THE EFFICACY OF SHORT TERM AMOXICILLIN THERAPY AND THE EFFECT OF FUROSEMIDE ON CONVENTIONAL ANTIBIOTIC THERAPY IN EXPERIMENTALLY INDUCED BACTERIAL LOWER URINARY TRACT INFECTION IN CATS

by

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(ABSTRACT)

The efficacy of short term (3 day) oral amoxicillin therapy was compared to conventional (14 day) oral therapy in an experimental model of bacterial lower urinary tract infection (UTI) in the cat. Chemical cystitis was induced using an infusion solution of salicylic acid, 70% ethanol, and normal saline via transabdominal cystocentesis. Cats were challenged with a Staphylococcus intermedius inoculum twenty-four hours later introduced via urethral catheterization. Serial quantitative aerobic bacterial urine cultures obtained via cystocentesis were used to evaluate groups of cats.
Eighteen adult cats (9 males and 9 females) were divided into 3 groups of 6 cats (3 males and 3 females): Group I = conventional amoxicillin therapy (14 day), Group II = control group (no treatment), and Group III = short term therapy (3 day). Results indicated the conventional therapy successfully eradicated infection, however, the short term therapy did not eradicate infection when compared to controls. During the study period the diuretic furosemide was used in some cats to facilitate cystocentesis procedures. Those cats were observed to exhibit less stranguria, which is a common sign of lower UTI.

The second study evolved from observations made in the first study and evaluated the effect of furosemide on conventional antibiotic therapy in an experimental model of bacterial lower UTI in the cat. A similar experimental design was utilized with Group I = control group (no treatment), Group II = oral furosemide (14 day), and Group III = oral furosemide and oral amoxicillin (14 day). Statistical analysis failed to demonstrate the efficacy of the furosemide and amoxicillin combination, but showed furosemide alone was not an appropriate therapy when compared to controls. It was again observed that those cats receiving furosemide showed fewer secondary signs of lower UTI such as stranguria which suggests a possible role for furosemide as adjunct therapy in the treatment of lower UTI in the cat.
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I. SECTION I. BACTERIAL LOWER URINARY TRACT INFECTION IN CATS

Definition

Except in the distal portion of the urethra, the urinary tract does not normally contain bacteria.\textsuperscript{1,2,3,4,5} Urinary tract infection (UTI) exists when a portion of the urinary tract that is normally sterile is colonized by bacteria. Infection of the urinary tract is usually caused by bacterial organisms that are microfloral constituents of the intestinal or lower urogenital tracts.\textsuperscript{5} Infection is usually caused by a single bacterial species, except in complicated infections which may be secondary to anatomic or functional abnormalities of the urinary tract.\textsuperscript{5}

A functional definition of the lower urinary tract divides the urinary system at the junction between renal parenchyma and renal pelves, thereby considering the entire excretory pathway (including renal pelvis, ureters, urinary bladder, and urethra) as the lower urinary tract.\textsuperscript{6} This classification matches the biologic division of basic functions within the urinary system. Lower urinary tract disease may affect the ureter and renal pelvis, although the bladder and urethra are the more common sites of involvement.\textsuperscript{6}
Incidence

Disease of the lower urinary tract is a common health problem in cats. Unlike dogs and humans, bacterial infections are an uncommon inciting cause of lower urinary tract disease in cats; however, cats commonly develop bacterial urinary tract infections as a complication of pre-existing lower urinary tract disease. The actual incidence of feline lower urinary tract disease is unknown. It appears to exceed 0.5% of the domestic cat population/year, but is less than 1%/year. The proportion of cases of bacterial urinary tract infection is also unknown due to inconsistencies in criteria used by various investigators for diagnosis of UTI in cats. As previously stated, it is generally accepted that bacterial UTI is rarely present at the outset of episodes of feline urinary tract disease, but is often present subsequently.

Pathogenesis

Bacteria usually invade the urinary tract by ascending through the lumen of the excretory pathway. Bacteria also occasionally invade via hematogenous spread or by extension from adjacent tissues. Development of UTI indicates an alteration in the host and bacterial flora interrelationship. Precipitating causes may include underlying disease of the urinary system, alterations in host immune competence, and an increase or change in bacterial virulence. Bacteria most commonly associated with feline UTI include Escherichia coli, staphylococci,
streptococci, *Proteus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp., and *Pasteurella* spp.\(^1-3,6,12,15-18\)

Three major sequential phases characterize development of bacterial UTI. The first of these, the contamination phase, begins when bacteria arrive in any part of the urinary tract that is normally sterile. If local conditions are favorable, bacteria survive, grow, and initiate the colonization phase. Colonization may initially involve either tissues of the urinary tract or urine, but will usually ultimately involve both.

Colonization by a single type of bacteria usually occurs, but multiple species may be involved on occasion. Recent evidence supports a role of bacterial adherence in colonization of vaginal and urethral surfaces\(^19,20\) and uroepithelial cells.\(^5,13,14,21-23\) When bacteria approach the uroepithelial surface they encounter a number of attractive forces which bring them into close apposition with the uroepithelium. Attractive forces due to fluctuating dipoles of similar frequency on both bacterial and epithelial surfaces are active over a relatively long distance. Long hydrophobic molecules extending from bacterial surfaces are attracted to the phospholipid epithelial cell membranes. Both of these forces tend to bring bacteria into close apposition with the uroepithelium; however, close apposition may be prevented by strong repulsive forces since both the bacterial cell wall and the uroepithelium are negatively charged. Bacterial adherence to epithelial surfaces is achieved by molecular binding between bacterial fimbriae or fibrillae and specific receptor sites on the uroepithelial surface. Fimbriae and fibrillae are long,
filamentous projections found on gram-negative and gram-positive bacterial cell walls, respectively. Specific molecular structures on the fimbriae and fibrillae, called adhesins, bind with complementary receptor sites on epithelial cell surfaces. Bacteria become permanently attached when a sufficient number of adhesin-receptor interactions occur. Bacterial adherence is essential to subsequent tissue invasion.  

Bacteria may multiply in their new habitat if urine provides a favorable medium for their growth. The final and symptomatic phase of UTI occurs when inflammation develops in urinary tract tissues because of infection. Pain, altered patterns of micturition (pollakiuria, stranguria), and abnormal urine (hematuria, pyuria, proteinuria) characterize this phase.  

However, urinary tract inflammation has causes other than bacterial infection.  

Urinary tract infections do not always produce clinical signs of inflammation. If bacterial growth is confined to urine it may produce little or no inflammation in surrounding tissues. This phenomenon has been termed asymptomatic bacteriuria.  

**Lower Urinary Tract Defense Mechanisms**

The status of host defense mechanisms is an extremely important factor in the pathogenesis of UTI. Feline bacterial UTI's may be uncommon because cats appear to have extremely effective local host defenses that serve as a barrier to urinary tract infection with bacteria. It is when disruption of these defense mechanisms occurs that bacterial infections are most likely to develop.
The hydrodynamics associated with voiding urine are thought to represent one of the most important natural defense mechanisms against infection of the urinary tract. Mechanical washout induced by unimpeded, frequent, and complete voiding of urine inhibits bacterial colonization of the urinary tract by rapidly eliminating organisms that reach the lumen of the proximal urethra and urinary bladder. Micturition also reduces the population of bacteria lining the urethral mucosa by flushing the urethral lumen with sterile urine. The effectiveness of voiding is dependent on the rate of urine production and flow, the frequency of voiding, the completeness of emptying, and the rate of bacterial multiplication.

