent et al, 1945b). Isolates with natural, or passage-induced, low virulence formed the basis for vaccination in North Africa, Europe, and the Middle East (Pipano, 1989). The ability to transfer infection using blood enabled in vivo culture of parasite strains for vaccination purposes (Pipano, 1974). The infection and treatment method developed successfully for *T. parva* was also found to be effective for *T. annulata* (Gill et al, 1977); however, it is unclear whether this was undertaken on a routine or commercial basis.

A breakthrough in the development of a more practical vaccine against *T. annulata* came with the successful culture of schizonts by Tchernomoretz (Tsur) (1945). Eugene Pipano (1989) credits unpublished work by Abraham Kimron for discovering a culture method that was amenable for use on a sufficient scale to form the basis of a commercial vaccine. The method was developed first in Israel. In vitro culture of *T. annulata* schizonts for longer than 10 months was found to lead to loss of virulence, and the attenuated parasites were found to be capable of inducing immunity against a virulent challenge (Pipano and Tsur, 1966). Efficient methods for cryopreserving infective parasites were developed by Cunningham et al (1973). Concerns similar to those mentioned previously for *T. parva* about the possibility of inducing a carrier state involving foreign vaccine-derived parasite strains led to production of *T. annulata* vaccines from locally acquired isolates. Commercial vaccines based on published production methods (Pipano, 1989) are manufactured and sold by government laboratories and/or private companies in Israel (Figure 9-7), Iran, Morocco, Tunisia, Turkey (Figure 9-8), India, and China (previously also Uzbekistan) (Shkap et al, 2007). When it is possible to use the vaccine within 4 days, it can be shipped refrigerated; otherwise it must be distributed under liquid nitrogen.

FIGURE 9-7. A vaccine for prevention of pathology due to infection with *Theileria annulata* in cattle. Originally developed by Eugene Pipano and colleagues in the Department of Parasitology, Kimron Veterinary Institute (KVI), Bet Dagan, Israel, during the 1960s, the vaccine contained live schizonts from parasites attenuated by prolonged culture in vitro. Unless the vaccine can be used within a few days, it must be distributed and maintained under liquid nitrogen until it is used. (Originally developed by Eugene Pipano and Colleagues in the Department of Parasitology, Kimron Veterinary Institute [KVI].)

FIGURE 9-8. An example of the numerous *Theileria annulata* vaccines developed locally in different countries based on the same methods used in development of the first vaccine, incorporating parasites attenuated by culture in vitro. This example is produced in government facilities in Istanbul, Turkey. (Courtesy Gulay Vural.)

CANINE LEISHMANIASIS

Three vaccines have been commercialized for canine visceral leishmaniasis: Leishmune and Leish-Tec marketed in Brazil, and CaniLeish in Europe. Visceral leishmaniasis is caused by members of the *Leishmania donovani* species complex. The nomenclature used for species causing animal and human leishmaniases is subdivided into species present in the "New World" (the Americas) and those in the "Old World" (Africa, Asia, and Europe). *Leishmania donovani* causes human visceral leishmaniasis in South Asia and Africa and is maintained in an anthroponotic cycle. *Leishmania infantum* causes human and canine visceral leishmaniasis in the "Old World," particularly in China and the Mediterranean basin (in Europe, especially Portugal, Greece, Spain, Italy, and Southern France), whereas *Leishmania chagasi* is described as the etiologic agent of visceral leishmaniasis in the "New World" (particularly Brazil). *L. infantum* and *L. chagasi* are the same parasite (Bañuls et al, 2007), nevertheless both species names continue to be used in publications. Taxonomic niceties aside (*L. infantum* has priority), the two species names will be used here in reference to vaccines and publications that exclusively use a particular nomenclature. Many animal species can be infected with the *Leishmania* parasites that cause visceral leishmaniasis; however, dogs are the principal reservoir host for *L. infantum/chagasi,* and the parasite is maintained as an anthropozoonosis. For this reason, vaccination against canine visceral leishmaniasis has two significant benefits: It potentially reduces an important and possibly fatal disease of dogs, and it may reduce transmission of the disease to humans. Other control measures that can be applied for *L. infantum/chagasi* include reducing the prevalence of dogs, especially non-owned dogs, treatment of infected dogs, and control of the sandfly vector. The World Health Organization (WHO) recommends euthanasia of dogs that are found to be infected with *Leishmania* (World Health Organization, 1990); this controversial practice is actually undertaken in Brazil and China, but it is regarded as unacceptable in Europe.

The first commercial vaccine for canine leishmaniasis, Leishmune, was registered in Brazil in 2003 by Fort Dodge and has been marketed since 2004; since the acquisition of Wyeth by Pfizer in 2009, the vaccine has been marketed as a Pfizer product. Until now, the vaccine has been marketed only in Brazil. Leishmune was the product of research undertaken by Clarisa Palatnik-de-Sousa and

colleagues at Universidade Ferdeal do Rio de Janeiro. As part of an investigation into parasite molecules that play a role in attachment and penetration of *L. donovani* into mouse macrophages, Palatnik et al (1989) described a complex glycoprotein aqueous extract of *L. donovani* (LD 1S/MHOM/SD/00-strain 1S—a Sudanese isolate) obtained from in vitro cultured promastigotes. This extract, which contained 10% fructose and 47% mannose, was found to be capable of inhibiting the internalization of promastigotes and amastigotes by macrophages (Palatnik-de-Sousa et al, 1993). The fraction was designated *fructose mannose ligand (FML).* A 36-kDa glycoprotein was identified as a major component of FML (Palatnik-de-Sousa et al, 1993). Studies in mice revealed that immunization with FML was capable of inducing up to 85% reduction in parasite load after a challenge infection with *L. donovani* (Santos et al, 1999). The *L. donovani* promastigote–derived FML was subsequently shown to be able to reduce canine visceral leishmaniasis in dogs naturally exposed to *L. chagasi* (Borja-Cabrera et al, 2002; da Silva et al, 2000).

