Pharmacokinetics and pulmonary distribution of Draxxin ® (tulathromycin) in healthy adult horses

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science
In
Biomedical and Veterinary Sciences

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June 15, 2021

Blacksburg, VA

Keywords: adverse drug reaction, antibacterial, equine, macrolides

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Academic Abstract

The objective of this study was to determine the pharmacokinetics and tolerance of tulathromycin (Draxxin®; 2.5 mg/kg once) after intramuscular (IM), subcutaneous (SC), and slow intravenous (IV) administration to six adult horses. A three-phase design and 4-week washout period were used. Drug concentrations in blood and bronchoalveolar lavage (BAL) samples were determined by ultra-performance liquid chromatography tandem mass spectrometry and pharmacokinetic parameters calculated using noncompartmental analysis. Following SC and IM administration, all horses exhibited sweating, discomfort, and periods of recumbency. As signs were more severe after SC administration this route was only used in 3/6 horses. Intravenous administration of tulathromycin was well tolerated in all horses. Mean bioavailability was 99.4% IM and 115% SC. Mean maximum plasma concentration was 645 ng/ml IM and 373 ng/ ml SC. Mean half-life was 59.8 h, 54.8 h, and 57.9 h for IV, IM, and SC administration, respectively. Mean clearance was 3.25 ml/kg/min, and mean volume of distribution was 16.8 L/kg following IV administration. Drug was detectable in plasma and BAL samples for 120 h following all routes; however, adverse effects may prevent IM use and SC use is not recommended. Tulathromycin may be a practical and affordable antibacterial for use in adult equine patients.

Keywords
adverse drug reaction, antibacterial, equine, macrolides

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Public Abstract

In human and veterinary medicine, antibacterial drugs are a mainstay of treatment. Antibacterials have been used for almost 100 years to prevent microbial organism infection, and as a treatment once there is an established infection. Although there are multiple “classes” of antibacterials that have different spectrums of activity and mechanisms of action, antibacterial resistance has become increasingly prevalent over time. The increasing rate of antimicrobial resistance has led to recommendations that medical practitioners be more judicious in the use of these drugs and to prescribe antibacterials to patients only when necessary. In equine medicine, once an antibacterial is deemed necessary, there are additional considerations, including administration method, frequency of administration, and availability and cost of antibacterial drugs. Tulathromycin, a long-acting semi-synthetic macrolide, is an antibacterial that is approved for use in cattle and swine and may have utility for equine patients for a variety of conditions. This study in healthy adult horses demonstrated that tulathromycin was detectable in plasma and the respiratory tract for up to 5 days after single dose administration. Thus, tulathromycin may be a practical and affordable antibacterial for use in equine patients.

Acknowledgements

I would like to sincerely thank the members of my graduate committee, Dr. Jen Davis, Dr. Katie Wilson, Dr. Harold McKenzie, Dr. Chris Byron, and Dr. Krista Estell for their endless guidance, advice, support, and encouragement in the preparation and execution of my research project and with my thesis. Your constant support and dedication to my graduate education and to my training throughout my residency and in the pursuit of this degree has been invaluable, and I am very appreciative for having had this opportunity to further my education and career. I am very grateful for the expertise and advice that Dr. Harold McKenzie, Dr. Krista Estell, and Dr. Jennifer Davis provided during the execution of the research project. Dr. Katie Wilson, Dr. Harold McKenzie, Dr. Krista Estell, and Dr. Jennifer Davis kept me calm and provided excellent guidance and mentorship throughout the collection phase of the project, even when the best laid plans went awry, as tends to happen during research projects. I am also grateful for Dr. Katie Wilson's willingness to assist with additional and necessary IACUC paperwork with little notice. I am also grateful to the faculty, staff, interns and residents at the Marion DuPont-Scott Equine Medical Center in Leesburg, VA for graciously allowing me use of the facilities, research horses, and laboratory space in order to conduct this research project and for all of the assistance provided during the study periods. The laboratory portion of this research project would not have been possible without the assistance of Dr. Jen Davis and McAlister Council-Troche, and I am forever grateful for the assistance in the lab. I would also like to thank Dr. Bill Huckle for graciously allowing me use of his lab and equipment for completion of the urea assay. Finally, I would like to thank Dr. Mark Crisman and Zoetis ® for providing the funding for this project.

To Dr. Harold McKenzie, Dr. Katie Wilson, Dr. Chris Byron, Dr. Virginia Maxwell, and Dr. Kent Scarratt: thank you for all of your guidance, assistance, mentorship, encouragement,

and support with clinical cases and emergency cases throughout my residency. I am eternally grateful for everything that you have taught me; I have grown as a veterinarian and as a person throughout this experience and cannot thank you all enough for believing in me and encouraging me every step of the way throughout my residency and time in graduate school. I am a better clinician because of everything that you all have taught me.

I would also like to thank my family for their love, support, and understanding as I have pursued my career goals and have constantly moved across the country in order to continue my education and training.

Attributions

The execution of the research project, laboratory analysis, pharmacokinetic analysis, and writing of the manuscript (chapter 3) of this thesis would not have been possible without the assistance from many colleagues and mentors. The contributions of each are described below.

Dr. Harold McKenzie, DVM, MS, MSc (Vet Ed), DACVIM (LAIM) is a Professor of Large Animal Internal Medicine at the Virginia-Maryland College of Veterinary Medicine. Dr. McKenzie was integral in designing the study, securing funding, preparing for sample collection, supervision of data collection and interpretation, and contributed to the preparation of the manuscript.

Dr. Krista Estell, DVM, DACVIM (LAIM) is a Clinical Assistant Professor of Equine Medicine at the Marion duPont Scott Equine Medical Center in Leesburg, VA. Dr. Estell was integral in designing the study, securing funding, supervision of data collection and interpretation, and contributed to the preparation of the manuscript.

Dr. Jennifer Davis, DVM, PhD, DACVIM (LAIM), DACVCP is an Associate Professor of Clinical Pharmacology at the Virginia-Maryland College of Veterinary Medicine. Dr. Davis was integral in designing the study, securing funding, collecting samples, supervised data analysis and interpretation, performed the laboratory analysis, was instrumental in the pharmacokinetic and statistical analysis, and provided immense input and guidance in the preparation of the manuscript.

McAlister Council-Troche was involved in the assay development, sample analysis, and manuscript preparation.

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Chapter 1: Thesis Organization

This thesis is compiled and formatted to discuss antibacterial use in equine medicine. The topic of antibacterial resistance is briefly discussed, which is followed by an explanation of the need for additional options that are effective, safe, and affordable for the commercial use in horses. Characterization of macrolides, and especially tulathromycin is included in the literature review. This thesis contains a journal publication as the central portion of the document. The publication is entitled "Pharmacokinetics and pulmonary distribution of Draxxin ® (tulathromycin) in healthy adult horses" and was published in the Journal of Veterinary Pharmacology and Therapeutics in spring 2021.

Chapter 2: Literature Review

Antibacterial drugs in horses:

Indications of current antibacterials in horses

In both human and veterinary medicine, antibacterial drugs are used to treat infectious diseases that are caused by bacterial organisms. Such infections often involve the respiratory, urinary, gastrointestinal, and musculoskeletal organs/tracts, and can rapidly spread and involve multiple organ systems and/or the systemic circulation when left untreated. In foals and adult horses, clinical respiratory abnormalities are a common concern leading to veterinary evaluation and treatment, potentially involving the use of antibacterial drugs. [1] Antibacterial drug classes commonly used in equine medicine include aminoglycosides, penicillins, cephalosporins, fluoroquinolones, macrolides, nitroimidazoles, phenicols, potentiated sulfonamides, and tetracyclines. The appropriate antibacterial selection is ideally based on a culture and sensitivity.[2,3] However, culture is not often feasible or affordable and therefore empiric antibacterial selection is often used in equine medicine. When selecting the appropriate antibacterial treatment for the equine patient, there are many things to consider, including the drug's spectrum of activity and the resistance patterns of the implicated organism, the route of administration, cost of the treatment, frequency of administration, as well as known adverse effects of the selected drug.

Antibacterial resistance

Many bacterial organisms have developed resistance to antibacterial drugs that are widely used. More worrisome is the emergence and increased prevalence of bacterial organisms resistant to multiple antibacterial drug classes and therefore have limited treatment options.[4]

Antibacterial resistance is a concern on an international scale, and has a significant global economic impact, with more than 700,000 human deaths reported per year due to antibacterial-resistant bacteria.[5,6] Similar concerns are seen in veterinary medicine prompting the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the World Organization for Animal Health (OIE) to provide guidelines to help mitigate the development and acceleration of resistance..[5,6] The overall economic impact and mortality rate in veterinary medicine is unknown, but is recognized as a severe clinical concern.

Patterns of multi-drug resistance have been recognized with both gram-negative and gram-positive pathogens, making these bacterial infections difficult, or even impossible, to treat with conventional antibacterial drugs.[7] Drug shortages, a lack of successful prevention measures, and few new antibacterial drugs in development reinforce the need for judicious use of the antibacterial drugs that are currently available in both human and veterinary medicine.[5-11]

The WHO has identified five classes of antibacterial drugs used in equine medicine as "critically important" to human medicine.[5] These include the commonly used third and fourth generation cephalosporins, macrolides, polymyxins, and quinolones.[3] Glycopeptides are also on the list but are used only rarely in horses. Moreover, the OIE produced a "list of antimicrobial agents of veterinary importance",[12] which provides recommendations in limiting the use of fluoroquinolones, third and fourth generation cephalosporins, and polymyxins, of which fluoroquinolones and cephalosporins are commonly used in equine medicine. The OIE guidance recommends that these drugs not be used as the first line treatment unless justified, and use as a second line treatment should be based on the results of bacteriological tests. Extra label use of these antibacterial drugs should be limited and reserved for instances with no alternatives available for treatment.[4,6,8,12]

Horses may serve as an important reservoir for antibacterial resistance. [10-12] Pathogens with high zoonotic potential include *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBL), methicillin resistant *Staphylococcus aureus* (MRSA), *Salmonella* spp., *Escherichia coli*, *Enterobacteriaceae* spp., *Klebsiella, Pseudomonas*, along with a wide range of other bacterial organisms.[10,13-19] The American Veterinary Medical Association (AVMA) has identified the Enterobacteriales, *Pseudomonas aeruginosa* and *Staphylococcus* spp. as organisms of heightened concern in equine medicine (Figure 2.1), and several retrospective studies have noted the development of resistance patterns among these isolates.[3,9-11,17,19-65] The development of bacterial resistance means that there is a need to study potential new treatments for these bacteria in horses.

	Aminoglycosides	Carbapenams	Cephalosporins	Fluoroquinolones	Macrolides	Penicillins	Tetracyclinees	Trimethoprim
Enterobacteriales	X		X	X		X	X	X
Pseudomonas aeruginosa	X	X		X				
Staphylococcus spp.					X	X	X	

Figure 2.1. Equine pathogens of heightened concern, derived from the Animal pathogens of heightened concern table in the AVMA Committee on Antimicrobial Resistance 2020 Full report. Derived from Vet09 Table 8 and Vet08 Appendix B, both of which are available from the Clinical Laboratory Standards Institute at www.CLSI.org

Adverse effects of antibacterial drugs in horses

There are adverse effects associated with the administration of antibacterials to horses beyond the development of antibacterial resistance. Antibacterial-associated diarrhea (AAD) is a common clinical concern. Presumptive diagnosis of AAD is made in the non-diarrheic patient developing acute colitis and diarrhea during or shortly after stopping antibacterial therapy, while simultaneously ruling out other causes.[66,67] The pathogenesis of AAD has been described in the literature as alteration of the normal gastrointestinal microflora and enteric environment as a result of antimicrobial therapy.[67] The horse is highly reliant upon the gastrointestinal microbiome, especially within the hindgut. When the microbiome is disrupted due to antibacterial administration, the normal metabolism of carbohydrates and volatile fatty acids is affected, altering water absorption and secretion in the lower gastrointestinal tract, and resulting in colitis and diarrhea. Additionally, opportunistic commensal enteropathogens, such as *Salmonella* spp., *Clostridium difficile,* and *Clostridium perfringens* can proliferate and cause disease.[22,66,68-76] Furthermore, some antibacterials have a prokinetic effect on intestinal motility, whereas others create an environment that allows for the proliferation of toxin production from commensal enteropathogens.[77-81]

One of the earliest reports in the literature regarding AAD described fatalities associated with oxytetracycline in horses following an orthopedic research study.[79] Subsequent reports described further instances of diarrhea after the administration of oxytetracycline or with tetracycline-contaminated sweet feed.[82,83] Erythromycin, a macrolide antibacterial, has been documented to cause diarrhea when administered directly to adult horses or when adult horses are exposed to drug administered to a foal housed in the same stall.[79,80] Baverud et al. reported that administration of ß-lactam antibacterials for a non-gastrointestinal disorder resulted in acute colitis in 54% of the horses.[75] Finally, potentiated sulfonamides, a commonly used and

prescribed class in equine medicine, have also been associated with AAD, although reported risk is less compared to other available antimicrobials.[84-86]

In the equine literature the incidence of individual antibacterials causing AAD varies from 0.6-52%.[66,69,75,78-80,82,85] It is important to point out that in many of these studies, the horses were hospitalized, which is an added stressor that may have contributed to the development of disease. Barr et al. performed a retrospective study at three US referral practices, in three different areas of the country, to identify non-hospitalized equine patients that developed diarrhea after being treated with antibacterials for non-gastrointestinal related conditions.[69] Of the 5251 horses identified as being treated with antibacterials during this time period, 32 were diagnosed with AAD for an overall prevalence in this study of 0.6%. Of the 32 AAD horses, there was an 18.8% mortality rate. In this study, the most common antibacterials associated with AAD were gentamicin in combination with penicillin (7 horses), enrofloxacin (7 horses), and doxycycline (4 horses). Four horses were positive on fecal samples for *Clostridium difficil*e, 2 of which died, and three horses were positive for *Salmonella.* Implications of AAD can be severe for the equine patient, and can include hospitalization or prolongation of hospitalization, increased cost of treatment and supportive care, endotoxemia as a result of colitis and the change in gastrointestinal microbial population, and an increased mortality risk.[69]

Cost of treatment

For many clients, the cost of the antibacterial is a determining factor when deciding a treatment regimen. The route and frequency of administration for antibacterial drugs plays a role in the overall cost of the treatment and management. Intravenous administration typically requires a veterinarian or trained technician, resulting in hospitalization or farm calls, which

increase costs. Feasible routes of administration for most authors include oral and intramuscular. Additionally, owners may have difficulty administering drugs more frequently than twice a day. Table 2.1 details the costs of various antibacterial medications available at the Virginia-Maryland College of Veterinary Medicine Veterinary Teaching Hospital, with prices current as of January 2021.

