High Dose Antimicrobial Protocols for Canine Urinary Tract Infections

Thesis

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Abstract

Background: Treatment for canine urinary tract infections (UTI) typically consists of 7-14 days of empirically chosen antimicrobial drugs. Enrofloxacin is a veterinary approved fluoroquinolone (FQ) antimicrobial and is useful for treatment of canine UTI. Ciprofloxacin, the primary metabolite of enrofloxacin, contributes additive antimicrobial activity. Higher doses of FQs may inhibit the emergence of antimicrobial resistance.

Objectives: 1) Determine if dogs with naturally occurring uncomplicated UTI have equivalent microbiologic cure with a high dose short duration protocol of enrofloxacin, compared to a standard antimicrobial protocol. 2) Measure urine concentrations of enrofloxacin, and ciprofloxacin, following a 20mg/kg single oral dose in healthy dogs (n=6).

Animals: Client-owned dogs with naturally occurring, uncomplicated UTI (n=38), and healthy dogs owned by students and staff of OSU-VMC (n=6).

Methods: A multi-center clinical trial was conducted. Dogs were assigned to 1 of 2 groups in a randomized blinded manner. Dogs in group 1 received treatment with 18-20mg/kg oral enrofloxacin (Baytril®) once daily for 3 consecutive days. Dogs in Group 2 were treated with 13.75-25mg/kg oral amoxicillin-clavulante (Clavamox®) twice daily

for 14 days. Urine and plasma concentrations of enrofloxacin and ciprofloxacin were measured following a single dose of 20mg/kg oral enrofloxacin in 6 healthy dogs.

Results: Thirty-eight dogs completed the clinical trial. No difference in microbiologic cure was found between the enrofloxacin or the amoxicillin-clavulanate groups (P= 1.0). In the 6 healthy dogs, mean peak urine concentrations were 138.7 µg/mL (range 73.0 µg/mL – 226.0 µg/mL) for enrofloxacin and 370.9 µg/mL (range 200.5 µg/mL – 638.9 µg/mL) for ciprofloxacin. Two-hour mean plasma levels were 3.4 µg/mL (range 0.7 µg/mL – 8.9 µg/mL) for enrofloxacin and 0.5 µg/mL (range 0.18 µg/mL – 0.96 µg/mL) for ciprofloxacin.

Conclusions and Clinical Relevence: The high-dose, short-duration enrofloxacin protocol was equivocally effective to the standard protocol in treating uncomplicated canine UTI in the sample patient population, and may represent a viable alternative therapeutic regime for similar patients. Ciprofloxacin contributes the majority of the antimicrobial activity in the urine after high dose enrofloxacin.

Dedication

Dedicated to my family: Mark, Janet, and Daniel Irom for all their years of support.

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1.1 Canine Urinary Tract Infections - General introduction

Canine lower urinary tract infections (UTIs) are a common reason for presentation to a veterinary hospital. UTI occurs when there is adherence, multiplication, and persistence of an infectious agent in the urinary system. [1] Normal host defenses that are important in prevention of UTI include normal micturition, mucosal defense barriers, antibacterial properties of urine, specific anatomic structures, and systemic immunocompetence. [2] The urinary tract from the kidney, ureter, bladder, and proximal urethra is normally sterile; there is a normal microflora in the canine distal urethra, prepuce, vagina, and vestibule. [3] Approximately 14% of dogs will be affected with UTI in their lifetime. [1]

In the largest retrospective study of canine UTI, which included 8,354 Canine Urinary Tract Infections**,** 3.9% of female dogs and 2.9% of male dogs examined at a teaching hospital had positive urine culture results during the 1969 to 1995 study period. In the initial study period prior to 1980, urine was only cultured if the urinalysis was abnormal. This practice likely lowered the detection rate. In this study, 37% of urine cultures from female dogs and 29% from male dogs were positive for UTI. [4] Female dogs are more commonly affected than males, presumably due to the shorter length of the

urethra. [4] Nevertheless, both sexes are represented in the literature, and two retrospective studies reported similar rates of occurrence between female and male dogs with recurrent or complicated UTI. [2, 5] UTIs are caused by similar bacterial organisms in both male and female dogs. [4] However, female dogs were more likely to have polymicrobial infections. [2, 5] Distributions of ages at UTI diagnosis tended to be similar between genders. Incidence of UTI increases with age, and peaks at 8 years of age, in both males and females. Some breeds may be predisposed to UTI at younger ages (0-4 years old); this is especially prevalent in large and giant breed dogs. [2, 4]

Clinical signs of UTI include pollakiuria (frequent urination), stranguria (straining to urinate), hematuria, and inappropriate urination. Dogs will occasionally show no clinical signs (i.e. "occult UTI"). This is more common in immunocompromised patients. For example, <5% of dogs with UTI and hyperadrenocorticism, diabetes mellitus, or both, have clinical signs of UTI. [6] Healthy dogs with UTI will commonly have mild clinical signs localizing to the lower urinary tract but systemic signs of fever, inappetence, or abdominal pain are uncommon. [7]

1.2 Canine Urinary Tract Infections - Diagnosis

Antepubic cystocentesis is the preferred urine collection method since it is least likely to cause sample contamination. [8] Contamination during urine collection varies with the sampling method used, and urinary catheterization and collection of mid-stream voided samples both are to different degrees, contaminated by the normal flora of the

lower urinary tract. [9] Sample contamination is more common with free catch samples compared with sterile catheterization. [10] Urine specimens collected for bacterial culture should be transported and stored in sealed and sterilized containers, and processing should begin as soon as possible since bacterial counts multiply quickly at room temperature. If laboratory processing is delayed by more than 30 minutes, it is recommended to refrigerate the specimen at 4 degrees C for up to 6-8 hours. [7]

Common urinalysis findings include: pyuria, hematuria, and/or bacteriuria. Urinalysis may also be within normal limits in dogs meeting bacteriological criteria for UTI. [4] In dogs with UTI and diabetes mellitus, hyperadrenocorticism, or both, urinalysis was normal in 20% of cases. [6] Pyuria and hematuria indicate inflammation and hemorrhage, respectively, within the urinary tract, but are not diagnostic for UTI alone. [10] Visible bacteria in the urine sediment may occur in the absence of UTI (aymptomatic bacteriuria) or from contamination during sampling. [11] Furthermore, the presence of bacteria in urine sediment may not correlate with culture results; varying amounts of amorphous debris in the sediment that can mask or that may be mistaken for bacteria may explain this. [10]

The diagnosis of UTI is based on quantitative urine culture. Quantitative cultures should be performed on all urine specimens so that the number of bacteria per milliliter of urine can be determined and expressed as colony-forming units per milliliter (cfu/ml). [10] The most common method uses a calibrated bacteriologic platinum loop that delivers 0.001 ml or 0.01 ml of urine onto culture plates. Suggested reference ranges are available

for bacterial growth in urine samples collected by free catch, catheterization, and antepubic cystocentesis (Table 1). Guidelines state substantial microbial growth (>1000 cfu/ml) of one or two species of potentially pathogenic bacteria in a sample obtained by antepubic cystocentesis represent clinical UTI. This suggests that bacterial numbers >1000 cfu/ml likely represent clinical infection and that lower bacterial numbers may represent contamination. [7] For example, growth of low numbers of bacteria that normally colonizes the skin, likely represents contamination. [8] More stringent guidelines are required for catheterization and free-catch urine, with >10,000 cfu/ml and 100,000 cfu/ml, respectively, of 1 or 2 target pathogens, likely representing clinical infection. [8]

Table 1: Interpretation of quantitative urine cultures in dogs (colony-forming units per milliliter of urine) [7]

	Significant	**Suspicious**	**Contaminant**
Cystocentesis	≥1000	100–1000	≤100
Catheterization	≥10,000	1000–10,000	≤1000
Midstream voiding	≥100,000b	10,000–90,000	≤10,000
Manual compression	≥100,000b	10,000–90,000	≤10,000

Contamination of midstream voided samples may cause growth of >10,000 colonyforming units per milliliter (ie, false-positive results); therefore, these samples should not be used routinely for diagnostic culture. [7] Growth of low numbers of multiple enteric bacteria in a urine sample may represent inadvertent puncture of the colon by cystocentesis. [8]

If there is bacterial growth present on culture plates (typically within 24 hours for common urinary pathogens), organism identification and antimicrobial susceptibility is performed. [7] Antimicrobial susceptibility can be performed by measuring minimum inhibitory concentrations (MIC), or the lowest drug concentration used to inhibit bacterial growth. Susceptibility can also be performed by agar disk diffusion (Kirby-Bauer). The agar disk diffusion method uses Mueller-Hinton agar plates that have been inoculated with a standardized suspension of the target bacteria. Paper disks impregnated with different antimicrobial drugs are placed on the plate. Eighteen to 24 hours after inoculation and incubation at 38 degrees C, antimicrobial susceptibility is estimated by measuring zones of inhibition of bacterial growth surrounding each disk. Zones of inhibition are then interpreted in light of established breakpoint standards that correlate to organisms MIC, and susceptibility is recorded as resistant, susceptible, or intermediate. [7] Interpretive categories, termed clinical breakpoints, were created to interpret antimicrobial susceptibility as resistant, susceptible, or intermediate for a specific bacterial isolate. [13] A "susceptible" result implies that this antibiotic used for this infection at approved dosages is likely to achieve clinical cure. A "resistant" result

implies that the isolate is not inhibited by clinically achievable drug levels, development of antimicrobial resistance is likely, or that clinical cure was not reliable in clinical studies. An "intermediate" result implies that the isolate may be treated in body sites where the drug concentrates or when a high dosage of the drug can be used. [13] Clinical breakpoints are determined by The Clinical and Laboratory Standards Institute (CLSI) committee using information specific to bacterial organism and antimicrobial drug or class of drug. [12] This information includes: data from pharmacokinetic, pharmacodynamic, microbiologic, and animal modeling studies, as well as clinical trials in humans and animals, if available. Most breakpoints are set based on drug levels achieved in plasma, but breakpoints are set for different sites of infection if supporting data is available. [13]

1.3 Canine Urinary Tract Infection – Predisposing Factors

UTIs are considered uncomplicated if the patient has no identifiable concurrent urinary or systemic diseases. Host factors that may predispose dogs to UTI include structural, functional, and metabolic abnormalities; UTI in the presence of these factors is considered complicated. Structural abnormalities such as recessed vulva, vestibulovaginal stenosis, ectopic ureters, bladder diverticuli, perivulvar dermatitis, and persistent urachus can all predispose dogs to UTI. In multiple retrospective studies, 64-68% of dogs with ectopic ureters have urinary tract infections at initial diagnosis. [14, 15] Pelvic bladder can be associated with urinary incontinence and UTI, with 65% of dogs with pelvic bladder

diagnosed with UTI. [16] Diabetes mellitus and hyperadrenocorticism predispose dogs to UTI due to local and systemic immunosuppression, respectively. [6]

Glucocorticoids inhibit some aspects of immune function, and 39% of dogs receiving chronic corticosteroid treatment were diagnosed with UTI. [17] In diabetic dogs, hyperglycemia and glucosuria inhibit leukocyte function and migration. [17] In one retrospective, 42% of dogs with diabetes mellitus, hyperadrenocorticism, or both, were diagnosed with UTI. [6]