Factors interfering with normal micturition, and thus predisposing to UTI, include mechanical obstruction to outflow (urooliths, urethral plugs and strictures, herniation of the urinary bladder, prostatic anomalies, obstructing urothelial neoplasms) and incomplete emptying of the excretory pathway (damaged innervation, anatomic defects). Decreased urine volume caused by a negative water balance (dehydration) or primary oliguric renal failure also adversely affect mechanical washout.

Certain anatomic features of the lower urinary tract help to prevent ascending bacterial infections. These include a normal zone of high resting pressure in the middle portion of the urethra, intermittent urethral and ureteral peristalsis, and the ureterovesical "flap valves" created by the oblique course of the ureters through the bladder wall. Scanning electron microscopy in dogs has revealed the presence
of microplicae, or folds, in the proximal urethra that may hinder further migration and colonization of bacteria gaining access to this area.\textsuperscript{4,30} The urethra of male cats appears to aid in defense against UTI. It has been shown that bacterial UTI is more prevalent in cats following perineal urethrostomies as compared to cats managed nonsurgically.\textsuperscript{6,31,32} Potential mechanisms include the use of urinary catheters post-operatively, post-operative stricture formation, licking and self trauma occurring post-operatively, and the anatomical alteration in the urethra itself.\textsuperscript{31} Other anatomic defects such as urethral anomalies, ectopic ureters, and urachal diverticuli may also predispose to UTI.\textsuperscript{2,24,33,34}

Studies of feline urinary bladders have revealed microscopic evidence of urachal remnants located in the wall of the vertex of the urinary bladder from the level of the submucosa to the subserosa. The mechanism responsible for atrophy of the urachus has not been defined; however, microscopic diverticula appear to represent risk factors for the development of macroscopic vesicourachal diverticula.\textsuperscript{34} Grossly visible (macroscopic) vesicourachal diverticula may be limited to the thickness of the bladder wall (intramural) or they may protrude beyond the serosal surface of the bladder (extramural). It is suggested they may be congenital or acquired and are likely to predispose to UTI. The most likely explanation of persistence of a portion of the urachal canal is abnormally high or sustained pressure within the bladder lumen. Possible causes of increased pressure include anatomic or functional (reflex dyssynergia) outflow obstruction of the lower urinary tract,
disorders associated with detrusor hyperactivity, and/or abnormal production of a large volume of urine.\textsuperscript{34}

Epithelial surfaces of the urinary tract have intrinsic defenses against infection that vary with the anatomic site.\textsuperscript{5,6,11,13,14,19,24,35,36} Colonization in the periurethral and distal urethral areas by a resident flora of nonpathogenic bacteria may exclude colonization by uropathogens and protect against UTI. The normal flora is bound to epithelial receptor sites which may limit the accessibility of the epithelial surfaces to pathogens.\textsuperscript{4} Substances produced by the normal flora (bacteriocins) may interfere with the normal metabolism of other strains of bacteria. In addition, the normal flora may consume essential nutrients the pathogenic bacteria require for survival.\textsuperscript{4}

Locally produced antibodies may assist in protection of the urinary tract, however their role is not entirely clear. Bacterial adherence may be prevented by antibody coating. Genital mucosa, urethral mucosa, and possibly other mucosal surfaces of the urinary tract appear to produce secretory immunoglobulin A (IgA).\textsuperscript{4,11,23,24} Defects in production of IgA may be a cause for UTI associated with long term corticosteroid administration or in association with uremia and hyperadrenocorticism.\textsuperscript{24,37}

The bladder mucosa secretes a protective mucopolysaccharide coating from its luminal surface. This layer contains glycosaminoglycans and prevents attachment of bacteria to the uroepithelium.\textsuperscript{4,6,11,14,19,24,35} Production and secretion of the normal glycosaminoglycan layer is under hormonal control by estrogens and progesterones
in some species. If injured, this coating can be replaced within 24 hours. Due to its hydrophilic nature, a layer of water forms at the surface and provides a barrier between the transitional epithelium and the urine, explaining in part why the bladder epithelium can tolerate constant exposure to urine. Disruption of the glycosaminoglycan layer by many mechanisms including urolithiasis, neoplasia, catheterization and other instrumentation, trauma, and irritants, may allow bacterial attachment to occur.

In addition to antibody production and the surface mucopolysaccharide layer, other bladder defense barriers include intrinsic antibacterial properties and normal exfoliation of uroepithelial cells. The desquamated cells and bacteria become trapped in amorphous and fibrin-like strands and are removed from the urinary tract by voiding. Furthermore, the effectiveness of bladder defenses against infection has been hypothesized to be a function of blood flow in the bladder wall. It is suggested that blood flow decreases as the bladder wall is stretched during filling. The resulting decreased blood flow in the bladder wall leads to tissue ischemia and impairs bladder defenses against infection.

The antibacterial properties of urine represent another host defense mechanism against UTI. Unfavorable physiologic conditions for bacterial growth include extremes (high or low) of urine pH, hyperosmolality of urine, high urea content of urine, and the presence of certain weak organic acids in urine derived from diet. If these conditions are altered, urine could become a
good bacterial culture medium. The major determinants of bacterial growth in urine appear to be pH and osmolality. It has been suggested that the infrequency with which bacterial UTI's occur in cats might be related to their typically high urine osmolality.²⁵,²⁹,³⁸

In general bacteria grow well in urine samples with a pH value in the range of 6.0-7.0. Urine pH affects the antibacterial activity of organic acids because an acid pH favors higher concentrations of undissociated acids in urine which reduces bacterial growth. As pH values depart from optimum toward either extreme, bacterial growth is progressively inhibited and the range of osmolality values tolerated diminishes. The range of pH values in which bacteria will grow also diminishes as osmolality departs from the optimum for bacterial growth.⁶,²⁵,⁵⁰

Bacteria are killed by extreme osmolality, particularly by high osmolality. Rod-shaped bacteria generally are less tolerant of high osmolality than are cocci. Bacteriuria in cats with concentrated urine appears to be mainly caused by staphylococci and streptococci.⁶ Bacteriuria that develops in cats with dilute urine is generally due to gram-negative rods.⁶,⁴⁶

**Diagnosis**

Diagnosis of UTI is made by identifying bacteria at a site in the urinary tract in which they normally do not exist. Urine culture is the principal technique used for this purpose, but microscopic examination or bacteriological cultures of affected
urinary tract tissue may also be considered.\textsuperscript{1,21} Cystocentesis is the preferred method of urine collection for bacterial culture, since lower genitourinary tract contamination is avoided.\textsuperscript{5,23,39,51-55} Bacteriuria in urine specimens obtained by cystocentesis is unequivocally abnormal, although inadvertent bowel penetration or skin contamination can occur which may inadvertently affect interpretation of results. Because of the possibility of inadvertent contamination, diagnosis is more certain if urine is cultured quantitatively as well as qualitatively. Quantitative urine culture includes determination of the number of bacteria per unit volume in addition to isolation and identification of the bacteria.\textsuperscript{1-3,5,6,23,29,39,51,56-61}

