Review

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Infectious Bovine Keratoconjunctivitis: A Review

Michael H. Brown, Alan H. Brightman, Bradley W. Fenwick, and Maureen A. Rider

The economic impact of infectious bovine keratoconjunctivitis (IBK) warrants continued investigation of the mechanisms by which *Moraxella bovis* survives on and colonizes the corneal surface. Virulent strains of *M bovis* produce hemolysin and exhibit different plasmid profiles than nonvirulent strains. Interactions among host, environment, vector, season, and concurrent infection influence the prevalence of IBK. *Mycoplasma* sp. or infectious bovine rhinotracheitis virus may enhance or hasten the disease process. The manifestations of IBK may range from mild conjunctivitis to severe ulceration, corneal perforation, and blindness. Treatment of IBK is dictated by economic considerations, intended animal use, and feasibility of administration. Antibiotic therapy is aimed at achieving drug concentrations in tears to meet or exceed the minimum inhibitory concentration for prolonged periods. At present, IBK is not a preventable disease. Affected animals must be separated from the herd and vector control vigorously instituted. Carrier animals must be identified and removed from the herd. Vaccination trials have been unsuccessful because of pili antigen cross-reactivity, variable strains, and uncontrolled environmental factors. Recent investigations have determined that *M bovis* may utilize host iron sources via iron-repressible outer membrane proteins and siderophores for growth. Elucidation of normal defense mechanisms of the bovine eye may lead to new strategies to enhance the immune response against *M bovis*.

Key words: Bovine pinkeye; IBK.

The first reports of infectious bovine keratoconjunctivitis (IBK) appeared in 1889[1] and nearly a century later, the mechanisms *Moraxella bovis* uses to survive in the ocular environment of cattle are poorly understood. Therapeutic and preventative measures have limited success, therefore, continued investigation is warranted. The detrimental effects of IBK are documented by regional and national surveys.[2,3] Kansas cattle ranchers in 1993 reported IBK to be the 2nd most common disease. The clinical effects of IBK in male and female calves are long-standing; affected calves have adjusted 205-day body weights decreased by 17–18 kg compared to body weights in healthy calves.[2] Postweaning animals have lower performance parameters in average daily gain, weight per day of age, 365-day weight, and final weight.[4,5] Bilateral disease produces greater losses than unilateral disease, wherein final weight is reduced an average of 15.9 kg (35 pounds).[6] Missouri cattle ranchers report endemic IBK in 45.4% of all herds, with an average prevalence of 8.75/100 cattle in affected herds.[7] The economic impact of IBK is not restricted to North America. In an Australian postal survey 81.3% of participating cattle owners reported the occurrence of IBK, and 75% observed reduced weight gain in affected cattle.[8] In Australia, more than 22 million dollars were estimated to be lost because of reduced production, with 1.5 million dollars spent for treatment in 1979.[9] The worldwide distribution and economic impact of IBK requires veterinarians to be familiar with the latest information regarding this complex disease.

From the Veterinary Referral Centre, Little Falls, NJ (Brown); the Department of Clinical Sciences (Brightman), and the Department of Diagnostic Medicine/Pathobiology (Fenwick, Rider), College of Veterinary Medicine, Kansas State University, Manhattan, KS.

Reprint requests: Michael H. Brown, DVM, MS, Veterinary Referral Centre, 48 Notch Road, Little Falls, NJ 07424;
e-mail: Muro128@aol.com.

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Epizoology

Although *M bovis* is the pathogen most commonly isolated from IBK, various factors are involved with the pathogenesis of this disease. The environment, season, concurrent pathogens, *M bovis* strain, and host immune system play integral roles in the occurrence and clinical severity of IBK. IBK is a highly contagious disease that spreads rapidly within a herd.[6] The yearly and seasonal prevalence of the disease in different geographic regions varies greatly.[10,11] The isolation rate of *M bovis* infection gradually increases during the spring (21.4%) and summer (29.3%) months to reach a maximum in the fall at 45%.[12] Peak IBK prevalence is preceded by the highest values of ultraviolet (UV) radiation.[13,14] IBK can occur during any time of the year, but outbreaks are more common during summer months. Nonetheless, heavy snowfall and UV radiation have been individually associated with outbreaks of IBK.[15–17]

M. bovis is spread by direct contact, nasal and ocular discharges, and by mechanical vectors. The most important vector is considered to be the face fly (*Musca autumnalis*).[17] The house fly (*Musca domestica*) and barn fly (*Stomoxys calcitrans*) may also transport the organism. In addition to physically irritating the eye, insects may harbor the organism on their legs for up to 3 days.[18] A positive correlation exists between the number of flies per animal and *M bovis* infection,[18] and disease prevalence is reduced by rigorous fly control programs.[17] Various forms of insect control (face dust bags, back rubbers, and insecticide-impregnated ear tags) have proven equally effective.[18] Regardless of the aggravating factors, *M bovis* has to be transmitted in sufficient quantities for IBK to occur,[17] and preventing transmission remains the single most important factor in controlling the disease. Other management factors such as dry tall weeds, dust, and lack of mineral supplementation have been suspected in the pathogenesis of disease, but not proven.[18] Long-distance transport of cattle may represent a stress factor that is associated with the carrier state of the disease.[19] Higher numbers of *M bovis* isolates are obtained by bacterial culture from nasal secretions from cattle after shipment than before shipment. Cattle represent the only known

natural reservoir for *M bovis* and subclinically infected carrier animals may harbor the organism year-round.[20] *M bovis* may be isolated throughout the year from ocular and nasal secretions of cattle naturally affected with IBK.[19]

