PROCEEDINGS

Myths & Truths on Antibiotics
International Symposium
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## INTRODUCTION

Ralf Ebert, José Mottet (GER)

## PROCEEDINGS

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INTRODUCTION

At Bayer, we are continually striving to make scientific information relevant for the veterinary clinic in keeping with our mission statement “Science For A Better Life”.

In both human and veterinary medicine, the battle against bacterial infections is continuously evolving. While some bacteria have almost been eradicated, new ones are emerging. Some older antimicrobials are becoming less effective, while the development and use of new drugs is becoming increasingly challenging.

Current discussions on the use of antimicrobials have many facets. As different stakeholders are involved, diverging opinions arise. We are left with a plethora of national and international guidelines and recommendations. Which one do we choose at the moment of truth? And do we still have a choice?

In human medicine it is well accepted that a clinical cure is not a good measure for treatment success. A bacteriological cure, on the other hand, has been shown to be associated with fewer recurrences as well as less selection for resistance. And the faster the bacteriological cure is achieved, the shorter the treatment plan can be. Does this also apply to veterinary medicine? And what data do we have to support this?

This 3rd International Symposium on Antibiotics tries to answer these and many other questions on the use of antibiotics. We thank our distinguished lecturers for helping and guiding us to a better understanding of what the prudent use of antibiotics means, how the use of Veraflox® and Baytril® fits in, as well as for answering all our questions. We thank them for contributing to “Science For A Better Life”.

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INTRODUCTION
Recently, there have been several antimicrobial use consensus statements produced by various groups in order to provide guidance on antimicrobial administration and improve stewardship of these agents in veterinary practice. The consensus guidelines for empirical treatment of common infections in animals focus on selecting an antimicrobial agent with a high likelihood of success for the suspected clinical infection without increasing the risk of emergence of resistant bacteria. Many guidelines have appeared in published proceedings, review papers, consensus documents, and textbooks. Consensus statements have been produced by committees from the International Society for Companion Animal Infectious Diseases (http://www.iscaid.org/), the British Small Animal Veterinary Association (www.BSAVA.com), and the AFVAC (https://afvac.com/). Guidelines have been published by veterinary journals such as Veterinary Record.1, 2 National organizations such as the American Veterinary Medical Association (AVMA) have provided recommendations from their Task Force on prudent use of antibiotics (www.AVMA.org). The American College of Veterinary Internal Medicine (ACVIM) also has published guidelines on responsible use of antimicrobials.3

Although these guidelines vary somewhat in their scope and specific recommendations, the overall theme is consistent. The guidelines promote careful and responsible use of antimicrobials in companion animals in order to reduce the risk of resistance and produce a favorable therapeutic outcome. The guidelines generally advocate for the use of approved antibiotics and discourage the administration of other drugs that are ordinarily reserved for drug-resistant infections. In a few instances (for example, third-generation cephalosporins and fluoroquinolones), the guidelines discourage the use of antibiotics that are actually approved to treat infections in small animals and recommend the administration of antibiotics that are not approved by some regulatory authorities.

Whether the treatment is for skin infections (pyoderma or bacterial folliculitis),4 urinary tract infection,5 or respiratory infections (publication pending), the recommendations from the guidelines are to utilize the “first choice” or “first tier” initially for empirical treatment of routine infections. They are listed in these guidelines as a first choice because most are approved by regulatory authorities for treating common infections and usually will be active against the wild-type population of bacteria that cause infections in these sites. These guidelines provide
recommendations for empirical antibiotic treatment, but do not guarantee a cure. Empirical choices for initial treatment are based on the assumption that the infection is not complicated and the infection is caused by susceptible bacteria. Wild-type strains of bacteria are those that have an absence of acquired and mutational resistance mechanisms, whereas non-wild-type strains of bacteria are those that have the presence of an acquired or mutational resistance mechanism to the drug in question. Wild-type strains may include bacteria that have inherent resistance to antimicrobials. For example, wild-type anaerobic bacteria are inherently resistant to aminoglycosides by virtue of a lack of an oxygen-dependent drug entry to the bacteria. Gram-negative bacteria of the Enterobacteriaceae and Pseudomonas aeruginosa are inherently resistant to macrolide antibiotics.

Susceptible strains of bacteria may or may not respond clinically to antimicrobial treatment. Likewise, occasionally the infections in some patients resolve, despite treatment with antibiotics to which the organism may be resistant. The prediction of whether the infection will or will not respond to treatment has been referred to in human medicine as the “90/60 rule”. The 90/60 rule was derived from the observation that, in general, bacteria treated with antimicrobials to which the strain is susceptible will have a favorable therapeutic response in approximately 90% of the patients. On the other hand, when the bacteria is resistant to the antimicrobial administered, despite the susceptibility test result, approximately 60% of patients will respond to therapy. In veterinary medicine, we have no data to confirm or challenge the 90/60 rule. The authors emphasize that these observations apply to immunocompetent patients with infections caused by a single bacterium, when the drug is expected to penetrate to the site of infection adequately. These criteria may not apply to every case and veterinarians must evaluate each patient to determine if there are complicating factors. Many patients have polymicrobial infections treated with more than one antibiotic, have pathologic changes that may affect drug distribution (e.g., protein-binding changes), have received oral antibiotics that are insufficiently absorbed, are immunocompromised patients, or have infections at sites that are either poorly penetrated or diluted, or for which antibiotics are concentrated (for example, from topical treatment or by tubular concentration prior to clearance by the kidneys).
CONSENSUS STATEMENT
RECOMMENDATIONS

Skin infections
Susceptibility of the most common isolates has been documented well enough to make recommendations for empirical antimicrobial drug choices based on past experience, evidence-based studies, and antimicrobial susceptibility information.1, 2, 4 The guidelines often include agents that drug manufacturers have marketed to treat these common infections encountered in small animals. For example, recommendations for empirical treatment of pyoderma caused by *Staphylococcus pseudintermedius* consist of administration of cephalosporins, amoxicillin-clavulanic acid (also known as potentiated amoxicillin), clindamycin, or trimethoprim-sulfonamides. If the lesion is amenable to topical treatment, veterinarians can use shampoos, spray disinfectants, and ointments that contain medications active against these pathogens. These bacteria are also susceptible to the β-lactam antibiotics cloxacillin, dicloxacillin, or oxacillin, the lincosamide lincomycin, and the macrolide erythromycin. However, these alternatives are rarely used today because of poor oral absorption, lack of known efficacy, better alternatives within the same class, or lack of available formulations for animals.

Some skin infections are caused by bite wounds (especially in cats), abrasions, or trauma. These infections may be caused by *Pasteurella* species, *Streptococcus* species, and/or *Actinomyces*. These are also typically susceptible to the antibiotics listed above.

Urinary tract infections
For urinary tract infections (UTI), empirical treatment assumes that the infection is uncomplicated. In the document produced by the ISCAID working group,5 uncomplicated UTI was defined as a bacterial infection of the bladder “in an otherwise healthy individual with normal urinary tract anatomy and function”. In these patients, the urine is concentrated and normal patient defense mechanisms exist that will assist in eradicating the infection. Ordinarily, the low pH of the urine, high osmolarity, and presence of salts, urea, and organic acids inhibit bacterial colonization and growth. Empirical drug selection in these patients can utilize oral treatment with amoxicillin, amoxicillin-clavulanate, or trimethoprim-sulfonamides. Some guidelines have also considered administration of cephalosporin antibiotics for these cases in countries in which they are approved by regulatory authorities. In some of the published guidelines, a long half-life cephalosporin, cefovecin (Convenia) may be allowed if pet owner compliance is a problem. The guidelines also provide helpful information on diagnosis, duration of treatment, and other aspects of management.

The success of these empirical choices for initial treatment is based on the assumption that the drugs will concentrate sufficiently in the urine. A pathogen that may be ordinarily resistant to systemic concentrations of drugs (e.g., plasma drug concentration) can be suppressed in the high concentration of drugs attained in the urine.7 The Clinical and Laboratory Standards Institute (CLSI) has a higher breakpoint for some drugs used in urinary tract infections because of the drug concentration effect.8

OTHER ANTIBIOTIC CHOICES
If the infection is complicated, or if a drug-resistant bacteria is suspected, antibiotics beyond the “first tier” may be necessary. When empirical treatment fails, or when resistance is suspected, a culture and susceptibility test is recommended to guide therapy. Susceptibility testing should be performed using standards established by the CLSI.8
In some instances, other drugs are considered appropriate first-choice agents. For example, if the patient is a male dog and prostate involvement is suspected, a fluoroquinolone antimicrobial (for example, enrofloxacin, marbofloxacin, orbifloxacin, pradofloxacin) is appropriate for treating a urinary tract infection. If a patient treated for a skin infection cannot tolerate the “first-tier” agents, a fluoroquinolone may be considered. If *Pseudomonas aeruginosa* is suspected as a pathogen, a fluoroquinolone may be appropriate because it is the only oral drug active against this organism.

For skin infections, methicillin-resistant *S. pseudintermedius*, or other methicillin-resistant *Staphylococcus* species may be identified on a susceptibility test result. These are often multi-drug-resistant strains. In these cases, either topical treatment – if the patient is amenable to topical therapy – or other drugs may be considered. Before using the alternative drugs ("second-tier" or "third-tier" agents), it is the veterinarian's obligation to check with local regulations to determine if the use of these drugs is allowed. Some of the choice that may appear on a susceptibility test are unapproved drugs and restricted by some regulatory bodies.

For urinary tract infections, it is also possible that meticillin-resistant *Staphylococcus* species are identified and drugs active against this strain may be considered. Other resistant bacteria in the urinary tract are likely to be from the Enterobacteriaceae (for example, *Escherichia coli*, *Klebsiella* spp., or *Proteus* spp.). For these bacteria, it is important that a susceptibility test is performed to identify the most appropriate treatment, because some of these isolates can be multi-drug resistant. As noted above for skin infections, it is important when selecting a treatment for these cases to be familiar with local regulations for antibiotic use in companion animals.

Some agents identified on the susceptibility test may not be approved in some countries or are not allowed for use.

**OTHER COMPONENTS OF CONSENSUS STATEMENT GUIDELINES**

In addition to providing recommendations for antibiotic selection for common infections, these guidelines also contain information on proper terminology, pathology, diagnostic tests, and procedures. The guidelines often include antimicrobial stewardship recommendations to prevent unnecessary use of antibiotics in order to reduce the emergence of bacterial resistance.

**Efforts in guidelines to decrease resistance**

The consensus statement guidelines indicate areas in which it is appropriate to not administer antibiotics because clinical signs do not warrant treatment or because the infection may spontaneously resolve without drugs. For example, the urinary tract guidelines emphasize that antibiotics for subclinical (asymptomatic) bacteriuria are not necessary. The guidelines indicate that for treatment of skin infections topical agents may be used to avoid systemic exposure. The guidelines also place emphasis duration of treatment. Often, veterinarians treat animals for an inappropriately long duration. Shorter courses of treatment may be just as effective as long courses. Short courses of treatment do not increase the risk of resistance emerging, and actually decrease the likelihood of persistent resistant strains. Skin infection (pyoderma) may be treated for as little as two weeks in many patients. Urinary tract infections may be treated for 3–5 days, and no longer than 7–10 days.

Many of these guidelines have been produced only in the last few years. It is too early to tell of these
will have an impact on antimicrobial use or trends in antibacterial drug resistance. Continued monitoring of antibiotic use in animals and susceptibility data from bacteria isolated from animals will be important to measure the effect of these efforts.

REFERENCES


06 Doern GV, Brecher SM. The clinical predictive value (or lack thereof) of the results of in vitro antimicrobial susceptibility tests. J Clin Microbiol 2011; 49(9):11–14.


PURPOSE OF ANTIMICROBIAL SUSCEPTIBILITY TESTING

The purpose of antimicrobial susceptibility testing is to determine the susceptibility – also the term sensitivity is used sometimes – of a bacterium to an antimicrobial substance. The susceptibility is expressed as the minimum inhibitory concentration (MIC) in µg/ml of an antimicrobial or concluded from the diameter of the zone of inhibition around an antimicrobial test disk in the disk diffusion method.

METHODS FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING

The methods for antimicrobial susceptibility testing are described in detail in document VET01-A (currently in the 4th edition) of the Clinical and Laboratory Standards Institute (CLSI). The standard agar used for disk diffusion and agar dilution is Mueller-Hinton. For broth dilution, cation-adjusted Mueller-Hinton II broth is used. Media may be adjusted based on the special growth requirements of certain bacterial species such as fastidious organisms. CLSI has also established interpretive criteria for antimicrobial susceptibility testing that have been published in document VET01S. Based on these criteria, i.e. clinical breakpoints, bacterial strains are categorized as susceptible “S”, intermediate susceptible “I” or resistant “R” to an antimicrobial agent.

The current methods for susceptibility testing comprise disk diffusion, agar dilution and broth dilution. Due to the higher accuracy and the result being a defined MIC, the agar and broth dilution methods are preferred over the disk diffusion method for most applications of antimicrobial susceptibility testing. However, disk diffusion is still widely used by laboratories for establishing antibiograms due to its easiness and convenience. For all methods, it is crucial to use the appropriate quality control strains in every test run. Only if the test results of the quality control strains lie within the quality control ranges published by CLSI (VET01S), the susceptibility test results are considered valid. If the control strains perform outside of these ranges, the test needs to be repeated and the laboratory is advised to check their processes in detail to avoid further invalid test results in the future.

DISK DIFFUSION

For the disk diffusion method, a bacterial suspension prepared from well-defined bacterial colonies or an overnight culture is adjusted to a 0.5 McFarland turbidity standard. A cotton swab is soaked with the suspension and streaked onto an appropriate agar plate. After excess moisture has been absorbed by the agar plate, the antimicrobial test disks are placed onto the agar. It is important that only disks are used...
that contain the correct amount of antimicrobial in μg as specified by CLSI. The plates are then incubated at 35 ± 2 °C for 16–24 hours. The diameter of the inhibition zone is measured through the center of the disk and the bacterial strain classified according to the zone diameters published in CLSI VET01S.