Poorly concentrated urine, such as that found in patients with chronic kidney disease, supports the growth of bacteria. [18] There are no studies reporting the incidence of UTI in dogs with chronic kidney disease (CKD). Nevertheless, this is likely higher than the general population. Twenty percent of feline patients with stable CKD have UTI and CKD is thought to be a risk factor for UTI in humans. [19, 20] UTI was present in 30% of Boxer dogs at the time of diagnosis with juvenile nephropathy. [21] Urine acidity, normal urine flow, and urine concentration are inherent defenses against ascending infection in addition to organic acids, and urea concentration. Urinary tract infection is also more likely to occur in conjunction with diseases such as urolithiasis and bladder neoplasia, which damage the normal uroepithelium. [22, 23] Chemical irritants, such as cyclophosphamide may inflame the bladder wall and predispose it to bacterial infection. [24] Neurologic disease, causing retained volumes of urine or obstruction to flow, can also predispose dogs to UTI. [25] Indwelling urinary catheters also predispose dogs to UTI. The prevalence of UTI was 48% in dogs with indwelling urinary catheters in one study and

the incidence of UTI increased by 27% per day increase in duration of catheterization. [25] Factors that predispose catheterized dogs to UTI include mucosal damage during catheter insertion and manipulation, and contamination of the catheter by fecal microorganisms. Retrograde flow of urine from the collection system to the urinary bladder, residual urine, and urinary bladder dysfunction also contribute to the predisposition to UTI. [26] The risk of UTI in dogs with indwelling urinary catheters also increased with each year increase in patient age and also with concurrent administration of antimicrobials. [25]

Complicated UTIs occur with conditions that increase the risk of serious complications or treatment failure, such as with immunosuppression, lack of normal micturition, concurrent pyelonephritis, or damaged uroepithelium. [27] For dogs with persistent UTI, the prognosis was better in dogs where an underlying disorder was identified and corrected. Clinical resolution was achieved with treatment of the underlying disorder in addition to treatment with antibiotics. [2] Dogs with UTI that are younger than 3 years may be less likely to respond to antibiotics alone since anatomic abnormalities are more common in this age group. Dogs with congenital anatomic abnormalities may have the highest risk of persistent UTI. [2, 5] Routine screening for UTI is therefore recommended for dogs with structural, functional and metabolic abnormalities. [28]

1.4 Canine Urinary Tract Infection - Pathogens

Most UTIs are associated with a single bacterial pathogen, which usually originates from the gastrointestinal tract or the distal urinary tract. [29] UTIs typically involve bacterial pathogens, but rarely fungi or viruses can cause infections of the urinary tract. [4] Approximately 75% of bacterial UTIs in dogs are caused by a single species of pathogen, approximately 20% are caused by two species, and approximately 5% are caused by three species. [4] *E. coli* is the most common canine uropathogen, and comprised greater than 40% of all laboratory isolates in the largest retrospective series on canine UTI. No other single pathogen represented more than 11% of the laboratory isolates. [4] The available retrospective studies include urinary isolates both from healthy dogs and those with underlying predisposing disease. There are currently no large studies on UTI solely in healthy dogs. Healthy young women have a high incidence of UTI; a large prospective study identified an incidence of 0.5 per person per year. In this population, 80% of UTIs are caused by *E. coli*. [30] While healthy dogs often present to primary care veterinarians for acute onset of lower urinary signs and are suspected of UTI, empiric antibiotics are often prescribed without a urine culture. A urine culture is often recommended only after a treatment failure or recurrent infection occurs. Other commonly isolated bacteria from canine UTI include *Proteus, Staphylococcus, Klebsiella, Enterococcus*, and *Streptococcus.* Less common bacterial isolates include *Mycoplasma, Pseudomonas*, and *Pasteurella*. [4] *Corynebacterium urealyticum* has also been reported as an uncommon cause of canine UTI and a cause of encrusting cystitis. [31] *C. urealyticum* is reported most often in conjunction with other abnormalities of the

urinary tract such as repeated catheterization, recent urogenital surgery, or neurologic defecits. [31] Fungal UTI are rarely reported in small animals but *Candida spp*, *Cryptococcus spp*, *Blastomyces* spp., *Aspergillus* spp., *Histoplasma* spp., and *Rhodotorula* have been reported in the dog. [32, 33] *Candida albicans* was the most common isolate (48%) in a recent retrospective on fungal UTI in cats and dogs. [32]

Antimicrobial agents are necessary for treatment of bacterial UTI, but functional host resistance mechanisms are required for successful treatment and prevention. [2] Experimental models of UTI were conducted in healthy dogs, during which *E. coli* was directly inoculated into the bladder. These dogs were able to clear the infection without administration of antimicrobial drugs. [34] The same phenomenon occurred in rats when *E. coli* was experimentally inoculated into their bloodstream or bladder; all normal rats cleared the bacteria without administration of antibiotics. [18]

Consequences of untreated UTI include pyelonephritis, prostatitis, and infertility. [1] Infection with coagulase positive staphylococci is the main causative factor for struvite urolithiasis in dogs. [23] Additionally, septicemia and discospondylitis can be consequences of untreated UTI in dogs. [1]

1.4.1 Uropathogenic E Coli

The risk of UTI increases with exposure to virulent *E. coli*. [35] Specific virulence factors appear to influence the ability of *E. coli* to overcome host defenses and successfully colonize the host urinary tract, causing UTI. [36] A high frequency of specific

virulence factors are present in *E. coli* strains isolated in canine UTI. [37] Strains carrying these specific urovirulence factors are referred to as uropathogenic *E. coli* (UPEC). UPEC were included in the recently defined pathotype ExPEC (extraintestinal pathogenic *E. coli*) that classifies a variety of *E. coli* pathogenic to specific extraintestinal body sites. [38] The urinary tract is the most common site for colonization and infection with ExPEC. [39] Although UPEC typically originate from the gut flora, they do not appear to cause disease in the gastrointestinal tract. [40]

Dogs spontaneously develop UTIs with strains of *E. coli* carrying urovirulence factors genetically similar to UPEC in humans. [37] Like human UPEC, canine UTI isolates are in phylogenetic group B2 and express O antigens on the outer surface of cell surface LPS, which are important for host evasion. These strains also possess numerous extraintestinal virulence-associated factors (VFs). Strains lacking these phylogenetic and urovirulence characteristics are more likely to behave as commensals. [37] In both dogs and humans with UTI, the UTI strain is often isolated from the feces concurrently, consistent with host fecal strains as the primary source for UTI pathogens. [41] Nevertheless, in a study of dogs with naturally occurring *E. coli* UTI, concurrent fecal cultures were performed to determine the predominant fecal strain of *E. coli* in each dog. In the majority of dogs, the predominant fecal *E. coli* was not the infecting strain causing the UTI. Instead, the UTI strain contained particular urovirulence factors by DNA analysis, while the fecal *E. coli* strain did not. In the cases where the predominant fecal strain was identical to the UTI strain, both contained urovirulence factors. [37] This supports the hypothesis that UTIs

occur from microbial pathogens with enhanced intrinsic virulence capability rather than quantitative presence of organisms (the special pathogenicity hypothesis). [42]

Specific UPEC virulence factors include fimbriae to enhance epithelial adherence, iron uptake systems, and cytotoxins. [36] Bacterial adherence structures require a complex organization of multiple proteins for assembly of fimbriae. The adhesive organelles, type 1, P, S, and F1C fimbriae promote adherence to host cells and tissues. UPEC also characteristically carry siderophores that sequester iron from the host. Secreted toxins include alpha haemolysin, cytotoxic necrotizing factor-1 (CNF1), and secreted autotransporter cytotoxin (Sat). These toxins alter host cell signaling cascades, modulate inflammatory responses, and stimulate apoptosis. [38] Experimental and epidemiological data strongly suggest that no single virulence factor is sufficient for UPEC to cause disease. Rather, expression of multiple, putative virulence factors, in concert with host factors, contributes to establishment of UTIs. [36, 43] Some virulence factors frequently occur together in the same strains due to their association with pathogenicity-associated islands (PAIs). PAIs are discrete genetic units incorporated into the genome of pathogenic microorganisms. PAIs are likely horizontally acquired due to flanking sites that allow for direct insertion into DNA, and may allow for simultaneous spread of multiple virulence factors among bacterial populations. [41]

UPEC also invade epithelial cells of the urinary tract; this has been demonstrated both *in vitro* and *in vivo*. [36] This may provide a survival advantage within the urinary tract and allow evasion of some host immune mechanisms, as well as contribute to treatment

failure when antimicrobial agents do not reach therapeutic levels in cytoplasm. [36] Recent studies of uropathogenic *E. coli* in superficial epithelial cells of the mouse bladder have identified intracellular bacterial biofilms. [44] Persistence of *E. coli* in biofilms is conjectured to be one source of recurrent *E. coli* UTI. Recurrent *E. coli* UTI in dogs is often due to genetically identical strains (confirmed by PFGE), suggesting that some cases of chronic *E. coli* UTI in dogs occur secondary to relapsing infections. [45] Antibiotic susceptibility profiles, often used as a proxy for DNA fingerprinting methods do not provide sufficient resolution to reliably distinguish relapsing or persisting infections from reinfections. [45]

E. coli strains genetically identical to canine UPEC have been isolated in human UTI. [42] One study showed that 26% of healthy dogs sampled shed *E. coli* containing urovirulence factors in their feces. [42] Thus, healthy dogs may serve as potential reservoirs for such pathogenic *E. coli*. [37]

1.5 Canine Urinary Tract Infection – Therapy

There are a multitude of antimicrobial therapies available for canine UTI. Antimicrobials appropriate for treatment of UTI include those that are able to attain high urine drug concentrations, have oral formulations, are easy to administer with few side effects, are inexpensive for owners and have minimal effects on intestinal flora. [46] Cure of urinary tract infections depends on antimicrobial concentrations in the urine rather than in the serum, and therapy based on urinary drug levels is recommended. [47] Higher drug

levels in the tissues are essential for infections that are deep seated in the bladder wall or have ascended to the kidneys (pyelonephritis). Longer duration of therapy for these infections may also be necessary. For uncomplicated canine UTIs, antimicrobial therapy has traditionally been recommended for ten to fourteen days. [7, 48] This is based on experimental studies in dogs using a UTI model created by instilling sulfosalicylic acid into the bladder of healthy dogs (12 males and 12 females) and then innoculating a single *E. coli* isolate (10^{10} bacteria). [49] In this study, single and 3 consecutive daily doses of amikacin (20mg/kg) and trimethoprim-sulfadiazine (TMS, 30mg/kg) were given to 12 dogs each. Only the 3-day regimen used in female dogs was successful in clearing UTI in 4/4 dogs, and no dosage was successful in male dogs. [49] Nevertheless, treatment of naturally occurring UTI in dogs may not be equivalent to that for induced UTI. Standard of care for women with acute, uncomplicated cystitis, is 3 days of empiric antimicrobial treatment. Empiric antibiotic therapy for 3 days is equivalent in efficacy to longer durations of therapy, while therapy for 1 or 2 days is associated with a lower cure rate. [27]

Many antimicrobials chosen for the treatment of UTIs have relatively short half-lives or time-dependent dosing; therefore, repeated dosing and client compliance during treatment is necessary for treatment outcomes to be successful. For example, Amoxicillin (Amoxi-tabs®, Pfizer Animal Health) is a veterinary approved antimicrobial appropriate for canine UTI. This is a time-dependent antimicrobial and recommended dosage frequency is every 8-12 hours. [50] Cephalexin is another antimicrobial commonly used for uncomplicated UTI in dogs; there are no veterinary approved products available

but dosage frequency recommended for use in dogs is every 8-12 hours (Keflex®, Dista). [51] Evidence from clinical practice suggests that in many cases, owners fail to comply with dosing regimens. Poor owner compliance and inadequate antimicrobial dosing can contribute to persistence of UTIs. [1] Several authors have attempted to assess veterinary owner compliance with short and long term medication dosing. In one study of veterinary compliance, 14% of enrolled dogs were excluded when their owners failed to fill the initial prescription. Of those clients that remained in the study, only 44% had 100% compliance with the short term of antimicrobial drug prescribed. [52] Compliance was higher in a recent study in which owners enrolled in a program to monitor compliance with short-term antimicrobials. [53] In this study, the dosage regimen was found to significantly influence compliance. Compliance was lower with TID dosing compared to SID or BID dosing. [53] Additional studies are recommended to assess whether owner compliance increases with shorter duration antimicrobial therapy.

