Antimicrobial Therapy

[1]John K. House and [2]Bradford P. Smith

[1]Ruminant Health and Production, University of Sydney, Australia
[2] Department of Medicine and Epidemiology, University of California Davis, USA

Antimicrobials play an important role in the prevention and therapy of many diseases in ruminants. Constraints on the use of antimicrobials in food producing animals include consideration of current and future efficacy, availability, cost, and potential for drug residues. With the increasing public and regulatory attention on food safety there is a global call for 'prudent or judicious' drug use to preserve the efficacy of antimicrobials and to avoid introduction of antimicrobial resistant bacteria into the human food supply. Rational use of antimicrobials calls for selection of an antibiotic with known pharmacokinetic and pharmacodynamic properties for use in a patient or group of animals with a known disease process caused by a bacterial pathogen known to be susceptible to the administered dose of the chosen antibiotic. In practice, antimicrobial selection is often complicated by the fact that through necessity many decisions are based on incomplete information.

Considerations for antimicrobial use include:

1. Criteria for selection of patients requiring antibiotic therapy.
2. Knowledge of major bacterial pathogens and antimicrobial susceptibility.
3. Pharmacological and toxicological properties of the antibiotics to be used.
4. Physiological effects of age and the disease process on drug disposition.
5. Potential for drug residues
6. Economics (therapeutic cost and prognosis).

Criteria for selection of patients requiring antimicrobial therapy

Bovine veterinarians are faced with two scenarios that require establishing criteria for selection of patients requiring antimicrobial therapy. The decision to instigate antimicrobial therapy of individual sick cows is based on the animal's history, physical findings, and the results of ancillary diagnostic tests in a similar manner to companion animal medicine. With the advent of increasing herd size, veterinary involvement in the management of individual sick cows is decreasing, with a gradual transition toward veterinarians serving as consultants, educators, and evaluators of herd management protocols. In large herds the day-to-day decisions regarding therapy of sick cattle are often made by herdsman, in this situation the veterinarian's role is to establish a protocol that may be used by the herdsman that provides criteria for selection of patients requiring antimicrobial therapy. Drug use protocols are commonly employed for common disease problems including calf scours, calf pneumonia, mastitis, and metritis. Ideally treatment protocols should reflect microbial isolates and antimicrobial susceptibility data from the herd. Examples of herd monitoring programs include the fresh cow monitoring program that emerged in the late 90's that calls for daily measuring of rectal temperature for 10 days following parturition. While the logic behind such programs is reasonable no studies have been conducted to critically determine which criteria are most useful in predicting sepsis in the post partum cow. Therefore the current programs reflect individual opinions rather than a science based methodology. Establishing a good record keeping system is an important component of implementing treatment protocols as it allows for retrospective assessment of outcomes and in the event of excessive drug use provides an indicator (albeit late) of nutritional and or husbandry problems during the transition period.

Bacterial pathogens and antimicrobial susceptibility

A large proportion of antimicrobial drug use in cattle is directed at a handful of common disease problems that include calf diarrhoea, pneumonia, metritis, mastitis, and lameness. The major pathogens that cause these diseases are well described and there are numerous studies that have determined their antimicrobial susceptibility, this data is provided in Table 1. A notable feature of the different studies is the spatial and temporal differences in minimal inhibitory concentrations observed. These differences in MIC illustrate the importance of local ongoing surveillance data for the practice of rational antimicrobial therapy.

There are numerous ways to measure the susceptibility of bacteria to antimicrobial agents. The two methods used most often in veterinary medicine are the disk diffusion method and the tube dilution method. The disk diffusion method involves measuring a zone of inhibition around a disk impregnated with the antimicrobial agent. A concentration gradient is created as the antimicrobial diffuses out of the disk into the agar. The size of the zone of inhibition correlates with the susceptibility of the agent. This method of assessing antimicrobial resistance provides a qualitative but not quantitative measure of antimicrobial susceptibility. The tube dilution method of determining antimicrobial susceptibility involves inoculating media containing serial dilutions of the test antimicrobial. The tube dilution method provides a quantitative measure of antimicrobial susceptibility that may be reported as a specific drug concentration. For clinical reporting the results of in vitro antimicrobial susceptibility tests are often reported as susceptible, intermediate, or resistant based on obtainable serum concentrations of the drug. While classification of susceptibility is useful it is not infallible, failure of antimicrobial susceptibility tests to predict clinical outcomes may reflect application of misleading interpretive criteria. For example, the current NCCLS susceptible breakpoint for ampicillin is 8.0 μg/ml when testing enterobacteriaceae however the Cmax of ampicillin in cattle dosed at 11 mg/kg is <4 μg/ml. The source of the discrepancy reflects the fact that the source of the breakpoint for ampicillin is derived from human studies. Predictive failure of susceptibility tests may also fail due to variables associated with the bacterium, inconsistencies between the in-vitro test and the in-vivo environment, and or failure of the dosage regimen.

When a specific pathogen is identified the use of narrow spectrum drugs has less potential to select for antimicrobial resistance. Consideration should also be given to the disposition of antimicrobials. Drugs that are excreted via bile or that undergo enterohepatic circulation tend to disturb the intestinal flora more than those excreted via the kidneys and have the potential to select for antimicrobial resistance in commensal flora. Antimicrobial resistance determinants may be passed between bacterial species.

Pharmacological and toxicological properties

The objective of antimicrobial therapy is to provide an effective drug, in sufficient concentration and maintained for sufficient time at the site of infection to allow host defenses to clear the pathogen. Potential risks associated with antimicrobial treatment include host toxicity, drug residues, selection for antimicrobial resistance, adverse interactions with other drugs, and compromised intestinal flora.

There are no criteria that universally predicts *in vivo* efficacy of the various antimicrobial compounds that are available. The characteristics of antimicrobial compounds that may be related to efficacy are described by their pharmacokinetic properties. Specific pharmacokinetic properties correlating with efficacy vary among antimicrobial compounds. Two variables that strongly relate to antimicrobial efficacy are time and concentration. For time dependent anti-bacterial drugs efficacy is related to the period for which drug concentration exceeds the minimal inhibitory concentration (MIC) of the target pathogen. For these drugs (e.g. beta lactams and macrolides) there is a point of diminishing returns in as much as exceeding MIC values several fold is not required for optimal efficacy. Concentration dependent microbial killing is observed with other antimicrobials (e.g. aminoglycosides and fluroquinolones), with these drugs efficacy is related to peak concentration and the area under the curve. These drugs continue to inhibit microbial growth even when the concentration of the drug drops below MIC (post antibiotic effect).

Pharmacodynamic properties related to efficacy include MIC, minimum bacteriacidal concentration, concentration dependent killing, first-exposure effect, post antibiotic effect, sub-MIC post antibiotic effect, and post antibiotic leukocyte enhancement effect. The first exposure effect refers to the effect an antimicrobial agent has on a bacterial pathogen at the time of the initial therapy. Ideally, initial exposure will kill the organism. However, for bacteria that survive there may be a time in which they will be unaffected by a repeat exposure to the drug. For example re-exposure to an antimicrobial that inhibits protein synthesis will have little effect on a bacterium that already has compromised protein synthesis capabilities. The length of the post antibiotic effect is dependent on the microorganism, the class of antimicrobial agent, the concentration of drug to which the bacteria are exposed, and the duration of exposure. Antimicrobial agents that inhibit DNA or protein synthesis have prolonged post antibiotic effects.

