Bovine mastitis therapy and why it fails

J H du Preez[a]

ABSTRACT

Treatment of bovine mastitis depends on the cause, the clinical manifestation and the antibiotic susceptibility of the agent. Mastitis therapy is commonly unsuccessful owing to pathological changes that occur in the udder parenchyma as a result of the inflammatory reaction to mastitogenic bacteria, pharmacokinetic properties of antimicrobial mastitis drugs, mastitogenic bacterial and related factors, and poor animal husbandry and veterinary interventions.

Key words: infectious bovine mastitis, mastitogenic bacterial factors, mastitis therapy, pathological udder changes, pharmacokinetic properties, poor animal husbandry.

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INTRODUCTION

Mastitis can occur at any stage of a cow's productive life. Microbiological investigations of clinical bovine mastitis reveal a causative agent in 75–95% of cases. There is good evidence that microorganisms are involved in almost all cases[36,40,49]. At least 137 infectious causes of bovine mastitis are known, but the majority of cases are caused by only a few common bacterial pathogens, namely staphylococci, streptococci, coliforms and *Arcanobacterium pyogenes.*

For antibacterial mastitis therapy to be successful, the active drug must attain and maintain concentrations exceeding the minimum inhibitory concentration (MIC) at the focus of infection for long enough to break the production and toxin-producing cycle of the causative pathogen[3]. This may be prevented by a number of factors that include udder pathology and poor selection of antimicrobials, and is also influenced by the route of administration. Therapy may have poor results owing to tissue damage and introduction of new infections during treatment, and/or failure to eliminate the management factors that predispose to mastitis. Although there is an underlying assumption that the primary goal of antibacterial mastitis therapy is to kill bacteria and that the normal udder is sterile, usually the best that can be achieved is temporary reduction or suppression of the bacterial population to allow the host to overcome the infection. The mechanisms to cleanse the udder are evidently poorly developed in some cows, as relapses and re-infections commonly follow antimicrobial therapy. Udder infections tend to be dynamic, and stress may contribute to udder infections becoming clinically apparent[48,49].

The purpose of this article is to provide an update on bovine mastitis therapy and reasons for its failure.

PRINCIPLES OF MASTITIS THERAPY

The success of mastitis therapy depends on correct diagnosis, appropriateness of the route of administration and the drug selected, stage at which treatment is initiated, severity of udder pathology, supportive treatment, and elimination of predisposing factors. There is no standard treatment for mastitis, but it is advocated that the clinical forms should be treated according to the severity of the udder inflammation[12].

Bovine mastitis is most commonly treated by intramammary infusion of drugs[29,64]. This is the route of choice in subclinical, mild or moderately severe mastitis, and is used as an adjunct to parenteral administration in severe mastitis. Some clinicians prefer intramammary administration, without systemic administration, even in severe disease[40]. For effective intramammary treatment, drugs should distribute throughout the udder and be rapidly absorbed into the general blood circulation (Table 1). Significantly better results can be obtained when the drug is administered intracisternally in 1 ℓ of 0.5 % glucose solution, rather than in 50 mℓ saline[23].

The disadvantage of local application of antimicrobials is the slow and uneven distribution of certain drugs in the infected udder (Table 1). In acute, severe disease, distribution through the udder may be impaired by inflammation or blockage of milk ducts by debris. Parenteral administration may overcome these problems, although it is usual to administer agents concurrently by the intramammary route[17]. Severely inflamed udders should be milked out frequently, with the aid of oxytocin if necessary. In peracute or acute clinical mastitis cases with systemic signs, combined systemic and intramammary treatment with compatible antibiotics, supplemented with supportive therapy, is recommended[64].

It is always desirable to treat infectious mastitis according to the antimicrobial drug sensitivity pattern of the pathogens and clinical experience[40]. The basic rule in selecting the drug is to opt for one with as narrow a spectrum as possible, to focus treatment on a specific pathogen and minimise side-effects. Because it takes time to do sensitivity determinations, broad-spectrum antibiotics must be given initially for practical reasons, based on knowledge of the pharmacokinetic properties of the drugs and the formulation[9,29,32,65,66,67]. In general, narrow-spectrum antibiotics are bacteriocidal and those with a broad spectrum are bacteriostatic[58].

Response to treatment increases with persistence of the antibiotic in the udder. The concentration of the drug used must at least exceed the MIC-value for the pathogen, but preferably also the MBC-value (minimum bacteriocidal concentration) in the udder. If bacteriostatic drugs are used, the need to maintain high concentrations in the target organ is increased.

Successful intravenous or intramuscular mastitis therapy depends on effective passage of the drug from blood into milk to reach foci of infection, which is largely governed by lipid solubility, degree of ionisation (dependent on the dissociation constant (pKa)), and the extent of protein-binding of the drug with plasma[44,45,53], since drugs cross the blood–milk barrier by passive diffusion[30]. Only the unbound or free drug can diffuse through the blood–milk barrier and exert pharmacological or antimicrobial

[a]Technology Transfer Division, ARC - Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa.

Table 1: **Classification of antibacterial drugs according to their potential distribution throughout the udder after parenteral and intramammary administration[40,49,67].**

Distribution	Parenteral[a]	Intramammary[b]
Good	Clindamycin, erythromycin, florfenicol, norfloxacin, oleandomycin, oxytetracycline, penethamate, rifampin, spiramycin, sulfanilamide, tiamulin, trimethoprim, tylosin	Amoxycillin, ampicillin, cephalexin, dapsone, erythromycin, hetacillin, lincomycin, nitrofurans, novobiocin, oleandomycin, penethamate, quinolines, rifamycin SV, spiramycin, sulfanilamide, tiamulin, trimethoprim, tylosin
Limited	Ampicillin, amoxycillin, cephapirin, cloxacillin, fusidic acid, novobiocin, penicillin G, rifamycin, sulfadoxin, sulfadimidine, tetracyclines	Cephoxazole, cephalothin, cephapirin, cefoperazone, cephacetrile, ceftiofur, cloxacillin, oxacillin, penicillin G, sulfadimidine, tretracyclines, vancomycin
Poor	Aminosidine, cephalexin, cefoperazone, colistin, dihydrostreptomycin, gentamicin, kanamycin, spectinomycin, vancomycin	Aminosidine, bacitracin, colistin, dihydrostreptomycin, kanamycin, neomycin, polymyxins, spectinomycin, tyrothricin

[a]As determined experimentally from the milk/serum drug concentration ratios.
[b]As determined experimentally from the rate of drug absorption from the udder or from physicochemical properties of the drug.

activity[2]. However, the blood circulation through the udder of a cow producing 20 kg of milk per day is approximately 10 000 ℓ/d, or 7–10 ℓ/min[22]. Systemic administration of rationally selected antibacterials can therefore be used as the sole treatment in acute mastitis. Following systemic administration, basic drugs (if sufficiently lipid-soluble) tend to concentrate in milk. The non-ionised fraction reaches equal concentrations on either side of the the blood–milk barrier [29], even when marked udder changes are present.