Variation exists between the prevalence of disease and breeds affected. In South America, Aberdeen Angus and Charolais have a higher prevalence of IBK than do the same breeds in North America and Australia.[7,8,21] In the United States, Hereford cattle appear to be predisposed to IBK, whereas Brahma and Zebu or their crosses (*Bos indicus* breeds) are less frequently affected.[7] In Australia, Channel Island breeds are more often affected than is the Hereford, a surprising finding considering the high prevalence of the disease in Herefords in other studies.[9] In South America, periocular pigmentation is suspected to be an important risk factor for IBK, because pigmented Angus cattle have a higher prevalence of the disease than do Aberdeen Angus and Charolais cattle.[5] In addition to breed variation, susceptibility to IBK may be age-related. The recovery rate of *M bovis* from ocular secretions of younger and older cows is similar; however, older animals have a lower prevalence of clinical disease.[5,22] A genetic or age-related susceptibility has been suggested because increased numbers of *M bovis* can be isolated from younger animals lacking periocular pigment[23] than from older animals with periocular pigment. Overall, the role of periocular pigmentation is not well defined.[23–25] Older cattle appear less susceptible to *Moraxella* ocular infection (4.5–19.4%) when compared to susceptibility of cattle less than 2 years of age (10–62.5%).[8] Calves from older dams appear less susceptible to disease than calves from dams less than 3 years of age.[5] No gender predilection has been definitely determined,[26] although a higher prevalence has been reported in males.[26]

Etiology

M. bovis, a gram-negative bacillus, is generally regarded as the etiologic agent of IBK.[27] *M bovis* represents the only organism capable of partially fulfilling Koch's postulates and is the agent most commonly isolated from cattle with clinical disease.[28] The morphologic description of *M bovis* colonies grown in vitro is either rough or smooth. Bacteria isolated from clinical cases of IBK form colonies that are rough, flat, dry, firm, and umbonate. The rough colony phenotype is associated with cell surface pili, autoagglutination in distilled water, and hemagglutination. Following culture, rough colonies may spontaneously transform into smooth, moist colonies that do not adhere to growth medium.[29,30] The specific bacterial strain and culture conditions govern the transition from rough to smooth colonies. *M bovis* isolates exhibit pleomorphism as the culture ages, occurring in pairs or chains as short, plump rods with rounded ends.[31] On blood agar, colonies are approximately 1–3 mm in diameter, with a zone of beta hemolysis extending approximately 1 mm from the colony edge. Characteristic growth patterns occur at the interface between agar and a polystyrene petri dish, further aiding in bacterial differentiation.[32] *M bovis* isolates do not require complex growth media.[33] Nonhemolytic strains of *M bovis* are not generally associated with clinical disease.

Routine laboratory techniques may aid in the diagnosis of *M bovis.*[31] *M bovis* is nonmotile and mostly hemolytic. The bacteria does not ferment carbohydrates or reduce nitrates, and is oxidase-positive. A characteristic 3-zone reaction is noted in litmus milk, which later changes into a homogeneous purple. The surface of liquid media does not support growth of *M bovis,* but instead produces coarse, flocculent sediment with little turbidity.

The literature is replete with pathogens other than *M bovis* that are associated with clinical IBK.[34–37] Infectious bovine rhinotracheitis (IBR) virus and *Mycoplamsma* spp. are the two most commonly associated organisms with clinical IBK. IBR virus and adenovirus-associated conjunctivitis, keratitis, and secondary anterior uveitis may be confused with IBK and careful distinction is necessary as these conditions may exist concurrently.[38,39] Experimental inoculation of *M bovis* into healthy cattle eyes is not always successful in reproducing the clinical signs of IBK. A higher prevalence of the disease is noted when *M bovis* is instilled into the eye that has been exposed to UV radiation or concurrent *Mycoplasma* infection.[26,37] Whether *Mycoplasma* alone can cause clinical IBK or whether *Mycoplasma* enhances the disease caused by *M bovis* is controversial.[34–36] One study failed to demonstrate a difference in *Mycoplasma* isolation between normal and naturally occurring IBK.[12] Instillation of *Mycoplasma* alone into healthy bovine eyes may cause conjunctivitis, but is unable to produce clinical IBK. Although *Mycoplasma* conjunctivitis and IBK are separate clinical entities, *Mycoplasma bovoculi* has strong cell association with corneal epithelium and may increase infection rate and duration of infection by *M bovis* and *Moraxella ovis* in calves.[34–36] *M bovoculi* is the only pathogen isolated in epizootic conjunctivitis.[35] In an epidemiologic study of 8 herds, 6 had epizootic conjunctivitis; *M bovis* and *M bovoculi* were isolated from cattle with IBK.[35] Latency of *M bovis* occurs, evidenced by isolation of the organism more than 4 weeks after infection, and frequent recurrence of disease.[35] Recovery rates of *M bovis* and *Mycoplasma* sp. from ocular secretions are low when both are instilled into healthy eyes and may not necessarily precipitate clinical IBK.[40] The interval between instillation of the 2 organisms may be important to the clinical disease. Clinical IBK cases have been associated with an unidentified *Mycoplasma* sp.[40] A positive relationship exists between the severity of infection and the isolation of *M bovis* and/or *Mycoplasma.* Other cases of IBK have been associated with *Ureaplasma* sp., a reported cause of bovine conjunctivitis.[40] When calves infected with *Ureaplasma* were challenged with *M bovis,* a prolonged colonization period or keratitis did not occur.[34]

Pathophysiology

The pathophysiology associated with *M bovis*-induced IBK is not completely understood. The virulence of different bacterial strains is associated with capsular pili and rough colony appearance. In addition to colony morphology, crystal violet staining can further characterize virulence of the bacteria. Colonies that stain with crystal violet (rough) contain pili.[30] Pili are important structural attributes that allow bacteria to adhere to the corneal surface, thus enhancing their ability to defeat host defense mecha-

nisms.[41–44] The pilated strain of *M bovis* is the only form able to cause infection, and nonpilated forms appear to be nonpathogenic.[45] A unified pili serotyping scheme has been introduced to address the differences in pili antigens (United States, Australia, United Kingdom).[46] Pili have been arranged in 7 groups (A–G) to decrease confusion with the previous numbering system. The 7 pili groups exhibit little antigenic variation when comparing isolates from Europe, Australia, and the United States.[46] Tears from animals vaccinated with pili bacterin are able to inhibit adherence of homologous *M bovis* to bovine corneal endothelial cells.[42] Epizootic IBK has been associated with the emergence of a new pilus type that reacts weakly with antibodies against the classical strains.[47] Various types of pili have been described, and each is associated with the production of specific antibodies.[41–44] Failure of commercial pili vaccines for *M bovis* may be due to low cross-reactivity among variant pili types.