Plasma concentrations are often the focus of pharmacokinetic studies and may be used to provide a guide as to predicting likelihood of efficacy. Most infections however occur within tissues or even within cells that may not contain the same drug concentration as plasma. Antimicrobial agents generally distribute into tissues and cells by passive diffusion. Concentration gradient, lipid solubility, and degree of ionization of a drug largely influence the extent of absorption, distribution, and rate of elimination.

The volume of distribution of a drug reflects its dispersal within the body. Lipid soluble drugs tend to have a high volume of distribution whereas charged polar molecules have a low volume of distribution. Drugs that are highly protein bound generally have a low volume of distribution, even when they are lipid soluble. It is generally believed that only the non-protein bound drug is active against micro-organisms. Conversely reversible binding to inflammatory proteins can provide a means of delivering drug to infected sites. Rational antimicrobial therapy calls for selection of a drug that is likely to achieve therapeutic concentrations at the site of infection. High lipid solubility is desirable for treatment of intracellular pathogens and for treatment of infections of the meninges and mammary gland. The dissociation constant, pK_a, of drugs influences the distribution of drugs in tissues and body fluids by determining the degree of ionization at any given pH. The majority of antimicrobial agents are weak acids or bases, a few like fluoroquinolones and tetracyclines are amphoteric compounds. Lipid solubility is a requirement for passive diffusion of drugs across cell membranes. It is the non-ionized form of weak organic acids and bases that is lipid soluble. When the pH of a body fluid differs from the pH of blood (pH 7.4) there is a potential for ion trapping. This phenomenon is relevant to systemic antimicrobial therapy of mastitis. Milk is acidic relative to blood. Basic drugs that diffuse into milk dissociate and are then unable to diffuse back into plasma leading to a higher concentration of drug in milk.

Lipid soluble drugs are primarily cleared by hepatic metabolism and to a lesser degree renal excretion. Ruminants are well endowed with oxidative enzyme systems such as the cytochrome P450 group. Consequently, the half life of lipid soluble drugs is generally shorter in ruminants than in man. Drugs that are poorly lipid soluble (penicillins and aminoglycosides) are eliminated predominantly by renal excretion. Drugs which are poorly reabsorbed from the renal tubule (aminoglycosides) achieve high urine concentrations, and those which are additionally secreted across the proximal tubule (penicillins) can achieve even higher concentrations. These mechanisms of renal excretion do not vary significantly between mammalian species. However renal excretion of drugs is often altered by disease states that affect renal perfusion.

Drug dose

Most antimicrobials licensed for use in livestock have been developed and evaluated for treatment of bovine respiratory disease. Consequently the antimicrobial dose and dosing frequency is formulated to maintain therapeutic drug concentrations against *Mannheimia hemolytica*, *Pasteurella multocida*, and *Hemophilus somnus*. Because of the high costs associated with obtaining drug approval, many drugs are not approved or may be approved only for narrowly specified purposes at dosages that may be based more on the potential for drug residues or economics of treatment rather than on optimal clinical efficacy. Off label drug use may be employed to optimize clinical efficacy however the liability rests with the

prescribing veterinarian. To some extent, drug dosage can be tailored to the susceptibility of the organism, the site of infection, and the pharmacokinetic and pharmacodynamic properties of the selected antimicrobial agent. The UK's Veterinary Medicines Directorate has proposed two criteria for defining the appropriate dosing of antimicrobial compounds. The first is that the maximum concentration following dosing (Cmax) should be at least twice the minimal inhibitory concentration of the 90th percentile for the target pathogen (MIC_{90}) and that the plasma concentration should exceed the MIC_{90} for at least half the inter-dose interval. While this recommended dosing schedule may be effective for some drugs it is not necessarily optimal for all antimicrobials.

The interval for IV administered drugs required to maintain therapeutic plasma concentrations should not usually exceed twice their elimination half-life. Because elimination half life is based on IV dosing, however, administering appropriate formulations by other routes can be an effective way to lengthen the interval between doses, since absorption may be delayed.

The pharmacokinetic profile of long acting formulations of antimicrobials are characterized by flip-flop pharmacokinetics i.e. elimination rate exceeds absorption rate and the declining phase of the concentration-time curve provides a measure of absorption half-life. An essential feature of prolonged release preparations is that the rate of drug release be adequate to maintain effective plasma concentrations for the duration of the dosage interval. For acute infections a response to therapy should be observed within 2 days of initiating therapy. Treatment should be continued for at least 2 days following clinical and or microbiological resolution of infection. For potentially life threatening infections treatment should last 7 to 10 days. Treatment of chronic disease processes generally requires more protracted therapy.

Off label drug use - Currently the regulations regarding off label drug use in Australia are handled by each of the states. This is soon to change with the coming implementation of national guidelines that will cover antimicrobial use in livestock. The following information regarding drug use in New South Wales is supplied courtesy of Lee Cook from New South Wales Agriculture. Under the NSW Stock Medicines Act, registered veterinary surgeons have *two major privileges* not available to anyone else. They can:

- Use or prescribe or supply for use an unregistered stock medicine—Section 38(2).
  These must be human pharmaceuticals or mixtures made up by the vet, but only for non food-producing animals actually under their care.

- Vary a label direction—Section 39(2).
  This includes using a product registered for use in one food-producing species in some other food-producing species, e.g. using oral Ivomec for goats not sheep, or changing the dose on the label.

Use of human pharmaceuticals or companion animal products for food-producing species is NOT permitted. Neither is use of *any unregistered product* for food-producing species. These freedoms carry with them a responsibility to provide *written directions for use* of the product. Section 40(2) specifies that the following written directions must be supplied if label directions are varied, and that they must be *signed and dated by the vet*, and include the name and address of the veterinarian (practice) supplying them –

ANIMAL SPECIES
WITHHOLDING PERIOD
DOSE RATE
FREQUENCY OF TREATMENT
LENGTH OF TREATMENT
MANNER OF ADMINISTRATION

Note that all "off-label" use is at the veterinarian's professional discretion. *Liability* for such use also rests with the veterinarian. Residue problems can occur when medicines are used at higher doses, for longer periods than the label allows or by different routes. Information on such proposed uses may sometimes

be supplied by the manufacturers. Most manufacturers are listed in the back of the IVS Annual, published each year by MIMS Australia (phone 0061 2 9902 7770 or 1800 800 629).

Residues - Maximum residue limits are established for all registered antimicrobials. The maximum residue limit (MRL) is determined by assays in laboratory animals to assess the no observable effect level (NOEL) in chronic toxicity studies and via assays to determine the no microbiological effect level (NMEL). This involves determination of concentrations of the drug producing no effect against the most sensitive species of bacteria, with emphasis on the normal flora of the human alimentary tract. The acceptable daily intake (ADI) is then determined by applying safety factors (normally 100). Finally, a food withholding period is established, this being the time between administration of therapeutic doses of the drug and when food may be consumed with negligible risk.