The second of these field trials was a double-blind, placebo-controlled trial in which there were 8 cases of fatal visceral leishmaniasis among 41 control animals and 1 case among 44 vaccinated animals (Borja-Cabrera et al, 2002). Examination of 4 control and vaccinated dogs 41 months after the start of the trial revealed evidence of *Leishmania* infection in 3 controls but in none of the vaccinated animals. The ability of the vaccine to reduce the potential for transmission of *L. chagasi* was confirmed by using the commercial product (Nogueira et al, 2005). A subsequent study, also carried out in Brazil, involved 550 vaccinated dogs and 588 nonvaccinated controls. Over a 2-year period during which the groups were followed, 1% of the vaccinated animals died of visceral leishmaniasis and 1.2% of the remainder showed symptoms of infection with *L. chagasi*, whereas in the control group 39% of the dogs died due to leishmaniasis and 20.6% of remaining dogs had symptoms of infection (Borja-Cabrera et al, 2008). Although control and vaccinated dogs resided in different localities, which was not ideal for comparative purposes, the results do support the conclusion that the vaccine is effective in reducing canine visceral leishmaniasis.

Leishmune contains 1.5 mg of FML and 0.5 mg Riedel De Haën saponin as adjuvant and is given subcutaneously as a 1-mL injection. Vaccination is recommended for dogs after 4 months of age, with the program involving three separate immunizations, each given 3 weeks apart. Annual revaccination is recommended on the anniversary of the first injection. Dogs are required to be serologically negative and clinically negative for leishmaniasis at the time of the first vaccination. In relation to this requirement, an intriguing finding has been that use of the Leishmune vaccine may have a therapeutic effect on infected dogs (Borja-Cabrera et al, 2010). In studies of the therapeutic effectiveness of the vaccine, researchers have increased the saponin quantity used with the vaccine to 1 mg saponin. Leishmune is not registered for use as a therapeutic; however some practitioners have been using a 2× dose of the vaccine (hence 1 mg saponin) for this purpose. Initial assessments suggest that use of the vaccine in dogs is associated with a decreased incidence of visceral leishmaniasis in humans living in the same areas (Palatnik-de-Sousa et al, 2009).

A second vaccine against canine leishmaniasis, Leish-Tec, was licensed in Brazil in 2007 and has been marketed by Hertape Calier Animal Health since 2008. The vaccine is historic in that it was the first, and remains the only, recombinant antigen vaccine against a protozoal parasitic infection that has been commercialized. It incorporates an amastigote-specific antigen, A2, encoded by a multigene family and present in *Leishmania* parasites causing visceral disease (Charest and Matlashewski, 1994). The A2 target protein appears to play a role in the parasite's stress responses, contributing to survival of the parasite at elevated temperatures associated with spread of the parasite to internal organs (Zhang and Matlashewski, 1997, 2001). Vaccine protein is expressed in *E. coli* and has an N-terminal, 6× histidine tag used for affinity purification of the antigen from *E. coli* lysates (Carvalho et al, 2002). In a laboratory-based trial, the vaccine provided protection to dogs against a challenge infection with *L. chagasi* (7/5 controls symptomatic for leishmaniasis, 2/7 vaccinates symptomatic) reduced the level of symptoms in those vaccinated animals that did succumb to infection compared with controls, and delayed the appearance of symptoms (3 to 6 months in controls, 1 year in vaccinates) (Fernandes et al, 2008). Fernandes et al (2012) cite unpublished data from a randomized, placebo-controlled field trial of the vaccine in dogs, which achieved 71% protection against infection. Leish-Tec comprises 0.1 mg affinity purified recombinant A2 protein and 0.5 mg saponin. Vaccination is recommended for animals that are seronegative for *Leishmania* infection and older than 4 months. An initial vaccination course is applied as three 1-ml subcutaneous injections, given at 3-week intervals. An annual booster immunization is recommended on the anniversary of the first dose. Dogs vaccinated with Leish-Tec are able to be differentiated serologically from infected dogs, which is an important aspect given that euthanasia is recommended for animals showing evidence of infection, including serologic evidence of infection.

The only canine leishmaniasis vaccine to be commercialized outside Brazil is CaniLeish, which was licensed in Europe by Virbac in 2011. The vaccine was developed by the Virbac subsidiary Bio Véto Test in collaboration with scientists at the Institut de Recherche pour le Développement, in Montpellier, France. In 2005 Lemesre et al (2005) achieved promising results in an immunization trial against *L. infantum* infection in dogs using antigens excreted or secreted by *L. infantum* promastigotes in in vitro culture (*Li*ESAp). Subsequently the preparation was assessed in a field trial undertaken in the south of France in vaccinated and control dogs, where one of 165 vaccinated dogs became infected, whereas 12 of 175 control dogs were found infected after a 2-year follow-up (Lemesre et al, 2007). This trial encouraged further commercial development, leading to the CaniLeish vaccine.

Much of the detailed information about the effectiveness of the commercial product is not available in the scientific literature, although information is in the public domain, courtesy of the European Medicines Agency. CaniLeish was developed using *Li*ESAp, with the commercial vaccine comprising 100 µg or more of promastigote excreted/secreted antigens plus 60 µg of the purified QA-21 component of Quil A. Similar to the other canine leishmaniasis vaccines, dogs from 6 months of age receive three 1-mL subcutaneous injections, 3 weeks apart. Booster immunizations are recommended annually after the last vaccination. Vaccination is not recommended for dogs that are already infected with *L. infantum* because the vaccine has not been assessed for benefit in this circumstance; however, the vaccine has been found to show no specific adverse reactions when applied to infected animals, other than the occasional reactions that may occur also in noninfected dogs. Virbac describes the vaccine as providing 3.6× less risk for development of an active *L. infantum* infection in dogs, and 4× less risk for development of clinical disease compared with nonvaccinated animals; this information is based on a trial wherein dogs were submitted to a very high level of natural exposure to the parasite in outdoor kennels over a 2-year period. CaniLeish is licensed in all European Member states plus Switzerland. It has been launched in most of these countries already (in the endemic area as a key preventive tool, and in the nonendemic areas for traveling

FIGURE 9-9. Toxovax from MSD as marketed in New Zealand. This vaccine contains a live, attenuated form of the agent *Toxoplasma gondii*.

dogs) and will be available throughout Europe in the near future. The vaccine has been shown to induce a Th1-profile cell-mediated response, which effectively reduces the parasite load in preinfected macrophages in vitro (Moreno et al, 2012).