1.6 Fluoroquinolone Antibiotics – Enrofloxacin

Fluoroquinolone (FQ) antimicrobial drugs have a broad spectrum of activity, and are used to treat a variety of bacterial infections, including urinary tract infections. Fluoroquinolone antimicrobial agents block bacterial DNA replication by inhibiting DNA gyrase. DNA gyrase is responsible for relaxing torsional strain on DNA supercoils; this is necessary for DNA replication, transcription, and recombination. [54]

Enrofloxacin (Baytril®, Bayer Animal Health) is a synthetic antibacterial agent, developed for veterinary use, and was approved by the Food and Drug Administration in 1989 for use in dogs. [55] Enrofloxacin has nearly 100% bioavailability after oral administration and reaches high tissue concentrations. [56] Enrofloxacin has activity against many gram-negative and gram-positive bacteria that are associated with canine infections. Its spectrum includes the majority of bacterial genera isolated from UTI in dogs. Enrofloxacin has a labeled dosage range of 5-20mg/kg/day, and may be administered once daily or divided into two doses. Antimicrobial cure after fluoroquinolone therapy is associated with a Cmax/MIC ratio of 8 to 10 or AUC relative to MIC (AUC/MIC) ratio of > 100, where Cmax is the maximum concentration of the drug, AUC is the area-under-the-curve, and the MIC is the minimum inhibitory concentration required to prevent growth of the bacteria. [57] In dogs, enrofloxacin is metabolized by the liver and deethylated to ciprofloxacin, which also has bactericidal activity. [58] A study of enrofloxacin plasma pharmacokinetics in 4 dogs following a 5mg/kg oral or intravenous dose, suggested that a significant portion of antimicrobial activity was due to ciprofloxacin since antimicrobial levels of ciprofloxacin were reached in the plasma as well. [59] Enrofloxacin is eliminated by the kidneys and high concentrations of both enrofloxacin and ciprofloxacin are reached in urine compared to plasma following oral dosing. [50] Urine concentrations of enrofloxacin and ciprofloxacin were measured 2 hours following 20mg/kg IV dose of enrofloxacin to anesthetized dogs. [58] High concentrations of both enrofloxacin and ciprofloxacin were present in the urine

compared to plasma, with ciprofloxacin contributing 50% to total antimicrobial concentration. [58] Ciprofloxacin is available and approved for use in humans but is not approved for use in animals. Ciprofloxacin can be used off-label by veterinary prescription in dogs, but has inconsistent bioavailability in dogs. Bioavailability of ciprofloxacin after oral administration in dogs ranges from 42-97% depending on the report. [60, 61]

1.6.1 Fluoroquinolone Antibiotics - Mechanisms of Antimicrobial Resistance

Antimicrobial resistance to fluoroquinolones occurs primarily from the accumulation of specific point mutations in the FQ target genes leading to inhibition of bacterial DNA topoisomerases (DNA gyrase and topoisomerase IV) or decreased drug uptake by changes in the outer membrane and increased efflux. [62] The DNA topoisomerases II (DNA gyrase) and IV are essential for bacterial replication by controlling DNA supercoiling and chromosome partitioning. They consist of two subunits, A and B, which are encoded by gyrA and gyrB for DNA gyrase, and parC and parE for topoisomerase IV, respectively. [63] The primary mechanism of bacterial resistance to FQ antimicrobials has been elucidated in *Escherichia coli* due to a mutation in *gyr*A, the gene encoding the A subunit of the enzyme DNA gyrase. [64] This is consequently designated the quinolone resistance-determining region (QRDR). Mutations in gyrB are of less significance, whereas mutations in parC are the predominant cause of FQ resistance in Gram-positive bacteria. [54, 65] Single mutations in gyrA confer low-level

FQ resistance; several mutations are necessary for the development of high level FQ resistance. Most isolates with high level FQ resistance have an additional mutation in parC. Topoisomerase IV (encoded by the *par*C and *par*E genes) is a secondary target of the fluoroquinolone antimicrobials, and mutations in *par*C and *parE* contribute to quinolone resistance. [54] Decreased bacterial cell wall permeability to FQ antimicrobials causing decreased cytoplasmic drug accumulation also contributes to resistance. [54] Efflux-related mutations are also common in highly resistant strains. [63] These efflux-related mutations inactivate marR or acrR, thus up-regulating the activity of the drug efflux pump, and reducing the antimicrobial concentration in bacterial cytoplasm. In addition to this mechanism, a reduction of the OmpF porin channels that can reduce intracellular FQ concentrations has been described. [62]

E. coli is one of several pathogens for which elevated mutation frequencies have been described among natural isolates. These hypermutator strains have up to 1000-fold higher mutation frequencies compared to normal strains. [63] When bacteria acquire two or more of these mutations, they will survive despite the presence of antimicrobial drugs at concentrations many times higher than what is achievable in a patient. These point mutations occur spontaneously in bacterial populations at a rate of 1 in 10^6 to 1 in 10^8 cells, and mutants can be further amplified under selective pressure during antimicrobial therapy. [66] Furthermore, mutations in bacterial DNA are essentially irreversible and resistance is transmitted to all daughter cells of the resistant isolate, potentially leading to large populations of resistant bacteria. [5]

Plasmid mediated mechanisms of resistance to FQ antimicrobials have also been described. Q*epA*, a plasmid-associated gene responsible for reduced FQ susceptibility, has been isolated from multiple individuals with *E coli* infection worldwide. *QepA* encodes an efflux pump that confers FQ resistance. [67] MICs of norfloxacin, enrofloxacin, and ciprofloxacin are 32- to 64-fold higher in experimentally tranformed strains expressing QepA than nontransformed isogenic controls. [68] Multidrug resistant *E. coli* occur in both human and veterinary medicine, and minimize available therapeutic options. [63] The production of extended-spectrum Beta-lactamases (ESBL) by multidrug resistant *E. coli* strains has caused increasing concern over the last decade. Plasmids encoding extended-spectrum β-lactamases that additionally encode plasmid-mediated quinolone resistance (PMQR) genes have been isolated from clinical isolates of *E. coli* in humans and dogs. [68] Multi-drug resistant *E. coli* with extended-spectrum beta-lactamase activity and fluoroquinolone resistance are reported in clinical infections in dogs. [64]

1.6.2 Fluoroquinolone Antibiotics – Increasing Antimicrobial Resistance

Bacterial resistance to FQ antimicrobials in human and veterinary medicine is increasing, and parallels increased antimicrobial use. [62, 69] During routine administration, antimicrobial agents apply selective pressure on populations of bacteria, leading to an increase in bacterial populations with antimicrobial-resistance. This occurs with FQ antimicrobials both *in vitro*, and in animal models, demonstrating that resistant mutant bacterial populations are selectively enriched after exposure to FQs. [62] Widespread use of

fluoroquinolones in humans has selected for bacterial resistance to fluoroquinolones. [70] Several investigators have associated increased antibiotic usage in practice with increased antibiotic resistance. There is a significant increase in the incidence of enrofloxacin-resistant *E coli* isolated in urine from dogs with UTI in recent years. This corresponds to increased use of enrofloxacin, as documented by veterinary pharmacy records. [54] A retrospective study of bacterial isolates from canine patients with urinary tract infections documented an increase in the prevalence of resistance to commonly used FQ antimicrobials from 1992-2001. [71] An increase in the incidence of FQ resistant canine UTI *E. coli* isolates was documented from 1996-1998 in a veterinary teaching hospital. This was shown by pulsed field gel electrophoresis to not be associated with clonal populations of *E. coli*. [54] An increase in the resistance to ciprofloxacin in hospitalized human patients over time was also seen from 1990-1996, and this increase in the prevalence of FQ resistance was parallel to an increase in ciprofloxacin usage. [69] Additionally, FQ resistance in *E. coli* is not restricted to clinical isolates, but is commonly present in surveys of the healthy human and animal feces. These may serve as reservoirs for pathogenic *E.coli* and contribute to spread between animals and humans. [72] These findings question whether the FQ-resistant *E. coli* encountered in dogs arise through mutation of FQ-susceptible canine resident strains to resistance, or instead originate from an external source.