The systemic availability of drugs is influenced by the route of administration. Intravenous injection of antimicrobials provides high plasma concentrations rapidly however effective plasma concentrations generally persist for a shorter duration than following extra vascular administration. Absorption from IM and SC injection sites is determined by drug formulation, vascularity of the injection site, and by the volume of drug administered. Most formulations recommend that no more than 10 ml be injected in any one site. ***Dividing larger doses increases the surface area promoting rapid absorption of drug. Conversely administering a large volume in a single site may delay absorption and increase the risk of drug residues.*** Subcutaneous administration of drugs tends to provide slower absorption of drugs than intramuscular administration and has been associated with protracted variable drug residues when product formulated for intramuscular administration was administered subcutaneously.

Significant variation in drug absorption has been observed between different intramuscular sites of administration. Drugs administered in the shoulder and neck region are generally absorbed better than when administered into the gluteals or semimebrinosus muscles. The neck is the preferred site for intramuscular injections because drug absorption is better and the losses due to carcass damage are less than those associated with rump injections. Unfortunately the logistics of administering drugs in the neck is often more difficult, particularly in poor facilities.

Antimicrobials registered for therapeutic use in livestock in Australia

Penicillin

Beta lactam antibiotics exert a bactericidal effect by inhibiting penicillin-binding proteins and therefore the synthesis and incorporation of peptidoglycan into the cell wall of susceptible bacteria. The antimicrobial spectrum of penicillin G includes most gram positive aerobes including *Streptococcus spp*, *Bacillus anthracis*, *Actinomyces spp*, *Corynebacterium spp*, *Arcanobacterium pyogenes*, and *Listeria monocytogenes*. Approximately 50% of coagulase positive *Staphylococcus spp* are resistant to penicillin due to production of beta lactamase enzymes. Susceptible anaerobes include *Clostridia spp*., *Fusobacterium spp*., and some *Bacteroides spp*. Susceptible gram negative bacteria include *Haemophillus somnus* and some *Pasteurella spp*. Good susceptibility is reflected by a MIC $\leq$ 0.12 μg/ml, moderate susceptibility by an MIC of 0.25 – 2 μg/ml and resistance by an MIC $\geq$ 4 μg/ml. The relative lack of efficacy against gram negative organisms may result from an inability of the antibiotic to penetrate the gram negative cell wall, lack of available binding site (penicillin binding proteins), or enzymatic inactivation (beta lactamases).

Acid hydrolysis precludes oral medication of penicillin in ruminants. Products registered for use in ruminants are all labeled for parenteral administration. Penicillins are weak acids (pK_a 2.7) that are strongly ionized in plasma and have relatively short half-lives (0.5 – 1.2 hours). The poor lipid solubility of penicillins limits their distribution to the extracellular fluid space. The limited distribution of penicillins across membranes is reflected by the relatively low concentration observed in milk following systemic administration (1/5 of plasma). Entry across biological membranes is enhanced by inflammation (increased vascular permeability) however the activity of penicillins is reduced in acid environments such as occur in

abscesses and sites of tissue necrosis. Excretion is almost exclusively through renal excretion. The subsequent high concentration found in urine may be sufficient to treat organisms not normally considered susceptible such as *E. coli* associated with pyelonephritis.

Formulations of sodium benzyl penicillin G include benzyl, procaine benzyl, and tribenzyl ethylenediamine (benzathine). Frequent dosing of benzyl penicillin is required because of the drug's rapid excretion. Depot forms that prolong drug levels by delaying absorption include procaine and benzathine penicillin. The activity of penicillin G may be expressed in units. The recommended dose for procaine penicillin is 25,000 units (15.6 mg) per kilogram once a day. This provides effective serum concentrations against susceptible bacteria for at least 12 hours and generally for 24 hours. For moderately susceptible bacteria (for example *Pasteurella hemolytica*) dosing should be repeated twice a day or the daily dose increased to 45,000 IU per kg. Benzathine penicillin is a long acting, slow release formulation that is administered every 72 hours. Serum concentrations are usually so low that it is only recommended for extremely susceptible bacteria and is not recommended for serious infections. Adverse reactions to penicillin are relatively uncommon in cattle. Intravenous administration of procaine penicillin may induce an anaphylactoid knock down like reaction. Animals that are hypersensitive to penicillin may develop urticaria.

Attachment of an acidic radical to the amino group of 6-aminopenicillanic acid has been used to alter the spectrum of activity of penicillin derivatives. Anti-staphylococcal isoxazolyl penicillins used in bovine practice include cloxacillin. Cloxacillin, by virtue of its chemical structure resists cleavage and inactivation by many beta lactamases including most of those elaborated by coagulase positive *Staphylococcus spp*. Extended-spectrum penicillins include aminobenzylpenicillins (ampicillin, hetacillin, and amoxicillin). Amoxicillin and ampicillin have increased activity against gram negative organisms including *E. coli* and *Salmonella spp*. due to their capacity to penetrate the outer membrane of gram negative bacteria. The activity of amoxicillin and ampicillin against gram positive bacteria are slightly reduced compared to penicillin G and both drugs are susceptible to beta lactamases. Plasmid mediated resistance to aminobenzylpenicillins is common and is mediated by promotion of beta lactamase production.

Amoxicillin trihydrate formulations are registered for intramuscular injection in cattle in Australia. The trihydrate formulation provides a depot effect similar to procaine penicillin. Plasma concentrations following administration of amoxicillin trihydrate formulations are variable with most preparations producing low drug concentrations. The low serum concentrations achieved limit the spectrum of activity. Long acting preparations of ampicillin trihydrate have also been produced which are recommended for every other day dosing. The associated low plasma concentrations may limit distribution of antibiotic to sites of infection. Cut points for expressing antimicrobial susceptibility for ampicillin and amoxicillin are MIC $\leq$ 1 for good susceptibility, MIC = 2 – 4 μg/ml for moderate susceptibility, and MIC $>$ 4 μg/ml for resistant organisms. Although amoxicillin and ampicillin have a broader spectrum of activity and better distribution in the body than penicillin relatively high doses are required to treat gram negative infections and acquired resistance is common.

Cephalosporins

Cephalosporins have a six membered dihydrothiazine ring attached to a beta lactam ring. Cephalosporins are more resistant to beta lactamases than penicillin. Two cephalosporins are registered for use in cattle in Australia Cephalothin and ceftiofur. Cephaolthin is a first generation cephalosporin it has high activity against gram-positive bacteria, including beta lactamase producing *Staph. aureus* and moderate activity against some gram negative bacteria. Preparations registered for use in cattle include intramammary preparations and ocular formulations for treatment of pink eye. Acquired resistance is common in gram negative but rare in gram positive bacteria.

Ceftiofur is a third generation cephalosporin that has high antibacterial activity against gram positive, gram negative, and anaerobic bacteria. It is generally resistant to beta-lactamases however over the last couple of years there has been emergence of resistance in a number of strains of salmonella in the United States.

