Effect of food on the pharmacokinetics of minocycline in healthy research dogs and minocycline and doxycycline susceptibilities of methicillin-resistant *Staphylococcus pseudintermedius* isolates using current and revised breakpoints

THESIS

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Abstract

*Staphylococcus pseudintermedius* (SP) is the most common pathogen isolated from canine superficial pyoderma. Methicillin-resistant *S. pseudintermedius* (MRSP) is isolated with increasing frequency from these lesions.\(^1\) Doxycycline is the member of the tetracyclines that has been most commonly used to treat MRSP infections in dogs,\(^2\) while the use of minocycline has only been sporadically reported.\(^3\)

Clinical and Laboratory Standards Institute (CLSI) human tetracycline breakpoints to predict minocycline and doxycycline susceptibility of SP isolates from dogs are not appropriate because they do not meet pharmacokinetic/pharmacodynamic data using a standard dose. New breakpoints have been approved for doxycycline and proposed for minocycline and are four dilutions lower than tetracycline breakpoints, providing a more conservative standard for classification of isolates.

The objectives of this study were to measure MICs of minocycline and doxycycline of 100 canine MRSP clinical isolates, compare their susceptibilities to minocycline and doxycycline based on current and revised standards, and document their tetracycline resistance genes. MICs were determined with E-test strips. PCR was used to identify *tet* genes. Using the human-derived tetracycline breakpoint of MIC\(\leq 4\) μg/mL, 76 isolates were susceptible to minocycline and 36 isolates were susceptible to doxycycline. In contrast, using the proposed minocycline breakpoint (MIC\(\leq 0.25\) μg/mL) and approved...
doxycycline breakpoint (MIC ≤ 0.125 μg/mL), 31 isolates were susceptible to both minocycline and doxycycline. Thirty-one isolates carried no tet genes, two had tet(K), and 67 had tet(M). Use of human-derived tetracycline breakpoints for doxycycline and minocycline susceptibility testing misclassified 45 of 76 (59%) canine MRSP isolates susceptible to minocycline and 5 of 36 (14%) susceptible to doxycycline demonstrating the importance of using the proposed minocycline and approved doxycycline canine specific breakpoints.

Minocycline could serve as a reliable alternative to doxycycline for treating dogs with infections caused by SP. Many pet owners prefer to administer oral medications with food; however, this may alter absorption. Since the pharmacokinetics of minocycline in unfed dogs has not been evaluated, the objective of this study was to determine the pharmacokinetics of minocycline after administration of a single oral dose in dogs with and without food.

Ten research hounds were administered oral minocycline (approximately 5 mg/kg) with and without food, in a crossover study, with a one-week washout between treatments. Blood samples were collected immediately prior to minocycline administration and over 24 hours. Minocycline plasma drug concentrations were measured using high-performance liquid chromatography and were analyzed to determine primary pharmacokinetic parameters. Wilcoxon signed-rank test was used to compare the two groups. A population pharmacokinetic modeling approach using nonlinear mixed effects modeling for primary parameters for the population as fixed effects and the difference between subjects as a random effect was performed. Covariate analysis was used to identify the source of variability in the population.
No significant difference was found between treatments for AUC (P=0.0645), although AUC was higher in fasted dogs. A significant difference was found for $C_{\text{MAX}}$ (P=0.0059), with fasted dogs attaining a higher $C_{\text{MAX}}$. As the covariate of feeding versus fasted accounted for a significant variation in the pharmacokinetics we recommend administration of minocycline without food.
Dedication

Dedicated to Mom, Dad, Walt, and Paco
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During my residency, I’ve realized that it takes a village to raise a dermatology resident. I appreciate all the help and support I’ve received from each and every one of you along the way!
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Chapter 1

Introduction

*Staphylococcus pseudintermedius* (SP) is the most common cause of canine superficial pyoderma. Methicillin-resistant *S. pseudintermedius* (MRSP) organisms are characterized by the presence of the *mecA* gene. This gene encodes an altered penicillin binding protein 2a (PBP2a) that confers resistance to all beta-lactam antibiotics including cephalosporins and amoxicillin-clavulanate, which are the most commonly utilized antibiotics for treatment of SP in the dog. In addition, these bacteria often carry coresistance to many non-beta-lactam antibiotics, limiting treatment options for these infections. Over 90% of these isolates are multi-drug resistant.

With the emergence of MRSP, the number of antibiotics to which isolates are susceptible has decreased leading to antibiotic choices that may have profound side effects or are reserved for human infections. Antibiotics that have been considered for the treatment of MRSP include aminoglycosides, chloramphenicol, clindamycin, fluoroquinolones, rifampin, and tetracyclines. The aminoglycosides are rarely used to treat MRSP since they are administered parenterally; chloramphenicol can cause common side effects (vomiting, diarrhea) as well as rare but severe side effects (liver toxicity, bone marrow suppression, and suspected myositis in dogs; fatal aplastic anemia in humans); MRSP organisms are variably susceptible to clindamycin and the fluoroquinolones. Rifampin may cause liver injury in a high proportion of treated dogs.
and there is a risk of rifampin resistance if it is not administered with other antimicrobials.²,³

While doxycycline is the tetracycline that has been most often used to treat canine MRSP infections,² there is new interest in minocycline as an alternative for treatment. This interest is primarily due to the rising cost of doxycycline,⁵ but is also based on research that suggests minocycline can be used to treat certain MRSP isolates that are resistant to doxycycline.⁶

The pharmacokinetics of minocycline have been determined in dogs administered minocycline hydrochloride intravenously and well as orally, however, none of these studies have been performed where the dogs were administered food with the minocycline.⁷,⁸ It is often easier for pet owners to administer pills with food. In addition, there is evidence to suggest that PK can differ depending on the breed of dog used in the analyses.⁹ Therefore, the objective of our study was to determine the pharmacokinetics of minocycline after administration of a single oral dose of minocycline in dogs other than Beagles with and without food. As antibiotic absorption may be affected by the presence of food,¹⁰⁻¹⁵ we hypothesized that administering minocycline with food would affect absorption.

Resistance of SP to the tetracyclines is mediated through the acquisition of tetracycline resistance genes (tet genes). The most commonly identified tet genes in SP isolates are tet(M) and tet(K).¹⁶,¹⁷ Staphylococcus spp. that possess only the tet(K) gene, retain susceptibility to minocycline, and are resistant to other tetracyclines, thus minocycline may be useful against isolates possessing tet(K).¹⁸,¹⁹ On the other hand, Staphylococcus spp. that possess the tet(M) gene are resistant to both doxycycline as well
as minocycline.\textsuperscript{18}

Previous work suggests that MRSP isolates are more susceptible to minocycline than to doxycycline.\textsuperscript{6} However, the human-derived tetracycline breakpoints were used to evaluate minocycline and doxycycline susceptibility.\textsuperscript{6} Maaland \textit{et al.}\textsuperscript{7} proposed that the use of canine-specific doxycycline breakpoints in place of the human-derived tetracycline breakpoints for determining doxycycline susceptibility. These canine-specific breakpoints have been approved by the Clinical Laboratory and Standards Institute (CLSI), but the new standard has not yet been published. This means that commercial broth microdilution plates still be produced using the human-derived tetracycline breakpoints for doxycycline susceptibility. Since the human-derived tetracycline breakpoints are not as conservative as the approved canine doxycycline breakpoints, some isolates may be classified as susceptible to doxycycline when they are actually resistant.

In light of these discrepancies that may occur with the use of the human-derived tetracycline breakpoints, we aimed to evaluate minimum inhibitory concentrations (MIC) of minocycline and doxycycline for clinical MRSP isolates from dogs to determine susceptibilities based on canine-specific doxycycline and minocycline breakpoints compared to the human-derived tetracycline breakpoints. In addition, we aimed to document the presence of tetracycline resistance genes in those isolates. We hypothesized that fewer MRSP isolates would be susceptible to doxycycline and minocycline based on the canine-specific breakpoints than with the human-derived tetracycline breakpoints and those isolates susceptible to minocycline would possess the \textit{tet}(K) gene, while those found to be resistant to both minocycline and doxycycline would possess the \textit{tet}(M) gene.
Chapter 2

Literature Review

2.1 Pharmacology and Pharmacokinetics

2.1.1 Pharmacology of Drug Distribution

In order for a drug to be absorbed into the circulation it must enter the body either by the oral or intravenous (IV) route. The percentage of an administered dose of a drug that reaches the systemic circulation is known as bioavailability. Drugs that are administered IV are 100% bioavailable which is denoted as F, while those that are administered orally reach the systemic circulation after absorption in the gastrointestinal (GI) tract. The rate and extent of drug absorption in the GI tract is dependent on a number of factors including GI pH, surface area, motility, epithelial permeability, and intestinal blood flow. In addition, bioavailability of orally administered drugs can be reduced if the drug is metabolized by the liver, microbes, or intestinal epithelial cells. All orally administered drugs enter the portal vein then the liver after being absorbed from the GI tract. Some drugs will be almost completely removed from the blood by hepatocytes during this first pass through the liver and these drugs may not achieve high enough concentrations to be pharmacologically active and thus will need to be administered IV.

In order for the drug to impart a pharmacologic effect, after reaching the systemic circulation, it must be distributed to tissues. Factors that influence drug distribution include the binding of the drug to plasma proteins, drug lipid solubility, and organ blood
flow. As the pharmacologically active component of a drug is the free drug, the extent of protein binding of a drug will limit distribution of the free drug from the systemic circulation to the tissues. If greater than 80% of a drug is protein-bound, the drug is considered to be highly protein-bound. The volume of distribution (Vd) of a drug is used to estimate the amount of tissue to which a drug is distributed or in other words, the ease by which the drug leaves the systemic circulation.

Drug metabolism mainly occurs in the liver and can occur in two phases. Drugs and their metabolites may undergo phase I and phase II or only phase II metabolism. Phase I metabolism occurs mainly by the hepatic microsomal enzymes, which are the cytochrome-P450 enzymes and involves oxidative reactions. This phase generally renders the drug more water-soluble and more readily able to undergo phase II metabolism. The end result is usually inactivation of the drug. However, in some cases these phase I metabolites may be more or equally as active or toxic as the parent drug. In these drugs, the phase II metabolism is important to protect the liver from these damaging metabolites. Phase II metabolism, or conjugation, generally results in inactive metabolites, which will then be eliminated from the body by renal excretion (most common) or biliary excretion (least common). Renal excretion occurs through the active transport in the proximal tubules of the glomerulus. Passive reabsorption from the renal tubules into the peritubular capillaries will slow renal excretion. Drugs that are not passively reabsorbed will become concentrated in the renal tubules, which can be beneficial or result in toxicity.²⁰
2.1.2 Pharmacokinetics

2.1.2.1 Pharmacokinetic Modeling

Pharmacokinetics is the study of the time course of the concentration of a drug as it moves through the body or stated another way, it is what the body does to the drug and involves the entry of the drug into the body, and the absorption, distribution, metabolism and elimination of the drug or its metabolites. Pharmacokinetic modeling is useful to predict the biological behavior of a drug in the body. The drug of interest is administered orally or intravenously as a single dose and blood samples are collected at specific time points for at least three elimination half-lives. Additional studies can also be performed including bioavailability studies administering the drug IV, as well as, multiple dosing studies. Analysis involves initially plotting the plasma drug concentration versus time data on semilogarithmic paper to linearize the data. The models used for pharmacokinetic analysis include compartmental and noncompartmental analysis as well as population pharmacokinetic analysis.

2.1.2.1.1 Compartmental Analysis

The compartmental model allows one to describe a drug’s movement in compartments over time. Specifically, the model divides the body into compartments where the rate of drug disappearance is similar between all of the compartments and the percent of drug eliminated per unit of time is constant. In the one-compartment model, the body is considered one compartment or space where the drug dissolves. The volume of distribution is the volume in the one compartment to which the drug distributes. There is only one elimination rate.
The two-compartment model is more complex than the one-compartment model. The body is divided into two compartments each with a different rate of drug distribution. The two compartments consist of the central compartment and a peripheral compartment. The central compartment may be composed of the intravascular space and the extracellular fluid of the heart, lungs, liver and kidneys. The peripheral compartment could be the drug distributing to other body tissues (these are less highly perfused than the heart, lungs, liver and kidneys). In this model, there is one distribution rate and one redistribution rate. The distribution rate is the rate of the drug distributing from the central compartment to the peripheral compartment and the redistribution rate is the drug distributing from the peripheral compartment back to the central compartment. There is one rate of elimination from the central compartment. The one- and two-compartment models are the most common models in veterinary medicine.

The three-compartment model is even more complex than the two-compartment model. The body is divided into three compartments. The drug diffuses into a central compartment and two peripheral compartments. The central compartment is still the intravascular space and extracellular fluid of the heart, liver, kidneys and lungs. But in this model, the drug can also diffuse into two peripheral compartments that each have a different distribution rate and redistribution rate. There is still only one elimination rate from the central compartment.

2.1.2.1.2 Noncompartmental Analysis

There are patients whose pharmacokinetic data do not fit into a compartmental model. In these patients, a noncompartmental analysis may be used. Noncompartmental
models are based on probability density functions, which evaluate the probability that a drug is at a specific body location. In contrast to compartmental models where parameters include absorption and elimination rates, noncompartmental models are evaluated using mean residence time (MRT), area under the curve (AUC), area under the moment curve (AUMC) and mean absorption time (MAT).

The MRT is the AUMC divided by the AUC. AUC is calculated using a trapezoidal rule where the area of multiple trapezoids is determined in the area under the concentration versus time curve. The area of each trapezoid is combined for the total area under the curve. Thinking of MRT in another way, half-life is equal to 0.693(MRT) so the MRT also evaluates the amount of time the drug is persisting in the body.

Another parameter used in noncompartmental analysis is mean absorption time (MAT), which is used to determine the rate of drug absorption in studies of bioequivalence. MAT is the time it takes for the bioavailable drug to get into circulation.

2.1.2.1.3 Population Pharmacokinetics

Population pharmacokinetics is the study of the sources and correlates of variability in drug concentrations among individuals who are the target patient population receiving clinically relevant doses of a drug of interest. It has been used in human medicine for defining dosing strategies for medications and allows one to be able to identify variation between members of a population with regard to their drug concentration. Variability can be affected by age, genetic mutations that may affect drug metabolism, and interactions between drugs, illness, and feeding among other causes.
There are certain advantages to population pharmacokinetics. These can include evaluating pharmacokinetics of a population where many samples cannot be obtained, such as in neonates or critically ill patients, or when collecting fewer samples is less expensive.\textsuperscript{23,24} Another advantage is the ability to identify significant sources of variation. Variability includes random and fixed effects. Fixed effects are the means of the population pharmacokinetic parameters.\textsuperscript{24} Random effects include interindividal, intra-individual, inter-occasion and residual variability.\textsuperscript{24} In addition, data can be pooled from different populations with different sampling times for the same pharmacokinetic analysis. This can increase the sample size and also increase power.\textsuperscript{24} However, one disadvantage to population pharmacokinetics is that a pharmacokineticist with knowledge of performing the pharmacostatistical analysis must be involved in the study.\textsuperscript{24,25} Determining which analysis to use, what data collect as well as compiling results from many patients, possibly from multiple studies, and analyzing the data can be time-consuming.\textsuperscript{25}

The use of population pharmacokinetic analyses in veterinary studies is lagging behind that of human medicine due to the few veterinary pharmacokineticists, who have an in-depth knowledge of population pharmacokinetics. There are several methods of estimation involved in population pharmacokinetics. They include naïve average data approach, naïve pooled data analysis, two-stage approach, and nonlinear mixed-effects model approach.\textsuperscript{26}

For the naïve average data approach, all individuals have the same sampling times so the average value can be calculated for each sampling time. The advantage to this approach is its simplicity compared to other analyses. However, while averaging the
values for the sampling times is helpful in compiling data, averages can obscure the actual variation in the data between individuals. Therefore, this type of analysis should be used in populations with small variation between individuals. This approach is often not useful for studies in humans where there can be large variation between individuals.

The naïve pooled data analysis pools the data from individuals and determines estimates of the parameters as if they came from one individual. This analysis can deal with more data types, such as nonstandard data, than the naïve average data approach. However, like the naïve average data approach, this type of analysis can still obscure variation between individuals and is not useful when there is large variation between individuals, thereby limiting its use in human pharmacokinetics.

The two-stage approach estimates the individual’s parameters in the first stage and estimates the population parameters in the second stage. There are several two-stage approaches. Two of which are the standard two-stage approach and the global two-stage approach. The standard two-stage approach is one of the more simple two-stage approaches, but the global two-stage approach provides a better estimate of the population mean parameters.

The nonlinear mixed-effects model approach is typically used when only a small number of samples (one to six) are obtained for each individual. With this approach, the data is pooled due to problems evaluating the parameters in each individual, like the standard two-stage approach, with such a small number of samples. Pooling data in the other approaches could obscure variation between individuals, but the nonlinear mixed-effects approach is able to separate out variation between subjects despite pooling the data. The non-linear mixed effects approach is able to provide an estimate of the
population’s parameters using a maximum likelihood approach. This approach evaluates different sets of parameters, but the parameters that are the best estimate are those that make the observed data more probable than other parameters.\(^{26}\)

### 2.1.2.2 Pharmacokinetic Parameters

Pharmacokinetic parameters include the dose, absorption rate constant \((k_a)\), volume of distribution \((V_d)\), clearance \((Cl)\), area under the curve \((AUC)\), maximum concentration \((C_{MAX})\), time to the maximum concentration \((t_{MAX})\), terminal or elimination half-life \((t_{1/2})\), bioavailability \((F)\) and elimination rate constant \((k_{el})\). The primary pharmacokinetic parameters are \(t_{1/2}\), \(Cl\) and \(V_d\).\(^{22}\) Knowing the dose of the drug and the \(k_{el}\) allow one to calculate the primary parameters.\(^{22}\)

The dose is the amount of drug administered and can be administered by various routes, such as IV or oral routes. The absorption rate constant \((k_a)\) is the fractional rate of drug absorption into circulation. The rate of absorption of a drug will influence the peak plasma concentration \((C_{MAX})\) as well as the time to reach the peak plasma concentration \((t_{MAX})\). Thus, the rate of absorption will determine the time for the drug to reach an effective concentration.

Volume of distribution \((V_d)\) is a theoretical volume that would be required to contain the same amount of drug present in the body (tissue) at the same concentration as in the plasma. The \(V_d\) is calculated by dividing the dose of the drug by the maximum plasma drug concentration.\(^{20}\) Therefore, a drug that accumulates in tissues will have a low plasma concentration and thus a high \(V_d\), while drugs with a very small \(V_d\) will be
confined to the intravascular fluid. Vd of a drug is useful in estimating the dose required to achieve a specific plasma concentration.\textsuperscript{20}

Clearance (Cl) is the measure of the body’s ability to eliminate a drug and is the volume of plasma cleared of the drug per unit of time.\textsuperscript{20} Clearance can be calculated on the basis of compartmental modeling, from the Vd and the rate at which the drug is eliminated (elimination constant) from the body: \( \text{CL} = \frac{\text{Dose}}{\text{AUC}} \) or by dividing the dose of the drug by the area under the plasma drug concentration-time curve (AUC): \( \text{CL} = \frac{\text{Dose}}{\text{AUC}} \).

The AUC is the body’s exposure to the drug\textsuperscript{20} and is dependent on the rate of elimination of the drug and the administered dose. The AUC is inversely proportional to clearance. Therefore, the higher the clearance of the drug, the smaller the AUC, indicating less time the body is exposed to the drug.

\( t_{\text{MAX}} \) is the time to reach the maximum drug concentration (\( C_{\text{MAX}} \)) in the plasma. The \( t_{\text{MAX}} \) occurs when the rate of absorption is equal to the rate of elimination and is independent of the administered dose.\textsuperscript{28} The drug elimination or terminal half-life (\( t_{1/2} \)) is the amount of time it takes for the plasma drug concentration to decrease by 50%.\textsuperscript{20} The elimination rate constant (\( k_d \)) is the fraction of drug eliminated per unit time and this can be calculated by \( k_d = \frac{\text{Cl}}{\text{Vd}} \).\textsuperscript{20} Bioavailability (F) is the percentage of drug that reaches the site of action. For an IV administered drug, bioavailability is 100%, while for orally administered drugs, bioavailability if often less than 100%.\textsuperscript{29}
2.2 Tetracyclines

2.2.1 History and Source

The discovery of the tetracyclines began in the 1940s. Benjamin Minge Duggar, a retired professor of plant physiology and economic botany, was hired by American Cyanamid, one of the first companies involved in antibiotic research and development, to screen soil samples in an effort to identify those that would yield actinomycete bacteria to produce antibiotics. Yellow colonies isolated from a soil sample from Missouri showed promise as an antibiotic and was later named Aureomycin® (chlortetracycline). Chlortetracycline was the first member of the tetracycline family and was produced by Streptomyces aureofaciens. Duggar published the results of his discovery in 1948. Not long after this, Alexander Finlay discovered the compound oxytetracycline, which was produced by Streptomyces rimosus and named Terramycin®. Terramycin® was approved by the FDA (Food and Drug Administration) in 1950, rivaling Aureomycin® as a treatment for infectious diseases. By 1952, the chemical structures of Aureomycin® and Terramycin® were elucidated, allowing for production of a new family of semisynthetic antibiotics. Chemical modifications of Aureomycin®, resulted in the production of a more potent, more soluble, semisynthetic compound, tetracycline. In 1954, tetracycline, or Teracyn, was approved by the FDA. These three—chlortetracycline, oxytetracycline, and tetracycline—were the first-generation tetracyclines.

Further modifications of the tetracycline nucleus led to the development of the second-generation semisynthetic tetracyclines. In 1967, the first second-generation tetracycline was approved by the FDA, doxycycline, also known as Vibramycin®. Later, a bioengineered tetracycline strain, demeclocycline, was produced; however, it was not
bioactive and was chemically modified to yield an intermediate sancycline. Modifications of sancycline produced minocycline, which had greater pharmacologic and antibacterial activity than the first-generation tetracyclines and doxycycline. Minocycline was approved by the FDA in 1971.19,30

The most recently discovered tetracyclines are the third-generation semisynthetic tetracyclines referred to as glycycyclines. The third-generation tetracycline antibiotics were developed specifically to combat antibiotic resistance to the earlier tetracyclines.31 One of the members of this generation include tigecycline, which was approved for use in 2005 (Doan et al. 2006).32 Tigecycline was derived from the minocycline structure.

2.2.2 Chemistry and Stability

The basic chemical structure of the tetracyclines is four linearly fused rings (naphthacene rings), with the rings designated as D, C, B and A (Figure 1).19,30 The carbons of the naphthacene rings are numbered starting at C1 with the A-ring and moving counterclockwise. All skeletal and exoskeletal carbon atoms are labeled. Bridgehead tertiary carbon atoms have a number as well as a letter designation.