An ideal antibiotic for parenteral mastitis therapy should have a low MIC against the majority of udder pathogens, high bioavailability *via* the intramuscular route, sufficient lipid solubility, a low degree of serum protein binding, a long half-life in the body, similar clearance from organs, tissues and blood, no distribution to the gastrointestinal tract, and be weakly basic or otherwise highly non-ionised in serum[40,49,67]. To date, as far as we know, no single antibiotic meets all these requirements.

Therapy of acute mastitis should commence as soon as possible after diagnosis[12,58], using a highly bioavailable antibiotic, with the necessary supportive therapy[41]. The milder the pathology in the udder parenchyma, the better the prognosis.

The duration of treatment depends on the clinical symptoms, medical history of the cow and microbiological diagnosis. Lengthy treatment means a lengthy milk withdrawal period, which makes the treatment costly. If the cow has chronic mastitis that cannot be cured during lactation, culling is the obvious solution. However, sufficiently lengthy treatment of a cow with a good prognosis may lead, in addition to clinical recovery, to elimination of the bacteria from the udder.

All cows that have had clinical mastitis during lactation should be given drying-off therapy.

SPECIFIC THERAPY FOR THE VARIOUS FORMS OF MASTITIS

Teat canal infection (TCI)

Treating lactating cows with small quantities of antibiotic (1 to 2 droplets) introduced 3 times at 12-hourly intervals effectively eliminates teat canal infection[9,10]. Teat canal therapy is not advocated commercially. A high percentage of TCI (> 50 %) undergo spontaneous recovery (self-cure) when predisposing factors to mastitis are limited or eliminated[9].

Subclinical mastitis

For treatment to make a significant contribution to reducing the incidence of mastitis, it is necessary to treat subclinical as well as clinical cases. There will usually be 15–40 subclinical cases for every clinical case. Treatment at drying-off with a dry-cow antibiotic preparation is practical and inexpensive. Treatment of subclinical mastitis during lactation is not indicated[49], unless a very high infection rate endangers the marketing of milk. The cost of therapy and milk discarded during the withdrawal period seriously reduces the benefit of the therapy. The organism that can be treated most readily in lactation is *Streptococcus agalactiae,* with cure rates usually in the range of 90–95 %[36]. The cure rate for environmental streptococci and various staphylococci may be as low as 10 % and will rarely exceed 40–50 %. These infections are best treated at drying-off.

Usually only intramammary therapy, by infusion of antibiotic preparations 3 times at 12-hourly intervals, is recommended. Exceptionally, parenteral antibiotic therapy can be given when extremely virulent bacteria or very valuable dairy cows are involved[32,46,50].

It is only worth treating subclinical mastitis when the predisposing factors have been limited or eliminated[13,14,23,38]. Cows with chronic infections, evidenced by previous clinical episodes and persistent high somatic cell counts, may not respond to dry-cow treatment and should be culled[36].

Subacute clinical mastitis

This is the most prevalent form of clinical mastitis in dairy herds[15,36]. Duration of treatment depends on the causative organism and clinical improvement. Intramammary treatments are usually administered 4 times at 12-hourly intervals with drugs that are rapidly absorbed, or 3 times at 24-hourly intervals with slowly absorbed drugs[40]. Treatment should be continued for at least 24 hours after the disappearance of clinical signs[36], since the aim is to achieve bacteriological and not only clinical cure[15]. Initially, 2 syringes of intramammary antibiotic preparation may be infused into the affected quarter in high-yielding cows. Multidose intramammary syringes should be avoided for hygienic reasons. The introduction of cefoperazone for intramammary administration with single-dose treatment is a significant advance[59]. Parenteral antibiotic therapy is not routinely advocated but may be indicated in the circumstances mentioned for subclinical mastitis[1,22,32,46,47,50]. Table 2 lists drugs of choice for local and parenteral treatment of bovine mastitis.

Acute clinical mastitis

In acute mastitis, frequent failure of intramammary antimicrobial therapy is due, at least partly, to poor or uneven distribution of the drug in the intensely swollen udder parenchyma. Parenteral antibiotic therapy may be preferred[56,62]. Either the same antibiotic, or compatible antibiotics with identical or synergistic action are administered parenterally and locally for 3–5 days, depending on clinical cure. The intravenous route must be used to achieve maximum parenchymal diffusion[29,67]. Initially, a double infusion with

Table 2: **Antimicrobial drugs of choice, if pathogens are sensitive, in the local and parenteral therapy of bovine mastitis[12,40,49].**

Pathogen	Therapy	Antimicrobial drugs
Staphylococcus aureus	Local	Cephalosporins, cloxacillin, erythromycin, nitrofurans, penicillin-neomycin, rifampin
	Parenteral	Cephalothin, cloxacillin, erythromycin, procaine penicillin G, tylosin
Coagulase-negative staphylococci *e.g. S. hyicus, S. epidermidis*	Local	As for *S. aureus*
Streptococci	Local	Cephalosporins, cloxacillin, penicillin G, penicillin-neomycin, penicillin-streptomycin, oxytetracycline
	Parenteral	Macrolides, penicillin G, procain penicillin, oxytetracycline
Coliforms *e.g. Escherichia coli, Klebsiella* spp., *Proteus* spp.	Local	Ampicillin-cloxacillin, enrofloxacin, cephalosporins (cefamandole, cephalothin, cefotaxime), gentamicin, kanamycin, neomycin, nitrofurans,polymyxin B. trimethoprim-suphonamide
Pseudomonas aeruginosa	Local and/or parenteral	Carbenicillin, colistin, gentamicin, polymyxin B
Arcanobacterium pyogenes	Parenteral	Macrolides, penicillin
Clostridium perfringens and *Bacillus cereus*	Parenteral	Penicillin G, sodium benzylpenicillin
Anaerobic bacteria *e.g. Bacteroides fragilis, Eubacterium combesii, Peptococcus indolicus, Fusobacterium necrophorum*	Local and/or parenteral	Cefoxitin, clindamycin, erythromycin, metranidazole, penicillin G
Nocardia spp.	Local	Amikacin, minocycline, nitrofurantoin, trimethoprim-sulfamethoxazole (may be unrewarding)
Mycoplasma spp.	Local	Aminoglycosides, macrolides, nitrofurantoin (may be unrewarding)
Fungi (moulds and yeasts) *Candida, Saccharomyces, Cryptococcus neoformans*	Local	Amphoteracin B, clotrimazole, ketoconazole, natamycin, yeasts also nystatin

an intramammary antibiotic formulation into the affected quarters and a single infusion into each of the healthy quarters is recommended to prevent new intramammary infection. After the initial infusion of intramammary antibiotic preparations into the affected quarter, intramammary antibiotic preparations as recommended by the manufacturer should be infused 12-hourly and continued for 3–5 days. For increased bioavailability of the drug, a large volume should be administered intramuscularly at 2 sites[26,28,29,32,46,47,50,58,67]. For supportive therapy and optimal animal husbandry see Table 3.