TOXOPLASMOSIS

A single commercial vaccine has been licensed for toxoplasmosis in sheep. Toxovax (Intervet) was first marketed in New Zealand in 1988 and was launched in the UK in 1992 (Figure 9-9). The vaccine incorporates viable *T. gondii* tachyzoites of the attenuated S48 strain produced by cell culture in Vero cells. *T. gondii* strain 48 was originally isolated in New Zealand in 1958 from an aborted sheep fetus and was maintained in the tachyzoite stage by serial intraperitoneal passage more than 3000 times, twice weekly, through mice (Buxton, 1993; Wilkins et al, 1987). During the long period of passage, strain 48 lost both its ability to infect cats and produce oocysts and its ability to develop persistent infection in sheep tissues (Jonas, 1987). Vaccine trials in sheep with the live, attenuated parasite strain were begun in New Zealand as early as 1978 and clearly demonstrated a protective efficacy in sheep, with markedly improved lambing performance in vaccinated ewes that were exposed to *T. gondii* during pregnancy (Buxton et al, 1991, 1993; O'Connell et al, 1988; Wilkins et al, 1988).

The vaccine is marketed in New Zealand as Toxovax and as Ovilis Toxovax internationally. It is provided as a suspension of strain 48 tachyzoites, together with but separate from a proprietary diluent. Before use, the concentrated parasites are diluted to a working concentration containing at least 10^5 per 2-mL dose, and ewes receive a single intramuscular injection in the anterior muscles of the neck at least 4 weeks before mating. The vaccine must be used within 2 hours of dilution. Information about the product provided by MSD Animal Health indicates that a single injection provides lifetime protection. A number of precautions are recommended in relation to handling the product because it is a live vaccine that contains, albeit in attenuated form, what is a known human pathogen. It is recommended that pregnant women and persons whose immune system may be suppressed should not handle the vaccine. Use of personal protective equipment is suggested for those diluting or using the vaccine, to protect against accidental exposure. The actual level of risk to humans is unclear; Murray Wilkins and Elaine O'Connell (1992) refer to three instances in which humans were accidentally inoculated with the vaccine, and indicate that no reports have described any serious effects.

TRICHOMONAS INFECTION IN CATTLE

TrichGuard and TrichGuard V5L are marketed by Boehringer Ingelheim for the purpose of aiding in the prevention of disease caused by *Tritrichomonas foetus*. The vaccines were acquired by Boehringer Ingelheim after Pfizer was required to divest them after its takeover of Wyeth. Little information is available in the public domain about these vaccines, which seem to have been developed in the 1990s through collaboration with William Kvasnicka and colleagues at the University of Nevada. Initially, research in Australia showed that a whole-cell vaccine prepared from cultured *T. foetus* could prevent genital infection in most bulls up to 5 years of age (Clark et al, 1983). Subsequently Kvasnicka and colleagues (1989, 1992) achieved a significant degree of protection against experimental *T. foetus* infection in heifer calves acquired after mating with infected bulls. A larger trial, involving 65 vaccinated and 64 control heifers, was undertaken using a vaccine that combined *T. foetus* organisms added to the commercial Fort Dodge TriVib 5L vaccine. Vaccinated heifers mated with infected bulls showed double the pregnancy rate and a reduction in *T. foetus* infection rates compared with controls receiving TriVib 5L alone. Fort Dodge went on to commercialize both a stand-alone *T. foetus* vaccine, TrichGuard, and a vaccine incorporating *T. foetus* as well as *Campylobacter fetus* and five serovars of *Leptospira* (*L. canicola, L. grippotyphosa, L. hardjo, L. icterohaemorrhagiae,* and *L. pomona*) in an oil adjuvant, designated TrichGuard V5L. The *T. foetus* vaccine is not effective in bulls, which seems a little surprising given that the original Australian research was undertaken in bulls, not cows, and was quite successful. BonDurant (1997) suggests that this may be due to lack of recognition of particular vaccine antigens at the squamous epithelial cell surface of the penis or prepuce. Vaccinating female cattle in endemic areas (especially areas of shared grazing) with the current vaccine does not provide complete protection; however, it is better than taking no action, that is, some protection

against embryonic and fetal loss is provided. Vaccination is one arm of the approach to control; annual examination of bulls, especially bulls older than 3 years, for preputial infection with *T. foetus* is also important, as is culling of infected bulls.

TrichGuard is indicated to contain killed protozoa; no adjuvant is designated. Vaccination (2 mL) is applied subcutaneously and a second dose is applied 2 to 4 weeks later, with the recommendation that the last injection should be applied by 4 weeks before breeding. Annual revaccination is suggested, as is application 4 weeks before breeding. TrichGuard V5L includes an oil adjuvant and is injected (5 mL) according to a similar regime.

HELMINTH INFECTIONS

BOVINE PARASITIC BRONCHITIS

If we exclude situations involving deliberate exposure to virulent parasites to induce a state of immunity, the bovine bronchitis vaccine Dictol was the first antiparasite vaccine developed for use in either animals or humans. The same vaccine continues to be sold to this day.

Early scientific investigations of "hoose" or "husk" in cattle led to the discovery of an important role for naturally acquired immunity following past exposures to the etiologic agent, *Dictyocaulus viviparus* (Taylor, 1951). The disease was studied by a young veterinary graduate in Glasgow, William Fleming Hoggan Jarrett (1928-2011), who, apart from his work on parasitic bronchitis, came to be well known for discovering the fact that certain feline tumors were caused by a retrovirus, now known as *feline leukemia virus.* Jarrett first showed that immunity to *D. viviparus* in calves could be transferred to naive animals with gamma globulins (Jarrett et al, 1955), and subsequently showed that a substantial degree of immunity could be induced in calves with whole-worm extracts and Freund's adjuvant (Jarrett et al, 1960b). However immunity elicited using worm extracts was found to lead to the death of many worms from a challenge infection in the lungs or bronchi (Jarrett et al, 1958b; Jarrett et al, 1960b). Lesions caused by these dying worms induced a severe reaction around them—indeed a more severe reaction than would otherwise have been the case had the animals not been vaccinated at all (Jarrett et al, 1958b). Jarrett and colleagues were aware of the work of Tyzzer and Honeij (1916), who had shown that radium irradiation of *Trichinella spiralis* inhibited the parasite's development in rats, and surmised that presentation of *D. viviparus* metabolic products within the mesenteric lymph nodes by parasites incapable of completing their development and undergoing pulmonary migration might stimulate immunity without the risk of causing lung pathology (Jarrett et al, 1958b). They undertook experiments examining the ability of irradiated *D. viviparus* larvae to stimulate immunity in calves. The first data from their experiments appear to have been published in the discussion of a paper that Jarrett presented on the 29th of August, 1957, at the Annual Congress of the British Veterinary Association, held in Cambridge. Although Jarrett's formal presentation dealt with aspects of the natural history of parasitic bronchitis, including the results of vaccination with nonliving parasite extracts (Jarrett et al, 1957), publication of a general discussion of Jarrett's presentation was included as part of the same article and this contained substantial details about the effectiveness of protection generated by vaccination using irradiated larvae. Publication of full details of Jarrett's experiments followed (Jarrett et al, 1959, 1960a), and field trials confirmed the effectiveness of vaccination against natural exposure to the parasite (Jarrett et al, 1958a, 1961). A commercial product, Dictol, was rapidly brought to market by Allen and Hanburys Ltd of Hertfordshire, UK, and was released in 1959. It proved to be highly effective (Jones and Nelson, 1960). Poynter and colleagues (1970) evaluated field use of the vaccine over the period 1965 through 1968 and concluded that it provided 98.5% protection against clinical disease; Holzhaur and colleagues (2005) confirmed the efficacy of the current vaccine.