For a tetracycline to be active, it must at minimum have the tetracyclic nucleus; an A ring C1-C3 diketo substructure, exocyclic C2 carbonyl or amide group, a C10 phenol, and a C11-C12 keto-enol substructure and 12a-OH group.30

The structures of tetracycline and doxycycline differ by the placement of one hydroxyl group, which is on C6 in tetracycline and C5 in doxycycline.33 This structural change is responsible for doxycycline being more lipophilic than tetracycline.33
Figure 1. The structure of the naphthacene ring, tetracycline, doxycycline and minocycline.
Structural changes of the tetracycline molecule affects the activity of the tetracycline against microbes. The 4S and 4R isomers of the C4-dimethylamino group have varying activity with the 4S isomer providing the best activity against microbes.\(^\text{30}\) In general, there is a decrease in antibacterial activity if there are changes to the groups at positions 1, 3, 4a, 10, 11 or 12.\(^\text{19}\)

The tetracyclines are strong chelating agents and their chelation sites occur at several positions on the tetracyclic rings including positions 1 and 3 (enol groups), positions 11 and 12 (β-diketone system) and position 2 (carboxamide groups).\(^\text{19}\) The chelating property of the tetracyclines allows them to be incorporated into the teeth of developing humans and dogs, and to cause yellow tooth discoloration.\(^\text{20}\) The ability of the tetracyclines to chelate metal ions is thought to be the cause of decreased absorption of tetracycline and minocycline when administered with iron supplements in humans.\(^\text{34}\)

The tetracyclines’ ability to chelate divalent cations is involved in their inhibition of matrix metalloproteinases (MMP). MMPs have collagenolytic activity. Tetracyclines bind to the divalent cations (i.e. zinc, calcium) in the MMPs, inhibit the MMPs,\(^\text{35}\) thus reducing the breakdown of collagen.\(^\text{35,36}\)

Due to increasing resistance among tetracyclines, further research on semisynthetic tetracyclines resulted in the discovery of the glycylcyclines.\(^\text{31}\) The addition of a glycyl group to C9 increased the antimicrobial activity, even in those bacteria harboring tetracycline resistance genes.\(^\text{31,37}\) Tigecycline was approved by the FDA in 2005 and is still the only glycylcycline to receive FDA approval.\(^\text{38}\)
2.2.3 Mechanism of Action

2.2.3.1 Antibacterial

Tetracyclines have antibiotic properties and non-antibiotic properties. Antibiotic properties are related to their ability to treat bacterial infections. In general, tetracyclines are broad spectrum and bacteriostatic. They can target gram-positive, gram-negative and anaerobic microbes\textsuperscript{20} as well as \textit{Rickettsia}, \textit{Chlamydia}, \textit{Mycoplasma}, and spirochetes.\textsuperscript{39}

Many antibiotics target bacterial protein synthesis. The ribosome is responsible for the synthesis of proteins in cells and serves to convert mRNA into the amino acid chains that make up the proteins. The ribosome is made up of ribosomal RNAs (rRNAs; 16S, 23S, and 5S) and ribosomal proteins. Bacteria have 70S ribosomes, which form when the 30S ribosome and the 50S subunit associate. The 30S subunit has a 16S rRNA subunit bound to proteins, while the 50S subunit is composed of a 5S rRNA and 23S rRNA subunit bound to proteins. There are 3 sites within the ribosome: A (acceptor) site, P site and E site. The appropriate start codon, based on the mRNA sequence, must be in the P site. Then the aminoacyl-tRNA brings the next anticodon into the A site mediated by Ef-Tu, an elongation factor. A peptide bond is formed between the amino acid in the A site and the amino acid in the P site. The amino acid chain is shifted to the tRNA in the A site. The tRNA in the A site is moved to the P site and the tRNA in the P site is moved to the E site, thereby leaving the A site open for a new tRNA. Movement of the tRNA from the P site to the E site is done via another elongation factor, EF-G.\textsuperscript{40} The elongation factors are coupled to GTP.\textsuperscript{40} The GTP binding domain is near the N-terminus.\textsuperscript{19} When a
stop codon is reached, the bond between the peptide and tRNA is hydrolyzed, releasing the polypeptide into the cytoplasm where it folds.\textsuperscript{40}

In order to inhibit protein synthesis, tetracyclines must first find a way to cross the cell membranes. Tetracyclines enter gram-negative bacteria via passive and active transport mechanisms. The passive mechanisms include passing through cell membranes via porin proteins. The active mechanisms require energy to move tetracycline into the cell. For gram-positive bacteria, the mechanisms have not been fully elucidated, but it is thought that energy is needed for tetracycline transport.\textsuperscript{18}

Tetracycline may bind to the ribosome at either two or six sites.\textsuperscript{41} The main tetracycline binding site (Tet-1) is on the 30S subunit of the bacterial ribosome where the anticodon stem loop of the aminoacyl-tRNA fits into the A site.\textsuperscript{40,41} Tetracycline interacts with the rRNA via its sugar phosphate backbone,\textsuperscript{41} preventing both aminoacyl-tRNA binding to the A site and elongation of the peptide chain\textsuperscript{40,41} thus inhibiting bacterial protein synthesis.\textsuperscript{40,42}

The secondary tetracycline binding site (Tet-5) interacts with the 16S rRNA, and although in this position, tetracycline cannot directly interfere with tRNA binding, it may exert its inhibition by interfering with open and closed state of the 30S ribosome which monitors the base-pairing of the mRNA codon and tRNA anticodon for delivery of the correct tRNA.\textsuperscript{40,41} The other four tetracycline binding sites include Tet-2, Tet-3, Tet-4 and Tet-6 and are not as easily correlated with the inhibitory function of tetracycline.\textsuperscript{41}
2.2.3.2 Nonantibacterial

Tetracyclines have many non-antibiotic properties that are important in their ability to treat inflammatory and immune-mediated diseases. These include antioxidant properties and inhibition of inflammation, proteolysis, angiogenesis, apoptosis and bone metabolism.

2.2.3.2.1 Antioxidant

The antioxidant properties of tetracyclines arise from their structure, specifically the multi-substituted phenol ring, which is similar to that of vitamin E. Tetracycline and minocycline decrease production of reactive oxygen species. Minocycline can quench hydrogen peroxide and scavenge superoxide and peroxynitrate. Minocycline is superior to tetracycline in its scavenging ability due to the presence of a diethylamino group on the phenolic carbon, which is unique to minocycline.

2.2.3.2.2 Inhibition of Inflammation

Inhibition of inflammation is important for treatment of many diseases including acne vulgaris, rosacea, rheumatoid arthritis and inflammatory bowel disease. Tetracyclines have a wide spectrum of anti-inflammatory effects, due to their ability to interfere with the synthesis and activity of inflammatory mediators and their ability to interfere with cytokine production from immune cells.

Tetracyclines inhibit neutrophil chemotaxis as well as their migration to sites of inflammation. The inhibition involves chelation of intracellular calcium, which is necessary for the assembly of the microtubules involved in cell movement.
Overexpression of nitric oxide synthase, which is produced by nitric oxide, is involved in the inflammatory pathway of colitis, osteoarthritis, and rheumatoid arthritis. In addition, the overproduction of nitric oxide is involved in diseases of the central nervous system, such as Parkinson’s disease, cerebral ischemia, and Huntington’s disease.\textsuperscript{44,52} Nitric oxide can also upregulate MMPs. Minocycline and doxycycline have been shown to decrease expression of inducible nitric oxide synthase (iNOS).\textsuperscript{44,52}

Cyclooxygenases (COXs) are involved in inflammatory pathways and they are enzymes that convert arachidonic acid from phospholipids into prostaglandins.\textsuperscript{53} There are two COX isoforms - COX-1 and COX-2. COX-1 is expressed in most tissues and the prostaglandins it generates play a role in hemostasis and protection of the gastric mucosa. Whereas, COX-2 is inducible by numerous physiologic stimuli such as cytokines and mitogens and its derived prostaglandins play a role in inflammation.\textsuperscript{53} In vivo studies utilizing experimental models of brain ischemia and Alzheimer’s disease have shown minocycline to decrease COX-2 and prostaglandin production, while some in vitro studies have shown the opposite result.\textsuperscript{44,54,55} Therefore, the reduction in COX-2 and prostaglandin expression after treatment with minocycline in vivo may be due to an improvement in the inflammatory response.

Phospholipase A\textsubscript{2} (PLA\textsubscript{2}) is a key enzyme in the biosynthesis of inflammatory mediators.\textsuperscript{56} After arachidonic acid is liberated by PLA\textsubscript{2} the free arachidonic acid undergoes enzymatic metabolism by lipooxygenases, leukotrienes, and cyclooxygenases (COX-1, COX-2), which generate thromboxanes and prostaglandins. The extracellular form of PLA\textsubscript{2} is secreted PLA\textsubscript{2} (sPLA\textsubscript{2}) which plays a role in inflammatory processes.
such as rheumatoid arthritis and rosacea. Minocycline and doxycycline inhibit PLA$_2$ likely by binding to the active site of PLA$_2$ and inhibiting enzymatic activity.

Tetracyclines reduce the production of inflammatory cytokines and inhibit their release. Tetracycline acts on the signaling pathway involved with protease-activated receptor 2 (PAR2), which is activated by proteases. When activated, PAR2 causes an increase in intracellular calcium levels that leads to downstream signaling that allows for gene transcription via NF-$\kappa$B. Specifically, genes are transcribed that are important for production of proinflammatory cytokines. PAR2 can also stimulate the expression of MMPs, such as MMP-1, MMP-2, MMP-3, MMP-9, MMP-13. Tetracycline acts to decrease levels of proinflammatory cytokines and MMPs by chelating the calcium, which reduces the PAR2 signaling pathway. Tetracyclines can also decrease the activity of MMPs by chelating the zinc in their active site.

### 2.2.3.2.3 Inhibition of Apoptosis

In addition to their anti-inflammatory properties, tetracyclines have anti-apoptotic properties. Apoptosis is the process of programmed cell death and occurs via the extrinsic or death receptor pathway, intrinsic or mitochondrial pathway and perforin/granzyme pathway. These three pathways lead to the activation of the caspase cascade, triggering the execution pathway.

The extrinsic pathway starts when death ligands bind to transmembrane receptors called death receptors. Examples of some of the best-defined death ligands and death receptors include FasL/FasR and TNF-$\alpha$/TNFR1. The death receptors contain death domains that are rich in cysteines that facilitate the upregulation of the signaling
The binding of a death ligand to a death receptor causes binding of adaptor proteins leading to the activation of caspase-8. Caspase-8 then activates Caspase-3, triggering the execution phase of apoptosis.

The perforin/granzyme pathway is mediated by cytotoxic T lymphocytes. The cytotoxic T-cells release perforin, which is a transmembrane protein that forms pores in cells (Elmore 2007). Once the pore is formed, the cytotoxic T cells release granules into the pore that contain granzyme B. Granzyme B is a serine protease that can cleave pro-caspase-10 to form caspase-10, which activates caspase-3 as well as directly activating caspase-3.

The intrinsic pathway is mediated by specific apoptotic stimuli, such as free radicals or hypoxia, that cause changes in the inner mitochondrial membrane. The Bcl-2 family of proteins and p53 play a role in changes in the mitochondria during apoptosis. A mitochondrial permeability pore forms in the mitochondrial membrane, and cytochrome c and second mitochondrial-derived activator of caspase (Smac/DIABLO) leak into the cytoplasm. Cytochrome c binds to apoptotic protease activator factor-1 (Apaf-1) and can activate caspase-9, which activates caspase-3. Rather than activating caspases, Smac/DIABLO promote apoptosis by inhibiting inhibitors of apoptosis proteins (IAPs) activity.

The execution phase of apoptosis begins with the activation of caspase-3, which activates endonucleases and proteases to degrade components of the cell resulting in characteristic morphological changes of the cell including cell shrinkage, condensation of chromatin, formation of apoptotic bodies with resultant phagocytosis of the apoptotic bodies by macrophages, neoplastic cells or adjacent parenchymal cells.
Minocycline can decrease the expression of caspase-1 and caspase-3, and prevents Smac/DIABLO from leaving the mitochondria. Minocycline can also upregulate Bcl-2 and Bcl-xl, which are involved in inhibiting apoptosis, and downregulate Bax, Bak, BID and FAS, which enhance apoptosis. When bound to minocycline, Apaf-1 cannot activate caspases.

The previous pathways relied on activation of caspases to mediate apoptosis. However, there is a caspase-independent pathway, which is precipitated by granzyme A. Granzyme A is found within granules of cytotoxic T lymphocytes and NK cells and enters the cytoplasm of cells via pores formed by perforin as did granzyme B. Granzyme A activates DNases, which form breaks in the DNA, and it can also cleave poly(ADP-ribose) polymerase-1 (PARP-1), which is responsible for repairing DNA. Minocycline inhibits apoptosis-inducing factor in the cytoplasm and prevents it from moving into the nucleus and decreasing its ability to cause DNA fragmentation. Minocycline also inhibits PARP-1.

**2.2.3.2.4 Inhibition of Angiogenesis**

Angiogenesis occurs in many diseases including rosacea and cancer. Angiogenesis is facilitated by matrix-degrading enzymes such as MMPs present in the extracellular matrix, to allow for these new blood vessels to penetrate the tissue. While it was previously thought that doxycycline and minocycline inhibit angiogenesis via inhibition of MMPs, it is now known to be a non-MMP-dependent mechanism. Furthermore, minocycline was found to inhibit hypoxia inducible factor-1α expression and transcription, which is involved in angiogenesis in cancer cells.
2.2.4 Tetracycline Resistance

For the tetracyclines, three different mechanisms of tetracycline resistance have been identified: tetracycline efflux, ribosome protection and tetracycline modification. The first two mechanisms are the most common and resistance of bacteria to the tetracyclines is a result of the acquisition of genetically mobile tetracycline resistance genes (tet) via transferable plasmids or transposons.\textsuperscript{31} Forty-five tetracycline and oxytetracycline resistance genes have been characterized to date.\textsuperscript{66} Twenty-nine genes encode for efflux pumps, twelve genes encode for ribosomal protection, three genes encode for enzymatic modification and one gene has an unknown function.\textsuperscript{66,67} Of the three oxytetracycline (otr) genes, two encode for efflux pumps\textsuperscript{67} and one encodes for ribosomal protection.\textsuperscript{66}

The efflux pumps are membrane-associated proteins that are about 46 kDa which pump tetracyclines out of bacteria.\textsuperscript{18,19} The pump moves a tetracycline-cation complex out of the bacteria and moves a proton into the bacteria.\textsuperscript{19} These efflux pumps have been divided into two groups based on similarity among amino acid sequences (Chopra and Roberts 2001). The members of group 1 are: \textit{tet}(A), \textit{tet}(B), \textit{tet}(C), \textit{tet}(D), \textit{tet}(E), \textit{tet}(G), \textit{tet}(H), \textit{tet}(Z), \textit{tet}(I), \textit{tet}(J), and \textit{tet}(30). All are found in gram-negative bacteria except for \textit{tet}(Z). These \textit{tet} genes encode for doxycycline and tetracycline resistance, but not minocycline or tigecycline resistance with the exception of \textit{tet}(B) that also confers resistance to minocycline.\textsuperscript{18} The members of group 2 are \textit{tet}(K) and \textit{tet}(L). Group 2 efflux pumps are primarily located in gram-positive bacteria. \textit{tet}(K) and \textit{tet}(L) confer resistance to tetracycline and doxycycline, but not minocycline or tigecycline.\textsuperscript{18}
Ribosomal protection proteins (RPP) are soluble cytoplasmic proteins involved in tetracycline resistance. RPP are similar in structure to the elongation factors involved in protein synthesis (Ef-Tu and Ef-G) and compete with these elongation factors for binding on the ribosome.\textsuperscript{19} RPP cause tetracycline to dislodge from the ribosome, protecting the bacteria from tetracycline’s activity, allowing for protein synthesis and bacterial growth.\textsuperscript{19,41,68,69}

The ribosomal protection proteins have been divided into three groups based on similarity in amino acid sequence. The members of group 1 include \textit{tet}(M), \textit{tet}(O), \textit{tet}(S) and \textit{tet}(W). The members of group 2 include \textit{otr}A and \textit{tet}B(P). The members of group 3 include \textit{tet}(Q) and \textit{tet}(T). All of these genes confer resistance to tetracycline, doxycycline and minocycline.\textsuperscript{19} The most-studied RPP, are \textit{tet}(O) and \textit{tet}(M) while those less studied RPP include \textit{tet}(S), \textit{tet}(T), \textit{tet}(Q), \textit{tet}B(P), \textit{tet}(W) and \textit{otr}A.

The third mechanism of tetracycline resistance involves a cytoplasmic protein that chemically modifies tetracycline. This reaction takes place only in the presence of oxygen and NADPH\textsuperscript{19} and occurs with \textit{tet}(X)\textsuperscript{70} and \textit{tet}(37).\textsuperscript{71}

\textbf{2.2.5 Preparations, Routes of Administration}

\textbf{2.2.5.1 Preparations for Oral Administration}

Minocycline tablets, capsules and a suspension are all available for oral administration. There is also an extended-release minocycline tablet. The tablets (not extended release) are available in 50 mg, 75 mg and 100 mg strengths while the extended release tablets are available in 45 mg, 55 mg, 65 mg, 80 mg, 90 mg, 105 mg, 115 mg and 135 mg strengths. These are marketed as Solodyn\textsuperscript{®} and Dynacin, however there are
generic tablets and extended release tablets available. The tablets are coated and not scored. The tablets should not be broken or crushed.

Minocycline is also available in a capsule. Some forms of the capsule are pellet-filled. Capsule strengths include 50 mg, 75 mg and 100 mg. The capsules are marketed as Minocin® or Dynacin, but there are now generic formulations available.

2.2.5.2 Preparation for Topical Administration

A minocycline topical formulation is available for human patients with periodontal disease Arestin®, which contains minocycline hydrochloride microspheres. Arestin® is supplied in individual disposable cartridges and when attached to the handle mechanism, powder is dispensed into periodontal pockets (based on manufacturer’s instructions). However, there are currently no other available topical minocycline formulations for use in patients.

2.2.5.3 Preparation for Parenteral Administration

Parenteral minocycline is also available in 100 mg per vial. It is marketed as Minocin® IV. No generic formulations of this product exist.

2.2.5.4 Aquaculture Preparations

Minocycline is also used in aquaculture for treatment and control of septicemia, bacterial fin and tail rot and cotton wool disease in tetras, cichlids, livebearers, catfish and goldfish. It is supplied as Maracyn® Two, which contains 10 mg powder packets of minocycline. This form of minocycline is not approved by the FDA.
2.2.6 Untoward Effects

2.2.6.1 Gastrointestinal

Gastrointestinal (GI) signs are common side effects of oral antibiotics. GI signs are likely related to absorption of antibiotics in the duodenum\(^{72}\) or due to changes in the gut microflora.\(^{73}\) In humans treated with doxycycline or minocycline, the most commonly reported side effects are GI signs.\(^{74}\) Vomiting (18.3%), diarrhea (7%) and anorexia (2.5%) have been noted in dogs receiving oral doxycycline.\(^{75}\)

Older dogs treated with doxycycline have been found to be at increased risk for vomiting, although it may not be entirely due to doxycycline, but rather that older dogs have concurrent diseases or may be on concurrent medications that could result in vomiting.\(^{75}\) GI side effects appear to be less common in dogs treated with minocycline than doxycycline. Minocycline administered at dosages of 30 mg/kg orally daily to dogs for 30 days, resulted in one to three episodes of vomiting per dog.\(^{76}\) However, when the dose administered was 10 mg/kg orally daily, no adverse GI effects were observed.\(^{7,8}\)

There are few reports on the use of doxycycline in horses.\(^{77-80}\) Gastrointestinal effects are rare in horses and with diarrhea being the only sign.\(^{77}\)

2.2.6.2 Phototoxic

Photosensitivity is an abnormal sensitivity to sunlight. Photosensitivity can be idiopathic, occur after exposure to medications or chemicals, or be due to a systemic disease. Some medications can contribute to sun sensitivity and can cause two types of photosensitive reactions, phototoxicity and photoallergy. Tetracyclines can induce
phototoxicity and this occurs via photosensitization of the tetracycline, generation of phototoxic products and by formation of singlet oxygen.\textsuperscript{81,82} Chlortetracycline and demeclocycline exhibit the greatest phototoxicity of the tetracyclines while minocycline is the least phototoxic.\textsuperscript{81,82} The complement system may also be involved in development of phototoxicity.\textsuperscript{83}

Reported symptoms of phototoxicity in humans include tingling, burning feeling, papules, and onycholysis.\textsuperscript{82} Doxycycline-induced phototoxicity appears to be dependent on the dose of doxycycline administered as patients taking higher doses are more at risk for developing phototoxic reactions.\textsuperscript{84}

Some of the tetracyclines, such as demethylchlortetracycline, doxycycline, oxytetracycline, lymecycline, and methacycline, but not minocycline, when added to human RBCs exposed to ultraviolet radiation (UVA) cause \textit{in vitro} photohemolysis which correlated with the degree of phototoxicity of the tetracycline. Photohemolysis can be an effective tool to study phototoxicity in newly introduced tetracyclines.\textsuperscript{85}

Phototoxicity in dogs, cats or horses being treated with tetracyclines has not been reported. Guinea pigs develop phototoxic skin lesions (erythema, induration and erosions) after receiving intradermal injections of demethylchlortetracycline and being exposed to ultraviolet A light.\textsuperscript{83} Tetracycline caused photohemolysis in mouse RBCs after exposure to UV light.\textsuperscript{86}

\textbf{2.2.6.3 Hepatic Toxicity}

In humans, the tetracyclines cause two forms of drug-induced liver injury, microvesicular steatosis and liver failure occurring after 4 to 10 days with high doses of
parenteral tetracyclines and an idiosyncratic liver injury that occurs with the oral agents; doxycycline causes a cholestatic injury and minocycline a hepatocellular injury which may or may not be associated with autoimmune features.\(^{87}\)

The accumulation of lipid in the liver leads to the development of microvesicular steatosis.\(^{88,89}\) The hepatocytes of mice treated with IV tetracycline contained cytoplasmic vacuoles, which scarlet red stain revealed as fat.\(^{90}\) Vacuolation was also present in the liver of dogs who were treated with IV Aureomycin\(^{8}\).\(^{90}\) Tetracycline inhibits mitochondrial oxidation of fatty acids. Free fatty acids have been found to cause oxidative stress, whereas, triglycerides may act to protect the liver.\(^{91}\) Tetracycline also decreases the ability of lipids to be transported out of rat hepatocytes.\(^{92}\)

Minocycline can cause a drug induced systemic lupus erythematous (SLE), autoimmune hepatitis, and hypersensitivity reactions. Minocycline-induced SLE is characterized by a positive ANA (antinuclear antibody) test, at least one clinical sign associated with SLE and the suspected cause to be minocycline.\(^{93}\) Humans with minocycline-induced SLE experience arthralgia, arthritis, fever, rash and pleuritis.\(^{93}\) Hepatitis can be a component of this disease, which may improve when the patient is taken off of the drug or it may continue to progress.\(^{94}\)

Minocycline-induced autoimmune hepatitis is characterized by high levels of transaminases which are not reported in patients with minocycline-induced SLE.\(^{93}\) Patients with autoimmune hepatitis also report having arthralgia, arthritis, fever, rash and jaundice.\(^{93}\) Improvement in autoimmune hepatitis may take longer when discontinuing minocycline than minocycline–induced SLE.\(^{93}\)
The least common cause of hepatic damage due to minocycline is to a hypersensitivity reaction. The condition is typically associated with an eosinophilia and desquamation of the skin in addition to liver abnormalities. Minocycline treatment was shorter in duration for patients developing a hypersensitivity reaction compared to an autoimmune hepatitis.

In 386 dogs treated with doxycycline, 39.4% and 36.4% had elevations in alanine aminotransferase (ALT) and alkaline phosphatase (ALP), respectively. The medical records of these patients did not reveal the reason for which bloodwork was performed while being treated with doxycycline, or if the clinician had a concern for an underlying disease that could have caused the elevated liver enzymes. Dogs were more at risk for developing ALT elevations if they were older. It was theorized that older dogs may be more at risk for hepatopathies because of concurrent disease or that older dogs may be more likely to be on other medications that could contribute to hepatotoxicity. Dogs treated with higher doses of doxycycline were more likely to have an elevated ALP, but not ALT. Minocycline induced-hepatotoxicity has not been reported in the dog.

2.2.6.4 Renal Toxicity

Tetracycline is excreted in the urine and feces. There have been reports that tetracycline and oxytetracycline may cause a decrease in the glomerular filtration rate in patients with chronic renal failure. It was also proposed that tetracycline’s anti-anabolic effects on protein synthesis could cause an increase in amino acid metabolism with an overload of metabolites requiring renal excretion leading to worsening of kidney function. Administering an anabolic steroid with tetracycline had less of an increase in
BUN than when tetracycline was administered alone. In one case series, seven human patients with a history of renal failure were placed on tetracyclines (oxytetracycline, tetracycline) for various infections and within two to eight days developed worsening of their renal function.97

Doxycycline is also excreted in the urine and feces. Similar to tetracycline, some patients in renal failure treated with doxycycline had a decline in their renal function.99 However, another report suggests that doxycycline can be administered to patients with renal failure as the half-life is not prolonged.100 Minocycline is cleared via hepatic metabolism with only about 10% excreted unchanged in the urine.101 Minocycline has been evaluated in normal patients and those with mild uremia. There was no significant difference between normal patients and those with mild uremia after a single intravenous dose of minocycline. Minocycline was also given orally for five days with no change in the BUN of patients with mild to moderate uremia.101

Outdated and improperly stored tetracycline administered to humans has been reported to cause Fanconi Syndrome.102 Degradation of the tetracycline was believed to be responsible for causing this syndrome. In a study in laboratory rats and dogs, anhydro-4-epi-tetracycline hydrochloride one of the tetracycline degradation products, caused cortical and tubular necrosis103 similar to the Fanconi Syndrome observed in humans.102

2.2.6.5 Effects on Calcified Tissues

Tetracyclines can cause discoloration of teeth as well as aveolar and cortical bone. Females administered tetracycline during the second or third trimester of pregnancy may give birth to a child with discolored teeth. Furthermore, mineralization of the permanent
teeth is not complete until the child is at least 8 years old, as such, tetracyclines should not be administered to any child under the age of 8.\textsuperscript{104,105} However, adult-onset tooth discoloration from tetracyclines has been noted to occur.

The mechanism of tooth discoloration during mineralization of permanent teeth occurs when tetracyclines travel via systemic circulation to the capillaries of teeth and finally the capillary loops near predentin.\textsuperscript{106} Tetracycline binds to calcium and the tetracycline-calcium complex diffuses to the dentin where tetracycline calcium orthophosphate complex develops.\textsuperscript{106} Initially, there is a yellow fluorescence of teeth when teeth are exposed to UV light.\textsuperscript{106} Over time, the teeth develop brown discoloration, which may be due to oxidation of the tetracycline calcium orthophosphate complex.\textsuperscript{106}

Adults treated with doxycycline very rarely develop tooth discoloration.\textsuperscript{107} The first reported case of tooth discoloration in an adult taking doxycycline was a 20-year old woman who had taken doxycycline for one month and the tooth discoloration was reversed after the drug was discontinued.\textsuperscript{107} There have been other reports of adults with yellow discoloration of the permanent teeth after taking doxycycline.\textsuperscript{108} In these patients, sun exposure and poor oral hygiene were also suspected to play a role in the tooth discoloration. Doxycycline therapy was continued and the discoloration resolved with abrasive dental cleaning.