M. bovis isolates may also be characterized by their plasmids, which presumably carry virulence factors.[30] In vivo and in vitro studies confirm the divergent behavior of strains with different types of plasmids.[30] An *M bovis* isolate with 5 plasmids had low virulence in cattle, and little ability to destroy macrophages in vitro. A strain with 3 plasmids produced IBK in an experimental animal (bovine) and was cytotoxic in vitro. Three basic groups of plasmid patterns, containing between 1 and 6 plasmids, are recognized in the United States.[48] The plasmid patterns are exhibited by smooth and rough isolates of the same strains. Isolates from large herds with outbreaks contain multiple plasmid patterns, whereas isolates from small herds with clinical IBK have an identical plasmid pattern.[48]

The pathophysiology of IBK is likely associated with collagenase release from epithelial cells, fibroblasts, and neutrophils.[49] Hydrolytic enzymes of *M bovis* possess the ability to degrade lipids, mucopolysaccharides, and matrix proteins, which may contribute to corneal ulceration.[49] However, the ability to hydrolyze collagen could not be proven among 13 reference strains of *M bovis*.[49] The initial production of corneal ulcerations, however, appears to be attributed to direct cytotoxicity of *M bovis* and not endogenous inflammatory factors from neutrophils.[50] *M bovis* likely releases a necrotizing factor that kills corneal epithelial cells.[50] Living *M bovis* and sterile filtrates from shaker cultures of *M bovis* kill corneal epithelial cells in vitro, further demonstrating a cytotoxic factor of *M bovis*.[51]

The ability to produce hemolysin may be an important virulence factor of *M bovis*. A positive correlation exists between the percentage of hemolytic strains isolated and prevalence of clinical disease. Nonhemolytic strains of *M bovis* were isolated in 26% of animals tested when clinical IBK was not observed.[47] When clinical disease subsides, the percentage of nonhemolytic strains returns to original values. Increased levels of UV radiation in warmer months, when hemolytic isolates are most frequently recovered, may aid in the transformation from nonhemolytic to hemolytic strains.[47] A hemolytic fraction of *M bovis* containing outer membrane-bound vesicles is cytotoxic to calf corneal epithelial cells in vitro and in vivo.[52]

Preexisting infection with *Mycoplasma* may act as a predisposing factor for clinical IBK. *M bovoculi* preinfection extends *M bovis* ocular colonization time.[34] Pathogenicity may be enhanced by extended time for replication and expression of virulence factors by *M bovis*. *Mycoplasma* spp. are highly associated with corneal and conjunctival cells,[53] and *M bovoculi* is cytotoxic in vitro to bovine corneal cell monolayers.

Nuclear fragmentation, loosening of bovine corneal epithelial cells, and epithelial degeneration occur with increased UV irrradiation.[54] As the integrity of the corneal epithelium is disrupted, *M bovis* may penetrate and multiply.[55] The effects of UV on the cornea include keratitis, corneal edema, and corneal ulceration.[56]

The precorneal tear film is an integral part of ocular physiology and is essential in ocular defense. Tears wash away pathogens by mechanical flushing, whereas tear proteins are integral to the protective mechanisms of tear film. Tear proteins identified in humans include prealbumin, immunoglobulins (Igs), lysozyme, lactoferrin, transferrin, complement, β-lysin, and antiproteinases.[57–60] Protective defense mechanisms of the bovine tear film are poorly understood.[61,62] Bovine lacrimal secretions contain IgA (secretory), IgGa, IgGb, and IgM.[63] Standard spectrophotometric and plate assays failed to identify lysozyme in bovine tears.[61] A paucity of information exists concerning the iron-binding proteins in tears of domestic animals.[61,62] Human tears are known to contain lactoferrin,[64,65] whereas rabbit tears are reported to contain transferrin.[66] Both lactoferrin and transferrin have been identified in guinea pig tears.[66] The importance of host iron-binding proteins in external secretions is derived from their antimicrobial properties.[67–69] Recently, these protiens have gained attention as a potential source of iron for pathogenic bacteria,[70] including *M bovis*.[71,72]

In the absence of lysozyme, lactoferrin and secretory IgA likely represent integral components of bovine ocular defense. Lactoferrin has recently been demonstrated in bovine tears.[73] Whole bovine tears were analyzed using size exclusion high-performance liquid chromatography (HPLC), sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and amino acid sequencing to determine the presence of lactoferrin. Based on HPLC chromatograms of purified lactoferrin, peak collection was performed to recover a protein from the bovine tear film with chromatogram characteristics nearly identical to purified bovine lactoferrin. SDS-PAGE and silver staining of this protein provided a band consistent with bovine lactoferrin (estimated mass of 78 kd). The first 13 amino acid residues of this protein were identical to the amino acid sequence of bovine lactoferrin. Lactoferrin is found in very high concentrations in ocular, oral, and genital secretions, where it may exert bacteriostatic effects. Bacteria are controlled at these mucosal sites by lactoferrin, which deprives them of iron, an essential element for their growth. A greater understanding of how *M bovis* overcomes the bacteriostatic effects of bovine tear lactoferrin may provide valuable information with respect to bovine ocular health and *M bovis*-associated IBK.

A virulent strain of *M bovis* may utilize bovine lactoferrin as a sole source of iron for required growth.[72] *M bovis* strain Epp63 was iron-starved by repeated passages in iron-restricted media (RPMI 1640) to adapt the bacteria to growth in iron-restricted conditions. Chelex® 100 resin

(Biorad Laboratories, Richmond, CA) was added to tryptic soy broth (TSB) to remove iron. The deferrated TSB was then mixed with agarose. Purified bovine milk lactoferrin, colostral lactoferrin, and serum transferrin were added to deferrated filter paper discs and applied to the TSB–agarose plates to determine if *M bovis* could utilize different iron sources for growth. Growth in TSB was inhibited by the addition of Chelex® 100 resin to the media. *M bovis* was able to grow around filter paper discs supplemented with purified bovine milk lactoferrin, but not around discs supplemented with colostral lactoferrin or serum transferrin. The ability of *M bovis* to grow in iron-restricted media after successive passages and to utilize bovine lactoferrin as a source of iron for growth suggests an important virulence mechanism of *M bovis*.