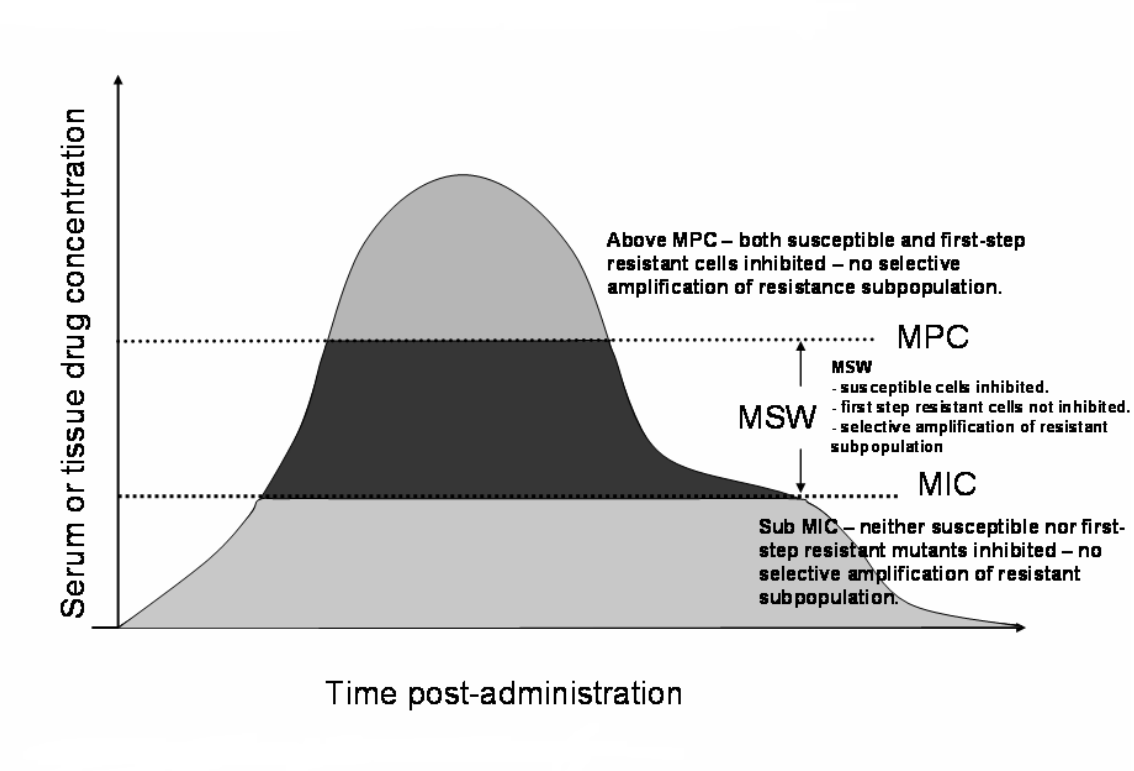
1.7 Mutant Prevention Concentration

To combat the increasing prevalence of antimicrobial resistance, recent studies have highlighted the need for optimal dosing regimens of antimicrobials that consider bactericidal efficacy, as well as the potential for selection of resistant mutants. Standard dosing regimens relying on interpretations of minimum inhibitory concentration (MIC) take into account only the clinical laboratory definition of *in vitro* inhibition of growth, and are not relevant to prevention of mutant selection. New dosing strategies using Mutant Prevention Concentration (MPC) guidelines consider both goals of curing bacterial infections as well as preventing formation and selection of resistant mutant bacteria. These dosing regimens may help to combat the emergence of resistant mutant bacterial strains, and are most applicable to FQ antimicrobials. [73] Mutations arise spontaneously in a microbial population, and some of these mutations confer antimicrobial resistance. These mutant subpopulations may be present in an infection site, or in other sites in the host, prior to initiation of antimicrobial treatment. Certain dosing strategies may select for mutant subpopulations when antimicrobial concentrations are high enough to kill susceptible bacteria in the population, but are not high enough to kill some mutants. This is referred to as the mutant selection window hypothesis, and the dose range in which mutants may be selected is referred to as the mutant selection window. [74]

The mutant selection window hypothesis contends that ideally, drug levels should be kept above the mutant prevention concentration (MPC) to restrict selection for resistant mutants. (Figure 1) MPC is defined as the minimum inhibitory concentration

(MIC) of a first step resistant mutant. [74] Mutant selection window (MSW) is defined as the concentration range between MIC and MPC that provides an *in vitro* measurement of the range of drug concentration where resistant mutants are thought to be most intensely selected (Figure 1). [74] For bacteria to grow at the MPC, they would have to acquire two or more resistance mutations. [75] If the frequency of FQ resistance mutations in *E. coli* is in every 10^7 bacteria, a bacterial population of $>10^{14}$ cells would be needed for two in every 10^7 bacteria, a bacterial population of $>10^{14}$ cells would be needed for two

Figure 1 : Mutant Prevention Concentration [76]



in every 10^7 bacteria, a bacterial population of $>10^{14}$ cells would be needed for two concurrent resistance mutations to arise. [71] Such large bacterial population sizes are considered unlikely in clinical infections. [75]

Mutant subpopulations have been isolated from patients treated with antimicrobial doses that fall within the MSW. [73] Although MPC is a laboratory assessment, it may become an effective tool when applied *in vivo*. Notably, the selection of FQ resistant strains of *Streptococcus pneumoniae* was effectively prevented in rabbits when MPC criteria were used. [77] In a different study, doses using only MIC criteria were insufficient to prevent the selection of resistant mutants of *E. coli in vitro* when serum concentrations of some commonly used FQs at standard doses were simulated. Mutant growth was prevented only using *in vitro* ciprofloxacin concentrations that simulated the highest labeled dose of ciprofloxacin approved in humans. [73] MPC appears to be valid for evaluation of enrofloxacin against *E. coli* isolated from chickens. All single-step mutants that occurred for *E. coli* during *in vitro* incubation with enrofloxacin had MICs equal to or lower than the MPC for enrofloxacin. [74] Ciprofloxacin has better efficacy *in vitro* using MPC criteria, meaning that MPC values were lower for all isolates when using ciprofloxacin compared to enrofloxacin. [73, 74] This may be an advantage *in vivo* since ciprofloxacin is the main metabolite of enrofloxacin and has antimicrobial activity. [74] Another method, the agar-plate mutant accumulation assay is an *in vitro* assessment of propensity for clinical isolates to acquire mutations that confer fluoroquinolone resistance. [78] This also may be useful when designing antimicrobial protocols.

The MPC method has not yet been validated in clinical patients. [79] MPC data for common veterinary pathogens are necessary before doses can be adjusted to target MPC guidelines in clinical patients. [80] This has been explored in *E. coli* isolates from chickens, and in *E. coli* isolated from human UTI, but not in dogs or cats. [66, 81] Furthermore, pharmacokinetic parameters that correlate with clinical efficacy using MPC criteria have been suggested *in vitro* but not tested *in vivo*. [79] The AUC_{24}/MPC and t>MPC may be useful pharmacokinetic parameter for predicting prevention of the emergence of resistant bacteria in vivo. [74, 80] From initial studies in non-neutropenic mice, it appears that antimicrobial concentrations would not need to remain above MPC levels for the duration of treatment in animals with an intact immune response, but the duration antimicrobial concentrations would need to remain above MPC for clinical success is unknown. [82] For example, with a local *Staphylococcus aureus* infection of rabbits, levofloxacin concentrations needed to be above MPC for only 20% of the dosing interval to restrict mutant amplification. [80] MPC is not predictable based on established susceptibility points, such as MIC. [81] High dose antimicrobial therapy protocols may be more efficacious in prevention of antimicrobial resistant populations by targeting MPC guidelines. MPC is an in vitro laboratory tool and is time-consuming and technically demanding. New ways to estimate MPC for clinical isolates are necessary before application to clinical practice.

Chapter 2: Evaluation of the Efficacy of a High Dose Short Duration Baytril® Treatment Regimen for Uncomplicated Urinary Tract Infection in Dogs.

2.1 Introduction

The urinary tract is a common site for bacterial infection in dogs, and it is estimated that 14% of dogs will be affected by a urinary tract infection (UTI) in their lifetime. [1] It is important to differentiate between uncomplicated and complicated UTI for diagnostic and treatment purposed. UTIs are considered uncomplicated if the patient has neither 1) concurrent disease affecting the urinary tract nor 2) systemic disease with known comorbidity with bacterial cystitis. Uncomplicated canine UTI is routinely treated with a 7-14 day course of orally administered antimicrobial agents. [7, 48] Poor owner compliance and inadequate dosing can contribute to treatment failure in canine UTI. A shorter duration of therapy may increase client compliance and decrease cost. [53] Furthermore, therapy using high doses of antimicrobial agents with concentration-dependent pharmacodynamic targets will increase bactericidal activity; this may decrease the development of antimicrobial resistance due to high drug concentrations achieved. [69]

Fluoroquinolones are frequently used to treat canine bacterial infections because of their broad antimicrobial spectrum and relatively wide therapeutic margin. Baytril®

(enrofloxacin) is a fluoroquinolone antimicrobial drug developed specifically for veterinary use and approved by the FDA for use in dogs at 5-20mg/kg orally. Enrofloxacin is active against most major pathogens that cause UTI in dogs, including *E. coli*, other coliform bacteria, and *staphylococci* spp. The bactericidal effect of fluoroquinolones is concentration dependent. Fluoroquinolones also exert a post-antibiotic effect on Gram positive and Gram negative bacteria. It is conceivable that high-doses of FQs used once daily for fewer than 7 days may be an efficacious regimen for treatment of UTIs in dogs.

The objective of this study is to evaluate the effectiveness of a high dose - short duration enrofloxacin (Baytril®) dose regimen in the treatment of naturally-occuring lower urinary tract infections in dogs. We hypothesized that in naturally occurring canine uncomplicated urinary tract infections, neither microbiologic cure rate nor clinical resolution rate will be significantly different between two treatment conditions: 1) enrofloxacin administered at the highest FDA-labelled dosage for 3 days and 2) A standard and common treatment regimen of antimicrobial therapy (amoxicilln-clavulanate 13.75-25mg/kg for 14 days).

2.2 Materials and Methods

2.2.1 Clinical Drug Trial Design

A multicenter clinical drug trial was designed to evaluate a high dose - short duration enrofloxacin (Baytril®) dose regimen for the treatment of UTI in dogs using

Baytril® (enrofloxacin) tablets. This study was conducted as a prospective, controlled, randomized, and blinded clinical trial. The study was conducted starting February 2009 and aimed to enroll 100 dogs between 3 study sites. A midpoint analysis was conducted in March 2010 for Masters thesis analysis.

2.2.2 Planned Enrollment

Client owned dogs presented to study site veterinary clinics by their owners for lower urinary tract signs and considered for enrollment. Study sites included: The Ohio State University Veterinary Medical Center (Columbus, OH), The University of California-Davis Veterinary Teaching Hospital (Davis, CA), and Bradford Park Veterinary Hospital, a private referral practice (Springfield, MO). Adult dogs, 5-50kg were enrolled to ensure appropriate dosing based on tablet sizes used in the study. The dogs were classified as adults based on the following criteria: >9 months in small or medium sized dogs 5 - 25.9kg, >1 year in large breed dogs 26 -45kg or >18 months in giant breeds >45kg. The dogs were deemed otherwise healthy based on owner history, physical exam performed by one of the investigator veterinarians, a complete blood count, and serum biochemical analysis. Dogs were enrolled into the study when provisional enrollment criteria were met and when target pathogens were identified on urine culture.

For definitive eligibility, diagnosis of bacterial UTI was based on quantitative urine culture obtained by antepubic cystocentesis on the study Day 0. UTI was

diagnosed when ≥ 1000 cfu/ml of 1 or a mixture of up to 2 target pathogenic bacteria was grown. Target pathogenic organisms included *E. coli, Proteus mirabilis, Klebsiella pneumonia, Pseudomonas aeruginosa, Enterobacter* spp., *Staphylococcus aureus, Staphylococcus pseudointermedius, Streptococcus* spp. (alpha hemolytic), and *Enterococcus* spp.

We aimed to enroll a total of 150 dogs over 12 months, estimating that 1/3 of dogs initially enrolled would be excluded due to study criteria and lost to follow-up. This would leave 100 dogs followed to study endpoint. After the first 3 months, only 3 dogs had been followed to study endpoint. Due to slow enrollment of clinical cases, recruitment of dogs with uncomplicated UTI from primary care veterinarians was performed. The clinical trial was advertised to primary care clinics in areas surrounding the study cite centers. Advertisements consisted of posters, mailings, phone calls, and personal visits by investigators. A stipend was given to primary care veterinarians for referral of adult, otherwise healthy dogs with lower urinary tract signs to study site centers without initiating treatment.

2.2.3 Exclusion Criteria

Dogs with persistent or recurrent UTI were excluded. Persistent UTI was defined as more than 3 positive urine cultures in succession with no intervening sterile cultures and an elapsed time period of 1-8 weeks between cultures. [83] Recurrent UTI was defined as more than 3 successfully treated UTI episodes within a single year in which each

episode is followed by a period of sterile urine. [83] Dogs with suspected pyelnonephritis, prostatitits, urinary tract neoplasia, or urinary calculi were excluded as well. Animals with uncontrolled systemic disorders and animals with spinal cord injuries were also excluded. Dogs treated within 7 days with a short duration antimicrobial, or within the last 14 days with a long-term antimicrobial were excluded. Dogs treated within the last 14 days with a systemic short-acting steroid or within the last 28 days with a sustained release steroid were also excluded. Dogs administered any medication that could interfere with systemic absorption, such as antacids and other compounds containing metal cations, were also excluded.