AGAR DILUTION
The agar dilution method uses agar plates containing defined concentrations of the antimicrobial agent, which are freshly prepared by the laboratory for each test. A serial two-fold concentration range centering on 1 μg/ml is used for each antimicrobial substance, e.g., depending on the expected susceptibility of the tested bacteria, 0.002, 0.004, 0.008, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256 μg/ml. As for the disk diffusion method, bacterial suspensions are adjusted to a 0.5 McFarland turbidity standard. Such a suspension contains 1–2 x 10^8 cfu/ml and is further diluted 1:3 in order to obtain the required inoculum of 3.3–6.6 x 10^7 cfu/ml. Using automated multi-pointers, 0.3 μl of each bacterial suspension are inoculated onto the agar plate giving the final inoculum of 1–2 x 10^4 cfu/spot. Up to 100 MIC values can be determined on one dilution series of agar plates. The plates are incubated at 35 ± 2 °C for 20–24 hours and the results interpreted according to the MIC breakpoints published in CLSI VET01S. The MIC is recorded as the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism, disregarding a single colony or a faint haze inherent in the inoculum.

BROTH DILUTION
Broth dilution can be performed both as a macro-dilution and microdilution method. The macrodilution requires relatively large volumes of broth, typically 2 ml in 10 ml test tubes, and as a consequence relatively large amounts of antimicrobial substances. Furthermore, the workload is higher than for the microdilution so that broth macrodilution is not widely used. The broth microdilution typically uses broth volumes of 100 μl in 96-well microtiter plates. Serial two-fold dilutions of the antimicrobial drug are prepared in the microtiter plates in a way that 50 μl of appropriate broth contain twice the required final concentration of the active. The bacterial suspensions are adjusted to a 0.5 McFarland turbidity standard and further diluted 1:100 to reach 1 x 10^6 cfu/ml. The final inoculum is 5 x 10^3 cfu/ml equivalent to 5 x 10^4 cfu per well as 50 μl of the bacterial inoculum are added to each well containing 50 μl of the anti-
microbial dilution. In this way, also the final required concentrations of the antimicrobial are achieved in each well. The microtiter plates are incubated at 35±2 °C for 20 hours. The MIC is recorded as the lowest concentration of an antimicrobial agent that prevents visible growth of a microorganism, i.e. the absence of both turbidity of the broth and a button of ≥2 mm at the bottom of the well. Results are interpreted according to the MIC breakpoints of CLSI VET01S.

ETEST
A commercially available test system that is frequently used is the Etest (BioMerieux). A CLSI standard procedure for the Etest is not available and the test needs to be performed according to the manufacturer’s instructions. The Etest consists of a predefined gradient of antibiotic concentrations on a plastic strip and is used to determine the Minimum Inhibitory Concentration (MIC) of antibiotics by placing the strip onto an agar plate inoculated with a bacterial suspension similar to the aforementioned disk diffusion method. When considering to use the Etest, it should be ensured that the test has been validated for the bacterial species to be investigated, in particular if anaerobes are involved. Results are interpreted according to the MIC breakpoints of CLSI VET01S.

INTERPRETATION OF THE RESULTS
CLSI establishes clinical breakpoints following the detailed standard procedure of document VET02-A (currently in the 3rd edition). Setting a clinical breakpoint takes into consideration pharmacodynamic, pharmacokinetic and clinical data and, hence, the clinical breakpoint is the best guidance for predicting the efficacy of an antimicrobial drug in treating a clinical disease. The CLSI breakpoints published in VET01S are currently the only validated set of breakpoints for bacterial infections in veterinary medicine and should be used for guidance of treatment decision. A bacterial infection caused by a strain classified as susceptible “S” is likely to respond to treatment with the antimicrobial at the normal recommended dosage regimen. If a bacterial strain is classified as intermediate “I”, care should be taken that only infections in body sites will be treated, in which clearly elevated drug concentrations are reached. In the majority of cases it is advisable to select a different antimicrobial drug to which the bacterial strain is classified as “S”. If the test result is resistant “R”, it is highly likely that the infection will not respond to treatment with the antimicrobial drug and a different substance must be selected. In the case of methicillin resistance (MRSP, MRSA), none of the beta-lactam antibiotics will be effective and a substance from a different chemical class needs to be selected.

Many publications use a further interpretation criterion, the so-called epidemiological cut-off value (ECV). ECVs are useful for detecting the presence of resistance mechanisms in bacteria that lead to reduced susceptibility and distinguish such bacteria from the susceptible wild-type population. If used for that purpose, the ECV is a valuable tool for early detection of shifts in susceptibility of a bacterial population. However, the ECV is often misused for categorizing bacteria with reduced susceptibility as resistant, although they would be classified as “S” according to CLSI and still be expected to respond to antimicrobial treatment. Therefore, the treatment decision and selection of an appropriate antimicrobial drug should always be based on the CLSI clinical breakpoints. Table 1 shows the CLSI breakpoints for enrofloxacin (Baytril®) and pradofloxacin (Veraflo®) for bacterial infections in dogs and cats.
APPLICATIONS OF ANTIMICROBIAL SUSCEPTIBILITY TESTING

The most important application of antimicrobial susceptibility testing is to provide guidance on the antimicrobial therapy of clinical bacterial infections. If the severity of infection allows, the prepared antibiogram will give a recommendation to the veterinary practitioner what antimicrobial drugs can be used before starting treatment of infection. If therapy has to be started in severe infections before the result of the susceptibility testing becomes available, the antibiogram will help to select a different antimicrobial for continuation of therapy should the initial treatment not have improved the disease after a few days or the causative strain be classified as “I” or “R”. It should be remembered that state-of-the-art collection of the diagnostic sample from a bacterial infection is a prerequisite for isolation and identification of the causative bacterial pathogen and the subsequent selection of the best antimicrobial treatment.

Furthermore, antimicrobial susceptibility testing is widely used in surveillance studies that establish resistance rates in bacterial populations and often monitor changes of resistance rates over prolonged periods of time. This is done both for target animal pathogens and zoonotic and commensal organisms, the latter in order to detect potential public health risks arising from antimicrobial resistance. Most studies involving target animal pathogens are interpreted based on the CLSI clinical breakpoints, whereas ECVs are often used to detect changes of the proportion of the wild-type population for early detection of potential changes in resistance patterns. However, conclusions on ECV-based studies

<table>
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<th>Antimicrobial Agent</th>
<th>Disk Content</th>
<th>Zone Diameter (mm)</th>
<th>MIC Breakpoint (µg/ml)</th>
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<tr>
<td></td>
<td></td>
<td>S</td>
<td>I</td>
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<td><strong>Enrofloxacin</strong></td>
<td></td>
<td></td>
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<td>Dogs (skin, soft tissue, UTI, respiratory)</td>
<td>5 µg</td>
<td>≥23</td>
<td>17–22</td>
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<tr>
<td>Enterobacteriaceae, <em>Staphylococcus</em> species, <em>Streptococcus</em> species</td>
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<tr>
<td><strong>Cats (skin, soft tissue)</strong></td>
<td>5 µg</td>
<td>≥23</td>
<td>17–22</td>
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<tr>
<td>Enterobacteriaceae, <em>Pseudomonas aeruginosa</em>, <em>Staphylococcus</em> species, <em>Streptococcus</em> species</td>
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<tr>
<td><strong>Pradofloxacin</strong></td>
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<tr>
<td>Dogs (skin, UTI)</td>
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<tr>
<td><em>Staphylococcus pseudintermedius</em>, <em>Escherichia coli</em></td>
<td>5 µg</td>
<td>≥24</td>
<td>20–23</td>
</tr>
<tr>
<td><strong>Cats (skin, respiratory)</strong></td>
<td>5 µg</td>
<td>≥24</td>
<td>20–23</td>
</tr>
<tr>
<td><em>Staphylococcus pseudintermedius</em>, <em>Staphylococcus aureus</em>, <em>Staphylococcus felis</em>, <em>Escherichia coli</em></td>
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<tr>
<td><em>Pasteurella multocida</em>, <em>Streptococcus canis</em></td>
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Table 1: Approved CLSI interpretive criteria for enrofloxacin and pradofloxacin
need to be drawn with caution as it is well known that decreased susceptibility does not directly and proportionally translate into clinical resistance. Further intensive research into this complex and multifactorial issue is warranted until robust conclusions and predictions are possible.

The determination of MICs also plays an important role in the research and development of novel antimicrobial drugs. MICs are needed for early profiling and selection of promising candidates, to establish the antimicrobial spectrum of activity and also play a crucial role in the prediction of resistance robustness of a compound, e.g., by means of conducting multi-generation resistance development studies.

MIC values are also indispensable in PK/PD modeling for prediction of an effective clinical dose before clinical studies are started in animals. In the later clinical development susceptibility testing is utilized for determination of the baseline susceptibility of bacterial populations before a product is marketed and for detection of rapid resistance development under normal practice conditions in clinical field studies in naturally diseased patients. Only if all the data indicate high clinical and bacteriological cure rates along with a low risk for resistance selection, a novel antimicrobial will make it to the market.

Whenever a new antimicrobial compound is included into CLSI standards, several laboratories need to provide MIC values for the relevant quality control strains so that the quality control ranges for future susceptibility testing can be established. Also for setting a clinical breakpoint MIC values are a central element used for establishing susceptibility distributions, epidemiological and PK/PD cut-off values, correlation between MICs and clinical and bacteriological cure and the correlation between disk diffusion zone diameters and MICs in scattergrams.

**WHEN SHOULD SUSCEPTIBILITY TESTING BE USED IN ANTIMICROBIAL THERAPY OF DOGS AND CATS?**

The answer to this question is: whenever possible. Ideally, a susceptibility test for the causative pathogen should always be conducted before antimicrobial treatment is initiated. However, this will not be possible in severe infections that are life-threatening or may result in lasting harm to the animal. In such cases, the susceptibility test should be performed in parallel to treatment initiation in order to confirm the susceptibility of the causative pathogen to the chosen antimicrobial or to be able to switch to a more appropriate alternative drug. In this context, a treatment guidance should be developed listing all bacterial infections for which the degree of severity or the course of infection allows to wait for the result of the antibiogram before treatment initiation.

Examples that come to mind are pyoderma, wound infections without systemic signs, uncomplicated acute and chronic UTI, feline upper respiratory tract infections without systemic depression. If antimicrobial susceptibility testing is used for all infections, either before or at least in parallel to the start of antimicrobial treatment, it will be an important element of responsible use of antimicrobials and contribute to safeguarding the long-term utility of these vital therapeutics also within the scope of the One Health Concept.
REFERENCES


ABSTRACT
Pathogenic bacteria cause infectious diseases for which antimicrobial therapy is usually warranted. When bacteria enter normally sterile sites (blood, urine, lung), bacterial replication, endotoxin (Gram-negative organisms) and the liberation of virulence factors such as exotoxins contribute to symptomatic disease. Inflammatory reactions and activated macrophages also contribute to symptomatic infections.

Antimicrobial agents selectively inhibit bacterial pathogens by interfering with essential or critical pathways/functions necessary for bacterial replication/survival. Antimicrobial agents can be divided into those considered bactericidal and those bacteriostatic. Bactericidal agents kill bacteria whereas bacteriostatic agents may reversibly inhibit a bacterial function and through inhibition prevent bacterial replication and allow the body’s immune system to ultimately kill and eliminate bacteria from sterile spaces. Considerable debate over the use of cidal versus static agents in various clinical conditions and diseases severity has been ongoing for years. In vitro kill data clearly show differences in the rate and extent of bacterial killing between cidal versus static antimicrobial agents and such observations are likely important clinically. Recently statistically significant differences were shown between the kill rate and extent of bacterial killing for cidal versus static agents.

Killing bacteria (bacteriological cure) is relevant to clinical cure. One study investigating bacteriological failure in children with acute otitis media focused on those that were culture-positive before treatment. By day 4–5 of antibiotic therapy, 46% of children remained culture-positive and 66% showed bacterial eradication. Of the children culture-positive at day 4–5, 37% showed clinical failure by day 10 compared to 13% that showed bacterial eradication by days 4–5. Some 91% of clinical failures at day 10 were culture-positive on days 4–5 of therapy. In some instances, clinical improvement may be seen, however, in patients where bacteria were not cleared, relapse may occur. Other studies comparing a bacteriostatic agent with a bactericidal agent found that eradication of the target pathogen was achieved in 33% of patients receiving a macrolide (static) as compared to 87% of those receiving a beta-lactam/beta-lactamase inhibitor (cidal) antimicrobial agent.

A dated study from the 1970s examined urinary tract infection in a human general practice population. An
interesting aspect of the study was a question asked specifically to patients regarding the treatment and if it made them feel better. The correlation between a patient responding cured and bacteriological cure was poor and of 134 patients responding “cure”, 74 (55%) had bacteriological cure and 60 (44%) had bacteriological failure. The explanation is likely temporary symptom relief. Patients who responded “failure” had a 91% (21/23) bacteriological failure and the answer to the question was likely influenced by persisting symptoms.

In human patients, 1-day short course therapy for urinary tract infection (UTI) is associated with a higher likelihood of relapse than are longer durations of therapy – most likely due to failure to eradicate the organism. Spontaneous clinical cure was found to occur in 25 (42%) of untreated women or women treated with a drug without activity against the infecting pathogen for UTI. Untreated UTI is associated with prolonged symptoms and the risk for disease progression. In one Scandinavian study, use of varying dosages of a beta-lactam antimicrobial for treatment of UTI was statistically significantly better than placebo. Of 96–99% of patients free for symptoms at follow-up, they were also free of bacteria; for patients with symptoms, 10–30% had persisting bacteria. At study follow-up, 93–98% of antimicrobial-treated patients were also bacteriologically cured, whereas 25–48% of patients with symptoms also had persisting bacteria.

Bacteriological cure is a prerequisite of clinical cure. Clinical cure in the absence of bacteria eradication may lead to persistence or relapse of infection.

REFERENCES
References available upon request.
ABSTRACT
Antibiotics, like all veterinary medicinal products, are regulated on the basis of safety, efficacy and quality. Regulatory guidelines are in place and updated as necessary, to ensure that the approval of licensed products is consistent with current scientific understanding; this is especially true for antimicrobials where antibiotic resistance presents an ongoing challenge in terms of safety and efficacy and resultant public health implications.