In vitro studies suggest that *M bovis* possesses iron-acquisition systems such as siderophores and outer membrane receptors that bind bovine lactoferrin.[74,75] *M bovis* (strain Epp63) was grown in iron-restricted media to induce the expression of iron-repressible outer membrane proteins (IROMPs). The ability of these outer membrane proteins to bind bovine lactoferrin was determined by SDS-PAGE and immunoblotting. Bovine milk lactoferrin binds a 34- and 70-kd IROMP of *M bovis*. The bacteria were also cultured with gold-labeled bovine milk lactoferrin and transmission electron microscopy was utilized to determine the ability of *M bovis* to bind bovine milk lactoferrin. Gold-labeled lactoferrin particles bind to the surface of *M bovis* when grown in iron-restricted media. *M bovis* grown in iron-supplemented media bound very few, if any, gold-labeled particles. It is important to determine whether iron acquisition from lactoferrin in bovine tears by *M bovis* enhances infection. Immunologic recognition of the IROMPs (iron-acquisition systems) of *M bovis* may represent an effective means of preventing IBK.

Clinical Signs

Many clinical descriptions of IBK are available.[11,17,55,76–79] Frequently only 1 eye is affected initially; however, cross-infection from the 1st eye may then lead to bilateral disease.[11] The initial description of the clinical disease includes 5 stages of IBK based on severity.[79] In the acute form, mild conjunctivitis and keratitis are noted. Subacute stages are characterized by corneal ulceration. Clinical signs of chronic IBK include severe keratoconjunctivitis with descemetocele formation and possible ocular rupture. Severe ulceration, panophthalmitis (generally bilateral), blindness, and death due to ascending infection depict the rare, fulminating form of the disease.[80] The carrier form occurs in cattle with intermittent or chronic excessive lacrimation; however, many show no signs of infection. Killinger and Helper[81] described an alternative method of classifying the severity of disease on the basis of lesion appearance.

Experimental infections demonstrate variation in clinical disease.[10] Cattle may spontaneously recover from the disease at any point. The onset of clinical signs in experimental infection occurs between 1 day and 2 weeks postinoculation.[28,54,82] The first signs of IBK are profuse lacrimation and/or photophobia with varying degrees of blepharospasm. Inability to closely observe cattle often precludes diagnosis at this stage. A serous or mucopurulent discharge is usually noted as the disease progresses.[28,54,82] Within 24–48 hours after the onset of lacrimation and photophobia, corneal lesions usually develop, although ulceration may be delayed for up to 2 weeks.[69] Ulceration may be preceded by the development of small corneal vesicles.[17,54] The axial cornea develops 0.25- to 1-mm epithelial defects that may not be noticed without close observation.[83] As the disease progresses, size of corneal ulcers increases up to or greater than 25 mm in diameter with subsequent loss of corneal stromal integrity. The epithelial defect leads to an ingress of fluid into the corneal stroma resulting in corneal edema. Occasionally, corneal opacities are noted prior to epithelial loss and ulceration. As the disease progresses, conjunctivitis of varying severity develops. Conjunctival vessels are dilated, and mild to moderate chemosis and hyperemia develop. Eyelid abnormalities include generalized edema and blepharitis.[84]

As the corneal stroma becomes affected, inflammatory by-products and proteolytic enzymes compromise corneal integrity. The patient may lose vision due to severe corneal edema, photophobia, or blepharospasm. Corneal rupture may be spontaneous or result from blunt trauma. If perforation occurs, panophthalmitis and phthisis bulbi may result, but more commonly, uveal prolapse and fibrin seal the wound and the shape of the globe is retained. In contrast to other species, wherein corneal perforation is an emergency to prevent loss of the globe or vision, cattle may retain all or partial vision after corneal rupture.[84] As early as 2 days after ulcer development, the healing process may begin with corneal neovascularization. Superficial or deep corneal neovascularity depends on the depth and nature of the corneal lesion. Superficial vessels are bright red, arborizing, and originate at the limbus and may extend to the axial cornea. Deep corneal vascularity occurs as dark red thin "paint brush" vessels originating at the limbus. Neovascularization may occur over 360° of the cornea or may be restricted to the limbus opposite the lesion. Granulation tissue may form at the ulcer site, depending on the degree of corneal stromal involvement. Once severe stromal defects have healed, a dense axial corneal scar (leukoma), lasting months to years, will result. In milder cases of IBK, with superficial corneal involvement, corneal opacities may completely resolve within 2–4 weeks.

Treatment

Although antimicrobial therapy is the treatment of choice for IBK, no regimen will ensure 100% success. Antimicrobial therapy may not eradicate the carrier state or improve the clinical disease.[85,86] Maintaining consistent therapeutic drug concentration in tear film is difficult because of economic and practical considerations.[87]

M. bovis is typically susceptible to gentamicin, 1st generation cephalosporins, trimethoprim-sulfonamides, nitrofurans, tetracycline, and sulfonamides. Increasing resistance has been shown against tylosin, lincomycin, streptomycin, erythromycin, and cloxacillin.[27,28,83,84] Susceptibility patterns to procaine penicillin are inconsistent.[27] Regional and strain differences in susceptibility patterns necessitate culture and sensitivity testing prior to selection of antibiotic treatment.