Exclusion criteria also included animals with known or suspected central nervous system (CNS) disorders (quinolones, in rare instances, have been associated with CNS stimulation that may lead to convulsive seizures). Young dogs, based on the aforementioned criteria, were also excluded since quinolone-class drugs have been associated with cartilage erosions in weight bearing joints of immature animals.

2.2.4 Treatment Groups

Amoxicillin-clavulante (Clavamox®) was chosen as the positive control because it is a suitable comparative antimicrobial indicated for use in canine UTIs, and is considered to be highly efficacious for UTI in dogs. Dogs were randomized to group 1 or group 2 by randomly generated numbers to ensure 1:1 ratio of dogs between groups. A veterinary technician was assigned to enroll dogs and dispense medications. The

dispenser instructed all pet owners not to discuss the treatment regimen with anyone other than the dispenser. All veterinarians, technicians, and staff responsible for clinical observations were blinded to the treatment. Dogs in Group 1were treated with 18-20mg/kg oral enrofloxacin (Baytril®) once daily for 3 consecutive days. Dogs in Group 2 were treated with 13.75-25mg/kg oral Amoxicillin-clavulante (Clavamox®) twice daily for 14 days.

2.2.5 Timeline and Assessment Criteria

All dogs were examined on Day 0 (initial day of presentation and treatment), Day 10 (+/- 2 days) and Day 21 (+/- 2 days). At Day 0, clinical signs were recorded (presence or absence of pollakiuria, stranguria, hematuria), and a physical exam, including bladder palpation, was performed by one of the study investigators. A complete blood count, serum biochemical analysis, urinalysis (by cystocentesis), and quantitative urine culture (by cystocentesis) with susceptibility by MIC were also performed at Day 0. Treatment was initiated on Day 0. Quantitative culture and susceptibility results were available 3-5 days after Day 0. At Day 10 and Day 21, clinical signs were again recorded (presence or absence of pollakiuria, stranguria, hematuria), and a physical exam, including bladder palpation, was performed by one of the study investigators. A urinalysis and quantitative urine culture +/- susceptibility by MIC were also performed.

Due to slow enrollment, an amendment was made to the study protocol allowing dogs to be enrolled up to 3 days after initial presentation to a study site if a positive urine culture was obtained. Day 0 was defined as the day of initial treatment for these dogs.

For definitive enrollment, ≥ 1000 cfu/ml of 1 or a mixture of up to 2 target pathogenic bacteria was required, isolated from a urine sample collected by cystocentesis. All enrolled dogs were followed to study endpoints 1) microbiologic cure 2) microbiologic failure 3) withdrawal due to adverse effects. The clinical cure rate (based on resolution, if present, of pollakiuria, stanguria, hematuria, and pain on bladder palpation) was also recorded. A treatment failure was defined as post-treatment culture positive for the same pathogen as the pre-treatment urine sample. Isolation of colonies of any pathogen different from the pre-treatment urine sample was considered a contaminant or a re-infection.

2.2.6 Statistical Analysis

An unpaired t-test was used to determine difference in treatment response between the two groups. A necessary sample size of 17 in each group was determined by power analysis using an alpha error 0.05 and a desired power of 0.80. This analysis was powered to detect a 10% difference between groups.

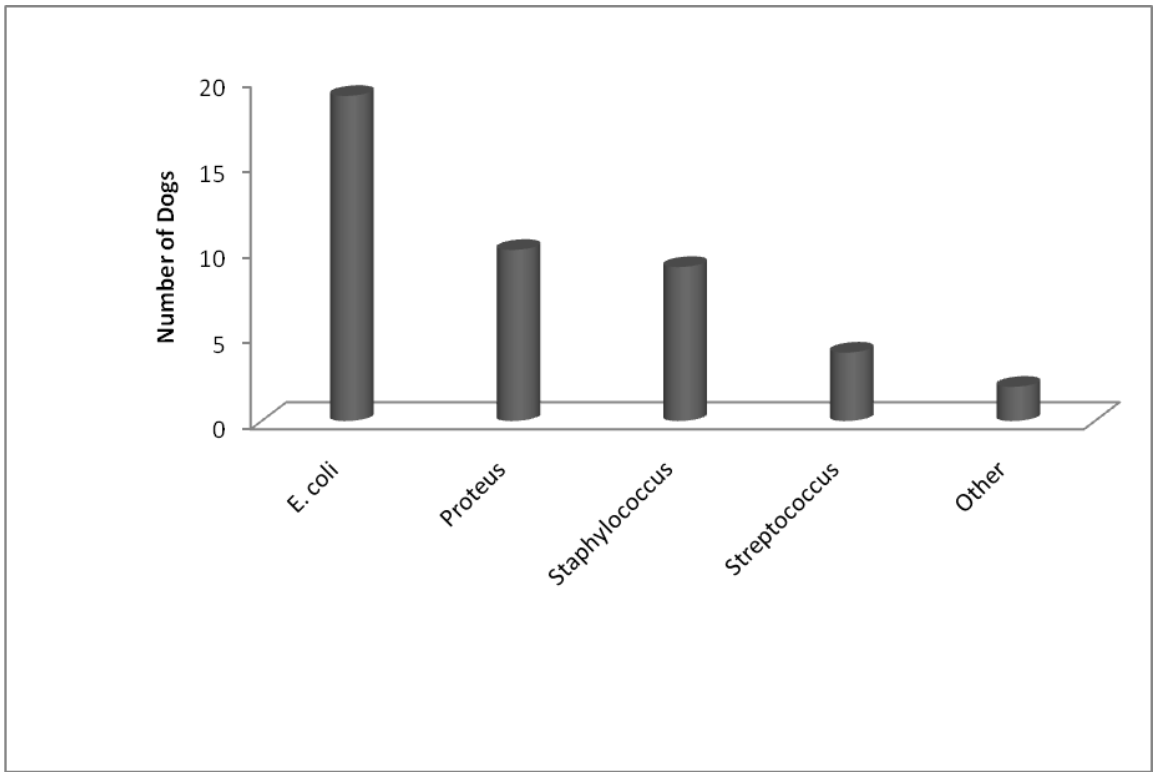
2.3 Results

Due to the unanticipated slow enrollment of clinical cases of uncomplicated UTI, midpoint study analysis was conducted for MS thesis publication. At midpoint, 77 dogs were preliminarily enrolled in the study. 34 dogs were excluded based on negative culture results at D0, and 3 dogs were lost to follow-up after D0. 40 dogs were definitively enrolled and followed to study endpoint. Of these, 2 (5%) were intact females, 5 (12%) were neutered males, and 33 (83%) were spayed females. No intact males were enrolled. The most common breeds (and number) included Labrador retriever (5), Mixed (5), Dachshund (4), Boxer (2), Pug (2), and Golden retriever (2). Mean and median weights were 21kg and 22 kg, respectively (range 5-50kg). Mean and median ages were both 7 years (range, 1-13 years). Mean and median duration of clinical signs prior to presentation were 9.6 days and 4 days, respectively (range, 1-28 days).

Presenting complaints were: pollakiuria in 38 dogs (95%), dysuria in 22 dogs (55%), and hematuria in 21 dogs (53%). 2 (5%) dogs presented with no clinical signs but were enrolled based on positive urine cultures. Common physical examination abnormalities included pain on bladder palpation (24%), vulvar hooding or recession (14%), obesity (5%) and concurrent pyoderma (5%). Other physical exam abnormalities were considered incidental to the study analysis. Complete blood count and biochemical profile abnormalities were considered mild and clinically insignificant to study analysis. Upon urinalysis, 25 dogs (60%) had clinically significant pyuria (>3WBC/hpf) and 34 dogs (81%) had bacteriuria; 24 dogs had both pyuria and bacteriuria (57%). Mean urine specific gravity (USG) was 1.027, median 1.030 (range 1.002-1.052). Mean urine pH was

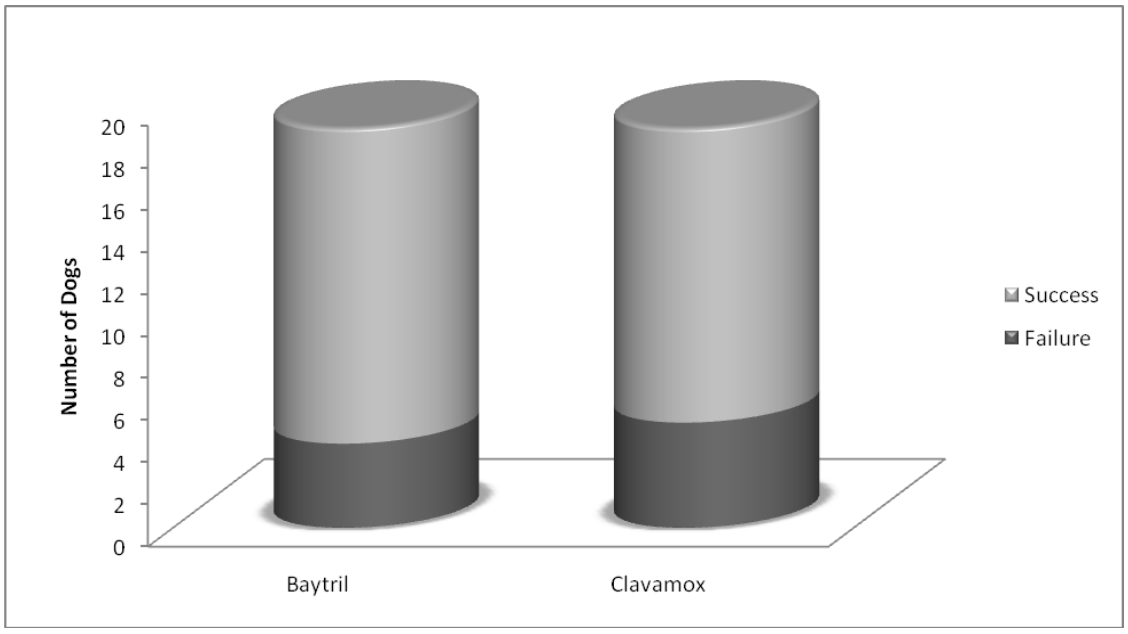
7.2, median 7.0 (range 5.0-9.0). Upon initial culture (D0) 34 dogs yielded one bacterial isolate and 6 dogs yielded two bacterial isolates. *E. coli* was the most frequently isolated urinary tract pathogen in the dogs enrolled (42%) followed by *Proteus* spp. (23%), *Staphylococci* (21%), and *Streptococcus* (8%). *Enterococcus* spp. and *Citrobacter* spp. were isolated in 1 dog each. Culture results are shown in Figure 2.

Figure 2: Canine UTI Isolates



A total of 19 dogs treated with enrofloxacin and 19 dogs treated with amoxicillin-clavulante were followed to study completion. 2 dogs were withdrawn due to adverse effects. Bacteriological cure was achieved in 15 dogs (83%) treated with enrofloxacin and 14 dogs (79%) treated with amoxicillin-clavulante, respectively. (Figure 3) There was no statistical difference between groups ($p = 1.0$). Clinical cure was observed in all dogs with microbiologic cure, except one: this dog was initially diagnosed with an *E. coli* UTI but signs of polyuria and polydypsia persisted despite sterile urine. This dog was

Figure 3: Microbiologic Cure Rate



subsequently diagnosed with pyelonephritis. One dog with an initial *Proteus* spp. UTI had a positive culture (*Enterococcus* spp.) from enrichment broth only on Day 10. No treatment was initiated since the dog was asymptomatic and D21 urine culture was negative.