Drug	Class	Route	Dose	Cost for one dose	Frequency	Cost per day
Potassium Penicillin (K-Pen)	Beta-lactam	IV	22,000 IU/kg	$43.50	Q 6 h	$174
Procaine Penicillin G (PPG)	Beta-lactam	IM	22,000 IU/kg	$3.50	Q 12 h	$7
Gentamicin	Aminoglycoside	IV	6.6 mg/kg	$22.50	Q 24 h	$22.50
Enrofloxacin	Fluoroquinolone	IV	5.5 mg/kg	$25.00	Q 24 h	$25.00
Enrofloxacin	Fluoroquinolone	PO	7.5 mg/kg	$34	Q 24 h	$34
Naxcel ® (Ceftiofur sodium)	Cephalosporin	IV or IM	2.2 mg/kg	$30	Q 12 h	$60
Excede ® (Ceftiofur crystalline free acid)	Cephalosporin	IM	6.6 mg/kg	$45	Q 96 h	$11.25
Oxytetracycline	Tetracycline	IV	6.6 mg/kg	$12.31	Q 12-24 h	$24.62 (+ fluids to dilute)
Minocycline	Tetracycline	PO	4 mg/kg	$16	Q 12 h	$32
Doxycycline	Tetracycline	PO	10 mg/kg	$35	Q 12 h	$35
Trimethoprim-sulfamethoxazole	Sulfonamide	PO	30 mg/kg	$11.50	Q 12 h	$23
Chloramphenicol	Phenicol	PO	50 mg/kg	$27.50	Q 4-6 h	$110.00

Table 2.1. Cost of antibacterial drugs at the Virginia Maryland College of Veterinary Medicine Veterinary Teaching Hospital as of January 2021. The estimated cost is based upon a 500 kg horse patient. IV= intravenous; IM= intramuscular; PO= *per os* (oral administration)

Use of long acting antibacterial drugs in horses

Presently, the availability of FDA-approved antibacterials that are safe, effective, and affordable in horses is limited. In order for a drug to receive FDA approval, the safety and effectiveness must be proven. The FDA defines safety of a drug to include safety to the animal receiving the drug, safety of any food products derived from the animal receiving the drug, and

safety to the individuals handling and administering the drug to the animal. The FDA further defines a drug's efficacy as consistently and uniformly doing what the label of the product describes that it will do. If the drug is to be provided to a food-producing animal, the residues of the drug in any food products must be established as safe for human consumption. Supporting scientific data, information on the drug's chemistry and composition, the drug's ingredients, manufacturing methods, labeling, analytical methods for drug residues, and environmental assessment must also be provided.[87] Long-acting antibacterial formulations are beneficial in that they require less frequent administration, which decreases cost and improves client compliance. Currently, the only long acting antibacterial that is licensed for use in horses is Excede® (ceftiofur crystalline free acid), a third-generation cephalosporin labeled for use in horses with lower respiratory tract infections caused by *Streptococcus equi* subsp. *zooepidemicus*. Because Excede® is a long-acting product, many practitioners elect to use this as an empiric treatment despite recommendations to decrease use of third-generation cephalosporins.

Long-acting antibacterial drugs are particularly useful in food animal medicine, due to the difficulty in treating these animals. Tulathromycin, a long-acting semi-synthetic macrolide, is FDA approved for the treatment of respiratory disease in cattle and swine. In veterinary medicine, extra label or off-label drug use for patients is allowed if the following conditions are met:

- a valid veterinarian-client-patient-relationship exists;
- there is no animal drug approved for the intended use; or
- there is an animal drug approved for the intended use, but the approved drug does not contain the necessary active ingredient; or

- there is an animal drug approved for the intended use, but the approved drug is not in the required dosage form; or
- there is an animal drug approved for the intended use, but the approved drug is not in the required concentration; or
- the approved drug is clinically ineffective when use as labeled.

In non-food producing animals, approved human drugs can be used extra-label even if an approved animal drug is available, but this is not true for food-producing animals; if there is a drug approved for food-producing animals, that must be used first before prescribing a drug approved for human use.[88]

Extralabel use of tulathromycin has been investigated in foals for treatment of bacterial respiratory diseases with a prolonged dosing interval. The main adverse effect observed in foals after administration was injection site reaction after intramuscular administration. [89-92] There are currently no studies that examine the use pharmacokinetics of tulathromycin in adult horses.

Macrolide antibacterials

Macrolides are a group of chemically related compounds that are characterized by a central 12- to 16- membered lactone ring, with one or more deoxy sugars attached (Figure 2.2).[93,94] Differences in the chemical structures of macrolide drugs result in differences in absorption, distribution, metabolism, and elimination *in vivo*.[95] In human medicine these drugs are important treatments for *Campylobacter*, *Chlamydia*, *Legionella*, and *Mycobacterium* species. Widespread use and the resulting decreased efficacy of the original macrolides has led to the development of semisynthetic drugs with an expanded spectrum of activity, more favorable pharmacokinetic parameters, and reduced adverse reactions.[94] The macrolide class is also widely used in veterinary medicine.[96-100]

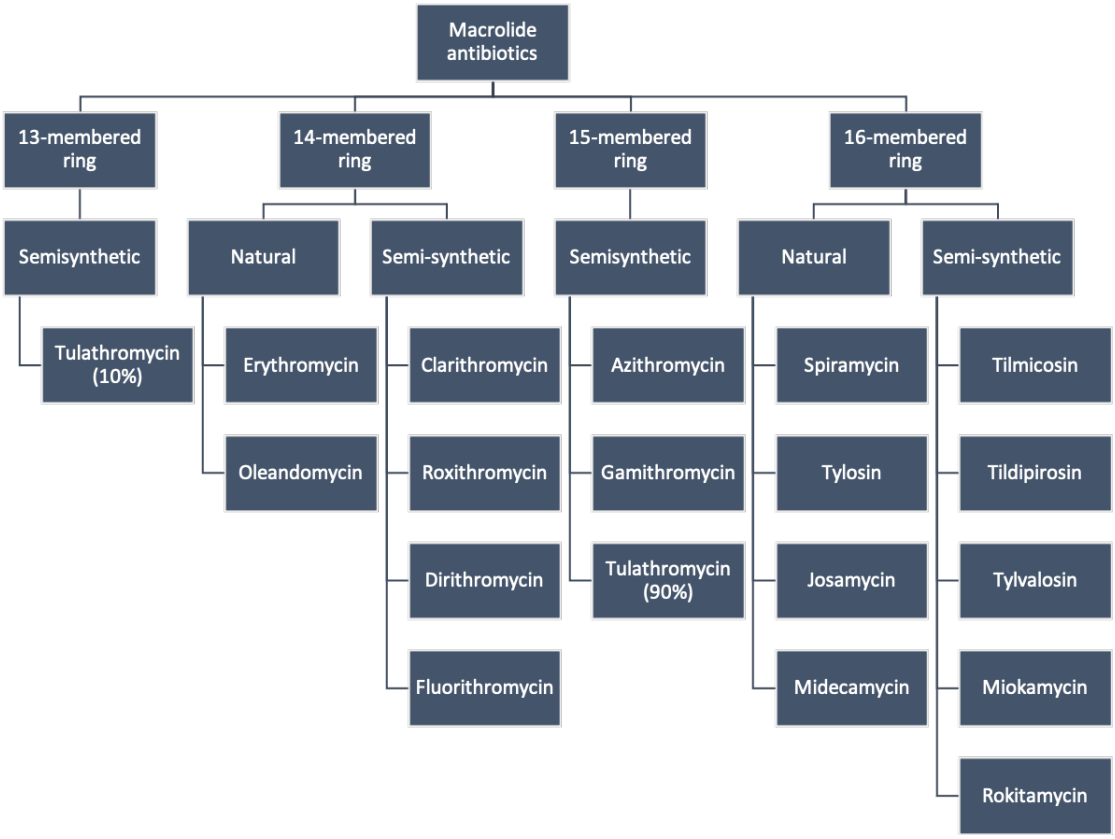

Figure 2.2 Chemical structure and classification of macrolide antimicrobials according to the macrocyclic lactone ring size. Derived from Giguère, S., Prescott, J.F., & Dowling, P.M. (eds). (2013). Antimicrobial therapy in veterinary medicine. ProQuest Ebook Central.

Mechanism of action

Macrolides work via inhibition of protein synthesis by way of reversibly binding to the 50S subunit of the ribosome resulting in inhibition of transpeptidation and translocation processes, causing premature detachment of incomplete polypeptide chains. The macrolide binding sites on the 23S rRNA of the 50S ribosomal subunit overlap with other classes of antibacterial drugs, including lincosamides, strepogramins, ketolides, and oxazolidinones but are different from those of chloramphenicol within the phenicol class. Macrolides accumulate within phagocytes, making them excellent choices for intracellular pathogens.[91] They are bacteriostatic against most pathogens, although bactericidal activity can be seen at high concentrations with a

low inoculum of highly susceptible bacteria.[94] Gamithromycin, unlike other macrolides, has consistent bactericidal activity against *Histophilus somni, Mannheimia hemolytica,* and *Pasteurella multocida* (all of which are associated with bovine respiratory disease complex and swine respiratory disease).[101-103]

Macrolides approved for use in veterinary medicine

Eight of the eighteen macrolide drugs are currently approved by the FDA for use in veterinary medicine, including a mix of synthetic and semi-synthetic drugs. There are also numerous reports of off-label use of macrolide drugs in other species. Currently, erythromycin is the only approved 14-membered ring drug, gamithromycin an approved 15-membered ring drug, and tylosin, spiramycin, tilmicosin, tildipirosin, and tylvalosin of the 16-membered ring classification are approved for use in veterinary species.[91] Due to the novel chemical structure of tulathromycin, a semi-synthetic macrolide with three nitrogen and amine functional groups in the molecule, it is in the triamilide subclass of macrolides and has both a 13-member and a 15-member ring.[104-106]

Mechanism of resistance

There are three different mechanisms that account for bacterial resistance to macrolides: 1) rRNA methylation; 2) active efflux; and 3) enzymatic inactivation. Active efflux and rRNA methylation are better investigated and responsible for the majority of resistance in bacterial isolates.[94,107] Most genes of macrolide resistance are associated with mobile elements, allowing for easy spread between strains and species of bacterial organisms.[94] Resistance as a result of rRNA methylation is encoded by erythromycin-resistant methylase (*erm*) genes, the outcome of

which is cross-resistance to multiple classes of antibacterial drugs, including macrolides, lincosamides, and streptogramin B (MLSB resistance).[94,107] As of 2013, 35 different rRNA methylases have been characterized and reported. These rRNA methylase genes are present in both gram-positive and gram-negative bacteria and are located on plasmids or transposons. The expression of *erm* genes is constitutive or inducible, with constitutive resistance occurring when the methylase enzyme is intrinsically produced and inducible resistance occurring during exposure of the bacterial organism to 14- or 15- member ring macrolides. However, exposure of a bacterial organism to a 16-member ring macrolide does not have the same outcome.[94] Recently, Anastasi and colleagues identified a novel *erm* gene, known as *erm*(46), that was present in all macrolide resistant *R. equi* isolates, and absent from the susceptible isolates from foals studied in the United States as confirmed by PCR screening of 124 clinical isolates. The expression of *erm*(46) in a *R. equi* strain that was macrolide-susceptible induced high levels of resistance to macrolides, lincosamides, and streptogramins B, but did not induce resistance to other classes of antibacterial drugs. Transfer of the *erm*(46) to macrolide susceptible isolates of *R. equi* occurs, allowing further spread of the resistant isolates.[107]

Active efflux of macrolides is mediated by various members of the ATP binding cassette family of proteins or by major facilitator superfamily transporters. The ATP binding cassette family of proteins effectively work to pump the macrolide antibacterial agent out of the cell, effectively allowing the bacterial ribosomes to function again.[94,107] As of 2013, there have been 20 different efflux genes identified and characterized. Of these, some contribute to resistance to the 14- and 15-member ring macrolides while not at all interfering with susceptibility to 16-member ring macrolides, ketolides, lincosamides, and streptogramin B. Other efflux genes have been characterized as allowing for different resistance patterns, including MLSB. Active efflux

genes have been identified in an assortment of both gram-negative and in gram-positive bacterial species.