To achieve therapeutic drug concentration, topical antibiotic administration is required several times per day; however, daily multidose therapy is not practical for most producers.[78,79] Administration of benzathine cloxacillin is effective for the treatment of experimentally induced and naturally acquired cases of IBK.[88] Powders and dyes, once commonly used for the treatment of IBK, are not widely used due to painful irritation of crystals, which causes lacrimation, thus reducing effectiveness. Oral medication in feed is not possible unless affected animals are separated from the herd. Parenteral (subconjunctival, subcutaneous, intramuscular, and intravenous) antibiotics are commonly used.[86,87,89] High concentrations of antibiotic may be achieved in tear film by subconjunctival administration, although local irritation may result. Subconjunctival injection of tetracyclines is effective but may cause necrosis at the injection site. Intramuscular oxytetracycline is more effective than topically applied furazolidone.[85]

Efficacy of antimicrobial drugs for treatment of IBK is influenced by the pharmacologic properties of the drug. Long-acting tetracycline is effective after parenteral use, even with relatively low tear drug concentrations, because concentrations are maintained above the minimum inhibitory concentration for a prolonged period.[90] High tear concentrations of kanamycin of short duration are more effective in eliminating *M bovis* than are lower, longer duration concentrations after intramuscular treatment.[27] Recurrence and transmission of the disease occur during the carrier stage of the disease. Duration of the carrier stage (subclinical infection) is reduced by 2 intramuscular injections of long-acting tetracyclines at 20 mg/kg.[91] In addition to shortening the duration of the carrier stage, this treatment reduces progression of lesions and healing times in affected animals. Given at 20 mg/kg body weight, long-acting oxytetracycline distributes selectively to the epithelium of the conjunctiva and lacrimal gland ductules, reaching higher concentrations than in serum.[92] Mean peak lacrimal fluid concentrations less than 1 μg/mL were reached after intramuscular administration. Subconjunctival injections may increase tear oxytetracycline concentrations to greater than 2.0 μg/mL for 72 hours; however, severe local reactions may preclude this method of treatment. In comparing treatment regimens in calves with naturally acquired IBK, those treated with parenteral long-acting oxytetracyline had shorter periods with corneal ulcers than did nitrofurazolidone-treated calves.[93] A single dose of sulfadimidine at 100 mg/kg eliminates the organism from ocular and nasal secretions after experimental infection.[94]

Treatment selection is a function of the type of management practice and intended use of the animals. The goal of a producer of purebred animals is cosmetic healing, whereas dairy producers are concerned with milk withdrawal times. Dairy operators choose procaine penicillin because of short milk withdrawal time (3 days).[27] Commercial beef producers factor the cost of medication, labor, and decreased weight gain. For these reasons, long-acting tetracyclines are often the treatment of choice for beef producers. Because an optimal treatment does not exist and losses are often underestimated, the commercial beef producer may not treat affected animals.[9] Preventative medication of the entire herd in an outbreak has been suggested,[55] but may not be effective when cattle in neighboring pastures are untreated. Investigations concerning collagen ocular inserts have had mixed results.[95] Release rate, duration of release, antibiotic selection, and vehicle are important characteristics to be considered. Although the combination of erythromycin estolate and soluble collagen produced a sustained drug-delivery system, the intrinsic physical properties of collagen and poor retention of the ocular insert necessitate further studies using this treatment modality.[95]

To reduce the spread of IBK, affected animals should be isolated. Irritating factors such as sunlight and flies should be limited by providing shaded shelters or eye patches. Third eyelid flaps or temporary tarsorrhaphy may facilitate healing in cases with severe ulceration or descemetoceles. Little has been reported regarding the efficacy of periocular artificial pigmentation with sprays, dyes, and tattooing.

Prevention

M. bovis is ubiquitous; therefore, elimination of the organism is impossible and prevention of disease is required. Immediate detection through careful visual inspection and isolation of affected cattle is of paramount importance. Carriers should be removed and efforts to control the most important vector, *M autumnalis,* are warranted. Repeated occurrence of disease is possible, even though some animals develop protective immunity.[17] Current vaccines provide limited, if any, protection against clinical disease.[11] Chemically inactivated bacterins containing pili antigens are available, but their protective value is controversial.[45] Endemics of IBK have been associated with *M bovis* isolates possessing novel pili types.[96] Vaccination with commercially prepared autologous *M bovis* bacterin administered into the 3rd eyelid failed to prevent disease.[47]

The severity and prevalence of IBK vary greatly in vaccination trials.[16,42,97] Vaccination with homologous strains reduces the prevalence and severity of disease, although infection occurs.[16,42,98] Heterologous strain vaccines against IBK are not effective.[16] Protection against heterologous and homologous challenge represents an area that warrants further study because most efforts have proven ineffective.[97] Multivalent pili vaccines may prove promising if the serotype causing clinical IBK is included in the vaccine.[99] An *M bovis* pili vaccine produced by recombinant DNA technology was able to protect calves challenged with *M bovis* Dal2d.[100] The outcomes of vaccination studies are influenced by environmental conditions where housing, management, and other environmental factors are not controlled.[22]

Herds vaccinated with modified-live IBR virus have an increased risk for IBK.[7] Calves infected with *M bovis* 4 days after vaccination with modified-live IBR virus had more severe clinical signs and higher numbers of corneal ruptures than did calves infected with *M bovis* alone. IBR virus-vaccinated animals with IBK had increased numbers of *M bovis* isolates from tear film and higher total white blood cell counts. As such, vaccination with modified-live IBR virus should be avoided during the spring, summer, and fall months. Attempts to culture IBR virus from IBK-affected animals have been unsuccessful.[78]

After vaccination, natural challenge, or experimental ex-

posure, concentrations of lacrimal and serum antibodies to *M bovis* vary. The protective value of IgG and IgA remains controversial.[101] Protection against a 2nd episode of IBK appears more related to lacrimal IgA than to serum IgG.[101] High concentrations of anti-*M bovis* IgG, IgM, and secretory IgA do not protect against infection and clinical disease.[101] Maternal antibody titers to *M bovis* decrease in calves between 3 and 4 months of age.[102] The concentration of lacrimal and serum antibodies is influenced by the method of vaccination. The highest degree of protection occurs when tear film antibodies (IgA) are induced by mucosal vaccination.[103] Serum antibody titers appear to correlate poorly with protection.[103] Ocular challenge with virulent *M bovis* and clinical IBK produces high lacrimal IgA concentrations and greater resistance to reinfection. Interestingly, cattle that recover from milder cases of IBK are not protected against reinfection.[103]

Conclusions

IBK is a challenging disease for the veterinary practitioner. The multifactorial influences that enhance the disease frustrate both the practitioner and researcher. Treatment is often guided by management practices and preventative measures are limited. Continued research is necessary to further characterize factors that allow *M bovis* to overcome host defenses and cause disease. Methods to more successfully treat and reliably prevent IBK may be discovered as the defense mechanisms of the bovine eye are further characterized.