FIGURE 9-10. Vaccine against *Dictyocaulus viviparus* infection in cattle. The vaccine, which contains live, irradiation-attenuated L3 larvae, was developed in England and was originally marketed as Dictol. This was the first commercial vaccine designed for use against any parasitic disease.

In time, vaccine manufactured at a single site came to be marketed by two different companies under two brand names, Dictol and Huskvac, and the vaccine is now marketed only as Bovilis® Huskvac (Figure 9-10). The vaccine is currently marketed under the Intervet label, but it seems likely this will be altered to MSD Animal Health following regulatory approval. It is manufactured in the UK and marketed in Switzerland, Holland, and Belgium, as well as in the UK. Vaccination of at-risk calves is recommended from 8 weeks of age and involves administration of two 25-mL doses of oral vaccine, approximately 4 weeks apart. Each dose comprises 1000 viable, irradiated third-stage *D. viviparus* larvae. Use of anthelmintics in vaccinated animals is to be avoided from 8 weeks before vaccination until 14 days after the second dose, with sustained-release anthelmintic boluses avoided completely until 14 days after the second dose. Protection lasts for a year and is boosted by subsequent natural exposure of vaccinated animals; annual vaccination with a single vaccine dose is recommended.

Bain and Urquhart (1988) demonstrated that the Dictol vaccine was effective if injected subcutaneously. They considered that the larvae they injected did migrate to the lungs, but that experiments showed that it was not necessary for the irradiated parasites to penetrate the intestine and migrate via the mesenteric lymph nodes, to stimulate immunity. Use of the vaccine does not stimulate sterile immunity to a natural exposure to *D. viviparous,* and although they show no clinical signs, vaccinated animals may shed small numbers of larvae after natural exposure. This is considered to be an advantage because continuing exposure of vaccinated animals to low levels of larval challenge heightens and prolongs immunity to clinical disease and may reduce the need to vaccinate older animals (Urquhart, 1985).

HAEMONCHOSIS

Scientists working on immunity to ticks came up with the idea that a vaccine directed toward the gut of a blood feeding parasite could damage the parasite and provide protection for the host (discussed

later). Some nematodes, such as hookworms and *Haemonchus contortus,* are also blood feeders. After success had been achieved with ticks and gut-associated antigens, similar investigations were undertaken with *H. contortus* and, later, with other blood-feeding parasites. Although no commercial vaccine against *H. contortus* is presently available, development of a new commercial vaccine appears to be nearing fruition following the submission of a package for regulatory approval by Dr. W.D. (David) Smith at the Moredun Research Institute in Edinburgh, Scotland, and colleagues. This vaccine is based on gut-associated antigens.

The first gut-associated antigen to be investigated as a potential vaccine for *H. contortus* was identified during ultrastructural studies of the intestinal epithelial cells of the parasite. An abundant, extracellular, helical protein was found to be loosely associated with the intestinal surface (Munn, 1977). The protein could be obtained in relatively pure form through differential centrifugation and was given the name *contortin.* In 1987 Edward Munn and colleagues at the Institute of Animal Physiology in Babraham, England, published the results of vaccine trials in sheep using contortin, which suggested that the protein was able to induce up to 70% protection against a challenge infection with *H. contortus* (Munn et al, 1987). Attention shifted, however, to minor contaminants of the contortin preparation (Smith and Munn, 1990) and, in particular, to integral membrane protein components of the intestinal microvilli that migrated in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) as a doublet at 110 kDa and were termed *H110D,* abbreviated to *H11* (Munn, 1993). Vaccine trials with semipurified, native H11 have consistently induced high levels of protection in lambs against *H. contortus*. Associated proteins have been characterized extensively and expressed as recombinants; however, protection has been achieved only with the native protein preparations (reviewed by Munn, 1997; Newton, 1995; Newton and Meeusen, 2003). Following a decade or so of intense investigation and substantial investment in H11, work on its development as a recombinant antigen vaccine appears to have all but ceased. No clear explanation has been provided about why vaccine investigations also ceased with contortin itself, and it is unclear whether that protein complex per se does actually have efficacy as a vaccine. Contortin has been found to comprise two prolyl-carbopeptidases and very likely plays a role in the parasite intestine as an anticoagulant (Geldhof and Knox, 2008).

While research in Babraham was progressing, a little more than 300 miles to the north David Smith and colleagues in Moredun, Scotland, instigated a research program that sought to identify protective gut-associated vaccine antigens for *H. contortus* that were different from contortin or H11. They undertook studies of integral membrane proteins prepared using differential detergent solubilization methods and identified the presence of new protective antigens (Smith, 1993). Further investigations using lectin affinity chromatography identified an integral membrane protein fraction with affinity for *N*-acetylgalactosamine, which was effective as a vaccine and did not contain H11 (Smith et al, 1994). The fraction was designated H-gal-GP. Many components of the H-gal-GP complex have been characterized (reviewed in Knox et al, 2003; Newton and Meeusen, 2003), and many have been expressed as recombinant proteins; however, no protection has been reported for the recombinants (Geldhof and Knox, 2008).

Although efforts to produce recombinant *H. contortus* vaccine proteins have yet to be successful, evidence confirming the effectiveness of the native proteins has continued to accumulate. In a field trial of a vaccine prepared from worm extracts and comprising a combination of both H11 and H-gal-GP, lambs were protected against haemonchosis as reflected by the prevention of death, reduced anemia, and reduction in pasture contamination with larvae (LeJambre et al, 2008).