Whilst the increase in prevalence of resistance genes and reports of novel resistance mechanisms continue apace, there is a need to step back and not only reflect on the public health consequences of the use of antibiotics in veterinary medicine, but also ask whether our present regulatory guidance is fit for purpose. This presentation argues that it is and will consider a way forward consistent with existing protocols, so we can protect our existing antibiotics and encourage sponsors to move forward with confidence in the development of novel treatment options. To do so, we must operate under the auspices of existing guidance; treatment must be based on established diagnosis and choice of antibiotics must be dictated by approved claims and treatment regimens. Within these constraints, veterinarians must have the freedom to choose the most appropriate drug for therapy; to do so, they will need access to clinical breakpoints commensurate with label claims in order to be able to adequately interpret susceptibility testing data and be set free from political ideologies.
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Dr. Silley has extensive experience of regulatory systems with regard to microbiological requirements for successful registration of antimicrobial compounds and feed additives. Working in Europe and the USA as well as Japan, Australia, Canada, and Brazil gives him a valuable insight into how to meet respective worldwide regulatory requirements. With significant experience of public health and infectious disease, the antimicrobial resistance issues and involvement in risk analysis Dr. Silley is able to provide expertise on the right approach to address safety and efficacy issues in today’s onerous regulatory climate.
INTRODUCTION
Pradofloxacin is an advanced-generation fluoroquinolone antibacterial agent developed specifically for and approved for use in veterinary medicine in companion animals. Pradofloxacin shows increased potency (lower minimum inhibitory concentration [MIC] values) against clinically important Gram-positive pathogens than does other fluoroquinolone agents. Against susceptible strains of Gram-negative pathogens, pradofloxacin in vitro activity is similar or equivalent to other fluoroquinolones. By mutant prevention concentration (MPC) testing, pradofloxacin had the lowest MPC values against canine strains of Escherichia coli and Staphylococcus pseudintermedius than did other quinolones. Pradofloxacin has a favourable pharmacokinetic (PK) and pharmacodynamic (PD) profile with a mean maximum serum drug concentration in dogs of 1.5 µg/ml (based on 3 mg/kg) and is widely distributed to various tissues – in some instances with drug concentrations in excess of serum concentrations. Pradofloxacin is a bactericidal drug. In clinical trials, pradofloxacin was found to be clinically equivalent to comparator drugs but had superior microbiology cure rates.

Drug distribution to various compartments/tissues allows for consideration of drug inhibiting/killing target pathogens at those specific anatomical locations. Renally excreted drug has higher urine drug concentrations than those in serum or other compartments. Unfortunately, antimicrobial concentrations achievable and/or sustainable in many compartments (normal or inflamed) are unknown for many drugs depending on the anatomical location. As such, determining optimal versus adequate versus inadequate therapy is challenging. This has become especially critical in today’s environment where suboptimal or inadequate therapy is thought to precipitate antimicrobial resistance.

The traditional in vitro measurement of susceptibility or resistance is based on measuring the minimum inhibitory concentration (MIC) – a standardized assay using 105 colony forming units (CFU)/ml of bacteria. The lowest drug concentration blocking growth is the MIC. MIC measurements are useful, however, the results may be misleading when higher densities of bacteria are present during infection. The mutant prevention concentration (MPC) was described in 1999 and defines the antimicrobial drug concentration threshold blocking the growth of the least susceptible bacterial cells present in higher density bacterial populations. In essence, MPC is the drug concentration blocking growth of resistant subpop-
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He has published in excess of 160 peer-reviewed manuscripts, more than 240 abstracts at international meetings and 5 books. Dr. Blondeau has given more than 600 invited presentations around the world. Dr. Blondeau is a reviewer for many prestigious journals. He serves as section editor for anti-infective agents for the journal Expert Opinion on Investigational Drugs. He is also an associate editor for the journal Expert Reviews in Clinical Pharmacology. Dr. Blondeau was the University of Saskatchewan’s nominee for the Henry Friesen Award and Lecture, an award given by the Royal College of Physicians and Surgeons of Canada and the Canadian Society for Clinical Investigation. Dr. Blondeau was twice nominated for a University of Saskatchewan student union teacher of the year award.

From in vitro measurements with pradofloxacin against key veterinary pathogens, MPC values were found to be low for S. pseudintermedius strains and E. coli strains at 0.125 µg/ml. These values are well below the reported drug concentration values for pradofloxacin in skin (4.5 µg/ml), urine (3.4 µg/ml) or serum (1.2–1.5 µg/ml). When considering PK/PD parameters, Cmax/MIC and AUC/MIC ratios are very favourable for clinical cure and bacteriological cure. Such values suggest a low propensity for pradofloxacin to select for drug-resistant subpopulations given that the skin drug concentrations exceed the MPC values by 36-fold and in urine by 27-fold.

From recently published/presented studies, pradofloxacin was found to rapidly kill bacteria when canine isolates of S. pseudintermedius and E. coli were exposed to drug. Comparator agents were cefazolin, cefovecin and doxycycline. Kill experiments were run as either 3-hour or 24-hour experiments. Kill experiments were conducted using clinically relevant drug concentrations: MIC, MPC, maximum serum and maximum tissue drug concentrations. Bacterial densities tested ranged from $10^5$ (100,000) colony forming units (CFU)/ml to $10^9$ (1 billion) CFU/ml. This range of bacterial densities covers low and high bacterial burdens that may be seen during infection.

From recently published investigations (Veterinary Dermatology), statistically significant differences were seen in favor of pradofloxacin versus the comparator agents for killing of the bacterial strains tested as well as the speed in which the organisms were killed and over the range of the bacterial densities tested. For example, at the maximum tissue drug concentration killing of S. pseudintermedius strains by pradofloxacin was statistically superior (p value – 0.5–0.001) to comparator drugs (cefovecin, cefazolin, doxycycline) following 10 minutes of drug exposure. Statistically significant differences were seen for pradofloxacin versus comparators at multiple time points, varying drug concentrations and over a range of bacterial densities tested.

In vitro data show pradofloxacin to be bactericidal and rapidly kills key companion animal pathogens. Such observations impact bacteriological cure and clinical cure and reduce the likelihood for relapse and resistance selection.
REFERENCES


ABSTRACT
Deep pyoderma has long been recognised as a debilitating and potentially serious disease in dogs. Treatment is often lengthy, challenging for veterinary surgeons and can be frustrating and costly for owners. More recently, the growing threat to public health from increasing antimicrobial resistance in bacterial pathogens has added another dimension to treatment considerations. A renewed focus on responsible use of antimicrobials is required in order to maximise efficacy of our currently available valuable drugs. Correct diagnosis, including early recognition and confirmation of bacterial involvement is critical to make appropriate initial treatment choices. Identification of underlying and concurrent diseases is vital to support resolution of infection and to prevent relapses. Aspects of antimicrobial drug selection, the urgent need to reduce empirical drug selection, dosing, decisions on treatment duration, progress monitoring and owner education will be discussed, as all contribute to treatment success and responsible use of antimicrobials.

BACKGROUND
Bacterial skin infections are one of the most common skin conditions seen in small animal practice. Pyoderma is categorised histologically into surface, superficial and deep infections with each category associated with several clinically distinct entities. Deep pyoderma is a serious, debilitating infection extending into the dermis and possibly subcutis, and can be acute or chronic and may lead to systemic disease through haematogenous spread. It may occur less frequently than surface and superficial pyoderma, but deep infections are serious for the patient, challenging to treat for veterinarians and costlier for owners.

Deep pyoderma always occurs secondary to a primary cause and successful management of the patient mandates antibacterial therapy and in parallel, identification and correction of the underlying cause. Follicular damage (e.g., demodicosis!) and reduced immune surveillance (e.g., hyperadrenocorticism) are likely the most relevant underlying triggers, but trauma (including, e.g., wrong clipping) and virtually any skin disease, including allergy, can predispose to deep infection.

Infection is thought to arise from deep infection in hair follicles. These ruptures are leading to furunculosis with more discrete suppurative and eventually pyogranulomatous inflammation around remnants of follicle or hair shafts (concurrent foreign body reaction to keratins). If destruction of the hair follicle is permanent, it will heal with scarring. Cellulitis
arises from confluent lesions extending through tissue planes and into the panniculus. **Cutaneous signs** seen with deep pyoderma include some of the lesions occurring in superficial pyoderma such as papules and pustules, but hallmark lesions such as haemorrhagic crusts, haemorrhagic bullae, pus from draining sinus tracts, bloody exudate will indicate involvement of dermal tissue. Nodules, plaques, ulcers, surface necrosis, more diffuse swelling and pain also occur and may be associated by regional or generalised lymphadenopathy.

The terminology for different clinical presentations of deep pyoderma is extensive. Some clinical types reflect the location as in muzzle, pedal, callus deep pyoderma (or “folliculitis and furunculosis”) and these may be associated with certain breed predispositions (e.g., callus pyoderma in larger breeds, muzzle pyoderma in short-coated breeds). Another distinct clinical type is pyotraumatic folliculitis and furunculosis. Superficial ulceration with substantial inflammation in the cheek and neck region, most often seen in golden and labrador retrievers, St. Bernards and Newfoundlands, closely resembles acute moist dermatitis (“hot spots”, pyotraumatic dermatitis). It can be differentiated as deep pyoderma through recognition of satellite lesions (papules, pustules) surrounding the central larger erosion/ulcer and indicates haematogenous spread. Acral lick dermatitis in most cases represents a deep pyoderma although the primary aetiology of the disease remains unclear. German shepherd pyoderma is well established in dermatology texts as a distinct entity of deep pyoderma that is severe and slow to resolve with a familial and immunologically mediated background. However, in the author’s experience, this disease seems to have disappeared over the past fifteen years. Nasal deep pyoderma is described mostly in German shepherd dogs and bull terriers. In all breeds though, nasal lesions can present as acute and cytologically/histologically eosinophilic and often represent an aetiology of hypersensitivity as in a suspected arthropod hypersensitivity of ‘eosinophilic furunculosis of the face’ rather than deep pyoderma.

Deep pyoderma may resemble its differential diagnoses or concurrent diseases very closely. Demodicosis and calcinosis cutis in particular may easily be misdiagnosed as deep pyoderma on appearance but will often concur. Diagnosis needs to include confirmation of bacterial involvement by cytology (or histopathology) but should always include hair plucks/skin scrapes for Demodex mites and further diagnostic tests as appropriate to investigate differential diagnoses such as sterile granulomatous disease (e.g., reactive histiocytosis) and neoplasia.
or concurrent problems such as other infectious disease, endocrinopathies or immune-mediated disease (e.g., vasculitis).

*Staphylococcus pseudintermedius* remains the major pathogen as for other pyodermas but only accounts for 60–80% of deep pyoderma pathogens rather than for the >92% as in superficial pyoderma. Infections due to Gram-negative organisms (e.g., *Escherichia coli*, *Pseudomonas* spp.), anaerobes or mixed bacterial populations are more common and identification of pathogens and their susceptibility patterns requires laboratory testing. If bullae, pustules or draining tracts are present, these can be sampled for bacterial culture and susceptibility testing. Biopsy and submission for tissue culture (posted in plain containers, not in formalin) is recommended in order to grow representative pathogens from drier or very deep lesions. Culture and particularly tissue culture (often combined with histopathology and special stains to obtain a diagnosis) will also be essential to guide treatment in cases of anaerobic cellulitis, deeper abscesses that are not amenable to lancing and topical therapy alone, bacterial pseudomycetoma (granulomatous inflammation around a bacterial centre), actinomycosis, and mycobacterial skin disease (public health implications!).

**TREATMENT**

**General comments**

Management of deep pyoderma requires a two-fold approach: The infectious component needs to be resolved with antibacterial therapy while the underlying triggering cause needs to be corrected to support resolution but most importantly to prevent relapse.

**Owner education** is important for successful management of deep pyoderma as a better understanding will improve compliance. Since treatment is often lengthy and likely to require prolonged co-operation and funding from owners, the severity of the disease, treatment approach, requirement for long courses of antibiotics and the need to identify and correct triggering causes in parallel need to be explained to owners carefully. Treating the bacterial infection with antibiotics is often the more straightforward part to explain while the importance of searching for underlying causes may seem less pressing to owners. The **prognosis** for deep pyoderma varies and depends on susceptibility of pathogens involved, location and severity of infection and importantly on underlying or contributing diseases.

For deep pyoderma, after bacterial involvement has been confirmed, ideally by cytology (cocci and/or rods seen within pyogranulomatous inflammation), **systemic antibacterial treatment** is always indicated. However, in view of the urgent need for responsible use of antimicrobials, prescriptions need to be preceded by careful consideration. Speculative use, empirical drug selection or medication based on the “need to do something” are no longer acceptable. Antibiosis will kill or stop the growth of susceptible bacterial pathogens at infection sites but will not always eliminate all pathogens, particularly where chronic changes have led to fibrosis and scarring. To maximise the effect of antimicrobial agents and hopefully speed up clinical cure, antibacterial treatment needs to be supported by measures aimed at reversing skin pathology and at strengthening the patient’s immune defences by correcting accompanying problems. Topical antibacterial therapy, particularly chlorhexidine or povidone iodine-containing soaks twice daily, are advocated by some dermatologists as an adjunct for systemic treatment. However, pain may preclude such measures, at least initially. Efficacy of systemic antibacterial therapy depends predominantly on susceptibility of the organism but will also be determined by correct drug administration including accurate dosing, owner
compliance and clinical variables such as severity of disease and causative and concurrent diseases.

Drug selection
High-quality evidence on efficacy of individual drugs for deep pyoderma is sparse.\(^2\) In contrast to superficial pyoderma where empirical selection of drug may still be appropriate under certain conditions,\(^3\) drugs should always be chosen based on bacterial culture and antimicrobial susceptibility testing for deep pyoderma. This is important for several reasons: i) Involved pathogens are less predictable in deep infections compared to superficial pyoderma and will more frequently involve Gram-negative bacteria with a tendency for multidrug resistance. ii) Deep pyoderma may involve systemic disease including sepsicaemia, so that escalating antimicrobial treatment may become necessary. iii) Cost of laboratory testing will be off-set by the cost for potentially the wrong medication prescribed empirically as treatment duration and time to expected first clinical improvement (typically two weeks) are longer in deep pyoderma. iii) Most broad-spectrum antimicrobials that were licensed for use in dogs more recently require culture and susceptibility testing prior to use as per data sheet. Care is needed when choosing a diagnostic laboratory for bacterial culture and susceptibility testing nowadays. Substantial progress has been made during the past ten years with regard to accuracy of bacterial species identification. Furthermore, clinical breakpoints specific for animal pathogens and veterinary drugs are now widely available and should be used to support correct prescribing and improve prognoses.