References

1. Billings F. Keratitis contagiosa in cattle. Bull Agrc Exp Stn Nebr 1889;3:247–252.
2. Troutt F, Schurig G. Pinkeye: Facts and figures. Anim Nutr Health 1985;28–35.
3. Simms A, Langemuir L, Udder S, et al. Results of a production analysis survey of cow herds in Kansas. Cattleman's Rep 1993.
4. Thrift F, Overfield J. Impact of pinkeye (infectious bovine keratoconjunctivitis) on weaning and postweaning performance of Hereford calves. J Anim Sci 1974;38:1179–1184.
5. Ward J, Nielson M. Pinkeye (bovine infectious keratoconjunctivitis) in beef cattle. J Anim Sci 1979;8:361–366.
6. Killinger A. Economic impact of infectious bovine keratoconjunctivitis in beef calves. Vet Med Small Anim Clin Agric Pract 1977; 618–620.
7. Webber J, Selby L. Risk factors related to the prevalence of infectious bovine keratoconjunctivitis. Am J Vet Res 1981;179:823–826.
8. Slatter D, Edwards M. A national survey of the occurrence of infectious bovine keratoconjunctivitis. Aust Vet J 1982;59:65–68.
9. Slatter D, Edwards M. A national survey of the clinical features, treatment and importance of infectious bovine keratoconjunctivitis. Aust Vet J 1982;59:69–72.
10. Aikman J, Selman A. Experimental production of infectious bovine keratoconjunctivitis. Vet Rec 1985;117:234–239.
11. Punch P, Slatter D. A review of infectious bovine keratoconjunctivitis. Vet Bull 1984;54:193–257.
12. Schoettker-Wegner H, Binder A. Nachweis von Mycoplasmen in Augentupferproben von Rindern. J Vet Med Ser B 1990;37:436–441.
13. Smith P, Blakenship T. Effectiveness of two commercial infectious bovine keratoconjunctivitis vaccines. Am J Vet Res 1990;51: 1147–1150.
14. Hughes D, Pugh G. A five-year study of infectious bovine keratoconjunctivitis in a beef herd. J Am Vet Med Assoc 1970;157:443–451.
15. Lepper A, Barton I. Infectious bovine keratoconjunctivitis: Seasonal variation in cultural, biochemical and immunoreactive properties of *Moraxella bovis* isolated from the eyes of cattle. Aust Vet J 1987; 64:33–39.
16. Hubbert W, Hermann G. A winter epizootic of infectious bovine keratoconjunctivitis. J Am Vet Med Assoc 1970;157:452–454.
17. Kopecky K, Pugh G. Infectious bovine keratoconjunctivitis: Contact transmission. Am J Vet Res 1986;47:622–624.
18. Gerhardt R, Allen J. The role of face flies in an episode of infectious bovine keratoconjunctivitis. J Am Vet Med Assoc 1982;180: 156–159.
19. Pugh G, McDonald T. Identification of bovine carriers of *Moraxella bovis* by comparative cultural examinations of ocular and nasal secretions. Am J Vet Res 1986;47:2343–2345.
20. Pugh GW. Infectious bovine keratoconjunctivitis: Comparison of a fluorescent antibody technique and cultural isolation for the detection of *Moraxella bovis* in eye secretions. Am J Vet Res 1977;38: 1349–1353.
21. Gil-Turnes C, Bischoff H. Comparison of the prevalence of infectious bovine keratoconjunctivitis in Aberdeen Angus and Charolais cattle. 14th World Congress on Diseases of Cattle, Dublin, Ireland, 1986.
22. Hughes D, Pugh G. Effects of vaccination with a *Moraxella bovis* bacterin on the subsequent development of signs of corneal disease and infection with *M. bovis* in calves under natural environmental conditions. Am J Vet Res 1976;37:1291–1295.
23. Pugh GW. Infectious bovine keratoconjunctivitis: Evidence for genetic modulation of resistance in purebred Hereford cattle. Am J Vet Res 1986;47:885–889.
24. Caspair EL, Wood PP. Eyelid pigmentation and the incidence of infectious bovine keratoconjunctivitis in Hereford–Friesian crossbred calves. Br Vet J 1980;136:210–212.
25. Clegg FG, Goodall M. Breed susceptibility to infectious bovine keratoconjunctivitis. Vet Rec 1982;110:617–621.
26. Powe T, Nusbaum K. Prevalence of nonclinical *Moraxella bovis* infections in bulls as determined by ocular culture and serum antibody titer. J Vet Diagn Invest 1992;4:78–79.
27. George L, Wilson W. Antibiotic treatment of *Moraxella bovis* infection in cattle. J Am Vet Med Assoc 1984;185:1206–1209.
28. George L. Clinical infectious bovine keratoconjunctivitis. Compend Cont Educ Pract Vet 1984;6:S712–S720.
29. Sandhu T, White F. Association of pili with rough colony type of *Moraxella bovis*. Am J Vet Res 1974;35:437–439.
30. Schurig G, Lighfoot D. Genotypic, phenotypic, and biological characteristics of *Moraxella bovis*. Am J Vet Res 1984;45:35–39.
31. Pugh G, Hughes D. The isolation and characterization of *Moraxella bovis*. Am J Vet Res 1966;27:957–962.
32. McMichael J. Bacterial differentiation within *Moraxella bovis* colonies growing at the interface of the agar medium with the petri dish. J Gen Microbiol 1992;138:2687–2695.
33. Riley W. Growth requirements for *Moraxella bovis*. Vet Microbiol 1984;9:593–598.
34. Rosenbusch R. Influence of *Mycoplasma* preinfection on the expression of *Moraxella bovis* pathogenicity. Am J Vet Res 1983;44: 1621–1624.
35. Rosenbusch R, Knudtson W. Bovine mycoplasmal conjunctivitis: Experimental reproduction and characterization of the disease. Cornell Vet 1980;70:307–320.
36. Rosenbusch R, Ostle A. *Mycoplasma bovoculi* infection increases ocular colonization by *Moraxella ovis* in calves. Am J Vet Res 1986;47:1214–1216.
37. Weech G, Renshaw H. Infectious bovine keratoconjunctivitis: Bacteriologic, immunologic, and clinical responses of cattle to experimental exposure with *Moraxella bovis*. Comp Immunol Microbiol Infect Dis 1983;6:81–94.