Macrolide antibiotics are potentially best for the treatment of Gram-positive bacterial mastitis, and gentamicin, or polymyxin B for Gram-negative infections*. Penicillin G is the drug of choice for *Clostridium perfringens* and *Bacillus cereus* infections[64]. Mastitis caused by *S. agalactiae, S. dysgalactiae* and penicillin-sensitive coagulase-negative staphylococci is usually cured by penicillin treatment for 2–3 days. *S. uberis* may be more problematic and some authors recommend that treatment should be continued for 3–5 days. *Staphylococcus aureus* is known to be therapy-resistant, and 5-day treatment has produced better results. Different drugs should be administered together only if synergism or at least no antagonism exists; when using combinations the risk of residues is increased. Use of more than 2 antimicrobial drugs together in routine treatment of mastitis is not pharmacologically justified[41]. Because relatively small amounts of drug are required for intramammary treatment of mastitis, new and otherwise expensive antibiotics, such as 3rd-generation cephalosporins, are not excluded when administered by this route. The cost of treatment is determined mainly by the cost of milk discarded rather than the cost of the drug. Third-generation cephalosporins give broad-spectrum coverage and render antibiotic combinations unnecessary[40].

For intramammary treatment of mild clinical mastitis, bacteriocidal drugs that act only against Gram-positive bacteria are preferred. If the microbe is sensitive to natural penicillin, there is no reason to use semi-synthetic penicillins, as their MIC-values for streptococci and penicillin-sensitive staphylococci are higher. The frequency of application of intramammary preparations depends on the pharmacokinetics of the drug selected. One daily application of drugs that are absorbed slowly (*e.g.* procaine penicillin) is sufficient; drugs that are rapidly absorbed must be given twice a day (*e.g.* cephalexin, erythromycin)[41].

The drug of choice for acute, severe disease in many countries remains penicillin, as the MIC and MBC values for the most common mastitis pathogens are very low[49]. Repeated intramammary administration of slow-release formulations is more effective than using rapid-release dosage forms once or twice[40]. Philpot and Nickerson[36] recommended continuing treatment until at least 24 hours after the disappearance of clinical signs, failing which the infection might only be suppressed to subclinical level.

There are very few antimicrobial drugs suitable for treating coliform mastitis. High concentrations of some drugs (tetracyclines, chloramphenicol, novobiocin-penicillin, streptomycin) have been found to suppress phagocytosis in the udder. This effect is enhanced if corticosteroids are combined with the drugs. These considerations are important when treating severe coliform mastitis, in which the local defence mechanism is of major significance[41]. The most frequently-used drugs are enrofloxacin and

*Chloramphenicol is effective but its use in food animals is not permitted. Florfenicol is registered for treatment of bovine respiratory conditions but not mastitis.

Table 3: **Supportive therapy for cows with acute and peracute mastitis**[22,26,36,40,41,46].

Therapeutic	Dosage	Route of administration	Interval between doses	Comments
1a. Isotonic sodium bicarbonate or sodium chloride	5–10 ℓ (20–30 ℓ during 1st day)	iv[b] per os	4–8 hours 24 hours	Essential to restore circulating volume quickly with endotoxic/hypotensive shock[a]
1b. Isotonic saline	25 ℓ			Possible alternative to above
2. Oxytocin Frequent milking	5–30 IU	iv or im[c]	12 hours Every 1–4 hours	Facilitates stripping Keeps milk ducts patent, removes toxins, bacteria and inflammatory products
3. 20% calcium borogluconate	400–800 mℓ	iv	–	If indicated (coliform mastitis), give slowly and diluted in saline
4. Glucose	2–5 ℓ	iv	12 hours	Reverse hypoglycaemia and for glycogen depletion
5. Etamiphylline camsylate or theophylline	1400 mg	iv or im	8 hours	Helps cardiac output and increases the stroke volume of the heart and improves pulmonary function
6. Salicylates (*e.g.* acetylsalicylic acid)	30 g	per os	8 hours	Reduces pain and temperature and inflammation, restores appetite
7. Concentrated multivitamins especially B-complex group	30 mℓ	iv or im	24 hours	May help liver, supplement enzyme systems
8. Corticosteroids (*e.g* dexamethasone)	1–3 mg/kg	iv or im	6 hours	Cost may be prohibitive, impairs defence systems
9. Immunoglobulin m/v of total protein (gamma globulin, 75,0%)	20–50 mℓ	iv	12 hours, daily	Neutralise toxins, stimulate and improve phagocytosis
10. Non-steroidal anti-inflammatories (*e.g.* flunixin meglumide)	As prescribed	iv or im	12 hours	Anti-inflammatory, counteract shock, help to prevent deleterious pathological changes
11. Ruminotorics	Depends on drug	per os	12 hours	Stimulate the rumen function
12. Lysostaphin or nisin	As prescribed			Antibacterial enzyme. Effective against both growing and latent bacteria

[a]Often, supportive therapy with fluids is more important than the antibiotic selected; this is certainly true in endotoxic coliform mastitis.
[b]iv = intravenous, [c]im = intramuscular.
Animal husbandry
1. Water: fresh, cool drinking water must be freely available.
2. Food: soft digestible and nutritious food must be available *ad lib.*
3. Rest: enough rest without disturbance is of the utmost importance for quick recovery.
4. Protection against exogenous (*e.g.* sun, wind, noise) and endogenous (decreased drug induced defence mechanism) stressors as far as possible.

trimethoprim-sulphonamide.

Causal agents of yeast mastitis are usually sensitive to fungicidal drugs such as amphoteracin B. Many fungicides are, however, toxic to udder tissue and may be more damaging to the udder than the fungus itself. Spontaneous recovery from fungal mastitis is relatively common, and frequent milking out enhances recovery. A cow that persistently has yeasts in her milk should be culled[49].