The label dose of ceftiofur for treatment of respiratory disease in cattle in Australia is 1.1 mg/kg. The target pathogen, *Mannheimia hemolytica*, has an MIC_{90} of 0.015 – 0.06 ug/ml (Watts et al, 1994; Salmon et al, 1996). As with other Beta lactam drugs ceftiofur does not have a post antibiotic effect so the therapeutic objective is to maintain drug concentration above the MIC of the target pathogen for the duration of therapy. In vivo, ceftiofur is rapidly metabolized to desfuroylceftiofur (DFC), a microbiologically active metabolite retaining the beta-lactam ring. Desfuroylceftiofur has an exposed sulfhydryl moiety that is involved in reversible binding to cysteine moieties, glutathione, sulfhydryl and itself (desfuroylceftiofur-dimer), through formation of disulfide bonds. The exposed sulfhydryl moiety of DFC is also responsible for reversible, covalent binding of DFC to plasma and tissue proteins, with the formation of disulfide or thioester bonds. The protein binding extends the biological half-life of DFC, since it protects the β-lactam ring from cleavage and reduces the rate of excretion by the liver and kidney. These conjugates all have intact β-lactam rings and can be reduced back to desfuroylceftiofur. The desfuroylceftiofur MIC is not detectably different from the ceftiofur MIC (Salmon et al, 1996). The combined concentration of ceftiofur and desfuroylceftiofur may be expressed as ceftiofur equivalents. The reversible protein binding of ceftiofur and its metabolites facilitates its distribution to sites of inflammation. Bacteria are classified as susceptible when they have an MIC $\leq$ 2 μg/ml, moderately susceptible MIC $\leq$ 4 μg/ml and resistant when the MIC $\geq$ 8 μg/ml. Ceftiofur is labeled for treatment of respiratory disease, footrot, and metritis in cattle. *Haemophilus somnus*, *Mannheimia hemolytica*, *Pasteurella multocida*, and *Fusobacterium necrophorum* are exquisitely sensitive to ceftiofur. The efficacy of ceftiofur for treatment of metritis has been demonstrated however the therapeutic dose selected for these trials is low considering the mixed microbial population associated with metritis in cattle. According to the reported susceptibility of the pathogens associated with metritis a higher dose of ceftiofur would be expected to be more efficacious.

Aminoglycosides

Aminoglycosides are used relatively infrequently in food producing animals due to their propensity for producing protracted meat residues. Persistent drug residues reflect selective binding to renal tubules in the kidney cortex. Neomycin is the only aminoglycoside licensed for use in cattle in Australia. Oral neomycin preparations may be useful in management of outbreaks of enterotoxigenic *E. coli* infections in calves. Aminoglycosides are poorly absorbed from the gastrointestinal tract with only 3% of orally administered neomycin absorbed systemically.

Aminoglycosides are basic drugs that are ionized in plasma, have little binding to plasma proteins, and a limited capacity to pass through cellular membranes. Although parenteral neomycin formulations are registered for use in livestock in Australia they are not recommended as safer, less toxic, more efficacious alternative drugs available. The most common manifestation of aminoglycoside toxicity observed in ruminants is acute tubular necrosis. Deafness has also been reported following administration of neomycin. Excretion is by renal excretion. Compromised renal function decreases the rate of excretion and may contribute to drug build up and toxicity. Toxicity may be observed with prolonged therapy or excessively high trough serum concentrations. Dehydration, underlying renal disease, and or concurrent therapy with other potentially nephrotoxic drugs are variables that increase the risk of aminoglycoside toxicity. Neomycin is the most toxic of the aminoglycosides.

As a class of drugs aminoglycosides have a rapid dose related bacteriacidal action on susceptible micro-organisms. Aminoglycosides have high antibacterial activity against aerobic gram negative bacteria, a limited spectrum of activity against gram positive bacteria and they are inactive against anaerobic bacteria due to the requirement for an oxidative transport mechanism to penetrate the cell envelope. The synergy observed between aminoglycosides and beta lactam antibiotics reflects improved uptake of aminoglycosides following damage to the cell wall. Once inside the cell aminoglycosides bind to the 30S subunit of the ribosome inducing misreading of messenger RNA inhibiting protein synthesis.

The efficacy of aminoglycosides is compromised by the presence of purulent debris and resistance to aminoglycosides is carried on R plasmids that code for enzymes that inactivate the drug.

Macrolides

Macrolides are a group of antibiotics that are characterized by a macrocyclic lactone ring attached to two or more sugar moieties. They are basic, lipid soluble, bacteriostatic drugs that compromise bacterial protein synthesis by binding to the 50S ribosome inhibiting the translocation step. Spectrum of activity is predominantly gram positive aerobes with some activity against select gram negative aerobes, anaerobes, and mycoplasma spp.

Erythromycin – Parenteral formulations are the only erythromycin products registered for use in cattle. These products generally come in an oily base and are administered by deep intramuscular injection. Tissue reaction and pain at the site of injection are often observed and may limit repeated drug administration. Good susceptibility (MIC $\leq$ 0.5 μg/ml) is observed with gram positive aerobes: *Bacillus spp*, *Corynebacterium spp*, *Listeria spp*, Staphylococci, and Streptococci. Gram negative aerobes susceptible to erythromycin include *Brucella spp*, *Campylobacter spp*, and *Leptospira spp*. Anaerobic bacteria: *Actinomyces spp*, *Bacteroides spp*, *Clostridium spp*, and some *Fusobacterium spp*. *Haemophilus spp* and *Pasteurella spp* are moderately susceptible (MIC 1 – 4 μg/ml). Enterobacteriaceae, mycoplasma, and mycobacterium spp are resistant (MIC $\geq$ 8 μg/ml). Clinical application of erythromycin in cattle is limited by its associated tissue reaction and injection site pain in the face of efficacious alternatives. Antimicrobial resistance may also be a limitation as a one step chromosomal mutation to high level resistance develops fairly readily and plasmid resistance is also common.

Tylosin - Tylosin is a macrolide antibiotic isolated from *Streptomyces fradiae*, it has a similar spectrum to erythromycin however it is generally less active against bacteria and more active against mycoplasma spp. Tylosin is registered for parenteral and enteral administration in cattle. Therapeutic applications include pneumonia, foot-rot, metritis, pinkeye, and mastitis. Resistance to tylosin develops quickly. Due to the advent of more efficacious newer generation antimicrobials tylosin is rarely used for therapeutic purposes bar treatment of mycoplasma infections. The most common commercial application of tylosin in cattle is as a feed additive to improve weight gain and feed efficiency and to prevent liver abscess. Tylosin (pK_a = 7.1) is 67% non-ionized at serum pH of 7.4 whereas only 20% non-ionized at the normal bovine milk pH of 6.5. Since the non-ionized fraction is diffusible, passive diffusion from serum to milk is favored over diffusion from milk to serum.

