# Rational Dosing of Antimicrobial Agents for Bovine Respiratory Disease: The Use of Plasma Versus Tissue Concentrations in Predicting Efficacy

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## ABSTRACT

Factors associated with identifying an appropriate dose and dosing regimen for the treatment of bovine respiratory disease include the activity of a given drug against common respiratory bacterial pathogens and the ability of the drug to gain access to the site of infection. Although randomized prospective field trials are the best means of determining the relative efficacy of antimicrobial agents for the treatment of bovine respiratory disease, it is impossible to conduct such trials for every possible dose and dosing interval. Based on limited data in cattle, traditional pharmacokinetic/pharmacodynamic models focused strictly on plasma concentrations appear to be adequate surrogate markers of efficacy for the treatment of susceptible extracellular bacterial respiratory pathogens with β-lactams and fluoroquinolones. However, plasma concentrations of macrolides and azalides, such as gamithromycin, tilmicosin, and tulathromycin in cattle, are considerably lower than their respective minimum inhibitory concentrations (MIC) against the pathogens for which they are approved. Nonetheless, multiple studies have demonstrated the efficacy of these drugs in the treatment of bovine respiratory disease, indicating that drug concentrations at the site of infection provide more clinically relevant information than simple reliance on plasma concentrations. Measurement of drug concentration in lung tissue homogenates does not distinguish between free drug available for bacterial killing and drug bound to various extra- or intracellular biological material. For macrolides and azalides, recent findings suggest that measurement of drug concentrations in pulmonary epithelial lining fluid is a better predictor of efficacy than either lung or plasma concentrations for the treatment of pulmonary infections caused by extracellular pathogens.

## INTRODUCTION

Bovine respiratory disease is the most common cause of morbidity and mortality in beef cattle[1] and the second most common cause of slaughter condemnation in dairy calves.[2] The bacterial pathogens most frequently recovered from cattle with respiratory disease include *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni,* and *Mycoplasma bovis.* Administration of an effective antimicrobial agent is one of the

most effective methods of preventing respiratory disease and the only effective method of treatment. Several antimicrobial agents are approved for the treatment or control of respiratory disease in cattle.

The plasma pharmacokinetics of most of these antimicrobial agents have been studied extensively in cattle. Optimal dosing of antimicrobial agents is dependent not only on the pharmacokinetics, but also on the pharmacodynamics of the drug. Pharmacodynamic properties of an antimicrobial drug address the relationship between drug concentration and antimicrobial activity. Pharmacokinetic/pharmacodynamic studies play an important role in drug development and drug evaluation. At the moment, the most widely used pharmacokinetic/pharmacodynamic approaches for antimicrobial agents rely on plasma concentrations as the pharmacokinetic input and minimum inhibitory concentration (MIC) as the pharmacodynamic input value. For many drugs, microorganisms, and disease processes, measurement of plasma concentration is an adequate surrogate marker of efficacy. However, for some drugs and disease states, concentration at the site of infection is more relevant and directly related to the desired therapeutic effect, which is bacterial eradication. This manuscript reviews current information regarding pulmonary disposition of antimicrobial agents with special emphasis on bovine respiratory disease.

**Minimum Inhibitory Concentration and Interpretation of *in vitro* Susceptibility Data**

*In vitro* bacterial susceptibility is determined by disk diffusion, concentration-gradient agar diffusion, or broth dilution methodologies. Disk diffusion provides qualitative susceptibility data, whereas broth-dilution methods and the concentration/gradient tests generate a minimum inhibitory concentration (MIC) expressed quantitatively in µg/mL. The MIC is the lowest concentration of an antimicrobial agent, which inhibits the growth of the target bacteria. Both the disk diffusion and methods generating MIC values assess inhibition of bacterial growth rather than killing of the pathogen as the endpoint.

Susceptibility designations are determined by comparing the microorganism's MIC (or zone of inhibition if the disk diffusion method is used) to clinical breakpoints established by the Clinical Laboratory Standards Institute (CLSI). Breakpoints or interpretive criteria are the concentration (or zone diameter) above and below which specific bacterial isolates are categorized as susceptible, intermediate, or resistant. Clinical breakpoints are determined by:

- The range of *in vitro* MICs of an antimicrobial for representative populations of specific bacterial pathogens
- Pharmacokinetic-pharmacodynamic parameters established on the basis of the relationship between physiologic drug concentrations and a microbial susceptibility parameter, generally the minimum inhibitory concentration relationship (MICR).
- Results of clinical trials in the target species versus MIC of the isolates recovered from diseased animals, the ultimate standard of efficacy.[3]

Results of *in vitro* susceptibility tests are presented to the clinician by designating the pathogen as susceptible, intermediate, or resistant. The CLSI defines the three susceptibility designations as follows:

- Susceptible – An infection caused by the specific isolate may be successfully treated with the recommended dosage regimen of an antimicrobial agent approved for that specific disease process and infecting microorganism
- Intermediate – An infection by the isolate can be treated at body sites where drugs are physiologically concentrated or when a high dosage can be used; also indicates a "buffer zone" that should prevent minor technical factors from causing major discrepancies in interpretations

• Resistant – An infecting isolate is not inhibited by typically achievable concentrations of a given drug with normal dosage regimens and/or clinical efficacy has not been reliable in treatment studies.[3]

**Interpreting MIC values**

Clinical breakpoints are relevant only for a specific bacteria, specific drug, and specific organ system infected. When species-specific breakpoints are used, pathogens with a MIC below an antimicrobial's susceptibility breakpoint have a higher probability for treatment success, and organisms with an MIC above the resistance breakpoint have a lower probability of treatment success. However, there is no evidence that efficacy increases the further the MIC is below the breakpoint. Conversely, it should be noted that a relatively high MIC in itself is not necessarily an indicator of resistance. Some resistance breakpoints have been set at ≥ 64 µg/mL or higher (eg, the resistance breakpoint for *Mannheimia haemolytica* against tulathromycin for bovine respiratory disease is ≥ 64 µg/mL).

For the practitioner, an important limitation in interpreting the results of *in vitro* susceptibility data is that breakpoints for only a small number of drugs have been established for bovine respiratory disease in cattle (Table 1). For all other antimicrobials, the breakpoints have been adapted from humans or other domestic animal species. For these antimicrobials, a result indicating susceptibility is unquestionably preferable to one indicating resistance. However, there are no data correlating the results to clinical efficacy and there is no guarantee that the breakpoint in one species is valid for a given pathogen or site of infection in a different species.