Minocycline-induced tooth discoloration appears to occur more frequently than doxycycline, but is still rare.\textsuperscript{109} One report of adults taking minocycline found that four of 72 (5.5\%) individuals developed tooth discoloration, which did not resolve when minocycline was discontinued.\textsuperscript{109}
There are several possible mechanisms for staining of adult teeth. One mechanism may be that doxycycline or minocycline binds to the tooth pellicle, which is a proteinaceous film on the surface of teeth, via glycoproteins, then oxidized by UV light or bacteria, and lastly, chelates iron. The second mechanism may occur when doxycycline or minocycline binds to glycoproteins on the pellicle. This leads to cycles of demineralization and remineralization and then the oxidation of doxycycline or minocycline degrades its structure leading to the formation of insoluble black quinone.

Doxycycline- or minocycline-induced tooth discoloration has not been reported in animals. However, there are several reports of tetracycline causing tooth discoloration in animals. Tetracycline administered to a pregnant dog three weeks pre-partum and then to the puppies post-partum, resulted in discoloration of the primary dentition that fluoresced with UV light. It was also found in the enamel and dentin in puppies treated with daily tetracycline from weaning to five months of age. These puppies also had enamel hypoplasia.

Bone discoloration can occur in other calcified tissues besides the teeth. Pigmentation of the jawbone, thought initially to be gingival pigmentation, has reported in a human patient with a six-year history of minocycline therapy. Yellow discoloration but not fluorescence was found in the femur and calvarium of rats treated with oral minocycline. Tetracyclines have not been reported to cause bone discoloration other than the teeth in dogs.
2.2.6.6 Vestibular

Vestibular signs have been reported in humans taking minocycline and occur more frequently than in patients taking other tetracyclines.\textsuperscript{18,115} The prevalence of vestibular signs was reported as 7.2% prior to 1974.\textsuperscript{107} During 1974, there was a reported increase in patients experiencing vestibular signs with a range of 12 to 90% of patients affected.\textsuperscript{107} In 12% to 52% of patients, the vestibular signs were so debilitating that they had to stop taking minocycline or quit their jobs.\textsuperscript{107} Vestibular signs include nausea, vomiting, vertigo, difficulty ambulating and a change in hearing.\textsuperscript{116} In general, vestibular signs can begin as early as after the second dose of minocycline and reportedly resolve within two days of discontinuing minocycline.\textsuperscript{116} Vertigo is a unique side effect of minocycline, occurs primarily in women (70% of patients with vertigo), and resolves within several days of stopping minocycline.\textsuperscript{37} There are no reports of doxycycline- or minocycline-induced vestibular signs in dogs.

2.2.6.7 Esophageal Erosion

Tetracycline and doxycycline become more acidic in solution (pH<5) and can cause ulcers when in contact with the esophageal lining.\textsuperscript{117} Doxycycline hyclate reportedly becomes more acidic than doxycycline monohydrate.\textsuperscript{118} Esophagitis and subsequent esophageal ulcers have been reported in humans taking doxycycline and tetracycline.\textsuperscript{119} The risk of esophagitis is worse if these medications are taken just before lying in a recumbent position or without drinking much liquid.\textsuperscript{119} Symptoms include retrosternal pain, heartburn and odynophagia (pain when swallowing).\textsuperscript{120} About 24% of patients taking tetracycline antibiotics developed symptoms of esophagitis.\textsuperscript{120} Endoscopy
is used to diagnose esophageal ulcers and the ulcers most commonly occur in the middle third of the esophagus. Esophagitis resolves after discontinuing the tetracycline as well as with supportive care. In humans, these ulcers heal after stopping the tetracycline antibiotic and do not form strictures. Esophageal ulcers have not been reported in humans taking minocycline.

Esophageal erosions, ulcerations and strictures have been reported in cats taking orally administered tetracyclines. In cats, unlike humans, as the ulcers heal, secondary strictures can form due to fibroblastic proliferation and wound contraction. Doxycycline and oxytetracycline have been reported to cause esophageal strictures.

Many of the reported cases of esophageal strictures have been in cats receiving doxycycline hyclate rather than doxycycline monohydrate. The predominant clinical sign in cats with esophageal strictures is regurgitation, while on doxycycline. Diagnosis is made on endoscopy or fluoroscopy. Treatment for esophageal stricture is balloon dilation of the stricture and in some patients, multiple balloon dilation procedures are required for resolution. To prevent esophageal strictures, it is recommended that water be used as an oral flush after doxycycline administration in cats. Doxycycline-induced esophageal injury has not been reported in the dog.

2.2.6.8 Hemolytic Anemia

Hemolytic anemias are rarely caused by tetracyclines (tetracycline and minocycline). There are no reports of doxycycline causing a hemolytic anemia in humans. Both immune mediated and non-immune mediated hemolytic anemias have
been reported. A positive Coombs test is diagnostic for an immune-mediated hemolytic anemia. There are two possible mechanisms of tetracycline-induced immune-mediated hemolytic anemias. Tetracycline immune-mediated hemolytic anemias can occur due to the production of IgG against tetracycline with the subsequent binding of the drug to the red-cell membrane and then binding of the IgG molecule (hapten phenomenon) causing extravascular hemolysis. On the other hand the immune-mediated hemolytic anemia could be due to the antibody binding to the free drug in the plasma, with the immune complex binding to the red-cell membrane, causing intravascular hemolysis.

Even less common to occur are non-immune mediated hemolytic anemias. In one patient with non-immune mediated hemolytic anemia, the diagnosis was based on a positive Heinz-body preparation thus the hemolysis was caused by oxidative denaturation of hemoglobin.

Hemolytic anemia has been reported in dogs after intravenous treatment with minocycline. In dogs treated with a 5 mg/kg daily dose of minocycline, no hemolytic anemia was observed, while in dogs treated with 10 mg/kg, 20 mg/kg, and 40 mg/kg daily dose of minocycline for 27 to 29 days, varying degrees of anemia were noted ranging from trace to slight to moderate, respectively. None of these dogs were icteric or developed a hemolytic crisis. Noble et al. found hemolytic activity evidenced by dose-related decreases in packed cell volume, hemoglobin concentration, and erythrocyte cell count in dogs receiving similar intravenous doses.

Variable sample hemolysis was noted after one dose in six beagles receiving 5 mg/kg minocycline intravenously while only mild sample hemolysis was noted in two beagles receiving 10mg/kg orally. Hemolytic anemia was not reported in these dogs.
Neither sample hemolysis or hemolytic anemia were noted after administration of 10 mg/kg minocycline orally to five healthy greyhounds.\textsuperscript{8}

\textbf{2.2.6.9 Pigmentation}

Pigmentation of various body sites has been reported with the use of tetracyclines, mainly minocycline, including the thyroid gland, nails, skin, heart valves, oral mucosa and sclera. The pigmentation is caused by polymerization of the oxidation products, 9-hydroxy and N-demethylated derivatives, to form an iron-binding melanin-like molecule. Oxidation of minocycline is facilitated by thyroid peroxidase in the presence of iodide.\textsuperscript{126} In humans, thyroid pigmentation resulting in a black thyroid gland can occur rarely with minocycline treatment.\textsuperscript{126} There has been some concern that papillary thyroid carcinoma may be linked to thyroid pigmentation.\textsuperscript{126} There are only rare reports of thyroid gland pigmentation due to tetracycline or doxycycline.\textsuperscript{127}

Thyroid gland pigmentation associated with minocycline administration has been described in rats,\textsuperscript{114} dogs,\textsuperscript{114} and monkeys.\textsuperscript{114} The mechanism of pigment deposition in animals is similar to humans, in that minocycline interferes with thyroid peroxidase in the thyroid gland and is degraded to the black pigment by an enzymatic reaction. At autopsy, thyroid pigmentation was found grossly and microscopically in beagles administered intravenous doses of minocycline at 5 mg/kg, 10 mg/kg, 20 mg/kg and 40 mg/kg daily for one month.\textsuperscript{114} Thyroid hyperplasia was also observed microscopically in the same beagles.\textsuperscript{114} The degree of thyroid hyperplasia appeared to increase with the dose of minocycline such that beagles receiving 5 mg/kg daily had only slight hyperplasia and those receiving higher doses had moderate to marked hyperplasia.\textsuperscript{114} One beagle
receiving 40 mg/kg daily had a pigmented thyroid gland, but the thyroid was not hyperplastic.\textsuperscript{114}

Thyroid pigmentation was found in six monkeys administered minocycline orally daily for one month at a dose of 30 mg/kg.\textsuperscript{114} While all monkeys had a grossly pigmented thyroid gland, the pigmentation was only visible microscopically in three monkeys.\textsuperscript{114} Thyroid hyperplasia was not present in the monkeys.\textsuperscript{114}

Thyroid pigmentation was not observed in mice receiving 250 mg/kg orally daily for 28 days.\textsuperscript{114} The researchers could not find a reason for the lack of thyroid pigmentation in mice compared to other species.\textsuperscript{114}

Tetracyclines can cause pigmentation of the nails.\textsuperscript{128} Rarely, tetracycline can cause a yellow discoloration of the lunulae (white crescent shape on nails) that fluoresces with a UV light.\textsuperscript{128} The fluorescence distinguishes the tetracycline-induced pigmentation from other diseases causing nail discoloration and the incorporation of the tetracycline into the nail matrix is responsible for the fluorescence.\textsuperscript{128} The yellow discoloration can occur within one month of starting tetracycline and resolves over several months after discontinuing tetracycline. Interestingly, when tetracycline was administered during pregnancy, the teeth of the adults showed no UV fluorescence but their nails fluoresced, and the children developed tooth discoloration and fluorescence with UV light, but there was no UV fluorescence of the nails.\textsuperscript{129}

Doxycycline-induced nail pigmentation is very rare.\textsuperscript{130,131} Two pediatric patients developed discomfort of the nail beds and discoloration of the nails after taking doxycycline.\textsuperscript{130} Another pediatric patient developed nail discoloration, but no nail discomfort.\textsuperscript{131}
Nail discoloration caused by minocycline appears to be more common than that caused by tetracycline or doxycycline, but is still uncommonly reported.\(^{132}\) The entire nail can be affected or longitudinal lines of pigment (longitudinal melanonychia) may be observed.\(^{133}\) Longitudinal melanonychia develops when there is melanization of the nail caused by increased activity of the melanocytes of the nail matrix or when there are more melanocytes than are normally present.\(^{134}\) There are no reports of the tetracyclines causing nail pigmentation in dogs.

Skin pigmentation is more common than nail pigmentation with the use of minocycline.\(^{133}\) This can occur as early as eight weeks after starting minocycline, but may also develop after taking minocycline for years.\(^{132}\) There are three forms of minocycline-induced skin pigmentation. The first includes blue to black macules in the area of scars or inflammation. The second includes pigmentation that is not associated with skin inflammation, which occurs on the lower legs or in areas exposed to the sun. The third includes a generalized brown to gray pigmentation of the skin. There are several possible pigments that could cause minocycline-induced skin pigmentation. Hemosiderin, melanin, minocycline degradation product have all been implicated.

There is one case report of doxycycline causing blue-black pigmentation of the skin.\(^{135}\) A 36 year old, man who had taken doses of doxycycline that exceeded those of the normal dose range for over 12 years, developed pigmentation of his distal legs and dorsal feet. The basal keratinocytes and histiocytes in the superficial dermis were positive for melanin using Fontana Masson staining. The histiocytes in the deeper dermis stained positive for calcium (von Kassa stain) and iron (Perls stain). This patient’s pigmentation
faded over the course of one year. Tetracyclines have not been reported to cause skin pigmentation in dogs.

Osteoma cutis are localized calcium deposits that are a common complication of acne vulgaris. Osteoma cutis rarely occur in patients treated with tetracycline for their acne. Tetracycline forms a complex with calcium and is deposited in the new bone formation of the osteoma, resulting in the brown discoloration. Clinically, the brown osteomas appear blue.

Pigmentation of heart valves caused by minocycline is rare. In the reported cases, the pigmentation was discovered during heart surgery, and microscopic examination of the tissue revealed finely dark granular pigment that stained positive for melanin with Fontana-Masson.

Minocycline can cause pigmentation of the soft tissues of the oral cavity, but this has not been observed in patients treated with tetracycline or doxycycline. Minocycline can cause pigmentation of the gingiva, lips, and tongue. Pigmentation of the soft tissues of the oral cavity is less common than pigmentation of the alveolar bone. This has not been reported in dogs.

The tetracyclines can cause pigmentation of the structures of the eye and surrounding soft tissues. Tetracycline can cause brown to black pigmented conjunctival deposits that on closer examination consisted of calcium and fluoresced yellow with UV light. The conjunctival deposits can also be dark green. There are no reports of tetracycline causing scleral pigmentation. Doxycycline has not been reported to cause pigmentation of the sclera or conjunctiva.
Minocycline can cause scleral or conjunctival pigmentation as well as cause conjunctival pigmented cysts.\textsuperscript{140} The scleral pigmentation can be a blue, gray, brown or black band and can be found in patients who also have pigmentation in other body sites.\textsuperscript{141} There may be permanent scleral pigmentation or it may resolve slowly and is more likely to resolve if minocycline is discontinued early.\textsuperscript{141} It is not known if the pigment is due to metal ion or melanin.\textsuperscript{141} There are no reports of tetracycline, doxycycline or minocycline causing ocular pigmentation in dogs.

\textbf{2.2.6.10 Hypersensitivity Reactions}

Anaphylactic reactions are type I hypersensitivity reactions that have been rarely reported in humans taking tetracycline,\textsuperscript{142} doxycycline\textsuperscript{143} and minocycline.\textsuperscript{144} Tetracyclines were identified as the precipitating cause by prick test\textsuperscript{142} and drug challenge.\textsuperscript{144} In one case of doxycycline anaphylaxis, the patient’s disease (Q fever) was responsive only to doxycycline, so a decision was made to perform a rapid IV desensitization to doxycycline. The desensitization treatment was successful as the patient was able to tolerate oral daily doxycycline.\textsuperscript{143} Hypersensitivity reactions to minocycline can include hepatitis and acute eosinophilic myocarditis.\textsuperscript{145}

There is a rare minocycline-induced drug reaction with eosinophilia and systemic symptoms syndrome otherwise known as DRESS syndrome.\textsuperscript{146} Systemic signs can include skin eruptions, myocarditis, thyroiditis, acute renal failure, facial edema, fever, lymphadenopathy, hepatitis, cerebral edema, pneumonitis, and pericardial effusion.\textsuperscript{146} Skin lesions can include erythematous papules and plaques as well as desquamation and fissuring of the soles of feet. Patients present with these symptoms within two weeks to
two months of beginning minocycline therapy\textsuperscript{146} and can improve within three to six weeks of stopping minocycline.\textsuperscript{147} Beside discontinuing minocycline, other treatments include supportive care and corticosteroids.\textsuperscript{147} There is a reported 10\% mortality rate with DRESS syndrome.\textsuperscript{146} People of African American descent may have a genetic predisposition to developing DRESS when treated with minocycline.\textsuperscript{146} No gender predisposition has been reported.

Other reported minocycline reactions include Sweets syndrome (acute febrile neutrophilic dermatosis),\textsuperscript{148} Stevens-Johnson syndrome\textsuperscript{149} and immune thrombocytopenic purpura.\textsuperscript{150}

While similar reactions have not been reported in dogs, Nobel \textit{et al.}\textsuperscript{76} reported that six of eight beagle dogs receiving intravenous minocycline daily for one month developed an erythematous, papular dermatitis occurring mainly around the eyes, muzzle and ears but only rarely on the abdomen or limbs. With more severe reactions, the papules coalesced. Variable swelling of the face and ears occurred with the erythematous reaction. At the 5 mg/kg dose, dogs only developed transient, mild erythema during the initial dose. At the 10 mg/kg dose, dogs developed erythema of the muzzle and ears, which resolved by the end of the injection. At the 20-40 mg/kg dose, dogs developed moderate to severe reactions. Erythema was not observed in other studies evaluating minocycline in dogs.\textsuperscript{7,8,114}

\textbf{2.2.6.11 Intracranial Hypertension}

In people, benign intracranial hypertension (pseudotumor cerebri syndrome) has been reported in patients treated with minocycline\textsuperscript{18}, doxycycline\textsuperscript{151} and tetracycline.\textsuperscript{151}
Intracranial hypertension is rare. Tetracyclines may interfere with the secretory or filtration function of the choroid plexus leading an accumulation of CSF. As minocycline is more lipophilic than other tetracyclines and it may more easily cross the blood-brain barrier, thus be more likely to cause an increase in CSF.

This disease can cause symptoms such as vomiting, headache and bilateral papilledema (swelling of the optic disk due to intracranial hypertension) with this symptom being diagnostic for the disease. Intracranial hypertension is most common in young women. Symptoms may resolve as early as one month of stopping the tetracycline antibiotic. While most cases of intracranial hypertension are mild, there are reports of more severe symptoms. In these cases, there has been severe papilledema as well as vision loss than can persist despite stopping minocycline. In cases where vision loss is progressive, surgical intervention may be necessary. In one case series of twelve patients being treated with minocycline for acne vulgaris, nine of twelve (75%) patients developed signs of intracranial hypertension within eight weeks of starting minocycline. Two of twelve patients developed intracranial hypertension after being treated with minocycline for one year. One of these patients was treated with 50 mg minocycline daily for the first year and then the dose was increased to 100 mg daily after which she developed intracranial hypertension. The second patient had a chronic history of sinus headaches and after one year of minocycline developed changes in vision.
2.2.7 Therapeutic Uses

2.2.7.1 Leptospirosis

Leptospirosis is a disease caused by a spirochete bacteria with zoonotic significance. Humans, similar to dogs, are at risk of developing leptospirosis when exposed to fresh water or moist soil. Leptospires may survive better in stagnant water, but may also be found in areas of quickly moving water adherent to rocks and debris. Leptospires can also be transmitted to dogs in contact with infected urine or infected tissue as well as via bite wounds.

Hawaii has the highest number of human cases of leptospirosis in the United States. Leptospira interrogans serovars Icterohemorrhagiae and Australis were the most common causes of human infections. In dogs, L. interrogans serovar pomona and bratislava, as well as Leptospira kirschneri serovar grippotyphosa were the most common causes of canine infections. However, there are other serovars that can cause infections. The host for L. interrogans serovar canicola is the dog, but all other serovars have rodents, farm animals, deer or other wildlife species that are the hosts.

Infections in humans and dogs can range from mild to severe. Clinical signs in humans include fever, headaches, myalgias and progress to jaundice and bleeding tendencies. Clinical signs in dogs include fever, uveitis, pulmonary hemorrhage, fever, abortion, and liver or kidney failure. Diagnosis in humans and dogs is based on the microscopic agglutination test (MAT). This test involves incubation of varying concentrations of the patient’s serum with live spirochetes to assess antibody agglutination of the spirochetes. In dogs, MAT cannot distinguish between exposure and vaccination. Also, it may be falsely negative.
the first seven days after exposure so a repeat test is often needed.\textsuperscript{155} Other methods of diagnosis in dogs include blood and urine cultures of leptospires (gold standard), and PCR.\textsuperscript{155}

Treatment of human leptospirosis includes the use of penicillin, azithromycin or doxycycline.\textsuperscript{155} A consensus panel on canine leptospirosis concluded that treatment with doxycycline should be administered at 5 mg/kg twice daily for two weeks.\textsuperscript{157} If the patient has gastrointestinal signs such that oral medications cannot be administered, then intravenous ampicillin is recommended.\textsuperscript{157}

A leptospirosis vaccine is available for dogs against serovars Icterohemorrhagiae, Canicola, Grippotyphosa and Pomona for prevention of canine leptospirosis.\textsuperscript{157} The consensus on canine leptospirosis recommends that at risk dogs receive the vaccine annually.\textsuperscript{157}

\textbf{2.2.7.2 Borreliosis}

\textit{Borrelia burgdorferi} is a spirochete that causes Lyme disease and is transmitted by ticks in the \textit{Ixodidae} family.\textsuperscript{158} Clinical signs of lyme disease in humans include erythema migrans skin lesions. These skin lesions are an area of erythema that can expand and develop a bull’s eye lesion with an area where the erythema lessens or resolves between the central area of erythema and the outer edge of erythema.\textsuperscript{158} Erythema migrans can develop within seven to 14 days after the tick bite.\textsuperscript{158} Other clinical signs include arthralgia, myalgia, fever, headache, lethargy.\textsuperscript{158} Extracutaneous signs include a carditis, seventh cranial nerve palsy, meningitis, and arthritis.\textsuperscript{158} Antibiotic therapy for suspected Lyme disease should be started in a patient with an
erythema migrans skin lesion. For patients without erythema migrans, serologic testing is recommended. Serologic testing includes an ELISA and if the ELISA is positive, then IgM and IgG immunoblotting are recommended.\textsuperscript{158} Doxycycline, amoxicillin or cefuroxime axetil are recommended treatments for Lyme disease in humans.\textsuperscript{158}

Clinical signs of Lyme disease in dogs include fever, anorexia and arthritis.\textsuperscript{159} Dogs may also develop signs associated with an immune-mediated glomerulonephritis, specifically membranoproliferative glomerulonephritis secondary to Lyme disease.\textsuperscript{159} The C6 assay detects antibodies against the C6 peptide (which is a protein on the surface of the borrelia organism) and when positive indicates exposure.\textsuperscript{159} ELISA, IFA antibody tests and Western Blot immunoassays are also available.\textsuperscript{159} Based on the consensus for canine Lyme disease if a dog is asymptomatic, but positive for Lyme disease no treatment is necessary.\textsuperscript{159} If a dog is symptomatic, then doxycycline is administered at 10 mg/kg daily to treat arthritis or the nephropathy.\textsuperscript{159}

\textbf{2.2.7.3 Yersinia}

\textit{Yersinia pestis} is the causative agent of the plague and is an anaerobic, gram-negative bacteria.\textsuperscript{39} The reservoir hosts are rodents, particularly prairie dogs, and rock and ground squirrels.\textsuperscript{39} The primary vector are fleas.\textsuperscript{39} Cats and dogs may be infected when ingesting a rodent or when bitten by a flea with \textit{Y. pestis}.\textsuperscript{39} Fleas acquire \textit{Y. pestis} when they bite an infected host.\textsuperscript{39} \textit{Y. pestis} proliferate in the flea’s proventriculus and midgut eventually forming an obstruction, which prevents fleas from digesting blood so the fleas bite the host more frequently and with each bite, \textit{Y. pestis} is expelled into the host’s tissue.\textsuperscript{39}
The three forms of plague are bubonic, septicemic and pneumonic plague. Bubonic and septicemic plague occur after a bite from an infected flea or from a scratch on the skin from an infected animal. Polymorphonuclear cells phagocytize *Y. pestis* and travel to the lymph nodes forming an abscess. *Y. pestis* then travels to other organs. The incubation period is two to six days. Pneumonic plague occurs after the inhalation of infected aerosols containing encapsulated *Y. pestis* organisms and is transmitted via coughing. The incubation period is one to three days.

In humans, symptoms of bubonic plague include lymphadenomegaly, fever and headache. In one-quarter of cases, a papule or vesicle may be found at the site of infection. Signs of septicemic plague are similar to those of septic shock and include abdominal pain and skin necrosis of the extremities. Pneumonic plague is characterized by respiratory signs, such as coughing and chest pain, but other signs can be present such as a fever, headache and weakness. Treatment in humans includes streptomycin, gentamicin, tetracycline or doxycycline.

In cats, bubonic plague is associated with signs of fever, lymphadenomegaly and in some cases draining abscesses of the lymph nodes. If untreated, bubonic plague can spread throughout the body to become the septicemic form. Signs of septicemic plague are the same as those of sepsis. Bubonic and septicemic plague undergo hematogenous spread, whereas, pneumonic plague can undergo hematogenous or lymphatic spread. In dogs, clinical signs include fever, anorexia, vomiting, diarrhea, lymphadenopathy, lethargy and dyspnea.

Treatment options include gentamicin, doxycycline, chloramphenicol and sulfonamides. Treatment is administered for ten to 21 days. If an animal is exposed to
the plague, a seven day course of a tetracycline antibiotic is recommended.\textsuperscript{39} Prevention in cats and dogs is aimed at reducing exposure to rodent’s habitat, preventing cats and dogs from hunting and applying regular flea prevention.\textsuperscript{161}

2.2.7.4 Brucellosis

Brucellosis is caused by gram-negative coccobacillary organisms.\textsuperscript{39} \textit{Brucella canis} can be differentiated from other species of \textit{Brucella} based on its rough colony morphology.\textsuperscript{39} The rough colonies are formed due to decreased amounts of smooth lipopolysaccharide in the cell wall.\textsuperscript{39} Dogs are the primary host and humans can be infected after exposure to infected dogs.\textsuperscript{39} Domestic animals can be infected by six known species of \textit{Brucella}. These include \textit{B. canis}, \textit{Brucella suis}, \textit{Brucella abortus}, \textit{Brucella melitensis}, \textit{Brucella neotomae} and \textit{Brucella ovis}. \textit{Brucella abortus} affects cattle, \textit{Brucella melitensis} affects goats and sheep, \textit{Brucella neotomae} affects rodents while \textit{Brucella ovis} affects sheep. \textit{Brucella suis} is the primary species that causes infection in pigs, but it can also affect other animals.