Rifampin, which is often paired with macrolides when administered to foals, is an inducer and modulator of efflux and uptake transporters.[108-112] Rifampin regulates efflux transporter genes, including ABCB1 (P-glycoprotein) transporter, ABCC2 (multidrug resistance protein 2) transporter, and CYP3A4 transporter via the nuclear PXR-receptor induction pathway.[108,109] Rifampin has been shown to reach high concentrations in the lung and penetrates into septic lesions, abscesses, and phagocytes, in addition to killing intracellular pathogens. [108,111] Therefore, multimodal antibacterial therapy using macrolides together with rifampin has become an effective treatment protocol while increasing the survival rate of respiratory infections in foals between 2-6 months of age from 20% to 90%.[113] Venner demonstrated that prolonged treatment with tulathromycin and rifampin in foals influences expression of ABCB1 and ABCC2 in bronchoalveolar cells and concentration of tulathromycin in epithelial lining fluid and bronchoalveolar cells.[108]

The final mechanism of resistance is enzymatic inactivation. As of 2013, there have been 2 esterase and 6 phosphorylase inactivating enzymes reported as contributing to macrolide resistance, but clinical relevance is unknown.[94]

It has been estimated that anywhere from 1-4% of the macrolide-resistant gram-positive bacteria do not carry any of the three-known acquired macrolide resistance mechanisms or genes. This small percentage of isolates typically have mutations in the rRNA genes or in the ribosomal protein genes.[94]

In equine medicine, macrolides are most frequently used for *Rhodococcus equi* infections in foals and are frequently combined with rifampin.[113-118] Erythromycin quickly became the

treatment of choice globally for the treatment of *R. equi* pneumonia in foals, but within one decade of using the erythromycin-rifampin combination, resistance to erythromycin emerged, with the first published report in a 10 month old Standardbred filly.[119,120] Erythromycin-derived macrolides azithromycin and clarithromycin were investigated in foals as a possible alternative therapy due to excellent activity against *R. equi* and a more favorable pharmacokinetic and safety profile.[121-123] Recently, Erol and colleagues published a retrospective analysis of the antibacterial susceptibility patterns of *R. equi* from 256 necropsied foals with 256 *R. equi* isolates. From the 256 isolates, there were high rates of resistance exhibited to rifampin (22.65%), azithromycin (16.01%), clarithromycin (14.84%), and erythromycin (15.23%).[124]

Spectrum of activity

The macrolides have good activity against a variety of gram-positive aerobes and a few gram-negative aerobes (Table 2.2), although susceptibility may vary between drugs.

Organisms	Erythromycin	Tylosin	Spiramycin	Tilmicosin	Gamithromycin	Tulathromycin	Tildipirosin	Roxithromycin	Clarithromycin	Azithromycin
Gram-positive aerobes										
Arcanobacterium pylogenes	2	2	4	0.05*		8		≤ 0.03	≤ 0.016	≤ 0.016
Erysipelothrix rhusopathiae	0.13	<0.13	0.25	<0.13				0.13	0.06	0.03
Listeria monocytogenes	0.25							0.5	0.13	1
Rhodococcus equi	≤ 0.25	64	128	32	1	>64		0.25*	0.06*	1*
Staphyloccocus querus	0.25	2	8	1				0.25	0.25	0.25
Streptococcus agalactiae	≤ 1	1		4				0.13	0.06	0.13
Streptococcus uberis	≤ 0.5	1	0.5*							≤ 0.12
Streptococcus equi subsp. zooepidemicus	≤ 0.25				0.125				≤ 0.06	
Gram-negative aerobes										
Actinobacillus pleuropneumoniae	8	32	32	2		32	8			
Escherichia coli	>4								>4	>8
Histophilus somni	2	8	128	8	0.5	4	4			
Klebsiella spp.	>4								>4	>8
Mannheimia hemolytica	16	128		4	1	2	1			
Pasteurella multocida	16	128		16	1	1	1	4	2	1
Pasteurella spp. (equine)	1								1	0.25
Salmonella enterica	>4								>4	4
Bordatella bronchiseptica				16		8	4			
Haemophilus parasuis	2*			8*		2	1			
Moraxella bovis	1	16		4		0.5				
Moraxella bovoculi		16		≤ 4		4				
Brucella spp.	16							16	8	2
Gram-negative: other										
Bartonella henselae	0.13							0.13	0.03	0.016
Campylobacter spp.	2							2	2	0.5
Helicobacter pylori	0.5							0.125	1	0.25
Anaerobes										
Dichelobacter nodosus	0.25	1	1							
Bacteroides fragilis	32	0.25*	>64						16	4
Fusobacterium necrophorum	8	4	64	4		64		16	8	1
Brachyspira hyodysenteriae	>128	>128	>128	>64						
Clostridium perfringens	4	2		4					4	4
Peptostreptococcus spp.	>32							>32	>32	>32
Mycoplasma										
Mycoplasma bovis	0.5	0.5	4	>128	4	1				
Mycoplasma hyorhinis	128	1	0.5	4		>32				
Mycoplasma hyopneumoniae	4	1	1	0.5		>32				
Mycoplasma mycoides subsp. *mycoides*	0.06	0.06	0.5	0.06						
Ureaplasma spp.		0.13	0.5							
Leptospira spp.	0.06	0.06								
Lawsonia intracellularis	0.5	64		2						

Table 2.2 *In vitro* activity (MIC_{90}) of veterinary macrolides and erythromycin derivatives clarithromycin and azithromcyin (ug/mL) against selected bacterial and mycoplasma pathogens. Adapted as reported in Giguère, S., Prescott, J.F., & Dowling, P.M. (eds). (2013). Antimicrobial therapy in veterinary medicine. ProQuest Ebook Central.

*Some reports show resistance

Pharmacokinetics and pharmacodynamics

As a class, macrolides are time-dependent and have a large volume of distribution (>1 L/kg), reaching therapeutic concentrations intracellularly and within tissues, but not the cerebrospinal fluid.[94] Bioavailability is low to moderate following oral administration.[125,126] Drugs within the macrolide class are lipophilic with a molecular weight of less than 1000 Da and are basic molecules with pKa > 7.0.[126,127] The physiochemical factors and characteristics of macrolides favor their accumulation within the lung.[126,128,129] Constant dissociation of macrolides coupled with the low degree of ionization at plasma pH is believed to favor accumulation of macrolide drugs in an acidic environment, allowing for ion trapping in the alveolar epithelial lining fluid as well as the airways of patients with pneumonia[126,130-132] Macrolides are metabolized and excreted mostly in the bile with a small amount of small intestinal reabsorption. Some macrolides have more renal metabolism and excretion than others, but the majority is still hepatic.[94]

Adverse effects

The incidence of adverse effects and level of toxicity among the various macrolides is dependent upon the species being treated and the route of administration. A common finding with intramuscular administration is injection site reactions causing pain and swelling. Thrombophlebitis and periphlebitis have been reported in a variety of species after intravenous

administration, and intramammary administration may result in inflammatory reactions in cattle. Dose-dependent gastrointestinal disturbances, including nausea, vomiting, diarrhea, intestinal pain and colic, have been reported in most species treated with erythromycin.[94] Additionally, erythromycin and tulathromycin bind motilin receptors, stimulating gastrointestinal smooth muscle and causing diarrhea and fatal colitis in adult horses.[80,94] Severe and fatal colitis as the result of *Clostridium difficile* infection has also been reported in the dams of foals that were treated orally with erythromycin and rifampin for *Rhodococcus equi* infection. This is believed to be associated with ingestion of small quantities of the drug from the feces of the foals.[79] Fatal typhlocolitis has also been reported in rabbits that have received erythromycin, and severe diarrhea has been reported in ruminating calves.[94,133] In humans, the estolate form of erythromycin has been associated with self-limiting cholestatic hepatitis and jaundice, with abdominal pain, especially with prolonged use or repeated dosing in patients that had pre-existing hepatic disease.[94] Finally, erythromycin, azithromycin, and clarithromycin have been associated with hyperthermia that can result in respiratory distress in foals, especially those that do not have access to shade and are exposed to direct sunlight and/or kept in high environmental temperatures (i.e. during the summer).[134] As few as two doses of erythromycin can induce severe sweating dysfunction and vulnerability to heat stress, the severity of which depends on duration of treatment, amount of time since treatment, and environmental temperature.[135] The mechanism for erythromycin-induced anhidrosis has not been completely elucidated, although it is believed that the ability of 14- and 15-membered macrolide drugs to inhibit chloride secretion from epithelial cells and inhibition of calcium-activated chloride channels may contribute to increases in intracellular calcium concentration and affect the ß-2 adrenergic receptor on equine sweat

glands.[135-140] Foals receiving intravenous clarithromycin, erythromycin, and tilmicosin have exhibited respiratory distress in conjunction with hyperthermia. [141-144]

Tylosin has similar adverse effects as erythromycin, with irritation to tissues reported when administered intramuscularly to cattle and swine or subcutaneously to cattle.[94,145] There are reports of pigs with injection site reactions that include pruritis, rectal mucosal edema, and mild anal protrusion..[94,146-149] Tylosin has reportedly caused fatal diarrhea in a horse after intravenous administration.[94] Accidental feeding to dairy cows at a concentration of 7-20 ppm of tylosin resulted in ruminal stasis, inappetence, malodorous manure, and decreased production of milk, with some cows exhibiting hyperesthesia and others developing recumbency. [150] The intravenous administration of tylosin produced shock, dyspnea, and depression in affected cattle. Tylosin has also been reported to induce contact dermatitis in veterinarians exposed to the drug during administration.[94]

Tilmicosin has the potential to be cardiotoxic, although the effects are species dependent. Swine can tolerate oral administration, but intramuscular administration can be fatal.[94,151,152] Similarly, tilmicosin has been fatal to humans after accidental injection.[94] In goats, tilmicosin has been toxic and fatal after both subcutaneous and intravenous administration,[94] and subcutaneous and intramuscular administration to horses has resulted in severe reactions and diarrhea.[126,142,153] There has been one equine death reported after consumption of 2000 ppm of tilmicosin in the diet.[142] The cardiotoxic effects of tilmicosin are believed to be mediated through direct effects on the cardiac myocytes via rapid depletion of calcium.[154]

Gamithromycin and tildipirosin use has been reported to be safe in cattle, with the exception of transient discomfort and mild to moderate swelling at the injection site after administration.[94,155-157] When administered to swine, tildipirosin administration caused shock in 2

of 1048 animals.[94,158] Safety has not been evaluated nor reported in any other species. Very recently, it was reported that intravenous and subcutaneous administration of a single dose of tildipirosin to horses ranging in age from 6 months to 15 years did not result in any systemic adverse effects, although subcutaneous administration did cause a self-limiting local tissue reaction for 72 hours post-injection.[159]

The newer macrolides, clarithromycin and azithromycin, are better tolerated and cause fewer gastrointestinal disturbances than erythromycin. These drugs still have the potential to cause enterocolitis in foals after administration, but are safer for the dams of these foals.[94] Direct administration to adult horses is still a concern for the development of adverse gastrointestinal affects.[79,80,94] Additionally, macrolide administration to foals with pneumonia has been reported to cause diarrhea.[98,141,160] Hyperthermia and lack of sweating can also occur in foals administered azithromycin and clarithromycin, although the effects are less severe than seen with erythromycin. The intramuscular administration of tulathromycin and tilmicosin caused pain, discomfort, and swelling at the injection site in foals, which may be associated with the drug vehicle.[98]

Drug interactions

When studied *in vitro*, erythromycin has antagonistic effects when combined with other macrolides, lincosamides, or chloramphenicol. Erythromycin has been combined with aminoglycosides to prevent or to treat peritonitis in humans, but this combination is not as effective as clindamycin and metronidazole combined with an aminoglycoside.[94,126,161,162] Combining a macrolide with a fluoroquinolone or aminoglycoside when treating mixed bacterial infections may result in synergy, depending on the bacterial species being treated.[94,163] In equine

medicine, the most common macrolide synergistic relationship is with rifampin in the treatment of *R. equi* in foals.[94]

Erythromycin and some other macrolides are inhibitors of cytochrome P450 (CYP) enzymes. This may increase the concentration of drugs that are dependent upon the CYP3A (the most abundant and clinically important enzyme) metabolic pathways such as theophylline, midazolam, omeprazole, or ranitidine. Clarithromycin has a lower affinity for the CYP450 system than erythromycin, while azithromycin does not affect CYP enzymes and is not associated with drug interactions.[94]

Concurrent administration of clarithromycin with rifampin reduces the bioavailability of clarithromycin in foals. When both drugs were administered for a period of 11 days, the bioavailability of clarithromycin was decreased by more than 90%, which likely resulted from induction of hepatic and intestinal CYP3A4 and intestinal ABCB1 and ABCC2 pathways.[126,164] When comedication of tulathromycin and rifampin was evaluated in foals, the concentration of tulathromycin within the lungs of foals was significantly decreased, which was attributed to extrapulmonary mechanisms leading to lower plasma concentrations or induction of a minor metabolic elimination pathway of tulathromycin in the liver.[108,165] When multiple doses of gamithromycin and rifampin were administered concomitantly to healthy foals, rifampin administration significantly increased plasma concentration of gamithromycin with a decrease in total body clearance attributed to inhibition of hepatic elimination.[166]

Anti-inflammatory and prokinetic activities of macrolides

In addition to antibacterial properties, macrolides are known to have beneficial immunomodulatory effects in humans suffering from inflammatory pulmonary diseases such as

cystic fibrosis, idiopathic bronchiectasis, and chronic obstructive pulmonary disease.[94,167] The immunomodulatory effects are likely independent of the antibacterial activity. Erythromycin, azithromycin, clarithromycin, and roxithromycin inhibit chemotaxis and infiltration of neutrophils into the airway and decrease mucus secretion through an unknown mechanism.[94,168] Anti-inflammatory effects have been reported in foals receiving erythromycin and cattle and pigs receiving tilmicosin or tulathromycin.[94,169-172] Specifically, macrolides suppress transcription factor nuclear factor kappa B or activator protein 1, which inhibits production of proinflammatory cytokines including interleukin (IL)-1, 6, 8, and tumor necrosis factor alpha.[94,172,173] In addition, macrolides prevent formation of adhesion molecules necessary for neutrophil migration by inhibiting formation of leukotriene B4, which functions to attract neutrophils and inhibits the release of superoxide anion release from neutrophils.[173] Macrolides may influence adaptive immunity as well. Finally, macrolides of the 14- and 16-member class have exhibited prokinetic effects on the gastrointestinal tract by acting on the motilin receptor as an agonist. Prokinetic effects have been reported with erythromycin in horses and dogs, and with tylosin and tilmicosin in cattle.[94,174-176]

Clinical use in horses

Erythromycin has limited utility in adult horses due to the potential to induce diarrhea and/or fatal colitis. Erythromycin with rifampin was previously used to treat experimentally induced *Neorickettsia risticii* infection,[177] and was suggested as a treatment for *Lawsonia intracellularis* infections in foals and weanlings,[94,178] and has been investigated for use in intravenous regional limb perfusion.[179,180] Erythromycin is occasionally still used with rifampin

to treat *Rhodococcus equi* infections, although it has been mostly replaced with clarithromycin and azithromycin which have fewer adverse effects and are administered less frequently.