For example, the CLSI breakpoint for susceptibility to doxycycline of ≤4 µg/mL is based on human pharmacokinetic and clinical efficacy data. Administration of oral doxycycline to an adult horse at the recommended dosage of 10 mg/kg results in peak

***Table 1****.Minimal concentration inhibiting at least 90% of common bovine respiratory tract pathogens (MIC90), peak plasma concentrations (Cmax) at recommended dosages, and CLSI breakpoints for interpretation of in vitro susceptibility testing.*

| Drug | $MIC_{90}$ (µg/mL) | | | Plasma | Breakpoint (µg/mL) | | |
|---|---|---|---|---|---|---|---|
| | *Mannheimia haemolytica* | *Pasteurella multocida* | *Histophilus somni* | $C_{max}$ (µg/mL) | S | I | R |
| Ceftiofur | 0.015 | 0.004 | 0.004 | 1.25 | ≤2 | 4 | ≥8 |
| Danofloxacin | 0.06 | 0.015 | 0.06 | 1.69 | ≤0.25 | | |
| Enrofloxacin | 0.06 | 0.05 | 0.02 | 1.20[c] | ≤0.25 | 0.5-1 | ≥2 |
| Florfenicol | 1 | 0.5 | 0.5 | 3.07 | ≤2 | 4 | ≥8 |
| Oxytetracycline[a] | 32 | 1 | 2 | 5.7[d] | ≤2 | 4 | ≥8 |
| Tilmicosin | 4 | 8 | 8 | 0.55/1.06[e] | ≤8 | 16 | ≥32 |
| Gamithromycin[b] | 1 | 1 | 0.5 | 0.43 | | | |
| Tulathromycin | 2 | 1 | 4 | 0.28 | ≤16 | 32 | ≥64 |

*S = susceptible; I = intermediate; R = resistant*

*[a] Breakpoint derived from pharmacokinetic and pharmacodynamics data. Results of clinical trials in the target species versus MIC of the isolates recovered are not available.*

*[b] Breakpoint not yet established.*

*[c] Sum of enrofloxacin and its active metabolite ciprofloxacin after administration of a dose of 8 mg/kg SC.*

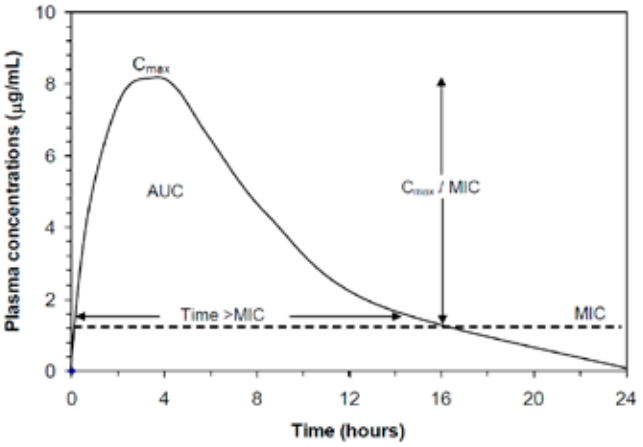
*[d] Dose of 20 mg/kg of a 200 mg/mL formulation IM; Cmax will vary according to the dose and formulation*

*[e] Doses of 10/20mg/kg SC, respectively.*

serum, synovial fluid, and peritoneal fluid concentrations of approximately 0.5 µg/mL.[4] A pathogen with a MIC of 4 µg/mL, isolated from the synovial fluid of a horse would be reported as susceptible even though such concentrations are far from achievable in horses. Based on pharmacokinetic/pharmacodynamic data in horses, a breakpoint of ≤0.25 µg/mL would be more appropriate as a susceptibility standard for doxycycline.[4,5] Thus, the lack of species- and disease-specific interpretive criteria is one factor that may explain discrepancies between *in vitro* susceptibility and clinical response.

By itself, *in vitro* susceptibility of a specific pathogen does not guarantee clinical outcome. Other factors, such as the host animal's age, immune status, severity of disease at the time of initiation of therapy, distribution of the drug at the site of infection, microenvironment at the site of infection, and presence of mixed infections can all contribute to individual clinical response.

***Figure 1*** *– Plasma concentration (vertical axis) versus time (horizontal axis) profile of an antimicrobial agent. The MIC of a hypothetical pathogen is indicated by the horizontal broken line. Efficacy of concentration-dependent antimicrobials is best predicted by the Cmax-to-MIC ratio. Efficacy of time-dependent antimicrobials is associated with maintaining a plasma concentration >MIC for the majority of the dosing interval (T > MIC). For other antimicrobial agents the ratio of AUC-to-MIC best predicts in vivo efficacy.*




**Pharmacokinetic/Pharmacodynamic Data to Determine the Optimal Dosage Regimen**

Determination of the appropriate dose and dosing intervals of an antimicrobial agent requires knowledge of and integration of its pharmacokinetics and pharmacodynamic properties. The pharmacokinetic properties of a drug describe its movement and disposition within the body and include drug absorption, distribution, metabolism, and excretion. Pharmacodynamic properties address the relationship between drug concentration and antimicrobial activity. Drug pharmacokinetic features such as maximum plasma concentrations, the time concentrations remain above a target concentration and area under the plasma concentration-time curve (AUC), when integrated with MIC values, can predict the probability of bacterial eradication and clinical success.[6-8] These pharmacokinetic and pharmacodynamic relationships may also play an important role in preventing the genetic selection and spread of resistant strains.[9] Pharmacodynamic studies can be done in vitro, in vivo in laboratory animal models of infection, or using experimental challenge models or natural disease in the species of interest.

The most significant parameter predicting the efficacy of β-lactams, trimethoprim-sulfonamide combinations, and most bacteriostatic agents such as macrolides and tetracyclines is the length of time that plasma concentrations exceed the MIC of the pathogen (T > MIC; Figure 1).[6] Increasing the concentration of the drug several-fold above the MIC does not significantly increase the rate of micro-

***Table 2.*** *Classification of antimicrobial agents according to their pharmacodynamics properties.*

| Time (T > MIC) | Concentration ($C_{max}$ / MIC) | Both (AUC / MIC) |
|---|---|---|
| β-lactams | aminoglycosides[b] | azalides |
| tetracyclines | fluoroquinolones[c] | fluoroquinolones[c] |
| macrolides | metronidazole[a] | glycopeptides[a] |
| lincosamides | | |
| chloramphenicol[a] | | |

[a] *Illegal to use in food-producing animals*
[b] *Voluntary ban in food-producing animals*
[c] *Off-label use illegal in food-producing animals*

bial killing. Rather, it is the length of time that bacteria are exposed to concentrations of these drugs above the MIC that dictates killing effect. Therefore, optimal dosing of such antimicrobial agents typically involves frequent administration. However, how much above the MIC the plasma concentration should be maintained and for what percentage of the dosing interval therapeutic concentrations should be maintained, are sources of debate and likely vary depending on a given drug and specific microorganisms.