The effectiveness of native gut–associated antigens as vaccines encouraged David Smith and colleagues to embark on a project to develop a commercial vaccine based on antigens prepared directly from worms. Some details of progress in development of the vaccine have been presented at scientific conferences and have been provided by David Smith (personal communication). Key to the feasibility of the approach has been the finding that reliable protection can be obtained with as little as 5 μg of parasite extract, together with Quil A as adjuvant. Vaccine production under Good Manufacturing Practice–compliant conditions has been established in Western Australia in collaboration with Dr. Brown Besier.

Kilogram quantities of adult *H. contortus* are obtained by purchasing lambs from local farmers that are drenched and subsequently infected with a known dose of *H. contortus* L3. A few weeks later, the animals are sold for slaughter and are processed normally, except that their abomasa are retained. These are transported to a facility and are processed by NemESys, a patented worm harvesting machine that has certain affinities to a small cement mixer lashed to a couple of sieves and shower heads! The isolated worms are homogenized, their membranes are extracted with a nonionic detergent, and a subfraction of the resulting integral membrane proteins are prepared by column chromatography and used as vaccine antigen. The vaccine contains predominantly H11 and H-gal-GP, but other potentially protective antigens are also present. Pilot field trials of the vaccine have been completed in sheep in the UK, Australia, Uruguay, and Brazil, and the vaccine has been tested in grazing goats in South Africa, as well as in housed calves challenged with *H. contortus* or *H. placei* (Bassetto et al, 2011). In all trials, the vaccine provided a high level of protection, which has encouraged continued commercial development. A registration package has been submitted in South Africa and submission of another appears likely in Australia in the near future. As would be expected, protection is associated with antibodies against gut antigens. Protection is evident after two 1-mL immunizations containing 5 μg of antigen protein and 1 mg Quil A, injected subcutaneously at an interval of 4 to 6 weeks. To maintain immunity, further immunizations are required at 6-weekly intervals thereafter. Discussions with farmer focus groups in Australia have apparently supported the acceptability of this requirement. It is believed that the vaccine will be known as Barbervax or, possibly, Polevax.

In David Smith's first publication concerning vaccination of sheep against *H. contortus* almost 20 years ago (Smith, 1993), in which substantial protection was demonstrated with parasite extracts, the author states, "It would be completely uneconomic to produce a commercial vaccine for *H. contortus* using native protein antigens whether derived from dissected intestines or whole worms." He is now attempting to do just that. Time will tell whether the vaccine produced achieves fruition and commercial success.

CESTODES

By far the greatest degree of scientific success in development of recombinant vaccines against parasitic infections has been achieved for cestode parasites. A defining feature of the host-parasite relationship in the intermediate hosts of taeniid cestodes is a solid immunity that is induced by an initial infection that prevents parasites establishing from subsequent challenge infections; however, this immunity does not affect parasites established as a result of the primary infection (Lightowlers, 2010b). This type of immunity is known as *concomitant immunity.* The phenomenon was discovered

in taeniid cestodes by Harry M. Miller, Jr, and colleagues working at Washington University in St Louis, Missouri, during the 1930s. While investigating immunity to *Taenia taeniaeformis* (then known as *Cysticercus fasciolaris*), Miller noticed that sometimes individual rats were refractory to an experimental infection. He necropsied these animals and discovered that they invariably had a small number of mature strobilocerci already in the liver (i.e., they had become contaminated by infection before they were delivered to him from his animal supplier) (Miller, 1931b). Miller undertook experimental studies and found that an initial infection led to solid resistance to a subsequent challenge infection (Miller, 1931b).

Miller credits Vogel (1888) with recognizing this phenomenon previously; however, Miller was the scientist who first demonstrated it in controlled experiments. This was the first instance wherein immunity had been demonstrated scientifically against a metazoan parasite. Miller went on to establish most of the important tenets related to immunity to this group of parasites, and showed that he could vaccinate animals with nonliving antigens to achieve a very high level of protection (Miller, 1931a). He showed that immunity could be transferred with antibody in serum or colostrum from infected or vaccinated donors (Miller and Gardiner, 1932). Subsequently, vaccination proved extremely successful against other taeniid cestode species, including those of economic and medical importance (reviewed by Rickard and Williams, 1982). Until the advent of recombinant DNA technology, however, and its first application to the field of parasitology after 1983, there was no way that practical vaccines could be produced for this group of parasites. The larval stage contained within parasite eggs, known as an *oncosphere,* was identified as the target of protective antibodies (Heath and Lawrence, 1981; Kyngdon et al, 2006) and was found to be the richest source of vaccine antigens (Rajasekariah et al, 1980). With the application of genetic engineering methods, highly protective recombinant antigens were produced and were shown to protect against infection with *Taenia ovis* in sheep (Johnson et al, 1989), *Taenia saginata* in cattle (Lightowlers et al, 1996b), *Taenia solium* in pigs (Flisser et al, 2004; Gonzalez et al, 2005), and *E. granulosus* in sheep (Lightowlers et al, 1996a). All of these vaccines were developed by a single research group at the University of Melbourne, together with their collaborators.

The EG95 vaccine against *E. granulosus* was tested in experimental trials undertaken independently by different research groups in six different countries (Lightowlers, 2006; Lightowlers et al, 1999), and 94% to 100% protection was achieved in all experiments. Two immunizations with 50 μg of recombinant protein expressed in *E. coli* plus 1 mg Quil A, delivered subcutaneously 4 weeks apart, induced immunity lasting at least a year (Heath et al, 2003). The vaccine was registered for commercial use in China in June 2007 and in Argentina in February 2011.

The TSOL18 recombinant vaccine against *T. solium* infection in pigs has been tested by independent research groups in four different countries and has achieved almost total protection (>99%) in each trial (Lightowlers, 2006). The experimental vaccine comprises 200 μg recombinant protein expressed in *E. coli* and Quil A adjuvant. The vaccine has been the subject of successful field testing in Cameroon and Peru (Assana et al, 2010; Jayashi et al, 2012). A combination TSOL18 vaccination and a single anthelmintic treatment of young pigs with oxfendazole at the time of vaccination achieved the complete elimination of parasite transmission under field conditions (Assana et al, 2010). Scale-up of TSOL18 production and its registration as a commercial vaccine are being undertaken under the sponsorship of the Global Alliance for Livestock Vaccines and Medicines, in collaboration with Indian Immunologicals Limited, in Hyderabad. *T. solium* is one of six diseases identified by the International Task Force for Disease Eradication (Schantz et al, 1993) as having the potential to be eradicated. Development of the TSOL18 vaccine appears to have brought that goal closer to being feasible (Lightowlers, 2010a).