If the bladder is not palpable and blind cystocentesis fails to obtain a urine sample, urine can be collected via bladder catheterization, however, this usually requires sedation or anesthesia in the cat.\textsuperscript{5} At least $10^3$ colony forming units (CFU) of bacteria/ml should be present to rule out contamination. Midstream collection could also be used to obtain a sample although it is usually difficult to obtain from the cat. Greater than $10^5$ CFU/ml in voided samples would be suggestive of infection if contamination during collection was minimal.\textsuperscript{1,3,5,6,23}

If circumstances prevent rapid processing of samples for culture following collection, immediate refrigeration is recommended. Padilla, et al showed that culture samples refrigerated for 2 hours showed no difference in quantitative counts as compared to samples cultured immediately following urine collection.
Refrigeration for 2 to 6 hours resulted in differing quantitative counts but did less to the identical interpretation of the results.\textsuperscript{62}

The most accurate results of quantitative bacterial cultures are obtained by dilution pour plate methods and surface streaking of media plates with calibrated loops.\textsuperscript{58} Pour plates are prepared by mixing a diluted aliquot of urine with a measured volume of molten agar. After incubation bacterial colonies that appear deep in the agar can be counted. This technique must be combined with surface streaking for organism identification. The use of calibrated bacteriologic loops is less time consuming and allows identification of organisms as well as colony counts. Screening techniques that may be used are either based on bacterial growth on bacteriological cultures such as the dip-slide technique or are based on bacteria-induced chemical reactions such as the pad culture technique and nitrite test.\textsuperscript{23,58}

In order to evaluate a cat for bacterial infection by analysis of urine a complete urinalysis including sediment examination should be performed. Findings suggestive of bacterial UTI include pyuria, hematuria, proteinuria, and bacteriuria, although these may be greatly affected by method of urine collection and nature of the particular infection. Because of the inconsistencies of urine sediment findings, urine culture remains the definitive test for bacterial UTI.\textsuperscript{1,39} Since bacterial UTI is a relatively uncommon primary cause of lower urinary tract disease in cats, detection should arouse suspicion that an underlying cause is present. Further diagnostic
procedures that may be considered include exfoliative cytology of urine sediment, survey and contrast radiography, ultrasonography, and biopsy.2,63

Principles of Antibacterial Therapy

The principles of therapy of UTI in cats are the same as those in other species. Besides recognition and correction of impairments of host defenses, treatment of bacterial UTI requires administration of appropriate antibacterial therapy.15 The treatment must control bacterial growth in the urinary system. This is accomplished by sustaining a concentration of the antibacterial drug in the immediate environment of the bacteria that is sufficient to kill the organism or at least prevent growth until host defense mechanisms may eradicate them. Secondly, control of microbial growth must be maintained until host defenses are capable of preventing colonization of the urinary tract without further administration of the drug.4,12 Formulation of a rational course of therapy ultimately involves selection of an effective antibacterial drug, the proper route of administration, and adequate duration of treatment. Treatment should also be safe and economical.1,2,6,15

Urine concentrations of antimicrobial drugs rather than blood concentrations correlate best with the efficacy of agents used to treat UTI.12,64-66,70,71 The preferred method for selecting drugs for treatment of UTI requires measurement of minimum inhibitory concentration (MIC). The least concentration of a drug that is sufficient to prevent growth of bacteria in vitro is called the MIC of that drug for that
organism. Efficacy of a therapeutic regimen largely depends on its ability to provide a drug concentration in the environment of the bacteria (in vivo) that exceeds the MIC of the drug for the infecting organisms.\textsuperscript{6,12,15,66,69-71} Each drug that probably will be effective is identified by observing that its MIC is less than a susceptibility break point value defined as one-fourth of the mean drug concentration expected in the urine during treatment. Susceptibility break point values have been recommended for numerous drugs on the basis of urinary concentrations of drugs that have been found in otherwise healthy dogs after treatment with specific regimens.\textsuperscript{6,12,54,72,73} Although urinary concentrations of drugs during treatment of cats may be different than what has been found in dogs, relying on canine data is necessary until urinary concentrations of drugs are determined in cats.\textsuperscript{6}

Standardized disk diffusion methods, such as the Kirby-Bauer test, are often used to evaluate bacterial susceptibility to antibacterial agents. Such methods have an important pitfall because results are usually interpreted on the basis of expected blood concentrations of the drugs rather than on the basis of urine concentrations.\textsuperscript{12,74} These methods are qualitative interpretations of susceptibility that will underestimate bacterial susceptibility in sites where the drugs are concentrated physiologically (eg, the urinary tract) or where local infusions or topical applications result in concentrations that exceed usual blood concentrations.\textsuperscript{75}

In healthy animals antibacterial agents may need to be given every 8 to 12 hours to maintain appropriate drug concentrations in the urine. Amoxicillin
maintains concentrations in the urine that are adequate to control bacterial growth when given every 12 hours. Most drugs for the treatment of lower bacterial UTI's are given orally and treatment for 10-21 days is usually adequate for uncomplicated cases.6,12,54,66,72

Recent studies in humans have shown some success with short term or single-dose treatment regimens for lower UTI's in women.76-82 Similar studies in dogs83,84 have shown less promising results although one study did suggest that a three day trimethoprim-sulfa regimen might be a reliable therapeutic regimen for some female dogs with bacterial UTI.83

Although antibacterial therapy is the mainstay of treatment for bacterial UTI, ancillary therapy is sometimes used to complement the beneficial effects of antimicrobial drugs. Ancillary treatments include use of urinary pH modifiers, antiseptics, analgesics, and antispasmodics. These agents are controversial and may have unfavorable side effects in cats.2,6,15,24,26 The induction of diuresis has been proposed to enhance mechanical washout and thereby eliminate organisms in the lumen of the proximal urethra and urinary bladder. Because the dilution of urine may affect antibacterial agent concentration, this mode of therapy remains controversial.29,50,26,85 Newer treatment approaches that may have future applications include analogues that block molecular sites of attachment on bacterial or mucosal cell walls, suppressing the production of bacterial structures such as fimbriae, and
agents that promote development of an immunologic response to the bacterial organisms.$^{4,15,19,86}$
II. SECTION II. AN EXPERIMENTAL MODEL OF BACTERIAL LOWER URINARY TRACT INFECTION IN CATS

Introduction

Experimental models of bacterial urinary tract infection (UTI) in the dog have been reported, and have been used successfully by various investigators. The method used most commonly involves the induction of chemical cystitis followed by inoculation of bacteria.\textsuperscript{83,84,87-89} The principle of this model is to alter the individual's local host defense mechanisms to produce a favorable environment for bacterial growth. Development of chemical cystitis appears to be a critical step in the creation of this model.

An experimental model of bacterial UTI may also be successful in the cat in mimicking natural disease since bacteria primarily invade the urinary tract as a sequela to interruption of the local host defense mechanisms by naturally occurring or iatrogenic events.\textsuperscript{6-10} The induction of chemical cystitis may disrupt the cat's host defense mechanisms and thus allow bacteria to colonize. Similar experimental models of bacterial UTI for the cat have not been reported. The purpose of this study was to create an appropriate model of bacterial lower UTI for use in cats.
Materials and Methods

Cats - Twelve cats (7 females and 5 males) were studied. Prior to entering the study each cat received a physical examination, complete blood count (CBC), serum biochemical profile (albumin, total protein, urea nitrogen, creatinine, total bilirubin, alanine aminotransaminase, serum alkaline phosphatase, sodium, potassium, chloride, carbon dioxide, calcium, phosphorus, glucose), FeLV test (ELISA), urinalysis and quantitative aerobic bacterial urine culture. All urine samples were obtained by transabdominal cystocentesis. All cats entered in the study were free of bacterial urinary tract infections and were normal based on physical examination and laboratory tests.