Other antimicrobial agents, such as the aminoglycosides, fluoroquinolones, and metronidazole, exert concentration-dependent killing characteristics.[6-8] Their rate of killing increases as the plasma drug concentration increases above the MIC for the pathogen. As a result, it is not necessary or even beneficial to maintain drug levels above the MIC between doses. Thus, optimal dosing of concentration-dependent drugs involves administration of high doses with long dosing intervals [8-10]. Some drugs exert characteristics of both time and concentration dependent activity. The best predictor of efficacy for these drugs is the 24-hour plasma AUC-to-MIC ratio where AUC is the AUC calculated over a 24-hour period following treatments. Glycopeptides, rifampin and, to some extent, fluoroquinolones, fall into this category (Table 2).[8]

Very few studies have explored the pharmacokinetic/pharmacodynamics of antimicrobial agents as it relates specifically to bovine respiratory disease. In two studies, the plasma AUC/MIC ratio was the best predictor of the efficacy of danofloxacin against *Mannheimia haemolytica* in models of infected subcutaneous tissue cages in calves.[10,11] Using a similar model, T > MIC was the best predictor of the efficacy for penicillin against *M. haemolytica*.[12] In calves infected intrabronchially with *M. haemolytica*, a single bolus of danofloxacin was significantly more effective than the same dose administered by continuous infusion, indicating that danofloxacin may exhibit concentration dependent activity.[13]

**Limitations of Current Pharmacokinetic/ Pharmacodynamic Models Relative to Bovine Respiratory Disease**

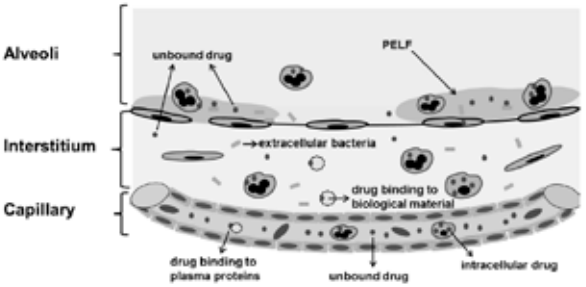
As explained previously, current pharmacokinetic/pharmacodynamic approaches for antimicrobial agents, such as T > MIC, Cmax/MIC, and $AUC^{24}$/MIC, rely exclusively on plasma concentration as the pharmacokinetic input. However, most infections occur in tissues rather than in the blood stream. For an antimicrobial to affect target pathogens and provide clinical efficacy against disease, it is axiomatic that antimicrobial agents reach the primary site of infection. While drug concentration in plasma is clearly a driving force for penetration to the site of infection, the actual drug-concentration time profile at a peripheral site may be quite different from that of plasma. The rate and extent of penetration of a drug

into most sites outside the vascular space are also determined by the extent of plasma protein and tissue binding, the drug's molecular charge and size, lipid solubility, and by blood flow at the site of infection.[14] Understanding the relationship between plasma concentrations and concentrations at the site of the antimicrobial activity, therefore, may be complex and not easily measured.

Nevertheless, the use of plasma concentration has been shown to be a good surrogate marker of clinical efficacy for most drug types and for many tissue infections. As only protein-free drug is microbiologically active, plasma concentrations should be corrected for protein binding to only represent the unbound fraction of the drug.[15] For most drug classes, concentrations in plasma are sufficiently high that enough unbound drug is driven into the extracellular fluid to eradicate the pathogen, especially if the MIC against the target pathogen is low. However, for intracellular pathogens and when drug diffusion to the site of infection is further restricted by tight junctions between cells (central nervous system, prostate, and eye), plasma concentration are of less value in predicting outcomes. The same is true for drugs which preferentially accumulate within cells such as macrolides and azalides for which plasma concentrations do not predict clinical efficacy.

Plasma concentrations of macrolides or azalides such as gamithromycin, tilmicosin, and tulathromycin in cattle are in general considerably lower than their respective MIC against the pathogens for which they are approved (Table 1).[16-19] Nonetheless, multiple studies have demonstrated the efficacy of these drugs in the treatment of bovine respiratory disease[20,21] indicating that drug concentrations at the site of infection provide more clinically relevant information than simple reliance on plasma concentrations.

***Figure 2**. Schematic representation of antimicrobial drug diffusion across the blood-alveolar barrier. The capillary wall and bronchial wall are separated by a fluid filled interstitial space. PELF represents the secretions present on the interior surface of the alveolar wall and smaller bronchi. The antimicrobial agents measured in PELF represent the portion of the drug which diffuses across the capillary wall, the interstitial fluid, and the alveolar epithelial cells. Cells can also carry antimicrobial agents to the PELF. Assessment of drug concentrations in tissue homogenates would measure total drug concentrations including drug bound to plasma protein and extracellular biological materials as well as intracellular drug. However, only unbound drug in the interstitium or PELF is available to kill or inhibit extracellular bacteria.*




## Concentrations of Antimicrobial Agents in the Lungs

For pulmonary infections caused by extracellular bacteria such as *M. haemolytica* and *P. multocida*, concentrations of antimicrobials in the extracellular or interstitial space within the lungs would provide additional relevant information. Several methods have been used to estimate the distribution of antimicrobial agents in the respiratory tract. These methods include measurement of drug concentration in tissue homogenates, microdialysis, nasal secretions, bronchial secretions, and pulmonary epithelial lining fluid (PELF). Measurement of antimicrobial drug concentration in bronchoalveolar cells is also relevant to the treatment of pulmonary infections caused by intracellular pathogens.