38. Timoney PJ. An outbreak of the conjunctival form of infectious bovine rhinotrachetitis virus infection. Vet Rec 1971;89:370.

39. Bowen JM. Experimental immunization of cattle against bovine keratoconjunctivitis. Tex Rep Biol Med 1970;28:531–533.

40. Langford E, Dorward W. A *Mycoplasma* isolated from cattle with infectious bovine keratoconjunctivitis. Can J Comp Med 1969; 33:275–279.

41. Ruehl W, Marrs C. Infection rates, disease frequency, pilin gene rearrangement, and pilin expression in calves inoculated with *Moraxella bovis* pilin-specific isogenic variants. Am J Vet Res 1993;54: 248–253.

42. Lepper A, Moore L. The protective efficacy of pili from different strains of *Moraxella bovis* within the same serogroup against infectious bovine keratoconjunctivitis. Vet Microbiol 1992;32:177–187.

43. Jayappa H, Lehr C. Pathogenicity and immunogenicity of piliated and nonpiliated phases of *Moraxella bovis* in calves. Am J Vet Res 1986;47:2217–2226.

44. Ruehl W, Marrs C. Q pili enhance the attachment of *Moraxella bovis* to bovine corneas in vitro. Mol Microbiol 1993;7:285–288.

45. Jayappa H, Lehr C. Pili of *Moraxella bovis* as virulence factors and their successful use as antigens in an *M. bovis* bacterin. Proc Symposium Western Vet Conf, Las Vegas, NV, 1987.

46. Moore L, Lepper A. A unified serotyping scheme for *Moraxella bovis*. Vet Microbiol 1991;29:75–83.

47. Arora A, Killinger A. Bacteriologic and vaccination studies in a field epizootic of infectious bovine keratoconjunctivitis in calves. Am J Vet Res 1976;37:803–805.

48. McDonald T, Pugh G. Plasmid profiles of *Moraxella bovis* isolates. Am J Vet Res 1986;47:964–967.

49. Frank S, Gerber J. Hydrolytic enzymes of *Moraxella bovis*. J Clin Microbiol 1981;13:269–271.

50. Kagonyera GM, George LW. Light and electron microscopic changes in corneas of healthy and immuno-modulated calves infected with *M. bovis*. Am J Vet Res 1988;49(3):386–395.

51. Kagonyera GM, George LW. Cytopathic effects of *M. bovis* on cultured bovine neutrophils and corneal epithelial cells. Am J Vet Res 1989;50(1):10–17.

52. Beard M, Moore L. Reproduction of bovine keratoconjunctivitis with a purified haemolytic and cytotoxic fraction of *Moraxella bovis*. Vet Microbiol 1994;42:15–33.

53. Annuar B, Wilcox G. Adherence of *Moraxella bovis* to cell cultures of bovine origin. Res Vet Sci 1985;39:241–246.

54. Vogelweid CM, Miller RB. Scanning electron microscopy of bovine corneas irradiated with sun lamps and challenge exposure with *M. bovis*. Am J Vet Res 1986;47(2):378–384.

55. Troutt F, Schurig G. Pinkeye facts and figures. Norden News 1985;28–35.

56. Hughes DE, Pugh GW. Ultraviolet radiation and *M. bovis* in the etiology of infectious bovine keratoconjunctivitis. Am J Vet Res 1965;26:1331–1338.

57. Stanifer R, Kretzer F. Tear film. In: Anderson R. ed. Biochemistry of the Eye. San Francisco, CA: American Academy of Ophthalmologists. 1983:723.

58. Holly F, Lemp M. Tear physiology and dry eyes. Surv Ophthalmol 1977;22:69–87.

59. Cotlier E. Resistance to infection of the external dry eye: The role of tears. Surv Ophthalmol 1979;24:33–38.

60. van Haeringen N. Clinical biochemistry of tears. Surv Ophthalmol 1981;26:84–96.

61. Brightman A, Wachsstock R. Lysozyme concentrations in the tears of cattle, goats, and sheep. Am J Vet Res 1991;52:9–11.

62. Padgett G, Hirsch J. Lysozyme: Its absence in tears and leukocytes of cattle. Aust J Exp Biol Med Sci 1967;45:569–570.

63. Arora A, Killinger A. Detection of *Moraxella bovis* antibodies in infectious bovine keratoconjunctivitis by a passive agglutination test. Am J Vet Res 1976;37:1489–1492.

64. Masson P, Heremans J. An iron binding protein common to many external secretions. Clin Chim Acta 1966;14:735–739.

65. Broikhuyse R. Tear lactoferrin: A bacteriostatic and complexing protein. Invest Ophthalmol Vis Sci 1974;13:550–554.

66. Boonstra A, Kijlstra A. The identification of transferrin, an iron-binding protein in rabbit tears. Exp Eye Res 1984;38:561–567.

67. Jensen O, Gluud B. The concentration of lactoferrin in tears of normals and of diabetics. Acta Ophthalmol 1986;64:83–87.

68. Seyfert H, Tuckoricz A. Structure of the bovine lactoferrin-encoding gene and its promoter. Gene 1994;143:265–269.

69. Smith J, George L. Treatment of acute ocular *Moraxella bovis* infections in calves with a parenterally administered long-acting oxytetracycline formulation. Am J Vet Res 1985;46:804–807.