Peracute clinical mastitis

In principle, treatment is the same as for acute mastitis, but more aggressive, and special attention must be given to shock[26,28,46,47,50,56,58]. When the response to antibiotic and other supportive therapy for acute and peracute mastitis cases is insufficient, additional measures may be required to prevent death or to treat a severely affected quarter. When the affected quarter is gangrenous or severely damaged, intramammary treatment will be ineffective, and teat amputation may help drainage[46] if it is economically feasible. Tying off the mammary veins has also been advocated in severe cases to reduce the uptake of toxins into the bloodstream[46]. Severely affected cows that have survived the peracute stage may not recover completely, and may develop persistent pyrexia and evidence of damage to vital organs such as the liver. The prognosis is poor in these cases[46].

Chronic mastitis

It is usually necessary to cull the cow or to destroy the affected quarter/s by means of an infusion of 25–40 mℓ of concentrated ether to eliminate an important potential source of bacterial infection for healthy quarters[12,15]. Parenteral and intramammary antibiotic treatment for 3–5 days may be used in conjunction with anti-inflammatory products, but the prognosis remains poor. Infusion of 100–250 mℓ of a 5 % or 10 % dextrose solution in combination with antibiotics to which the bacteria are sensitive into the affected quarter 3 times at 12-hourly intervals has been recommended[46,50,62].

COMMONLY AVAILABLE MASTITIS DRUGS

Penicillins

Penicillin is a weak organic acid with pKa 2.7 and it is therefore largely ionised in plasma, so that milk levels are always lower than plasma levels[44,45], but its activity is decreased only slightly in milk. While the absorption rate is only moderate, penicillin is well-distributed throughout the udder and diffuses relatively well into mammary tissue in both normal and mastitic glands, except where extensive necrosis has occurred[20,55,63]. Good diffusion is probably due to moderate lipid-solubility[62]. Moreover, penicillin concentrations in mastitic milk are higher than in normal milk[20]. Penicillin is non-irritating following local infusion, and has a low degree of protein binding. An intramammary penicillin dose of 11 000 IU per

kg twice daily, continued for 3–5 days for mastitis therapy, has been recommended[32]. Pyörälä[41] recommended a parenteral administration of procaine penicillin of at least 20 000 IU/kg (20 mg/kg) per day, but residues are a major concern when administering this drug. Water-soluble potassium penicillin can reach high concentrations in milk, but owing to the shorter half-life the dose must be at least 15 000 IU/kg twice a day. A marked advantage of administering potassium penicillin is its rapid elimination from the body; even after several days of treatment the risk of residues is considerably smaller than when using procaine penicillin.

Sodium benzylpenicillin: most isolates from mastitis cases caused by *S. aureus* are resistant whereas virtually all streptococci are sensitive[28,29].

Cloxacillin: a narrow spectrum semi-synthetic penicillin, highly lipid-soluble and resistant to staphylococcal penicillinase. In lactating cows infusion of cloxacillin is as effective as benzylpenicillin against streptococcal mastitis infections[27–29].

Ampicillin: a semi-synthetic penicillin with a broad spectrum of activity, which diffuses into the udder slightly better than benzylpenicillin[69]. The dose necessary to maintain milk levels that are effective against microorganisms, *i.e.* with MICs in the range of 0.5–1.0 mcg/mℓ for 25 hours, is around 10–20 mg/kg[62]. This dosage is several times higher than that usually recommended[28]. Moore and Heider[32] recommended an intramuscular ampicillin dose of 10 mg per kg, twice daily, and an intramammary dose of 62.5 mg twice daily, continued for 3–5 days.

Benzathine penicillin: parenteral doses as high as 9 million units per cow are unlikely to produce bacteriostatic levels in milk[51], which reflects the low blood levels obtainable[28].

Aminoglycosides

Dihydrostreptomycin is a base with pKa 8.0 and a low lipid solubility and is therefore unsuitable for systemic treatment of mastitis[29]. Systemic administration of aminoglycosides is in any case not recommended for production animals owing to the occurrence of long-term residues.

Dihydrostreptomycin is bacteriocidal at concentrations approximately 3–4 times higher than the MIC[61]. However, the activity of dihydrostreptomycin is markedly decreased in the presence of milk[61], possibly owing to extensive binding to milk protein[71]. It is very unevenly distributed in the udder parenchyma, taking up to 8 hours to achieve wide distribution[63], and is unlikely even at very high doses to reach therapeutic levels in milk[70]. Ziv[62] suggested that dihydrostreptomycin at 10–20 mg/kg every 6–12 hours might be suitable for treatment of Gram-negative udder infections. The aminoglycosides have fairly low MICs for staphylococci and for some Gram-negative mastitis pathogens, but their bacteriocidal activity against streptococci is low[33]. Dihydrostreptomycin is usually used in combination with penicillin[27] for intramammary treatment, but clinical trials have demonstrated no advantage over penicillin alone[41].

Ziv and Sulman[68] reported that neomycin passed only poorly into milk in both normal and mastitic mammary glands, and suggested that its limited penetration was due mainly to poor lipid solubility, which would curtail its potential usefulness in the parenteral treatment of mastitis. Milk markedly decreases the activity of neomycin, the MICs being up to 500 times higher when tested in milk[39]. However, neomycin has been used as the chief ingredient in combination drugs for intramammary mastitis therapy owing to its wide antimicrobial spectrum[47].

Tetracyclines

Oxytetracycline and chlortetracycline are partially inactivated in milk by chelation with magnesium and calcium ions and by combination with casein[31]. Injectable oxytetracycline has limited bioavailability and does not reach therapeutic levels in milk when administered intramuscularly[2,41,66], and should therefore be administered intravenously if used for treating mastitis[66]. Large doses (in the order of 10 mg/kg) administered intravenously will maintain milk concentrations of oxytetracycline above 1.0 mcg/mℓ for 24 hours. Moore and Heider[32] suggested an intravenous oxytetracycline dose of 20 mg per kg daily. The average MIC of oxytetracycline for mastitogenic staphylococci and streptococci is about 1.0 mcg/mℓ. Gram-negative organisms require higher concentrations, 2.44 mcg/mℓ being the mean MIC for sensitive *Escherichia coli*[37]. Oxytetracycline should not be infused into the udder, as it is an irritant, absorption is very poor and it is very unevenly distributed in normal udder tissue[63].