In canine brucellosis, dogs become infected when a mucosal surface comes in contact with infected fluids or tissues (placenta).\textsuperscript{39} The organisms are phagocytized by macrophages and neutrophils and brought to the lymph nodes and spleen,\textsuperscript{39} Bacteremia develops and can persist for up to 64 months.\textsuperscript{39} There is hematologic spread of the infection leading to discospondylitis, uveitis, glomerulonephritis, prostatitis, and epididymitis. Abortion can occur between 45 to 60 days gestation of pregnancy with a brown to green vaginal discharge for up to six weeks\textsuperscript{39} If the puppies are not aborted, some may die hours to weeks after birth.\textsuperscript{162} Puppies that survive can develop generalized
lymphadenopathy as well as leukocytosis, fevers and seizures. A pyogranulomatous dermatitis has also been observed in a dog with brucellosis.

Bacterial culture to identify Brucella provides definitive diagnosis. Cultures can be performed from blood, urine, semen or tissue. With chronic infection, there are lower numbers of organisms so a culture may be negative despite infection. Serology can also be performed using a rapid slide agglutination test, tube agglutination test, agar gel immunodiffusion, ELISA testing and indirect immunofluorescence.

Treatment for canine brucellosis is difficult as it is an intracellular bacteria, therefore, combination therapy should be used. The most often recommended treatment regimen is doxycycline or minocycline for four to eight weeks with streptomycin. A culture or agar gel immunodiffusion test should be performed when finished with treatment and every three months thereafter until obtaining two negative serologic test results. Since this can be a problem in breeding kennels, strict measures of control must be taken and all positive dogs should be neutered and removed from the kennel.

Humans can be infected with B. canis, B. abortus, B. suis and B. melitensis. The most common symptoms include uveitis, conjunctivitis, spondylitis, and polyarthritis. Symptoms of B. canis include fever, weight loss, lymphadenomegaly and lethargy. Dog owners should be warned that B. canis is zoonotic. Veterinarians and laboratory technicians working with Brucella should wear personal protective equipment. Combinations of doxycycline and rifampin, or doxycycline and streptomycin have been used to treat human brucellosis.
2.2.7.5 Wolbachia

*Wolbachia pipiens* is a gram-negative bacteria that can reside in *Dirofilaria immitis*. Clinical signs of *W. pipiens* are difficult to distinguish from those of *D. immitis*. Treatment for *W. pipiens* in infected dogs is doxycycline at 10 mg/kg orally twice daily for 4 weeks. Use of heartworm prevention will help prevent infection with *W. pipiens*.

2.2.7.6 Rickettsial Infections

Rocky Mountain spotted fever (RMSF) is caused by *Rickettsia rickettsia* an obligate intracellular organism. Common tick vectors include *Dermacentor andersoni* in the western states and *Dermacentor variabilis* eastern and southern states. Within affected ticks, transstadial and transovarial transmission can occur. Larval and nymphal ticks can feed on infected rodents and then become infected. Adult ticks can then feed on dogs or humans and can transmit infection.

Clinically, canine RMSF is characterized by fever, petechiae, ecchymoses and peripheral edema. Canine bloodwork abnormalities include thrombocytopenia, leukocytosis and hypoalbuminemia. Treatment options are tetracycline, doxycycline, enrofloxacin and chloramphenicol. Minocycline has not been evaluated as a treatment for RMSF.

Symptoms in humans include fever, headaches, myalgia, vomiting, cough, sore throat, chest and abdominal pain. A skin rash starts as a macular lesion and then progresses to maculopapular and then petechial. Diagnosis is made by biopsy of skin lesions and immunofluorescent antibody assays. A serum antibody titer rise of at least
four fold indicates infection with RMSF. Bloodwork abnormalities can include hyponatremia and thrombocytopenia. Other diagnostic tests include latex agglutination titers and ELISAs. Doxycycline is used for treatment and should be given at least three days post-fever with the length of treatment ranging from five to ten days.

Canine ehrlichiosis is caused by several species of *Ehrlichia* including *Ehrlichia canis, Ehrlichia chaffeensis* and *Ehrlichia ewingii*. *Ehrlichia canis* is transmitted by the primary vector tick, *Rhipicephalus sanguineus*. Immature ticks are infected when they feed upon an infected dog, which is the primary host. The tick harbors the infection and then passes it to other dogs during feeding as a nymph or adult. The primary vector tick for *E. chaffeensis* is *Amblyomma americanum*. The infected nymph or adult tick feeds on a dog and infects it. Other ticks that may be able to transmit *E. chaffeensis* include *Dermacentor variabilis* and *R. sanguineus*. The primary vector tick for *E. ewingii* is *A. americanum*.

German shepherds may be predisposed to developing ehrlichiosis. Clinical signs of canine ehrlichiosis include lethargy, anorexia, weight loss and possibly dermal or mucosal petechiae, ecchymoses or epistaxis. Blindness or changes in eye color can also occur. Seizures, ataxia, vestibular signs, cerebellar dysfunction, and polyarthritis. Canine ehrlichiosis causes thrombocytopenia, pancytopenia, hypoalbuminemia, lymphocytosis, increased serum alanine aminotransferase.

A consensus statement on ehrlichial disease lists tetracycline, oxytetracycline, chloramphenicol, amicarbalide and imidocarb dipropionate as drugs that have been successful in the treatment of ehrlichiosis. However, doxycycline and minocycline are used more frequently with doxycycline still considered the treatment of choice. The
The consensus statement recommended doxycycline 10 mg/kg orally once daily for 28 days.

There are two types of ehrlichiosis in humans: monocytic and granulocytic. Human monocytic ehrlichiosis (HME) is caused by *Ehrlichia chaffeensis*, which is similar to *Ehrlichia canis*. *E. chaffeensis* forms morulae in monocytes. Human granulocytic ehrlichiosis (HGE) forms morulae in neutrophils.

Tick vectors for HME include *A. americanum* and *D. variabilis*, and the reservoir host is the white-tailed deer. Natural hosts for HME are humans, dogs, goats and captive lemurs. The tick vector for HGE is *Ixodes scapularis* and the reservoir hosts are the white-tailed deer and white-footed mouse. Natural hosts are humans, dogs, cats, horses, sheep, goats, cattle and llamas.

Symptoms in humans include skin rash, fever, headache, vomiting, diarrhea, abdominal pain, arthralgia, lymphadenopathy and cough. The development of a skin rash is much less common with ehrlichiosis than with RMSF. A skin rash is also more common with HME. The skin rash can be transient and presents as primarily a macular, papular or maculopapular rash, but petechiae, purpura and erythema have also been described. In severe cases, hepatitis, meningitis, pericarditis, renal failure and organ failure can occur.

Common bloodwork abnormalities in humans include anemia, thrombocytopenia, leukopenia as well as increase liver enzymes (alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase). Diagnosis can also be made with the identification of morulae within monocytes or neutrophils on blood smears. Other diagnostic tests include serology (indirect fluorescent antibody test) and PCR for the 16S
Tetracycline antibiotics, primarily tetracycline or doxycycline, are the main treatment for ehrlichial diseases and it is recommended that they be started if ehrlichiosis is suspected. Other treatment options include chloramphenicol or rifampin. The length of treatment should be at least five to seven days. Treatment should be continued three days past resolution of a fever.

Canine anaplasmosis can be caused by *Anaplasma phagocytophilum* or *Anaplasma platys*. *Anaplasma phagocytophilum* is primarily transmitted via *Ixodes* species and *A. platys* is primarily transmitted by *R. sanguineus*. Clinical signs of *A. phagocytophilum* include fever, lethargy and anorexia. However, polyarthritis, gastrointestinal signs and bleeding tendencies also occur. Many of these dogs develop thrombocytopenia. *A. platys* causes infectious cyclic thrombocytopenia and causes inclusions within platelets. Clinical signs of *A. platys* include fever, uveitis, petechiae, and ecchymoses. Treatments include tetracycline antibiotics or enrofloxacin.

Q fever, is a zoonosis caused by *Coxiella burnetii*, and is within the gamma subdivision of *Proteobacteria*, whereas, *Rickettsia* are within the alpha subdivision of *Proteobacteria*. *C. burnetii* is a small, obligate intracellular gram-negative bacterium that occurs as a small-cell variant and and a large-cell variant. The large-cell variants are the active stages and the small-cell variants are spore-like forms released when the cells are lysed and remain in the environment for extended periods. Q fever is a concern among people who work with cattle, sheep or goats, which are the primary reservoirs. Other reservoirs are birds and ticks.

Humans acquire Q fever mostly by inhalation of infected aerosols, but it can also be acquired by ingestion of raw milk. Dogs and cats can also be reservoirs and human
infections have been reported after contact with affected dogs and cats.\textsuperscript{170} The disease in humans can be mild or progress to have serious complications.\textsuperscript{170}

Symptoms in people include fever, lethargy, headache, myalgia, cough, vomiting, diarrhea, chest pain and skin rash.\textsuperscript{170} The skin lesions are truncal macular to papular lesions.\textsuperscript{170} Complications can include meningoencephalitis, endocarditis, hepatitis, joint infections or osteoarthritis, and pulmonary infections.\textsuperscript{170} The infections can be acute or chronic.\textsuperscript{171} About 40\% of infected people are symptomatic and 60\% are asymptomatic.\textsuperscript{171} Tetracycline antibiotics are the treatment of choice. Doxycycline can be administered for two weeks for acute infections, but a longer course (>1 year) may be needed for chronic infections.

Most animals with \textit{C. burnetii} infection are asymptomatic.\textsuperscript{171} Q fever can cause abortion and the aborted tissues contain infectious organisms.\textsuperscript{171} Infected animals can shed the organism in urine, feces and milk.\textsuperscript{171} Diagnosis is made by examining smears from placenta, immunohistochemistry, PCR or serology.\textsuperscript{171} Treatment of ruminants with Q fever includes two injections of oxytetracycline during the month prior to parturition.\textsuperscript{171}

Salmon poisoning disease or Elokumin Fluke Fever in dogs is caused by \textit{Neoricketttsisa helminthoeca}. Dogs become infected by ingesting fish that are infected with a trematode, \textit{Nanophyetus salminocola}, that harbors \textit{N. helminthoeca}.\textsuperscript{39,172} Clinical signs include diarrhea, lethargy, vomiting, inappetance and neurological signs.\textsuperscript{172} Bloodwork changes include thrombocytopenia, neutrophilia and lymphopenia.\textsuperscript{172} Effective treatments include doxycycline (parenterally and orally), oxtetracycline (parenterally), and tetracycline (orally).\textsuperscript{172}
2.2.7.7 **Mycoplasma**

Mycoplasmas are bacteria that are enclosed by a lipid bilayer, but do not have a cell wall. There are hemotropic and nonhemotropic mycoplasmas. Hemotropic mycoplasmas are gram-negative and non-acid fast. Hemotropic mycoplasmas that infect that cat are *Mycoplasma haemofilis, Candidatus Mycoplasma haemominutum* and *Candidatus Mycoplasma turcensis*. The cause of canine hemotropic mycoplasma is *Mycoplasma haemocanis*. Both *M. haemofelis* and *M. haemocanis* used to be classified as *Haemobartonella felis* and *Haemobartonella canis*, respectively. In cats and dogs, hemotropic mycoplasmas are attached to the surface of the erythrocyte, and can appear as cocci, rods or rings.

Clinical signs of feline hemotropic mycoplasmosis in cats include anemia, hepatosplenomegaly, lymphadenopathy, icterus, dyspnea. Dogs usually do not have clinical signs associated with *M. haemocanis*. Diagnosis for canine and feline hemotropic mycoplasmosis is made via blood smears, however, PCR can be used in cases where no organisms are identified on a blood smear.

Feline hemotropic mycoplasmosis is treated with pradofloxacin, marbofloxacin, enrofloxacin or doxycycline. Treatment of canine hemotropic mycoplasmosis is with doxycycline or tetracycline.

Nonhemotropic mycoplasmosis in cats is due to *Mycoplasma felis*. *Mycoplasma felis can* cause conjunctivitis, and in some cases bronchitis, pneumonia, and polyarthritis.
Several *Mycoplasma* species have been found in the eye of dogs, but have yet to be found to cause ocular pathology.\(^{39}\) These mycoplasmas are part of the normal flora of the upper respiratory tract in dogs and about one-fourth of dogs have mycoplasmas in their lower respiratory tract.\(^{173,174}\) *Mycoplasma cynos* is associated with pneumonia in dogs.\(^{173}\) Canine infectious respiratory disease (CIRD) or kennel cough is caused by *Bordatella bronchiseptica*, *Streptococcus equi* subspecies *zooepidemicus*, canine respiratory coronavirus and *M cynos*.\(^{173}\) CIRD is common and very contagious among dogs.\(^{175}\) It can cause mild clinical signs, such as a hacking cough that is self limited, but it can also cause severe signs of bronchopneumonia.\(^{175}\) Initially, it was thought that *Bordatella*, canine adenovirus and canine parainfluenza virus were the only causes of CIRD, but research has shown that other pathogens are also involved.\(^{173}\)

*Mycoplasma canis* can be found in dogs with reproductive disease, such as endometritis, epididymitis and infertility.\(^{39}\) It is not known if mycoplasmas are causative agents in urogenital diseases or infertility.\(^{39,174}\) Large numbers of *M. canis* were cultured in a dog with chronic prostatitis, but it is unknown if *M. Canis* was the causative agent.\(^{176}\)

Some species of *Mycoplasma* have been isolated from animals other than dogs and cats. *M. felis* has been found in horses, servals and humans.\(^{174}\) *Mycoplasma arginini* has been found in goats, sheep, camels and humans.\(^{174}\) *Mycoplasma bovigenitalium* and *M. canis* have been found in cattle.\(^{174}\) *Mycoplasma canis* and *Mycoplasma maculosum* has also been isolated from humans.\(^{174}\)

Mycoplasmas are resistant to β–lactams due to their lack of a cell wall.\(^{177}\) Overall, doxycycline is the treatment of choice for acute *M. haemocanis* infection.\(^{174}\) However, tetracycline or doxycycline can be used to treat other mycoplasmas.\(^{174}\) Other antibiotics
to treat nonhemotropic mycoplasmas include azithromycin, clarithromycin, chloramphenicol, lincomycin, clindamycin, fluoroquinolones, nitrofurantoin and aminoglycosides.\textsuperscript{39}

**2.2.7.8 Chlamydiosis**

Chlamydiae are gram-negative parasites that divide by binary fission within cytoplasmic vacuoles of their host.\textsuperscript{39} They have intracellular stages called reticulate bodies, which are involved in the process of binary fission, and extracellular stages that are called elementary bodies, which act to infect new cells.\textsuperscript{39} *Chlamydia* and *Chlamidophila* are the genera that make up Chlamydiaceae.\textsuperscript{39} There are many species that infect cattle, sheep, koalas, horses, guinea pigs, rodents, swine, humans, dogs and cats.

*Chlamydophila felis* can infect cats and humans.\textsuperscript{39} In cats, it causes conjunctivitis, inflammation of the nictitating membrane, fever, sneezing and ocular discharge.\textsuperscript{39} Diagnosis is by culturing *C. felis*, visualization of the intracytoplasmic inclusion by Giemsa staining of a smear from a conjunctival swab or by commercial antigen detection enzyme immunoassay kits, PCR, and serology.\textsuperscript{39} Treatment includes amoxicillin-clavulanate, doxycycline, enrofloxacain or pradofloxacin.\textsuperscript{39} Doxycycline can either be administered at a dose of five to ten mg/kg orally twice daily or ten mg/kg orally once daily.\textsuperscript{39}

*Chlamydophila psittaci* can cause infections in humans and birds.\textsuperscript{178} It invades and proliferates in the epithelial cells of the respiratory tract, then spreads to other tissues.\textsuperscript{178} Birds may show respiratory or gastrointestinal signs as well as liver disease.\textsuperscript{178}
In cockatiels, flaccid paresis and paralysis can develop.\textsuperscript{178} Avian psittacosis is treated with doxycycline.\textsuperscript{179} This can be administered in their feed, water, orally, or via intramuscular injection.\textsuperscript{179} Interestingly, birds can develop signs of doxycycline toxicity, which include decreased appetite and activity level, elevated liver enzymes and green/yellow urine.\textsuperscript{179} Human psittacosis is caused by \textit{C. psittaci} and can cause headaches, myalgia and respiratory signs, but can progress to pneumonia and encephalitis.\textsuperscript{180} Human infection occurs by inhalation of infected aerosols (urine, feces, respiratory secretions. Treatment of human psittacosis is with doxycycline or tetracycline.\textsuperscript{181}

\textit{C. psittaci} is rarely isolated from dogs with exposure to affected birds. In general, chlamydiosis is not well understood in dogs.\textsuperscript{39} There are reports of it being isolated from dogs with keratoconjunctivitis, polyarthritis, encephalitis and reproductive failure.\textsuperscript{39}

\textbf{2.2.7.9 Bacillary Hemoglobinuria}

Bacillary hemoglobinuria or red water disease is caused by \textit{Clostridium hemolyticum}.\textsuperscript{182} The disease has a short clinical course and is rapidly fatal if not treated.\textsuperscript{182} It produces phospholipase C, beta-toxin that causes hemolysis and hepatocyte necrosis.\textsuperscript{182} The hemoglobinuria is caused by the toxic effects of phospholipase C on the capillary endothelium.\textsuperscript{182} The disease can occur in cattle and sheep, but is rare in dogs. Clinical signs in cattle include hemoglobinuria, abdominal pain, fever and depression. Edema, anemia and jaundice may also occur. Treatment is with penicillins or tetracyclines.
2.2.7.10 Staphylococcal Infections

*Staphylococcus pseudintermedius* is the most common cause of superficial pyoderma in dogs. Clinical signs include papules, pustules, crusts, patches of alopecia and epidermal collarettes. A superficial pyoderma is diagnosed by observing intracellular cocci on skin cytology. If no bacteria are observed on skin cytology, it does not completely rule out a superficial pyoderma and a bacterial culture would be recommended.

Methicillin-resistant *S. pseudintermedius* (MRSP) has been isolated more frequently than in the past. Methicillin-resistance occurs when the staphylococcal bacteria harbor the *mecA* gene, which encodes the penicillin binding protein 2a (PBP2a). PBP2a confers resistance to all beta-lactam antibiotics, including penicillins and cephalosporins. A bacterial culture and susceptibility panel is needed to determine if an isolate is methicillin-susceptible or methicillin-resistant. Methicillin-susceptible isolates can be treated with first tier antibiotics, such as first generation cephalosporins, amoxicillin-clavulanate, clindamycin or lincomycin.

Antibiotics that can be used to treat MRSP include tetracyclines, clindamycin, fluoroquinolones, chloramphenicol, rifampin and aminoglycosides. Several of these antibiotics have concerning side effects. Chloramphenicol can cause gastrointestinal signs, but can also cause severe side effects, such as liver toxicity, bone marrow suppression, and a suspected myositis in dogs. Owners need to take precautions when handling chloramphenicol due to the risk of fatal aplastic anemia. Rifampin can cause liver injury in dogs and there is a risk of rifampin resistance if it is not administered with other antimicrobials. Aminoglycosides are parenteral and can be nephrotoxic.
Understandably, an oral antibiotic with the mildest side effects would be the best for treating an MRSP infection.

Doxycycline is the tetracycline that has been most commonly used to treat MRSP infections in dogs, while only sporadic reports of the use of minocycline have been documented in the veterinary literature. Based on a recent publication, a minocycline dosage of 5 mg/kg given orally twice daily would be sufficient to treat a S. pseudintermedius with an MIC of 0.25 μg/mL and that a minocycline dosage of 10 mg/kg given orally twice daily would be sufficient to treat a S. pseudintermedius with an MIC of 0.5 μg/mL. Minocycline could serve as a reliable alternative to doxycycline for treating dogs with infections caused by SP.

In veterinary medicine, the human-derived tetracycline breakpoint is used to determine doxycycline and minocycline susceptibility of MRSP isolates in dogs. Using the human-derived tetracycline breakpoint overestimates the number of susceptible isolates. As such, the Clinical Laboratory and Standards Institute (CLSI) has approved new canine-specific doxycycline breakpoints.

Weese et al. using the human-derived tetracycline breakpoints, evaluated 107 MRSP isolates for susceptibility to tetracycline, doxycycline and minocycline. There was a significant difference (p=0.0004) in the frequency of resistance with 65% of isolates susceptible to minocycline, 38% susceptible to doxycycline and 36% susceptible to tetracycline. These results suggest that minocycline may be superior to doxycycline for the treatment of MRSP, but as the human-derived tetracycline breakpoints were used, the actual percentage of susceptible isolates may be lower.
2.2.7.11 Urinary Tract Infections

Urinary tract infections are divided into uncomplicated and complicated infections. Complicated infections are those where there is an underlying disease that may predispose the patient to developing urinary tract infections.

The most common human\textsuperscript{185} and canine\textsuperscript{186} urinary pathogen is \textit{E. coli}. In humans, tetracyclines are not recommended for treating urinary tract infections. Treatment options for uncomplicated infections include trimethoprim-sulfamethoxazole, nitrofurazone, fosfomycin, while fluoroquinolones are used in complicated infections.\textsuperscript{187}

Other canine urinary pathogens include \textit{Pseudomonas} species (spp.), \textit{Enterococcus} species, \textit{Klebsiella} spp., \textit{Staphylococcus} spp., and \textit{Proteus} spp.\textsuperscript{186} In dogs and cats, doxycycline is currently not recommended for routine treatment of urinary tract infections due to low concentrations in the urine.\textsuperscript{188} However, Wilson \textit{et al.}\textsuperscript{189} found that the tetracyclines may be useful in treating some urinary tract infections because the urine concentration of doxycycline after 4 hours was higher than that in the serum and 78\% of urinary tract isolates were susceptible to doxycycline.

In humans, chronic bacterial prostatitis is common and can negatively impact a patient’s quality of life.\textsuperscript{190} Clinical signs include pain during urination and lower urinary tract signs.\textsuperscript{190} Antibiotics that have been used for treatment include ciprofloxacin, levofloxacin, azithromycin, doxycycline and clarithromycin.\textsuperscript{190} The fluoroquinolones are the treatment of choice.\textsuperscript{190}

In dogs, prostatitis can be acute or chronic.\textsuperscript{191} Common bacterial organisms that are isolated in prostatitis are \textit{E. coli}, \textit{Mycoplasma}, \textit{Staphylococcus} spp., \textit{Streptococcus} spp., \textit{Klebsiella} spp., \textit{Proteus} spp., \textit{Pseudomonas} spp. and \textit{Brucella canis}.\textsuperscript{191} Clinical
signs of acute prostatitis are abdominal pain, stiff gait, vomiting and preputial discharge.\textsuperscript{191} In contrast to dogs with acute bacterial prostatitis, dogs with chronic bacterial prostatitis may not have any clinical signs.\textsuperscript{191} Diagnosis of bacterial prostatitis occurs via culture of prostatic fluid.\textsuperscript{191} For acute prostatitis, the antibiotic would ideally be chosen based on the susceptibility panel of the prostatic fluid.\textsuperscript{192} As there is a breakdown of the blood-prostate barrier due to the inflammation in acute prostatitis, many antibiotics are effective.\textsuperscript{192} The blood-prostate barrier is intact in chronic prostatitis so antibiotic choices would include trimethoprim, clindamycin, chloramphenicol and erythromycin.\textsuperscript{192} Doxycycline and minocycline likely do not reach prostatic fluid concentrations that would treat prostatitis.\textsuperscript{193}

2.2.7.12 Acne

Acne vulgaris is the most common skin disease in humans in the United States.\textsuperscript{194} It is caused by inflammation of the hair follicle and sebaceous glands, as well as build up of excess sebum and dead skin cells in the follicle.\textsuperscript{195} The primary bacteria involved in acne is \textit{Propionibacterium acnes}.\textsuperscript{195} Minocycline was considered a primary treatment for acne, however, due to adverse effects and expense, the use of minocycline has decreased and is no longer considered a primary treatment.\textsuperscript{196,197} If oral antibiotics are needed to treat acne, doxycycline, lymecycline or oxytetracycline are preferred.\textsuperscript{197}

In addition to their antibiotic properties, tetracyclines have several non-antibiotic properties that help decrease inflammation and treat acne. Due to their lipophilicity, tetracyclines become concentrated in the pilosebaceous units. Tetracyclines decrease
neutrophil chemotaxis, decrease levels of inflammatory cytokines and decrease matrix metalloproteinases (MMPs), which decrease the inflammatory response.