Induction of Experimental Infection - An isolate of Staphylococcus intermedius (obtained from a cat with a naturally occurring UTI)\(^a\) was used to induce UTI in all cats. The bacteria were grown in trypticase soy broth\(^b\) to a density of \(10^9\) colony forming units (CFU)/ml (mid-log phase), washed twice in phosphate buffered saline (pH = 7.4), and suspended in saline to a final concentration of \(2 \times 10^8\) CFU/ml for the inoculum. A modification\(^c\) of a procedure used previously in dogs to induce UTI was used.\(^83,84,87-89\) The cats were anesthetized with 30 mg of ketamine\(^d\) (IM). A single dose of furosemide\(^e\) (2.2 mg/kg IM) was given to some of the cats to facilitate bladder infusion. A solution containing 0.17% salicylic acid, 70% ethanol and 0.2% sterile normal saline was infused into each cat's bladder via transabdominal cystocentesis. The apparatus used to perform the cystocentesis consisted of a 22-
gauge 1-inch needle attached to a 3-way stopcock valve. A 12-ml syringe was attached to the 3-way stopcock and was used to initially remove a small amount of urine from the bladder to verify correct placement of the needle. Four mls of the solution was slowly injected into the bladder via another syringe attached to flexible IV tubing through the other access of the 3-way stopcock. At the end of each infusion, a small amount of urine was again aspirated to verify needle position.

Twenty-four hours after induction of the chemical cystitis, 5 mls of the bacterial inoculum (creating a final concentration of $1 \times 10^9$ CFU/5 mls) were introduced into each cat's bladder via urethral catheterization with a polypropylene catheter. The cats were anesthetized with ketamine (30 mg IM) for this procedure. Forty-eight hours post challenge, urine was obtained via cystocentesis, and quantitative aerobic bacterial cultures were done to confirm the presence of a bacterial urinary tract infection. A surface streaking technique using a 10 microliter calibrated bacteriologic loop was used to prepare the cultures on blood agar and MacConkey media. Any growth of *S. intermedius* was considered significant based on collection of urine via cystocentesis. If no growth occurred in 48 hours, the cultures were deemed negative.

_Urine collections and analyses_ - Quantitative aerobic bacterial urine cultures were performed 48 hours post challenge and every 5 days thereafter for a minimum of 20 days. All urine samples were obtained by transabdominal cystocentesis.
Results

All cats were healthy and free of bacterial UTI upon initiation of the study based on physical examination and laboratory data. Quantitative urine cultures indicated that 10/12 cats had growth of $\geq 10^4$ CFU/ml *S. intermedius* 48 hours post challenge. Two cats (#7312, 3174) had $10^3$ CFU/ml *S. intermedius*. Two cats (#3186, 3174) had growth of $10^3$ CFU/ml *E. coli* in addition to that of *S. intermedius* on a single culture during the course of the study. One cat (#7312) had only *Aeromonas* growth on culture 5 and another cat (#3174) had only *E. coli* growth on culture 6. Four of seventy-two total urine cultures showed contamination with organisms other than *S. intermedius*. (Table 1)

Seven of twelve cats maintained positive *S. intermedius* urine cultures ($\geq 10^4$ CFU/ml) for the entire study period (minimum of 30 days). One cat (#3183) maintained positive *S. intermedius* growth ($10^5$ CFU/ml) for 21 days and two cats (#7576, 3175) maintained positive *S. intermedius* growth ($\geq 10^4$ and $\geq 10^3$ CFU/ml, respectively) for 14 days. The two cats having only $10^2$ CFU/ml *S. intermedius* growth on culture 1 (48 hours post challenge) did not have urinary bacterial growth after this initial culture. (Table 1)

One male cat developed urethral obstruction early on in the study and was immediately excluded and euthanized by lethal injection. This cat was replaced by a female cat since no extra male cats were available. Necropsy examination of the obstructed cat showed erosive, ulcerative, hemorrhagic cystitis and secondary reactive
fibroplasia of the urinary bladder. Histologically, the process was confined to the mucosal layer and did not penetrate deeper into the bladder wall.

Most cats were subjectively noted to exhibit signs of lower urinary tract disease such as pollakiuria, stranguria, and hematuria. All of the cats maintained adequate appetites and did not appear to be adversely affected by the procedures.

Discussion

The model of bacterial lower urinary tract infection used in this study was effective in producing a lower UTI which persisted for a minimum of 14 days with \( \geq 10^4 \text{ CFU/ml} \) *S. intermedius* growth in 10/12 cats. Two cats having an initial post challenge (culture 1) bacterial growth of only \( 10^2 \text{ CFU/ml} \) cleared their infections at the time of the second culture (5 days later). This may indicate that an initial post challenge urinary bacterial concentration of \( <10^4 \text{ CFU/ml} \) may not be sufficient to maintain infection long term.

Growth of *E. coli* and *Aeromonas* was most likely iatrogenically introduced via bladder catheterization, through inadvertent penetration of bowel during cystocentesis, or from skin contamination of the needle. It would have been preferable to introduce the bacterial inoculum via cystocentesis, however the inflammatory response created by the chemical cystitis caused frequent voiding and therefore resulted in a small urinary bladder which did not lend itself to the performance of this procedure on the cats in this study.
The use of furosemide in cats #7312 and 7576 may help to explain early clearance of the organism; although, other cats having early clearance of the organism (#3175, 3183, 3174) did not receive any furosemide. An important natural defense mechanism against bacterial UTI is thought to be the mechanical washing of the lower urinary tract by voiding of urine. This may serve to reduce the population of bacteria lining the urethral mucosa.26

With the exception of one cat that developed urethral obstruction, the experimental model did not appear to create any significant side effects in the cats other than those commonly seen in naturally occurring lower urinary tract disease, such as pollakiuria, dysuria, and hematuria. The obstructed cat could have developed an underlying condition, such as urolithiasis that predisposed him to urinary obstruction. It may therefore be a successful model of bacterial lower UTI in cats and dogs since UTI’s tend to occur in these species most commonly as a complication of pre-existing disease or iatrogenically.6-10 Induction of chemical cystitis may alter the host’s local defense mechanisms enough to allow persistent colonization of bacteria within the lower urinary tract. However, we did not attempt to challenge cats that did not have induction of chemical cystitis prior to bacterial inoculation. In the future this model may be beneficial in providing a method with which to evaluate antibiotics and/or new therapeutic protocols for the treatment of bacterial lower urinary tract infection in cats.
III. SECTION III. THE EFFICACY OF SHORT TERM AMOXICILLIN THERAPY IN EXPERIMENTALLY INDUCED BACTERIAL LOWER URINARY TRACT INFECTION IN CATS