### Tissue Homogenates

Measurement of antimicrobial drug concentration in homogenized lung tissue has been, until recently, the most widely used method to assess pulmonary concentration of antimicrobial agents in cattle. This approach does not take into account the fact that lungs are made of distinct compartments such as interstitial fluid, bronchial secretions, and various types of cells (Figure 2). The homogenization procedure disrupts cell membranes and produces a suspension containing both intracellular and extracellular fluid and particles.[22,23] If a drug is distributed mainly in the extracellular environment such as β–lactams and aminoglycosides, tissue homogenates dilute the drug by releasing intracellular content resulting, in underestimation of drug concentration in the extracellular space. This phenomenon has been documented in cattle after administration of ceftiofur crystalline free acid. Drug concentrations in lung tissue were consistently lower than concurrent concentrations in *M. haemolytica*-infected or non-infected tissue chamber fluid as an indicator of extracellular fluid concentrations.[24]

Conversely, for drugs reaching high intracellular concentrations such as macrolides, tissue homogenates will overestimate extracellular concentrations. An example of how overall drug concentration in tissue homogenates overestimate concentrations in PELF, but underestimates intracellular concentrations are presented in Figures 3A and 3B. The concentrations of gamithromycin and tulathromycin in lung tissue homogenate of cattle considerably overestimate PELF concentrations and underestimate intracellular concentrations in BAL cells.[25,26] In addition, only free or unbound drug is available for antimicrobial activity at the site of infection. Measurement of drug concentration in tissue homogenate does not distinguish between free drug and drug bound to various extra- or intracellular biological material.[23] As a result, the use of drug concentration from tissue homogenates to predict efficacy is misleading and this approach is not recommended.[27] Measurement of drug concentration in mucosal biopsy samples is likely subject to the same limitations. In one cattle study, concentrations of danofloxacin and enrofloxacin in bronchial mucosa, although slightly lower than in whole lung tissue, were considerably higher than those measured concurrently in respiratory secretions.[28]

***Figure 3.A****- Mean ± SD gamithromycin concentrations in serum, BAL cells, PELF (*μg/mL*), and lung tissue (*μg/g*) of cattle following a single SC dose of gamithromycin (6 mg/kg of body weight). The dotted line represents the MIC of an isolate of M. haemolytica. From: Am. J. Vet. Res. 2011;72:328 with permission.*[26]

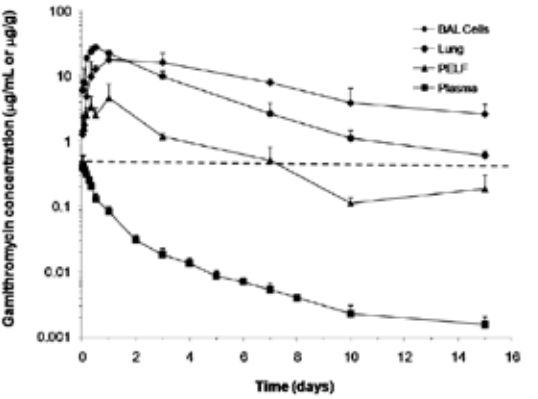
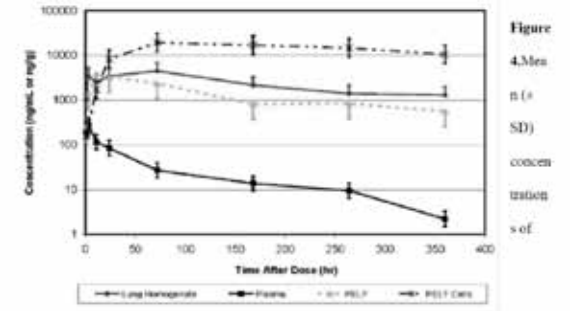



***Figure 3.B****- Least squared mean tulathromycin concentrations and 95% confidence intervals for the 36 h following a single 2.5 mg/kg IM dose to cattle. From Intern. J. Appl. Res. Vet.Med. 2010;8:134 with permission.*[25]



**Microdialysis**

Microdialysis is a relatively recent technique that allows continuous monitoring of unbound or free antimicrobial agents directly in the extracellular fluid of a tissue of interest. Microdialysis mimics the function of a capillary by perfusing a small dialysis probe implanted into lung tissue with a physiological solution.[29] The dialysate reflects the composition of the extracellular fluid over time because of diffusion across the semipermeable membrane. Because large molecules such as proteins are excluded from the dialysate, only free-drug concentrations are measured. The technique has been used to investigate the pulmonary distribution of multiple antimicrobial agents in laboratory animals and in humans.[23,29] The main disadvantages of microdialysis are the need for thoracotomy to insert the probe and the fact that the technique is not suitable for sampling lipophilic drugs and for simultaneously measuring intracellular concentrations of the drug being investigated.[29] To the authors' knowledge, microdialysis has not been used to evaluate pulmonary distribution of antimicrobial agents in cattle.

**Nasal and Bronchial Secretions**

Many methods have been used to measure antimicrobial concentrations in respiratory tract secretions of cattle. These methods include measurement of drug concentrations in nasal, tracheal, or bronchial secretions collected using an absorbent tampon[28,30] and in tracheobronchial fluid collected after injection and immediate aspiration of saline.[31] Some investigators separate the cells from the fluid, whereas others measure drug concentrations in cell-containing secretions.

Measurement of drug concentration in cell-containing secretions releases intracellular drug and has some of the same limitations as those explained previously for tissue homogenates. Measurement of drug concentration in tracheobronchial fluid obtained after injection and aspiration of saline considerably underestimate drug concentrations unless dilution is accounted for. These differences in methodology complicate data interpretation across studies. Nasal secretions, although easily obtained, are less representative of the target site of infection, which is the lower respiratory track. In one cattle study, measurement of danofloxacin in nasal secretions considerably overestimated concentrations in bronchial secretions.[32] Similar comparisons between nasal and bronchial secretions are not available for other drugs approved for use in cattle. Similarly, comparison between concurrent drug concentrations in tracheobronchial secretions obtained using the methods listed above and concentrations measured in PELF obtained by bronchoalveolar lavage (BAL) are not available in cattle.

**PELF**

Measurement of drug concentration in pulmonary epithelial lining fluid (PELF) collected by bronchoalveolar lavage (BAL) is the most widely used approach and it is considered one of the better methods to estimate antimicrobial concentrations at the site of infection for antimicrobial agents intended to treat lower respiratory tract infections caused by extracellular bacterial pathogens in humans.[33-35] The technique has also been used extensively to study the pulmonary distribution of various antimicrobial agents in horses.[36-42] More recently, the technique has been adapted to the study of antimicrobial drug distribution in the lungs of cattle.[25,26]

PELF represents the secretions present on the interior surface of the alveolar wall and smaller bronchi. The capillary wall and bronchial wall are separated by a fluid filled interstitial space (Figure 2). The antimicrobial measured in PELF represents the portion of the drug which diffuses across the capillary wall, the interstitial fluid, and the alveolar epithelial cells.

Most unbound drugs will readily cross the fenestrated pulmonary capillaries. However, to reach the PELF, the antimicrobial agent must also pass through the tight junction of the alveolar epithelial cells. Several factors will impact entry of antimicrobial agents into the PELF. Because only the

free fraction of the drug will equilibrate between plasma and interstitial fluid, the degree of protein-binding influences the drug concentrations in interstitial fluid and PELF considerably. In addition, the extent of drug passage through the alveolar epithelium depends on the lipophilicity and diffusibility of the drug, similar to drug entry through the blood brain barrier.