Tilmicosin – Tilmicosin is a semisynthetic macrolide derivative of tylosin developed exclusively for use in livestock. The antibacterial activity of tilmicosin is greater than tylosin and its anti-mycoplasma activity greater than erythromycin. As with other macrolides, tilmicosin is highly lipid soluble producing low serum concentrations and a large volume of distribution (> 2 l/kg). Tilmicosin is registered for treatment of bovine respiratory disease. Following a dose of 10mg/kg administered via subcutaneous injection serum concentrations do not exceed 0.35 μg/ml, whereas the concentration in lung tissue ranges from 8 – 17.8 ug/g within 8 hours of dosing. Tilmicosin also distributes well into consolidated lung and the drug is concentrated within alveolar macrophages (651 μg/g) (Shryock et al, 1995). A single SC dose of 10 mg/kg provides drug levels in the lungs that exceed the MIC of *Mannheimia hemolytica* for 72 hours. The NCCLS approved breakpoints for the MIC dilution testing are resistant $\geq$ 32 μg/ml, intermediate 16 μg/ml, and susceptible $\leq$ 8 ug/ml. Mechanisms of resistance to tilmicosin is similar to other macrolides and cross resistance between macrolides is observed. Tilmicosin exhibits species dependent cardiovascular toxicity. Cardiovascular toxicity has been observed in cattle following intravenous administration. The drug may be fatal to horses and swine by IM injection. Caution is advised when administering the drug to avoid accidental human injection. Tilmicosin is not recommended for use in lactating cattle as it produces prolonged (2 – 3 week) residues in milk.

Florfenicol

Florfenicol is a derivative of chloramphenicol, in which the p-nitro group that is associated with the induction of irreversible aplastic anemia has been replaced by a sulfomethyl group and the hydroxyl group has been replaced with fluorine. Substitution of the hydroxyl group for fluorine provides resistance to inactivation by chloramphenicol transacetylases. Florfenicol inhibits microbial protein synthesis by

binding irreversibly to a receptor site on the 50S subunit of the bacterial ribosome interfering with the formation of peptides by blocking the action of peptidyl transferase.

Bacteria susceptible to florfenicol include gram-positive, many gram-negative, and anaerobic bacteria. Susceptible organisms (MIC ≤ 8 μg/ml) include *Arcanobacterium pyogenes*, *Corynebacterium spp*. *Listeria monocytogenes*, *Staphylococcus spp*, *Streptococcus spp*., *E. coli*, *Salmonella spp*., *Haemophilus spp*., *Leptospira spp*., *Moraxella bovis*, *Mannheimia hemolytica, Pasteurella multocida* and all anaerobes. Florfenicol also suppresses rickettsial and chlamydial growth however it is not efficacious against *Mycoplasma spp*. Florfenicol is registered for treatment of respiratory disease and pink eye. The recommended dosing regimen is 20 mg/kg administered intramuscularly twice with a dosing interval of 48 hours. This dosing regimen provides adequate drug levels for treatment of respiratory pathogens (*Pasteurella*, *Haemophillus*, and *Mannheimia*) that have a MIC ≤ 1 ug/ml. Infections caused by less susceptible bacteria such as *E. coli* are likely to require a more frequent dosing interval.

Serum florfenicol concentrations are limited by absorption kinetics following intramuscular dosing. Despite its high lipid solubility florfenicol is excreted unchanged in the urine. Reversible bone marrow suppression may be observed following prolonged florfenicol administration and transient diarrhea and inappetence have been reported.

Tetracyclines

The tetracyclines include a group of products produced by *Streptomyces spp*. They are bacteriostatic drugs inhibiting protein synthesis by binding irreversibly to the 30S subunit of the ribosome, preventing addition of amino acids to the elongating peptide chain. The tetracyclines are amphoteric compounds that are excreted unchanged in urine and to a lesser extent bile. Enterohepatic circulation prolongs the half life of tetracyclines and contributes to gastrointestinal disturbances following administration of larger doses. Tetracyclines utilized in bovine medicine include chlortetracycline, oxytetracycline, and tetracycline. Differences in clinical efficacy among tetracyclines are largely attributable to characteristics of absorption, distribution, and excretion, not to differences in microbial susceptibility.

The antibacterial spectrum of activity of tetracyclines includes *Coxiella burnetti*, *Ehrlicia spp*., *Mycoplasma spp*. *Anaplasma spp*., *Babesia spp*., *Rickettsia spp*., gram positive, gram negative, and anaerobic bacteria. The breakpoint for classifying bacteria as susceptible is an MIC ≤ 4 ug/ml. Organisms that often fall into this category include *Corynebacterium spp*, *Listeria monocytogenes*, *Streptoccoci spp*., *Haemophilus spp*., *Pasteurella multocida*, *Campylobacter fetus*, *Leptospira spp*., *Fusobacterium*. *Coxiella burnetti*, and *Anaplasma*. Variable susceptibility is reported with *Staphylococci spp*., enterobacteriaceae, *Bacteroides spp*. and *Clostridia*. Antimicrobial resistance mediated by reduced active transport of tetracycline into and increased efflux from the cell is widespread.

Adverse side effects associated with the administration of tetracyclines include injection site reactions, fatal anaphylactoid reactions associated with rapid IV infusion, renal toxicity, hepatic toxicity, and yellow discoloration of teeth and bone in developing foetuses (chelation with calcium). The degree of tissue reaction at the injection site varies according to the drug formulation. Oxytetracycline is poorly soluble in water. Many formulations are available as hydrochlorides which are acidic. Organic solvents are used in commercial oxytetracycline preparations. These organic solvents are in themselves irritants and contribute to tissue damage at the site of injection. The more irritating products have lower bioavailability and greater drug persistence at the injection site. The long acting formulations of oxytetracycline have their long-acting effect because of both the high dosage used and the prolonged drug persistence at the injection site. Long acting formulations provide serum concentrations of oxytetracycline above 0.5 μg/ml for 48 hours when administered intramuscularly at 20 mg/kg, similar drug concentrations may be achieved for a similar duration using the same dose of 'short acting' formulation. Of note is the discrepancy between the MIC susceptible cutoff (≤ 4 μg/ml) and the drug concentration maintained with this dosing regimen. Subcutaneous injection in cattle maintains serum concentrations similar to those following IM administration and reduces the amount of tissue necrosis. Different products should be used according to their labeled directions. The least reactive oxytetracycline formulations contain

polyvinylpyrrolidone, a large molecular weight polymer that forms a stable complex with the magnesium salt of oxytetracycline.

Clinical applications of tetracyclines include metritis, pneumonia, footrot, papillomatous digital dermatitis, anaplasmosis, babesiosis, Q fever, pink eye, and mastitis. Oxytetracycline achieves higher concentrations in diseased lung than healthy lung and milk drug concentrations are higher than plasma concentrations when there is inflammation of the mammary gland (Nouws et al, 1983).