Macrolides

Erythromycin, tylosin, lincomycin and spiramycin are macrolide antibiotics. They achieve effective passage from blood into udder. They are logical candidates for parenteral treatment to eliminate persistent Gram-positive udder infections[6,41,67]. For treatment of acute mastitis caused by Gram-positive udder pathogens, combined parenteral and intramammary application of the macrolide antibiotics is bacteriologically and pharmacokinetically rational[67]. Erythromycin is a highly lipid-soluble base with pKa 8.862. After systemic administration, it normally reaches levels in milk 4–5 times higher than in plasma but, with rising pH, as occurs in mastitis, levels in milk are decreased[42,43]. MICs of erythromycin range from 0.025 to 0.04 mcg/mℓ for streptococci, and from 0.2 to 0.39 mcg/mℓ for sensitive strains of *S. aureus.* Erythromycin at a dose of 12.5 mg/kg repeated at 24-hour intervals, was shown to maintain milk levels of more than 11 mcg/mℓ over the dosage interval[3]. An intramuscular dose of 5 mg/kg twice daily, and an intramammary dose of 300 mg/kg, twice daily, for 3–5 days have been recommended[32].

The minimum dose of spiramycin is 10 mg/kg, preferably administered intravenously. The problems associated with spiramycin include persistence in tissues, with very long withdrawal periods. Milk must always be tested for residues before delivery to the dairy. Intramuscular administration of spiramycin causes irritation and residues remain even longer.

Sulphonamides and trimethoprim

The average range of MICs of sulphonamides for sensitive streptococci is between 2 and 16 mcg/mℓ and for sensitive staphylococci between 8 and 64 mcg/mℓ[21]. Sulphonamide concentrations of 50–150 mcg/mℓ are considered therapeutic[5,29]. After intravenous injection, the sulphonamides are distributed evenly throughout the mammary gland in both normal and indurated udder tissue[43]. Sulphadimidine, when given intravenously at a dose of 200 mg/kg, will maintain milk levels of more than 50 mcg/mℓ for 20 hours[51]. Moore and Heider[32] recommended an initial dose of sulphamethazine of 100 mg per kg followed by 50 mg per kg daily, administered intravenously, for 3–5 days. Trimethoprim has a short half-life in cattle that varies between 50 and 100 minutes, which limits its usefulness, as very high doses are necessary[7,29,43]. The suspension of trimethoprim in sulphadiazine, at a dose rate of 48 mg/kg, is absorbed more slowly and might be expected to provide effective milk levels for 12 hours[57].

Enrofloxacin

An alternative drug for treating Gram-negative infections is enrofloxacin, at a dose of 5.0 mg/kg daily. This dose is sufficient to maintain concentrations that are

clearly higher than the MIC-values for the coliforms in milk[41].

Cephalosporins

The cephalosporins, which possess broad-spectrum activity against many Gram-negative udder pathogens and beta-lactamase-producing staphylococci, may replace antibiotic combinations[52]. Cephalosporins have a limited distribution in the udder after parenteral and intramammary administration[67]. Cephoxazole is bacteriocidal and resistant to destruction by staphylococcal penicillinase. Cephoxazole and penicillin are mutually potentiating[25].

Polymyxins

Polymyxins (polymyxin B, colistin) are basic cyclic decapeptides. They disorganise the outer membrane of Gram-negative bacteria by binding to phospholipids (endotoxin), through direct interaction with the anionic lipid A region. They bind moderately to plasma proteins, diffuse poorly through biological membranes, and attain low concentrations in milk. The milk:serum ratio for polymyxin B is 0.25:1. Polymyxin is poorly absorbed from the udder. Penetration from blood to milk is correspondingly poor, but is increased by inflammation. However, polymyxins are useful for the treatment of coliform and *Pseudomonas* mastitis, and their potential to inactivate endotoxin may be of particular use in the treatment of coliform mastitis. An intramuscular dose of 5.0 mg/kg of polymyxin B should give milk concentrations exceeding 2 µg/mℓ for 4 hours, sufficient to eliminate the more sensitive coliforms[40].

REASONS FOR FAILURE OF MASTITIS THERAPY

Bacterial factors

Tissue invasion: tissue-invading bacteria such as staphylococci become walled off in the udder parenchyma by thick, fibrous scar tissue[29] so that the antibiotic cannot reach the pathogen, and bacteriological failures may occur even when the organisms are sensitive to the antibiotics used[38]. *S. aureus* udder infections promote development of localised scar tissue that lacks a blood supply, so that intramuscular and intravenous injections probably provide little benefit. Therapy may kill the bacteria that are not walled off, but the bacteria within the scar tissue can break out, multiply, cause additional damage to the udder secretory tissue and promote more scar tissue formation[35]. *S. aureus* and other mastitis pathogens can survive, in some instances, within leukocytes, and may not come into contact with the drug[35].

L-forms of bacteria: many antibiotics such as penicillins and cephalosporins kill bacteria by preventing synthesis of cell walls when bacteria multiply. Sometimes certain bacteria develop an acapsular or L-form that is contained only in a cell membrane, is not susceptible to antibiotics that attack the cell wall, and can revert to the normal form[35,36,48,60].

Bacterial mechanisms that overcome antibacterial effects in milk: bacteria can escape from endogenous antibacterial factors by capsule or slime formation, receptor-mediated absorption of host proteins into bacteria, interference with phagocyte function and leukocidin production. Other mechanisms include adherence of bacteria to tissue linings that results in avoidance of the wash-out effect of milking, upward flotation of bacteria with cream, and increase in bacterial replication rate[6,48,49].

Bacterial dormancy: non-multiplying bacteria are not sensitive to most antibiotics. Bacteria are most susceptible to antibacterials during their logarithmic growth phase. All bacterial populations contain organisms that are not in the active growth phase.

Host factors

Teat canal infection: standard methods of antibiotic administration into a mastitis quarter and intramammary antibiotic therapy at drying off do not necessarily eliminate TCI, which serves as a potential source of bacteria for infection of the udder parenchyma[9,11,16], and may cause mastitis[9].

Failure of endogenous antibacterial factors: the effect of antibacterials depends on interaction with the antibacterial host factors and their efficacy[48]. All endogenous antibacterial factors are greatly diluted in milk. Lactoperoxidase does not have sufficient substrate (thiocyanate, hydrogen peroxidase) to produce an effective antimicrobial system in milk. The lactoferrin-dependent iron-binding system does not effectively withhold iron from bacteria if the milk contains haemoglobin or other haem compounds usually present in mastitic milk. Components in milk mask or inactivate the effect of activated complement. Effective phagocytosis is hampered by too-long milking intervals, pH too low for optimal granulocyte function, wastage of phagocyte protective capacity in endocytosis of fat and casein, covering of the receptors for opsonins by casein, deprivation of phagocyte energy by low glucose content in mastitic milk, oxygen tension too low for phagocytes to operate effectively, non-specific stimulation of phagocytes by casein split products, dilution of opsonins, and blockage of the normal functions of phagocytes by immune complexes[48,49].