Introduction

Short term antibacterial therapy has been used in the treatment of uncomplicated bacterial lower urinary tract infections (UTI's) in women. Reports on studies of humans cite the effective use of a single day and three day therapeutic regimens.\textsuperscript{76-82} Obvious advantages to short term therapy include increased client compliance, decreased expense, decreased risk of bacterial drug resistance, and diagnostic value in identifying patients with renal infections that fail to respond to an abbreviated regimen.\textsuperscript{76-82} Two recent studies in the veterinary literature describe investigation of the use of short term antibiotic regimens for the treatment of experimentally induced bacterial UTI's in dogs.\textsuperscript{83,84} Turnwald, et al evaluated a single oral dose (60 mg/kg and 90 mg/kg) versus conventional oral antibiotic therapy (15 mg/kg BID x 21 days) using trimethoprim-sulfadiazine (TMP-S) in a group of female dogs infected with \textit{Staphylococcus intermedius}. The study did not support the efficacy of single dose therapy in the female dog. However, it was suggested that the experimental model of bacterial UTI did not adequately reproduce naturally occurring bacterial cystitis in the dog.\textsuperscript{84}
Rogers, et al have more recently compared the efficacy of a single oral dose (30 mg/kg) and three day (15 mg/kg BID) oral TMP-S and single parenteral (20 mg/kg SC) and 3 day parenteral (10 mg/kg SC) amikacin regimens for the treatment of *Escherichia coli* UTI's in a group of male and female dogs.\textsuperscript{83} Short course treatment regimens failed to eradicate UTI in male dogs. This was attributed to the prostate gland being an important reservoir of infection in male dogs with bacterial UTI\textsuperscript{90}, thus rendering them inappropriate candidates for short term therapy. This study did suggest, however, that a three day TMP-S regimen might be a reliable therapeutic regimen for some female dogs with bacterial UTI. Response to single dose therapy was not significant.\textsuperscript{83}

The use of short term antibiotic therapy for UTI has not previously been reported in the cat. Because the cat's local host defense mechanisms are generally effective in preventing the occurrence of spontaneous bacterial UTI's\textsuperscript{6,10}, (resulting in <1% of the domestic cat population affected per year as opposed to 14% of the canine population affected/year\textsuperscript{5,54}) one could speculate that eradication of spontaneous or iatrogenic infections would be easier in this species. This would appear to make the cat an ideal candidate for short course therapy. An experimental model of bacterial UTI may also be successful in the cat in mimicking natural disease since bacteria primarily invade the urinary tract as a sequela to interruption of the defense mechanisms by naturally occurring or iatrogenic events.\textsuperscript{6,10} The purpose of this study was to evaluate the efficacy of three day amoxicillin therapy as compared
to a conventional fourteen day regimen in an experimental model of bacterial lower UTI in cats.

Materials and Methods

Cats - Eighteen cats (9 males and 9 females) were studied. Prior to entering the study each cat received a physical examination, complete blood count (CBC), serum biochemical profile (albumin, total protein, urea nitrogen, creatinine, total bilirubin, alanine aminotransaminase, serum alkaline phosphatase, sodium, potassium, chloride, carbon dioxide, calcium, phosphorus, glucose), FeLV test (ELISA), urinalysis and quantitative aerobic bacterial urine culture. All urine samples were obtained by transabdominal cystocentesis. All cats entered into this study were free of bacterial urinary tract infection and were normal based on physical examination and laboratory data.

Induction of Experimental Infection - A description of the methods used may be found in materials and methods, Section II. The organism’s susceptibility to amoxicillin was confirmed by a minimum inhibitory concentration of <4ug/ml. Furosemide⁶ was used occasionally in some cats to obtain urine cultures via cystocentesis during the course of the study.

Study Groups - Study groups were randomly divided into 3 groups of six cats each (3 males, 3 females per group) via the selection of random lots.
Group I (Conventional Therapy Group) cats were treated with oral amoxicillin at a dosage of 10 mg/kg every 12 hours for 14 days. Quantitative urine cultures were performed 48 hours post challenge, 48 hours after the treatment period ended, and every 5 days thereafter for at least 20 days to determine efficacy of the treatment protocol.

Group II (Control Group) cats received no treatment. Quantitative urine cultures were performed 48 hours post challenge and every 5 days thereafter for at least 20 days.

Group III (Short Term Group) cats were treated with a 3-day course of oral amoxicillin at a dosage of 10 mg/kg every 12 hours. Quantitative urine cultures were performed 48 hours post challenge, 48 hours after the treatment period ended, and every 5 days thereafter for at least 20 days to determine efficacy of the treatment protocol.

Urine collection and analyses - Quantitative aerobic bacterial urine cultures were performed on all cats 48 hours post challenge and at intervals previously mentioned for each treatment group. Procedures are described in Section II.

Statistical analysis - Results from bacterial urine cultures (quantitated in CFU's/ml) were analyzed statistically. A two-way analysis of variance and a multivariate analysis were used to evaluate interactions between groups regarding response to treatment, gender of the cat, and interaction between gender and
response to treatment. A Duncan's multiple range test\textsuperscript{91} was also used to compare response to treatment among groups. A p-value of < 0.05 was considered significant.

**Results**

All cats were healthy and free of UTI upon initiation of the study based on physical examination and laboratory data. Urine cultures indicated that initially 14/18 cats had \( \geq 10^4 \) CFU/ml growth of *S. intermedius* 48 hours post challenge. One cat (#7312 of Group II) had only \( 10^2 \) CFU/ml of *S. intermedius* and another cat (#7191 of Group III) had \( 10^3 \) CFU/ml of *E. coli* only. Two cats (#7573 and #5015 of Group III) had negative cultures after the initial challenge, but were successfully re-infected and showed \( \geq 10^4 \) CFU/ml of *S. intermedius* 48 hours after the second challenge. (Table 2)

Bacterial growth was not observed from any of the urine samples taken from cats in the conventional therapy group (Group I) after treatment was concluded. (Table 2) Four cats (#7586, 7453, 7533, 1526) in Group II (Control group - no treatment) maintained positive *S. intermedius* urine cultures (\( \geq 10^4 \) CFU/ml) for the entire study period (with the exception of one \( 10^2 \) CFU/ml growth of *S. intermedius* on day 22 post challenge for cat #7586). One cat (#7576) in Group II did not have bacterial growth observed on cultures 4, 5, 6 (days 17, 22, and 27 post challenge). The sixth cat's (#7312) urine cultures had no growth on cultures 2, 3, 4, and 6 (days
7, 12, 17, and 27 post challenge) and a positive culture of *Aeromonas* spp. (10^3 CFU/ml) only was obtained on culture 5 (22 days post challenge). (Table 2)

In the group that received short term therapy (Group III), one cat (#5345) maintained positive *S. intermedius* urine cultures throughout the study period (all ≥10^2 CFU/ml). A second cat's (#7536) urine cultures were all positive for *S. intermedius* (≥10^4 CFU/ml) with the exception of culture 2 (48 hours after the end of the treatment period) which showed no growth. Three cats (#5015, 5553, 7191) were negative for bacterial growth on all post treatment cultures; however, one of these cats (#7191) had an initial post challenge urine culture growth of *E. coli* (10^3 CFU/ml) only. A sixth cat (#7573) had all negative post treatment cultures except for culture 6 (22 days after the end of the treatment period) which showed growth of *E. coli* (10^2 CFU/ml) only. (Table 2)

Bacterial growth of *S. intermedius* expressed in CFU/ml (ie., 10^5 = 100,000 CFU/ml) was used for statistical analysis. Initial statistical analysis of the data compared each culture (2-6) as a group (ie., culture 2 and the data from all 18 cats for that particular culture served as a group) using a p-value of <0.05 as significant. A two way analysis of variance (ANOVA) showed a difference in response to treatment among the cats in culture groups 2-6 (p-value of culture 2 <0.0001, culture 3 <0.05, culture 4 <0.03, culture 5 <0.03, culture 6 <0.01). This analysis did not identify the specific differences among groups (ie., whether Group I was similar to Group II, etc.); however, it did show no interaction between gender of the cat and
its response to treatment (p-value of culture 2 < 0.4, culture 3 < 0.2, culture 4 < 0.2, culture 5 < 0.4, culture 6 < 0.2).