2.4 Discussion:

This study reports a high microbiologic and clinical cure for canine uncomplicated UTI using a novel high-dose, short-duration protocol for enrofloxacin (Baytril®). Overall bacterial elimination rate in enrofloxacin treated dogs (83 %) was comparable to rates reported recently for other relatively new antimicrobial agents, including the third generation injectable cephalosporin, cefovocin, given once SQ and pradofloxacin given orally for at least 10 days. [84, 85] The clinical cure rate for HDSD Baytril® was also similar to that reported for marbofloxacin given orally for 10 days for UTI. [86] Amoxicillin/clavulanate given orally for 10 days also had a similar cure rate in a previous study (85%). [86]

Resolution of clinical signs in all treated dogs mirrored microbiologic cure except for the 1 dog with persistent polyuria and polydypsia who was considered a clinical failure and withdrawn from the study. This dog was subsequently diagnosed with pyelonephritis. Pyelonephritis can be difficult to diagnose due to intermittent shedding of bacteria into the bladder. A longer course (4-6 weeks) of antimicrobial therapy is recommended for treatment of pyelonephritis due to sequestration of bacteria within the

kidneys. [2] One dog with an initial *Proteus* spp. UTI had a positive culture (*Enterococcus* spp.) from enrichment broth only on Day 10. No treatment was initiated since the dog was asymptomatic. Treatment of asymptomatic UTI in dogs due to *Enterococcus* spp. is controversial. The urine culture on Day 21 was negative in this dog even after no antimicrobial treatment was given.

Demographics of dogs enrolled showed a wide distribution of ages, weight, and breed. This is a small analysis compared to previous retrospectives, such as the largest which enrolled 8,354 dogs. Female spayed dogs were over-represented, consistent with previous reports. The bacterial species identified during this study were also typical of those described previously for canine UTI. [2, 4, 5] All dogs in this study were otherwise healthy. Complete blood count and biochemical profile were overall unremarkable. This is consistent with previous data, supporting the idea that uncomplicated lower UTI does not usually perturb routine hematologic and biochemical parameters. [7]

The Clinical and Laboratory Standards Institute (CLSI) set MIC breakpoints for enrofloxacin as ≤ 0.5 ug/ml (susceptible), 1–2 ug/ml (intermediate) and ≥ 4 ug/ml (resistant). [12] No antimicrobial resistance to enrofloxacin was observed in any dogs treated with enrofloxacin our study. Thus, empiric use of high dose, short duration enrofloxacin appears to be a rational therapy for uncomplicated UTI in dogs. Efficacy for FQ antimicrobials is dependent on the maximum drug concentration at the site of infection – often described as the Cmax (maximum drug concentration) or AUC (area under the time concentration curve). The duration of time the drug remains above the

minimum inhibitory concentration (MIC) seems less important. The dogma for FQ pharmacodynamics is that clinical cure is based on achieving a Cmax/MIC ratio of at least 8-10 or an AUC/MIC ration of at least 100. This is based solely on achieving clinical cures in a neutropenic mouse model. [57]

Enrofloxacin is nearly 100% bioavailable, when administered orally to dogs. It has a relatively high volume of distribution, and is mainly eliminated by the kidneys, allowing high levels of active drug to be reached in urine. [58] In addition, its primary metabolite, ciprofloxacin also exerts bactericidal effects, and is also renally eliminated. Efficacy of treatment of UTI is logically correlated with urine drug concentrations. Applying MIC interpretive breakpoints for antimicrobial susceptibility, as defined by the Clinical Laboratory Standards Institute, which are based on plasma drug concentrations may be too conservative in UTIs. [47] Furthermore, higher doses of antimicrobials limit the growth of mutant subpopulations *in vitro* and may decrease emergence of antimicrobial resistant bacteria *in vivo*. [57] Future studies are necessary to test this hypothesis in clinical patients.

In conclusion, 20mg/kg Baytril administered orally once daily for 3 consecutive days, was as effective as amoxicillin-clavulanate administered at 13.75-25mg/kg twice daily for 14 consecultive days, in the treatment of uncomplicated bacterial UTI in dogs. Additionally, HDSD Baytril offers the benefit of potentially limiting selection for antimicrobial resistance and increasing owner compliance.

Chapter 3: Urine Concentrations of Enrofloxacin and Ciprofloxacin in Healthy Dogs

3.1 Introduction

Enrofloxacin is a Fluoroquinolone antimicrobial drug approved for use in dogs with a flexible dose range of 5 to 20 mg/kg once daily for treatment of urinary, skin, and soft-tissue infections. Enrofloxacin is one of four Food and Drug Administration (FDA) approved fluoroquinolones in the United States for use in dogs. Enrofloxacin is rapidly and widely distributed in the dog, and it achieves high intracellular and tissue concentrations. [59] The volume of distribution for enrofloxacin is 3.63 ± 1.17 L/kg after 1.24 mg/kg/hour IV infusion in dogs. [55] A volume of distribution above 1.0 L/kg implies a higher drug concentration outside of the plasma compartment. [59] Enrofloxacin is distributed in all body fluids and tissues and reaches particularly high concentrations in urine and bile, as well as in the liver and stomach, relative to plasma. [58] Its spectrum of activity also makes it a useful antimicrobial in dogs because it has bactericidal activity against many Gram-negative aerobes as well as some Gram-positive and intracellular bacteria. Enrofloxacin is nearly 100% bio-available after oral dosing. [59, 87] The half-life of enrofloxacin and its post-antibiotic effect, make it suitable for once-daily oral dosing. [85] Furthermore, enrofloxacin has a high safety profile with a low incidence of side effects.

[88] Ciprofloxain is the primary active metabolite of enrofloxacin, and contributes in an additive fashion to overall antimicrobial activity. [56]

The bactericidal activity of fluoroquinolones is concentration dependent and thus their efficacy is dependent on maximizing drug concentration at sites of infection. [57] The pharmacodynamic parameters of maximum plasma drug concentration (Cmax) and area under the concentration time curve (AUC_{0-24}) is correlated with clinical efficacy in a neutropenic mouse model. In this model, neutropenia (<100 neutrophils/μL^3) was induced in 6 mice by intraperitoneal injection of cyclophosphamide, and then 10^6 colony forming units of *E. coli* were injected into 1 or both thigh muscles of each mouse. Statistical analysis (Two-way analysis of variance) revealed that drug efficacy was significantly increased with increasing enrofloxacin total dose, but not with increasing dose frequency. [57]

Plasma concentrations of enrofloxacin are available for 2.5, 5, 7.5, 10, and 20mg/kg doses of oral enrofloxacin in healthy dogs. [50, 56] Information in the drug monograph is limited to plasma and tissue drug concentrations after 2.5mg/kg doses in 2 healthy dogs. [50] Information regarding drug concentrations in other body tissues following higher doses is sparse. [56, 88] Therefore, treatment recommendations are often based on plasma drug concentrations regardless of the site of infection. [58] Dosage regimens that optimize antimicrobial concentrations at the infection site may be better for predicting clinical success. [58] The primary route of excretion of enrofloxacin is in the urine, making it effective for treating UTIs because of the high drug concentrations

attained within the urinary tract. [54] Other antimicrobial agents commonly used in the treatment of canine UTI also attain urine drug concentrations that are exponentially higher than in plasma. This is the reason why successful antimicrobial treatment for UTI can occur with an infecting microbe that would be classified as resistant when considering concentrations of drug attained in other tissues. This has been shown with antimicrobials such as penicillin, and provided the basis for measuring urine concentrations of other antimicrobial agents commonly used for canine UTI. [1]

Despite the common use of enrofloxacin for canine urinary tract infections, there is limited information on urine concentrations of active drug following oral enrofloxacin dosing. Enrofloxacin, and its main active metabolite ciprofloxacin, are both found in higher concentrations in tissues and urine relative to plasma following oral and intravenous enrofloxacin administration. [50, 58, 87, 88] Urine levels following oral administration of 2.5mg/kg and 5mg/kg enrofloxacin have been measured in 1 study each. [50, 87] Following a dosage of 2.5mg/kg, mean urine enrofloxacin concentrations, measured in 2 dogs, were 43µg/ml at 2 hours and 55µg/ml at 8 hours post oral administration. [50] This is markedly higher than the 2 hour plasma concentration of 0.67µg/ml. [50] The time to reach peak serum levels after oral administration in fasted dogs varies but generally occurs between 1 to 3 hours after administration. [88] In one study, enrofloxacin and ciprofloxacin peak urine levels were over 100 times higher than in the plasma following 50mg IV and oral administration of enrofloxacin in 8 healthy Beagles (mean weight 8.6 ± 0.2kg). [87] Peak urine drug levels were measured 6 hours post

administration. In this study, both enrofloxacin and ciprofloxacin levels were measured by HPLC, but only calculated totals of enrofloxcain + ciprofloxacin are published. Methods used, exact measurements, and time-points chosen for urine levels were not provided. [87]

It has been suggested that urine concentrations of enrofloxacin and ciprofloxacin increase linearly with increasing doses similar to plasma. If so, urine drug concentrations following higher doses of enrofloxacin could be extrapolated based on the existing studies performed using 2.5mg/kg and 5mg/kg dosages. [59] Mean urine enrofloxacin and ciprofloxacin concentrations of two dogs were measured two hours following intravenous enrofloxacin administration, and were approximately equal (44 µg/ml of enrofloxacin and 43 µg/ml of ciprofloxacin). [50] One study evaluated enrofloxacin and ciprofloxacin concentrations in tissues and body fluids after 20mg/kg enrofloxacin was given intravenously in 4 anesthetized dogs. Enrofloxacin concentration in urine was 7 times higher than in plasma, while ciprofloxacin concentration in urine was 13 times higher than in plasma. [58] These findings are not consistent with the hypothesis that urine drug concentrations increase in a linear fashion according to dosage, as the levels measured after a 20mg/kg dose were comparable to those attained following a 2.5 mg/kg dosage. [50] However, the quantitative methods employed by each of these studies were different; the drug levels following the 2.5 mg/kg dosage in these earlier studies were conducted by bioassay versus high-performance liquid chromatography after a 20mg/kg dose. Drug levels measured by bioassay are expected to be higher since this assay utilizes a

quantified decrease in susceptible bacterial numbers to measure the total amount of active drug; a bioassay would measure combined enrofloxacin + ciprofloxacin antimicrobial killing. In contrast, HPLC measures each drug metabolite separately (ie individual enrofloxacin and ciprofloxacin concentrations). In addition, drug concentrations measured after the 20mg/kg dose were performed in anesthetized dogs. [58] The extent to which the drug levels attained in anaesthetized dogs apply to a UTI patient population is unknown. General anesthesia can lower cardiac output and blood pressure, re-distribute blood flow from the splanchnic organs to the brain and heart, and cause tissue hypoxia and hypercarbia. [58] This is particularly relevant to drug metabolism, urine production, and drug clearance. Furthermore, urine concentrations were only evaluated at 1 point in time after 20mg/kg enrofloxacin (2 hours post-administration). [58] To the author's knowledge, urine concentrations in dogs following 20mg/kg oral administration of enrofloxacin are not published.