Milk duct obstruction: in all cases of mastitis, oedema and inflammatory products obstruct diffusion of antibiotics to some extent by compression or blockage of the milk duct system, rendering antimicrobial contact with mastitis-causing bacteria difficult, especially with intra-mammary therapy. Many cases of mastitis are thus resistant to treatment even when the agent is fully sensitive to the drug used[29,56,67]. Milking at 1- to 2-hour intervals is recommended to remove toxins, debris, bacteria and other inflammatory products and to maintain milk duct patency[1,8].

Udder tissue necrosis: udder tissue necrosis leads to a poor blood supply to the affected areas[22] that results in a decreased redox potential that favours anaerobic bacteria[19]. There is no effective passage of drug into necrotic udder tissue.

Drug factors

Low bioavailability: Table 1 lists the drugs that are slowly absorbed and poorly distributed throughout the udder after intramammary and/or systemic treatment.

Weak passage of drugs across the blood–milk barrier: the degree of transfer of drugs from blood into milk is directly proportional to the concentration gradient across the membrane and inversely proportional to the extent to which the drug is ionised[28,29].

Antibiotic resistance: biochemical resistance of bacteria to antimicrobial agents may occur by mutation, natural selection, transformation, transduction or conjugation[60]. Bacteria initially sensitive to an antimicrobial agent may become resistant, and another antimicrobial agent must then be used. If there is no clinical improvement within 24–48 hours of initiation of intramammary and/or parenteral therapy, changing to another antibiotic is advisable. Although bacterial resistance receives much attention, more practical problems such as localised scar tissue in the udder and blockage of the milk ducts probably have a greater effect[35].

Beta-lactamase-producing *S. aureus* and *B. fragilis* destroy penicillin. Some mastitogenic strains of *B. fragilis* are resistant to ampicillin, cephalotine and amoxycillin[11,13], and some strains produce cephalosporinase, which renders them resistant to cephalosporin[19]. *P. aeruginosa* is not susceptible to ampicillin, cefamandole, cefoxitin, cephalothin or oxacillin. Carbenicillin and ticarcillin are not affected by chromosomal, inducible beta-lactamase of *P. aeruginosa* but are sensitive to the plasmid-mediated PSE type beta-lactamase[40].

Aminoglycoside antibiotics: some of the

aminoglycoside antibiotics possess a weak degree of activity against anaerobic mastitogenic bacteria[8,13,30], but the fact that they are basic and poorly lipid-soluble render them unsuitable for systemic treatment of mastitis.

Inadequate local tissue concentrations: antimicrobials do not reach the site of infection in adequate concentrations due to problems in maintenance of adequate levels of antibiotic over a required period of time, too low a dose, too long a dosing interval and too short a treatment period. Pharmacokinetic limitations of antibiotics such as absorption, disposition, elimination, sequestration due to ionisation, diffusion barriers, and intracellular parasitism *(S. aureus)* may prevent antimicrobials from reaching the site of infection in adequate concentrations[48,49].

Combined use of bacteriocidal and bacteriostatic antimicrobials: bacteriocidal (*e.g.* penicillins, cephalosporins, aminoglycosides, polymyxin B, trimethoprim-sulfonamide) and bacteriostatic (*e.g.* tetracyclines, macrolides, lincosamides) antimicrobials, when combined may be antagonistic. Penicillin should not be combined with sulphonamides or tetracyclines. The bacteriostatic effect of sulphonamides slows down bacterial cell wall synthesis and decreases the effect of penicillins, which are active against rapidly multiplying bacteria synthesising cell walls. Many penicillin products contain procaine. When procaine becomes hydrolysed, para-aminobenzoic acid (PABA) is released, which reduces the effect of sulphonamides[48,49].

Side-effects: expressed side-effects minimise the usefulness of drugs[52]. Large intramuscular doses of certain tetracyclines lead to severe swelling and oedema at the site of injection and consequent poor bioavailability.

High degree of milk and serum protein antibiotic binding: both oxytetracycline and chlortetracycline are partially inactivated in milk by chelation with magnesium and calcium ions and by combination with casein[31,49].

Half-life of the drug: the short plasma half-life of trimethoprim (50–100 minutes) in cattle limits the usefulness of this drug, which in solution is eliminated too rapidly to be useful for mastitis therapy[7,49].

Management and iatrogenic factors

Inaccurate diagnosis: incorrect clinical diagnosis of the aetiology of mastitis results in faulty therapy.

Delayed initial treatment: treatment should be commenced within hours of the 1st appearance of mastitis, since this determines the prognosis[36].

Inadequate supportive treatment: lack of supportive treatment[67], *e.g.* no shock treatment for peracute coliform mastitis, can result in failure of antimicrobial therapy.

Insertion of infusion cannula: treatment efficacy in quarters infected at drying off with partial insertion of infusion cannula into the teat canal is higher than that of full insertion of infusion cannula into the teat cistern[4].

Trauma: tissues lining the teat duct are very delicate and any unnatural manipulation of this structure, such as cannula insertion, may jeopardise antibacterial function[34], and predispose the quarter to infection or reinfection.

Superinfection and reinfection: introduction of a 2nd pathogen by insertion of a contaminated cannula into the teat[55] or with intramammary drug infusion where the teat tips are not thoroughly cleaned and disinfected before treatment[34]. Reinfection of the udder also occurs *via* the teat canal from external sources owing to inadequate milking procedures or teat canal infections.

Achieving clinical but not bacteriological cure: clinical recovery is more common than bacteriological cure with return to normal somatic cell count, and the cows do not recover full milk production after mastitis. Treatment for too short a period may result in clinical but not bacteriological cure. Systemic treatment as an adjunct to intramammary treatment has been advocated[54].

Therapy usually results in clinical cure of streptococcal mastitis and some cases of staphylococcal mastitis, but the bacteriological cure rate is low[38,47]. It is more difficult to obtain a bacteriological cure for mastitis caused by *S. aureus* than for streptococcal mastitis. Bacteriological cure rates are reduced with increasing age of the cow, in early lactation, by the severity of infection, by the number of quarters affected, by infection in the hind quarters, and by different strains of *S. aureus*[40]. A true cure, whereby all infecting microorganisms are eliminated from the affected quarter, occurs in only 10–50 % of cases.