Protease-activated receptor 2 (PAR2) is expressed on keratinocytes and is activated by the release of proteases from *P. acnes*. Activation of PAR2 leads to the production of IL-8, which is expressed in lesions associated with acne vulgaris to a significantly greater extent than in normal skin. It is believed that PAR2 increases IL-8 production by causing an increase in the keratinocyte calcium levels, leading to intracellular signaling and ultimately allowing the transcription factor, NF-κβ to bind to DNA. Tetracycline may play a role by chelating the calcium ions, thereby reducing the intracellular signaling pathway that leads to the production of IL-8. IL-8 is involved in neutrophil chemotaxis. Other inflammatory mediators whose levels are increased by PAR2 include IL-1α, TNF-α, MMP-1, MMP-2, MMP-3, MMP-9, MMP-13.

Toll-like receptors, specifically TLR-2 and TLR-4, are also increased in acne lesions. *P. acnes* activates the toll-like receptors and leads to production of pro-inflammatory cytokines and MMPs. MMPs breakdown the extracellular matrix. Tetracyclines can chelate the zinc on the active site of MMPs to decrease their activity.

While dogs and cats do not develop acne vulgaris, they can develop chin acne, which is due to a defect in keratinization of follicles. Chin acne is common among cats and there is no breed or gender predisposition. Lesions typically occur on the chin, but the lips can also be affected. Clinical signs include comedones, crusts, alopecia, papules, erythema and pruritus. Severe feline chin acne can have signs associated with folliculitis, furunculosis and cellulitis. Scarring may occur due to the severity of some lesions.
Diagnosis of chin acne is by observing lesions on the chin. Diagnostic tests to look for infections and parasites include skin cytology, deep skin scrapings, fungal culture and bacterial culture. Histopathology can help to rule out an eosinophilic granuloma of the chin.201

There can be a bacterial infection associated with chin acne and isolates cultured from chin lesions include Pasteurella multocida,201 beta-hemolytic streptococci,201 coagulase-positive Staphylococcus,201 alpha-hemolytic streptococcus,200 Micrococcus spp.,200 E. coli,200 and Bacillus cereus.200 Dermatophytosis may also be associated with chin acne in some cats.

In mild cases, no treatment is needed.201 Topical therapy is beneficial and can include Epsom salt compresses, medicated wipes with chlorhexidine or mupirocin ointment.201 Oral antibiotics are often based off of bacterial culture.

Canine chin acne is also called chin or muzzle folliculitis and furunculosis and is mainly found in short-coated breeds.201 The underlying cause for canine chin acne is unknown.201 Initially the lesions are sterile, but with time may become secondarily infected. Treatment for canine chin acne includes reducing trauma to or rubbing of the muzzle, benzoyl peroxide topical therapy, antibiotic therapy and topical corticosteroids once the infection has resolved.201

Rosacea is characterized by facial flushing and erythema.202 Other lesions can include papules, pustules, edema, telangiectasias and ocular lesions.202 Rosacea can be broken down into subtypes including erythematotelangiectatic rosacea, papulopustular rosacea, phymatous rosacea and ocular rosacea.202 While the pathogenesis of rosacea is not well understood, it is thought to involve an increase in blood flow to the face as well
as an increase in the number of facial blood vessels. Many precipitating factors have been examined including spicy foods, changes in environmental temperature, solar exposure, and *Demodex*. Tetracyclines can also be used to treat rosacea. While, the anti-inflammatory properties of tetracyclines may be involved in treating rosacea, anti-angiogenesis properties may also play a role.

### 2.2.7.13 Autoimmune Skin Diseases

Pemphigus foliaceus (PF) is an autoimmune skin disease whereby there are antibodies produced against the desmoglein 1 autoantigen in humans. Pemphigus vulgaris (PV) is an autoimmune vesicobullous disease due to production of autoantibodies mainly against desmoglein 3 but also desmoglein 1. Treatment of PF and PV is often aimed at immunosuppression therapy using corticosteroids, azathioprine, mycophenolate mofetil, cyclophosphamide as well as other immunomodulatory medications.

The best treatment option for humans with pemphigus is oral glucocorticoids. However the combination of tetracyclines (tetracycline, doxycycline or minocycline) and niacinamide has been used as a steroid-sparing therapy for the treatment of PF and PV. In one retrospective study 46 of 51 patients (90%) with pemphigus were in remission with tetracycline and niacinamide therapy. Initially, all patients were treated with oral corticosteroids in addition to tetracycline and niacinamide. The corticosteroids were tapered over two to three months. Occasionally, an oral steroid was necessary to treat flare-ups. Despite this report, a recent evidence based review on treatments for pemphigus vulgaris did not list tetracycline and niacinamide as a therapeutic option.
Canine PF is characterized by a crusting and pustular dermatitis affecting the face, trunk and limbs as well as hyperkeratosis of the paw pads.\textsuperscript{206} It may be idiopathic or drug-induced, and is the most common pemphigus in dogs.\textsuperscript{206} Autoantibodies are formed against desmocollin-1.\textsuperscript{207} Akitas and Chow chows appear predisposed to developing PF.\textsuperscript{206} Other forms of pemphigus in dogs include pemphigus erythematosus (PE) and PV.\textsuperscript{206} Panepidermal pustular pemphigus and paraneoplastic pemphigus have also been reported.\textsuperscript{206}

Treatment for canine pemphigus is aimed at immunosuppression and glucocorticoids and azathioprine are the two most common treatments.\textsuperscript{206} Tetracycline and niacinamide has been used to treat canine PF and PE.\textsuperscript{206} Some clinicians report that dogs with localized forms of PF or PE respond better to this therapy than dogs with generalized disease.\textsuperscript{206} It can take up to two months to start seeing an improvement.\textsuperscript{206} Due to the difficulty in obtaining tetracycline, doxycycline has replaced its use in veterinary medicine.

Bullous pemphigoid (BP) is an autoimmune vesiculobullous ulcerative disease.\textsuperscript{208} In BP, there are tissue-bound and circulating autoantibodies directed against components of the basement membrane zone. The pathogenicity of these autoantibodies is due to their role in complement fixation, recruitment of inflammatory cells (such as neutrophils and eosinophils), the release of proteolytic enzymes (such as MMPs), and interference with adhesion of the dermoepidermal junction with subsequent blister formation.\textsuperscript{208} BP is a very common autoimmune bullous disease in humans. A mucosal surface may be involved in up to one-fourth of patients with the disease.\textsuperscript{208} In the initial phase the lesions are papular to urticarial.\textsuperscript{208} The lesions may be static or progress to form large blisters.\textsuperscript{208}
The blister forms within the lamina lucida of the basement membrane. Tetracyclines, including tetracycline, doxycycline and minocycline with niacinamide or nicotinamide have been used to treat BP. Tetracycline inhibits neutrophil and eosinophil chemotaxis, as well as inhibits MMP-2 and MMP-9. Since tetracyclines and niacinamide (nicotinamide) have less side effects than corticosteroids, they are an attractive treatment option for patients in which corticosteroids would be contraindicated. Improvement may be seen within one to four weeks of starting treatment.

BP is a very rare autoimmune disease in dogs, cats and horses. It can affect the skin and mucous membranes. Ulcers rapidly develop when delicate bullae and vesicles rupture. In dogs, Collies and Doberman Pinschers appear to be predisposed. It has been reported in Quarter horses. Diagnosis is based on histopathology of intact vesicles and bullae. Treatment for BP in the dog is usually with topical and systemic glucocorticoids, but has also been successfully treated with tetracycline and niacinamide.

2.2.7.14 Inflammatory Bowel Disease (IBD)

The bacterial organisms in the gut comprise the host’s microbiome. There is a symbiotic relationship between the host microbiome and the host. The microbiome is formed at birth and can vary with disease states of the gut. Inflammatory bowel disease is characterized by an inflammatory response to intestinal microbes.

Similar to the epithelium of the skin, the intestinal epithelium acts as a barrier to infection. There are goblet cells and Paneth cells that serve to protect the intestines. Goblet cells produce mucus, which acts as another layer between the host microbiome
and the intestinal epithelium. Paneth cells secrete α-defensins. The lamina propria lies on the basilar side of the intestinal epithelium. Within this layer, there are dendritic cells that can sample the intestinal lumen and act as antigen-presenting cells. These antigen-presenting cells can migrate to Peyer’s patches or lymph nodes to present antigen to T cells.\(^{210}\)

In inflammatory bowel disease, the intercellular junctions are not as tight as those of a normal epithelium.\(^{210}\) This may be part of the pathogenesis of inflammatory bowel disease or may be secondary to inflammation caused by inflammatory bowel disease.\(^{210}\) Inflammatory bowel disease leads to the formation of erosions and ulcers of the intestines, as well as decreased production of α-defensins. These changes cause a weakening of the epithelial barrier and increase exposure of the gastrointestinal immune system to microbes, which incites further inflammation of the gut.\(^{210}\)

There is an increase in dendritic cells, neutrophils, macrophages, NK cells, B cells and T cells in the lamina propria of people with inflammatory bowel disease, indicating that the innate and adaptive immune systems are both involved in the pathogenesis of the disease.\(^{210}\) The increase in inflammatory cells leads to an increase in inflammatory cytokines, such as TNF-α, IL-1β and IFN-γ.\(^{210}\) MMPs levels have been found to be elevated in inflammatory bowel disease.

In humans, inflammatory bowel disease is divided into Crohn’s disease and ulcerative colitis.\(^{210}\) Crohn’s disease often affects the wall of the intestine, whereas, ulcerative colitis affects the mucosa.\(^{210}\) The intestines and colon are affected in patients with Crohn’s disease while ulcerative colitis affects the colorectum.\(^{210}\)
Clinical signs of Crohn’s disease vary based on the level of the gastrointestinal tract that is affected. If the upper gastrointestinal tract is affected, there are signs that can mimic those of an ulcer. If the ileum is affected, there are signs of abdominal pain and weight loss. Colonic signs include bloody diarrhea. Perianal signs can include abscesses or fistulas.

Treatments include corticosteroids, biological treatments such as anti-tumour necrosis factor alpha monoclonal antibodies, and enteral nutrition. For severe disease that is refractory to medical treatments, gastrointestinal surgery may be needed.

Ulcerative colitis is characterized by bleeding from the rectum, diarrhea and tenesmus. Treatments are similar to Crohn’s disease and include corticosteroids, 5-aminosalicylates (topical and oral), biological treatments such as anti-tumor necrosis factor alpha monoclonal antibodies as well as cyclosporine.

Minocycline has also been used in the treatment of inflammatory bowel disease. The beneficial effects of minocycline are associated with decreased expression of iNOS and MMP as well as its antibiotic properties.

Dogs can also develop inflammatory bowel disease. Lymphoplasmacytic enteritis, eosinophilic enteritis and eosinophilic gastroenteritis can affect the small intestine. Lymphoplasmacytic colitis, eosinophilic colitis, histiocytic ulcerative colitis and regional granulomatous colitis can affect the large intestine. The exact pathogenesis of canine inflammatory bowel disease is unknown, but it is thought to occur due to the loss of tolerance to antigens in the gut. In dogs, a diagnosis of inflammatory bowel disease is made when other causes of inflammation have been ruled out. Biopsies of the intestinal tract are needed for definitive diagnosis. Treatment options include diet changes,
probiotics, metronidazole, corticosteroids, azathioprine, cyclosporine, chlorambucil, cyclophosphamide and 5-aminosalicylates.\textsuperscript{214} Minocycline may be a treatment option for inflammatory bowel disease in the dog, but its use has not been reported.

\textbf{2.2.7.15 Anti-Tumor Effects}

Minocycline has been shown to have antitumor properties, such as suppression of tumor growth by inhibition of matrix metalloproteinases and inhibition of tumor angiogenesis.\textsuperscript{215} Minocycline’s anti-tumor effects have been evaluated in ovarian cancer,\textsuperscript{216} laryngocarcinoma\textsuperscript{215} and prostate cancer (Regen 2013) cells.\textsuperscript{217} Further studies are needed on the use of minocycline to evaluate minocycline’s effects on other tumor types.

\textbf{2.2.7.16 Prevention of Gentamicin-Induced Ototoxicity}

Aminoglycoside antibiotics are extremely useful antimicrobial agents, however, they have the potential to cause ototoxicity and nephrotoxicity. Outer hair cells are more susceptible to damage by aminoglycosides than inner hair cells. The proposed mechanism of aminoglycoside ototoxicity is due to hair cell damage due to apoptosis. Caspases play a central role in apoptosis. It is believed that aminoglycosides lead to the activation of caspases in hair cells. Specifically, caspase 8 and caspase 9 initiate the caspase signaling cascade. Caspase 9 is activated when cytochrome c leaves the mitochondria and enters the cytoplasm. Currently, it is thought that there is a mitochondrial permeability transition pore that allows cytochrome c to pass out of the mitochondria.\textsuperscript{218} Mitochondrial membrane destabilization may be another method of cytochrome c release.\textsuperscript{218}
Aminoglycosides interact with and cause the opening of the mitochondrial membrane transition pore, but may also disrupt the mitochondrial membrane, thereby releasing cytochrome c independently of the mitochondrial membrane transition pore. Minocycline protects hair cells by inhibition of the p38 MAPK phosphorylation, preventing cytochrome c release and inhibiting caspase 3 activation. When cytochrome c exits the mitochondria, it activates caspase 9 leading to apoptosis. Minocycline prevents the activation of caspase 9 by preventing the opening of the mitochondrial permeability transition pore.

Minocycline has been shown to have protective effects on inner and outer hair cells that were treated with gentamicin. When cell cultures were treated with varying concentrations of gentamicin there was a significant decrease in the number of hair cells in the gentamicin treated groups compared to the control group that was not treated with gentamicin. However, when minocycline was added to the gentamicin-treated cell cultures, there was a dose-related increase in the number of hair cells that survived. While these studies were performed \textit{in vitro} using hair cell cultures, it is possible that minocycline may help prevent aminoglycoside ototoxicity \textit{in vivo}.

2.2.7.17 Prevention of Cisplatin-Induced Ototoxicity

Cisplatin is a chemotherapy agent that acts by crosslinking guanine residues between strands of DNA. Besides its antitumor effects, it can cause neurotoxicity, nephrotoxicity and ototoxicity. Signs of ototoxicity include high-frequency hearing loss, deafness and tinnitus. Ototoxicity is thought to be caused by outer hair cell degeneration in the Organ of Corti as well as damage to the stria vascularis.
Mechanisms of cisplatin-induced ototoxicity include accumulation of cisplatin in the inner ear affecting inner ear metabolism, as well as oxidative stress-induced injury and apoptosis. Minocycline can inhibit apoptosis by blocking the release of cytochrome c and inhibiting the activation of caspases as well as by inhibiting PARP-1 (poly (ADP-ribose) polymerase).

Studies in guinea pigs and rats have been conducted that provide *in vivo* evidence of the protective effect of minocycline against cisplatin ototoxicity. In one study, the effects of minocycline on hearing threshold in cisplatin-treated guinea pigs was evaluated by auditory brainstem response (ABR) testing. Of the six guinea pigs that were treated with minocycline and cisplatin, two had no change in hearing threshold, which the authors attributed to a protective effect of minocycline. Also, the threshold shift was less in the group treated with minocycline than in the group that was only treated with cisplatin, with a significant difference in threshold shift between the two groups only at 16 kHz. Scanning electron microscopy was performed on the harvested guinea pigs’ cochleae. Outer hair cell survival was greater in those guinea pigs treated with minocycline.

When the cochlear basilar membrane of Wistar rats was cultured minocycline alone, there were similar numbers of hair cells and neurons as the control basilar membranes. When the basilar membranes were cultured with cisplatin, the numbers of hair cells and neurons were reduced compared to control membranes or membranes treated with minocycline. Treating membranes with minocycline and cisplatin, there were more hair cells and neurons than when treated with cisplatin substantiating the protective effect of minocycline against cisplatin ototoxicity.
2.2.8 Tetracycline Pharmacokinetics (Doxycycline, Minocycline)

2.2.8.1 Absorption, Distribution, Metabolism, Elimination

2.2.8.1.1 Humans

Absorption of doxycycline occurs in the duodenum. Doxycycline is nearly completely absorbed with an oral bioavailability of 90-100% in the fasted state, with no difference in absorption between the various salts. Absorption is linearly related to dose. Food or milk may decrease absorption by 20% but this effect is not clinically significant. The absorption half-life (t₁/₂ abs) is 0.85 ± 0.41 h. Peak concentration (C_MAX) is dependent on dose and for an orally administered dose of 500 mg is 15.3 mg/L with a t_MAX of 4 hours. Doxycycline has excellent tissue distribution due to its lipophilicity with a volume of distribution (Vd) of 0.7 L/kg. Highest concentrations are found in the liver, kidney and digestive tract. Doxycycline is highly protein-bound with an extent of protein-binding of 82-93%, which is the highest among the tetracyclines. Doxycycline is not significantly metabolized and as such has no metabolites. Doxycycline is eliminated unchanged by both renal and biliary routes; 35-60% is excreted in urine and the remainder in feces. Doxycycline has a long elimination half-life (t₁/₂) of 12 to 25 h, which is the longest of all the tetracyclines.

Like doxycycline, minocycline is almost completely absorbed with an oral bioavailability of 95 to 100% in the fasted state. Absorption occurs mainly in the stomach, duodenum, and jejunum. In two studies evaluating the effect of food on absorption of minocycline, conflicting results were reported, with one finding no change in the AUC (Smith) while the other found a significant difference in AUC as well as
C_{MAX} with a corresponding percent decrease in AUC of 46% and C_{MAX} of 42%.\textsuperscript{34} In addition, minocycline absorption is significantly decreased when administered with an iron supplement or milk\textsuperscript{34} as evidenced by an 81% and 77% decrease in AUC and C_{MAX} when administered with the iron supplement and a 65% and 58% decrease in AUC and C_{MAX} when administered with milk. In general peak concentrations for minocycline are obtained earlier than doxycycline after administration and they are slightly lower than those observed with doxycycline – C_{MAX} for an orally administered dose of 200 mg is 2.1 to 5.1 mg/L at a t_{MAX} of 2 hours.\textsuperscript{96,211} Peak concentration increases with increasing the dose and after multiple dosing.\textsuperscript{96} Minocycline has excellent tissue distribution due to its lipophilicity with a volume of distribution of 1.17 L/kg.\textsuperscript{96,211} Minocycline has been found to penetrate the liver, bile, duodenum, gall bladder and thyroid.\textsuperscript{96} Other organs, such as the bladder, prostate, uterus, breast, lymph nodes and colon are penetrated to a lesser extent.\textsuperscript{96} Plasma protein binding of minocycline ranges from 70 to 80%.\textsuperscript{211} In contrast to doxycycline, minocycline is metabolized in the liver to 6 active metabolites.\textsuperscript{37} When minocycline is metabolized, it undergoes hydroxylation and N-demethylation. The main metabolite is considered to be 9-hydroxyminocycline.\textsuperscript{224} Minocycline has also been found to undergo epimerization in urine to form 4-epiminocycline.\textsuperscript{224} This is considered a consequence of degradation rather than of metabolism.\textsuperscript{224} In contrast to doxycycline, only 5-12% of minocycline elimination occurs in the urine and 20-35% occurs unchanged in the feces.\textsuperscript{96,223} Minocycline is primarily eliminated as metabolites in the feces or urine.\textsuperscript{101,223} Only about 10% of minocycline in the urine has not been metabolized.\textsuperscript{101} Minocycline has an elimination half-life (t_{1/2}) of 16 hours.\textsuperscript{211}
2.2.8.1.2 Dogs

Absorption of doxycycline in dogs occurs in the duodenum. In dogs, doxycycline has a mean oral bioavailability in the fasted state of 61.847%.16 Doxycycline’s bioavailability is less in the dog than in humans.37 While food or milk may decrease absorption of doxycycline in humans,37,211 the affect of food or milk on the absorption of doxycycline in the dog has not been reported. However, when milk was instilled into the ileum of dogs, doxycycline accumulated in the milk to a significantly greater extent than the other tetracyclines (tetracycline, oxytetracycline, and minocycline) studied, possibly due to its high lipophilicity enabling it to pass across the intestinal mucosa and its tendency to chelate calcium.

The absorption half-life of doxycycline in dogs (1.180 ± 0.233 h)16 is longer than that of humans (0.85 ± 0.41 h). The peak concentration (C_MAX) is 4.526 ± 1.789 μg/mL for a dose range of 5 to 10 mg/kg with a t_MAX of 3.682 ± 0.971 h. Doxycycline has a long elimination half-life (t_1/2) of 14.475 h,16 but it is still shorter than that observed in humans of 15-24 h.37 The long t_1/2 in dogs suggests that doxycycline should allow for twice-daily dosing.

Similar to humans, doxycycline is highly lipophilic and distributes to many tissues in the body.225 After intravenous administration in dogs, doxycycline was observed in the kidneys, liver, ileum, cerebrospinal fluid, brain, aqueous humor and vitreous humor.225 Doxycycline, as well as minocycline, were in greater concentrations in the brain and eye than other tetracyclines.225 Similar to humans,211 the highest doxycycline levels in dogs were found in the kidney, liver and ileum.225 The volume of distribution (Vd) in dogs is 1.690 ± 0.559 L/kg.16
Doxycycline has the highest protein binding of tetracyclines in dogs (82%)\textsuperscript{225} and the level of protein binding is similar to that of humans.\textsuperscript{37,211} As in humans, no metabolites of doxycycline have been identified in dogs.\textsuperscript{96,211} About 25% of doxycycline is eliminated in the urine of dogs and the rest is eliminated via the feces.\textsuperscript{226}

In dogs, minocycline is also absorbed via the intestines.\textsuperscript{225} It is less orally bioavailable in fasted dogs (50.33%)\textsuperscript{7} than it is in fasted humans (95-100%).\textsuperscript{96,211} Similar to that in humans, the C\textsubscript{MAX} for minocycline (3.44 ±1.09 μg/mL) in dogs is slightly lower than the C\textsubscript{MAX} for doxycycline (4.526 ± 1.789 μg/mL) and the peak concentrations occur earlier for minocycline (2.34 ± 0.77 h) than for doxycycline (3.682 ± 0.971 h).

Similar to humans,\textsuperscript{96,211} minocycline has excellent tissue distribution in the dog due to its lipophilicity and Vd of 2.52 ± 0.34L/kg.\textsuperscript{7} Minocycline distributes to the kidneys, liver, ileum, cerebrospinal fluid, brain, eye and thyroid gland as well as to many other body tissues. Minocycline has a similar level of protein binding in dogs (65.8%) to that observed in humans (70-80%).\textsuperscript{37,211} No minocycline metabolites have been identified in canine feces, however, 4-epiminocycline was identified in canine urine (Kelly 1967).\textsuperscript{227} The primary route of elimination of minocycline in the dog is via the feces (Kelly 1967).\textsuperscript{227} The elimination half-life (t\textsubscript{1/2}) of minocycline in dogs is 4.14 ± 0.50 h (Maaland 2014),\textsuperscript{7} which is about one-fourth that of humans.\textsuperscript{211} Table 1 compares the pharmacokinetic parameters of doxycycline and minocycline in dogs.
Table 1. Oral doxycycline and minocycline pharmacokinetics in the dog

<table>
<thead>
<tr>
<th>Drug</th>
<th>No of subjects</th>
<th>Dose (mg/kg)</th>
<th>Route admin</th>
<th>Fed/ Fasted</th>
<th>terminal t1/2 (h)</th>
<th>kel (h⁻¹)</th>
<th>V/F (L/kg)</th>
<th>CL/F (L/kg/h)</th>
<th>AUC (μg.h/L)</th>
<th>CMAX (μg/mL)</th>
<th>Abs t 1/2 (h)</th>
<th>tMAX (h)</th>
<th>F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxycycline</td>
<td>27</td>
<td>5 to 10</td>
<td>PO</td>
<td>NK</td>
<td>14.475 ± 3.066</td>
<td>0.015</td>
<td>0.559</td>
<td>0.012</td>
<td>33.651</td>
<td>1.789</td>
<td>0.233</td>
<td>0.971</td>
<td>61.847</td>
</tr>
<tr>
<td>(Maaland 2013)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.690 ± 0.077</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>30.95</td>
<td>3.44</td>
<td>0.80</td>
<td>0.75</td>
<td>2.34</td>
</tr>
<tr>
<td>Minocycline</td>
<td>6</td>
<td>10</td>
<td>PO</td>
<td>Fasted d</td>
<td>4.14 ± 0.50</td>
<td>0.17</td>
<td>2.52 ± 0.50</td>
<td>0.085</td>
<td>10.84</td>
<td>1.09</td>
<td>0.75</td>
<td>0/77</td>
<td>50.33</td>
</tr>
<tr>
<td>(Maaland 2014)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.328</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>10.1</td>
<td>2.2</td>
<td>±</td>
<td>±</td>
<td>±</td>
</tr>
</tbody>
</table>
2.2.8.2 Pharmacokinetic Physiologic Variations

2.2.8.2.1 Age

In older human patients on doxycycline, serum and tissue concentrations are elevated while the apparent volume of distribution is reduced compared to younger patients.\(^{228}\) The apparent volume of distribution has been shown to be positively correlated with the α₂-globulin content of the serum and serum iron level. As doxycycline forms stable complexes with iron, and iron levels decrease in the elderly, a possible explanation for these changes could be that there would be a greater amount of freely diffusible doxycycline, resulting in higher serum levels and lower apparent volume of distribution.\(^{228}\) As doxycycline has a wide margin of safety, dosage adjustment in the elderly is not usually necessary.\(^{228}\) On the other hand, concentrations of minocycline are lower in children than in adults, and dose adjustments are usually necessary.\(^{229}\)

2.2.8.2.2 Gender

In humans, no changes in pharmacokinetics have been identified due to gender with the administration of doxycycline.\(^{228}\) No data are available for minocycline.