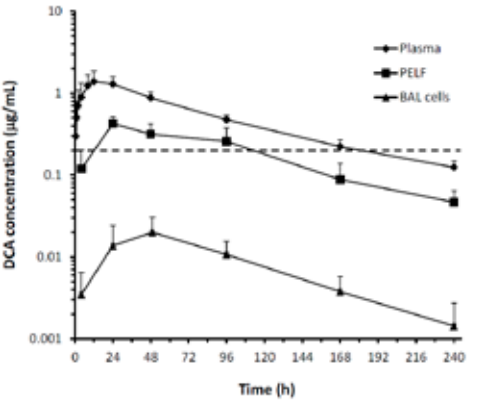
**Methodology**

The sample used for measurement of antimicrobial drug concentration is BAL fluid obtained through an endoscope or a commercially available BAL tube wedged into a bronchus. Aliquots of saline (50-100 mL) are injected and immediately aspirated for a total volume of 200-250 mL. The resulting fluid is centrifuged immediately. The cell pellet can be used to measure intracellular concentrations (see below) and the fluid is used to measure drug concentration in PELF. The volume of PELF sampled by BAL and the amount of drug contained in the fluid must be corrected for the dilution with saline that occurred during the BAL procedure. This correction is usually performed using the urea dilution method.[43]

Urea is used as an endogenous marker of PELF because it is a small and relatively nonpolar molecule that travels freely across membranes. The assumption is that urea concentration in PELF should be the same as in plasma because of complete diffusion. The volume of PELF ($V_{PELF}$) is adjusted for the dilution with saline using the following equation: $V_{PELF} = V_{BAL} \times (Urea_{BAL}/Urea_{PLASMA})$, where $V_{BAL}$ is the volume of recovered BAL fluid and $Urea_{BAL}$ and $Urea_{PLASMA}$ are the concentrations of urea in BAL fluid and in plasma, respectively. The concentration of drug in PELF ($DRUG_{PELF}$) is then derived from the following relationship: $DRUG_{PELF} = DRUG_{BAL} \times (V_{BAL}/V_{PELF})$, where $DRUG_{BAL}$ is the measured concentration of drug in BAL fluid.

Measurement of antimicrobial drug concentrations in PELF collected by BAL is relatively easy, minimally invasive, and allows for repeated sampling of the same animals over time. However, the technique has some limitations. For antimicrobial drugs that achieve high intracellular concentrations, release of intracellular drug to the BAL fluid may occur if there is cell lysis prior to or during centrifugation, which would result in artificially increased drug concentrations in PELF. In addition, estimation of PELF volume by use of the urea dilution method may result in falsely increased BAL fluid urea concentration by diffusion of urea from the interstitium and blood if BAL fluid dwell-time is prolonged.[44] The urea concentration in BAL fluid could also be increased if there is blood contamination during the BAL procedure. Overestimation of urea concentrations in BAL fluid would falsely increase the volume of PELF, which would in turn result in an underestimation of drug concentrations in PELF.[44] These limitations can be minimized by using a rapid dwell-time and by separating the cell pellet as soon as sample collection has been completed.

***Figure 4.*** *Mean (± SD) concentrations of desfuroylceftiofur and related metabolites in plasma, BAL cells, and PELF of 6 healthy foals after a single IM dose of ceftiofur crystalline free acid (6.6 mg/kg of body weight). The dotted horizontal line represents the MIC[90] of S. equi subsp. zooepidemicus (0.2 µg/mL).From Credille et al., 2011.[59]*




**Interpretation of Drug Concentrations in PELF and Bronchial Secretions**

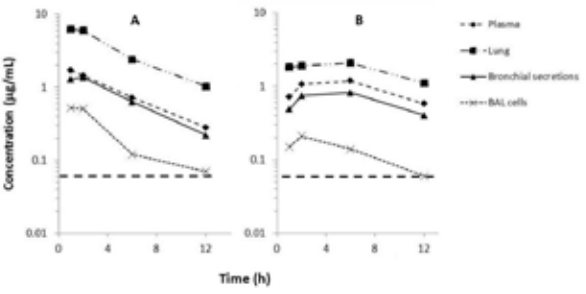
For many antimicrobial agents such as β-lactams and fluoroquinolones, concentrations in PELF or bronchial secretions are lower than concurrent plasma concentrations, but the drug follows a similar pattern of distribution at both sites. For example, administration of ceftiofur crystalline free acid to weanling foals results in PELF drug concentrations that are slightly lower than concurrent plasma concentrations (Figure 4).[57] Nevertheless, concentrations at both sites are above the MIC of the drug for *Streptococcus equi* subspecies *zooepidemicus*. Similarly, concentrations of danofloxacin and enrofloxacin in bronchial secretions of calves challenged with *M. haemolytica* are similar to concurrent plasma concentrations and well above the MIC of the drug against the pathogen (Figure 5).[28]

In these examples, traditional pharmacokinetic/pharmacodynamic models based on plasma concentrations would be expected to be good surrogate markers of efficacy for the treatment of susceptible extracellular respiratory pathogens. For other drug classes such as macrolides and azalides, simple reliance on plasma concentrations would be extremely misleading. For example, plasma concentrations of gamithromycin and tulathromycin are considerably below their respective MIC against *M. haemolytica* (Figure 3).[25,26,43] Yet, both drugs have been shown to be effective for the control and prevention of bovine respiratory disease.[20,21,25,26,43] Concentrations of both drugs in PELF are considerably above plasma concentrations and exceed the MIC of the most common pathogens causing bovine respiratory disease. Although concentrations of tilmicosin in PELF of cattle have not been measured, a similar phenomenon is likely to occur because plasma concentrations of the drug in cattle are considerably below the MIC against *M. haemolytica* and *P. multocida* despite documented clinical efficacy.[45]

The high ratios of PELF to plasma concentrations achieved with macrolides and azalides considerably exceed that which would be predicted solely on the basis of their high lipophilicity and good penetration across the alveolar epithelium.[34] Macrolides and azalides are potent weak bases that become ion-trapped within acidic intracellular compartments, such as lysosomes and phagosomes. A beneficial consequence of macrolide accumulation within cells is increased activity against intracellular pathogens (see Bronchoalveolar Cells section below).[46,47] In addition, phagocytes have been shown to act as a vehicle for the delivery of macrolides to the site of infection.[48,49] Therefore, the higher than predicted PELF concentrations measured are likely the results of drug delivery to the respiratory tract by white blood cells in vivo.