A Duncan's multiple range test\textsuperscript{91} was also used to compare cultures 2-6. This analysis identified any significant differences between the means of treatment groups I, II, and III and compared their response to treatment. Results showed no significant differences between the means of Groups I and III or between Groups II and III. Group II was significantly different than Group I. (Table 2) These results indicated the control group (Group II) and conventional therapy group (Group I) were different, however the short term therapy group (Group III) was not significantly different from the control group (Group II) or the conventional therapy group (Group I).

A multivariate ANOVA\textsuperscript{92} was also used to evaluate interaction between gender and response to treatment. The data was analyzed in a different manner by evaluating each cat and its cultures (2-6) as a separate group. This analysis also showed no interaction between a cat's response to treatment and its gender (all p-values > 0.17).

In summary, statistical analysis of data showed that cats receiving short term antibiotic therapy (Group III) had no different response than those cats in the control group (Group II) receiving no treatment or in the conventional therapy group (Group I). It also showed that cats receiving conventional antibiotic therapy (Group
1) had significantly different results than those cats receiving no therapy (Group II). Whether a cat was male or female did not affect its response to treatment.

Discussion

The model of UTI used in this study appeared to be effective in producing a bacterial lower UTI which persisted in most cats in the control group for an average of 27 days. Conventional (14 day) antibiotic therapy using amoxicillin was successful in eradicating the experimentally induced UTI. However, there was no statistical difference between cats receiving short term (3 day) antibiotic therapy (Group III) and cats in the control group (Group II) that received no treatment or in the conventional therapy group (Group I) that received 14 days of amoxicillin. The inability to show the 3 day treatment group to be different from the control group would suggest that a short term antibiotic therapy regimen may be inappropriate for the management of bacterial lower UTI's in cats or that the small number of cats used in the study made it impossible to distinguish its effects. The fact that the short term group was not statistically different from the conventional therapy group does not completely rule out its effectiveness as a therapeutic regimen; however, further studies using larger numbers of cats would be needed to re-evaluate its efficacy. The presence of *E. coli* and *Aeromonas* spp. growth was most likely due to iatrogenic contamination during urethral catheterization or via penetration of bowel or skin contamination during cystocentesis.
During induction of the chemical cystitis and bacterial inoculation some of the cats were given the diuretic furosemide to facilitate the procedure. Those cats were subjectively noted to have less severe stranguria than those cats not given furosemide; however, observations were not controlled as to time of day, amount of time observed, identity of the observer, nor were observations blinded. Furosemide was also used occasionally to aid urine collection by cystocentesis for subsequent culture from cats that did not allow their bladders to get sufficiently full to enable the procedure. This may have been the reason why cultures of urine samples at some times were negative (due to dilution of bacteria) and positive at other times in the same cat. The two cats (#7312, 7576) in the control group (Group II) who cleared their infections were very difficult to perform a cystocentesis on, and tended to receive furosemide on a more frequent basis than the other cats. This may have facilitated early clearance of the organism. The use of furosemide may certainly have affected culture results.

The cat (#7191) in Group III that had an initial culture of *E. coli* only (10^3 CFU/ml) should have been either excluded from the study or re-infected. Inclusion of this cat's data may have altered statistical analysis. Likewise, the cat (#7312) in Group II with an initial post challenge culture of 10^2 CFU/ml *S. intermedius* should probably have been re-infected. As discussed in Section II, initial post challenge urine bacterial concentrations of <10^4 CFU /ml may be insufficient to maintain infection long term in this experimental model.
The results of this study do not support the efficacy of short term antibiotic therapy for this experimental model of bacterial UTI in the cat; however, because the short term group was similar to the 14 day group as well as the control group its efficacy cannot be completely ruled out. Based on these results it is recommended that a 14 day vs short term (3 day) amoxicillin therapy be used in the treatment of bacterial lower urinary tract infections in cats. Interestingly, there was no difference in response to treatment between males and females. This might indicate less colonization of the prostate gland in male cats with bacterial UTI’s in contrast to what has been observed in canine males where the prostate is commonly the focus of urinary tract infection and a cause of chronic or recurrent urinary tract infections.\textsuperscript{90} Bacterial prostatitis in the domestic cat has not been reported in the current literature.\textsuperscript{90,93,94} Observations made during the course of the study suggest furosemide administration may have decreased the severity of signs of UTI such as stranguria. Further investigation is necessary to assess a possible role for furosemide as an adjunct therapeutic agent in the treatment of bacterial lower UTI’s in the cat.
IV. SECTION IV. EFFECT OF FUROSEMIDE ON THE
CONVENTIONAL ANTIBIOTIC TREATMENT
OF EXPERIMENTALLY INDUCED BACTERIAL
LOWER URINARY TRACT INFECTION IN CATS

Introduction

Lower urinary tract disease is a common health problem in cats. Although bacterial lower UTI is not a common primary problem in cats, it is often a complication of pre-existing lower urinary tract disease. Improvements in the management of cats with bacterial UTI would be of great benefit.

In a study that evaluated the efficacy of short term antibiotic therapy in experimentally induced bacterial lower UTI in cats (Section III), the diuretic furosemide was used to facilitate cystocentesis procedures. It was suspected that furosemide may have altered bacterial culture results by diluting bacterial numbers in the samples and may have facilitated early clearance of bacteria by mechanically washing out the lower urinary tract and potentially reducing numbers of bacteria. It was also subjectively observed that cats receiving furosemide exhibited fewer secondary signs of UTI such as stranguria, than cats that did not receive furosemide.

The hydrodynamics associated with voiding urine are thought to represent one of the most important natural defense mechanisms against infection of the urinary
tract. Mechanical washout induced by unimpeded, frequent, and complete voiding of urine inhibits bacterial colonization of the urinary tract by rapidly eliminating organisms in the lumen of the proximal urethra and urinary bladder. Micturition also reduces the population of bacteria lining the urethral mucosa by flushing the urethral lumen. The benefit of increased urine volume in preventing urolith formation, urethral obstruction, hematuria, or dysuria in cats with lower urinary tract disease has not yet been established by controlled studies. Theoretically, furosemide may enhance a cat’s local host defense mechanisms and possibly serve as an adjunct therapy in the treatment of lower urinary tract disease. A potential unfavorable effect of diuresis may include decreasing the osmolality of urine which serves as an important host defense mechanism. Antibiotic concentration in the bladder may also be reduced. The purpose of this study is to evaluate the effect of furosemide on the treatment of experimentally induced bacterial lower UTI’s in cats.

**Materials and Methods**

*Cats* - Eighteen cats (8 males and 10 females) were studied. Prior to entering the study each cat received a physical examination, complete blood count (CBC), serum biochemical profile (albumin, total protein, urea nitrogen, creatinine, total bilirubin, alanine aminotransaminase, serum alkaline phosphatase, sodium, potassium, chloride, carbon dioxide, calcium, phosphorus, glucose), FeLV test (ELISA), FIV test, urinalysis and quantitative urine culture. All urine samples were obtained by
transabdominal cystocentesis. All cats entered into this study were free of bacterial urinary tract infection and were normal based on physical examination and laboratory data.