When treatment of clarithromycin-rifampin was compared with azithromycin- rifampin and erythromycin-rifampin in *R. equi* foals, clarithromycin-rifampin was the most effective drug combination.[100] The practice of screening foals via ultrasound examination for early identification and mass treatment of foals with pneumonia was first described in 2001 and was then implemented at several farms in central Kentucky in the proceeding foaling seasons.[25,90,91,96,98,181,182] As with other antibacterials and other macrolides in particular, there is always a risk of foals treated with clarithromycin developing diarrhea, although often times the diarrhea is mild and self-limiting.[94,100,122,143,183] Despite the negative pharmacokinetic interactions between clarithromycin and rifampin, the combination is still frequently used due to its synergistic effects.[184,185]

With increasing resistance of *Rhodococcus equi* strains to macrolides, the related ketolide drug telithromycin has been evaluated *in vitro* and *in vivo* as a possible alternative to the currently available macrolides. The pharmacokinetics of telithromyin are similar to those of clarithromycin and azithromycin, with accumulation in the pulmonary epithelial lining fluid and bronchoalveolar cells. In macrolide-resistant isolates of *Rhodococcus equi*, telithromycin was more active than macrolides, but clinical use is not likely to be effective.[186] The MIC_{90} of telithromycin in the macrolide-resistant *Rhodococcus equi* isolates was 8 μg/mL, whereas the MIC_{90} of the macrolide-susceptible isolates was 0.25 μg/mL, which indicates that at least one of the macrolide-resistant mechanisms in *Rhodococcus equi* confers resistance in ketolides, such as telithromycin, as well.[94]

Gamithromycin has been investigated in foals following IM administration.[156,166,187-189] It has a wide volume of distribution (25 L/kg), slow clearance, and a prolonged half-life, particularly in pulmonary epithelial lining fluid (PELF) and BAL cells.[156,187-189] The PELF concentrations remained above MIC_{90} for *Streptococcus equi* subsp. *zooepidemicus* and above the MIC_{90} for *Rhodococcus equi* in BAL cells for 7 days following a single dose.[189] Gamithromycin administration has not been evaluated in adult horses, and safety and efficacy at this time are unknown.

Tulathromycin

Tulathromycin has been available for use in veterinary medicine since 2004. Tulathromycin is a unique macrolide due to its mixture of a 13-membered ring (10%) and 15-membered ring (90%) and a novel chemical structure that is comprised of three polar amine groups.[93,190,191] In July 2019, the OIE produced a list of antimicrobial agents of veterinary importance, which included macrolides (and tulathromycin) as veterinary critically important antibacterials (https://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/AMR/A_OIE_List_antimicrobials_July2019.pdf)

Differences/similarities to other macrolides

Tulathromycin has similar antibacterial activity to that of tilmicosin including activity against many gram-negative pathogens, including *Mannheimia hemolytica, Pasteurella multocida, Histophilus somni, Moraxella bovis, Fusobacterium necrophorum, Actinobacillus pleuropneumoniae, Hemophilus parasuis,* and *Bordetella bronchiseptica*.[190,192] Just as with other macrolides, tulathromycin has demonstrated *in vitro* activity against *Mycoplasma* species, although resistance has been reported.[193-198] Tulathromycin has activity against *Arcanobacterium*

pyogenes and poor activity against *R. equi*, with a MIC_{90} > 64 µg/mL (Table 2.2).[94] In cattle with respiratory disease, *Histophilus somni, Mannheimia hemolytic,* and *Pasteurella multocida* isolates are considered susceptible if MIC is $\leq$16 µg/mL and resistant if $\geq$ 64 µg/mL.[94]

Tulathromycin has a PKa of 8.6-9.6 and therefore is ionized and accumulates in acidic environments.[126] The MIC of tulathromycin for some bacterial organisms has been reported as inversely related to the pH of the tissue environment *in vivo*.[199]

When administered to cattle, swine, goats, and foals, tulathromycin is rapidly absorbed from the site of injection, extensively distributed, slowly eliminated, and achieves prolonged and high pulmonary concentrations.[104,108,126,200-208] When tulathromycin is administered subcutaneously to cattle and intramuscularly to swine, bioavailability is about 90% with an accompanying elimination half-life of approximately 90 hours in cattle and 76 hours in swine.[94,207,209] When administered enterally to swine, bioavailability of tulathromycin is approximately 50%.[200] In cattle, the volume of distribution after intravenous administration is 12 L/kg, with peak lung concentrations of 4 µg/g reported. When healthy adult meat goats received a single subcutaneous dose of tulathromycin, plasma elimination half-life was 110 hours, and similar to other species was rapidly absorbed and widely distributed with volume of distribution of 33 L/kg.[204] In a follow-up multi-dose study, absorption was rapid with plasma terminal elimination half-life of 61.4 hours after the second dose and tissue half-lives ranging from 2.4 days in muscle up to 9 days in lung tissue.[210]

Lung concentrations of tulathromycin, like other macrolides, have been reported to be 25-180 times higher than concurrent serum concentrations.[94,205,207,208] In calves that received tulathromycin, the plasma, and interstitial fluid concentrations of tulathromycin were lower than pulmonary fluid concentrations for the duration of the study.[205,208,211] Pulmonary epithelial lining

fluid (PELF) is a main component of the immune defense system with continuous distribution throughout the respiratory tract from the conducting airways to the alveoli, and this fluid is a possible site of bacterial contamination and subsequent colonization.[126,212] As with other macrolides, tulathromycin PELF concentration compared to plasma concentration contributes to improved antibacterial activity in the respiratory tract.[121,126,142,143,164,186,189,213] In calves, PELF concentration was more than nine times greater than plasma.[209] At most time points evaluated from 24-288 h post tulathromycin administration, plasma and interstitial tulathromycin concentrations were lower than the reported MIC of susceptible bacteria.[209]

The elimination half-life of tulathromycin from pulmonary tissue in cattle is approximately 11 days.[94,205,207,208] In calves, age of the animal affects plasma pharmacokinetics. Six-month-old calves had greater tulathromycin plasma concentrations and slower clearance of the drug. There was no difference between age groups with respect to PELF and interstitial fluid (ISF) concentrations.[214]

Tulathromycin accumulates within inflammatory cells. Venner et al. (2010) reported that bronchoalveolar lavage (BAL) cells had a greater concentration of tulathromycin compared to plasma at 24 and 192 h post tulathromycin administration.[108] Macrolides, including tulathromycin, also tend to persist in BAL cells and PELF longer than they do in plasma, which may be due to the degree of ionization and trapping within the acidic environment of the cell, encouraging higher accumulation and slower rate of exit.[126,128] When administered to foals and to cattle, tulathromycin concentrates in and is slowly eliminated from bronchoalveolar cells.[92,205]

No serious adverse effects or fatalities as a result of tulathromycin administration have been reported in cattle and swine.[215-220] At doses up to 10 times the label dosage, injection site pain, swelling, and discoloration have been reported.[94,221] Safe administration has been reported

in goats.[222] Injection site reactions have been reported in foals.[98] The safety of tulathromycin when administered to adult horses has not previously been studied.

The effects of tulathromycin on the GI microbiome have been evaluated in humans. *In vitro* testing in humans showed that high concentrations of tulathromycin (10 and 100 µg/mL) disrupted the colonization resistance of the human gastrointestinal microbiota and selected for antibacterial resistant *Enterococcus faecalis*.[223] The resistant *E. faecalis* always carried the *ermB* gene and was capable of horizontal gene transfer.[223] In commercial feedlot cattle receiving metaphylactic treatment with tulathromycin compared to untreated cattle, antibacterial resistance genes were identified that encompassed 9 classes of antibacterial and encoded for 24 unique mechanisms. However, the authors attributed the change more to the transition into the feedlot, diet changes, geography, and environment than to antimicrobial therapy.[224] In a randomized field trial with commercial dairy calves, tulathromycin administration had minimal impact on the microbiota over the 112 day study period.[225] When adult beef cattle received tulathromycin 2 days after transportation to another facility, significant changes of the fecal microbiota were appreciated for 5 days after administration of the drug.[226] In this study, antibacterial treatment increased the relative abundance of several antibacterial resistance determinants at day 12 and 34. There was also a large shift in the fecal microbiota after the initial transport of the cattle to the feedlot facility.[226]

In pre-weaned piglets, the changes in fecal microbiota between tulathromycin treated and control groups was not statistically different.[227] Fecal microbiota composition and antibacterial resistance gene abundance were changed significantly between sampling days 0, 5, and 20, however. Ultimately, this study demonstrated that the administration of tulathromycin

metaphylaxis does not have any detrimental effects on the fecal microbiota structure or on the abundance of antibacterial resistance genes in pre-weaned piglets.[227]

Clinical use of tulathromycin

Food animals and current label/indications

Presently, tulathromycin is FDA approved to treat bovine respiratory disease complex in cattle and calves associated with *Histophilus somni, Mannheimia hemolytica, Pasteurella multocida,* and *Mycoplasma bovis*.[94,104,228] In cattle, tulathromycin is also approved to treat infectious bovine keratoconjunctivitis associated with *Moraxella bovis* and bovine foot rot associated with *Fusobacterium necrophorum* and *Porphyromonas levii*.[94] In feedlot cattle, the administration of tulathromycin as a treatment for bovine respiratory disease complex (BRDC) resulted in less mortality and decreased cost to producers overall and produced significantly higher rumen pH and increased overall average weight gain of 8.6 kg.[229] When dairy calves at risk for development of BRDC were treated with one dose of tulathromycin at 3 and 46 days of age, body weight and hip width gain were increased compared to the control calves.[230] When calves were experimentally infected with *Mycoplasma bovis*, tulathromycin was effective as a treatment, regardless of the MIC of the challenge strain.[215,231]

Tulathromycin has been shown to be more effective than florfenicol in the prevention or the treatment of bovine respiratory disease complex.[226,227,232] An increased risk for re-treatment has been seen with gamithromycin and enrofloxacin compared to tulathromycin for treatment of BRDC.[202,219,225] When tulathromycin treatment was compared to tildipirosin treatment in calves, the tildipirosin treated calves had significantly less lung consolidation, a significantly lower clinical score, and fewer organisms isolated from bronchial secretions compared to those treated

with tulathromycin, suggesting tildipirosin was more effective in minimizing clinical disease and lung lesions in calves with BRDC caused by *Histophilus somni*.[233] However, in a separate study in which calves were experimentally infected with *Mycoplasma bovis,* treated calves had a lower percentage of lung lesions, lower mortality, fewer days with clinically observed quiet and dull demeanor, and a higher body weight compared to their cohorts that had received tildipirosin.[230]

Tulathromycin has been used in an extra label fashion for treatment of *Leptospira borgpetersenii* serovar *hardjo* type *hardjo-bovis*, as well as being used as a prokinetic. In vitro studies demonstrate activity against *Babesia bovis, Babesia bigemina,* and *Theileria equi.*[234,235] When the administration of tulathromycin via pneumatic dart was compared to the more traditional method of administration via subcutaneous injection to calves, a reduced total body exposure to tulathromycin was seen along with increased acute stress, increased muscle damage, and increased pain at the injection site.[236,237]