The preferential activity of macrolides against extracellular pathogens in the lung has been demonstrated conclusively in mice infected with *S. pneumoniae* isolates with efflux-mediated macrolide resistance where consistent bacterial kill is observed in a lung infection model while no drug effect is seen in a thigh

***Figure 5****.A- Mean concentrations of danofloxacinin plasma, lung tissue, bronchial secretions, and BAL cells of calves after administration of a single subcutaneous dose of 6 mg/kg of body weight. B- Mean concentrations of the sum of enrofloxacin and ciprofloxacin in calves after a single subcutaneous dose of enrofloxacin (8 mg/kg of body weight). n = 6 at each time point. The horizontal interrupted lines represent the MIC90 for M. haemolytica. Adapted from Terhune et al., 2005.[28]*



infection model.[50] These differences in bacterial activity between sites are explained by the much higher drug concentrations reached in PELF than in plasma.[50] The results of the aforementioned study demonstrate that drug concentration in PELF for macrolides and azalides is a better predictor of outcome than plasma concentrations for respiratory tract infection.

In a recent study, groups of 8-10 calves were infected with *M. haemolytica* 1, 5 and 10 days after a single injection of gamithromycin (6 mg/kg, SC).[51] A group of calves was used as untreated controls.[51] The proportion of calves with complete bacterial eradication was significantly greater in calves infected 1 and 5 days after administration of gamithromycin (times at which drug concentration in PELF would be expected to be above the MIC of *M. haemolytica*) compared to calves infected 10 days after treatment (time at which only lung and BAL cells but not PELF concentrations would be expected above the MIC of *M. haemolytica* based on Figure 3A). These findings support the notion that drug concentrations in PELF is a better predictor of bacterial killing than either lung tissue homogenate or plasma concentrations in pulmonary infections caused by extracellular pathogens such as *M. haemolytica*. Clinical scores of all treated groups were statistically significantly improved when compared to the control group, even in the calves infected 10 days after treatment, but there was no difference seen in lung lesion scores.[49]

**Bronchoalveolar Cells**

Although pharmacodynamic variables based on plasma or PELF concentrations may be used to estimate the optimal dosage regimen of antimicrobial drugs against extracellular pathogens, they cannot be applied to the treatment of facultative intracellular pathogens. For example, the high and sustained intracellular concentrations achieved by azithromycin explain its in vivo efficacy against several intracellular pathogens such as *Rhodococcus equi*, *Legionella spp*, *Salmonella spp*, *Shigella spp*, and *Mycobacterium avium*, despite *in vitro* MIC considerably higher than achievable peak plasma concentrations. [52-57] In addition, phagocytes have been shown to act as a vehicle for the delivery of macrolides and azalides to the site of infection. [48,49] The rates of intracellular penetration and subsequent release of antimicrobial agents from BAL cells vary widely even within the same class of drugs. For example, in foals, clarithromycin reaches higher concentrations than azithromycin in BAL macrophages.[39] However, clarithromycin is eliminated rapidly from BAL cells whereas azithromycin is released very slowly.[39] The slow and sustained release of azithromycin from cells explains the sustained antimicrobial activity of the drug in the lungs despite long dosing intervals and rapid disappearance of the drug in plasma. In contrast, clarithromycin is given twice daily. The half-lives of gamithromycin and tulathromycin in BAL cells of cattle are also very long.[25,26] High intracellular concentrations combined with slow and sustained release of the drugs in PELF likely contribute to their efficacy despite administration as a single injection and rapid disappearance of the drug from the plasma.

**Effect of Lung Inflammation on Antimicrobial Drug Distribution**

While our knowledge of drug concentrations in bronchial secretions and PELF of cattle has increased considerably over the last decade, the relevance of assessing drug concentrations in healthy lung has rightly been questioned. It could be argued that drug concentrations in inflamed tissues may be different and might be more appropriate as a predictor of therapeutic efficacy. The presence of inflammation may increase membrane permeability and increase penetration of drugs that do not readily cross membranes such as β-lactams.[58] Accumulation of inflammatory cells at the site of infection may also increase concentrations of drugs that are transported by phagocytes such as macrolides and azalides. Very few studies have investigated the effect of inflammation on antimicrobial drug concentrations

in extracellular fluid or bronchial secretions of cattle. In one study, concentrations of florfenicol in tracheobronchial secretions of calves experimentally infected with *P. multocida* (Cmax: 7.62 ± 0.26 μg/mL[mean ± SD]) were significantly higher than those of healthy calves (Cmax: 6.88 ± 0.23 μg/mL).[31] Concentrations of danofloxacin and enrofloxacin in respiratory secretions of calves experimentally infected with *M. haemolytica* have been measured (Figure 5) but non-infected controls were not available for comparison.[28]

## CONCLUSIONS

The factors associated with identifying appropriate doses and dosing regimens for the treatment of bovine respiratory disease include the activity of a given drug against common respiratory bacterial pathogens and the ability of the drug to gain access to the site of infection. Based on limited data in cattle, traditional pharmacokinetic/pharmacodynamic models based strictly on plasma concentrations appear to be good surrogate markers of efficacy for the treatment of susceptible extracellular respiratory pathogens with β-lactams and fluoroquinolones. However, plasma concentrations of other drugs such as macrolides and azalides are not useful surrogate markers of efficacy. For macrolide and azalides, recent findings support the notion that drug concentrations in PELF are better predictors of efficacy than either lung or plasma concentrations for the treatment of pulmonary infections caused by extracellular pathogens. Ultimately, the results of randomized prospective field trials are the best means of determining the relative efficacy of antimicrobial agents for the treatment of bovine respiratory disease in cattle.