CONCLUSIONS

Despite the widespread use of various antibiotics and other chemotherapeutic agents, antibacterial treatment of mastitis has generally achieved less than the desirable result[64]. The pathology of the udder tissue caused by mastitis and its effect on the pharmacokinetic properties of mastitis drugs, rather than widespread antibiotic resistance, appears to be a major cause of therapy failure. Success of mastitis therapy is lower in lactating dairy cows than in dry cows, especially for staphylococcal mastitis. Poor animal husbandry and veterinary practice also contribute to a less favourable prognosis. The answer to the mastitis problem lies in eliminating or minimising the predisposing factors to mastitis and in the prevention of new intramammary and teat canal infections, rather than the treatment of existing mastitic cases and infections.

REFERENCES

1. Anon. 1977 Report of the panel of the colloquium on bovine mastitis. *Journal of the American Veterinary Medical Association* 170: 1119–1123
2. Baggot J D 1977 Bioavailability and drug disposition in domestic animals. Part 1. *The Veterinary Review* 24: 66–76
3. Baggot J D 1977 *Principles of drug disposition in domestic animals. The basics of veterinary clinical pharmacology.* W B Saunders, Philadelphia.
4. Boddie R L, Nickerson S C 1986 Dry cow therapy: effects of methods of drug administration on occurrence of intramammary infection. *Journal of Dairy Science* 69: 253–257
5. Brandler G C, Pugh D M 1977 *Veterinary applied pharmacology and therapeutics* (3rd edn). Ballière Tindall, London
6. Burvenich C, Vandeputte-Van Messon G, Hill A W 1991 *New insights into pathogenesis of mastitis.* Rijksuniversiteit, Gent
7. Davitiyananda D, Rasmussen F 1974 Half-live of sulphadone and trimethoprim after a single intravenous infusion in cows. *Acta Veterinaria Scandinavica* 15: 356–365
8. Du Preez J H 1981 Die prevalensie aard en betekenis van anaerobiese bakterieë in die melkkoei-uier. M Med Vet (Hyg) dissertation, University of Pretoria
9. Du Preez J H 1985 Speenkanaalinfeksies by melkkoeie: diagnose, voorkoms, aard, terapie, omvang en betekenis. DVSc thesis, University of Pretoria
10. Du Preez J H 1985 Teat canal infections. *Kieler Milchwirtschaftliche Forschungsberichte* 37: 267–273
11. Du Preez J H 1986 Comparison of various criteria for determining the health status of the bovine udder. *Journal of the South African Veterinary Association* 56: 191–194
12. Du Preez J H 1989 Reasons why mastitis therapy is unsuccessful. *South African Journal of Dairy Science* 21: 21–29
13. Du Preez J H 1989 The role of anaerobic bacteria in bovine mastitis: a review. *Journal of the South African Veterinary Association* 60: 159–168
14. Du Preez J H 1999 Moenie dié mastitis behandel nie. *Landbouweekblad* 1093: 20–22
15. Du Preez J H 1999 Planne teen hoë SST in kuddes. *Landbouweekblad* 1094: 24–26
16. Du Preez J H, Greeff A S, 1985 Comparison of the effect of antibiotic dry cow teat canal and intramammary dry cow therapy of dairy cows on the prevalence of teat canal and intramammary infections at calving. *Journal of the South African Veterinary Association* 56: 191–194
17. Du Preez J H, Giesecke W H 1994 Mastitis. In Coetzer J A W, Thomson G R, Tustin R C (eds) *Infections diseases of livestock with special reference to southern Africa,* Vol. 2. Oxford University Press, Cape Town: 1564–1595
18. Elliot R E W 1971 Antibiotic resistance amongst haemolytic staphylococci isolated from bovine milk samples in New Zealand.