Induction of Experimental Infection - A description of the methods used may be found in materials and methods, Section II. This was altered by eliminating the use of furosemide during the induction of chemical cystitis. Furosemide was also not used for the purpose of collecting samples via cystocentesis. The organism's susceptibility to amoxicillin was confirmed by a minimum inhibitory concentration of <4μg/ml.

Study Groups - Groups were randomly divided into 3 groups of six cats each (3 males, 3 females per group) via selection of random lots. One male cat in Group I developed urethral obstruction and was excluded from the study and euthanized by lethal injection. This animal was replaced by a female cat which was the only additional cat available. Group I was therefore composed of 4 females and 2 males.

Group I (Control Group) cats received no treatment. As described in Sections II and III quantitative urine cultures were performed 48 hours post challenge and every 5 days thereafter for a minimum of 20 days.

Group II (Furosemide Group) cats were treated for 14 days with 2.2 mg/kg oral furosemide every 12 hours. Quantitative urine cultures were performed 48 hours post challenge and every 5 days for a minimum of 20 days.
Group III (Furosemide and Amoxicillin Group) cats were treated for 14 days with 2.2 mg/kg oral furosemide\(^e\) and 10 mg/kg oral amoxicillin\(^l\) every 12 hours. Quantitative urine cultures were obtained 48 hours post challenge and every 5 days for a minimum of 20 days.

All cats were subjectively observed for secondary signs of UTI such as stranguria, hematuria, decreased appetite, and lethargy throughout the course of the study. Observations were made and recorded by caretakers on a daily basis. No specifications such as time of day observed, amount of time observed, etc. were made, however all cats were observed during the same time intervals.

Urine Collection and Analyses - Quantitative aerobic bacterial urine cultures were performed on all cats 48 hours post challenge and at intervals previously mentioned for each treatment group. Procedures are described in Section II.

Statistical Analysis - Results from bacterial urine cultures (quantitated in CFU's/ml) were analyzed statistically. A two-way analysis of variance\(^91\) and a multivariate analysis\(^92\) were used to evaluate interactions between groups regarding response to treatment, gender of the cat, and interaction between gender and response to treatment. A Duncan's multiple range test\(^91\) was also used to compare response to treatment among groups. A p-value of <0.05 was considered significant.

Results

All cats were healthy and free of UTI upon initiation of the study based on
physical examination and laboratory data. Urine cultures indicated 16 cats had ≥10^4 CFU/ml of *Staphylococcus intermedius* 48 hours post challenge (culture 1). Two cats (#3182, 3188) had 10^3 CFU/ml of *S. intermedius* for culture 1 and one additional cat (#3174) had only 10^2 CFU/ml of *S. intermedius* for culture 1. (Table 3)

In the Control Group (Group I) 3/6 cats (#3186, 3190, 3182) maintained 10^5 CFU/ml of *S. intermedius* throughout the course of the study. Two cats (#3175, 3183) maintained 10^5 CFU/ml of *S. intermedius* at least 2 weeks into the study and one cat (#3174) with only 10^2 CFU/ml of *S. intermedius* at the 48 hour post challenge culture was negative on cultures 2-7. (Table 3) A male cat in this group developed urethral obstruction and was excluded from the study. This animal was replaced by a female cat.

In the furosemide group (Group II) 4/6 cats maintained 10^5 CFU/ml of *S. intermedius* throughout the course of the study. One of these cats (#3188) had negative cultures for 2, 3, and 4, but had 10^5 CFU/ml of *S. intermedius* for cultures 5, 6, and 7. Two cats (#3173, 3177) cleared their infections by culture 3. (Table 3)

No bacterial growth was present from any of the urine samples taken from cats in the furosemide and amoxicillin group (Group III) after treatment was initiated. One cat in this group developed urethral obstruction prior to culture 4 and was excluded from the study. (Table 3)

*E. coli* contamination in addition to growth of *S. intermedius* was noted in 4 cats (#3186, 3174, 3189, 3191 - involving all 3 groups). *E. coli* growth only was noted
for cat #3174 (Group I) on culture 6. (Table 3) Subjectively, cats receiving furosemide (Groups II and III) were observed to show less stranguria than cats in Group I that did not receive furosemide. This observation was most dramatic during the first few days following induction of the experimental model.

Bacterial growth of *S. intermedius* expressed in CFU/ml (ie., $10^5 = 100,000$ CFU/ml) was used for statistical analysis. Initial statistical analysis of the data compared each culture (2-7) as a group (ie., culture 2 and data from all 18 cats for that particular culture served as a group) using a p-value of $<0.05$ as significant. A two-way ANOVA$^9$ showed a difference to response in treatment among the cats for culture groups 2-4, but not for cultures 5-7. This analysis did not, however, identify the specific differences among groups (ie., whether Group I was similar to Group II, etc.). The analysis showed no interaction between gender of the cat and its response to treatment (p-value of culture 2 $<0.7$, culture 3 $<0.3$, culture 4 $<0.6$, culture 5 $<0.8$, culture 6 $<1.0$, culture 7 $<0.8$).

Duncan’s multiple range test$^9$ was also used to compare cultures 2-7. This analysis identified any significant differences between the means of treatment groups I, II, and III and compared their response to treatment. Results showed no difference between groups I and II for cultures 2-7. Significant difference was observed between groups I and III for cultures 2-4, but not for cultures 5-7. There was no statistical difference between any of the groups for cultures 5-7.
A multivariate ANOVA\textsuperscript{92} was also used to evaluate interactions between gender and response to treatment in a different manner by evaluating each cat and its cultures as a separate group. This analysis showed no interaction between how a cat responded to treatment and its gender (all p-values > 0.05).

In summary, statistical analysis of data showed that for cultures 2-4 the furosemide and amoxicillin group (Group III) was different from the control group (Group I). However, for cultures 5-7, there was no difference between any of the 3 groups. If the means of bacterial culture results (in CFU’s/ml) expressed in Ln\textsuperscript{e} from the furosemide and amoxicillin group (Group III) are compared with the means from the conventional therapy group (Group I) from the study evaluating the efficacy of short term antibiotic therapy (Section III) there are no differences. All means equal 1.386 for both groups. Since both studies were designed and evaluated similarly, this would indicate results from those two groups are the same. Whether a cat was male or female did not affect its response to treatment.

Discussion

Statistical analysis of data indicated that for half the cultures (cultures 2-4) the furosemide and amoxicillin group (Group III) was significantly different from the control group (Group I). However, for cultures 5-7 there was no significant difference between the two groups even though all cats in Group III were cleared of infection upon initiation of treatment. (Table 3) These results may be due to the
smaller number of cats in the last half of the study since cat #3184 (Group I) and cat #3202 (Group III) both developed urethral obstruction and were excluded from the study for cultures 3-7 and 4-7, respectively. Also for cultures 5-7 three of the control (Group I) cats had cleared bacterial infection further minimizing differences between Groups I and III. The use of an extra female cat creating a group with 4 females and 2 males may have also altered results, however, statistical analysis showed no interaction between gender and response to treatment. When means of bacterial culture results (expressed in CFU's/ml) expressed in Ln⁸ from cats in Group III are compared to means from cats in the conventional therapy group (Group I) of the previously performed study (Section III) there is no difference. This further suggests that the addition of furosemide to a conventional antibiotic treatment regimen did not adversely affect response to treatment of experimentally induced bacterial lower UTI.