While awaiting culture and susceptibility test results, antibiosis may be commenced based on cytology findings if clinical signs warrant immediate treatment. If cocci are seen on cytology, first-tier drugs or β-lactam antibiotics such as cephalaxin or amoxicillin-clavulanate would be appropriate choices based on the assumption that cocci are very likely *S. pseudintermedius* which should be susceptible to this class of antimicrobials, at least where there is a low risk of meticillin-resistant staphylococcal infections (MRSP). Identification of rods (typically in large numbers) amongst neutrophils and macrophages indicates the involvement of Gram-negative pathogens which are more likely to show multidrug resistance but may still be susceptible to fluoroquinolones. In those cases, a 5-to-7-day course of fluoroquinolone based on cytology may be appropriate. Once the laboratory report is available, more of the same drug can be dispensed or the drug choice can then be escalated or de-escalated.

Amongst antimicrobials for which *in vitro* testing has shown susceptibility of a particular isolate, the choice will be based on general considerations (bactericidal preferred, narrow spectrum vs. broad spectrum), clinical characteristics (accumulation in skin, penetration to inflamed areas, activity in pus, safety, previous adverse reactions), practicalities (dosing frequency) and cost as for other drugs. Tier systems or categories of first-, second- and third-line antibiotics are designed to reduce selective pressure on bacteria through responsible use of antibacterial drugs while allowing a degree of choice for clinicians. However, allocation of individual drugs to tiers varies depending on target disease and author group. There seems to be broad consensus on the purpose of categories though: Tier-one (first-line) antibiotics are those suitable for empirical therapy of strongly suspected pathogens, i.e. staphylococci in most skin infections. First-tier drugs include amoxicillin, ampicillin, the first-generation cephalosporins and amoxicillin-clavulanic acid, lincosamides, tetracycline, oxytetracycline and potentiated sulfonamides. Second-tier (second-line) antimicrobials should be used when first-tier drugs are not
appropriate and only after bacterial culture indicate susceptibility. Third-tier drugs are important for the treatment of some multidrug-resistant pathogens in human and animal medicine. In veterinary medicine, they should either only be used on a ‘restricted-use policy’, including proven in vitro susceptibility, and in collaboration with an infection control specialist so that the risk of spread of multidrug-resistant and often zoonotic bacteria. Or they should not be used at all for ethical reasons and because other alternatives are usually available. Drug allocation varies between tiers two and three even amongst pyoderma guidelines.\textsuperscript{3, 4} While fluoroquinolones are in the second tier in both recommendations, other, not widely authorised drugs, such as chloramphenicol, rifampicin and amikacin are either in the second tier\textsuperscript{3} or third tier.\textsuperscript{4} These latter drugs are rarely included in routine susceptibility testing and extended resistance testing may need to be requested from the laboratory. The use of third-line drugs such as rifampicin, amikacin or chloramphenicol in canine pyoderma has been described\textsuperscript{5, 6} but is best avoided due to their unlicensed status in most countries and increased risk of toxicity. Drugs that are always in the third tier due to their importance to human health and for which veterinary use is strongly discouraged include the glycopeptides (vancomycin, teicoplanin, telavancin) and linezolid and any new antimicrobials that have recently been introduced to human medicine such as some anti-MRSA cephalosporins. There was no consensus over the classification of cefovecin (first- or second-tier) within the ISCAID group,\textsuperscript{3} but it is included as second-tier antimicrobial by Beco et al., and as a third-tier antimicrobial at this author’s hospital policy.

Since involvement of Gram-negative bacteria and other non-staphylococci is seen more frequently in deep pyoderma (30–40 %), broad-spectrum second-tier drugs will be needed not infrequently. In the author’s clinic, fluoroquinolones are most often used when Gram-negative organisms complicate deep pyoderma. Several fluoroquinolones have become available for use in dogs since the 1980s in different countries (enrofloxacin, marbofloxacin, orbifloxacin, pradofloxacin) and studies have shown good efficacy for skin infections, including deep pyoderma, high bioavailability, good penetration to inflamed skin sites and a good safety profile in adult dogs. With fluoroquinolones being critically important drugs for human medicine (World Health Organisation), responsible use in small animal practice is essential to preserve their efficacy for people and pets. Pradofloxacin, as the most recently authorised veterinary fluoroquinolone, has been shown to kill \textit{Staphylococcus pseudintermedius} more rapidly than cefazolin, cefovecin and doxycycline in vitro\textsuperscript{7} and good efficacy was demonstrated in clinical cases of canine deep pyoderma.\textsuperscript{8} Furthermore, it may have the advantage of minimising the risk of selection for fluoroquinolone resistance due to its low mutant prevention concentration shown in \textit{E. coli} and \textit{Staphylococcus aureus} in vitro and it is currently the author’s preferred fluoroquinolone choice for dogs if in vitro susceptibility is shown. If meticillin-resistant staphylococci are isolated, second- or third-tier drugs might be indicated if systemic treatment is needed. For meticillin-resistant staphylococcal infections (MRSA and MRSP), including deep pyoderma, clinical consensus guidelines will soon be available (including MRSA and MRSP) through the World Association of Veterinary Dermatology (WAVD).

**Duration**

Recommendations on treatment duration for deep pyoderma have become highly controversial. In general, treatment of infection should continue until clinical and bacteriological cure has been achieved. However, bacteriological cure is difficult to ascertain in pyoderma (superficial and deep) as pathogens
tend to be opportunistic pathogens and can be sampled even from healthy skin. In addition, tissue culture after skin biopsies would be required to evaluate bacteriological cure for deep pyoderma cases. This approach would be challenging due to ethical and financial implications and difficult to justify due to a lack of supporting studies. At present, treatment has typically been recommended for at least 4 to 6 weeks and two weeks beyond clinical resolution. However, the latest trend in human medicine is that unnecessary treatment should be avoided after clinical resolution of signs and duration of antibiosis is generally shorter now in human medicine compared with veterinary medicine, particularly with veterinary dermatology. The rationale behind longer courses for deep pyoderma is to avoid sequestered foci of infection causing relapse but good evidence is lacking. In the absence of such data, clinicians should adhere to current recommendations but where the clinical course supports earlier discontinuation frequent monitoring after discontinuation would be advisable. It has been suggested that a relapse soon after stopping antibiotics is most likely due to inappropriately short treatment while later relapses would indicate an underlying cause that needs to be further investigated.

**Dosing and compliance**

General concepts of dosing for skin disease apply for deep pyoderma. Dosing should be at the higher end of the recommended dose range due to a limited cardiac output reaching the skin and to overcome potentially penetration-impeding effects of pyogranulomatous inflammation to some antimicrobials. Maintaining regular administration intervals is particularly important for time-dependent antimicrobials such as the β-lactams. Their clinical efficacy is affected if $T > \text{MIC}$ less than 40–50 % of the dosing interval. In general, the dosing window for antimicrobials used in dogs is much narrower than that in people.

In humans, dose recommendations tend to refer to a 70 kg adult according to WHO data, which leads to a wide range of dosing. While this discrepancy is striking, veterinarians should still weigh dogs prior to prescribing of antimicrobials for accurate dosing. Underdosing has been shown to occur commonly in first opinion practice and promotes resistance and risks reduced efficacy. Although adverse effects from antimicrobials are relatively frequent, they tend to be mild, but overdosing may increase the risk of adverse effects (and will occur unnecessary expense). With compliance being a major contributing factor to successful treatment, once daily dosing is likely to increase beneficial outcome. Cost may also be a limiting factor in the treatment of deep pyoderma, particularly where large-breed dogs may require long courses of broad-spectrum antimicrobials. Cheaper options, such as for example trimethoprim-sulfonamide, may be suitable based on sensitivity testing and availability in some countries, but contraindication and risks need to be evaluated carefully.

**Management of primary causes**

Treatment of underlying causes should always be initiated as soon as possible to support resolution of infection. For acaridal therapy, care may be required with topical products such as amitraz if ulcerations due to pyoderma are extensive. Although a recent study showed that systemic antimicrobials did not shorten the time to negative skin scrapes in dogs with generalised demodicosis, antibacterial treatment alone without stopping proliferation of mites in follicles is ineffective. Since some cases of deep pyoderma are due to allergic skin disease, rigorous flea control, elimination diet trials and anti-inflammatory treatment may be indicated. While glucocorticoids may be required to control allergic skin disease eventually, and even to prevent relapses of deep pyoderma ultimately, these should be withheld until pyoderma has resolved.
CONCLUSIONS
Deep pyoderma remains a challenging disease, but several effective and safe antimicrobial drugs are licensed for use in dogs. In order to preserve their efficacy for as long as possible, responsible use is indicated, more than ever before. Careful attention to early and correct diagnosis, including the use of cytology to confirm bacterial involvement, susceptibility-based drug choice without exception, and monitoring of progress are vital in the treatment of the infectious component. However, owner education to improve compliance and to raise awareness of the wider implications of antimicrobial therapy will support responsible use. And most importantly, identification and correction of the disease or condition that caused the deep pyoderma in the first place are paramount to successful resolution of infection and prevention of relapses.

REFERENCES

FURTHER READINGS

17 World Association of Veterinary Dermatology (WAVD) http://www.wavd.org/papers-for-review.html.
INTRODUCTION, EPIDEMIOLOGY, ETIOLOGY AND PATHOGENESIS

Bacterial urinary tract infections (UTIs) occur commonly in dogs, and it has been suggested that as many as 14% of all dogs develop at least one UTI during their lifetime. As many as 10% of hospitalized dogs with a variety of diseases have UTI without clinical signs. Female dogs have been reported to be at increased risk for a UTI, mainly due to anatomic difference (short urethra, recessed vulva) and lack of protective secretions from the prostate gland. Older dogs are predisposed to infections and the mean age of patients at diagnosis is 7 years old. Obesity has been associated to asymptomatic bacteriuria. Urinary tract infections induce inflammation by bacterial invasion of any part of the urinary system. Infections of the urinary tract are usually classified into two general anatomic categories: lower urinary tract infection (cystitis, urethritis and prostatitis) and upper urinary tract infection (pyelonephritis, renal abscess). Cystitis and urethritis are often mucosal infections, while prostatitis, pyelonephritis and renal abscess signify tissue invasion and usually require prolonged antimicrobial treatment.

Most UTIs are caused by bacteria emanating from the gastrointestinal tract crossing the perineum and colonizing the external genitalia prior to retrograde invasion of the urethra and bladder against the flow of urine. Gastrointestinal microbiota may also have a role in urinary homeostasis, influencing and regulating mucosal immunity.

Rarely, hematogenous spread of bacteria to kidney from cardiac septic embolism due to bacterial endocarditis may induce renal infection and subsequent UTI.

The status of host defense mechanisms appears to be important in the pathogenesis of UTI. The latter develops when there is a disturbance of anatomical integrity (ectopic ureters, ureterocele, urachal remnant, recessed vulva, fistula, stricture, urolithiasis, neoplasia, catheters or urethrostomy), functional integrity (bladder atony, incontinence, spinal cord lesions, vesicoureteral reflux), metabolic conditions (diabetes mellitus, hyperadrenocorticism, diluted urine due to renal failure) or immunological factors (immunosuppression, abnormal mucosal defense) that normally prevent microbial invasion of the urinary tract. Virulence factors (fimbria with adhesins that interact with the uroepithelial receptors, toxins) must also be present in the microbiological population, in order to achieve the capacity to colonize the urinary mucosa.
Uncomplicated UTIs occur in healthy dogs with no evidence of underlying disorders. Complicated UTIs occur in the presence of urinary tract structural or functional abnormality or systemic disorders such as immunosuppression or endocrine diseases. Recurrent UTIs imply three or more episodes of UTIs per year. Recurrent UTIs can be classified as relapsing, reinfection, refractory. A relapsing UTI means isolation of the same microorganism more than once with apparent clearance of the infection during antimicrobial treatment. This event suggests that bacteria may be deep-seated in tissues such as prostate, kidney, bladder submucosa, neoplasia or inside a stone, or the achieved concentration of the antimicrobial drug is suboptimal or the microorganism is not susceptible to the antibiotic.

Reinfection means isolation of a different microorganism within six months of apparent resolution of a previous infection. A refractory UTI occurs when the microorganism has not been eradicated even during administration of the appropriate antibiotic. Superinfection means the isolation of a new microorganism during antimicrobial treatment. Asymptomatic bacteriuria is a term to describe a positive bacterial culture without clinical signs or symptoms or cytological evidence of infection (pyuria or hematuria).

A single organism is isolated in 75% cases, two organisms are isolated in 20%, and three organisms are isolated in 5% of UTIs. Polymicrobial infections are more common in female dogs.

As many as 90% of male dogs with UTI have concomitant prostatic colonization.

Escherichia coli is the most common uropathogen in dogs (approximately 50% of all isolates), followed by Gram-positive cocci, and Proteus, Klebsiella, Pasteurella, Pseudomonas and Corynebacterium. The latter Corynebacterium urealyticum is associated with a unique form of relapsing UTI characterized by encrustations of urinary tissue with struvite and calcium phosphate. This kind of UTI called “encrusting cystitis” (Figure 1) cannot be cured with medical treatment alone, requiring debridement (endoscopic or surgical), submucosal resection or partial cystectomy.

The consequences of UTI are variable. In many cases, infection will be transient, signs will be minimal, and the condition responds readily to treatment. However, potential complications of persistent UTI include formation of struvite uroliths, polypoid cystitis (commonly caused by Proteus), emphysematous cystitis (commonly caused by E. coli or Clostridium,}

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especially in diabetic patients), chronic prostatitis, prostatic abscess, discospondylitis, pyelonephritis, renal failure, persistent or recurrent bacteremia. Furthermore, the emergence of antibiotic resistance in the causative agents (e.g., ESBLs extended-spectrum beta-lactamases *Escherichia coli*) could have an important zoonotic potential. For these reasons, a susceptibility test should be done in every case of UTI, instead of adopting an empirical drug selection.

Consequently, the microbiologic cure (negative urine culture or isolation of less than 1.000 cfu/ml of bacteria) instead of the clinical cure (relief of clinical signs or symptoms) must define our goal of treatment. For uncomplicated UTIs, the rate of microbiologic cure is approximately 75–80 %, whereas the rate of clinical cure can reach 85–90 %.

**CLINICAL SIGNS**
Symptomatic lower UTIs are associated with clinical signs that are similar to other urologic diseases like urolithiasis and neoplasia. Pollakiuria, dysuria, stranguria, hematuria, and inappropriate urination are the more common clinical presentations. In case of lower UTIs fever and leucocytosis are usually absent. Upper UTIs may result in systemic signs such as fever and leukocytosis, renal pain or kidney enlargement. However, the lack of systemic signs is insufficient to rule out upper UTI. Sometimes clinical or imaging distinction between lower UTI and upper UTI may be very difficult without performing a urine culture obtained from the renal pelvis by pyelocentesis. Prostatitis is frequently associated with fever, leucocytosis and lower urinary tract signs and sometimes prostatic pain can be confused with a locomotor abnormality because of a stiff gait. Physical examination of the prostate gland by rectal palpation is a fundamental step to assess prostatic diseases. In case of pyometra it’s not uncommon to observe a concomitant UTI.