New Zealand Veterinary Journal 19: 95–99
19. Finegold S M, Rosenblatt J E, Sutter V C, Atteberry H R 1976 Anaerobic infections. In Thomas B A (ed.) *Scope monograph on anaerobic infections* (3rd edn). The Upjohn Company, Kalamazoo, Michigan
20. Funke H 1961 The distribution of S35-labelled benzylpenicillin in normal and mastitis mammary glands of cows and goats after local and systemic administration. *Acta Veterinaria Scandinavica* 2: 1–88
21. Garrod L P, O'Grady F 1971 *Antibiotic and chemotherapy* (3rd edn). E & S Livingstone, Edinburgh
22. Giesecke W H 1979 *Bovine mastitis.* Department of Agricultural Technical Services, Republic of South Africa, Technical Communication No. 51: 1–37
23. Giesecke W H, Du Preez J H, Petzer I M 1994 *Practical mastitis control in dairy herds.* Butterworths, Durban
24. Gorzelewska K, Juszkiewicz T 1961 Studies to establish the dosage of chloramphenicol for domestic animals by means of serum and milk level determination. *Biuletyn Instytutu Veterynarii Pulawach* 5: 2–3
25. Harris A M, Davies A M, Marshall M J, Evans J M, Hendy P G, Watson D J 1977 The treatment of clinical mastitis with cephoxazole and penicillin. *The Veterinary Record* 101: 4–7
26. Jackson E, Bramley J 1983 Coliform mastitis. *In Practice* 5: 135–146
27. Le Loudec C 1978 Efficacité des antibiotiques contre les mammitis bovines staphylococciques et streptococciques. *Annales de Recherches Veterinaires* 9: 63–88
28. MacDiarmid S C 1978 Antibacterial drugs used against mastitis in cattle by the systemic route. *New Zealand Veterinary Journal* 26: 290–295
29. MacDiarmid S C 1980 Drugs used in the antibacterial therapy of mastitis. *Proceedings of a post-graduate short course. Palmerston North, Massey University, New Zealand,* 26–28 May 1980: 103–110
30. Miller G E, Banerjee N C, Stowe C M 1967 Diffusion of certain weak organic acids and bases across the bovine mammary gland membrane after systemic administration. *Journal of Pharmacology and Therapeutics* 157: 245–253
31. Mol H 1975 *Antibiotics and milk.* A A Balkema, Rotterdam
32. Moore G A, Heider L E 1984 Treatment of mastitis. *Veterinary Clinics of North America: Large Animal Practice* 6: 323–333
33. Muller R, Thaller M 1979 Antibiotikaresistenz bei mastitiserregern. *Schweiz Archiv für Tieremahrung* 121: 9–14
34. Nickerson S C, Boddie R L 1985 Method of intramammary drug infusion may be important. *Hoard's Dairyman* 130: 1175
35. Pankey J W 1986 Better udder health: why treatment doesn't always work. *Hoard's Dairyman* 131: 721
36. Philpot W N, Nickerson S C 1991 *Mastitis: counter attack.* Babson Bros., Naperville
37. Pilloud M 1973 Pharmacokinetics, plasma protein binding and dosage of oxytetracycline in cattle and horses. *Research in Veterinary Science* 15: 224–230
38. Platonow I, Blobel H 1963 Therapeutic failures in chronic staphylococcal mastitis. *Journal of the American Veterinary Medical Association* 142: 1097–1101
39. Plommet M, Le Loudec C 1975 The role of antibiotic therapy during lactation in control of subclinical and clinical mastitis *Proceedings of the International Dairy Federation Seminar on Mastitis Control, Square Vergote 41, Brussels, Belgium, Bulletin Document 85:* 265–281
40. Prescott J F, Baggot J D 1988 *Antimicrobial therapy in veterinary medicine.* Blackwell Scientific Publications, Boston
41. Pyörälä S 1995 Therapy of clinical mastitis. In Sandholm M, Honkanen-Buzalski T, Kaartinen L, Pyörälä S (eds) *The bovine udder and mastitis. Gummerus Kirjapaino Oy, Jyväskylä:* 201–208
42. Rasmussen F 1959 Mammary excretion of benzylpenicillin, erythromycin and penethamate hydriodide. *Acta Pharmacologica et Toxicologica* 16: 194–200
43. Rasmussen F 1964 Distribution of sulphonamides in the mammary gland of cows after intramammary and intravenous application. *Acta Veterinaria Scandinavica* 5: 347–361
44. Rasmussen F 1966 *Studies on the mammary excretion and absorption of drugs.* Carl F Mortensen, Copenhagen
45. Rasmussen F 1971 Excretion of drugs in milk. In Brodie B B, Gillette J (eds) *Handbook of experimental pharmacology.* Springer Verlag, New York: 390–402
46. Robinson T C 1980 Therapy for acute and peracute mastitis. In Bramley A J, Dodd F D, Griffen T R (eds) *Mastitis control and herd management.* Technical Bulletin 4, National Institute for Research in Dairying, Reading: 128–134
47. Sanderson C J 1966 The treatment of mastitis with intramammary infusions. *Australian Veterinary Journal* 42: 47–53
48. Sandholm M, Ali-Vehmas T, Nyholm K, Honkanen-Buzalski T, Louhi M 1991 Failure mechanisms in lactational therapy of staphylococcal mastitis. In Burvenich C, Vandeputte-Van Messon G, Hill A W (eds) *New insights into the pathogenesis of mastitis.* Vlaamse Diergeneeskundige Tijdschrift, Ghent: 171–178
49. Sandholm M, Honkanen-Buzalski T, Kaartinen L, Pyörälä S 1995 *The bovine udder and mastitis.* Gummerus Kirjapaino Oy, Helsinki
50. Schalm O W, Carroll E J, Jain N C 1971 *Bovine mastitis.* Lea & Febiger, Philadelphia
51. Schipper I A, Eveleth D F 1959 Rates and routes of sulfonamide excretion in the cow. 1. Milk levels following single intravenous and oral administration. *American Journal of Veterinary Research* 20: 714–717
52. Schluep J, Rosselet A, Heim H 1979 Comparative evaluation of cephacetrile in experimentally induced bovine mastitis. *Zentralblatt für Veterinärmedizin* (B)26: 304–324
53. Sjoqvist E, Borga O, Orme M L 1976 Fundamentals of clinical pharmacology drug treatment. In Avery G S (ed.) *Drug treatment.* Adis Press, Sydney: 1–42
54. Swarbrick O 1966 The use of parenteral erythromycin in the treatment of bovine mastitis. *The Veterinary Record* 79: 508–512
55. Ullberg S 1964 Studies on the distribution and fate of S35-labelled benzylpenicillin in the body. *Acta Radiologia Supplement:* 118
56. Ullberg S, Hanson E, Funke H 1958 Distribution of penicillin in mastitic udders following intramammary injection – an autoradiographic study. *American Journal of Veterinary Research* 19: 84–92
57. White G, Withnell C G 1973 Chemotherapeutic evaluation of trimethoprim and sulphonamide in experimental salmonellosis of sheep. *Research in Veterinary Science* 11: 245–254
58. Wilson C D 1980 Antibiotic therapy in mastitis control. In Bramley A J, Dodd F H, Griffen T K (eds) *Mastitis control and herd management.* Technical Bulletin 4, National Institute for Research in Dairying, Reading: 113–127
59. Wilson C D, Agger N, Gilbert G A, Thomasson C A, Tolling S T 1986 Field trials with cefoperazone in the treatment of bovine clinical mastitis. *The Veterinary Record:* 118:17
60. Youmans G P, Paterson P Y, Sommers H M 1975 *The biologic and clinical basis of infectious diseases.* W B Saunders, London
61. Ziv G 1969 Antibiotic sensitivity to *Staphylococcus aureus* strains isolated from bovine udders in Israel. *Refuah Veterinarith* 26: 104–113
62. Ziv G 1975 Pharmacokinetic concepts for systemic and intramammary treatment in lactating and dry cows. *Proceedings of the International Dairy Federation Seminar on Mastitis Control, Brussels, Belgium, Bulletin Document 85:* 314–340
63. Ziv G 1978 Distribution of several labelled antibacterial agents in the udder as measured by contact autoradiographic methods. *Refuah Veterinarith* 35: 32–33
64. Ziv G 1980a Practical pharmacokinetic aspects of mastitis therapy – 1: parental treatment. *Agri Practice:* 227–290
65. Ziv G 1980b Practical pharmacokinetic aspects of mastitis therapy – 2: practical and therapeutic applications. *Agri Practice:* 469–474
66. Ziv G 1980c Practical pharmacokinetic aspects of mastitis therapy – 3: intramammary treatment. *Agri Practice:* 657–670
67. Ziv G 1980d Drug selection and use in mastitis: systemic vs local therapy. *Journal of the American Veterinary Medical Association* 176: 1109–1115
68. Ziv G, Sulman F G 1972 Binding of antibiotics to bovine and ovine serum. *Antimicrobial Agents and Chemotherapeutics* 2: 206–213
69. Ziv G, Bogin E, Sulman F G 1973 Blood and milk levels of chloramphenicol in normal and mastitic cows and ewes after intramuscular administration of chloramphenicol and chloramphenicol sodium succinate. *Zentralblatt für Veterinärmedizin* (A)20: 801–811
70. Ziv G, Shani J, Pharm M, Sulman F G 1973 Pharmacokinetic evaluation of penicillin and cephalosporin derivatives in serum and milk of lactating cows and ewes. *American Journal of Veterinary Research* 34: 1561–1565
71. Ziv G, Rasmussen F 1975 Distribution of labelled antibiotics in different components of milk following intramammary and intramuscular administration. *Journal of Dairy Science* 58: 938–946