Trimethoprim and Sulfonamides

The sulfonamides interfere with the biosynthesis of folic acid in bacterial cells by competitively preventing para-aminobenzoic acid (PABA) from incorporation into folic acid. Bacterial cells must synthesize folic acid whereas mammalian cells use preformed folic acid. The action of sulfonamides is negated by tissue exudates and necrotic tissue that provide microbes with an alternate source of PABA. Trimethoprim interferes with the synthesis of tetrahydrofolic acid by combining with the enzyme dihydrofolate reductase. The combination of trimethoprim with a sulfonamide inhibits sequential steps in the synthesis of folic acid and subsequently purines required for DNA synthesis.

Sulfonamides are bacteriostatic. The antimicrobial spectrum of sulfonamides includes gram positive and negative bacteria, toxoplasma, and coccidia. An MIC of 8 – 32 μg/ml is considered susceptible and an MIC $\geq$ 64 μg/ml resistant. *Bacillus spp.*, *Brucella spp.*, *Listeria monocytogenes*, *Nocardia spp.*, *Streptococci spp.*, and coccidia are generally susceptible. Variable susceptibility because of acquired resistance is observed with *Staphylococci spp.* and enterobacteriaceae. Anaerobic bacteria susceptible in vitro may not be in vivo due to the presence of thymidine in purulent exudates. Microbial resistance to sulfonamides results from impaired drug penetration, production of an insensitive dihydropteroate enzyme, or hyperproduction of PABA. Resistance is widespread.

Sulfonamides are absorbed rapidly from the GI tract. Most parenteral preparations should be administered only by IV injection. Most dosing regimens call for a loading dose that is double the subsequent maintenance dose. The different sulfonamide preparations on the market differ in their duration of action. Sulfadiazine has a short half life (2.5 hrs) whereas sulfamethazine, sulfadimethoxine, and sulfadoxine have longer half lives of 8.2, 12.5, and 10.8 hours respectively. The degree of binding to plasma proteins is variable between compounds with extensive binding associated with a longer half life. Elimination is via renal excretion and biotransformation. Renal tubular reabsorption is determined by the pKa of the sulfonamide and the pH of the fluid in the distal tubules. Urinary alkalization increases renal excretion and the solubility of sulfonamides in urine. Hepatic metabolism includes acetylation, aromatic hydroxylation and glucuronide conjugation.

Trimethoprim is primarily eliminated by hepatic metabolism. The half life of trimethoprim in ruminants is particularly short due to rapid demethylation to produce inactive compounds. There is also a significant age associated change in the half life of trimethoprim. The half life in calves at 1, 7 and 42 days of age is 8.4, 2.1, and 0.9 hours respectively. The age associated change in the half life of trimethoprim in part reflects the rapid break down of the drug in the rumen.

The combination of trimethoprim with a sulfonamide (TMS) is bactericidal. Trimethoprim is approximately 20 – 100 times more active than the sulfonamides. Commercial preparations are formulated at a ratio of 1:5 to provide a 1:20 ratio of drug concentration (based on pharmacokinetics in humans). Where synergistic interactions occur, a 10 fold increase in activity of the trimethoprim component and a 100 fold increase in activity of the sulfonamide component are common. In contrast to the pharmacokinetics in humans the half lives of the TMS components do not coincide to provide prolonged synergy in ruminants. Administration of TMS to adult cattle maintains bacteriostatic concentrations of the sulfonamide, which for a time after each dose is enhanced by the synergistic bactericidal action of the combination. The route of administration also has a dramatic impact on the pharmacokinetics of TMS in cattle. Trimethoprim has reduced availability and slow absorption following intramuscular injection the prolonged half life

following intramuscular injection reflects absorption limited kinetics. Intramuscular administration of the labeled dose fails to achieve plasma drug concentrations that exceed the susceptible MIC breakpoint.

Susceptibility to TMS is reflected by an MIC $\leq$ 0.5 and 9.5 μg/ml to the trimethoprim and sulfonamide fractions respectively. *Staph. aureus*, *Streptococci spp.*, *Actinomyces spp.*, and enterobacteriaceae are often susceptible. Intermediate susceptibility is reflected by an MIC of 2 and 38 μg/ml respectively and resistance by an MIC $\geq$ 4 and 72 μg/ml. Synergism occurs at different drug concentration ratios with different bacterial species. Differences in the distribution and elimination of the trimethoprim and sulfonamide components may result in different concentration ratios in tissues and body fluids. While synergy occurs over a wide range it will not occur in some tissues since the trimethoprim is more widely distributed than the sulfonamide. Sulfonamides are weak acids whereas trimethoprim is a weak base. Trimethoprim is highly lipid soluble and as such is concentrated in tissues, whereas sulfonamides (weak organic acids) remain largely in extracellular fluid.

Trimethoprim sulfonamide formulations have been recommended for treatment of metritis and mastitis. Necrotic debris associated with metritis is likely to negate the antimicrobial action of these drugs. Treatment of coliform mastitis with parenterally administered TMS has been reported to improve clinical outcomes and reduce cow attrition. Cows in this trial were administered 48 mg/kg IV every 12 hours. The average milk to plasma ratio of trimethoprim is 3:1. Following an intravenous dose of 8mg/kg of trimethoprim and 40 mg/kg of sulphadiazine the concentration of trimethoprim achieved in milk is $>$ 0.5 μg/ml for approximately 6 hours. The concentration of sulphadiazine achieved in milk is $>$ 9.5 μg/ml for less than an hour. Unfortunately the labeled dose for trimethoprim sulfonamide formulations is 1/3 to 1/2 this dose and as such is unlikely to achieve therapeutic concentrations in milk.

Adverse reactions to trimethoprim and sulfonamides are uncommon with conventional dosing. Rare sporadic hypersensitivity reactions and nephrotoxicity have been reported with sulfonamides. Sulfonamides are not recommended for concurrent administration with procaine penicillin as procaine is an analog of PABA and will antagonize the antimicrobial action of sulfonamides.

References

1. Salmon SA, Watts JL, Yancey RJ, Jr. In vitro activity of ceftiofur and its primary metabolite, desfuroylceftiofur, against organisms of veterinary importance. *J Vet Diag Invest* 1996;8:332-336.

2. Watts JL, Yancey RJ, Jr., Salmon SA, et al. A 4-year survey of antimicrobial susceptibility trends for isolates from cattle with bovine respiratory disease in North America. *J Clin Microbiol* 1994;32:725-31.

3. Shryock TR, White DW, Werner CS, et al. Proposed quality control guidelines for antimicrobial susceptibility tests using tilmicosin. *J Clin Microbiol* 1995;33:331-5.

4. Nouws JF, van Ginneken CA, Ziv G. Age-dependent pharmacokinetics of oxytetracycline in ruminants. *J Vet Pharm Therapeutics* 1983;6:59-66.

5. Burrows GE, Morton RJ, Fales WH. Microdilution antimicrobial susceptibilities of selected gram-negative veterinary bacterial isolates. *J Vet Diagn Invest* 1993;5:541-7.

6. Post KW, Cole NA, Raleigh RH. In vitro antimicrobial susceptibility of Pasteurella haemolytica and Pasteurella multocida recovered from cattle with bovine respiratory disease complex. *J Vet Diag Invest* 1991;3:124-6.