**DIAGNOSIS**
Complete urinalysis and urine culture of a sample collected by cystocentesis are the most definitive means of diagnosing bacterial UTI. Presence of pyuria is important because other causes of lower urinary tract disease (stones and neoplasia) are associated with minimal pyuria. WBC (white blood cells) casts usually suggest pyelonephritis, but their absence cannot exclude the diagnosis. Identification of bacteria on urine sediment examination is helpful but not reliable and positive urine culture by cysto-
centesis is the gold standard for the diagnosis of UTI.

Diagnostic imaging (radiography, ultrasonography, endoscopy and CT scan) may reveal significant underlying anatomic abnormalities (obstruction, stones, strictures, ectopic ureters) and can help to define the origin of UTI such as prostatic abscess, pyelecstasy due to pyelonephritis or renal abscess.

SUSCEPTIBILITY TESTING AND THE MIC
The most common technique used for determining antimicrobial susceptibility is the Kirby-Bauer agar diffusion test.

The Kirby-Bauer test uses discs that contain concentrations of the antibiotics comparable to what can be achieved in human serum, assuming similarities between pharmacokinetics of different species. However, the concentration of the antimicrobial is likely to be much higher in urine than in serum. Consequently, in this kind of diagnostic test the microorganism may be reported as resistant to an antibiotic when in reality it is susceptible at concentrations of the drug that can usually be achieved in urine. The “90/60 rule” implies that approximately 90% of infections treated based on this test are likely to respond if the microorganism is susceptible to that antimicrobial, but up to 60% will respond even if a “resistant” drug is selected.

A more sensitive and specific test is to determine the minimum inhibitory concentration (MIC). The MIC is the lowest concentration of antibiotic required to inhibit bacterial growth. It is performed using a series of dilutions of each antibiotic to which a standard number of bacteria are added, or using strips containing varying concentrations of the antimicrobial on the surface of agar plates (the so-called Etest).

The MIC test is more reliable than Kirby-Bauer test, but it is more expensive.

Susceptibility data also does not take into account active metabolites of antimicrobials. For example, enrofloxacin is metabolized to ciprofloxacin and in this way bioactivity or the antimicrobial may increase up to 50% or more, underestimating the real efficacy of the antibiotic. Interestingly, oral ciprofloxacin is not recommended in dogs because of its low bioavailability.

Therefore, once susceptibility testing has been carried out, the results need to be carefully evaluated by the veterinarian and translated into effective therapeutic protocol.

Traditionally, if an antimicrobial reaches a mean urinary concentration (MUC) of at least 4 times the in vitro MIC, treatment with that drug has a high chance of therapeutic success.

THE MUTANT PREVENTION CONCENTRATION AND THE MUTANT SELECTION WINDOW (MSW)
Based on natural mutational frequencies, a mutation that leads to a single step in resistance to an antimicrobial can be observed in microbial populations whose density is at least $10^9$ cfu/ml.

Because MIC and Kirby-Bauer test use bacteria inocula of about $10^8$ cfu/ml, “first step” mutants microorganisms are not likely to be detected. Therefore, simply achieving the MIC of the cultured microorganism will inhibit the growth of the susceptible bacteria but not the first-step mutants.

The MPC (mutant prevention concentration) test is based on elevated numbers of bacteria (at least $10^9$ cfu/ml) and the lowest antimicrobial concentra-
tion preventing growth of all microorganisms (fully susceptible as well as first-step mutants) is the MPC value.

In the range between MPC and MIC values, the Mutant Selection Window (MSW) is delineated, a range of antibiotic concentrations that inhibits only susceptible bacteria but allows expansion of first-step mutants.

The Mutant Selection Window (MSW) delineates three profiles: antimicrobial concentration below MIC, between MIC and MPC and above MPC.

If the antimicrobial concentration doesn’t reach MIC, the patient is not cured but the resistance pattern is not altered. If the antibiotic concentration is between MIC and MPC, killing of susceptible bacteria is observed along with a successful clinical response, but “first-step” mutants are selected and allowed to grow favoring a resistance pattern.

If the antimicrobial concentration is above the MSW, corresponding to the MPC, first-step mutants and susceptible bacteria are inhibited or killed obtaining a more favorable outcome.

Full therapeutic efficacy of antimicrobials (concentration-dependent drugs like fluoroquinolones) is related to the achievement of the highest concentration that reach the MPC value. For time-dependent antimicrobial drugs it is more important to shorten the interval of time of administration. Pradofloxacin, a third-generation fluoroquinolone, outperformed other fluoroquinolones in terms of bactericidal activity and decreased propensity to select “first-step” mutants (low MIC, low MPC).

**TREATMENT**

The working group of the International Society for Companion Animal Infectious Diseases (ISCAID) has published in 2011 the guidelines for treatment of UTIs in dogs and cats and recommends treatment with antimicrobial drugs effective against more than 90% of the urinary isolates.

**UNCOMPROMICATED BACTERIAL UTI**

Before results of urine culture and sensitivity testing the patient should receive amoxicillin (11–15 mg/kg PO q 8h), cephalexin (12–25 mg/kg PO q 12h) or trimethoprim-sulfamethoxazole (15 mg/kg PO q 12h). Seven to 14 days of an appropriate antibiotic is often recommended. However, treatment duration is based on conventional experience over the years.

Two recent prospective randomized studies evaluated short-duration antimicrobial treatment (3 days) compared to standard antimicrobial treatment (7–10 days), as in human medicine where short duration antimicrobial treatment is recognized as the gold standard. In both studies, microbiologic and clinical cure were comparable between short-duration treatment and standard treatment. HDSD (high dose short duration) enrofloxacin (20 mg/kg PO q 24h for 3 days) treatment was not inferior to a conventional amoxicillin-clavulanate treatment (13.75–25 mg/kg PO q 12h for 14 days). However, furthers studies are needed in order to clarify the best treatment for uncomplicated UTIs in dogs.
COMPLICATED BACTERIAL UTI
Treatment must be guided by urine culture, and antimicrobial drugs are usually administered for 4–6 weeks.

Amoxicillin or trimethoprim-sulfamethoxazole can be prescribed initially, until the results of susceptibility tests are available to guide the selection of the more appropriate antimicrobial.

Pyelonephritis must be treated as a complicated bacterial UTI and a fluoroquinolone is the best choice before having the results of susceptibility tests.

Prostatitis is also considered a complicated UTI, so a prolonged treatment of 6–8 weeks has been suggested. In case of an intact blood-prostate barrier (e.g., chronic prostatitis) basic and lipid soluble antimicrobials (e.g., fluoroquinolones, sulfonamides) are the best option to penetrate prostatic tissue.

CATHETER-ASSOCIATED BACTERIAL UTI
Antimicrobial treatment during catheterization is discouraged because it might promote infection by multi-drug-resistant microorganisms. Bacterial UTI may be minimized by using intermittent catheterization, removing catheters as soon as possible and using a closed collection system.

Urine culture is indicated in case of appearance of lower urinary tract signs, but not in asymptomatic patients. Cystocentesis must be performed in order to obtain urine for bacterial culture. Other means for submitting urine cultures such as catheter tip culture or by catheter suction are not recommended.

ASYMPTOMATIC BACTERIURIA
Treatment is not usually recommended unless the patient is immunocompromised or has renal failure.

MONITORING
In uncomplicated UTIs urine cultures should be submitted after 5 to 7 days after cessation of the antimicrobial treatment. In complicated UTIs urine cultures are performed after one week of treatment, before therapy discontinuation (4–6 weeks) and one week and one month after cessation of treatment.

PREVENTION
Preventive therapy is not sufficiently supported by literature, and at the moment is not recommended.

REFERENCES/SUGGESTED READINGS


There are many causes of bacterial, viral, and fungal upper respiratory infections (URI) in cats. The primary objective of this presentation is to upper attendees in new developments in the treatment of the bacterial causes. The International Society on Companion Animal Infectious Diseases (www.iscaid.org) recently published antimicrobial guidelines for respiratory diseases in dogs and cats and is cited herein.

While the respiratory viruses feline herpesvirus 1 and calicivirus are the most common infectious agents detected in most studies of clinically ill cats, almost all cats with mucopurulent or purulent nasal discharge have a bacterial component to their disease. Primary bacterial disease is thought to occur most commonly with *Bordetella bronchiseptica*, *Mycoplasma* spp., *Chlamydia felis*, *Streptococcus canis*, and *Streptococcus equi* subspp. *zooepidemicus*. Recently it was shown that *Bartonella* spp. are not causes of rhinitis in cats. Both *B. bronchiseptica* and *Mycoplasma* spp. can be associated with bronchitis in cats. Chlamydiosis in general is a mild infection resulting only in conjunctivitis.

If primary bacterial infections are suspected and the disease duration is <10 days, the ISCAID Working Group recommends empirical use of doxycycline at 10 mg/kg, PO, once daily. Doxycycline can also be administered at 5 mg/kg, PO, twice daily. Minocycline pharmacokinetics suggest it can be prescribed at 50 mg per cat daily.1 Cats with acute disease only need to be treated for 7 to 10 days. The alternate drug recommended by the ISCAID Working Group was amoxicillin at 22 mg/kg, PO, twice daily when *Mycoplasma* or *C. felis* were not suspected. Other drugs should be used if needed after a diagnostic workup has been completed.

Most cases of bacterial rhinitis are secondary to other diseases including trauma, neoplasia, inflammation induced by viral infection, foreign bodies, inflammatory polyps, and tooth root abscessation. Thus, if routine antibiotic therapy fails, a diagnostic workup should be performed.

A culture of a nasal flushing or biopsies can then be performed to help guide treatment with other antibiotics if indicated.

Since bacterial rhinitis leads to chondritis and osteomyelitis, antibiotic therapy may need to be continued for weeks in cats with chronic disease. Drugs with an anaerobic spectrum that also penetrate bone and cartilage well are often effective. Clindamycin or
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Dr. Lappin graduated from Oklahoma State University and then completed an internship, internal medicine residency, and PhD program in Parasitology at the University of Georgia. His principal areas of interest are prevention of infectious diseases, the upper respiratory disease complex, infectious causes of fever, infectious causes of diarrhea, and zoonoses. Dr. Lappin is the Kenneth W. Smith Professor in Small Animal Clinical Veterinary Medicine at Colorado State University and he helps direct the shelter medicine program. Dr. Lappin is the director of the “Center for Companion Animal Studies”.

Recent awards include the Norden Distinguished Teaching Award, the European Society of Feline Medicine International Award for Outstanding Contribution to Feline Medicine, the Winn Feline Research Award, the ACVIM Robert W. Kirk Award for Professional Excellence, and the WSAVA Scientific Achievement Award.

amoxicillin-clavulanate are frequently used. Amoxicillin-clavulanate has the advantage of killing most *Bordetella* isolates. Clindamycin has the advantage of being effective against *Mycoplasma* spp. Azythromycin (10 mg/kg, PO, q 24–72 hrs) or fluoroquinolones can be used for cats with chronic disease. For cats that are difficult to treat, cephalosporin injections can be considered. In one study of shelter cats with suspected bacterial URI, the injectable cephalosporin, cefovecin was inferior to doxycycline or amoxicillin-clavulanate.²

Fluoroquinolones can be effective for treatment of cats with chronic bacterial rhinitis. Pradofloxacin is the only veterinary quinolone that also has an anaerobic spectrum. This suspension is well tolerated by cats and has 100% ocular safety.³

My laboratory completed a study of cats with upper respiratory disease complex that had two major objectives: to identify organisms associated with feline rhinitis in a natural setting and to compare the efficacy and safety of pradofloxacin and amoxicillin for the treatment of suspected bacterial rhinitis in cats residing in a humane society in North-central Colorado.² Forty humane-society cats with suspected bacterial upper respiratory infections were studied. Nasal discharges were collected for performance of infectious disease diagnostic tests prior to random placement into one of three treatment groups. Cats were administered amoxicillin at 22 mg/kg q 12 hrs, pradofloxacin at 5 mg/kg q 24 hrs, or pradofloxacin at 10 mg/kg q 24 hrs; all drugs were administered by mouth. Cats failing to initially respond to either pradofloxacin protocol were crossed to the amoxicillin protocol and cats that failed amoxicillin were crossed to one of the two pradofloxacin protocols.

The organisms most frequently isolated or amplified by polymerase chain reaction assays (PCR) pre-treatment were feline herpesvirus 1 (75%), *Mycoplasma* species (62.5%), *Bordetella* species (47.5%), *Staphylococcus* species (12.5%), and *Streptococcus* species (10.0%).

The initial treatment was amoxicillin for 15 cats, pradofloxacin at 5 mg/kg for 13 cats, and pradofloxacin at 10 mg/kg for 12 cats. Of the amoxicillin-treated cats, clinical signs resolved in 10 cats (66.7%) and five cats were switched to pradofloxacin (10 mg/kg for one cat and 5 mg/kg for four cats) after which clinical signs resolved in four. Of the pradofloxacin-treated cats (5 mg/kg), clinical signs resolved in 10 cats (76.9%) and three cats were switched to amoxicillin after which clinical signs resolved in all three. Of the pradofloxacin-treated cats
(10 mg/kg), clinical signs resolved in 11 cats (91.7%) and one cat was switched to amoxicillin after which clinical signs resolved. Overall, 73.7% of amoxicillin-treated cats resolved and 83.3% of pradofloxacin-treated cats resolved. However, differences in response rates between groups were not statistically different (P=0.2919), potentially because of the relatively small sample size. Drug toxicity was not noted and all cats were reported to tolerate the administration of the drug. We concluded in the manuscript that pradofloxacin can be a safe, efficacious therapy for some cats with suspected bacterial upper respiratory infections.4

In a separate study, our research group collaborated with researchers in the Department of Small Animal Internal Medicine, Veterinary Teaching Hospital, LMU, in Munich, Germany.5 In that study, we focused primarily on *Chlamydia felis* and *Mycoplasma* spp. with the purpose of finding a potentially effective therapy that could be used rather than doxycycline.