In swine, tulathromycin is approved for the treatment of swine respiratory disease caused by *Actinobacillus pleuropneumoniae, Bordatella bronchiseptica, Hemophilus parasuis, Mycoplasma hyopneumoniae,* and *Pasteurella multocida.* Tulathromycin is also approved for prophylactic use in herds where respiratory disease pathogens have previously been identified and diagnosed. In a study that compared treatment with saline, enrofloxacin, and tulathromycin for respiratory disease, tulathromycin treated pigs had significantly reduced lung lesion scores, coughing, and a significantly greater weight gain.[216] Enrofloxacin treated pigs compared to tulathromycin treated pigs had no significant differences in lung weight or weight gains in the tulathromycin group, although coughing and lung lesion scores were greater.[216] In a field study of naturally-occurring swine respiratory disease, the cure rate for tulathromycin-treated pigs was higher than the ceftiofur-treated group and the saline-treated group, demonstrating that

tulathromycin was a safe and effective treatment.[219] Compared to three daily doses of enrofloxacin, one single dose of tulathromycin was as effective for the treatment *Mycoplasma hyopneumoniae* in pigs.[218] Extra label uses include the treatment of *Streptococcus suis,* a cause of pneumonia, meningitis, and septic arthritis in pigs at higher than label doses.[238] Tulathromycin has demonstrated immunomodulatory effects in swine leukocytes *in vitro* and anti-inflammatory effects in pigs *in vivo* in experimental models of *Actinobacillus pleuropneumoniae* infection and nonmicrobial-induced pulmonary inflammation.[239,240]

Tulathromycin is not currently approved for use in small ruminants, although it is used on an extra-label basis, especially for respiratory pathogens in goats.[206] In sheep and goats with *Corynebacterium pseudotuberculosis* infection, the administration of intralesional or subcutaneous tulathromycin resulted in resolution of abscesses in the majority of cases.[94,241,242] The increased abomasal emptying rate in goats after administration of tulathromycin may reduce anorexia, encourage daily weight gain and growth, be used as a treatment for gastrointestinal hypomotility, and ultimately reduce economic loss for producers.[93] Additionally, in pregnant ewes that were experimentally inoculated with *Campylobacter jejuni* and treated with tulathromycin or a sham, the tulathromycin treated group had fewer ewes that demonstrated vaginal bleeding or abortion following experimental infection.[243-245]

Proposed use in horses

Tulathromycin has been used as a treatment for pneumonia with pulmonary abscessation due to *Rhodococcus equi* in foals, although it is not as effective compared to azithromycin or azithromycin-rifampin combinations.[89,98,126,246] Based upon previously reported MIC data in other macrolides (Table 2.2), possible uses of intravenous tulathromycin in equine medicine

include respiratory infections due to gram-positive bacterial infections such as *Streptococcus equi* subsp. *zooepidemicus* and *Streptococcus equi* subsp. *equi.* Other possibilities include treatment of *Actinobacillus equuli* pneumonia and pleuropneumonia, *Lawsonia intracellularis, Corynebacterium pseudotuberculosis,* and Rickettsial diseases. An additional use for tulathromycin may be in the treatment of pneumonia associated with anaerobic bacteria, such as *Fusobacterium necrophorum*. Tulathromycin may also work combined with other drugs for mixed bacterial infections. Moreover, the synergistic combination of tulathromycin combined with rifampin may prove effective for the treatment of gram-positive pneumonia in the presence of abscessation.

Reasons for study of tulathromycin in adult horses

There are few commercially available antibacterial formulations for horses. Products approved in other species may be used, but may not be safe, affordable, or convenient for use in horses. With the continued emergence and persistence of antibacterial resistance, new antibacterial options for horses are needed. Tulathromycin has an appropriate spectrum for treatment of some pathogens seen in equine medicine and its favorable pharmacokinetic properties in other species make it a potential antibacterial alternative for horses. While tulathromycin has been evaluated in foals, the pharmacokinetics of the drug have not previously been reported in healthy adult horses.

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Chapter 3: Pharmacokinetics and pulmonary distribution of Draxxin ® (tulathromycin) in healthy adult horses

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Published in the Journal of Veterinary Pharmacology and Therapeutics. 2021; 00: 1-10

DOI: 10.1111/jvp.12968

Abstract:

The objective of this study was to determine the pharmacokinetics and tolerance of tulathromycin (Draxxin®; 2.5 mg/kg once) after intramuscular (IM), subcutaneous (SC), and slow intravenous (IV) administration to six adult horses. A three-phase design and 4-week washout period were used. Drug concentrations in blood and bronchoalveolar lavage (BAL) samples were determined by ultra-performance liquid chromatography tandem mass spectrometry and pharmacokinetic parameters calculated using noncompartmental analysis. Following SC and IM administration, all horses exhibited sweating, discomfort, and periods of recumbency. As signs were more severe after SC administration this route was only used in 3/6 horses. Intravenous administration of tulathromycin was well tolerated in all horses. Mean bioavailability was 99.4% IM and 115% SC. Mean maximum plasma concentration was 645 ng/ml IM and 373 ng/ ml SC. Mean half-life was 59.8 h, 54.8 h, and 57.9 h for IV, IM, and SC administration, respectively. Mean clearance was 3.25 ml/kg/min, and mean volume of distribution was 16.8 L/kg following IV administration. Drug was detectable in plasma and BAL samples for 120 h following all routes; however, adverse effects may prevent IM use and SC use is not recommended. Tulathromycin may be a practical and affordable antibacterial for use in adult equine patients.

Keywords

adverse drug reaction, antibacterial, equine, macrolides

INTRODUCTION:

Availability of commercial antibacterial formulations that are safe, effective, and affordable for use in horses is currently lacking. In equine medicine, macrolides are most commonly used for the treatment of pneumonia and extrapulmonary conditions caused by *Rhodoccocus equi,* a gram-positive intracellular pathogen that affects foals.[1] Available macrolides include erythromycin, azithromycin, clarithromycin, tilmicosin, gamithromycin, and tulathromycin. Of these, clarithromycin, azithromycin, and erythromycin are more commonly used extra-label in foals, while tilmicosin, tulathromycin, and gamithromycin are more commonly administered to, and labeled for use, in cattle and swine. Chemical structures of the macrolides are similar, although there are small differences that contribute to differences in absorption, distribution, metabolism, and excretion.[2] In horses, adverse effects associated with administration of macrolides most commonly include diarrhea [3,4] and hyperthermia.[5,6] Injection site reactions have been reported in adult horses and foals with subcutaneous (SC) or intramuscular (IM) administration of tilmicosin and tulathromycin, respectively.[4,7]

Tulathromycin, a semi-synthetic long-acting macrolide, is currently approved in the United States and other countries for the treatment of respiratory disease in cattle and swine.[8] The pharmacokinetics and pharmacodynamics of tulathromycin have been previously evaluated in foals, and those studies supported the use of the drug in the treatment of bacterial respiratory disease with a prolonged dosing interval.[9,10] Extra-label use is also common in goats for the treatment of respiratory disease and caseous lymphadenitis.[11,12] Previous pharmacokinetic studies of tulathromycin in cattle, swine, deer, bison, and foals indicate that tulathromycin is rapidly absorbed following SC or IM injection and has a prolonged half-life in the pulmonary epithelial lining fluid (PELF).[9,13-15]

The pharmacokinetics and safety of tulathromycin in adult horses have not previously been reported, although there are anecdotal reports of its use in equine clinical cases. The objective of this study was to determine the plasma pharmacokinetics, pulmonary distribution and tolerance of tulathromycin after IV, IM, and SC administration to healthy adult horses.

MATERIALS AND METHODS:

Animals and experimental design

This study was approved by the Institutional Animal Care and Use Committee (IACUC) at Virginia Polytechnic Institute and State University (IACUC # 18-249, approval date November 15, 2018). Six healthy adult horses, (3 geldings and 3 mares; 4 Thoroughbreds, 1 American Paint Cross, and 1 Warmblood), with a mean body weight of 537 kg (range 448-577 kg) and a mean age of 14 years (range 9-19 years) were used. Horses were deemed healthy based on physical examination and baseline complete blood count and serum biochemical profiles performed prior to each drug administration. Weights were obtained the day prior to each drug administration and dosing adjusted accordingly. This study was conducted in three phases with a 4-week washout period between each phase. Six horses were treated in phases one and two, whereas only three horses were treated in phase three. Utilizing a random number generator, horses were assigned to receive either IM or SC tulathromycin in phase one. Based on adverse effects noted in the SC group, this route was not used in subsequent phases. In the second phase, horses then received either IM or IV tulathromycin, with the remaining horses receiving IV tulathromycin in the third phase.

Monitoring for adverse effects

Injection sites and catheter insertion sites were monitored daily for signs of swelling, pain, discharge, and heat. Number of days of swelling at a particular injection site, if noted, was recorded. Physical examinations were performed every 12 hr throughout the sampling period and were continued in any horse that developed adverse effects until normal. Fecal output and consistency were closely monitored in all horses for the duration of the sample collection period. Blood samples were collected for measurement of creatine kinase prior to drug administration and 24, 72, and 120 hr after each route of drug administration. During IV administration, horses were closely monitored for development of venous thrombosis and any indications of toxicity due to the drug vehicle (propylene glycol) during the administration period, including salivation, sweating, ataxia, and signs of pain.

Drug Administration

Horses were administered tulathromycin (Draxxin® 100 mg/mL; Zoetis US, Parsippany, NJ, USA) as a single dose of 2.5 mg/kg. For the IM and SC dosing, tulathromycin administration was performed in one site at the caudal aspect of the neck, without redirection of the needle, on the side opposite IV catheter placement. The total volume of injection was less than 15 mL for each horse and the needle was changed between drawing up the drug and injecting into the animal. Intramuscular injections were performed with a 22 ga 1.5" needle while subcutaneous injections were performed using a 23 ga 1" needle. The side of administration varied with each administration (i.e., if a horse received the SC injection in the left cervical neck, the IM administration site was the right cervical neck musculature). Due to adverse drug reactions, only three horses received the drug via the SC route; all 6 horses received IM and IV administration. For IV administration, a second IV catheter was placed on the side of the neck opposite the IV catheter used for blood sample collection. For the IV dosing, tulathromycin was diluted with

0.9% sterile saline (0.9% NaCl; Hospira, Lake Forest, IL, USA) to a volume of 60 mL and administered via syringe pump (Medfusion 3500, Smith Medex, Minneapolis, MN, USA) over a period of 15 minutes.

Collection of blood samples

Intravenous catheters (14 ga, 5 ¼", Mila International, Inc, Florence, KY, USA) were aseptically placed in the jugular vein following desensitization of the skin with 2% lidocaine hydrochloride SC. Blood samples were collected from an indwelling jugular catheter with a 7", extension set (Mila International, Inc, Florence, KY, USA). The total volume of the catheter and extension set was approximately 2 mL. Waste blood (10 mL) was collected and discarded prior to sample collection (6 mL). Samples were taken immediately prior to drug treatment (time 0) and at 15, 30, 45 minutes and 1, 1.5, 2, 4, 8, 12, 24, 48, 72, 96, and 120 hr after drug administration. An additional 5-minute sample was collected at the end of the IV administration. Jugular catheters were flushed prior to and immediately after sample collection with heparinized saline. Catheters were removed after 24 hr and all further samples were collected via direct venipuncture using 12 mL syringes and 20 ga 1.5" needles. Samples were placed in plastic blood tubes containing lithium heparin and centrifuged at 1200 x g for 10 minutes. Plasma was harvested and stored at -80°C until analysis. Catheter sites were monitored for the duration of the study for any discharge, heat, swelling, or pain.

Bronchoalveolar lavage sample collection and processing

Bronchoalveolar lavage (BAL) was performed at 24, 72, and 120 hr after IV and IM administration. Due to the fact that some horses were still exhibiting adverse effects 24 hours after SC drug administration, BALs were not performed after this route. For sample collection,

horses were sedated with xylazine (0.4-0.7 mg/kg) and butorphanol (0.01-0.016 mg/kg) IV and restrained appropriately. The BAL fluid was collected blindly using a cuffed BAL catheter passed through the ventral meatus and into the trachea while instilling 10 ml of 2% lidocaine hydrochloride diluted in 20 mL of saline (0.9% NaCl) solution. The catheter was then wedged in a distal bronchus and 240 mL of sterile isotonic saline solution, divided into 60 mL aliquots, was rapidly infused. Fluid was aspirated immediately after infusion of the last aliquot. The total volume of fluid recovered was recorded using a graduated cylinder, and the individual samples were then pooled and placed on ice until sample processing, which occurred within 1 hour of collection. Blood samples were collected immediately prior to performing the BAL for determination of plasma urea concentration.

After collection, a 5 mL sample of the fresh pooled BAL fluid was removed for determination of cell counts. Total nucleated cell count in BAL fluid was determined by use of an automated cell counter (Cellometer® Auto T4; Nexcelom Bioscience, Lawrence, MA, USA). Slides of the BAL fluid were prepared by cytocentrifugation, air-dried and then stained using a modified Wright-Giemsa stain. A differential cell count was determined by examination of 200 cells. Four 10 mL aliquots of the pooled BAL fluid were centrifuged at 200 x g for 10 minutes to separate BAL cells from BAL supernatant. Supernatant fluid was stored, in duplicate, at -80° C until assayed for tulathromycin concentrations. A separate aliquot was also stored for determination of urea concentration. The cell pellet was resuspended in 500 µL of Hanks balanced salt solution, vortexed for one minute, and frozen at −80°C until assayed.