7. Ewert KM. Food Animal Pharmacology III: Baytril. *Proc. 17th ACVIM Annual Convention, Chicago IL*. 1999:259-261.

8. Simmons RD, Varma KJ, Johnson JC. Food Animal Pharmacology II: Nuflor. *Proc. 17th ACVIM Annual Convention Chicago IL*. 1999:256-258.

9. Hannan PC, Windsor GD, de Jong A, et al. Comparative susceptibilities of various animal-pathogenic mycoplasmas to fluoroquinolones. *Antimicrobial Agents and Chemotherapy* 1997;41:2037-40.

10. Ayling RD, Baker SE, Nicholas RA, et al. Comparison of in vitro activity of danofloxacin, florfenicol, oxytetracycline, spectinomycin and tilmicosin against Mycoplasma mycoides subspecies mycoides small colony type. *Vet Record* 2000;146:243-6.

11. Lechtenberg KF, Nagaraja TG, Chengappa MM. Antimicrobial susceptibility of Fusobacterium necrophorum isolated from bovine hepatic abscesses. *Am J Vet Res* 1998;59:44-7.

12. Hirsh DC, Indiveri MC, Jang SS, et al. Changes in prevalence and susceptibility of obligate anaerobes in clinical veterinary practice. *J Am Vet Med Assoc* 1985;186:1086-1089.

13. Samitz EM, Jang SS, Hirsh DC. In vitro susceptibilities of selected obligate anaerobic bacteria obtained from bovine and equine sources to ceftiofur. *J Vet Diag Invest* 1996;8:121-123.

14. Jang SS, Hirsh DC. Broth-disk elution determination of antimicrobial susceptibility of selected anaerobes isolated from animals. *J Vet Diag Invest* 1991;3:82-84.

15. Yoshimura H, Kojima A, Ishimaru M. Antimicrobial susceptibility of Arcanobacterium pyogenes isolated from cattle and pigs. *Zentralblatt fur Veterinarmedizin. Reihe B* 2000;47:139-43.

Useful References Regarding Drug Residues

Food Animal Residue Avoidance Databank Comprehensive Compendium of Food Animal Drugs. Available from Publications, University of Florida, PO Box 110011, Gainsville, FL 32611-0011; or telephone 352-392-9861.

Helpful Tips for Extra-Label Drug Use, in Journal of American Veterinary Medical Association, vol 212, #5, March 1, 1998; #7, April 1, 1998; #9, May 1, 1998; #11, June 1, 1998.

Table 1. Antimicrobial susceptibility data for bacterial pathogens from bovine sources. From Smith BP. Large Animal Internal Medicine 3rd Ed. Vaala WJ and House JK. Disorders and Management of the Neonate, Mosby Co. St Louis

Organism (# isolates)	Antimicrobial	% Susceptible	MIC (μg/ml)		
			50%	90%	Range
Pasteurella hemolytica					
(n = 461)[2]	Ampicillin	60.5	0.25	32	≤ 0.03 - > 64
(n = 89)[5]	Ampicillin		4	>16	0.25 - > 16
(n = 421) [6]	Ampicillin		128	128	
(n = 461)[2]	Ceftiofur	100	≤ 0.03	0.06	≤ 0.03 – 0.13
(n = 50) [1]	Ceftiofur		0.0078	0.015	≤ 0.003 – 0.03
(n = 121)[7]	Enrofloxacin		0.06	0.06	0.03 – 0.12
(n = 461)[2]	Erythromycin	5.4	4	4	≤ 0.03 - > 64
(n = 89) [5]	Erythromycin		2	16	0.5 - > 16
(n = 421) [6]	Erythromycin		4	4	
(n = 243)[8]	Florfenicol			1	
(n = 89)[5]	Gentamicin		1	2	0.25 – 8
(n = 421) [6]	Gentamicin		2	4	
(n = 89)[5]	Kanamycin		4	>16	2 - >16
(n = 89)[5]	Penicillin G		8	>16	4 - >16
(n = 461)[2]	Spectinomycin	83.5	32.0	64	0.5 - > 128
(n = 89) [5]	Spectinomycin		12	>32	8 - > 32
(n = 421) [6]	Spectinomycin		8	16	
(n = 89) [5]	Sulfadimethoxine		200	>400	12.5 - >400
(n = 421) [6]	Sulfadimethoxine		> 256	>256	
(n = 461)[2]	Sulfamethazine	46.2	128.0	> 512	0.5 - > 512
(n = 461)[2]	Tetracycline	57	1.0	32	≤ 0.06 – 64
(n = 89) [5]	Tetracycline		> 16	> 16	0.5 - >16
(n = 421) [6]	Tetracycline		32	64	
(n = 461)[2]	Tilmicosin	69.1	4.0	8	0.06 – 16
(n = 89)[5]	Tylosin		> 16	> 16	8 - > 16
(n = 421) [6]	Tylosin		64	128	
Pasteurella multocida					
(n = 318)[2]	Ampicillin	88.1	0.25	8.0	≤ 0.03 - > 64
(n = 32)[5]	Ampicillin		1	> 16	0.25 - > 16
(n = 158) [6]	Ampicillin		2	4	
(n = 318)[2]	Ceftiofur	100	≤ 0.03	0.06	≤ 0.03 – 0.25
(n = 50) [1]	Ceftiofur		0.0078	0.0078	≤ 0.003 – 0.0078
(n = 108)[7]	Enrofloxacin		0.015	0.03	≤ 0.008 – 0.06
(n = 318)[2]	Erythromycin	16	2.0	8.0	≤ 0.03 - > 64
(n = 32)[5]	Erythromycin		4	>16	1 - > 16
(n = 158) [6]	Erythromycin		4	4	
(n = 183)[8]	Florfenicol			0.5	
(n = 32)[5]	Gentamicin		1	4	0.25->8
(n = 158) [6]	Gentamicin		4	8	
(n = 32)[5]	Kanamycin		4	16	1 - >16
(n = 32)[5]	Penicillin		4	> 16	0.12 - > 16
(n = 158) [6]	Penicillin		2	4	
(n = 318)[2]	Spectinomycin	76.4	32.0	>128.0	0.13 - > 128
(n = 32)[5]	Spectinomycin		12	>32	4 - > 32
(n = 158) [6]	Spectinomycin		8	16	
(n = 32)[5]	Sulfadimethoxine		>400	>400	100->400
(n = 158) [6]	Sulfadimethoxine		>256	>256	
(n = 318)[2]	Sulfamethazine	27.4	128.0	>512.0	0.5 - > 512