The furosemide group (Group II) was not significantly different from the control group (Group I) for cultures 2-7. This would suggest that furosemide by itself is not an appropriate treatment for bacterial lower UTI. Subjective observation of cats in Groups II and III that received furosemide suggested they exhibited fewer signs of bacterial lower UTI such as stranguria, when compared to the control group (Group I) that did not receive furosemide. This was particularly apparent during the first few days of treatment. Unfortunately these observations were only subjective and no specific data can be analyzed statistically. A preferred method of observation
would be to have neutral (blinded) observers and watch the cats for specified amounts of time each day. Preferably the cats would be observed for entire 24 hour periods at least for the first 1 to 3 days when the subjective observations made during this study showed the most differences among cats. To determine factors such as frequency of urination, computerized systems with photo beams might be used to measure activity of animals.

*E. coli* contamination was noted in some of the cats as in the previous studies (Sections II and III). It is suspected this was iatrogenically introduced during urethral catheterization or via penetration of bowel or skin contamination during cystocentesis. The clearance of infection in a male cat (#3174) in the control group (Group I) that only had an initial *S. intermedius* growth of 10^2 CFU/ml may indicate (as discussed in Section II) that a certain urine concentration of bacteria on the post challenge culture is necessary to sustain infection in this experimental model. This cat probably should have been re-infected.

There was no statistically significant interaction between gender of a cat and its response to treatment. Males and females of the same group responded similarly to the same treatment. As discussed in the previous study (Section III) this might indicate less involvement of infection of the prostate gland in feline lower UTI as contrasted to what has been observed in canine males.\(^9^0\) or alternatively, may indicate that not enough cats were evaluated.
Although statistical analyses did not definitively confirm that the furosemide and amoxicillin group (Group III) was successful in eradication of infection a direct comparison of the means suggests otherwise. The observation that cats receiving furosemide exhibited less stranguria and the indication that the addition of furosemide to conventional antibiotic therapy did not adversely affect success of therapy suggests that furosemide may potentially be a useful adjunct therapy in the treatment of bacterial lower urinary tract disease in cats.

Several improvements could be made in the study design of the previously discussed studies (Sections II, III, and IV). Ideally more cats should be evaluated. The use of only 18 cats in Sections III and IV may have compromised statistical analysis. Larger groups would also help to better evaluate the interaction between gender and response to treatment. Furosemide should not have been used in the studies discussed in Sections II and III. This was a major design flaw and made results difficult to interpret. Cats receiving furosemide should also have been specifically identified.

Growth of contaminants might be minimized if the cats' hair was shaved and surgically prepped prior to each cystocentesis or if cystocentesis was ultrasound-guided. The use of urethral catheterization to inoculate bladders with bacteria is less than optimal, but may be the only option. Furosemide would need to be used to create a bladder size suitable for cystocentesis. As previously discussed, furosemide may also alter culture results.
The inclusion of cat #7191 with a post challenge growth of only *E. coli* was perhaps an error. These results in addition to the results from any cat with growth of a contaminant only should not have been statistically analyzed with the rest of the data. As suggested by the studies, perhaps any cat with a post challenge urine bacterial concentration <10^4 CFU/ml should be re-infected. This would also improve continuity among cats.

The use of controlled observational procedures is definitely needed to provide data for statistical analysis. As previously discussed, blinded observers should be utilized as well as more specific criteria such as time of day observed, amount of time observed, etc. Round the clock observations for the first few days would be especially helpful since it appeared this was the most critical time for observation of signs of bacterial lower UTI. If further studies still suggest a potential role for furosemide as an adjunct therapy in the treatment of bacterial lower UTI's in cats, its use should be evaluated in naturally occurring infections.

The occurrence of urethral obstruction in two male cats was unfortunate. The inflammation created by the chemical cystitis and resultant bacterial infection of the lower urinary tract would certainly predispose a cat to problems such as obstruction. Abdominal radiographs, contrast radiography, and/or abdominal ultrasound could be used to help screen cats with factors predisposing to obstruction and bacterial infection such as uroliths; however, one could not totally rule out all the predisposing factors since potential etiologies are still being debated.
With improvements in the study design, this experimental model of bacterial lower UTI in cats could be used to evaluate other antibiotic protocols (including new antibiotics) for the treatment of bacterial UTI in cats. Other potential adjunct therapies such as analogues that block molecular sites of attachment on bacterial or mucosal cell walls and agents that alter immunologic response to bacterial organisms could also be evaluated.
V. REFERENCES


VI. FOOTNOTES

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b Trypticase Soy Broth, Difco Laboratories, Detroit, MI

c Lees GE. Texas A&M University. Personal communication. 1988

d Ketoset, Bristol Veterinary Pharmaceuticals, Syracuse, NY

e Lasix, Hoechst-Roche Pharmaceuticals, Syracuse, NY

f 3-Way Stopcock Valve, American Pharmaseal Co., Valencia, CA

g Extension Tube, American Pharmaseal Co., Valencia, CA

h TomCat Catheter, Sherwood Medical, St. Louis, MO

i Amoxi-Drops, Beecham Laboratories, Bristol, TN
TABLE 1
Quantitative aerobic bacterial culture results from cats with experimentally induced lower bacterial UTI

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Culture results are expressed on log10 CFU/ml.
PRETRX = pretreatment urine culture.
Culture 1 = 48 hr post challenge culture.
C = E. coli growth only.
* = Aeromonas spp. growth only.
* = E. coli growth in addition to S. intermedius.
TABLE 2

Quantitative aerobic bacterial culture results from cats in treatment Group I (14 day), Group II (Control), and Group III (3 day).

Group I - Conventional Therapy Group

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Sex</th>
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<th>Culture 6</th>
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<tbody>
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Group III - Short Term Therapy Group

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Culture results are expressed on log10 CFU/ml.
PRETRX = pretreatment urine culture.
Culture 1 = 48 hr post challenge culture.
\( ^0 \) = *E. coli* growth only.
\( ^* \) = *Aeromonas* spp. growth only.
\( ^+ \) = *E. coli* growth in addition to *S. intermedius*.
\( ^+ \) = cats requiring reinduction
TABLE 3

Quantitative aerobic bacterial culture results from cats in treatment Group I (control), Group II (Furosemide), and Group III (Furosemide and Amoxicillin).

Group I - Control Group

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Group III - Furosemide and Amoxicillin Group

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</table>

Culture results are expressed on log10 CFU/ml.
PRETRX = pretreatment urine culture.
Culture 1 = 48 hr post challenge culture.
6 = E. coli growth only.
* = E. coli growth in addition to S. intermedius.
+ = cats excluded from study due to urinary obstruction.

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VIII. VITA

Mary Ann Mann

Dr. Mann was born July 8, 1958 in Lewisburg, WV. She attended Duke University, Durham, NC (1976-1979) and West Virginia University, Morgantown, WV (1979-1980) where she received a B.S. in Animal and Veterinary Sciences. She attended veterinary school at The Ohio State University, Columbus, OH and received a D.V.M. degree in 1984. Following graduation from veterinary school she began a small animal medicine and surgery internship with Veterinary Specialists of Connecticut, West Hartford, CT and remained as an associate with that practice until 1988. In 1988 she began a residency in small animal internal medicine and a graduate program at the Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA. Dr. Mann completed her residency and Master of Science in 1991.

Mary Ann Mann, DVM