In this placebo-controlled, double-blind clinical trial, 39 cats with signs of bacterial upper respiratory infections or conjunctivitis were entered. The cats were randomly entered into 1 of 2 treatment groups: treated orally with either 5 mg/kg pradofloxacin q24 hrs or 5 mg/kg doxycycline q12 hrs for 42 consecutive days. Changes in health status and clinical scores were evaluated. The presence of *C. felis* and *Mycoplasma* spp. DNA was determined by quantitative polymerase chain reaction (PCR) and nested PCR of conjunctival swabs, respectively.

Prior to treatment, DNA of *C. felis* and *Mycoplasma* spp. was amplified from samples from 23 and 20 cats, respectively. Clinical signs improved markedly within the first week for cats of both groups. Complete elimination of *Mycoplasma* spp. DNA was achieved in both groups. During treatment with either drug, *C. felis* DNA copy numbers declined quickly, all cats administered doxycycline became *C. felis* DNA-negative and 4 cats treated with pradofloxacin remained *C. felis* DNA-positive.

In this study, it was concluded that both pradofloxacin and doxycycline have good efficacy against *C. felis* and *Mycoplasma* spp., resulting in a marked improvement of clinical signs. The study showed evidence that the pradofloxacin protocol studied may eliminate *Mycoplasma* spp. infections. However, since *C. felis* DNA was still amplified from samples from some cats after treatment with pradofloxacin, infection might not always be eliminated using this protocol.

REFERENCES


SELECTED READINGS


INTRODUCTION
Enrofloxacin is a veterinary broad-spectrum antibiotic approved for use in dogs and cats in bacterial infections caused by susceptible organisms. Both oral and parenteral formulations exist.

Enrofloxacin belongs to a class of antibiotics known as fluoroquinolones. Members of this antibiotic class act by inhibiting the process of DNA synthesis within the bacterial cell, which ultimately results in cell death.

Enrofloxacin has a broad spectrum of activity, with bactericidal activity against Gram-negative and Gram-positive bacteria, mycobacteria and rickettsia, with the exception of enterococci and anaerobes. Enrofloxacin is able to achieve high concentrations in most tissues.

It should be stressed that selection of antibiotics should be based on culture and susceptibility testing whenever possible.

In canine medicine, clinicians commonly use enrofloxacin to treat a variety of susceptible bacterial infections, especially skin and urinary tract infections. This is mainly due to its rapid bactericidal effect against a wide variety of clinically important organisms including Staphylococcus pseudintermedius and Gram-negative bacilli by virtue of interference with the supercoiling of bacterial chromosomal material. So enrofloxacin is very useful in the management of recurrent pyoderma, in chronic deep pyoderma with extensive scar tissue, in ear infections and in uncomplicated or complicated urinary tract infections in dogs.

However, are there other conditions beyond urinary and dermatological infections where enrofloxacin might also play a role? What about some gastrointestinal, hepatic or respiratory bacterial diseases?

GASTROINTESTINAL DISORDERS
Dogs with chronic intestinal disease typically present with clinical signs of diarrhea, weight loss and/or vomiting. Diarrhea that has lasted for 3 weeks or more is usually considered as being chronic. The diagnostic approach to chronic diarrhea includes a “step by step protocol” where infectious/parasitic agents, non-gastrointestinal disorders, exocrine pancreatic insufficiency and pathologies that require surgery need to be ruled out. The most common diagnoses of chronic enteropathy in dogs are diet responsive, antibiotic responsive, immunosuppressive drugs responsive and lymphangiectasia.
Within the group of antibiotic-responsive enteropathies we have two different clinical situations where enrofloxacin might be indicated:

1. **Secondary Small Intestinal Bacterial Overgrowth (SIBO) and idiopathic Antibiotic-Responsive Diarrhea (ARD)** are two separate syndromes with a different etiology and pathogenesis. SIBO can arise secondary to diseases that result in excess substrate in the intestinal lumen, diseases that affect the clearance of bacteria or to morphological or functional derangement of the mucosa. The increased numbers of bacteria compete for nutrients, produce ‘toxic substances’ and can damage the mucosal brush border with secondary clinical signs. ARD has several hypotheses regarding its etiology but the clinical responses of some dogs with idiopathic chronic diarrhea to antibiotics (such as tylosin or metronidazole) and the predisposition of certain breeds (e.g., German Shepherd) points to an interaction of host susceptibility and bowel bacterial population. The therapeutic approach to chronic enteropathies is influenced by different factors but is directed at correcting nutritional deficiencies and counteracting inflammation and changes in intestinal bacterial population. Treatment for secondary SIBO is best directed at the underlying disorder combined with an antibiotic treatment. The choice of antibacterial agent is controversial: most of the dogs with idiopathic ARD respond well to tylosin or metronidazole; however, for secondary SIBO, other antibiotics, like enrofloxacin, could be more appropriate because of its efficacy against Gram-negative organisms.

2. Enteropathies characterized by neutrophilic, histiocytic ulcerative or granulomatous inflammation are being diagnosed more frequently in dogs in the past few years. Some of these enteropathies are associated with bacterial pathogens such as *E. coli*, *Streptococcus*, *Campylobacter*, *Yersinia* or *Mycobacteria* spp. Culture of mucosal biopsies and intestinal lymph nodes should be performed in cases of granulomatous or neutrophilic enteritis to detect infectious organisms. It is imperative not to immunosuppress dogs with granulomatous or neutrophilic infiltrates until infectious causes have been ruled out. Successful treatment requires antibiotics that are effective against these aforementioned bacteria and penetrate intracellularly. Fluoroquinolones, such as enrofloxacin are widely regarded as the antibiotics of choice because of their ability to induce lasting clinical remission. Eradication of mucosal invasive *E. coli* in boxers, French bulldogs or other breeds of dogs with neutrophilic or granulomatous inflammations is associated with clinical cure, but antimicrobial resistance is common among enteropathies associ-
ated with *E. coli* and this factor will certainly impact clinical response. This means that antimicrobial treatment should be decided based on by mucosal culture and antimicrobial susceptibility test rather than empirical ‘wisdom’.

**HEPATIC DISEASES**

A recent publication demonstrated that bacterial cholangitis and cholecystitis occur more frequently than suggested by previous literature and should always be considered in the differential diagnosis of dogs presenting with clinical signs of jaundice, fever, abdominal pain, leukocytosis or with evidence of gallbladder or biliary tract abnormalities on abdominal ultrasound. The pathogenesis of these conditions is still poorly understood and little information is available on the relationship between clinical signs and presence of bacteria in the bile. One possible hypothesis is that bacteria ascend from the bowel into the biliary tract and the gallbladder and ultimately may progress to involve the biliary tree and liver. Bacteria most frequently isolated from bile, gallbladder wall or liver are *E. coli*, *Enterococcus*, *Clostridium*, coliforms, *Enterobacter*, *Klebsiella*, *Proteus* and *Bacteroides*. Treatment with a broad-spectrum antibiotic or with a combination of antibiotics to cover Gram-positive and Gram-negative aerobes and anaerobes could be indicated. But the choice of antibiotic(s) should always be based on culture and antimicrobial susceptibility test results. However, empirical coverage with enrofloxacin plus amoxicillin-clavulanate or enrofloxacin, metronidazole plus amino-penicillin combinations could be a good clinical decision, although resistance always remains a potential problem. This could suggest that repeat sampling is always important, both to confirm or refute infection and to ensure that antibiotic sensitivity remains.

**RESPIRATORY DISORDERS**

Canine infectious respiratory disease, from “kennel cough” to pneumonia, can be caused by many bacteria (such as *Bordetella bronchiseptica* or *Mycoplasma* spp.) and several viruses. All these canine respiratory pathogens cause similar clinical signs, especially in the first week of illness. Therefore, the cause of infection and the treatment decision cannot be made based solely on clinical signs. Bacteria are one of the most important causes of canine infectious respiratory disease and they typically enter into the lungs through the airways, either through inhalation of primary infectious agents or aspiration of oral, esophageal or gastric contents. If the presence of bronchopneumonia is detected, bacterial culture and sensitivity tests are necessary before deciding on a prolonged treatment course with antibiotics. There is no single antibiotic or drug of choice for treatment of canine infectious respiratory disease. For many dogs, treatment of these disorders is supportive and most are self-limiting. For dogs with evidence of bacterial disease or those at risk for secondary bacterial infection, antibiotic treatment is often indicated. Over the last years, most isolates in canine infectious respiratory disease were sensitive to doxycycline, enrofloxacin and amoxicillin/clavulanate. Doxycycline reaches reasonable airway concentrations in people, but is more protein-bound in dogs and may not penetrate into the epithelial lining fluid as readily. Amoxicillin/clavulanate is not thought to reach high concentrations in the epithelial lining fluid of healthy dogs. Enrofloxacin reaches high concentration in the epithelial lining fluid but could be reserved for more significant infections. However, since *B. bronchiseptica* is not the only bacterial pathogen that may be involved with canine infectious respiratory disease, and secondary infections subsequent to canine influenza or other viral infections may be seen, enro-
floxacin, amoxicillin/clavulanate or other broad-spectrum antibiotics could potentially be more effective than doxycycline. Puppies are more likely than adult dogs to have bacterial pneumonia from primary bacterial and viral infections. However, in both, young and adult dogs, it is necessary to evaluate the presence of underlying diseases or pathologies if there is relapse of clinical signs and bacterial infection.
ABSTRACT
This talk will discuss the increasing recognition of these important potentially zoonotic and nosocomial infections in pet cats (and dogs) in the UK. We now know that ~1% of all feline tissue biopsies sent for routine pathology in laboratories in the UK are found to have histopathology changes consistent with mycobacterial infections. Of these cases, ~35% are found to have tuberculosis, with ~20% caused by Mycobacterium microti and ~15% caused by Mycobacterium bovis. Which infection is present is highly regionally-dependent with M. microti found in cats from Scotland, the North of England and the South-East of England, while cats with M. bovis infections come from the areas of UK where infected cattle and badgers are found, i.e. the South-West and West of England and Wales.

The talk will cover how the cats are most commonly infected, which is particularly related to hunting small rodents, and what these infections can look like in the cat – which might not be as you expect. Most cases are cutaneous in nature and/or affect regional lymph nodes, especially the submandibular or popliteal lymph nodes. Only late on in infection does haematogenous spread to lungs eventually cause respiratory signs, typically dyspnoea. The talk will touch on tuberculosis in dogs, which while much less common than in cats, tends to present most commonly with fully disseminated disease.

Making a diagnosis can be complex and frustrating. There is no gold-standard test; while Ziehl-Neelsen staining can suggest that mycobacteria are present, currently available advanced diagnostics (specialist culture, PCR tests and the IFN gamma release assay [IGRA]) can only identify ~50% of these infections. In addition, specialist culture results are typically not available for two to three months, sometimes longer; current PCR diagnostics are expensive and not reliable when only a few mycobacteria are present; and the IGRA blood test is costly and can be tricky to interpret in some cases. The results from a number of tests may need to be pieced together before you are certain of what you are dealing with.

The discussion will review management options. While treating a cat of tuberculosis is always contentious – this statement belies a complex situation since many cats have only a single cutaneous lesion, and a confirmation of tuberculosis may take many months to be made. We need to look at each case in its entirety, considering the nature of the infection (once it is known) – since M. bovis is notifiable in the UK; both infections are potentially zoonotic, but only M. bovis has been shown to spread from cats.
to their owners, notably when a cat has a cutaneous lesion that is draining pus containing large numbers of mycobacteria; both infections can potentially cause nosocomial infections within a veterinary practice, but only *M. bovis* has been shown to do this so far, again when a cat has a cutaneous lesion that is draining pus containing large numbers of mycobacteria; the extent and severity of the cat’s clinical signs (especially the presence of cutaneous lesions that are draining pus containing large numbers of mycobacteria); the household within which the cat lives and whether or not there are any immunosuppressed individuals; the complexity, expense and potential toxicity of potential treatment; the need for prolonged treatment which requires cat and owner compliance; and the need to use drugs that are used to treat tuberculosis in humans, amongst other things.

Where appropriate cases (e.g., cases with non-ulcerated cutaneous lesions) are treated with two-to-three suitable drugs for up to six months the prognosis for an apparent cure or long-term remission can be as high as perhaps 70%. However, these are always complex and contentious cases to treat and the prognosis should be stated as guarded. Cases of canine tuberculosis are most typically severe and caused by *M. bovis*, so the dogs are euthanised.
INTRODUCTION

The haemotropic mycoplasmas (haemoplasmas) are small bacteria that parasitise red blood cells and can induce haemolysis, causing anaemia. They were formerly classified as rickettsial organisms (when they were named *Haemobartonella* spp.) but sequencing and resulting phylogenetic analysis showed that they were actually mycoplasmal in nature. Reclassification and renaming of these organisms within the genus *Mycoplasma* occurred, although recent work suggests they should reside in a genus of their own.\(^1\)

In most studies, feline haemoplasma infections are more common in male, non-pedigree cats with outdoor access. Infection with ‘*Ca. M. haemominutum*’ is usually more prevalent in older cats, presumably because the chance of acquiring chronic subclinical infection increases with time. Some studies have shown an association between haemoplasma infection and feline immunodeficiency virus (FIV) infection\(^3,4\) whereas others have not.\(^4\) Recent research suggests that the host phenotypic traits, such as being male and/or older, are more important in driving multiple exposures to pathogens compared to pathogen-pathogen interactions.\(^5\) *Felis catus* gammaherpesvirus 1 (FcaGHV1), a potential feline pathogen, has been found to be significantly associated with haemoplasma infection in a recent study,\(^6\) but the significance of FcaGHV1 in cats has not yet been elucidated.\(^7\)

OUTCOME OF HAEMOPLASMA INFECTION

*Mycoplasma haemofelis* is the most pathogenic of the feline haemoplasma species. Acute infection often results in severe haemolytic anaemia, although

<table>
<thead>
<tr>
<th>Haemoplasma species</th>
<th>Reported prevalence</th>
<th>Pathogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycoplasma haemofelis</em></td>
<td>0–46.6% (median 4.8%)</td>
<td>Acute infection often results in haemolytic anaemia</td>
</tr>
<tr>
<td>‘<em>Candidatus Mycoplasma haemominutum</em>’</td>
<td>0–46.7% (median 14.4%)</td>
<td>Acute infection can induce a drop in erythrocyte parameters but not usually severe enough to cause anaemia unless cat has concurrent disease or is immunocompromised e.g., chemotherapy</td>
</tr>
<tr>
<td>‘<em>Candidatus Mycoplasma turicensis</em>’</td>
<td>0–26% (median 2.0%)</td>
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</tbody>
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Table 1: Feline haemoplasma species, their prevalence and pathogenicity

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in some cases only mild anaemia results. Chronic infection is not usually associated with significant anaemia. Cats do not need to be immunocompromised or splenectomised to succumb to clinical disease with *M. haemofelis*. In the author’s experience, young cats may be more likely to develop severe clinical disease compared to older cats. Erythrocyte-bound antibodies have been demonstrated in anaemic cats with acute *M. haemofelis* infection. One study\(^8\) showed that cold reactive antibodies appeared a few days earlier than warm reactive antibodies, but that antibodies appeared only after the development of anaemia had started, suggesting that they appear as a result of haemoplasma-induced haemolysis rather than initiating it. Indeed, specific glucocorticoid treatment is not usually required for haemoplasmosis.