The purpose of this study was to measure the concentrations of enrofloxacin and its main metabolite, ciprofloxacin, in the urine of 6 healthy dogs, after oral administration of 20mg/kg Baytril® tablets (enrofloxacin, Bayer Animal Health).

3.2 Materials and Methods

3.2.1 Dogs and Treatments

Six healthy adult dogs were enrolled. These were otherwise healthy dogs owned by faculty/staff of the OSU-VTH and enrolled voluntarily with full disclosure of the

project protocol. All experimental protocols were approved by the University Laboratory Animal Care Committee. Dogs were considered clinically normal based on results of owner history, physical exam, complete blood count, serum biochemical analysis, and urinalysis. No concurrent medications were given during the study period.

Food was withheld for 12 hours prior to dosing to ensure an empty stomach and optimal oral absorption. Free access to water was allowed. Feeding has been shown to slow the absorption time but does not interfere with peak drug concentration in enrofloxacin. [56] Each dog was weighed immediately before treatment and a single oral dose of Baytril® flavored tablets was calculated (Bayer Animal Health) at 20mg/kg rounded down to the nearest multiple of 22.7mg to ensure FDA dosage approval. The dogs' bladders were emptied by voluntary voiding and confirmed as empty with palpation prior to dosing. Vomiting occurred after oral dosing in the first study dog, so the protocol was amended to give the drug dose with a small amount (estimated 1 tablespoon) of canned food. The exact amount and type of food given was not standardized. The dog that vomited was re-dosed 1 week later for study analysis according to the new protocol.

Urine was collected by free-catch sample for measurements of enrofloxacin and ciprofloxacin at 2, 8, and 24 hours post-administration. An additional blood sample (1 ml) was obtained 2 hours post-administration to coincide with the anticipated peak plasma concentration of the drug, to ensure that drug was absorbed from the gastrointestinal tract. After 2 hours, the dogs were allowed access to maintenance food

and water since this should no longer affect absorption; moreover we aimed to simulate realistic conditions in canine patients and document the associated variability in urine FQ concentrations in these animals. To minimize variability associated with clearance of the drug from the bladder itself, each dog was allowed to urinate at hour 2, 8, 14, and 24. The timing of urine collection was chosen based on plasma disappearance half-life for enrofloxacin (4.6-5.2 hours) and ciprofloxacin (8.8-10.7 hours) following oral enrofloxacin dosing since the drug is eliminated through the urine. The plasma disappearance half-life has been shown to be constant at doses 7.5, 10, and 20mg/kg of oral enrofloxacin. [77] The plasma and urine samples (1.0mL) were collected in heparin anticoagulated tubes. The samples were stored prior to shipment at -80°C. The samples were then shipped to North Carolina State University Department of Molecular Biomedical Sciences for drug analysis by overnight mail and packaged on ice. All samples were analyzed within 30 days of collection.

3.2.2 Determination of plasma drug concentrations

Reverse phase high-performance liquid chromatography (HPLC) was performed to determine urine and plasma levels of enrofloxacin and ciprofloxacin. Samples were centrifuged at 805 *g* for 10 min, after which the plasma was extracted and stored at –70 °C pending analysis. Plasma was assayed for enrofloxacin and ciprofloxacin simultaneously using reverse-phase high-pressure liquid chromatography with UV detection. [89, 90] The system consisted of a pump (Agilent series 1100 system quaternary

solvent delivery system, Agilent Technologies, Wilmington, DE, USA), an automated sampling system (Agilent Series 1100 series system, Autosampler, Agilent Technologies), and a UV light detector (Agilent Series 1100 series system variable wavelength detector, Agilent Technologies). A 4.6-mm × 15 cm reverse-phase column (Zorbax Rx-C8 Column, MacMod Analytical, Chadds Ford, MA, USA) was used to separate the drugs from other plasma components. The eluate was monitored using an UV detector at a wavelength of 279 nm. Each drug was extracted from the plasma using solid-phase extraction cartridges (Oasis, Waters Corp., Milford, MA, USA). The cartridges were conditioned with 1.0 mL of methanol followed by 1.0 mL of deionized water, and then each sample was washed with a mixture of deionized water:methanol (95 : 5). Each drug was eluted from the cartridge with 1.0 mL of methanol, which was evaporated under a flow of nitrogen at 45 °C for 25 min. The dried product was reconstituted with 200 μL of a 15 : 85 mixture of methanol:0.1% trifluoroacetic acid (TFA) in water. The isocratic mobile phase was 77% deionized water, 23% acetonitrile and 0.1% TFA, and the flow rate was 1 mL min−1. Retention time for enrofloxacin and ciprofloxacin was approximately 3.0 and 4.0 min, respectively. Chromatograms were integrated with a computer software program (Agilent Series 1100 Chem Station software, Agilent Technologies). Calibration curves of peak height versus concentration were calculated by use of linear-regression analysis. New calibration graphs for the range of 0.05 to 5.0 μg mL−1 for enrofloxacin and 0.05 to 2.0 μg mL−1 for ciprofloxacin were made for each assay by use of pooled canine plasma. Analytical reference standards for

enrofloxacin (enrofloxacin reference standard, Bayer Corporation) and ciprofloxacin (US Pharmacopeia, Rockville, MD, USA) were used to make calibration standards and to fortify quality control (QC) samples. The limit of quantification, defined as the lowest detected concentration resulting in a coefficient of variation < 20%, was 0.05 μg mL−1 for both enrofloxacin and ciprofloxacin.

3.3Results

The 6 dogs enrolled ranged in age from 4-9 years old (average 4.7 years), and were between 8 and 30kg (average 15.6 kg). They were sex mixed with 3 neutered males and 3 spayed females. Breeds included Boston terrier (2), Beagle (1), Cavalier King Charles Spaniel (1), Australian Cattle Dog (1), and Labrador retriever mix (1). All dogs were otherwise healthy. No significant abnormalities were present on physical examination. Complete blood count and biochemistry profile at time 0 were also within normal limits. Urinalysis at time 0 showed varying urine concentrations from 1.017 to 1.047 (average 1.037) and was otherwise within normal limits. Mean and median single oral enrofloxacin doses given were 19.2mg/kg and 19.4mg/kg, respectively (range 19.0-19.8 mg/kg).

The highest urine levels of enrofloxacin measured for each dog were present after 2 hours in 2 dogs, after 8 hours in 3 dogs, and after 24 hours in 1 dog. Enrofloxacin peak levels for each dog were averaged; mean peak urine enrofloxacin concentration was 138.7 μg/mL (range 73.0 μg/mL – 226.0 μg/mL). The highest urine levels of

ciprofloxacin measured for each dog were measured after 8 hours in 4 dogs, and after 24 hours in 2 dogs. Ciprofloxacin levels in the urine reached a mean value 370.9 µg/mL in 6 healthy dogs (range 200.5 µg/mL – 638.9 µg/mL). Values for enrofloxacin (Figure 4) and ciprofloxacin (Figure 5) at all timepoints are provided.

Figure 4: Enrofloxacin Urine Concentrations in 6 Healthy Dogs After 20mg/kg Single Oral Dose Enrofloxacin.

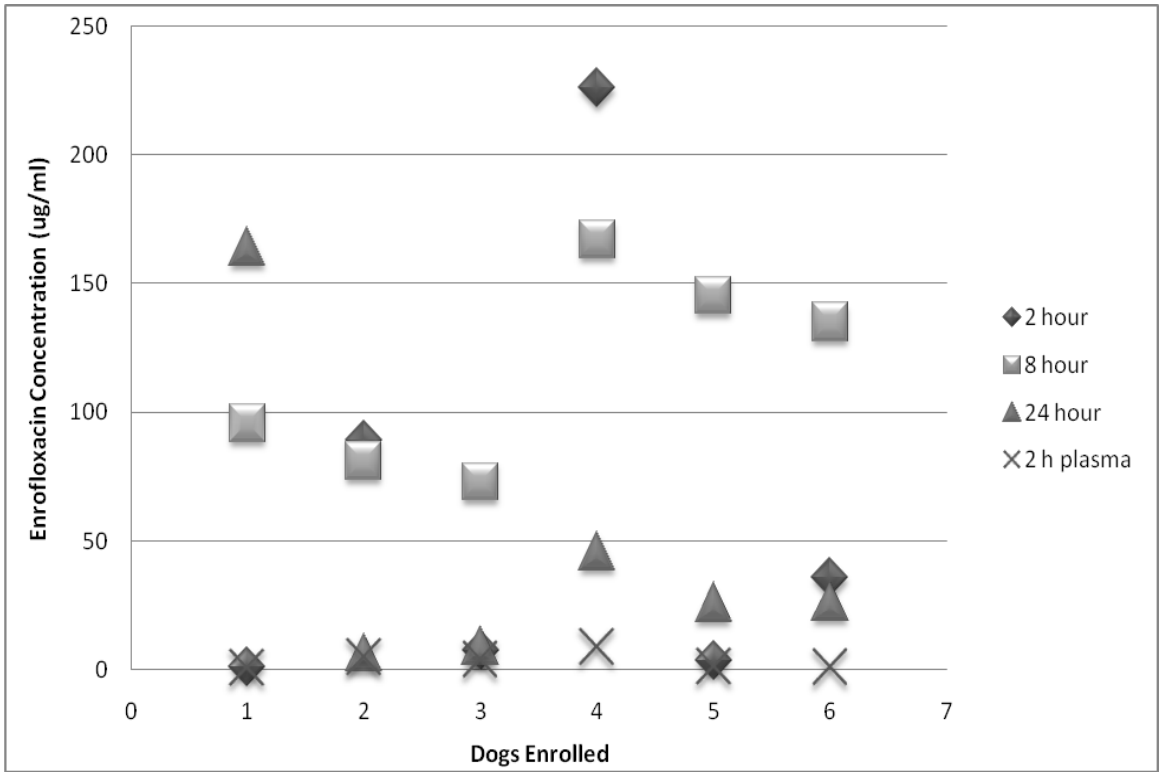
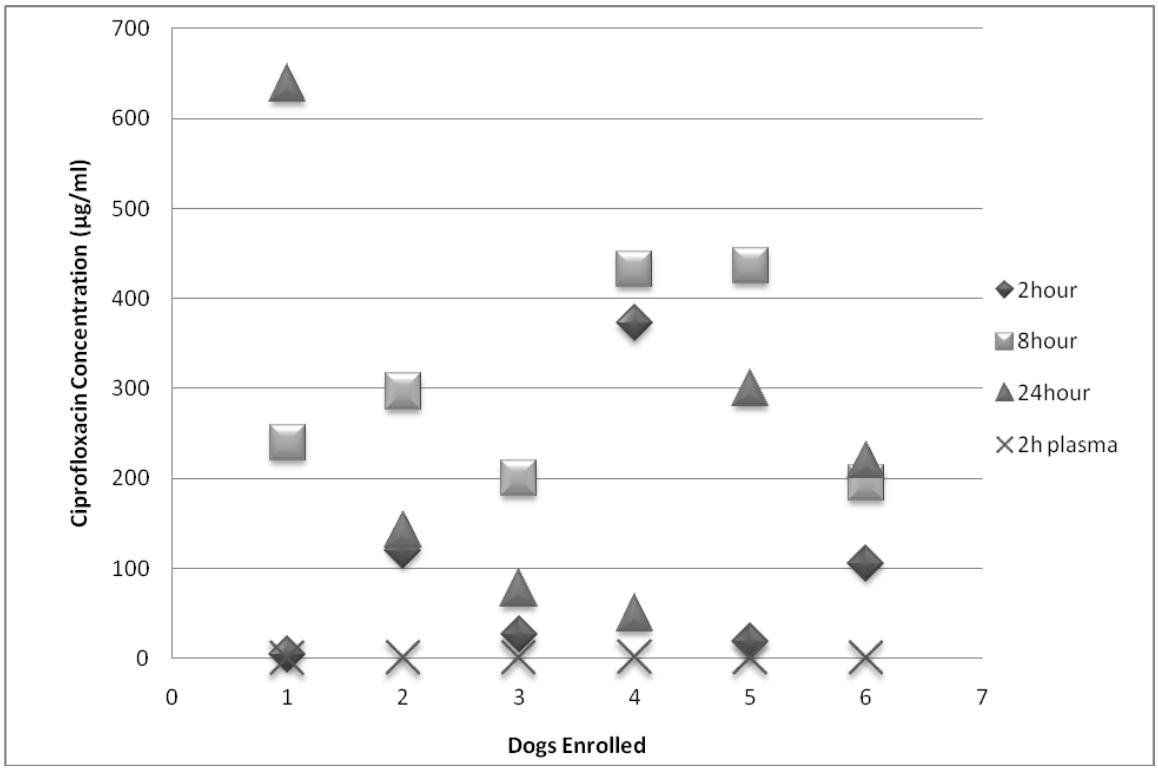