Organism (# isolates)	Antimicrobial	% Susceptible	MIC (μg/ml)		
			50%	90%	Range
(n = 318)[2]	Tetracycline	71.1	0.5	16.0	≤ 0.06 – > 32
(n = 32)[5]	Tetracycline		> 16	> 16	1 - > 16
(n = 158) [6]	Tetracycline		2	16	
(n = 318)[2]	Tilmicosin	58.9	4.0	8.0	0.25 – 32
(n = 32)[5]	Tylosin		>16	>16	16->16
(n = 158) [6]	Tylosin		32	64	
Haemophillus somnus					
(n = 109)[2]	Ampicillin	90.1	0.06	1.0	≤ 0.03 - > 64.0
(n = 109)[2]	Ceftiofur	100	≤ 0.03	0.06	≤ 0.03 – 0.13
(n = 59) [1]	Ceftiofur		≤ 0.0019	≤ 0.0019	≤ 0.0019
(n = 104)[7]	Enrofloxacin		0.015	0.03	≤ 0.008 – 0.5
(n = 109)[2]	Erythromycin	88.9	0.25	2.0	≤ 0.03 - > 32.0
(n = 34) [8]	Florfenicol			0.5	
(n = 109)[2]	Spectinomycin	87.1	8.0	32.0	≤ 0.13 - > 128.0
(n = 109)[2]	Sulfamethazine	35.8	256.0	>512.0	≤ 0.5 - > 512.0
(n = 109)[2]	Tetracycline	98.2	0.5	1.0	≤ 0.03 – 32.0
(n = 109)[2]	Tilmicosin	90.4	2.0	4.0	≤ 0.03 – 32.0
Mycoplasma bovis					
(n = 20)[9]	Enrofloxacin		0.1	0.25	0.05 - 1
(n = 100) [8]	Florfenicol			0.5	
(n = 20)[9]	Oxytetracycline		1.0	2.5	0.1 - 10
(n = 20)[9]	Tylosin		1.0	5	0.025 – > 100
Mycoplasma mycoides **subspecies mycoides small colony type**					
(n = 20)[10]	Florfenicol		1.0	2	0.25 – 8
(n = 20)[10]	Oxytetracycline		0.5	1	0.125 – 4
(n = 20)[10]	Spectinomycin		8	16	4 - > 128
(n = 20)[10]	Tilmicosin		0.015	0.03	< 0.008 – 0.25
Fusobacterium necrophorum					
(n = 21)[11]	Ampicillin		1.6	2.3	
(n = 68)[12]	Ampicillin	100			
(n = 17)[13]	Ceftiofur	100	≤ 0.062	≤ 0.062	≤ 0.062
(n = 68)[12]	Chloramphenicol	100			
(n = 21)[11]	Erythromycin		3.1	6.3	
(n = 12)[8]	Florfenicol			0.25	
(n = 21)[11]	Oxytetracycline		0.08	0.2	
(n = 365)[14]	Penicillin	96			
(n = 68)[12]	Penicillin	100			
(n = 21)[11]	Penicillin G		0.1	1.9	
(n = 365)[14]	Tetracycline	99			
(n = 68)[12]	Tetracycline	100			
(n = 21)[11]	Tylosin		3.1	6.3	
Clostridium perfringens					
(n = 67)[14]	Chloramphenicol	99			
(n = 67)[14]	Penicillin G	93			
(n = 67)[14]	Tetracycline	70			

Organism (# isolates)	Antimicrobial	% Susceptible	MIC (μg/ml)		
			50%	90%	Range
Other Clostridia					
(n = 109)[14]	Chloramphenicol	99			
(n = 109)[14]	Penicillin	90			
(n = 109)[14]	Tetracycline	77			
Fusobacterium necrophorum Subspecies fundiliforme					
(n = 16)[11]	Ampicillin		1.3	2.7	
(n = 16)[11]	Erythromycin		3.1	6.3	
(n = 16)[11]	Oxytetracycline		0.2	4.1	
(n = 16)[11]	Penicillin G		0.2	0.8	
(n = 16)[11]	Tylosin		4.7	21.3	
Bacteroides melaninogenicus					
(n = 11) [8]	Florfenicol			0.25	
Bacteroides fragilis					
(n = 29)[13]	Ceftiofur	69	1	16	≤ 0.0625 - ≥ 16
(n = 192)[14]	Chloramphenicol	99			
(n = 192)[14]	Penicillin G	15.9			
(n = 192)[14]	Tetracycline	77.3			
Non-*Bacteroides fragilis*					
(n = 12)[13]	Ceftiofur	58	2	16	0.125- ≥ 16
(n = 114)[14]	Chloramphenicol	100			
(n = 114)[14]	Penicillin G	89			
(n = 114)[14]	Tetracycline	96			
Peptostreptococcus anaerobius					
(n = 57)[12]	Ampicillin	100			
(n = 12)[13]	Ceftiofur	100	0.25	2	0.125 – 2
(n = 57)[12]	Chloramphenicol	100			
(n = 193)[14]	Chloramphenicol	100			
(n = 57)[12]	Penicillin	97			
(n = 193)[14]	Penicillin	96			
(n = 57)[12]	Tetracycline	100			
(n = 193)[14]	Tetracycline	100			
Escherichia coli					
(n = 24)[5]	Ampicillin		4	> 16	1 - > 16
(n = 40)[1]	Ceftiofur		0.25	0.5	0.13 – 1.0
(n = 24)[5]	Gentamicin		1	2	0.5 - > 8
(n = 24)[5]	Oxytetracycline		16	> 16	1 - > 16
(n = 24)[5]	Spectinomycin		16	> 32	8 - > 32
(n = 24)[5]	Sulfachlorpyridazine		200	> 400	12.5 - > 400
Arcanobacterium pyogenes					
(n = 42)[15]	Ampicillin		0.025	0.05	≤0.0125-0.05
(n = 42)[15]	Benzylpenicillin		≤ 0.0125	0.25	≤0.0125-0.05
(n = 42)[15]	Ceftiofur		0.78	1.56	0.39 – 1.56
(n = 42)[15]	Chloramphenicol		1.56	1.56	0.39-1.56

Organism (# isolates)	Antimicrobial	% Susceptible	MIC (μg/ml)		
			50%	90%	Range
(n = 42)[15]	Erythromycin		0.025	0.025	≤0.0125-0.025
(n = 42)[15]	Florfenicol		1.56	1.56	0.78-1.56
(n = 42)[15]	Gentamicin		1.56	1.56	0.2 - > 100
(n = 42)[15]	Oxytetracycline		6.25	25	0.2 – 25
(n = 42)[15]	Tilmicosin		0.05	0.05	≤0.0125-0.05
Salmonella spp.					
(n = 9)[5]	Ampicillin		> 16	....	2 - >16
(n = 48)[5]	Ampicillin		16	> 16	0.5 - > 16
(n = 28)[1]	Ceftiofur		1.0	1.0	0.6 – 2.0
(n = 9)[5]	Gentamicin		0.5	....	0.5 – 4
(n = 48)[5]	Gentamicin		0.5	2	0.25 – 8
(n = 9)[5]	Oxytetracycline		2	....	1 - > 16
(n = 48)[5]	Oxytetracycline		> 16	> 16	1 - > 16
(n = 9)[5]	Spectinomycin		12	....	8 - > 32
(n = 48)[5]	Spectinomycin		32	> 32	12 – 32
(n = 9)[5]	Sulfachlorpyridazine		150	....	50 - > 400
(n = 48)[5]	Sulfachlorpyridazine		400	> 400	12.5 - > 400
(n = 48)[5]	Sulfamethoxine		> 400	> 400	12.5 - > 400