Although ‘Ca. M. haemominutum’ infection can cause a drop in red blood cell parameters, anaemia is not usually induced except in cats with concurrent problems, e.g., FIV infection. However, cases of so-called primary ‘Ca. M. haemominutum’ anaemia, without any apparent concurrent disease or infection present, have also been reported.\(^9,10\) Thus infection with ‘Ca. M. haemominutum’ cannot be ruled out as a cause of anaemia in an individual case.

‘*Candidatus Mycoplasma turicensis*’ infection has resulted in anaemia or a small drop in red blood cell parameters in some experimental studies, but generally anaemia is uncommon following infection. Concurrent disease and immunosuppression are both thought to be involved in the pathogenesis of ‘Ca. M. turicensis’ disease, in a similar way to the pathogenesis described for ‘Ca. M. haemominutum’.

Different strains of each of the feline haemoplasma species may also exist, as well as these different species and these may also vary in pathogenicity. This might explain conflicting data in different studies. However, other factors, such as the health status of the cat, are also likely to play an important role in the outcome of haemoplasma infection. Long-term asymptomatic carrier status can occur, especially with ‘Ca. M. haemominutum’ and *M. haemofelis* infection.\(^4\) Clinical disease associated with reactivation has been reported.\(^10^-12\)

**TRANSMISSION**

Canine and feline haemoplasma DNA has been found in fleas and ticks\(^13^-18\), however this could reflect their haematophagous activity on infected hosts rather than signifying their role as a vector. The clustered geographical distribution of infection in some studies supports the role of an arthropod vector in...
haemoplasma transmission. The cat flea has been implicated in feline haemoplasma transmission, but only very transient *M. haemofelis* infection has been reported via the haematophagous activity of fleas, and clinical and haematological signs of *M. haemofelis* infection were not induced in the recipient cat. Additionally, a recent study found no evidence of haemoplasma transmission by fleas in an experiment involving the introduction of fleas into groups of cats housed together.

Some have suggested that cat fights may be involved in transmission. Subcutaneous inoculation of *Ca. M. turicensis*-containing blood resulted in infection transmission, whereas the same inoculation method using *Ca. M. turicensis*-containing saliva, did not. This suggests that haemoplasma transmission by social contact (saliva via mutual grooming etc.) is less likely than transmission by aggressive interaction (blood transmission during a cat bite incident). However, a recent study found evidence of horizontal transmission of *Ca. M. haemominutum*, but not *M. haemofelis*, by direct contact between cats in the absence of aggressive interaction and vectors. Vertical transmission has not been definitively shown using molecular methods with canine or feline haemoplasma infections but has been suggested for other haemoplasma species. Blood transfusion is another potential route of transmission, and blood donors should be screened for haemoplasma infection.

**DIAGNOSIS**

Haemoplasmas are currently unculturable *in vitro* despite numerous attempts in our and other laboratories. Recently, a number of haemoplasmas have been subjected to whole genome sequencing, including work performed by our group in sequencing two feline haemoplasma species; *M. haemofelis* strain Langford 1 and *Ca. M. haemominutum* strain Birmingham 1. These data have highlighted the limited metabolic capabilities of these important pathogens (glucose is their only energy source), which likely contribute to the haemoplasmas’ current uncultivable status. Such knowledge of haemoplasma metabolic capabilities has allowed us to direct *in vitro* cultivation attempts but successful growth has not yet been possible.

Cytology of blood smears may show haemoplasmas on the surface of erythrocytes, but this is known to be very insensitive for diagnosis, and cytology cannot differentiate between haemoplasma species. It can also be hard to distinguish stain precipitate and Howell-Jolly bodies from true haemoplasma organisms, although those confident in identifying haemoplasma organisms may be able to diagnose infection cat-side by examination of a blood smear as a screening tool, but organism numbers need to be extremely high in the blood to allow visualization on cytology.

Polymerase chain reaction (PCR) assays are now the diagnostic method of choice for haemoplasma infection. PCR is far more sensitive and specific than cytology. Real-time quantitative PCR (qPCR) assays allow quantification of haemoplasma DNA in the sample being analysed so we can monitor haemoplasma infection and evaluate response to treatment, e.g., a decrease in the level of haemoplasma DNA following starting effective antibiotic treatment. Quantitative PCRs have also enabled us to describe the *in vivo* kinetics of experimental haemoplasma infection. Cats experimentally infected with *M. haemofelis* can show great variation in blood copy numbers, especially in the first few weeks post-infection, but some do this longer term; this should be considered when interpreting qPCR results. In contrast, *Ca. M. haemominutum* - and *Ca. M. turicensis*-infected cats show little fluctuation in copy
(organism) number over time. The reasons for the marked fluctuations in blood *M. haemofelis* copy number over time is not known. Antigenic variation may mediate such fluctuations. Indeed, analysis has shown that a very large portion of the *M. haemofelis* genome encodes a set of uncharacterized hypothetical proteins arranged in multiple series of paralogous repeats; these could mediate antigenic variation through differing expression of haemoplasma surface proteins over time (we have confirmed *in vivo* expression of some of these proteins), thus enabling *M. haemofelis* to evade the host’s immune response.26

The development of haemoplasma protein-based serological assays has been limited by our inability to culture haemoplasmas *in vitro* preventing the easy acquisition of adequate amounts of haemoplasma proteins for use in such assays. Studies in our laboratory have evaluated the feline serological response to haemoplasma infection using an ELISA based on recombinant *M. haemofelis* DnaK.28 Experimentally infected cats became seropositive following infection, with a greater antibody response recorded in those cats inoculated with *M. haemofelis*, compared to ‘Ca. *M. haemominutum*’ and ‘Ca. *M. turicensis*’. This could be due to the humoral immune response being directed against conserved, haemoplasma clade-specific, and/or species-specific epitopes on *M. haemofelis* DnaK, or a measure of the degree to which the immune response to DnaK is triggered by the infecting haemoplasma species due to the severity of disease. Antibody levels were maximal in the early (~ 2 – 4 weeks) post-infection period, suggesting that antibody levels may help differentiate acute from chronic *M. haemofelis* infection. Such differentiation could be useful to the veterinarian trying to evaluate if a haemoplasma infection is likely to be the cause of disease in an anaemic cat, due to the existence of asymptomatic carrier cats and anaemia being more common in cats acutely infected with *M. haemofelis*. The cross-reactivity between the haemoplasma species seen on existing serological assays limits its usefulness, but since serology can be more sensitive than PCR in detecting haemoplasma exposure (PCR negative seropositive cats have been identified)29, the development of further serological assays should be investigated.

**HAEMOPLASMOSIS TREATMENT**

1. **Antibiotics**

Antibiotic treatment is indicated for cats with clinical signs and clinicopathological abnormalities consistent with haemoplasmosis. However, no antibiotic treatment regime that predictably eliminates haemoplasma infection with any species has yet been described, although only very limited studies have been done to evaluate for clearance of infection. Antibiotics are typically given for 2 – 4 weeks.

Doxycycline (10 mg/kg daily PO) is often used as first-line treatment for haemoplasmosis. This is usually adequate to induce a clinical response in *M. haemofelis* cases, but it has been shown that a 2-week course of doxycycline (5 mg/kg BID PO) does not consistently eliminate infection30, 31 with 4 of 4 cats still being *M. haemofelis* PCR-positive in the 3 weeks after doxycycline treatment had been stopped, although *M. haemofelis* copy numbers in the blood were significantly lower in the 4 doxycycline-treated cats cf. the 4 untreated control cats. Similar results were reported in another controlled study with doxycycline, where none of 5 doxycycline-treated cats became PCR-negative.32 Unfortunately, controlled antibiotic doxycycline treatment studies have not been performed for either ‘Ca. *M. haemominutum*’ or ‘Ca. *M. turicensis*’ infection, although one uncontrolled study reported the failure of 3 weeks of doxycycline to eliminate ‘Ca. *M. haemominutum*’ infection in 5 cats33, and a single ‘Ca. *M. turicensis*’-infected...
cat became PCR-negative after 2 weeks of doxycycline treatment in another observational study. Longer treatment courses are recommended by some to help eliminate infection, although controlled experiments to confirm this have not been performed. Care should be taken to ensure doxycycline pills or capsules do not lodge in the oesophagus as some formulations can be associated with oesophagitis and stricture formation, and this can be an issue for the effective treatment of cats, especially if treatment is required for several weeks.

Fluoroquinolones are usually used as second-line treatments for haemoplasmosis. A 2-week course of enrofloxacin (5 mg/kg q 24 hrs PO) has been successfully used to treat clinical *M. haemofelis* infection in controlled studies, with *M. haemofelis* copy numbers in the blood being significantly lower in the 4 enrofloxacin-treated cats cf. the 4 untreated control cats. However, 3 of 4 enrofloxacin-treated cats were still PCR-positive for *M. haemofelis* in the 3 weeks after enrofloxacin treatment was stopped, so clearance of infection was not documented. Diffuse retinal degeneration and acute blindness have been reported following enrofloxacin treatment in cats, although this is very rare.

In controlled studies using 4 weeks of marbofloxacin treatment (2 mg/kg q 24 hrs PO), significantly lower ‘Ca. *M. haemominutum*’ copy numbers were observed in 6 treated cats cf. 6 untreated control cats, with similar statistical results seen with *M. haemofelis* copy numbers in 6 treated cats cf. 6 untreated control cats. However, ‘Ca. *M. haemominutum*’ copy numbers only plateaued during treatment with no negative PCR results seen, and copy numbers rose back to near pre-treatment levels within 7–10 days of finishing marbofloxacin treatment. Conversely, the fall in *M. haemofelis* copy numbers was progressive during the treatment period with intermittent negative PCR results obtained at the end of the marbofloxacin treatment period and in the 6 weeks following it, although clearance of infection (as indicated by repeated negative PCR results) was not consistently documented in any cat. Another study comprising 6 cats given 2 weeks of marbofloxacin treatment (2.75 mg/kg q 24 hrs PO [N.B.: different doses of marbofloxacin are licenced for cats in different countries, resulting in variation in the doses used in studies]), and 6 untreated control cats, similarly documented that marbofloxacin was not effective at eliminating *M. haemofelis* infection, with no significant difference in conventional (non-quantitative) PCR results obtained between treated and control cats.

One controlled study has evaluated 2 weeks of pradofloxacin treatment for *M. haemofelis* infection; 6 cats were given 5 mg/kg q 24 hrs PO pradofloxacin and 6 cats a higher 10 mg/kg q 24 hrs PO pradofloxacin dose, whilst 5 cats received doxycycline (5 mg/kg q 12 hrs PO) for 2 weeks, and 6 cats were untreated controls. Copy numbers of *M. haemofelis* were significantly lower in all three treatment groups cf. the untreated control cats, and at various time points *M. haemofelis* copy numbers were significantly lower in the pradofloxacin-treated cats cf. the doxycycline-treated cats, with intermittent negative PCR results obtained in only the pradofloxacin- and not the doxycycline-treated cats. Thus this study suggested that pradofloxacin may be more effective at clearing *M. haemofelis* than doxycycline. The author has also found pradofloxacin to be effective in the treatment of ‘Ca. *M. haemominutum*’ infection in clinical observational cases, but again no controlled studies have yet been performed.

Azithromycin was not effective in the treatment of clinical haemoplasmosis in a partially controlled
study of cats infected with *M. haemofelis* and/or ‘*Ca. M. haemominutum*’.

Response to antibiotics can be monitored by qPCR to ensure copy numbers are decreasing appropriately with therapy, especially in severe cases, those in which a clinical improvement is not seen, and/or cases that have had previous antibiotic therapy (see below). A goal of treatment should be to eliminate infection, in view of the recrudescence of disease that can occur in carrier cats, although proving infection has been eliminated is difficult without performing PCR on the whole host! Repeatedly negative PCR results on blood samples are probably most reliable to indicate elimination, although recent studies have suggested that serology, as mentioned above, has increased sensitivity over PCR in the detection of infection. If negative PCR results do not result from treatment, control of clinical signs and a reduction of copy numbers in the blood indicate some efficacy of treatment even if elimination is not possible. However, recrudescence of disease remains possible in cats that remain haemoplasma-positive.

A few cases do appear to be refractory to standard antibiotic treatments. Occasionally, dual therapy (typically doxycycline and marbofloxacin or pradofloxacin) has been tried with variable success, so it may be worth considering introduction of an additional agent if monotherapy with doxycycline or a fluoroquinolone is inadequate. Variability in response to treatment in different studies may arise due to differences between haemoplasma species, strains and host factors.

2. Corticosteroids

Corticosteroids have been recommended in haemoplasmosis to treat any immune-mediated component of anaemia, although their efficacy has not yet been proven. In our experience, clinically ill cats, including those that are Coombs-positive, respond to antibiotic treatment and supportive care alone without the need for corticosteroids. Indeed immunosuppressive doses of corticosteroids have been used experimentally to exacerbate haemoplasma infection, so their routine use is not advised.

3. Supportive care

Supportive care is also required for acute haemoplasmosis treatment. This should include correction of dehydration with fluid therapy, and blood transfusion if the anaemia is severe.

WHEN SHOULD WE TREAT CATS WITH HAEMOPLASMA INFECTION?

Cats with clinical signs associated with infection with a haemoplasma species should be treated. As asymptomatic carrier cats exist, it is important that the clinician is as sure as possible that the haemoplasma infection diagnosed (usually by PCR) is responsible for the cat’s anaemia. The presence of the pathogenic *M. haemofelis* species is more likely to be associated with clinical haemoplasmosis than ‘*Ca. M. haemominutum*’ or ‘*Ca. M. turicensis*’ infection. However, the latter two species can be associated with clinical haemoplasmosis, usually in the presence of concurrent disease or immunocompromise. In such cases, the ‘*Ca. M. haemominutum*’ and/or ‘*Ca. M. turicensis*’ infections should be treated, as well as any treatable concurrent problems the cat has.