70. Ellison R. The effects of lactoferrin on gram-negative bacteria. In: Hutchens T, Rumball S, Lonnerdal B, eds. Lactoferrin: Structure and Function. New York, NY: Plenum Press; 1994:71–90.

71. Schryvers A. Characterization of the transferrin and lactoferrin receptors in *Haemophilus influenzae*. Mol Microbiol 1988;2:467–472.

72. Brown, MH. *Moraxella bovis* may use Bovine Lactoferrin as a Sole Source of Required Iron for Growth. Manhattan, KS: Kansas State University; 1995. Master's Thesis (Part III).

73. Brown M, Brightman A, Fenwick B, Rider M. Identification of lactoferrin in bovine tears. Am J Vet Res 1996;57(9):1369–1372.

74. Fenwick B, Rider M. Iron repressible outer membrane proteins of *Moraxella bovis* and demonstration of siderophore-like activity. Vet Microbiol 1996;48:315–324.

75. Brown, MH. *Moraxella bovis* Outer Membrane Proteins Bind Bovine Lactoferrin. Manhattan, KS: Kansas State University; 1995. Master's Thesis (Part IV).

76. Marioneaux S, Cohen E, Johnson A, et al. *Moraxella* keratitis. Cornea 1991;10:21–24.

77. Friis N, Pedersen K. Isolation of *Mycoplasma bovoculi* from cases of infectious bovine keratoconjunctivitis. Acta Vet Scand 1979; 20:51–59.

78. George K, Ardans A. Enhancement of infectious bovine keratoconjunctivitis by modified-live infectious bovine rhinotracheitis virus vaccine. Am J Vet Res 1988;49:1800–1806.

79. Jackson F. Infectious keratoconjunctivitis of cattle. Am J Vet Res 1953;14:19–25.

80. Bedford P. Infectious bovine keratoconjunctivitis. Vet Rec 1976; 98:134–135.

81. Killinger A, Helper L. A system for scoring lesions of infectious bovine keratoconjunctivitis. Vet Med Small Anim Clin 1976;71:1370–1382.

82. Bromberg N. Bovine keratoconjunctivitis. Compend Cont Educ Pract Vet 1981;3:S301–S309.

83. Cox P. Infectious bovine keratoconjunctivitis—Recent progress. Vet Annu 1984;24:75–79.

84. Miller R, Fales W. Infectious bovine keratoconjunctivitis: An update. Vet Clin North Am Large Anim Pract 1984;6:597–608.

85. George L, Mihalyi J. Topically applied furazolidone or parenterally administered oxytetracycline for the treatment of infectious bovine keratoconjunctivitis. J Am Vet Med Assoc 1988;192:1421–1435.

86. Punch P, Cosat N. Plasma and tear concentrations of antibiotics administered parenterally to cattle. Res Vet Sci 1985;39:179–187.

87. Abeynayake P, Cooper B. The concentration of penicillin in bovine conjunctival sac fluid as it pertains to the treatment of *Moraxella bovis* infection. (II) Topical application. J Vet Pharmacol Ther 1989;12:31–36.

88. Daigneault J, George L. Topically applied benzathine cloxacillin for treatment of experimentally induced infectious bovine keratoconjunctivitis. Am J Vet Res 1990;51:376–380.

89. Cooper B. Treatment of conjunctivo-keratitis of cattle and sheep with ethidium bromide. Vet Rec 1960;72:589–594.

90. George L, Smith J. Treatment of *Moraxella bovis* infections in calves using a long-acting oxytetracycline formulation. J Vet Pharmacol Ther 1985;8:55–61.

91. George LW. Antibiotic treatment of infectious bovine keratoconjunctivitis. Cornell Vet 1990;80(3):229–234.

92. George LW, Smith JA. Distribution of oxytetracycline in ocular tissues of calves. J Vet Pharmacol Ther 1985;8:47–54.

93. Edmondson AJ, George LW. Survival analysis of corneal ulcer healing times in calves with naturally acquired infectious bovine keratoconjunctivitis. Am J Vet Res 1989;50(6):838–844.

94. Pedersen K. Infectious Keratoconjunctivitis in Cattle. Copenhagen, Denmark: University of Denmark; 1973. Thesis.

95. Punch PI, Costa ND. The release of insoluble antibiotics from collagen ocular inserts in vitro and their insertion into the conjunctival sac of cattle. J Vet Pharmacol Ther 1987;10:37–42.

96. Vandergaast N, Rosenbusch R. Infectious bovine keratoconjunctivitis epizootic associated with area-wide emergence of a new *Moraxella bovis* pilus type. Am J Vet Res 1989;50:1437–1441.

97. Pugh G, McDonald T. Experimentally induced infectious bovine keratoconjunctivitis: Potentiation of *Moraxella bovis* pilus vaccine's immunogenicity by vaccination with *Mycobacterium paratuberculosis* bacterin. Am J Vet Res 1978;39:1656–1661.

98. Johansen K, Wannemuehler M. Biological reactivity of *Moraxella bovis* lipopolysaccharide. Am J Vet Res 1951;51:46–51.

99. Moore L, Rutter J. Attachment of *Moraxella bovis* to calf corneal cells and inhibition by antiserum. Aust Vet J 1989;66:39–42.

100. Lepper AD, Elleman TC. A *Moraxella bovis* pili vaccine produced by recombinant DNA technology for the prevention of infectious bovine keratoconjunctivitis. Vet Microbiol 1993;36:175–183.

101. Smith P, Greene W. Antibodies related to resistance in bovine pinkeye. Calif Vet 1989;14:7–9.

102. Maidment D, Batty D. Changes in titers of *Moraxella bovis*-specific IgG in growing cattle. Br Vet J 1986;142:65–70.

103. Nayar P, Saunders J. Infectious bovine keratoconjunctivitis II. Antibodies in lacrimal secretions of cattle naturally or experimentally infected with *Moraxella bovis*. Can J Comp Med 1975;39:32–40.