Urea determination in plasma and BAL fluid

The amount of pulmonary epithelial lining fluid (PELF) sampled from BAL fluid was estimated utilizing the urea dilution method as described previously.[16,17] Briefly, BAL fluid and

plasma samples were thawed to room temperature and processed all at once, in duplicate. Urea concentration in plasma and BAL fluid were determined using a commercially available quantitative colorimetric urea detection kit per manufacturer's instructions (Abnova, Walnut, CA, USA). In order to determine urea concentrations, the following calculations were utilized:

$$Urea_{PLASMA} = ((OD_{sample} - OD_{blank})/(OD_{standard} - OD_{blank})) \times 1 \times 50 \text{ mg/dL}$$

$$Urea_{BAL} = ((OD_{sample} - OD_{blank})/(OD_{standard} - OD_{blank})) \times 1 \times 5 \text{ mg/dL}$$

The volume of PELF (V_{PELF}) in BAL samples was then calculated as $V_{PELF} = V_{BAL} \times (Urea_{BAL}/Urea_{PLASMA})$, where V_{BAL} is the volume of fluid recovered during the BAL procedure. The concentration of tulathromycin in PELF (TUL_{PELF}) was then derived from the following relationship: $TUL_{PELF} = TUL_{BAL} \times (V_{BAL}/V_{PELF})$, where TUL_{BAL} is the measured concentration of tulathromycin in BAL fluid. The concentration of tulathromycin in BAL cells was calculated using the following relationship: $TUL_{CELL} = (TUL_{PELLET}/V_{CELL})$ where TUL_{PELLET} is the concentration of antibacterial in the cell pellet supernatant and V_{CELL} is the mean volume of BAL cells (1.20 μL/10^6 BAL cells).[18,19] Concentrations were corrected to a volume of 2.6 x 10^6 cells.

Chromatographic Assay

Concentrations of tulathromycin were measured using an Acquity H-class ultra-performance liquid chromatography with a Xevo TQD tandem mass spectrometer (UPLC-MS/MS; Waters Corporation, Milford, MA, USA). Calibration curves were prepared daily by fortifying blank equine plasma with stock solutions of tulathromycin and the internal standard tulathromycin-d7 (Td7). Concentrations were linear over a range of 20 – 3,920 ng/mL plasma.

Plasma standards and samples were all subjected to a protein precipitation method by adding 300 µL of acetonitrile (ACN) + 1% formic acid (FA) containing the internal standard (333 ng/mL solution) to 100 µL of plasma. Precipitated samples were vortexed before centrifugation. The supernatant was diluted by adding 100 µL to 900 µL of 1% formic acid in water and 5 µL of this solution was then injected onto the UPLC-MS/MS for analysis.

Tulathromycin was separated on an Acquity UPLC BEH Phenyl column (2.1 mm ID x 100 mm x 1.7 µm; Waters Corporation, Milford, MA, USA) and matching guard column. A gradient elution method was used with 1%FA in H2O and 1%FA in ACN, with a flow rate of 0.4 mL/min (see supplemental Table 3.1). Tulathromycin was quantified by multiple reaction monitoring (MRM) of its doubly charged parent ion $[M+2H]^{2+}$ with transitions of 403.9 > 158.1 (quant), 403.9 > 577.4 (qual) for tulathromycin and 407.5 > 158.2 (quant), 407.5 > 577.4 (qual) for tulathromycin-d7. Additional tuning parameters for the mass spectrometer can be found in supplemental Table 3.2. This method had a limit of quantification (LOQ) of 20 ng/mL and a limit of detection (LOD) of 5 ng/mL based on S/N = 3. The coefficient of determination (R^2) for all curves was >0.999. Accuracy and precision of the assay were determined using 5 standard curves and calculated at concentrations of 20, 400 and 3,920 ng/mL. Accuracy was within (mean ± SD) 2.67 ± 2.58% of the true value and intra-assay precision was within 2.46 ± 1.63% of the mean.

Bronchoalveolar lavage fluid samples were prepared by combining 350 µL of 1% (v/v) FA in water, 50 µL of Td7 internal standard addition solution (50 ng/mL), and 100 µL of the BAL fluid samples to the bottom half of a 0.2 µm syringeless filter vial (Separa®; GVS North America, Stanford, ME, USA). The filtered samples were vortexed to homogenize before being placed in the refrigerated autosampler of the UPLC-MS/MS for analysis. Cell pellet samples

were diluted with 1 mL of 18.2 M-Ohm deionized water and placed in an ultrasonic bath for 15 minutes to disrupt the cells, then extracted as described for the BAL fluid samples.

Calibration curves for determination of BAL fluid and cell concentrations were made up in blank BAL fluid collected from a healthy horse not used in the study. Calibration standards were prepared in the same manner as the samples, fortified with tulathromycin within a range of– 1 - 100 ng/mL. The coefficient of determination (R^2) for all curves was >0.999, and all standard values were within ±10% of the expected range. The system had a LOD of approximately 0.3 ng/mL BAL fluid and an LOQ of 1 ng/mL BAL fluid.

Pharmacokinetic analysis

Plasma tulathromycin concentrations were analyzed using noncompartmental methods on commercially available computer software (Phoenix WinNonlin, version 6.3, Certera, Raleigh, NC). Values below the LOQ were not included in the analysis. The maximum plasma concentration (C_{max}) after dosing and time to C_{max} (T_{max}) were reported directly from the data for extravascular routes. Terminal half-life ($T_{½\lambda z}$) was determined based on the slope of the terminal phase of the plasma concentration versus time curve. Area under the plasma concentration versus time curve (AUC) was determined using the linear trapezoidal rule. The AUC was calculated up to the last measured time point (AUC_{last}) and extrapolated to infinity using the constant (C_{last}/λ_z), where C_{last} is the plasma concentration at the last timepoint above the LOQ, and λ_z is the terminal rate constant. Systemic clearance (Cl) and apparent volume of distribution (Vd) were calculated following IV dosing. Absolute bioavailability for SC and IM administration were determined based on the ratio of the $AUC_{0-\infty}$ following extravascular administration to the $AUC_{0-\infty}$ following IV administration. Values are reported as geometric mean (range).

Statistical analysis

Statistical analysis was performed using SigmaPlot 14.0 (SysStat Software, Inc, San Jose, CA, USA). The Wilcoxon Rank Sum test was used to assess significant differences between the reported values following the IV and IM routes of administration, including λ_z, T $\frac{1}{2}\lambda z$, $AUC_{0-\infty}$ and AUC_{last}. For non-normally distributed data (CK activity), the Kruskal-Wallis one-way ANOVA on ranks was performed. Significance was set at $P<0.05$. Due to the small number of horses that received SC dosing (n = 3), data from this route of administration was excluded from all statistical analyses.

RESULTS:

Following SC administration, all horses exhibited signs of substantial discomfort, including tachycardia, pawing, flehmen response, full-body sweating, disinterest in hay, head shaking, and sternal or lateral recumbency. Signs began within 30 minutes of drug administration, occurred intermittently for up to 24 hr and were fully resolved by 36 hr after drug administration. One horse developed significant lameness in the forelimb corresponding to the side of the SC injection that persisted at 24 hr after administration. This horse was administered a single dose of flunixin meglumine (1.1 mg/kg IV) which resulted in substantial improvement, and no further treatment was required. No other horses were administered analgesic medications. Due to the consistent and notable adverse effects, only 3 horses were administered SC tulathromycin.

Following IM tulathromycin administration horses exhibited intermittent mild signs of discomfort, including pawing, sweating, disinterest in hay, and sternal or lateral recumbency beginning within 60 minutes of administration and fully resolving by 24 hr after administration. No horse required analgesic medications following IM drug administration.

Each horse (6/6 IM; 3/3 SC) that received SC or IM tulathromycin developed injection site reactions including focal sweating, edema and swelling at the injection site. Reactions were detectable for up to 36 hr. Creatine kinase (CK) values were elevated above the reference range for the laboratory (115-441 IU/L) at one or more time points in 6/6, 2/3 and 1/6 horses following IM, SC and IV administration, respectively (Figure 3.1). At 24 hr following IM administration, horses had significantly increased CK values (mean 1005 ± 288 IU/L) compared to all other time points after IV administration and compared to the baseline and 120 hr sample after IM administration ($p < 0.001$; Figure 3.1). Horses receiving IV tulathromycin did not exhibit any signs of adverse effects related to the drug or vehicle during or after administration. No horses in any administration group developed diarrhea and each horse remained afebrile and produced normal manure throughout the study period.

Plasma concentration (semi-log) versus time curves for tulathromycin are presented in Figure 3.2. Drug was detected at all time points in all horses, with the exception of 1 horse that had no detectable drug 120 hr following both IV and IM administration. Mean C_{max} was higher following IM versus SC administration (645 ng/mL versus 373 ng/mL, respectively) and T_{max} was greater with SC versus IM dosing (1.5 hr versus 0.75 hours, respectively). The half-life was long following administration via any route, with no significant difference detected between IV or IM administration ($p = 0.394$). Mean (range) half-life was 59.8 hr (49.6-70.1 hr), 54.8 hr (37.5-63.5 hr) and 57.9 hr (53.4-62.5 hr) for IV, IM and SC administration, respectively. No significant differences were detected between other pharmacokinetic values tested. Systemic plasma clearance after IV administration was 3.25 (2.79-3.60) mL/kg/min, and volume of distribution was 16.8 (12.6-20.9) L/kg. Mean absolute bioavailability was calculated as 99.4% (range 86.3-111%) and 115% (range 11-123%) for IM and SC administration, respectively.

Additional relevant pharmacokinetic parameters are summarized in Table 3.1 (Raw data is available in Supplemental Table 3.3).

Tulathromycin was detected for up to 5 days post administration following IV and IM administration in PELF and BAL cells. Following IV administration, the highest mean (± SD) concentration measured in PELF was 796.59 ± 311.36 ng/mL and occurred at 24 hr in 5/6 horses and 120 hr in one horse. Following IM administration, the highest concentration measured in PELF was 981.37 ± 452.9 ng/mL and occurred at 24 hr in 6/6 horses. Concentration of tulathromycin in PELF was significantly higher at 24 hr in the IM group compared to the 72 hr IM ($p = 0.003$) and IV sample ($p = 0.002$). Due to possible contamination of the IV BAL samples at 120 hr, comparisons of that time point were not made.

Mean percent recovery of BAL fluid and median alveolar cell differential counts are reported in supplemental Tables 3.4 & 3.5. Following IV administration, the highest concentration of tulathromycin measured in BAL cells was 36.01 ± 20.25 ng/mL and occurred at 72 ± 30.36 hr. Following IM administration, the highest concentration measured in BAL cells was 26.49 ± 6.06 ng/mL and occurred at 80 ± 19.6 hr. There was no significant difference in BALC concentration at any time point. The ratio of tulathromycin in PELF and BAL cells compared to corresponding plasma concentrations are presented in Table 3.3. Tulathromycin concentrations in PELF were up to 10.3 and 12.5 times greater than concentrations in plasma for IV and IM administration, respectively. There were no significant differences detected at any timepoint for the PELF to plasma ratio. The BAL cell to plasma ratio was <1 at all time points for both routes, with no significant difference detected at any time point.

DISCUSSION:

The pharmacokinetics of tulathromycin in horses determined in this study are typical of macrolide antibacterials in general and characterized by a long half-life, high volume of distribution and slow clearance. Bioavailability via both extravascular routes of administration was complete. Bioavailability following SC administration was >100% in all 3 horses that received the drug via this route, although the statistical significance of this was not determined due to the small number of horses receiving drug via this route. The use of $AUC_{0-\infty}$ to determine the bioavailability may contribute to bioavailability > 100% if the percent extrapolated differs greatly between routes of administration. This did not occur in the present study, and bioavailability was also > 100% when AUC_{last} was used in the calculation. Another potential explanation would include the lack of sampling during the infusion phase for IV administration resulting in an underestimation of the AUC via the IV route. This would also affect the bioavailability reported for the IM route, where some horses also had bioavailability calculated as > 100%. Additionally, 'flip-flop' kinetics, which occur when the rate of absorption of a drug is significantly slower than its rate of elimination from the body may be occurring.[20] This is supported by the prolonged T_{max} via the SC route, and the fact that the plasma concentration versus time curves for SC and IV administration are not parallel for up to 48 hr after administration (Figure 3.1). Flip-flop kinetics also typically result in a longer terminal half-life via the extravascular route. When the T½ for only the three horses that received drug via both the SC and IV routes are directly compared, the T½ is longer following SC administration (58.1, 62.5 and 53.4 hr for horse B, D and F, respectively) compared to IV administration (56.0, 54.6 and 49.6 for horse B, D and F, respectively). The longer absorption from the SC injection site may also explain the prolonged adverse effects noted via this route, assuming the adverse effects were due to local irritation at the injection site. Absorption was more rapid with IM compared to

SC administration and subsequently adverse effects were noted for a shorter time period following IM administration.

Intramuscular and SC administration of macrolides are associated with injection site reactions in foals and other species, potentially related to the drug vehicle.[7,21] When tulathromycin was administered to foals IM, swellings at the injection site were reported as an adverse effect,[4] similar to what was observed in this study with SC and IM injections. Subcutaneous injection in adult horses was associated with more patient discomfort than IM injection, while there were no adverse effects after IV administration, further supporting the theory that the adverse effects were due to pain at the injection site. Extravascular administration may be more tolerable when commercially available tulathromycin formulations are diluted in sterile water or saline; however, that would increase the total volume injected at the site and may ultimately require multiple injection sites in order to administer the proper dose. The degree of discomfort associated with subcutaneous administration was unexpected and considered unacceptable by the authors, therefore only 3 horses received drug via this route and BAL sampling was not performed.