2.2.8.2.3 Exercise

Interestingly, significantly increased serum levels of doxycycline as well as AUC were found during exercise in humans.\(^{230}\) Exercise significantly decreased excretion of doxycycline in the urine, but this decrease in excretion was not enough to explain the increase in serum levels.\(^{230}\) The reduction in urine excretion of the doxycycline may have been due to the diminished urine rate and reduction in urine pH, which have been shown
to reduce renal clearance of doxycycline. Therefore, physical activity should be controlled during drug studies with doxycycline.\textsuperscript{230}

\textbf{2.2.8.3 Effects of Pathologic States on Pharmacokinetics}

\textbf{2.2.8.3.1 Renal Disease}

There has been concern over the use of doxycycline in patients with renal failure. While doxycycline does not appear to accumulate in the blood of patients with renal failure and is generally considered safe,\textsuperscript{100} there has been a report of worsening of renal failure after starting doxycycline.\textsuperscript{99} In that patient, it was proposed that there was impairment of the non-renal excretion leading to nephrotoxic levels of doxycycline.\textsuperscript{99}

As only 5-12\% of minocycline elimination occurs in the urine\textsuperscript{96,223} and the elimination rate of minocycline is practically independent of kidney function, no change in dose or frequency is needed in patient with renal disease.\textsuperscript{231}

\textbf{2.2.8.3.2 Malnutrition}

In malnourished patients, there is lowered serum binding of doxycycline to proteins, since these patients have significantly lower serum albumin levels compared to normal individuals.\textsuperscript{232} This has been reported to cause a decrease in AUC and an increase in elimination.\textsuperscript{232} Although these pharmacokinetic changes were identified in undernourished patients, no change in doxycycline dose is recommended as undernourished patients likely have a lower body weight and will receive a higher dose per body weight and thus maintain plasma levels in the normal range. There are no reports on the pharmacokinetics of minocycline in undernourished human patients.
2.2.8.3.3 Hyperlipidemia

Due to the lipophilicity of tetracyclines, hyperlipidemia causes changes in the pharmacokinetics of these drugs. The clearance is reduced by about 50% for doxycycline and minocycline and there is an increase in AUC. As such, a reduction in dose of doxycycline and minocycline may be necessary in humans with hyperlipidemia.\textsuperscript{223,233}

2.2.8.4 Drug Interactions on Pharmacokinetics

2.2.8.4.1 Doxycycline and Warfarin

When given together, warfarin’s anticoagulant properties are potentiated by doxycycline.\textsuperscript{234} The specific mechanism of interaction is still unknown. However, it is thought that since both doxycycline and warfarin are protein-bound that doxycycline may competitively bind to proteins so there are less proteins to bind to warfarin. This leads to an increased concentration of free warfarin, which can increase its anticoagulant effects.\textsuperscript{234}

There is another possible mechanism of interaction between doxycycline and warfarin based on inhibition of the cytochrome P450 enzyme. This enzyme metabolizes warfarin. If doxycycline inhibits cytochrome P450, then warfarin will be metabolized more slowly and there will be an increase in free warfarin leading to changes in coagulation.\textsuperscript{234} Based on a current literature search, no articles were found describing interactions between minocycline and warfarin.
2.2.8.4.2 Tetracyclines and Antacids

Antacids contain divalent cations such as calcium, magnesium and iron. The divalent cations in antacids decrease the absorption of doxycycline\textsuperscript{235} because the tetracyclines chelate the ions. Specifically, aluminum magnesium hydroxide gel was found to significantly decrease the absorption of doxycycline.\textsuperscript{235} Thus when antacids are administered that contain these ions, the absorption and bioavailability of the tetracycline decreases.\textsuperscript{235}

The absorption of minocycline may also be affected by antacids. Antacids such as aluminum silicate and magnesium trisilicate have been found to decrease the dissolution of minocycline.\textsuperscript{236}

2.2.8.4.3 Tetracyclines and Iron

Iron is another divalent cation, which is not in antacids, but rather is taken as a supplement. It has been known for some time that iron can interfere with the absorption of tetracyclines. Ferrous sulphate decreases the absorption of doxycycline and thus it is recommended to avoid taking oral iron supplements when taking doxycycline. This is also true for minocycline hydrochloride.\textsuperscript{34}

2.3 Antimicrobial Susceptibility Testing Methods

2.3.1 Broth Dilution Tests

Broth dilution tests include the tube-dilution method and broth microdilution testing. The tube-dilution method consists of tubes containing two-fold dilutions of antibiotics in a growth medium inoculated with a standardized suspension of bacterial
organisms incubated overnight (Jorgensen 2009). The tube with the lowest concentration of antibiotic that inhibited bacterial growth represented the minimum inhibitory concentration (MIC) of that bacteria for that antibiotic. The advantage of this technique is obtaining a quantitative result. Disadvantages to the tube dilution test include the space needed for the tubes and the time to manually prepare the tubes.

Broth microdilution testing uses plates that are produced commercially and contain 96 wells. The wells contain two fold dilutions of antibiotics. As for the tube-dilution method, the wells are inoculated with bacterial suspensions and incubated overnight. The MICs are determined using automated or manual viewing device for evaluation of the bacterial growth in the wells. The advantage over the tube dilution test is that the plates are commercially prepared and less space is required due to the smaller size of the plates. If one is using an automated viewing device, these machines can also generate computer reports. The main disadvantage is not being able to test other antimicrobials not included on the commercial panel.

2.3.2 Antimicrobial Gradient (E-test)

The antimicrobial gradient method (E-test) uses diffusion of antibiotics across an agar medium to determine susceptibility. E-test strips are commercially produced and have gradually decreasing concentrations of antibiotics impregnated onto the strip. The strip is laid onto agar that has a standard bacterial suspension streaked onto it. The agar plate is incubated overnight, a symmetrical inhibition ellipse is produced, and the area where the zone of inhibition crosses the strip indicates the MIC. E-tests strips are particularly useful to evaluate MICs for antibiotics that are not on commercially available
plates or if testing of antibiotic concentrations is needed outside the range of dilutions available on a commercially available broth microdilution plate. However, E-test strips can be expensive if testing multiple antibiotics.\(^{237}\)

2.3.3 Disk Diffusion Test

The disk diffusion test uses similar principles as the antimicrobial gradient method. Just as with the antimicrobial gradient method, a standard bacterial suspension is streaked onto agar.\(^{237}\) Up to twelve paper disks impregnated with antibiotics are placed onto the surface of the agar and the agar plate is incubated overnight.\(^{237}\) The zone of growth inhibition looks like a concentric ring around each antibiotic disk and appears as a clearing in the lawn of the bacteria.\(^{237}\) The diameter of the zone of inhibition is measured in millimeters.\(^{237}\) The zone diameters are interpreted using the criteria published by the CLSI.\(^{237}\) The results however are qualitative and reported as susceptible, intermediate or resistant. This is the simplest and least expensive method of determining antimicrobial susceptibility, however, it can take longer than other automated methods.\(^{237}\) In veterinary medicine, there are some antibiotics for which there are no veterinary zone-diameter breakpoints, therefore, human-derived breakpoints are used.

2.3.4 Automated Instrument Systems

Use of automated instruments can standardize reading of susceptibility results and are faster than performing manual readings.\(^{237}\) There are four automated instrument systems approved by the FDA for use in the USA - The Microscan WalkAway (Siemens Healthcare Diagnostics), the BD Phoenix Automated Microbiology System (BD

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Diagnostics), the Vitek 2 System (bioMerieux), and the Sensititre ARIS 2X(Trek Diagnostic Systems).\textsuperscript{237}

\textbf{2.4 The Genus Staphylococcus}

\textbf{2.4.1 \textit{Staphylococcus intermedius} Group (SIG)}

Coagulase-positive staphylococcal organisms isolated from animals were originally identified as \textit{Staphylococcus aureus}. However, in 1976 Hajek identified a new species, \textit{Staphylococcus intermedius} (SI), based on an investigation of the cell wall structures and biochemical properties of 50 coagulase-positive staphylococcal isolates from the anterior nares of pigeons, dogs, mink and horses.\textsuperscript{238} Then in 2005, Devriese \textit{et al.} evaluated four staphylococcal isolates from clinical and necropsy specimens by 16S rRNA gene sequence analysis and found these isolates to constitute a distinct taxon, closely related to SI and \textit{Staphylococcus delphini} and named the novel species \textit{S. pseudintermedius} (SP).\textsuperscript{239} Further sequencing determined that previously described \textit{S. intermedius} was comprised of three genetically distinct species known as the \textit{S. intermedius} group (SIG): \textit{S. intermedius}, \textit{S. pseudintermedius}, and \textit{S. delphini}.\textsuperscript{240}

\textbf{2.4.2 Identification of \textit{Staphylococcus} species}

\textbf{2.4.2.1 Phenotypic identification}

\textbf{2.4.2.1.1 Morphology}

Staphylococcal organisms occur in clusters, pairs, tetrads and short chains. Colonies are typically 3 to 8 mm in diameter after incubation for 72 hours on blood agar, tryptic soy agar or brain heart infusion agar.\textsuperscript{241} Colonies are typically opaque and smooth,
with variable pigmentation and hemolysis based on species. S. pseudintermedius colonies are non-pigmented, raised, with two zones of hemolysis.

2.4.2.1.2 Gram Stain

Bacteria can be classified as gram-positive or gram-negative based on gram-staining characteristics. Gram-staining is performed by fixing a sample of bacteria on a slide, then staining the slide with crystal violet. The slide is rinsed with water and then mordant (iodine) is added. The slide is rinsed again with water and a decolorizing solvent (equal parts 95% ethyl alcohol and acetone) is added to remove any extra dye. The slide is then counterstained with safranin. Gram-positive bacteria stain blue since their cell wall contain a thick peptidoglycan layer which takes up the crystal violet stain. Gram-negative bacteria stain red, since they have an outer membrane that surrounds the cell wall and the decolorizing solvent degrades the bacterial surface so the crystal violet stain is lost and then is subsequently stained with safranin. All Staphylococcal organisms are gram-positive.

2.4.2.1.3 Coagulase Activity

Coagulase is an enzyme that clots of plasma and is used to differentiate between Staphylococcus species. To determine the presence of coagulase, two types of tests may be used - the slide test and the tube test. The slide test is used to detect bound coagulase also known a “clumping factor”. A drop of rabbit plasma is mixed with a drop of the emulsified bacteria. Clumping indicates the presence of coagulase. The tube test is used to look for free coagulase. Rabbit plasma is placed in a sterile tube,
inoculated with the bacteria and incubated and observed for clot formation. Members of the SIG group and *S. aureus* have a positive coagulase test.\(^{242}\)

### 2.4.2.1.4 Catalase Activity

The catalase test is used to differentiate staphylococci (catalase-positive) from streptococci (catalase-negative). The enzyme, catalase, is produced by bacteria that respire using oxygen, and protects them from the toxic by-products of oxygen metabolism. The presence of catalase enzyme in the bacteria is detected using hydrogen peroxide. If the bacteria possess catalase when a small amount of bacterial isolate is added to hydrogen peroxide, bubbles of oxygen are observed.\(^ {245}\)

### 2.4.2.1.5 Biochemical Tests

Some of the key biochemical tests used for the speciation of the clinically significant staphylococci include pyrrolidonyl arylamidase, \(\beta\)-galactosidase, acetoin production, arginine dihydrolyase, acid production from \(\square\)-gentobiose, resistance to polymixin B, and acid production from D-mannitol. While these tests are useful to differentiate SA from the members of the SIG group or differentiating SI from SP and SD, they cannot differentiate SP and SD.\(^{242}\) While phenotypic testing can differentiate SI from SP and SD, it cannot differentiate between SP and SD. Therefore, routine identification of SP from veterinary microbiology laboratories is based on the fact that SIG species other than SP are not found associated with canine bacterial infections.\(^{239,242}\) However, to truly differentiate SP from SD, molecular methods of identification are necessary.\(^{242}\)
2.4.2.2 Genotypic Identification

Genotypic methods of identification include PCR-restriction fragment length polymorphism (PCR-RFLP) based on an *MboI* restriction site in the *pta* gene,\textsuperscript{246} sequencing of the 16S rRNA gene,\textsuperscript{227} sequencing of the *hsp60* and *sodA* genes, and multiplex PCR using the thermonuclease (*nuc*) gene.

The *MboI* restriction site in the *pta* gene is found in SP, but not in SI or SD and PCR-RFLP has been used to identify SP based on this restriction site.\textsuperscript{246} The 16S rRNA gene has also been used to differentiate between members of the SIG group and was used in the first paper to identify SP as a new species.\textsuperscript{227} Sequencing of the *hsp60* and *sodA* genes was also able to differentiate between members of the SIG group.\textsuperscript{240,247} Multiplex PCR of the *nuc* gene was found to be a sensitive and specific method of identifying coagulase positive staphylococci and was able to differentiate between the members of the SIG group.\textsuperscript{234}

2.4.2.3 Proteomic Analysis

The matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) is a rapid, accurate and cost effective method for identification and characterization of microbes. This technology generates characteristic mass spectral fingerprints that are unique for each organism. This technique allows for identification of microbes at the genus and species level as well as strain typing and identification.\textsuperscript{248}

Preparation of samples for the MALDI-TOF begin with application of a sample of a colony of bacteria on a conductive metal plate in addition to quality control isolate,
such as *E. coli*. A matrix is applied to the target plate which leads to crystallization of the sample. After crystallization, it is placed in the machine. Laser pulses are directed at each sample on the plate and energy is absorbed by the matrix. This leads to desorption, vaporization and ionization of the sample. Ions are accelerated in an electrostatic field and then move through a flight tube with smaller ions traveling faster than larger ions until they reach the detector. The time of flight to reach the detector is based on the mass and charge of the analyte. The mass to charge ratio as well as the intensity of the ions is used to create the mass spectrum. The mass spectrum result is compared to a database for species identification.

Decristophoris *et al.* used MALDI-TOF to identify SI, SP and SD isolates that had been previously sequenced using the *hsp60* gene. Near perfect agreement was found between the two methods for identification of SI, whereas substantial agreement was found between the two methods for SP and SD, rating the overall efficiency of MALDI-TOF of 88% for SP and SD and 99% for SI. MALDI-TOF has been compared to PCR amplification of the *nuc* gene for identification of staphylococcal isolates from nasal and perineal samples from clinically normal Labrador retrievers. The authors found 100% agreement between the two techniques for identification of SA; however, the MALDI-TOF was only able to identify 69 of the 91 (77%) SP isolates. The overall performance of the MALDI-TOF to speciate organisms is directly related to the database and should improve as more highly characterized reference isolates are added. However, in regards to staphylococcal organisms, it should be remembered that SIG species other than SP are not found associated with canine bacterial infections.
2.4.3 Detection of Methicillin-Resistance

2.4.3.1 Phenotypic Identification

Identification of resistance to methicillin in staphylococcal organisms is important as methicillin-resistant organisms are characterized by the presence of the \( \text{mecA} \) gene, which encodes an altered penicillin binding protein 2a (PBP2a) that confers resistance to all beta-lactam antibiotics (with the exception of ceftaroline)\(^{252} \)) regardless of \textit{in vitro} susceptibility. Oxacillin has replaced methicillin for detection of the presence of methicillin-resistance in staphylococcal organisms as oxacillin is more stable \textit{in vitro} than methicillin and readily available.\(^{253} \) Methicillin-resistance can be detected by numerous methods.

Oxacillin salt agar (OSA) can be used to identify methicillin resistance in Staphylococcal isolates.\(^{254} \) Commercial OSA products are comprised of agar supplemented with oxacillin and 4% NaCL. The addition of the NaCL inhibits growth of bacteria other than staphylococci. The isolate is streaked onto the surface of the agar and incubated for 24 hours. If any colonies grow, they are methicillin resistant. Some product formulations will add aniline blue so the methicillin-resistant colonies will appear blue (Manufacturer’s instructions Oxoid).

Methicillin-resistance may also be determined by broth microdilution, oxacillin disk diffusion or the oxacillin E-test. There are commercially available broth microdilution plates that include oxacillin as well as other antibiotics. A SP isolate with an oxacillin MIC \( \geq 0.5 \mu\text{g/mL} \) is indicative of the presence of the \text{mecA} \) gene.\(^{255} \) The disk diffusion test and E-test strips are where either a disk or E-test strip, respectively, are placed on agar to which a standard bacterial suspension is streaked onto the agar and
incubated. For the disk diffusion test a zone of inhibition measuring ≤ 17 mm is consistent with the presence of the *mecA* gene for SP isolates (Bemis 2009). For the E-test strips, the breakpoint for oxacillin is the same as for the broth microdilution test of ≥ 0.5 μg/mL.

The use of cefoxitin disk diffusion tests for the detection of methicillin-resistance in SP isolates, based on 2004 CLSI guidelines of ≤ 24 mm as resistant and ≥ 25 mm as susceptible for methicillin were found to be very sensitive but not specific, underestimating methicillin-resistance. However, establishment of new cefoxitin disk breakpoints of ≤ 30 mm as resistant and ≥ 31 mm as susceptible resulted in improved sensitivity and specificity.

The PBP2a latex agglutination test evaluates isolates for the presence of the PBP2a antigen using anti-PBP2a monoclonal antibody sensitized latex particles. This test provides direct evidence that the *mecA* gene has been expressed and produced PBP2a, but the test does not evaluate if PBP2a is functional. Results of the PBP2a latex agglutination test should be interpreted with caution with SP isolates as false positive results can occur. It is possible that SP isolates may carry a *mecA* homologue that produces a protein that resembles PBP2a, but does not result in methicillin resistance.

### 2.4.3.2 Genotypic Identification

#### 2.4.3.2.1 *mecA* PCR

Detection of methicillin resistance can also be performed by PCR assay for *mecA*, which is the gold standard for evaluation of an isolate for methicillin resistance.
However, there can be false-positive results with mecA. A false positive reaction occurs when an isolate has a mutation in the mecA gene and forms PBP2a that is not functional.\textsuperscript{258} There can also be false negative results where a mecA gene inhibitor is suppressing expression of the mecA gene. mecA gene suppression has not been reported in SP isolates, but has been reported to occur in SA isolates by the presence of the mec regulator gene mecI blocking transcription of mecA.\textsuperscript{260} Although mecA PCR is the gold standard for detection of methicillin-resistance it is more time consuming and expensive to perform than the disk diffusion test, therefore, many clinical laboratories use oxacillin disk diffusion as a determinant of methicillin resistance.
Chapter 3

Evaluation of canine-specific minocycline and doxycycline susceptibility breakpoints for meticillin-resistant Staphylococcus pseudintermedius isolates from dogs

3.1 Abstract

**Background:** Clinical and Laboratory Standards Institute (CLSI) human tetracycline breakpoints to predict minocycline and doxycycline susceptibility of *Staphylococcus pseudintermedius* (SP) isolates from dogs are not appropriate because they do not meet pharmacokinetic/pharmacodynamic data using a standard dose. New breakpoints have been approved for doxycycline and proposed for minocycline. Revised breakpoints are four dilutions lower than tetracycline breakpoints, providing a more conservative standard for classification of isolates.

**Hypothesis/Objectives:** The objectives of this study were to measure MICs of minocycline and doxycycline of 100 canine meticillin-resistant SP clinical isolates, compare their susceptibilities to minocycline and doxycycline based on current and revised standards, and document their tetracycline resistance genes.

**Methods:** E-test strips were used to determine MICs. PCR was used to identify *tet* genes.

**Results:** Using the human tetracycline breakpoint of MIC\( \leq 4 \, \mu g/mL \), 76 isolates were susceptible to minocycline and 36 isolates were susceptible to doxycycline. In contrast,
using the proposed minocycline breakpoint (MIC$\leq$0.25 $\mu$g/mL) and approved doxycycline breakpoint (MIC$\leq$0.125 $\mu$g/mL), 31 isolates were susceptible to both minocycline and doxycycline. Thirty-one isolates carried no $tet$ genes, two had $tet$(K), and 67 had $tet$(M).

**Conclusions and clinical importance:** Use of the human tetracycline breakpoints misclassified 45 and five of the isolates as susceptible to minocycline and doxycycline, respectively. PCR analysis revealed that 43 and five of the isolates classified as susceptible to minocycline and doxycycline, respectively, possessed the tetracycline resistance gene, $tet$(M), known to confer resistance to both drugs. These results underscore the importance of utilizing the proposed minocycline and approved doxycycline canine breakpoints in place of human tetracycline breakpoints.

### 3.2 Introduction

*Staphylococcus pseudintermedius* (SP) is the most common pathogen isolated from canine superficial pyoderma lesions. In 2005, isolates from dogs that were previously identified as *Staphylococcus intermedius* (SI) were reclassified as SP.\textsuperscript{239,251} SP is considered a member of the SI group (SIG), which includes SP, SI and *Staphylococcus delphini*.\textsuperscript{240,248}

Meticillin-resistant *S. pseudintermedius* (MRSP) is being isolated with increasing frequency from lesional skin in dogs with superficial pyoderma.\textsuperscript{1} Meticillin-resistance is characterized by the presence of the $mecA$ gene that encode the penicillin binding protein 2a (PBP2a). This protein confers resistance to the beta-lactam group of antibiotics (with the exception of ceftaroline).\textsuperscript{252,261}
Doxycycline is the member of the tetracycline class of antimicrobial drugs that has been most commonly used to treat MRSP infections in dogs, while only sporadic reports of the use of minocycline have been documented in veterinary literature. There has been recent interest in using minocycline for MRSP infections due to a rise in the price of doxycycline. The increased price of the commercially formulated generic oral doxycycline has resulted in veterinarians resorting to prescribing compounded doxycycline, which while less expensive, has been shown to be unstable.

Two studies have evaluated minocycline susceptibility of canine SP isolates. In one, susceptibilities to seven antimicrobials, including minocycline, for 170 SP isolates from lesional skin of dogs with pyoderma were determined and 113 of those isolates were MRSP. Of the MRSP isolates, 39 (34.5%) were susceptible to minocycline. A second study compared the susceptibilities of tetracycline, doxycycline and minocycline of MRSP isolates from 79 dogs with clinical infections and isolates obtained via nasal or rectal swabs from 28 normal dogs. There was a significant difference (P=0.0004) in antimicrobial susceptibilities of the 107 isolates tested with 70 (65%) isolates susceptible to minocycline, 41 (38%) susceptible to doxycycline and 39 (36%) susceptible to tetracycline. These results suggest that minocycline may be useful for treatment of MRSP infections and superior to doxycycline for the treatment of MRSP.

It is important to note that the human interpretive susceptibility breakpoints for tetracycline were used in the previous studies. As of this writing, there are no published canine-specific interpretive breakpoints for tetracycline. The human minimum inhibitory concentration (MIC) breakpoints are \( \leq 4 \text{ \mu g/mL}, 8 \text{ \mu g/mL} \) and \( \geq 16 \text{ \mu g/mL} \) for susceptible, intermediate and resistant isolates, respectively. Pharmacokinetic data
indicate that these breakpoints are too high because they do not meet pharmacokinetic and pharmacodynamics (PK/PD) data using a typically administered dose. The Clinical and Laboratory Standards Institute (CLSI) recently approved canine-specific interpretive breakpoints for susceptible, intermediate and resistant SP organisms for doxycycline, which are ≤0.125 μg/mL, 0.25 μg/mL and ≥0.5 μg/mL, respectively; however the new breakpoints have not been published in a laboratory standards document up to now. For minocycline, the proposed susceptibility breakpoint for SP organisms is ≤0.25 μg/mL. Until new CLSI documents are published, many laboratories will continue to use the human-derived tetracycline breakpoints.

Resistance of SP to tetracyclines is mediated through the acquisition of tetracycline resistance genes (tet genes). Four tet genes have been identified among SP: tet(M), tet(O), tet(K) and tet(L). Ribosomal protection is conferred by tet(M) and tet(O), whereas, efflux pumps are encoded by tet(K) and tet(L). The most commonly identified tet genes in SP isolates are tet(M) and tet(K). Staphylococcus spp. that possess only the tet(K) gene, retain susceptibility to minocycline, and are resistant to other tetracyclines, thus minocycline may be useful against isolates possessing tet(K).