## REFERENCES


1. Griffin D, Chengappa MM, Kuszak J, Mcvey DS. Bacterial pathogens of the bovine respiratory disease complex. *Vet Clin North Am Food Anim Pract* 2010;26:381-394.
2. Biss ME, Alley MR, Madie P, Hathaway SC. Lesions in the carcasses and viscera of very young slaughter calves condemned at post-mortem meat inspection. *N Z Vet J* 1994;42:121-127.
3. Clinical and Laboratory Standard Institute. Development of *in vitro* susceptibility testing criteria and quality control parameter for veterinary antimicrobial agents; Approved guideline, CLSI document M37-A3. Wayne PA. 2008.
4. Bryant JE, Brown MP, Gronwall RR, Merritt KA. Study of intragastric administration of doxycycline: pharmacokinetics including body fluid, endometrial and minimum inhibitory concentrations. *Equine Vet J* 2000;32:233-238.
5. Davis JL, Salmon JH, Papich MG. Pharmacokinetics and tissue distribution of doxycycline after oral administration of single and multiple doses in horses. *Am J Vet Res* 2006;67:310-316.
6. Andes D, Anon J, Jacobs MR, Craig WA. Application of pharmacokinetics and pharmacodynamics to antimicrobial therapy of respiratory tract infections. *Clin Lab Med* 2004;24:477-502.
7. Craig WA. Does the dose matter? *Clin Infect Dis* 2001;33 Suppl 3:S233-S237.
8. Craig WA. Basic pharmacodynamics of antibacterials with clinical applications to the use of beta-lactams, glycopeptides, and linezolid. *Infect Dis Clin North Am* 2003;17:479-501.
9. Singh R, Tam VH. Optimizing dosage to prevent emergence of resistance - lessons from *in vitro* models. *Curr Opin Pharmacol* 2011;11:453-456.
10. Greko C, Finn M, Franklin A, Bengtsson B. Pharmacokinetic/pharmacodynamic relationship of danofloxacin against *Mannheimia haemolytica* in a tissue-cage model in calves. *J Antimicrob Chemother* 2003;52:253-257.
11. Shojaee AF, Lees P. Pharmacokinetic-pharmacodynamic integration of danofloxacin in the calf. *Res Vet Sci* 2003;74:247-259.
12. Greko C, Finn M, Ohagen P, Franklin A, Bengtsson B. A tissue cage model in calves for studies on pharmacokinetic/pharmacodynamic interactions of antimicrobials. *Int J Antimicrob Agents* 2003;22:429-438.
13. Sarasola P, Lees P, AliAbadi FS, McKellar QA, Donachie W, Marr KA, Sunderland SJ, Rowan TG. Pharmacokinetic and pharmacodynamic profiles of danofloxacin administered by two dosing regimens in calves infected with Mannheimia (Pasteurella) haemolytica. *Antimicrob Agents Chemother* 2002;46:3013-3019.
14. Nix DE, Goodwin SD, Peloquin CA, Rotella DL, Schentag JJ. Antibiotic tissue penetration and its relevance: impact of tissue penetration on infection response. *Antimicrob Agents Chemother* 1991;35:1953-1959.
15. Zeitlinger MA, Derendorf H, Mouton JW, Cars O, Craig WA, Andes D, Theuretzbacher U. Protein binding: do we ever learn? *Antimicrob Agents Chemother* 2011;55:3067-3074.
16. Godinho KS. Susceptibility testing of tulathromycin: interpretative breakpoints and susceptibility of field isolates. *Vet Microbiol* 2008;129:426-432.
17. Huang RA, Letendre LT, Banav N, Fischer J, Somerville B. Pharmacokinetics of gamithromycin in cattle with comparison of plasma and lung tissue concentrations and plasma antibacterial activity. *J*

*Vet Pharmacol Ther* 2010;33:227-237.

18. Modric S, Webb AI, Derendorf H. Pharmacokinetics and pharmacodynamics of tilmicosin in sheep and cattle. *J Vet Pharmacol Ther* 1998;21:444-452.
19. Nowakowski MA, Inskeep PB, Risk JE, Skogerboe TL, Benchaoui HA, Meinert TR, Sherington J, Sunderland SJ. Pharmacokinetics and lung tissue concentrations of tulathromycin, a new triamilide antibiotic, in cattle. *Vet Ther* 2004;5:60-74.
20. Schunicht OC, Booker CW, Guichon PT, Jim GK, Wildman BK, Pittman TJ, Perrett T. An evaluation of the relative efficacy of tulathromycin for the treatment of undifferentiated fever in feedlot calves in Nebraska. *Can Vet J* 2007;48:600-606.
21. Sifferman RL, Wolff WA, Holste JE, Smith LL, Drag MD, Yoon S, Kunkle BN, Tessman RK. Field efficacy evaluation of gamithromycin for treatment of bovine respiratory disease in cattle at feedlots. *Intern J Appl Res Vet Med* 2011;9:166-175.
22. Nix DE, Goodwin SD, Peloquin CA, Rotella DL, Schentag JJ. Antibiotic tissue penetration and its relevance: models of tissue penetration and their meaning. *Antimicrob Agents Chemother* 1991;35:1947-1952.
23. Liu P, Muller M, Derendorf H. Rational dosing of antibiotics: the use of plasma concentrations versus tissue concentrations. *Int J Antimicrob Agents* 2002;19:285-290.
24. Washburn K, Johnson R, Clarke C, Anderson K. Distribution of ceftiofur into Mannheimia haemolytica-infected tissue chambers and lung after subcutaneous administration of ceftiofur crystalline free acid sterile suspension. *J Vet Pharmacol Ther* 2010;33:141-146.
25. Cox SR, McLaughlin C, Fielder AE, Yancey MF, Bowersock TL, Garcia-Tapia D, Bryson L, Lucas MJ, Robinson JA, Nanjiani I, Brown SA. Rapid and prolonged distribution of tulathromycin into lung homogenate and pulmonary epithelial lining fluid of holstein calves following a single subcutaneous administration of 2.5 mg/kg bodey weight. *Intern J Appl Res Vet Med* 2010;8:129-137.
26. Giguère S, Huang R, Malinski TJ, Dorr PM, Tessman RK, Somerville BA. Disposition of gamithromycin in plasma, pulmonary epithelial lining fluid, bronchoalveolar cells, and lung tissue in cattle. *Am J Vet Res* 2011;72:326-330.
27. Mouton JW, Theuretzbacher U, Craig WA, Tulkens PM, Derendorf H, Cars O. Tissue concentrations: do we ever learn? *J Antimicrob Chemother* 2008;61:235-237.
28. TerHune TN, Skogerboe TL, Shostrom VK, Weigel DJ. Comparison of pharmacokinetics of danofloxacin and enrofloxacin in calves challenged with Mannheimia haemolytica. *Am J Vet Res* 2005;66:342-349.
29. Herkner H, Muller MR, Kreischitz N, Mayer BX, Frossard M, Joukhadar C, Klein N, Lackner E, Muller M. Closed-chest microdialysis to measure antibiotic penetration into human lung tissue. *Am J Respir Crit Care Med* 2002;165:273-276.
30. McKellar Q, Gibson I, Monteiro A, Bregante M. Pharmacokinetics of enrofloxacin and danofloxacin in plasma, inflammatory exudate, and bronchial secretions of calves following subcutaneous administration. *Antimicrob Agents Chemother* 1999;43:1988-1992.
31. Ramadan A, Abd El-Aty M. Pharmacokinetics and distribution of florfenicol in bronchial secretions of healthy and Pasteurella multocida infected calves. *Pharm Anal Acta* 2011;doi:10.4172/2153-2435.1000116.
32. Friis C. Penetration of danofloxacin into the respiratory tract tissues and secretions in calves. *Am J Vet Res* 1993;54:1122-1127.
33. Drusano GL. Infection site concentrations: their therapeutic importance and the macrolide and macrolide-like class of antibiotics. *Pharmacotherapy* 2005;25:150S-158S.
34. Kiem S, Schentag JJ. Interpretation of antibiotic concentration ratios measured in epithelial lining fluid. *Antimicrob Agents Chemother* 2008;52:24-36.
35. Drusano GL. What are the properties that make an antibiotic acceptable for therapy of community-acquired pneumonia? *J Antimicrob Chemother* 2011;66 Suppl 3:iii61-iii67.
36. Winther L, Baptiste KE, Friis C. Pharmacokinetics in pulmonary epithelial lining fluid and plasma of ampicillin and pivampicillin administered to horses. *Res Vet Sci* 2010;
37. Winther L, Baptiste KE, Friis C. Antimicrobial disposition in pulmonary epithelial lining fluid of horses, Part III. Cefquinome. *J Vet Pharmacol Ther* 2010;
38. Winther L, Guardabassi L, Baptiste KE, Friis C. Antimicrobial disposition in pulmonary epithelial lining fluid of horses. Part I. Sulfadiazine and trimethoprim. *J Vet Pharmacol Ther* 2011;34:277-284.
39. Suarez-Mier G, Giguère S, Lee EA. Pulmonary disposition of erythromycin, azithromycin, and clarithromycin in foals. *J Vet Pharmacol Ther* 2007;30:109-115.
40. Womble A, Giguère S, Murthy YV, Cox C, Obare E. Pulmonary disposition of tilmicosin in foals and *in vitro* activity against Rhodococcus equi and other common equine bacterial pathogens. *J Vet Pharmacol Ther* 2006;29:561-568.
41. Womble A, Giguère S, Lee EA. Pharmacokinetics of oral doxycycline and concentrations in body fluids and bronchoalveolar cells of foals. *J Vet Pharmacol Ther* 2007;30:187-193.
42. Womble AY, Giguère S, Lee EA, Vickroy TW. Pharmacokinetics of clarithromycin and concentrations in body fluids and bronchoalveolar cells of foals. *Am J Vet Res* 2006;67:1681-1686.
43. Rennard SI, Basset G, Lecossier D, O'Donnell KM, Pinkston P, Martin PG, Crystal RG. Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. *J Appl Physiol* 1986;60:532-538.
44. Baldwin DR, Honeybourne D, Wise R. Pulmonary disposition of antimicrobial agents: methodological considerations. *Antimicrob Agents Chemother* 1992;36:1171-1175.
45. McClary DG, Loneragan GH, Shryock TR,