Specific complement fixing antibodies are the dominant, if not the sole, protective immune mechanism induced by recombinant oncosphere antigen vaccines against *Taenia* species and *E. granulosus* (Kyngdon et al, 2006; Lightowlers, 2006). Investigations into the localization of protective antigens within the oncosphere indicate that they are associated with penetration of gland cells of the organism (Jabbar et al, 2010a, 2010c, 2011). An interesting discovery was made when localization of protective antigens in *T. ovis* during the parasite's early development was investigated (Jabbar et al, 2010b). The antigens were found not to be associated with the parasite surface until after approximately 3 days' development in culture. If it is assumed that the same occurs in vivo, the parasite would not be susceptible to attack by vaccine-induced specific antibody and complement until after some days of development. The potential significance of this finding relates to situations in which the oncosphere penetrates the brain. If a lethal effect were not delivered to the oncosphere before it penetrated the blood-brain barrier, the parasite would not be killed by vaccine-induced immune responses. This scenario is consistent with the finding that the only taeniid cestode in which vaccination with oncosphere antigens did not lead to a very high level of protection against infection is *Taenia multiceps* (Gauci et al, 2008), a species that infects the brain. If this interpretation were correct, the TSOL18 vaccine would not be effective if used directly in humans to prevent neurocysticercosis. Nevertheless, the vaccine is highly effective in preventing cysticercosis in the muscles of the natural animal intermediate of the parasite (pigs), hence control of *T. solium* could be affected indirectly by preventing transmission through pigs.

ARTHROPOD PARASITES

TICK VACCINES

The Bm86 vaccine against *Boophilus microplus** infection in cattle was the first commercially successful recombinant vaccine against any parasitic disease. Detailed information concerning the vaccine's production was published first in an International Patent (WO 88/03929) in June of 1988, and subsequently in the scientific literature in 1989 (Rand et al, 1989). Commercial development and registration of the product (TickGARD) by the Australian group that developed the technology saw the Australian vaccine first marketed in June 1994. On the basis of information published by the Australian group (Rodríguez et al, 1994; Willadsen et al, 1995), the technology was picked up by researchers in Cuba who developed, effectively, the same vaccine, with the product Gavac reaching the market in Cuba in 1993.

Exposure to persistent tick infestation in healthy animals may lead to a state of relative immunity, reflected both in a reduced number of ticks successfully feeding on an animal and in reduced fecundity of adult females fed on immune animals. The first scientific evidence to clearly support this phenomenon was provided by Johnson and Bancroft (1918) following their investigations of Peony, Clover, Tinkerbell, and other tick-resistant cattle in Queensland, Australia. The mechanisms underlying resistance were

*In 2003 it was suggested that there may be synonymy between the genera *Boophilus* Curtice, 1891, and *Rhipicephalus* Koch, 1844 (Murrell and Barker, 2003). This situation has not been universally accepted, and the nomenclature *Boophilus microplus* is retained here.

entirely unclear, with focus placed from the earliest times on products of the tick's salivary glands. An important subsequent development was the publication by William Trager of the Rockefeller Institute for Medical Research in Princeton (Trager, 1939). His experiments with *Dermacentor variabilis* infections in guinea pigs and rabbits confirmed the development of resistance following prior exposure, but also demonstrated that hosts could be immunized against a primary challenge infection by administration of intracutaneous injections of a saline extract of tick larvae. Although the numbers of animals in these experiments were small, the phenomena were shown to be repeatable.

The TickGARD vaccine uses a type of antigen that came to be known as *concealed* (Willadsen and Kemp, 1988) or *hidden* (Munn, 1993). Although Willadsen and Munn coined these terms, the strategy whereby antigens not normally exposed to a host's immune system could be effective as vaccine antigens against blood feeding organisms had been established by others. The first clear and specific indication that antigens associated with the gut of a blood feeding organism could be the source of effective vaccine antigens was provided by Nelda Alger and Edelberto Cabrera (1972) at the University of Illinois. Mosquitoes fed on rabbits immunized with a preparation derived from the mosquito mid gut were found to die at a substantially higher rate than mosquitoes fed on control rabbits. Alger and Cabrera suggested possible mechanisms to explain the phenomenon, including damage by antibody in the blood to cells in the digestive tract and inhibition of digestive enzymes. In relation to ticks, Galun (1978) speculated about the possibility that developmental hormones of the tick could serve as a potential target for vaccine development. Allen and Humphreys (1979) confirmed and extended Trager's observations from the 1930s using *Dermacentor andersoni* to successfully induce immunity in both guinea pigs and cattle. Allen and Humphreys' experiments used antigens obtained from tick midgut and reproductive organs, and they stated that this was done "based on the crude concept that ticks feeding on appropriately immunised hosts might ingest antibodies specific for antigens within the alimentary tract and reproductive organs of the tick, producing deleterious effects on the feeding and reproductive behaviour of the tick." Allen and Humphreys indicated that vaccination might be a particularly suitable method for control of one-host tick infections, such as *B. microplus.*

Not long after Allen and Humphreys published their findings, scientists at the Commonwealth Scientific and Industrial Research Organization in Queensland, Australia, began a research program investigating vaccination against *B. microplus.* By 1981 they had confirmed that protection could be achieved against *B. microplus* using crude antigen preparations derived from whole ticks (Tellam et al, 1992). Details of the results of these experiments were released in a series of articles published in 1986 (Agbede and Kemp, 1986; Johnston et al, 1986; Kemp et al, 1986). Fewer ticks from a challenged infection successfully fed on vaccinated cattle than on control cattle. Many of the ticks from vaccinated animals had damage to the gut, with erythrocytes present in the hemolymph. Evidence was obtained by feeding ticks in vitro on plasma from vaccinated cattle, implicating antibody and complement in mediating damage to the ticks. These discoveries came at the time that recombinant DNA technology was beginning to be applied in the field of parasitology, and the research program moved on to focus on identifying protective antigen(s) and using recombinant methods to produce a practical vaccine. It would not be hyperbole to describe the ensuing research program as heroic. A complex series of biochemical fractionation procedures were undertaken, each of which entailed assessment of various fractions in cattle vaccine trials along with examination of challenge infections with ticks (Willadsen et al, 1988, 1989). A glycoprotein of approximately 89 kDa was identified as the lead protective antigen (designated *Bm86*). Four consecutive preparations were made of the Bm86 antigen, each involving approximately 50,000 hand-picked, partially engorged female ticks weighing 20 to 30 mg, which yielded between 24 and 187 μg of the Bm86 antigen. Amino acid sequences were obtained following endoproteinase lys-c digestion of Bm86 (Willadsen et al, 1989); corresponding oligonucleotides were synthesized and synthesized elements were used to screen a cDNA library prepared in λgt11 (Rand et al, 1989). An insert sequence encoding Bm86 was sub-cloned into a plasmid expression vector, and a fusion protein comprising 651 amino acids of *E. coli* β-galactosidase and 599 amino acids of Bm86 (amino acids 31 to 629) was expressed in *E. coli*. Vaccination of cattle with insoluble inclusion bodies containing this antigen induced significant protective effects against *B. microplus,* reflected by a 77% decrease in eggs laid by ticks that fed on the vaccinated animals (Rand et al, 1989).