The level of haemoplasma DNA found in the blood by qPCR may also be helpful to determine the significance of the haemoplasma infection detected, as it is likely that higher levels of DNA are more likely to be associated with clinical disease, as seen in experimental studies. However, we know that *M. haemofelis* copy numbers can fluctuate markedly during acute infection, so interpretation of qPCR results
can be more difficult with this species. Monitoring of levels of haemoplasma DNA present in the blood via qPCR during and/or after treatment can be useful to document efficacy of treatment; especially if high levels have been documented at diagnosis as these levels should fall with effective treatment. If a clinical response is seen and the haemoplasma infection is not complicated by concurrent disease, monitoring may not be required, especially for cases of *M. haemofelis* infection that appear to respond quite predictably to antibiotic therapy. However, if infection with one of the other haemoplasma species is being treated and/or concurrent diseases or immunosuppression is present, documenting reduction (or a negative result) of haemoplasma DNA in blood is reassuring and ensures that appropriate treatment is being given and can help decide on the duration of therapy required.

Whether to treat asymptomatic carrier cats with subclinical infections (i.e. PCR-positive but with no clinical or laboratory evidence of disease) is less clear, especially as no treatment protocol exists that can reliably eliminate the carrier state. Additionally confirmation of elimination of infection is difficult. Such cats may be identified as a result of blood donor screening for example. The presence of *M. haemofelis* infection may be more concerning than the other feline haemoplasma species, but in most cases it is probably adequate to ensure that case records identify the cat as being a haemoplasma carrier so that if clinical signs consistent with haemoplasmosis appear in the future, the clinician is quickly alerted to the possible role of haemoplasma infection.

**PREVENTION**
Blood donors should be screened for haemoplasma infected by PCR to help prevent inadvertent transmission by blood transfusion from asymptomatic carrier cats. Keeping cats indoors is also likely to prevent infection, although this is not practical for many, as outdoor status has been identified as a risk factor and, in view of the potential for vector transmission, preventative flea and tick treatment is recommended. Recent work suggests that protective immunity develops following *M. haemofelis* infection\(^5\), opening the way for future haemoplasma vaccination.

**REFERENCES**


INTRODUCTION
The importance of bacterial diseases in exotics and wild animals is well documented. Writing about “exotic animal medicine” means dealing with thousands of species. Even within the same animal class, the anatomy and physiology of the digestive, renal or respiratory systems differ widely among species. However, after taking into account these several specific considerations, medicating exotics is accomplished by the same methods of administration used in domestic mammals. Following their arrival in clinical medicine in the later 1980s, the fluoroquinolones have become a widely used group of synthetic antimicrobials in veterinary medicine. They have provided small animal clinicians with a truly exciting new class of antimicrobials. Never before had veterinarians a drug with such a broad spectrum of activity, combined with the pharmacokinetic properties that allow for oral administration on a once-a-day basis. This has allowed clinicians to treat not only a larger number of patients, but also other species with more assurance.

We can see in the literatures, studies on pharmacokinetics of enrofloxacin on a large range of species, from sea turtle, crocodile to wild mammals. Even this year (2016), a study has evaluated the pharmacokinetics of enrofloxacin and its active metabolite ciprofloxacin in … the green sea urchin (Strongylocentrotus droebachiensis)!

Following some general considerations, this paper provides an overview of the author’s experience in some bacterial diseases encountered in exotic and wild species.

GENERAL OVERVIEW
The skills, medications and protocols used in medicine and surgery of domestic carnivores are applicable to exotics. Therefore, thorough physical examination should be followed by several clinical tests. Because most of the exotic species are highly dependent on their environmental conditions, husbandry records are extremely important. Reviewing information on both the sick individual and apparently healthy animals is part of the diagnostic process. Before any therapy is instituted, the clinician must carefully consider questions on the husbandry situation of the patient, especially its nutritional status. There is no sense in instituting antimicrobial therapy in exotic practice without correcting zoo-technical deficits. One should always have in mind that antimicrobial therapy is only a part of a more general therapeutic plan.
The practitioner must also be careful to avoid the unnecessary prophylactic use of antimicrobials that can result in antimicrobial resistance and in the emergence of infections that were previously subclinical, or that might interfere with experimental studies if the patients are experimental models.

Looking more specifically at antimicrobial therapy, the principles of antibiotic use in dogs and cats apply similarly to exotics. Ideally, before instituting antibiotics, the clinician should qualify the nature of the infection, predict the most likely pathogen(s), obtain a culture and a minimum database and choose the antibiotic based on those results. Establishing these basic parameters allows the clinician to form a reasonable prognosis and a clear therapeutic approach. However, in the author’s experience, many animals are often presented late in the disease process. Immunosuppression in clinically ill subjects, the rapid progression to life-threatening diseases, and the suspected presence of mixed infections are indications for empirical use of combination antibiotics while waiting for culture and susceptibility results. The clinician should select not only the most efficient drug but also the safest and will use the most efficacious route of administration, knowing that restraint is often difficult and parenteral therapy can have its own limitations. Many dosage regimens have been designed largely on an empirical basis.

In most of the cases, supportive care is as important as antimicrobial therapy, as well as correcting nutritional deficits. As a rule, optimal treatment of infectious diseases depends on accurate diagnosis, susceptibility to the selected drug, husbandry practices, and anatomical and physiological differences among the species.

EXOTIC MAMMALS

Ferrets

Medication and diagnostic process used in domestic carnivores can be applied in ferret medicine. There are no specific intolerances to any drugs described. In ferrets, primary bacterial infections seem uncommon and are rather secondary to another primary disease process. The empyema thoracis is a rare disease in ferrets but must be considered in the differential diagnosis of pleural effusions. With one case, we will remind the diagnostic process and treatment of empyema highlighting the specificities of the ferret.

Rabbits

Unlike ferrets, bacterial infections are common in rabbits. However, the clinician should keep in mind before starting an antibacterial therapy that nearly all important diseases in rabbits are directly or indirectly
related to diet and feeding practices or environmental conditions. The basic diagnostic approach first includes thorough investigation of the husbandry conditions. Bacterial infections induce an inflammatory reaction characterized by caseated pus and walled-off abscesses that are not necessarily associated with an increase in total white blood cell count. Many of them are attributed to *Pasteurella multocida*, even if numerous species of bacteria are cultured routinely from rabbits. Some additional selected bacterial species known to infect rabbits include pathogenic species of *Staphylococcus* and *Streptococcus*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Listeria*, *Actinomycoses*, and *Actinobacillus*. A study demonstrated that anaerobic bacteria, not *Pasteurella*, were common causes of bacterial disease in rabbit abscesses. As a rule, for abscess treatment, antibiotic administration alone will not cure the problem and surgical debridement of the area is always necessary.

We will mainly talk about dental abscesses, very common in rabbits. They may involve the mandibular or maxillary teeth. We will remind and underscore that antimicrobial therapy in rabbits (like in rodents) entails greater risk than in most other species because inappropriate therapy can result in death of the patient due to enterotoxemia. Some antibiotics provoke a disruption of the normal enteric flora in rodents and rabbits, which can be potentially fatal. This dysbiosis is caused by a sudden loss of microbial diversity in the cecum of affected animals, which subsequently leads to the overgrowth of opportunists such as *Clostridium* spp. and *Escherichia coli*.

**Birds**

Microbial diseases are common in companion and aviary birds. Bacterial infections may be primary, however, secondary infections due to poor husbandry conditions, stress, nutritional deficiencies, viral process or parasitic burden are the most common. Additionally, many secondary invaders are able to maintain a disease process independent of other infectious agents or predisposing conditions. Thus, with suspicion of bacterial infection, the clinician should always try to find the predisposing causes and should employ all practical diagnostic techniques to direct primary care before initiating any therapy.

The most common causes of primary and secondary bacterial infections in psittacine birds are Gram-negative bacteria (*E. coli*, *Klebsiella*, *Pseudomonas*) and *Chlamydophila psittaci*. Gram-negative bacteria are frequently resistant to routine antibiotics, however, most isolates are susceptible to enrofloxacin. Enterobacteriaceae (*Salmonella*, *Citrobacter*, *Proteus*, *Serratia*) and *Enterococcus faecalis* (canaries) are common as well. Less common infectious agents of psittacine birds are *Staphylococcus aureus* and *Streptococcus* spp., *Mycoplasma*, *Bordetella*, *Mycobacterium* and *Pasteurella multocida*. The causative organisms of mycobacteriosis are *Mycobacterium avium* spp. *avium*, *Mycobacterium intracellulare* and *Mycobacterium genavense*. The disease may be asymptomatic for long periods and the main clinical symptoms are chronic wasting, weakness, labored respiration, diarrhea, skin granulomas, lameness, and death. There is a zoonotic potential, particularly for immunocompromised individuals. *M. genavense* is of greatest zoonotic concern. Thus, therapeutic management must be considered with caution.

Once the antimicrobial therapy is decided, it is important to ensure the antibiotic reaches therapeutic levels in all target sites. For example, direct flushing or nebulization is needed to bring effective concentrations of antibiotics in the upper respiratory tract. Avian abscesses are usually presented with
solid pus and are completely unavailable to antibiotic penetration. Surgical excision or debridement followed by topical medication are often essential parts of the therapy. In all cases, antibiotic therapy is one part of the therapeutic process. Correcting husbandry and nutritional deficiencies, giving supportive care (placing the animal in its optimal thermal zone, fluid therapy and gavage if needed) are also essential.

Enrofloxacin has been extensively used in avian medicine because of their broad antimicrobial spectrum, which includes most Gram-negative bacteria, some Gram-positive bacteria (including staphylococci), some mycoplasma, and chlamydiae, as well as their bactericidal activity and relative lack of adverse effects. Enrofloxacin is one of the most common drugs within this group used in birds. Because of its antimicrobial properties, enrofloxacin has advantages for use in birds in treating common infectious diseases, such as Mycoplasma infection, colibacillosis, and pasteurellosis.

Reptiles
Bacterial infections play an important role as causes of disease and death in captive reptiles. It is now understood that most reptile bacterial pathogens are Gram-negative, although many of these pathogens could be part of the host’s normal flora, becoming pathogenic when the animal is immune-suppressed, after viral infection or stressed under the conditions of captivity.

Again, the therapeutic plan will begin first by correcting environmental and nutritional deficiencies, which are the most important predisposing causes of disease in reptiles. Without this first step, there is no sense in starting other treatments. Environmental temperatures should be maintained near the upper limit preferred by the species to enhance immune function. Higher metabolic rates of anorectic reptiles may necessitate force-feeding. Fluid therapy should be considered as well. Ideally, severely affected reptiles should be isolated and antibiotic therapy initiated. In general, good sanitation is paramount in prevention of all diseases. The enclosure should be set up to reduce stress, with addition of hide boxes. Arboreal animals should be furnished with a secluded branch on which to lay.

Although a wide variety of bacteria have been incriminated as either primary or secondary pathogens, infections caused by Gram-negative bacteria are most common. Aeromonas hydrophila, Klebsiella oxytoca, Morganella morganii, Providencia rettgeri, Pseudomonas aeruginosa, and Salmonella arizonae are prominent among the microorganisms isolated from healthy and ill captive reptiles. These bacteria can remain dormant and become invasive when conditions decrease the immune resistance of the host and/or follow primary viral infection. Anaerobic infections are more common than once thought and may be involved in up to 40% of all bacterial infections.

Amphibians
The amphibian patient is often presented late in the disease process and the most frequently apparent clinical signs are non-pathognomonic. Because amphibians are – more than any terrestrial vertebrate – very dependent on their environmental conditions, husbandry records are critical for the clinician.

Bacterial diseases have a high prevalence in amphibian facilities. Most of environmental bacterial agents become pathogens in stressed amphibians: transportation, bad husbandry, changes in the environment. Red-leg syndrome in amphibians is so named due to the hyperemia of the ventral skin of the thighs and abdomen of septicemia anurans, and
is now synonymous with any generalized bacterial infection in amphibians.

Safe, efficacious treatment for common anuran bacterial infections requires knowledge of specificity, pharmacokinetics and toxicity of antibacterial agents in frogs. Three readily available antibiotic agents – tetracycline, enrofloxacin, amikacin – which have specificity for common anuran bacterial pathogens were selected for investigation. Tetracycline was the first and most commonly recommended antibiotic for treatment of bacterial disease in frogs. However, tetracycline-resistant organisms from clinically ill amphibians have been isolated, and widespread bacterial resistance to tetracycline has also been reported in mammals and reptiles. On the other hand, bacterial resistance to enrofloxacin has only rarely been reported and amphibian pathogens have been uniformly susceptible.

Enrofloxacin and its active metabolite ciprofloxacin are frequently effective in inhibiting growth of pathogenic bacteria at serum levels of approximately 0.1 µg/ml. In bullfrogs (*Rana catesbeiana*), a study has shown that dosages of 5 and 10 mg/kg once daily maintained the plasma concentration above this level throughout the dosing interval. A single 10 mg/kg intramuscular dose did not induce significant hematological or biochemical abnormalities. Any route of administration is possible: PO, IM, percutaneous. All antibiotic therapy must last at least 7 days. In most amphibians, enrofloxacin is reported to be used at 5–10 mg/kg PO, SC, IM q 24 h. The weight is very variable depending on the state of hydration and one should not hesitate to reweigh the animal.

The percutaneous route by bath is certainly the less stressful. A dosage of 0.3 mg/ml waterbath for 15 days has demonstrated routinely favorable results.

**CONCLUSION**

Due to its bactericidal, wide distribution to tissues and the extracellular space, and because it can penetrate nearly every tissue in the body, enrofloxacin is among the most effective drugs for treating most bacterial infections in exotics. Enrofloxacin also offers the advantages of oral administration. Oral bioavailability of enrofloxacin is excellent in monogastric mammals and pre-ruminant calves, with up to 80% of the ingested dose being absorbed into systemic circulation. However, the metabolism and elimination half-life of enrofloxacin varies greatly between species. More pharmacokinetic studies are required in veterinary medicine for non-empiric use in more species.

Enrofloxacin is generally well tolerated. At therapeutic doses, it has proven to be relatively safe in all species, with few reported side effects. In addition, effective treatment with twice-daily, or once-daily in some species, administration is a clear advantage over some other antibiotics. The major disadvantage of parenteral administration is intramuscular pain and irritation at the site of injection.