The objectives of the study were (1) to measure minimum inhibitory concentrations of minocycline and doxycycline for 100 canine clinical MRSP isolates obtained from dogs with skin lesions or otic disease presenting to a veterinary tertiary referral center in the Midwestern U.S., (2) to determine minocycline and doxycycline susceptibility of these MRSP isolates based on the tetracycline and approved doxycycline/ proposed minocycline interpretive breakpoints and, (3) to document the tetracycline resistance genes in these isolates. Based on the more conservative
approved\textsuperscript{7,16} and proposed\textsuperscript{7} interpretative breakpoints for doxycycline and minocycline, respectively, we expect that fewer isolates will be susceptible to doxycycline and minocycline than with the currently used tetracycline breakpoints. We hypothesize that MRSP isolates that are susceptible to minocycline will possess the \textit{tet(K)} gene, and those MRSP isolates that are resistant to both minocycline and doxycycline will possess the \textit{tet(M)} gene.

\section*{3.3 Materials and Methods}

\subsection*{3.3.1 Bacterial Isolates}

MRSP organisms isolated from canine skin or ears (n=100), collected between January 2011 and August 2014 were selected for analysis from strains banked in 50\% buffered glycerol in a -80° C freezer bank from a clinical microbiology laboratory at a veterinary teaching hospital. Banked isolates included all SP from all cultures submitted to the clinical microbiology laboratory that were meticillin-resistant, based on oxacillin disk diffusion and broth microdilution tests (Sensititre COMPANIF Plate, TREK/Thermo Fisher Scientific; Waltham, MA) using CLSI standards.\textsuperscript{267,268} Isolates chosen for this study were obtained from dogs with a clinical history of skin lesions (pyoderma, wounds, and surgical site infections) or otic infections (otitis externa or otitis media). Surgical site infections included swab samples or biopsies from infected incisions or draining tracts near incisions. Isolates were selected beginning with those from 2014 and then from each prior year until 100 isolates were obtained. Each isolate originated from a unique patient. Organisms were speciated using conventional phenotypic and biochemical methods\textsuperscript{267} at the time of initial patient workups and subsequently re-
identified using Matrix-Assisted Laser Desorption/Ionization mass spectrometry (Biotyper, Bruker; Billerica, MA) during this study.\textsuperscript{248}

Minocycline MICs were measured using E-test strips (batch number 516058; bioMérieux; Durham, NC) according to manufacturer’s instructions. Doxycycline MICs were measured by broth microdilution (Sensititre COMPAN1F Plate, TREK/Thermo Fisher Scientific; Waltham, MA) according to CLSI standards.\textsuperscript{268} The range of MICs for doxycycline on the broth microdilution plate was 2 to 8 μg/mL. Because it was necessary to measure doxycycline MICs down to ≤ 0.125 μg/mL, given the accepted doxycycline breakpoints by Maaland et al,\textsuperscript{7,16} a doxycycline E-test strip (batch number 509750; bioMérieux; Durham, NC) was performed according to manufacturer’s instructions to extend the dilution range for isolates with MIC ≤ 2 μg/mL on the broth microdilution plate. Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 29213) were used as quality control isolates.

The MIC was recorded where the zone of inhibition overlapped on the E-test strip. If the MIC fell between two values, the higher MIC was recorded. All MICs were rounded up to the next doubling dilution.

\textbf{3.3.2 Genotyping}

Genomic DNA was isolated directly from bacterial colonies using a commercially available extraction kit (Qiagen DNeasy kit, Qiagen Inc, Valencia, CA) in a semi-automated device (QIAcube, Qiagen Inc, Valencia, CA). Briefly two to four bacterial colonies grown on Columbia Agar supplemented with 5% sheep blood were suspended in 180 μL of gram-positive lysis buffer (20 mg/ml Lysozyme in 20mM Tris-HCl, pH 8.0;
2 mM EDTA; 1.2% Triton) and placed into the QIAcube using the QIAcube gram-positive bacteria/yeast protocol with an elution volume of 100 μl per manufacturer’s instructions.

PCR for the tet(K) and tet(M) genes was performed using primers, cycling conditions and a commercial amplification mix (Platinum® PCR SuperMix Invitrogen™; Grand Island, NY) as previously described. Ethidium bromide stained 1% agarose (0.5X TBE [tris-borate-EDTA] buffer) gels were electrophoresed at 100V for 30 minutes. PCR products were visualized under ultraviolet light. Positive controls for tet(K) and tet(M) were obtained from D. Bemis (University of Tennessee).

3.4 Results

A total of 100 MRSP isolates were selected from the banked isolates; 20 from 2011, 37 from 2012, 19 from 2013 and 24 from 2014. Of these isolates, 79 were from pyoderma, five from wounds, 12 from surgical site infections and four from external (n=3) or middle ear (1) infections. The cultures were obtained from tissue biopsies (5), skin biopsies (6), ear swabs (4) or skin swabs (85).

The MIC distribution for minocycline appeared bimodal (Figure 2). The MIC\textsubscript{50} and the MIC\textsubscript{90} were 4 μg/mL and 8 μg/mL, respectively. Thirty-one isolates had an MIC ≤ 0.125 μg/mL, two isolates had an MIC = 0.5 μg/mL and 67 isolates had an MIC ≥ 2 μg/mL. Using the tetracycline breakpoint of MIC ≤ 4 μg/mL, 76 isolates would be classified as susceptible to minocycline while using the proposed minocycline breakpoint (MIC ≤ 0.25 μg/mL) only 31 isolates would be classified as susceptible to minocycline.
Use of the tetracycline breakpoint misclassified 45 isolates (59%) as susceptible to minocycline.

For doxycycline, the MIC distribution was bimodal (Figure 3). The MIC$_{50}$ and the MIC$_{90}$ were both 8 μg/mL. Thirty-one isolates had an MIC $\leq 0.125$ μg/mL and 69 isolates had an MIC $\geq 4$ μg/mL. Using the tetracycline breakpoint of MIC $\leq 4$ μg/mL, 36 isolates would be classified as susceptible to doxycycline, while using the approved doxycycline breakpoint (MIC $\leq 0.125$ μg/mL), 731 isolates would be classified as susceptible to doxycycline. Use of the tetracycline breakpoint misclassified 5 isolates (14%) as susceptible to doxycycline.

Thirty-one isolates carried no tet gene and 69 isolates carried either tet(K) or tet(M) (Table 2). No isolates with a minocycline or doxycycline MIC $\leq 0.125$ μg/mL carried tet(M) or tet(K) genes. All isolates with a minocycline MIC $\geq 2$ μg/mL carried tet(M). Two isolates with a minocycline MIC = 0.5 μg/mL and with a doxycycline MIC = 8 μg/mL carried tet(K). All other isolates with a doxycycline MIC $\geq 4$ μg/mL carried tet(M). No isolates carried both tet(K) and tet(M).

3.5 Discussion

In our study, the use of the human-derived tetracycline breakpoints for evaluation of MRSP susceptibility to minocycline and doxycycline lead to misclassification of some MRSP isolates as susceptible to those antimicrobial agents. The CLSI has approved new canine breakpoints for doxycycline; however, the new breakpoints have not been published and consequently, commercially available broth microdilution plates have not been revised to include the new doxycycline susceptibility
breakpoints. Using the tetracycline susceptibility breakpoint of ≤ 4 μg/mL rather than the new doxycycline or minocycline susceptibility breakpoints, 5 (5%) and 45 (45%) isolates, respectively, would have been misclassified as susceptible. Misidentification of MRSP organisms as susceptible to these antimicrobials can result in ineffective therapeutic choices, leading to treatment failures.

An earlier investigation used tetracycline breakpoints to determine the susceptibilities of MRSP isolates to doxycycline and minocycline and found that 38% of isolates were susceptible to doxycycline and 65% were susceptible to minocycline. This is in contrast to our work were only 31 (31%) isolates were susceptible to doxycycline and minocycline. The difference between the percentages of MRSP isolates susceptible to doxycycline and minocycline reflect that the new doxycycline and the proposed minocycline susceptibility breakpoints are more conservative than the tetracycline breakpoints.

Recently, the MICs of minocycline of 168 SP isolates were evaluated and 101 of 168 (60%) isolates had an MIC ≤ 0.25 μg/mL, which was approximately double the rate in our study (31%). This is reflected in the difference between the MIC$_{50}$ and MIC$_{90}$ in that report of 0.125 μg/mL and 4 μg/mL, respectively, with that of our study of 4 μg/mL and 8 μg/mL, respectively. Similarly, the MIC$_{50}$ and MIC$_{90}$ of doxycycline were higher in our study (both 8 μg/mL) than previously reported (MIC$_{50}$ = 0.063 and MIC$_{90}$ = 4 μg/mL). This difference may be due to a difference in the body sites sampled, type of lesion sampled (clinically affected versus carriage sites in asymptomatic dogs), the testing method (E-test versus broth microdilution), the resistance of the isolates (MRSP
versus meticillin-susceptible SP) as well as the geographic locations from which the samples were collected.

Based on the new doxycycline breakpoints\textsuperscript{7} and proposed minocycline breakpoints, our results indicate that if an MRSP isolate has an MIC $\leq 0.125$ \textmu{g}/mL for doxycycline, indicating a susceptible isolate, it will likely be susceptible to minocycline. If an MRSP isolate has an MIC $\geq 4$ \textmu{g}/mL for doxycycline, indicating resistance, it will likely be resistant to minocycline. Therefore, for laboratories that do not provide minocycline MICs, the use of the new doxycycline breakpoint is a reasonable surrogate for minocycline.

Two isolates had an MIC = 0.5 \textmu{g}/mL for minocycline, an MIC=8 for doxycycline and carried \textit{tet}(K), which indicates resistance to doxycycline, but susceptibility to minocycline. Based on the proposed minocycline breakpoint (MIC $\leq 0.25$ \textmu{g}/mL), these isolates would have been classified as resistant to minocycline. It could be that these isolates carry another \textit{tet} resistance gene, such as \textit{tet}(O), that would confer resistance to minocycline. As \textit{tet}(O) is very rarely identified in MRSP isolates,\textsuperscript{7} we did not evaluate our isolates for this \textit{tet} gene. In addition, based on their Monte Carlo simulation, Maaland \textit{et al.}\textsuperscript{7} concluded that the probability of achieving a target attainment value of over 98\% for isolates with a minocycline MIC = 0.5 \textmu{g}/mL, a minocycline dosage of 10 mg/kg is required. Therefore, performing MICs for minocycline would rarely be informative in dose selection given that this dataset as well as that of Maaland \textit{et al.}\textsuperscript{7} only had two of 100 (2\%) isolates and 0 of 168 (0\%) isolates with an MIC = 0.5 \textmu{g}/mL for minocycline, respectively, indicating that this level of minocycline resistance is currently uncommon.
Sixty-nine of the 100 (69%) MRSP isolates contained one of the tetracycline resistance genes, \( tet(M) \) or \( tet(K) \). No MRSP isolates carried both \( tet(M) \) and \( tet(K) \). \( Tet(M) \) has been reported to be the most common resistance gene in SP isolates.\(^{17,271} \)

While Weese \textit{et al.}\(^6 \) found that 33 of 103 (32.0%) MRSP isolates contained \( tet(M) \), 67 of 100 (67%) isolates in our study contained \( tet(M) \). Furthermore, Weese \textit{et al.}\(^6 \) identified 39 of 103 (37.9%) MRSP isolates with \( tet(K) \) and only two of 100 (2%) isolates contained \( tet(K) \) in our study. These differences may be attributable to differences in \( tet \) prevalence among MRSP between the geographical areas inhabited by the dogs in the two studies; 73% and 27% of isolates were from Canada and the United States,\(^6 \) respectively, whereas all isolates characterized in this work were from midwestern U.S. dogs. Further, the isolates characterized herein were exclusively from lesional skin, whereas 26% of samples in Weese \textit{et al.}\(^6 \) were sourced from clinically normal dogs.

In conclusion, tetracycline breakpoints adopted from human CLSI standards are too high to be used for susceptibility testing of minocycline and doxycycline for MRSP isolated from dogs, risking treatment failures. These results underscore the importance of utilizing the proposed minocycline and accepted doxycycline\(^7 \) breakpoints in place of human-derived tetracycline breakpoints. For laboratories that are unable to provide minocycline susceptibility testing against MRSP, the canine-specific doxycycline susceptibility breakpoint may be used as a substitute for minocycline susceptibility testing, as well as the commercially available E-test and disk diffusion test.
Table 2. Distribution of the minimum inhibitory concentrations (MICs) of doxycycline and minocycline for meticillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolates and associated tetracycline resistance (*tet*) genes for the isolates. MICs are in μg/mL.

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<tr>
<th>Doxycycline MIC (μg/mL)</th>
<th>Minocycline MIC (μg/mL)</th>
<th>≤ 0.125</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
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<th>8</th>
<th>16</th>
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<tr>
<td>≤ 0.125</td>
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<td>(no <em>tet</em> genes)</td>
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</table>
Figure 2. Minocycline MIC distribution of 100 meticillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolates. The number above the bars represents the number of isolates at that MIC. MICs are in μg/mL.
Figure 3. Doxycycline MIC distribution of 100 meticillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolates. The number above each bar represents the number of isolates at that MIC. MICs are in μg/mL.
Chapter 4

Effect of feeding on the pharmacokinetics of oral minocycline in healthy research dogs

4.1 Abstract

Background: The effect of food on minocycline oral absorption in dogs is unknown.

Hypothesis/Objectives: The objective of this study was to determine the pharmacokinetics of minocycline after administration of a single oral dose in fed and fasted dogs.

Methods: Ten research hounds were administered oral minocycline (approximately 5 mg/kg) with and without food, in a crossover study, with a one-week washout between treatments. Blood samples were collected immediately prior to minocycline administration and over 24 hours. Minocycline plasma drug concentrations were measured using high-performance liquid chromatography and were analyzed with compartmental modeling to determine primary pharmacokinetic parameters. Each dog was analyzed independently, followed by calculation of means and variation of the dogs. Wilcoxon signed-rank test (analyzing secondary pharmacokinetic parameters - peak concentration \([C_{\text{MAX}}]\), area under the concentration-versus-time curve \([\text{AUC}]\)) was used to compare the two groups. A population pharmacokinetic modeling approach using nonlinear mixed effects modeling for primary parameters for the population as fixed
effects and the difference between subjects as a random effect was performed. Covariate analysis was used to identify the source of variability in the population.

**Results:** No significant difference was found between treatments for AUC (P=0.0645), although AUC was higher in fasted dogs. A significant difference was found for C\(_{\text{MAX}}\) (P=0.0059), with fasted dogs attaining a higher C\(_{\text{MAX}}\). The covariate of feeding versus fasted accounted for a significant variation in the pharmacokinetics.

**Conclusions and clinical importance:** Since feeding was a significant source of variation for the population’s primary pharmacokinetic parameters and fasted dogs had higher minocycline concentrations, we recommend administering minocycline without food.

**4.2 Introduction**

*Staphylococcus pseudintermedius* (SP) is the most common cause of canine superficial pyoderma\(^1,4\) and meticillin-resistant *S. pseudintermedius* (MRSP) is being isolated more and more frequently from dogs with superficial pyoderma.\(^1\) MRSP organisms are characterized by the presence of the *meca* gene, which encodes an altered penicillin binding protein 2a (PBP2a) that confers resistance to all beta-lactam antibiotics including cephalosporins and amoxicillin-clavulanate, which are the most commonly utilized antibiotics for treatment of SP in the dog.\(^3,2,261\) In addition, these bacteria often carry coresistance to many non-beta-lactam antibiotics with over 90% of these isolates being multi-drug resistant.\(^255\)

With the emergence of MRSP, the number of antibiotics to which isolates are susceptible has decreased leading to antibiotic choices that may have profound side
effects or are reserved for human infections. Doxycycline is the tetracycline that has been most commonly used to treat MRSP infections in dogs, while only sporadic reports of the use of minocycline have been documented in the veterinary literature.3,6,7

Recently there has been a shortage of doxycycline leading to a substantial price increase resulting in veterinarians resorting to prescribing compounded doxycycline, which is less expensive but has been shown to be unstable in aqueous vehicles and risks administering an ineffective medication to pets. In the authors’ pharmacy, minocycline capsules are about half the cost of doxycycline capsules. Therefore, minocycline could serve as a reliable alternative to veterinarians for treating dogs with infections caused by SP.

The pharmacokinetics of minocycline as well as dosage recommendations have been determined in dogs administered minocycline hydrochloride intravenously and well as orally. However, a limitation cited was that all dogs were Beagles, and all oral doses were administered without food (fasted). For some drugs, Beagle dogs may have different pharmacokinetics that other breeds.9

Many pet owners prefer to administer oral medications with food; however, this may alter the absorption of oral medications. When four horses were administered oral minocycline, absorption after oral administration was similar when fed hay or hay and grain. In humans the oral absorption of minocycline was not significantly different when administered with food. However a decrease in the absorption of minocycline was found in people with the administration of iron, milk and food. The effect on minocycline oral absorption in dogs is unknown. Maaland et al. used a Monte Carlo
simulation and reported that a minocycline dosage of 5 mg/kg given twice daily had a high probability of treating infections due to SP with an MIC $\leq$ 0.25 μg/mL.\textsuperscript{7}

The objective of our study was to determine the pharmacokinetics (PK) of minocycline after administration of a single oral dose of minocycline in dogs other than Beagles with and without food. We hypothesized that the administration of food would not affect the absorption of a single oral dose of minocycline in dogs.

4.3 Materials and Methods:

4.3.1 Animals and Ethical Issues

All dogs were housed in a research facility at a veterinary college in individual runs with a temperature- and humidity-controlled environment. The University Laboratory Animal Resources (ULAR) staff cared for and supervised the dogs. They were socialized and exercised indoors daily.

An initial experiment (Study 1) with a sample size of six research dogs (hounds) was chosen based on previous pharmacokinetic studies.\textsuperscript{7,274} Based on results of these six dogs, a second experiment (Study 2) using an additional four dogs were selected for further evaluation to strengthen the power of this study. Both utilized a randomized crossover design with fed and fasted treatment allocation. All dogs were between one and two years of age, were heartworm negative, and were determined to be healthy on the basis of a physical exam performed by a veterinarian. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC).
4.3.2 Diet Composition

All dogs were fed a dry diet (2025 Teklad Global 25% Protein Dog Diet; Indianapolis, IN; Harlan®) which is designed and manufactured for research dogs, once daily for one week prior to the start of the study during the acclimation period and during the washout period. All dogs received water ad libitum.

For the fed portion of the crossover, it was necessary that the dogs ate their meal within 15 minutes. In Study 1, those dogs that were slow or reluctant to eat the dry diet were fed a maintenance canine canned diet (Pro Plan® Savor® chicken & rice entrée classic; St. Louis, Missouri; Purina) or the dry food mixed with the canned food. For Study 2, all dogs were fed the canned food.

Both diets were formulated to meet the nutritional levels established by the Association of American Feed Control Officials (AAFCO) Dog Food Nutrient Profiles for all life stages including maintenance. The iron and calcium contents for the canned and dry diets are shown in Table 3.

4.3.3 Experiment Design

A two-period, two treatment cross-over study design with a seven day washout was used so every dog received each treatment (fed and fasted). After a one-week acclimation period, a peripheral venous catheter (18- gauge) was placed in the cephalic vein by standard aseptic technique prior to oral administration of minocycline. For each study, dogs were randomized into one of two groups (group one and group two, with an equal number of dogs in each group) using the random function in Excel (Redmond, WA; Microsoft). Dogs were fasted for 12 h prior to minocycline administration.275-277
one received minocycline without food and group two received minocycline with food once. Minocycline 50 mg and 100 mg capsules were used in order to adjust the dose as close as possible to 5 mg/kg with rounding up to the nearest 50 mg capsule if needed. All dogs received at least 5 mg/kg. When minocycline was administered with food, it was administered orally within 15 min after the meal.

After the minocycline capsule(s) was administered, the oral cavity of each dog was inspected to make sure the capsule(s) was swallowed and each dog was given 12 mL of water by a syringe to ensure the capsule(s) was swallowed and flushed into the stomach. The dogs were monitored for vomiting for 24 h after administration of their dose. After the blood collection period, the dogs resumed their normal feeding schedule of dry food during the wash-out period until the onset of the cross-over.

For Study 1, blood samples were collected from the catheters immediately prior to minocycline administration and over a 24 h period at 20, 40 min and 1, 2, 3, 4, 6, 8, 10, 12, 16 and 24 h in the first six dogs. Based on the results of the six dogs, eight time points were selected for the additional four dogs in Study 2 based on peak minocycline levels as well as declining levels of minocycline over a 24 h period. Therefore, for the additional four dogs in Study 2, blood samples were collected from the catheters immediately prior to minocycline administration and over a 24 h period at 1, 2, 3, 6, 8, 12, and 24 h after minocycline administration.

A 4 mL blood sample was collected at each time point. The blood samples were collected in heparinized tubes (PST gel and lithium heparin; BD Vacutainer®; Franklin Lakes, NJ; BD), kept on ice in a closed container and centrifuged for 10 min at 1000 x g. Plasma was frozen at -80°C until analysis. Catheters were removed after the last blood
sample was collected. The frozen plasma samples were shipped overnight on dry ice to the North Carolina State University College of Veterinary Medicine Clinical Pharmacology Laboratory for high performance liquid chromatography (HPLC) analysis.

4.3.4 Minocycline Assay

High performance liquid chromatography (HPLC) was used to evaluate the plasma minocycline levels using a method previously validated. Briefly, minocycline concentrations were determined via HPLC with ultraviolet (UV) detection. The HPLC apparatus consisted of: a pump (Agilent 1100 Series solvent delivery system), with a mobile phase consisting of 85% oxalic acid buffer, 5% acetonitrile and 10% methanol with a flow rate of 1 mL/min; an autosampler (Agilent 100 Series) providing a 10 µl injection; UV detector (Agilent 1100 Series Variable Wavelength Detector) set at 350 nm; and computer for data collection and analysis (Agilent 1100 Series Chemstation 2D software). A Zorbax Rx C8 reverse-phase column was used for separation kept at a constant temperature of 40°C. The limit of quantitation of the assay was 0.01 mg/L.

4.3.5 Pharmacokinetics

Initial estimates of pharmacokinetic parameters were calculated from all dogs using a standard two-stage (STS) approach in which parameters for all dogs are calculated separately and averaged to obtain mean and standard deviation for the entire group. The dogs were sorted into fed and fasted for this analysis.

Plasma drug concentrations were plotted on linear and semilogarithmic graphs for analysis and for visual assessment of the best model for pharmacokinetic analysis. Analysis of the curves and pharmacokinetic modeling were then performed with a
commercial pharmacokinetic program (Phoenix® WinNonlin, Certara, St. Louis, MO).

Compartmental analysis of the data was performed using a weighting factor of $1/(\text{predicted } Y)^2$, where $Y$ is the plasma concentration. The primary parameters were calculated using the following formula:

$$C = \frac{k_{01} F D}{V(k_{01} - k_{10})} \left[ e^{-k_{10} t} - e^{-k_{01} t} \right]$$

(Equation 1)

Where $C$ is the plasma concentration, $t$ is time, $k_{01}$ is the non-IV absorption rate, assuming first-order absorption, $k_{10}$ is the elimination rate constant, $V$ is the apparent volume of distribution, $F$ is the fraction of drug absorbed, and $D$ is the non-IV dose. A lag-time was added to the model to account for dissolution of the oral product and stomach emptying. Secondary parameters calculated from the model included the peak concentration ($C_{\text{MAX}}$), time to peak concentration ($T_{\text{MAX}}$), area under the plasma-concentration versus time profile (AUC), and the respective absorption and terminal half-lives ($t_{1/2}$).

4.3.6 Population Pharmacokinetics

Initial STS analysis separated dogs into fed and fasted and mean estimates for parameters were determined for all dogs. Six dogs were studied in experiment one (Study 1), and four dogs studied in the later experiment (Study 2). Sampling times were different and more sparse in Study 2. Therefore, in order to analyze all dogs together, a population
pharmacokinetic analysis with nonlinear mixed effects modeling was performed using the Phoenix® NLME™ software (Certara, St. Louis, MO).

Various models and different error structures were tested to determine the best fit base model. The models were parameterized by first order input ($K_a$) and elimination ($K_e$) with a lag-time included for oral absorption. Lag-time allows for oral formulation dissolution and stomach emptying. The model was run with quasi-random parametric expectation maximization algorithm (QRPEM). Final model selection was based on goodness of fit plots, statistical significance between models using -2LL (twice the negative log likelihood), AIC (Akaike information Criterion - a goodness of fit measure based on the log likelihood adjusted for the number of parameters (degrees of freedom) in the fit), obtained in Phoenix NLME, and coefficient of variation (CV%) of parameter estimates. Secondary parameter estimates were obtained using standard compartmental equations.  