The characteristics of the Bm86 protein have been reviewed by Tellam et al (1992). Bm86 is an 89-kDa glycoprotein that is associated particularly with the surface membrane of digest cells in the tick gut. The full amino acid sequence is predicted to be 650 residues (M_r 71,716), with the sequence comprising 66 cysteine residues. Bm86 contains eight repeated domains with homology to epidermal growth factor, a predicted 19 amino terminal secretory signal sequence, and a 23 amino acid hydrophobic region, which has been confirmed to be a functional glycosyl-phosphatidylinositol membrane anchor sequence.

Commercialization of TickGARD and a subsequent variant with a modified adjuvant, TickGARD^{PLUS}, was undertaken by the Australian scientists in collaboration with Biotech Australia Pty, Ltd. TickGARD® was initially marketed by Hoechst Animal Health; however after the breakup of Hoechst AG and the sale of Hoechst Animal Health to Intervet International, the vaccine lost commercial support for a period. Although it was reintroduced on a limited scale by Intervet Australia Pty, Ltd., it is no longer being produced. Commercial production of Gavac (Figure 9-11) has

FIGURE 9-11. Recombinant *Boophilus microplus* vaccine for cattle, Gavac, manufactured by Heber Biotec S.A. Cuba, and marketed since 1993.

continued since its development by scientists at the Center for Genetic Engineering and Biotechnology in Havana and its commercialization by Heber Biotec S.A. in 1993. The vaccine was released in Colombia in 1994, in Brazil in 1995, and in Mexico in 1997 (de la Fuente et al, 2007). Gavac contains Bm86 expressed in the yeast *Pichia pastoris* (Canales et al, 1997) together with Montanide 888 adjuvant. Delivery of Gavac by deep intramuscular injection (2 mL) is recommended for cattle older than 1 month into the scapular area, or the gluteal region, on three occasions, with the second injection given 4 weeks after the first, and the third injection 7 weeks after the first. Booster immunization is recommended 6 months after the first injection and at 6-monthly intervals thereafter. In a 10-year review of the commercial use of TickGARD and Gavac, de la Fuente et al (2007) indicate that the vaccine is effective in controlling *B. microplus*. Use of the vaccine has been associated with a decrease in acaricide usage and reductions in anaplasmosis and babesiosis. The vaccine has been found to provide protection against *Boophilus annulatus* and *B. microplus*, but it does not protect against *Amblyomma cajennense* (Canales et al, 2009; de la Fuente et al, 2007). The vaccine provides protection for camels against *Hyalomma dromedarii* (Rodríguez-Valle et al, 2012).

The derivation of the nomenclature for the Bm86 antigen has not been explained. In the original patent documents and scientific publications, it was always recognized as being an 89-kDa native antigen. Hence, the seemingly likely scenario that initial size estimates suggested it was an 86-kDa antigen has no support. Peter Willadsen (personal communication) has provided information to solve the riddle. The antigen was named for the year in which it was first discovered—1986. The research group discovered another protective antigen in 1991, Bm91 (Riding et al, 1994), the size of which just happens to be 86 kDa in its native form!

FUTURE PROSPECTS

Progress has been slow in the development of effective commercial vaccines against many important parasitic infections of veterinary and medical importance. Nevertheless, new vaccines are coming to the market and the number of effective recombinant vaccines is increasing, albeit slowly. New technologies, including those in cell culture, stem cell technologies, and transgenics, may offer the prospect in the future of expressing antigens in their correct conformation with appropriate post-translational processing, so that better defined antiparasite vaccines come to commercial fruition. Veterinary vaccines, similar to vaccines for the neglected tropical diseases of man, face both formidable scientific hurdles in their creation and the harsh commercial realities of the marketplace, representing challenges and opportunities for the future.

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Derivations of Some Scientific Names and Terms