Carter BL, Guthrie CA, Corbin MJ, Mechor GD. Relationship of *in vitro* minimum inhibitory concentrations of tilmicosin against *Mannheimia haemolytica* and Pasteurella multocida and in vivo tilmicosin treatment outcome among calves with signs of bovine respiratory disease. *J Am Vet Med* Assoc 2011;239:129-135.

46. Fietta A, Merlini C, Gialdroni GG. Inhibition of intracellular growth of Staphylococcus aureus by exposure of infected human monocytes to clarithromycin and azithromycin. *J Chemother* 1997;9:17-22.
47. Labro MT. Intracellular bioactivity of macrolides. Clin Microbiol Infect 1996;1 Suppl 1:S24-S30.
48. Mandell GL, Coleman E. Uptake, transport, and delivery of antimicrobial agents by human polymorphonuclear neutrophils. *Antimicrob Agents Chemother* 2001;45:1794-1798.
49. Retsema JA, Bergeron JM, Girard D, Milisen WB, Girard AE. Preferential concentration of azithromycin in an infected mouse thigh model. *J Antimicrob Chemother* 1993;31 Suppl E:5-16.
50. Maglio D, Capitano B, Banevicius MA, Geng Q, Nightingale CH, Nicolau DP. Differential efficacy of clarithromycin in lung versus thigh infection models. *Chemotherapy* 2004;50:63-66.
51. Forbes AB, Ramage C, Sales J, Baggott D, Donachie W. Determination of the duration of antibacterial efficacy following administration of gamithromycin using a bovine *Mannheimia haemolytica* challenge model. *Antimicrob Agents Chemother* 2011;55:831-835.
52. Giguère S, Jacks S, Roberts GD, Hernandez J, Long MT, Ellis C. Retrospective comparison of azithromycin, clarithromycin, and erythromycin for the treatment of foals with Rhodococcus equi pneumonia. *J Vet Intern Med* 2004;18:568-573.
53. Butler T, Girard AE. Comparative efficacies of azithromycin and ciprofloxacin against experimental Salmonella typhimurium infection in mice. *J Antimicrob Chemother* 1993;31:313-319.
54. Frenck RW, Nakhla I, Sultan Y, Bassily SB, Girgis YF, David J, Butler TC, Girgis NI, Morsy M. Azithromycin versus ceftriaxone for the treatment of uncomplicated typhoid fever in children. *Clin Infect Dis* 2000;31:1134-1138.
55. Jonas D, Engels I, Daschner FD, Frank U. The effect of azithromycin on intracellular Legionella pneumophila in the Mono Mac 6 cell line at serum concentrations attainable in vivo. *J Antimicrob Chemother* 2000;46:385-390.
56. Koletar SL, Berry AJ, Cynamon MH, Jacobson J, Currier JS, MacGregor RR, Dunne MW, Williams DJ. Azithromycin as treatment for disseminated Mycobacterium avium complex in AIDS patients. *Antimicrob Agents Chemother* 1999;43:2869-2872.
57. Rakita RM, Jacques-Palaz K, Murray BE. Intracellular activity of azithromycin against bacterial enteric pathogens. *Antimicrob Agents Chemother* 1994;38:1915-1921.
58. Baldwin DR, Honeybourne D, Wise R. Pulmonary disposition of antimicrobial agents: in vivo observations and clinical relevance. *Antimicrob Agents Chemother* 1992;36:1176-1180.
59. Credille BC, Giguère S, Berghaus LJ, Burton AJ, Sturgill TL, Grover GS, Donecker JM, Brown SA. Plasma and pulmonary disposition of ceftiofur and its metabolites after intramuscular administration of ceftiofur crystalline free acid in weanling foals. *J Vet Pharmacol Ther* 2011;