In this study, IV administration of tulathromycin to healthy adult horses produced no clinically detectable adverse effects. Tulathromycin is not labelled for IV use in any species, however, and due to concerns over a potential reaction to the drug or drug vehicle when administered IV, the drug was diluted and administered as a slow IV infusion over 15 minutes. Propylene glycol, the vehicle in the tulathromycin formulation, has known and reported adverse effects when administered orally to horses, including depression, hypersalivation, sweating, ataxia, colic, abnormal breath odor, cyanosis, and dyspnea.[22-24] Intravenous administration of propylene glycol as a drug vehicle for chloramphenicol has also been reported to cause severe

adverse effects, including alterations in GI motility, diarrhea, ataxia and even death.[25] It remains unknown if more rapid administration of undiluted drug would be safe and therefore it should be given by slow IV injection and/or diluted in a larger volume of fluid for more rapid administration.

The most common and serious adverse effect of macrolide administration in horses is colitis, which may be fatal.[26] Diarrhea induced by macrolide antibacterials is thought to be related to a shift in the gastrointestinal flora, or a potential prokinetic effect.[27,28] Despite this, no horses in this study developed diarrhea, and they continued to pass normal amounts of manure throughout the sampling period. Effects on GI flora were not assessed.

Macrolides are often used for the treatment of respiratory infections in animals. There are several ways to determine antibacterial concentrations in the lung. For macrolides, the most common method reported is the determination of drug concentrations in the PELF, and this method was used in the present study. Pulmonary epithelial lining fluid is heterogenous across the respiratory tract and distributes continuously throughout the respiratory tract, including in the alveoli.[1,29,30] Additionally, it is a potential site of bacterial colonization and bacterial/drug interactions, therefore the pharmacokinetics of a drug within PELF may be relevant when evaluating a drug's antibacterial effect.[1] Previous work has reported that drug kinetics and accumulation of a drug within the PELF are the primary factors known to contribute to the antibacterial response.[31,32]

Tulathromycin administered to adult horses resulted in high concentrations in the PELF at all sampled time points, up to 5 days post-administration. Maximum PELF concentrations for the IM and the IV groups occurred at 24 hr in most horses, declining slowly over subsequent sample times. This is similar to the results reported with IM administration to foals.[10] Maximal

PELF drug concentrations in calves administered SC tulathromycin peaked later (approximately 72 hours).[33] PELF concentrations with SC administration were not determined in this study, but the longer T_{max} for plasma and potential prolonged absorption from the injection site suggest a similar phenomenon may occur in horses. As has been previously reported with other macrolide antibacterials, tulathromycin concentration was greater in PELF at all time points compared to plasma.[18,34-39] Tulathromycin is a weak base with a pKa of 8.6-9.6 that can accumulate in acidic environments, such as PELF, due to ion trapping.[40-42] The high concentration of drug in the PELF, a common site of bacterial colonization, suggests a potential therapeutic use for horses with pneumonia.

Many macrolides accumulate intracellularly. For example, mean azithromycin maximum concentrations in foals were reported to be 60 times higher in BAL cells than corresponding serum concentrations and to persist for at least 10 days after a single oral dose.[37] Similarly, that study reported clarithromycin concentrations in BAL cells 78.9 times higher than corresponding serum concentrations, although they did not persist as long following single dose administration. Erythromycin does not accumulate to the same extent as the newer macrolide antibacterials with a BAL cell to serum ratio of only 1.3, which is similar to that reported for tulathromycin following a single IM dose to foals[10,43] and closer to the ratios reported in the present study that show minimal accumulation of tulathromycin in BAL cells, with plasma to cell ratios of < 1. Venner et. al. (2010) showed that tulathromycin persisted for an extended period in BAL cells in foals, and drug accumulated in cells with multiple doses, resulting in cell concentrations approximately 7 and 4 times higher than serum at 24 and 192 hours, respectively, after the final dose.[10] The persistence of drug in BAL cells for at least 120 hours after dosing reported in the present study suggests accumulation would occur with multiple doses in adult horses as well.

The differences in intracellular drug concentration reached among macrolide antibacterials likely relates to the lipophilicity of the individual drugs; tulathromycin is readily soluble in water,[44] whereas azithromycin and clarithromycin are more lipophilic. Lipophilicity is a major determinant of drug transport across cellular membranes and therefore lipophilic drugs would be expected to reach higher intracellular concentrations. Despite this higher water solubility, tulathromycin still demonstrates a high volume of distribution and a slow clearance. Potential reasons for this may include extracellular tissue binding creating a drug depot, active transport mechanisms resulting in drug sequestration in tissues or non-inflammatory cells, or ion trapping of drug within acidic tissues.[15]

In most studies reporting BAL cell concentrations of macrolides in foals, it is assumed that the majority of cells in the sample are alveolar macrophages, which are the predominant cell found in BALF from horses.[45] The differential cell counts from BAL fluid in this study showed a higher lymphocytic cell population (supplemental Table 3.4) than expected (median 62.75%, range 34.5-76%) and a lower macrophage cell population (median 29.5%, range 17.5-43.5%). While this was unexpected, ranges are close to those reported by McGorum and Dixon (1994), with lymphocyte populations ranging up to 51.3% in normal horses and macrophage populations as low as 36%.[45] Tulathromycin BAL cell concentrations reported here are based on an average cell volume of 1.2 μL/10^6 cells, as determined in previous foal studies.[18] This altered cell population may have resulted in a lower reported BAL cell concentration. Overall, the BAL cell and PELF tulathromycin concentrations reported in this study are less than what was reported when tulathromycin was administered at the same dose intramuscularly to foals[10] which may be attributed to differences in physiology and anatomic size between foals and adult horses.

The pharmacokinetic-pharmacodynamic interactions necessary for therapeutic success with macrolide antibacterials are not well defined and plasma concentrations of drug are often below the MIC of the bacteria for all or a substantial part of the dosing interval. The therapeutic success for these drugs in treating respiratory disease is therefore thought to relate more to the wide distribution and high concentrations within the pulmonary compartment, including PELF, BAL cells, and neutrophils.[1] Nevertheless, as pulmonary samples are difficult to obtain in clinical cases, attempts have been made to determine effective drug dosing regimens using plasma concentrations as a surrogate. In calves, the optimal AUC_{24h}:MIC ratio reported for *Mannheimia hemolytica* and *Pasteurella multocida* is 24 hr for bactericidal activity.[46] Based on these criteria, tulathromycin at 2.5 mg/kg in horses would only be effective for bacteria with MICs < 0.25 μg/mL. It should also be noted that the $AUC_{0-\infty}$ reported by Toutain et al (2017) was almost 3 times higher than what has been reported in the literature for other calf studies, which may have affected results.[46] There is also some suggestion that the long half-life and persistence of macrolide antibacterials in the plasma and PELF mean that the exposure throughout the entire dose interval (τ) and therefore the AUC_{τ}:MIC ratio may be a better predictor of therapeutic success for newer macrolide antibacterials, however a specific ratio has not been reported for tulathromycin.[47]

The Central Laboratory Standards Institute (CLSI) uses a breakpoint MIC of ≤ 16 μg/mL for tulathromycin in cattle for treatment of respiratory disease pathogens. The breakpoint reported by the European Medicines Agency/Committee for Medicinal Products for Veterinary Use is ≤ 8 μg/mL. These breakpoints were determined using clinical efficacy endpoints, rather than PK-PD relationships. More recently, Toutain et al (2017) indicated that evaluation of susceptibility in Mueller-Hinton broth (MHB) rather than in serum results in higher MIC

values.[46] Based on a ratio for MHB to serum of 50, the corresponding breakpoint in calves would be 4 μg/mL. Currently, we have little data available on equine pathogens regarding susceptibility to tulathromycin. One study out of Germany determined MICs for tulathromycin against beta-hemolytic streptococci isolated from the respiratory or genital tract of horses and reported a MIC_{90} of 4 μg/mL.[48] Care needs to be taken when extrapolating this information to the possible MICs against similar organisms in other regions due to regional differences in animal husbandry and management, and bacterial susceptibility. Pharmacokinetic parameters determined in this study for horses are similar to those reported in cattle (Table 3.2), suggesting that tulathromycin may have utility for treating gram-positive bacterial infections caused by beta-hemolytic streptococci, such as *Streptococcus equi* var *zooepidemicus* and *Streptococcus equi* var *equi.* Based on clinical applications in other species, other potential uses for tulathromycin in horses include rickettsial infections, as well as internal infections with *Corynebacterium pseudotuberculosis*. Additional information is needed regarding clinical efficacy and susceptibility of equine specific pathogens.

In conclusion, the results of this study support further investigation of tulathromycin for use in adult horses. The drug exhibits a long half-life in plasma and high concentrations in the PELF after administration and, as a long-acting antibacterial, tulathromycin may have utility both in the field and clinic/hospital setting. The data represented in this study suggest a single dose of tulathromycin in adult horses may be effective for the treatment of susceptible bacteria, particularly those causing disease of the respiratory tract. A potential dosing interval of 5-7 days is suggested for further study to determine the pharmacokinetics and pharmacodynamics of multiple doses, based on the prolonged detection of drug in the respiratory tract. The adverse effects and injection site reactions from extravascular parenteral administration may, however

limit its use to slow IV infusion. Additional studies to determine the safety of multiple doses, as well as further information on bacterial susceptibility are needed.

FUNDING:

Funding for this study, as well as the Draxxin® used in this study, was provided by Zoetis.

ACKNOWLEDGEMENTS:

The authors would like to thank Jess Castellanos, Payton Lawrence, Karen Ingerson, Lisa Thomas, Kayla Turner, Charleez Simcik, Dr. Megan Marchitello, Dr. Jairo Perez, and Dr. Emily Schaefer at the Marion duPont Scott Equine Medical Center for the use of the facility and for their help with the sample collection and processing portions of this project.

DECLARATION OF ETHICS

The authors declare that they have adhered to the Principles of Veterinary Medical Ethics of the AVMA.

CONFLICTS OF INTEREST The authors express no conflicts of interest with this study.

AUTHOR CONTRIBUTION: Drs. Leventhal, McKenzie, Estell and Davis were all involved in study design, sample collection and manuscript preparation. Dr. Davis performed the pharmacokinetics and statistical analysis. Mr. Council-Troche was involved in assay development, sample analysis and manuscript preparation.

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Table 3.1. Noncompartmental plasma pharmacokinetic parameters reported as geometric mean (range) for tulathromycin following IV, IM or SC administration of 2.5 mg/kg to horses.

Parameter	IV (n = 6)	IM (n = 6)	SC (n = 3)
C_{max} (ng/mL)	---	645 (328-1050)	373 (297-427)
C_{5m} (ng/mL)	4030 (3070-5390)	---	---
T_{max} (hr)	---	0.750 (0.500-1.50)	3 (1.50-12.0)
λ_z (hr^{-1})	0.011 (0.010-0.014)	0.013 (0.011-0.018)	0.012 (0.011-0.013)
T ½ (hr)	59.8 (49.6-70.1)	54.8 (37.5-63.5)	57.9 (53.4-62.5)*
$AUC_{0-\infty}$ (hr*ng/mL)	12800 (11600-14900)	13000 (10800-15100)	14900 (14100-15800)
AUC_{extrap} (%)	19.0 (14.2-22.5)	16.6 (12.3-20.6)	14.4 (13.4-15.1)
AUC_{last} (hr*ng/mL)	10300 (8980-12200)	10800 (9510-12100)	12800 (12000-13400)
Cl (mL/kg/min)	3.25 (2.79-3.60)	---	---
Vd_z (L/kg)	16.8 (12.6-20.9)	---	---
F (%)	---	99.4 (86.3-111)	115 (111-123)

C_{max} = Maximum plasma concentration. C_{5m} = Plasma concentration at the first sample point after IV infusion. T_{max} = Time to maximum concentration. λ_z = Terminal rate constant. T ½ = Terminal-phase half-life. $AUC_{0-\infty}$ = Area under the concentration-time curve from time zero extrapolated to infinity. AUC_{extrap} = Percentage of AUC that was extrapolated. AUC_{last} = Area under the concentration-time curve to the last quantifiable concentration. Cl = Total plasma clearance after IV administration. Vd_z = Volume of distribution of the terminal phase after IV administration. F = Bioavailability. --- = Not applicable.

* May not represent a true elimination half-life due to possible flip-flop effect.

Table 3.2. Comparative plasma pharmacokinetic parameters for tulathromycin at 2.5 mg/kg in other species compared to the present study. See table one for definition of pharmacokinetic terms.

Species and route	***n***	**T1/2 (h)**	**T_{max} (h)**	**C_{max} (ng/mL)**	**AUC_{inf} (h*ng/mL or h*ng/g)**	**Reference**
Beef calves-SC	36	90.0	1.80 ±3.00	500 ± 400	18,700 ±1800	Nowakowksi et al. (2004)
Holstein calves-SC	24	64.0	3.00	277	-	Cox et al. (2010)
Foals*-IM	10	105	2.47 ± 4.09	464 ± 178	-	Venner et al. (2010)
Foals*-IM (steady-state)	10	140	0.360 ± 0.110	675 ± 174	-	Venner et al. (2010)
Horses-SC	6	57.9	3.00	373	14900	Present study
Horses-IM	6	54.8	0.750	645	13000	Present study
Horses-IV	6	59.8			12800	Present study

* Foals 50-71 days of age

Table 3.3. Mean ± SD tulathromycin concentration (ng/mL) in plasma, pulmonary epithelial lining fluid (PELF) and bronchoalveolar lavage cells (BALC) with the corresponding ratio of pulmonary to plasma concentration ratios. Different letters represent significant differences of the measured variable between routes of administration and different time points within those routes at $p < 0.05$ probability level.