ARTHROPODA	jointed foot
INSECTA	plural of Latin *insectum* (i.e., things cut into sections)
Diptera	two wings
Nematocera	thread-horn
Ceratopogonidae	horn beard
Culicidae	family of mosquitoes
Culicoides	mosquito-like
Phlebotomus	vein cutting
Simuliidae	family of things put together
Brachycera	short-horn
Chrysops	golden eye
Haematopota	drinking blood
Silvius	of woods or forests
Tabanus	gadfly
Cyclorrhapha	round suture
Auchmeromyia	unwashed or squalid fly
calcitrans	kicking with the heels
Calliphoridae	family beauty bearing
Chrysomyia	gold fly
Cochliomyia	screw fly
Cordylobia	lifelike swelling
Cuterebra	skin borer
Gasterophilus	stomach loving
Glossina	like a tongue
Haematobia	bloody life
Hippoboscidae	family of horse feeders
Hypoderma	under skin
Melophagus	sheep eater
Musca	housefly
Oestrus	gadfly
Sarcophaga	meat eater
Stomoxys	mouth sharp
Anoplura	no ribs
Haematopinus	blood pine-tree
Linognathus	thread jaw
Pediculus	little feet
Polyplax	many plates
P(h)thirus	louse
Solenopotes	pipe drink
Mallophaga	wool eaters
Damalinia	deer streak
Felicola	inhabiting cats
Heterodoxus	accepting the other
Trichodectes	hair receiver
Siphonaptera	tube no wing—wingless suckers
Ctenocephalides	cockle-like head
Echidnophaga	monster eater
Pulex	flea [in Latin]
Xenopsylla	host flea [in Greek]
Hemiptera	half-wing
Cimex lectularius	bug of little bed
Triatoma	cut into three
ARACHNIDA	spiders, family of
Metastigmata	punctures behind
Argas (= Argos)	named for the host, a pheasant (named for the 100-eyed monster, Argus, killed by Hermes, whose eyes were put on the peacock tail)
Ornithodoros	bird gift
Otobius	ear way of life
Amblyomma	dull eye
Boophilus	cattle lover
Dermacentor	skin puncturer
Haemaphysalis	blood bladder
Ixodes	sticky, like bird lime
Rhipicephalus	fan head
Mesostigmata	punctures in the middle
*Dermanyssu*s	skin piercer
Halarachne	salt spider
Liponyssoides	fat piercer
Ophionyssus	snake piercer
Ornithonyssus	bird piercer
Pneumonyssus	lung piercer
Raillietia	Railliet—famous parasitologist
Astigmata	no punctures
mange	from manger (French)—to eat
Chorioptes	membrane visible
Myocoptes	smiter of mice
Notoedres	back seat, referring to dorsal anus
Otodectes	ear receiver
Psoroptes	scab visible
Sarcoptes	flesh visible
Prostigmata	punctures in front
Cheyletiella	small lip
Demodex	tallow receiver
Lynxacarus	lynx mite
Myobia	lives on the mouse
Neotrombicula	new small twisted vein
PENTASTOMIDA	five mouths
Armillifer	bearing bracelet
Linguatula	small tongue
PROTOZOA	first animal
Acanthamoeba	spiny amoeba
Apicomplexa	referring to the apical complex
Babesia	Babes—famous protozoologist
Balantidium	small bag
Besnoitia	Besnoit—famous protozoologist
Blastocystis	germinating cyst
Bradyzoite	slow animal
Cryptosporidium	hidden small seeds
Cytauxzoon	hollow vessel (cell) increasing animal
Entamoeba	internal amoeba
Giardia	Giard—famous protozoologist
Hepatozoon	liver animal
Histomonas	tissue unit
Isospora	equal seeds
Leishmania	Leishman—famous protozoologist
Meront	part
Neospora	new seed
Sarcocystis	flesh cyst
Schizont	something splitting
Tachyzoite	swift animal
Toxoplasma	bow body (thing that is molded)
Trichomonas	hair unit
Trypanosoma	auger body

Derivations of Some Scientific Names and Terms—cont'd

HELMINTH	worm
PLATYHELMINTHES	flat worms
TREMATODA	like holes
Alaria	winged
Cercaria	tail
Clonorchis	confused testicle
Dicrocoelium	double-oared cavity
Fasciola	small band
Fascioloides	fasciola-like
*Gastrodiscoide*s	stomach like a disk
Heterophyes	different form
Mesocercaria	time period between tail and no tail
Metacercaria	time period after the tail
Metagonimus	posterior gonads
Metorchis	posterior testis
Miracidium	small child
Nanophyetus	dwarf form
Opisthorchis	poster genitalia
Paragonimus	side-by-side gonads
Paramphistomum	associated with mouths at both ends
Platynosomum	flat disease causing
Redia	Redi—father of parasitology
Schistosoma	split body
sporocyst	seed cyst
CESTODA	beltlike
Oncosphere	hairy ball
Strobilus or Strobila	anything twisted up
Pseudophyllidean	false leaves
Coracidium	little hooked engine of war
Diphyllobothrium	two-leaf trench
Plerocercoid	in the shape of a complete tail
Procercoid	the shape before the tail
Sparganum	swathing band
Spirometra	spiral uterus
Cyclophyllidean	round leaves
Anoplocephala	unarmed head
Coenurus	shared tail
Cysticercoid	shape of a tail with a cyst
Cysticercus	tail with a cyst
Dipylidium	two entrances
Echinococcus	spined kernel
Hydatid	pertaining to water
Mesocestoides	in-between cestode
Strobilocercus	twisted tail
Taenia pisiformis	intestinal worm (in Latin) and pea-shaped appearance of the proglottids
Taenia taeniaeformis	intestinal worm (in Latin) and tapeworm form of the adult
Tetrathyridium	four oblong shields
Thysanosoma	fringed body
NEMATODA	thread form
Aelurostrongylus	cat strongyle
Ancylostoma tubaeforme	curved mouth and straight trumpet shape
Angiostrongylus	vessel strongyle
Aonchotheca	sheath diminished in bulk
Ascaris	worm in the intestine
Baylisascaris	Baylis—famous parasitologist
Bursa	a purse
Bunostomum	mound mouth
*Coronocyclu*s	cyclic crown
Craterostomum	mixing bowl mouth
Crenosoma	notched body
Cyathostoma	cup-shaped mouth
Cyathostomum	ladle mouth
Dictyocaulus	latticework penis
Dioctophyme	twice eight tubercles
Dipetalonema reconditum	two-petaled thread and hidden
Dirofilaria immitis	dread thread and inexorable
*Dracunculu*s	small dragon
Filaroides	filarid-like
Globocephalus	ball head
Gnathostoma	jaw mouth
Gongylonema	nematode with bumps
Habronema	delicate thread
Haemonchus	blood spear
Halicephalobus	lobed head from salt
Hyostrongylus	hog strongyle
Mammomonogamus	married to one spouse in mammals
Nematodirus	terrible nematode
Oesophagostomum	esophagus mouth
Ollulanus	small jar anus
Onchocerca	barbed tail
Oxyuris	sharp tail
Physaloptera	bladder wing
Setaria	bristles
Skrjabinema	Skrjabin—famous parasitologist
Spicule	small shaft
Spirocerca	coiled tail
Stephanurus	encircling the urinary system
Stichocyte	cell of a row
Stichosome	row body
Streptopharagus	whirled chasm
Strongyloides	roundlike
Strongylus vulgaris	round and common
Syngamus	married together
Toxascaris	arrow ascaris
Toxocara	arrowhead
Trichinella	small hairs
Trichostrongylus	hair round
Trichuris	thread tail (original description thought the skinny end to be the tail end)
Triodontophorus	carrying three teeth
Uncinaria stenocephala	hooked nose and narrow head
ACANTHOCEPHALA	thorny head
Macracanthorhynchus	giant thorny trunk
Neoechinorhynchus	new spiny nose
Prosthenorchis	anterior strong testis