Figure 5: Ciprofloxacin Urine Concentrations in 6 Healthy Dogs After 20mg/kg Single Oral Dose Enrofloxacin



Average plasma level of enrofloxacin at 2 hours was 3.4 µg/mL (range 0.7 µg/mL – 8.9 µg/mL). Average plasma level of ciprofloxacin at 2 hours after oral dosing was 0.5 µg/mL (range 0.18 µg/mL – 0.96 µg/mL). Plasma measurements were performed to confirm systemic absorbtion post oral administration and were intended to approximate peak plasma concentrations.

3.4 Discussion

Urine concentrations of antimicrobials are more relevant than serum concentrations for predicting success of therapy for UTI in humans. [47] Efficacy of treatment of UTI in dogs is also logically correlated with urine drug concentrations. The data from these 6 dogs showed exponentially higher urine concentrations of enrofloxacin and ciprofloxacin compared to plasma concentrations, 2 hours after a single 20mg/kg dose of oral enrofloxacin was administered. The urine drug concentrations also continued to peak at 8 and 24 hours in most of the dogs. We chose to measure enrofloxacin and ciprofloxacin in plasma 2 hours post-administration to approximate peak plasma levels. Peak plasma levels require continuous drug measurements due to variability in individual dogs, and peak plasma enrofloxacin and ciprofloxacin concentrations are reported at a mean of 2 hours with an approximate range of 1-3 hours. We also compared our peak urine concentrations measured for each dog, to mean serum enrofloxacin and ciprofloxacin levels previously reported in dogs following a 20 mg/kg oral dose of enrofloxacin (previously reported maximum concentration (Cmax) mean of 4.7 µg/mL for enrofloxacin and 2.0 µg/mL for ciprofloxacin in 6 healthy dogs). [56] Our measured urine levels were also markedly higher than serum levels in these dogs. These results parallel previous data on urine concentrations following 5mg/kg doses of enrofloxacin in dogs, with additive peak urine concentrations of enrofloxacin and ciprofloxacin reaching over 100x that measured in plasma. [87]

Ciprofloxacin was the major antimicrobial agent in the urine after oral enrofloxacin administration during all the measured time-points. Ciprofloxacin is the major metabolite of enrofloxacin, and is antimicrobially active with the same mechanism of action as its prodrug; in a patient the activities of the two drugs are additive. [56] Ciprofloxacin contributes to antimicrobial activity following enrofloxacin administration, but there are discrepancies regarding the contribution of ciprofloxacin to total antimicrobial killing. Early studies approximated minimal contribution from ciprofloxacin. Two more recent studies found that ciprofloxacin contributed more than 30% of drug concentration in plasma after oral dosing, and 34% in serum after 20mg/kg was given IV to anesthetized dogs. [56, 58]

In our study, ciprofloxacin levels were lower than enrofloxacin levels in plasma. In the anaesthetized dog study, ciprofloxacin concentrations were lower than enrofloxacin concentrations in all tissues with the exception of urine and gall bladder. Ciprofloxacin is less lipophilic and thus cannot penetrate cell membranes as well as enrofloxacin. This could explain the higher ciprofloxacin urine concentrations since more ciprofloxacin would reside in extracellular fluid rather than being sequestered in intracellular fluid. Drug measurements by reverse phase HPLC allowed us to accurately determine concentrations of enrofloxacin and ciprofloxacin separately. Some of the earlier studies measured enrofloxacin concentrations via bioassay, which measures all of the active metabolites in the measurements. Such earlier studies cannot be used for accurate comparison of drug levels. [88]

We also noted marked variability in urine concentrations of enrofloxacin and ciprofloxacin between individual dogs and no single time-point for peak urine concentration was identified among the 6 dogs in the study. Therefore, we identified peak urine concentrations for each individual dog and used these for maximum drug levels in our analysis. Time of peak drug concentration in urine after oral administration was variable among the six dogs in this study, and likely differs widely in the general dog population as well as covarying with hydration status and rate of urine production. [1] Since it is not practical to conduct measurements of antimicrobial concentration of urine in every single patient, expected urine concentrations in dogs with normal renal function may be helpful in predicting therapeutic outcome.

Wide variation in plasma drug levels was also noted in our study. However, such wide variability was also appreciated in earlier studies, despite having standardized conditions and breed-standardized subjects. [59] Sources of variability likely include efficiency of intestinal absorption (with or without food), core metabolism, and elimination rates (how many times the patient is allowed to urinate, urine concentrating ability). These factors likely contributed in our study. We used volunteer dogs owned by OSU-VTH staff and students, and the study was performed using these dogs as outpatients. Furthermore, although the dogs were fasted for 12 hours prior to drug dosing, a non-standardized amount of dog food was given with the study drug to prevent drug-induced emesis. After 2 hours, the dogs were allowed free access to food and water. Although the study protocol elaborated on times allowed for urination, we relied on the

dog owners to comply with the study protocol. There may be differences in drug disposition between male and female dogs which is why we chose an equal mix of male and female dogs. [91] An additional weakness of the present study was that urine concentrations were measured only following a single dose. It is questionable whether enrofloxacin accumulates with repeated dosing. One study found no effect from repeated dosing after 15 days of consecutive dosing compared to a single dose in healthy dogs. [87] This study first measured drug levels achieved with one intravenous 50mg dose. After a drug-washout period, they administered 50mg enrofloxacin once daily for 15 consecutive days to the same 8 dogs. They compared drug levels from a single dose to those attained after 15 doses. Their analysis demonstrated no significant difference in drug levels after repeated doses, demonstrating that enrofloxacin levels may not accumulate with repeated dosing. [87] A different study found a higher Cmax when comparing drug concentrations after the seventh dose versus the first dose when 2.75, 5, and 11mg/kg enrofloxacin was given orally twice daily to 6 healthy dogs. [88]

Although more convenient, plasma drug concentrations are less valuable than determining active drug concentrations at the infection site. [58] Efficacy of treatment of UTI is logically correlated with urine drug concentrations. Applying MIC interpretive breakpoints for antimicrobial susceptibility, as defined by the Clinical Laboratory Standards Institute, which are based on plasma drug concentrations may be too conservative in UTIs. [47] The results from our study may be useful to guide enrofloxacin therapy for canine UTI since current clinical breakpoints are based on plasma drug

concentrations. Urine concentrations of ciprofloxacin and enrofloxacin greatly exceed clinical MICs of enrofloxacin for many specific bacteria cultured from the urinary tract of dogs. [92] This approach is already used in the clinical microbiology laboratory at the University of California-Davis, leading to less conservative clinical breakpoints for urinary pathogens.

The minimum inhibitory concentration (MIC) is the lowest concentration of an antibiotic that prevents visible growth after 18–24 hours of incubation. [47] The therapeutic strategy for antimicrobial drugs is to achieve drug concentrations above the minimal inhibitory concentration (MIC) during the dosing interval. This is simulated *in vitro* using drug concentrations known to be achieved in plasma. For the fluoroquinolones, studies integrating pharmacokinetics and pharmacodynamics (PK/PD) have shown that the area under the concentration-time curve (AUC)/MIC and the maximum concentration of drug in serum (Cmax)/MIC are the most important pharmacodynamic indices predicting bacterial killing. Maximizing the Cmax to MIC ratio has also been linked to decreased development of resistance, and a target Cmax of 8 to 10 times MIC was chosen on the basis of evidence of bacterial regrowth at ratios < 8:1. [57] In addition to killing bacteria in a concentration dependent manner, the fluoroquinolones also have a substantial postantimicrobial effect in which the rate of bacterial growth remains suppressed even after elimination of the antimicrobial, allowing for a longer dosing interval. [89]

The Clinical and Laboratory Standards Institute (CLSI) MIC breakpoints in µg/mL for enrofloxacin are ≤0.5 (susceptible), 1–2 (intermediate) and ≥4 (resistant). [12] It is recommended that the mean antimicrobial concentration available at the site of a urinary tract infection should exceed 4 x MIC for bacteria to be susceptible to that agent. [93] Nevertheless, MIC breakpoints for susceptible bacteria may be different for urinary tract infections, allowing enrofloxacin treatment for infections previously classified by CLSI as resistant. Data showing microbiologic and clinical cure from NCCLS "resistant" canine UTI with high doses of enrofloxacin would be necessary to support this hypothesis. Furthermore, new MIC standards using ciprofloxacin may be beneficial for optimal treatment of canine UTI.

3.5 Ongoing Research

Clinical and experimental studies indicate that suboptimal antibiotic dosage regimens may be a significant risk factor for emergence of resistance. [46, 57] Hence, it is important to optimize dosages, not only with respect to achieving a therapeutic effect, but also with respect to minimizing resistance development. If urine enrofloxacin concentrations achievable using the high limit of the labeled dosing range are sufficient to prevent residual growth of common canine uropathogens, even after first-step mutations have occurred, then this therapeutic strategy will prevent selection of resistance during treatment.

Additional studies are ongoing. We are comparing the urine ciprofloxacin concentrations attained after oral enrofloxacin administration to the minimum inhibitory concentrations (MICs) of 50 clinical isolates from canine *E. Coli* UTI with varying levels of susceptibility. We also plan to measure Mutant Prevention Concentration (MPC) for 10 of these isolates with a range of MIC values. Mutant prevention concentrations have not been evaluated across a diverse collection of canine urinary tract infection isolates. This information may be useful to help determine the appropriate doses of enrofloxacin for the treatment of canine UTI while considering mutant prevention guidelines for prevention of antimicrobial resistance. This may also change interpretation of MIC susceptibility testing since it is based on plasma drug levels. Establishing these criteria for canine UTI may provide guidance for veterinary practitioners using flexible dose-range FQ drugs.

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