Route	Time (hr)	Plasma	PELF	PELF: Plasma	BALC	BALC: Plasma
IV	24	72.7 ± 15.0	710 ± 320[a,b]	9.59 ± 3.09	15.2 ± 10.0	0.22 ± 0.16
	72	39.5 ± 5.40	402 ± 106[a]	10.3 ± 2.76	33.3 ± 23.6	0.83 ± 0.57
	120	28.3 ± 2.66	466 ± 458 [a,b]	NR	14.3 ± 7.64	0.42 ± 0.19
IM	24	123 ± 37.0	981 ± 453[b]	7.87 ± 2.06	15.6 ± 10.9	0.14 ± 0.09
	72	40.0 ± 10.2	431 ± 230[a]	11.0 ± 5.42	17.7 ± 10.8	0.48 ± 0.28
	120	28.3 ± 6.18	342 ± 207 [a,b]	12.5 ± 9.90	14.5 ± 10.6	0.57 ± 0.5

NR – not reported

Figures

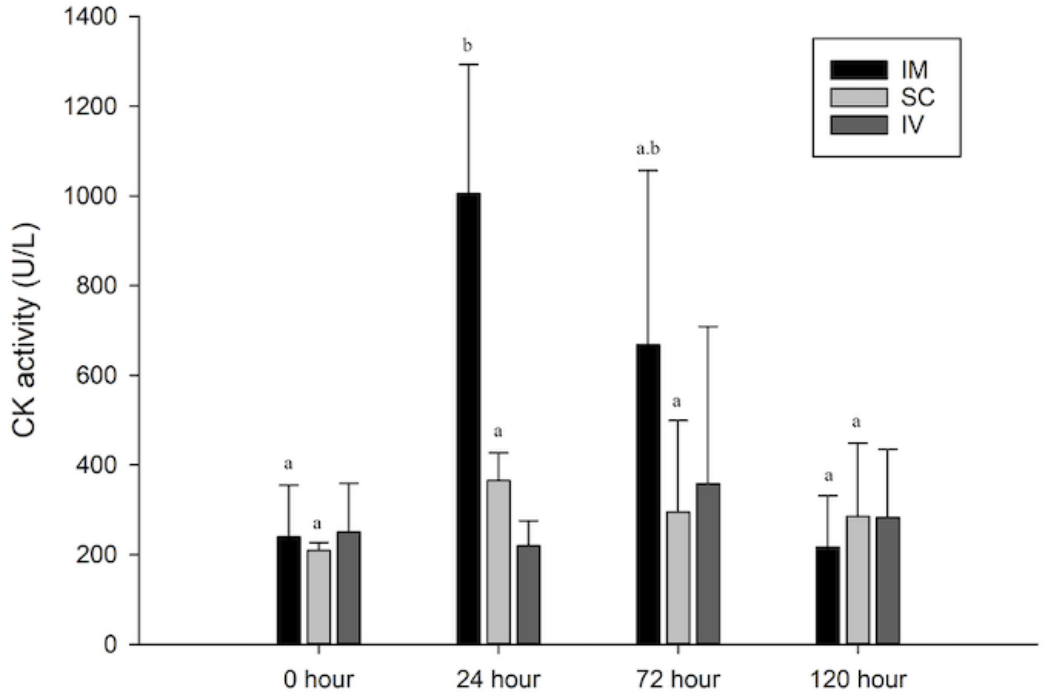

Figure 3.1. Creatine kinase values (U/L) reported as the mean (± standard deviation) values for each route of administration from 0 hr up to 120 hr post administration. Different letters represent significant differences compared to baseline and compared between routes at each time point at $p < 0.05$ probability level. Statistics were not performed on the SC data due to the small number of horses.

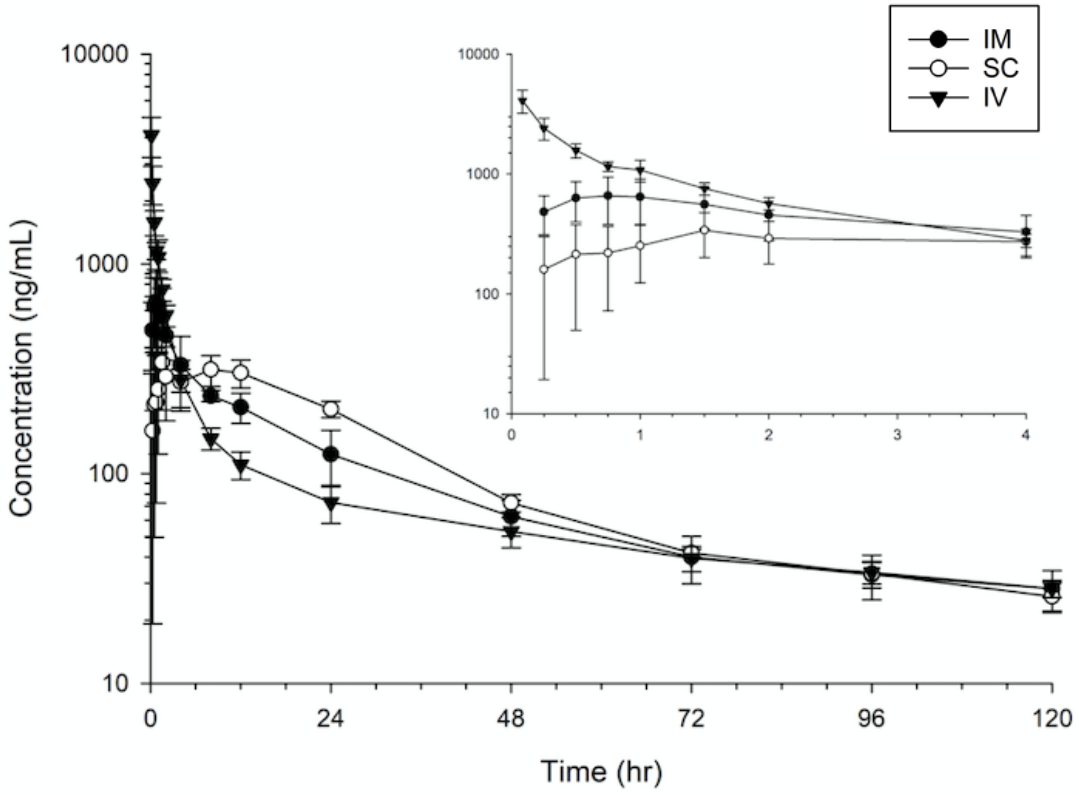

Figure 3.2. Semi-logarithmic graph representing plasma concentration versus time curves for IV (closed triangle; n=6), IM (closed circle; n = 6), and SC (open circle; n = 3) administration of tulathromycin at 2.5 mg/kg to healthy adult horses. Data are presented as mean ± standard deviation at each time point. The inset represents plasma concentrations during the initial 4 hours after to dosing to highlight differences in drug concentrations.

Supplemental Information

Time (mins)	%A (1%FA in H2O)	%B (1%FA in ACN)
0.00	85	15
0.25	85	15
2.00	2	98
3.00	2	98
3.01	85	15
5.00	85	15

Supplemental Table 3.1. UPLC gradient method used for the tulathromycin analysis.

Parameter	Value
Capillary (kV)	0.60
Cone (V)	30
RF (V)	2.50
Extractor (V)	3.00
Source Temperature (°C)	150
Desolvation Temperature (°C)	600
Cone Gas Flow (L/Hr)	10
Desolvation Gas Flow (L/Hr)	1000

Supplemental Table 3.2. Mass spectrometer tuning parameters for the detection of tulathromycin.

Intravenous									
	Horse A	Horse B	Horse C	Horse D	Horse E	Horse F	Geometric Mean	Min	Max
C_{5m} (ng/mL)	3070	3503	4950	4060	3680	5390	4030	3070	5390
λ_z (hr-1)	0.011	0.012	0.010	0.013	0.010	0.014	0.012	0.010	0.014
T ½ (hr)	63.9	56.0	70.1	54.6	67.2	49.6	59.8	49.6	70.1
$AUC_{0-\infty}$ (hr*ng/mL)	11900	12100	14900	12400	11600	14200	12800	11600	14900
AUC_{extrap} (%)	22.3	17.8	22.5	16.3	22.5	14.2	19.0	14.2	22.5
AUC_{last} (hr*ng/mL)	9280	9970	11600	10400	8980	12200	10300	8980	12200
Cl (mL/kg/min)	3.49	3.44	2.79	3.36	3.60	2.93	3.25	2.79	3.60
Vd (L/kg)	19.3	16.7	16.9	15.9	20.9	12.6	16.8	12.6	20.9
Intramuscular									
C_{max} (ng/mL)	664	901	440	790	1050	328	645	328	1050
T_{max} (hr)	0.5	0.5	0.5	1.0	1.0	1.5	0.8	0.5	1.5
λ_z (hr-1)	0.018	0.013	0.013	0.011	0.011	0.011	0.013	0.011	0.018
T ½ (hr)	37.5	55.1	54.4	61.6	63.5	61.5	54.8	37.5	63.5
$AUC_{0-\infty}$ (hr*ng/mL)	10800	13700	13000	13500	12100	15100	13000	10800	15100
AUC_{extrap} (%)	12.3	20.6	14.6	18.1	15.7	19.7	16.6	12.3	20.6
AUC_{last} (hr*ng/mL)	9510	10900	11100	11100	10200	12100	10800	9510	12100
F (%)	92.1	111	86.3	106	102	100	99.4	86.3	111
Subcutaneous									
C_{max} (ng/mL)		410		297		427	373	297	427
T_{max} (hr)		1.5		12.0		1.5	1.5	1.5	12.0
λ_z (hr-1)		0.012		0.011		0.013	0.012	0.011	0.013
T ½ (hr)		58.1		62.5		53.4	57.9	53.4	62.5
$AUC_{0-\infty}$ (hr*ng/mL)		14900		14100		15800	14900	14100	15800
AUC_{extrap} (%)		13.4		14.8		15.1	14.4	13.4	15.1
AUC_{last} (hr*ng/mL)		12900		12000		13400	12800	12000	13400
F (%)		123		113		111	115	111	123

Supplemental Table 3.3. Noncompartmental plasma pharmacokinetic parameters reported as geometric mean (range) for tulathromycin following IV, IM or SC administration of 2.5 mg/kg to horses. Cmax = Maximum plasma concentration. C5m = Plasma concentration at the first sample point after IV infusion. Tmax = Time to maximum concentration. λz = Terminal rate constant. T ½ = Terminal-phase half-life. AUC0–∞ = Area under the concentration-time curve from time zero extrapolated to infinity. AUCextrap = Percentage of AUC that was extrapolated. AUClast =

Area under the concentration-time curve to the last quantifiable concentration. Cl = Total plasma clearance after IV administration. Vdz = Volume of distribution of the terminal phase after IV administration. F = Bioavailability. --- = Not applicable.

	IV	IM
24 hr	107.5 (70-150)	117.5 (110-170)
72 hr	140 (90-175)	120 (65-175)
120 hr	117.5 (30-130)	120 (65-150)

Supplemental Table 3.4. Median (range) BAL fluid recovered. Volumes are reported as # mL recovered out of 240 mL infused. Samples were collected at 24, 72, and 120 hr post-administration of a single dose (2.5 mg/kg) of tulathromycin to 6 healthy adult horses via IV and IM routes.

	Macrophage	Lymphocyte	Neutrophil
24 hr	30.5% (17.5-42.5%)	57.25% (34.5-70%)	6% (2-12.5%)
72 hr	27.5% (14-35%)	66.5% (45.5-76%)	5% (3-10%)
120 hr	29.75% (13.5-42.5%)	62.25% (45%-72.5%)	6.5% (2.5-11.5%)

Supplemental Table 3.5. Median alveolar cell differential counts (percentages) with range recovered after single dose (2.5 mg/kg) administered via IV and IM routes at 24, 72, and 120 hr post-administration.

Chapter 4: Final Comments

Conclusions

The results of this study demonstrated that tulathromycin, like other macrolides, has a prolonged half-life in plasma and a high concentration in the pulmonary epithelial lining fluid (PELF) after a single dose administration to healthy adult horses. The persistence of the drug provides encouraging evidence that tulathromycin may have utility as a long-acting antibacterial drug for adult horses in both the field and in the hospital setting. The safety of multiple doses and the appropriate dose interval is unknown at this time and further investigation is warranted before appropriate dosing recommendations can be provided.

Due to the magnitude of the adverse effects that were exhibited after administration of subcutaneous or intramuscular injection of tulathromycin, those routes of administration are not recommended for use in clinical equine medicine. Furthermore, given the implausibility of administering tulathromycin via syringe pump in the field, further investigation into IV dosing is necessary, including the rate and concentration of drug administration. The vehicle of the commercially available cattle formulation, propylene glycol, can induce, sweating, salivation, ataxia, and pain. It is encouraging, however, that in this study, none of the horses developed diarrhea after administration of tulathromycin via any route.

Tulathromycin, compared to other options, may be an affordable treatment option: $12 per day for an antibacterial administered every 5-7 days is an attractive financial option.

Further investigation, including bacterial susceptibility and MIC data, is warranted. Ultimately, this study demonstrated that tulathromycin is a potential antibacterial option for use in equine medicine.