Inter-individual (between subject) variability (variance of a parameter among different subjects) were expressed using an exponential error model according to the equation:

$$P_i = P_{\text{pop}} \times \exp(\eta_i P), \quad \text{(Equation 2)}$$

where $P$ is the parameter of interest for the individual $i$, $P_{\text{pop}}$ is $\theta$ (theta), the typical value for the population estimate of the parameter of interest, and $\eta_i P$ is the $\eta$ (eta) for the individual and parameter of interest. The $\eta$ values were assumed to be independent and have a normal distribution with a mean of zero and variance of $\omega^2$. A multiplicative
model was chosen (among additive, log-additive, power, and mixed error models) to describe the residual random variability (\( \varepsilon \)) of the data for once daily dosing, where \( \varepsilon \) is the residual intrasubject (within subject) variability with a mean of zero and a variance of \( \sigma^2 \), according to the equation:

\[
C_{\text{obs}} = C_{\text{pred}} \times (1 + \varepsilon) \quad \text{(Equation 3)}
\]

where \( C_{\text{obs}} \) is the observed concentration for the individual and \( C_{\text{pred}} \) is the model predicted concentration plus the error value (\( \varepsilon \)).

Once the final model was obtained for the population, an examination of covariates was performed to determine if there are factors that may explain the variability in the primary parameters (\( K_a, K_{es}, V/F, \) and Lag time). The covariate examined was fed versus fasted, which were considered as a categorical covariate. A box plot of the covariate on each parameter showed that \( V/F \), which is the parameter most affected by extent of oral absorption, was the parameter most likely affected by the covariate.

The covariate of fed versus fasted was tested in a simple stepwise approach with forward inclusion and backward elimination. The effects of the covariate on a parameter was evaluated based on improvement in the -2LL (equivalent to the objective function value (OFV) in NONMEM). Results were considered statistically significant if the decrease was significant with a \( P \)-value <0.01. A backward elimination step was used to assess the significance of the covariate, and an increase in the -2LL with a \( P \)-value <0.001. After this covariate was considered significant, the covariate remained in the final model. The predictive accuracy of the final model was tested using the visual
predictive check (VPC) with 10 observations simulated for each subject stratified by the categorical covariate. The VPC was examined to compare observed quantiles with predicted quantiles predicted by the model.

4.3.7 Statistical Analysis

A Wilcoxon signed-rank test (analyzing secondary pharmacokinetic parameters - peak concentration \([C_{\text{MAX}}]\), area under the concentration-versus-time curve \([\text{AUC}]\)) was used to compare the two groups (fed versus fasted). A P-value < 0.05 was considered significant.

4.4 Results

The mean oral dose of minocycline administered was 5.99 mg/kg with a range of 5.08 mg/kg to 8 mg/kg. When dogs were fed during the PK study, in Study 1, two dogs were fed dry food only, 3 dogs were fed a mixture of dry and canned food, and one dog was fed canned food only. All dogs in Study 2 were fed canned food only.

One dog in Study 1 hypersalivated after receiving one dose of minocycline administered with food. This dog did not hypersalivate after receiving minocycline without food. No other adverse effects were observed in this dog or the other five dogs in Study 1 or the four dogs in Study 2. After administration of the minocycline capsule(s), in all dogs, the capsule(s) stuck to the base of caudal tongue and the capsule(s) had to be digitally pushed into the throat prior to the administration of 12 mLs of water.

For the STS analysis of these data, a one-compartment model with first order absorption and elimination was the best fit for the oral pharmacokinetic data, except for
one dog (No. 3833 in Study 1) where a compartment model could not be fit to these data because of an erratic terminal slope in the fed crossover. The data for this dog was analyzed using non-compartmental analysis. The pharmacokinetic parameter values and mean values for each experiment (Study 1, Study 2) and for the studies combined are shown in Table 4. The minocycline plasma concentration versus time data are shown in Figure 4 (Study 1) and Figure 5 (Study 2). Comparison of AUC values, and visual examination of Figures 3 and 4 shows that extent of absorption appeared higher in the fasted dogs than fed dogs, with a greater difference in Study 1 than Study 2.

4.4.1 The Population Model

The population base model was fit as a one-compartment model with first order absorption and elimination. The best fit was confirmed from an examination of the improvement in AIC and -2LL among different pharmacokinetic and error models, and estimation procedures (Phoenix Model Engines).

Including feeding versus fasted as a categorical covariate showed that this factor accounted for most of the intersubject (within subject) variation ($\eta$) for the parameter of volume of distribution ($V$). The volume of distribution in this model is actually $V/F$ for an oral dose, and is the primary parameter in this model most affected by extent of absorption, $F$. Because visual examination of the box plots of $\eta$ for each categorical variable (feeding versus fasted) showed $VD/F$ to be the parameter most affected, this was explored further. Fed versus fasted was added as a categorical variable and tested against the base model. We observed an improvement in the model with a decrease in the -2LL (equivalent to the objective function value (OFV) in NONMEM) considered statistically
significant with a P-value <0.01. A backward elimination step confirmed the significance of the covariate, and an increase in the -2LL with a P-value <0.001.

The difference in feeding in the model was also observed in the plots of individual subjects (spaghetti plots) shown in Figure 6. The left panel of Figure 6 shows all dogs fit using the base model, without a covariate, and the right panel shows the model fitted to the data with the covariate added. When the covariate was added to the model, a separation was observed in the plots, as seen in the right panel of Figure 6.

Pharmacokinetic parameters for the population model are listed in Table 5, with the typical values for the population (θ, theta) primary parameters and secondary parameters sorted by fed and fasted. This equation describes the covariate effect of feeding on the parameter of V:

\[ V_i = \theta V \times \exp^{dvd \text{ fed-fastened}} \times \exp^{(\eta V)}, \quad (\text{Equation 4}) \]

where \( V \) is the volume of distribution estimation for the individual, \( i \), \( \theta V \) is the population estimate for volume of distribution, \( dvd \text{ fed-fastened} \) is the value for the effect of feeding and is included if the individual is categorized as receiving the medication without food, and \( \eta V \) is the \( \eta \) of the individual \( i \) for the volume of distribution. (Note that volume of distribution included here is actually VD/F, because of oral administration.) No other covariates were explored, and based on visual analysis of the covariate on \( \eta \) using box plots, no other parameters were considered to be affected by feeding. The final model was confirmed by examining goodness of fit plots for the population predicted versus observed concentrations and individual predicted versus
observed concentrations. Visual Predictive Check (VPC) plots examining the 50\textsuperscript{th}, 5\textsuperscript{th} and 95\textsuperscript{th} percentiles of predictions validated the final model by showing that most of the observations fell within the 95\textsuperscript{th} and 5\textsuperscript{th} quartile range of the predicted concentrations.

4.5 Discussion

The only other pharmacokinetic study published on oral minocycline in dogs was performed in fasted Beagles administered 10 mg/kg of minocycline.\textsuperscript{7} The pharmacokinetics in other dog breeds and the effect of feeding was not explored. Studies have shown that Beagles can have different pharmacokinetics compared to other breeds\textsuperscript{9} and the effect of feeding has been shown to have variable effects on oral antibiotics in dogs.\textsuperscript{10-15} We used hounds that received a 5 mg/kg dose of minocycline and compared fed versus fasted conditions. Our hounds had lower peak absorption (C\textsubscript{MAX}), but somewhat longer elimination rate (K\textsubscript{10} T \(\frac{1}{2}\)) and an overall lower AUC than the Beagles (Table 6). Examination of a potential difference in the pharmacokinetics of minocycline when fed versus fasted is important because many pet owners prefer to administer medications with a meal or treat to dogs. In addition, some dogs with infections treated with antibiotics may be anorexic and reluctant to consume food with their medication.

In the initial experiment performed with 6 dogs (Study 1), there was a marked difference in the fed versus fasted dogs as observed by the plasma concentration differences (Figure 4) as well as a statistical difference in the AUC and C\textsubscript{MAX} (Table 4). To confirm the difference, and utilizing one type of food (canned diet) a second crossover study was conducted with 4 additional dogs (Study 2). Plasma concentration differences were still observed between fed versus fasted dogs in Study 2, but not as profound as in
Study 1 (Figure 5, Table 4). The overall effect in all dogs in both studies was that feeding diminished the extent of oral absorption, seen most prominently by the AUC and $C_{\text{MAX}}$, but also observed in the values of VD/F and CL/F, which are affected by the extent of oral absorption, F (Table 4). The elimination rate, as expected was not affected by feeding.

Population pharmacokinetic modeling is a tool used to identify the source of variation in the population. It can also be used for analysis of subjects from different studies, and with a variation in the sampling times. Our study was unique among canine pharmacokinetic antibiotic studies in that we used a population pharmacokinetic approach (NLME) to identify the source of variability in the study, which allowed for analysis of all the dogs and with variation in the sampling times. When the covariate of feeding versus fasted was included in the model, we showed that it accounted for a significant variation in the pharmacokinetics. Furthermore, the improvement in the model with the addition of the covariate was observed in the concentration versus time plots (spaghetti plots) of individual dogs (Figure 6).

One dog in this study (in Study 1) hypersalivated after receiving one dose of minocycline administered with food. This was the only adverse effect noted. It is possible that this episode was due to something other than minocycline administration as the dog did not hypersalivate when administered the other dose of minocycline without food. No adverse effects were previously reported in dogs receiving 10 mg/kg orally. In dogs receiving 30 mg/kg minocycline orally daily for 30 days, there were only a few reports of emesis. When the minocycline was administered, the capsules stuck to the base of the tongue of all dogs. Due to this, owners should be counseled to administer
water after administering the capsules and inspect the oral cavity to make sure the capsule is swallowed.

One limitation was the use of both canned and dry foods. Many of the dogs were used to slowly eating their dry food over a period of several hours. Because it is preferred in a pharmacokinetic study that all dogs eat at the same time, canned food was added to the dry food. In dogs that would not eat the canned/dry mixture, only canned food was fed. In Study 2, all dogs were administered canned food only during the pharmacokinetic study. Since feeding was a source of variation between treatments, it is possible that different foods may affect minocycline pharmacokinetics more than others. Both diets offered in this study were approved by the AAFCO so diets with nutrient profiles similar to these would likely be used to administer oral medications. The minocycline concentration was significantly higher in fasted dogs in Study 1, but was not significant for Study 2 where dogs were only fed canned food. This may be due to the higher iron and calcium levels in the dry food compared to the canned food. The iron in the foods are divalent cations, which can be chelated by tetracyclines. Iron supplements, milk and food have been reported to reduce the absorption of tetracycline and minocycline in humans.\textsuperscript{34} No studies on iron or food affecting the absorption of tetracyclines in dogs have been published. We cannot speculate further on the effect of diet composition on minocycline absorption without additional study.

In summary, population pharmacokinetics are useful to identify sources of variation in a population. We found feeding was a significant source of variation of minocycline pharmacokinetics in hounds. Based on these results we can recommend
when oral minocycline hydrochloride is administered to dogs, it should be administered without food.
Table 3: Iron and calcium content of study diets.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Iron (mg/1000 kcal)</th>
<th>Calcium (mg/100 kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canned diet*</td>
<td>60</td>
<td>290</td>
</tr>
<tr>
<td>Dry Diet**</td>
<td>86</td>
<td>486</td>
</tr>
</tbody>
</table>

* Pro Plan® Savor® chicken & rice entrée classic; St. Louis, Missouri; Purina

**2025 Teklad Global 25% Protein Dog Diet; Indianapolis, IN; Harlan®
Table 4: Pharmacokinetic parameters after oral administration of minocycline at ~5 mg/kg in fed and fasted hound dogs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Fed Dogs</th>
<th>Fasted Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Study 1 (n = 6 *)</td>
<td>Study 2 (n=4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Std. Dev.</td>
</tr>
<tr>
<td>AUC</td>
<td>hr*ug/mL</td>
<td>12.75**</td>
<td>4.64</td>
</tr>
<tr>
<td>CL/F</td>
<td>mL/hr/kg</td>
<td>502.22</td>
<td>114.56</td>
</tr>
<tr>
<td>CMAX</td>
<td>ug/mL</td>
<td>0.96‡</td>
<td>0.36</td>
</tr>
<tr>
<td>K01</td>
<td>1/hr</td>
<td>10.51</td>
<td>10.05</td>
</tr>
<tr>
<td>K01 T½</td>
<td>hr</td>
<td>0.27</td>
<td>0.41</td>
</tr>
<tr>
<td>K10</td>
<td>1/hr</td>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>K10 T½</td>
<td>hr</td>
<td>8.05</td>
<td>2.69</td>
</tr>
<tr>
<td>Lag time</td>
<td>hr</td>
<td>0.59</td>
<td>1.13</td>
</tr>
<tr>
<td>TMAX</td>
<td>hr</td>
<td>1.73</td>
<td>2.34</td>
</tr>
<tr>
<td>VD/F</td>
<td>mL/kg</td>
<td>5751.70</td>
<td>2110.36</td>
</tr>
</tbody>
</table>

(Continued)
Table 4: Continued

Table 4 Legend: AUC, area under the curve; CL/F, Clearance per fraction absorbed; C_{MAX}, Peak concentration; K_{01}, absorption rate; K_{01} T_{1/2}, absorption rate half-life; K_{10}, elimination rate; K_{10} T_{1/2}, elimination half-life; Lag-time, time allowed for dissolution and stomach emptying; T_{MAX}, time to peak concentration; VD/F, apparent volume of distribution per fraction absorbed. A Wilcoxon signed-rank test, analyzing C_{MAX} and AUC was used to compare fed versus fasted hound dogs in Study 1 (n=6), Study 2 (n=4) and for both studies (n=10). A P-value <0.05 was considered significant. A significant difference was found for AUC for Study 1 (** P=0.0313) and for both studies († P=0.0273); for C_{MAX} for Study 1 (§ P=0.0313) and for both studies (‡ P=0.0059).

* A compartmental model could not be fit to one dog in Study 1 (#3833), and data were analyzed using a noncompartmental analysis.
Table 5: Population pharmacokinetic parameters from nonlinear mixed effects model (NLME) analysis (n = 10 dogs) after oral administration of ~5 mg/kg minocycline in fed and fasted hound dogs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Units</th>
<th>Stderr</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta K_a$</td>
<td>5.26</td>
<td>1/hr</td>
<td>5.62</td>
<td>106.88</td>
</tr>
<tr>
<td>$\theta V$</td>
<td>5.33</td>
<td>L/kg</td>
<td>0.89</td>
<td>16.60</td>
</tr>
<tr>
<td>$\theta V$</td>
<td>0.09</td>
<td>1/hr</td>
<td>0.01</td>
<td>9.19</td>
</tr>
<tr>
<td>$\theta \text{Lag time}$</td>
<td>0.25</td>
<td>hr</td>
<td>0.01</td>
<td>4.10</td>
</tr>
<tr>
<td>$T_{\text{MAX}}$</td>
<td>1.03</td>
<td>hr</td>
<td>0.64</td>
<td>62.04</td>
</tr>
<tr>
<td>$\Delta$AUC</td>
<td>11.93</td>
<td>hr*µg/mL</td>
<td>2.30</td>
<td>19.26</td>
</tr>
<tr>
<td>$C_{\text{MAX}}$</td>
<td>1.01</td>
<td>µg/mL</td>
<td>0.15</td>
<td>15.31</td>
</tr>
<tr>
<td>$\text{Cl}$</td>
<td>0.49</td>
<td>L/kg/hr</td>
<td>0.09</td>
<td>19.26</td>
</tr>
<tr>
<td>$K_a T_{\frac{1}{2}}$</td>
<td>0.13</td>
<td>hr</td>
<td>0.14</td>
<td>106.88</td>
</tr>
<tr>
<td>$K_e T_{\frac{1}{2}}$</td>
<td>7.60</td>
<td>hr</td>
<td>0.70</td>
<td>9.19</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Units</th>
<th>Stderr</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta K_a$</td>
<td>5.15</td>
<td>1/hr</td>
<td>4.18</td>
<td>81.20</td>
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<tr>
<td>$\theta V$</td>
<td>3.03</td>
<td>L/kg</td>
<td>0.40</td>
<td>13.17</td>
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<tr>
<td>$\theta V$</td>
<td>0.10</td>
<td>1/hr</td>
<td>0.01</td>
<td>9.43</td>
</tr>
<tr>
<td>$\theta \text{Lag time}$</td>
<td>0.46</td>
<td>hr</td>
<td>0.08</td>
<td>18.00</td>
</tr>
<tr>
<td>$T_{\text{MAX}}$</td>
<td>1.24</td>
<td>hr</td>
<td>0.46</td>
<td>37.21</td>
</tr>
<tr>
<td>$\Delta$AUC</td>
<td>19.85</td>
<td>hr*µg/mL</td>
<td>2.78</td>
<td>13.98</td>
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<tr>
<td>$C_{\text{MAX}}$</td>
<td>1.78</td>
<td>µg/mL</td>
<td>0.27</td>
<td>15.02</td>
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<tr>
<td>$\text{Cl}$</td>
<td>0.29</td>
<td>L/kg/hr</td>
<td>0.04</td>
<td>13.98</td>
</tr>
<tr>
<td>$K_a T_{\frac{1}{2}}$</td>
<td>0.13</td>
<td>hr</td>
<td>0.11</td>
<td>81.19</td>
</tr>
<tr>
<td>$K_e T_{\frac{1}{2}}$</td>
<td>7.18</td>
<td>hr</td>
<td>0.68</td>
<td>9.43</td>
</tr>
</tbody>
</table>

Table 5 Legend: AUC, area under the curve; CL, Clearance per fraction absorbed; $C_{\text{MAX}}$, Peak concentration; $K_a$, absorption rate; $K_a T_{\frac{1}{2}}$, absorption rate half-life; $K_e$, elimination rate; $K_e T_{\frac{1}{2}}$, elimination half-life; $\Delta$ Lag-time, time allowed for dissolution and stomach emptying; $T_{\text{MAX}}$, time to peak concentration; V, apparent volume of distribution per fraction absorbed. The symbol $\theta$ (theta) indicates that this is the typical value for the population.
Table 6. Oral minocycline pharmacokinetics in fasted dogs.

<table>
<thead>
<tr>
<th></th>
<th>No. of dogs</th>
<th>Dose (mg/kg)</th>
<th>C(_{MAX}) (μg/mL)</th>
<th>K(_{10}) T(\frac{1}{2}) (h)</th>
<th>AUC (hr*μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maaland \etal. (^8)</td>
<td>6</td>
<td>10</td>
<td>3.44 ± 1.09</td>
<td>4.14 ± 0.50</td>
<td>30.95 ± 10.84</td>
</tr>
<tr>
<td>Hnot \etal.</td>
<td>10</td>
<td>5</td>
<td>2.16 ± 0.72</td>
<td>5.81 ± 0.65</td>
<td>25.84 ± 9.69</td>
</tr>
</tbody>
</table>

Table 6 Legend: C\(_{MAX}\), peak concentration; K\(_{10}\) T\(\frac{1}{2}\), elimination half-life; AUC, area under the curve.
Figure 4: Minocycline mean plasma concentrations (with standard deviation shown by bars) after oral administration of a single dose of minocycline (~5 mg/kg of body weight) with food (filled blue square) and without food (filled red circle) in six hound dogs.
Figure 5: Minocycline mean plasma concentration (with standard deviation shown by bars) after oral administration of a single dose of minocycline (~5 mg/kg of body weight) with food (filled blue square) and without food (filled red circle) in four hound dogs.
Figure 6: Population pharmacokinetic model plots (spaghetti plots) of fitted curves for all individual hound dogs (solid line) with observed data points (open circles) after oral administration of minocycline at ~5 mg/kg. Left panel, all hound dogs, fed and fasted in base model without addition of covariate. Right panel, all hound dogs, fed and fasted, with covariate of fed versus fasted added to the final model (note different y-axis scale for each plot).
Chapter 5

Conclusions and Future Directions

The results of our in vitro minocycline and doxycycline study concur with and validate the recommendations of previous authors\textsuperscript{7,16} for the need to have canine-specific breakpoints for these antibiotics. The use of human-derived tetracycline breakpoints for doxycycline and minocycline susceptibility testing misclassified 45 of 76 (59\%) canine MRSP isolates susceptible to minocycline and 5 of 36 (14\%) susceptible to doxycycline. To further substantiate the misclassification, 43 of the 45 isolates classified as susceptible to minocycline and all 5 isolates classified as susceptible to doxycycline by use of human-derived tetracycline breakpoints harbored \textit{tet}(M), known to confer resistance to both antibiotics. Misidentification of the susceptibility of bacterial organisms can result in the selection of an ineffective antibiotic, risking treatment failure. In addition, for those MRSP isolates found to be susceptible or resistant to doxycycline, they were also found to be susceptible or resistant to minocycline as well. Based on this, for veterinary laboratories that do not provide minocycline susceptibility testing, the use of the new canine-specific doxycycline breakpoint is a reasonable surrogate for minocycline susceptibility.

Four \textit{tet} genes have been identified among SP: \textit{tet}(M), \textit{tet}(O), \textit{tet}(K) and \textit{tet}(L).\textsuperscript{17,266} Ribosomal protection is conferred by \textit{tet}(M) and \textit{tet}(O), whereas, efflux pumps are encoded by \textit{tet}(K) and \textit{tet}(L).\textsuperscript{18} \textit{Staphylococcus} spp. that possess only the
tet(K) or tet(L) gene, retain susceptibility to minocycline and are resistant to other tetracyclines, while Staphylococcus spp. that possess the tet(M) or tet(O) gene are resistant to all tetracyclines. The most commonly identified tet genes in SP isolates are tet(M) and tet(K), which are the ones we evaluated.\textsuperscript{16,17} Interestingly, two isolates with an MIC = 0.5 μg/mL to minocycline and an MIC = 8 μg/mL to doxycycline harbored the tet(K) gene, which should have indicated minocycline susceptibility. There are two possible explanations for this discrepancy. It could be that once canine-derived minocycline breakpoints are established, MRSP with an MIC = 0.5 μg/mL for minocycline will actually be susceptible to minocycline, changing the breakpoint for MRSP isolates for minocycline to 0.5 μg/mL rather than 0.25 μg/mL. On the other hand, as we chose to perform PCR for the most common tetracycline resistance gene tet(M), it may be possible that in addition to those two isolates harboring tet(K) they also harbored tet(O), conferring resistance to both doxycycline and minocycline. Therefore, in addition to determining canine-derived minocycline breakpoints, future studies evaluating tetracycline resistance should be include evaluation of tet(O) in addition to tet(M).

Our \textit{in vivo} minocycline study is one of only a few pharmacokinetic studies in dogs utilizing population pharmacokinetics. By using population pharmacokinetics, we were able to combine data from two studies, with unequal blood collection time points to increase the sample size. As fasting dogs had higher minocycline concentrations, fed versus fasted was included as a covariate for analysis. Based on the population pharmacokinetic analysis, feeding introduces significant variation in the pharmacokinetics of minocycline in the dog. Thus we recommend that minocycline be administered to dogs without food. Future pharmacokinetic studies could be designed
investigating the use of minocycline in the dog using population pharmacokinetics. As traditional pharmacokinetics are performed on clinically normal dogs or research dogs, population pharmacokinetics can be performed in dogs with clinical disease being treated with minocycline as well as in many breeds of dogs. Pharmacokinetic studies looking at the effect of other diets, supplements, and treats can be performed to determine if these covariates affect minocycline pharmacokinetics. With population pharmacokinetics, associations between the characteristics of dogs and differences in pharmacokinetics of minocycline could then be used to customize the drug dosage in the face of other illnesses, such as renal or liver disease. As multiple samples are not needed, and sample times do not need to match, population pharmacokinetics would be useful to study pharmacokinetics of minocycline where obtaining numerous blood samples would be difficult, such as in small or miniature breeds of dogs. In addition, when designing pharmacokinetic studies, fewer blood samples would allow for flexible study designs as well as be cost effective. Multiple-dosing pharmacokinetic studies in dogs with clinical disease treated with minocycline can be performed much easier and economically as well.

In addition to its antibiotic effects, minocycline has been shown to exert additional biological actions including anti-inflammatory and anti-apoptotic activity, inhibition of proteolysis, as well as suppression of angiogenesis and tumor metastasis, and inhibition of bone metabolism. Minocycline is able to quench hydrogen peroxide and scavenge superoxide and peroxynitrate; inhibit neutrophil chemotaxis as well as their migration to sites of inflammation; suppress mediators of inflammation (nitric oxide, cyclooxygenases (COXs), phospholipase A2 (PLA2)); reduce the production of
inflammatory cytokines and inhibit their release such as protease-activated receptor 2 (PAR2) which can stimulate the expression of MMPs; and inhibit of apoptosis by decreasing the expression of caspase-1 and caspase-3.

As such, minocycline has been used in human medicine for treatment of numerous inflammatory diseases, autoimmune skin diseases as well as treatment of neoplasia and prevention of gentamicin-induced ototoxicity. Therefore, minocycline may be useful for the treatment of these types of diseases as well as others in the dog such as osteoarthritis, IBD, erythema multiforme, toxic epidermal necrolysis, canine and feline acne, neoplasia and canine neutrophilic dermatitis (Sweet’s syndrome) to name a few. Minocycline may be a less expensive alternative to doxycycline for treatment of canine autoimmune diseases including pemphigus folicaceus, pemphigus erythematosus, discoid-lupus erythematosus and bullous pemphigoid. There are numerous indications for the use of minocycline in the dog that have not been explored, opening up the future for further research of this drug.


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