

INVESTIGATIONS INTO THE URINARY TRACT

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Abstract

A urinary tract infection (UTI) is defined as a temporary or permanent breach in host defense mechanisms that allows microbes to adhere, multiply, and persist within the urinary tract. Development of a UTI is multi-factorial with bacterial number and virulence and the health status of the patient (normal urogenital tract anatomy and physiology and systemic immunocompetence) playing important roles in determining the outcome. A UTI can involve a single site, such as the renal pelvis, ureter, bladder, urethra, prostate or vagina, or can include multiple sites. Infection of any portion of the urinary tract may increase the likelihood of infection in other locations.

Diagnosis of a UTI incorporates findings from the history, physical examination, complete urinalysis, and urine culture. Proper classification and localization of the UTI are important when formulating a treatment regime as well as evaluating treatment success and failure. Most UTI can be successfully managed with appropriate antibiotic treatment; however, bacterial resistance and compromised host defense mechanisms can result in persistent or recurrent infections. In patients with recurrent UTI, identification of underlying predisposing conditions will often improve treatment success. In patients where underlying causes cannot be identified or treated, therapies designed to prevent recurrent UTI may be employed.

Proanthocyanidins found in cranberry juice inhibit *E. coli* attachment to human uroepithelial cells, impairing bacterial adherence and colonization. These characteristics have encouraged widespread usage of cranberry extract as a prevention strategy for woman predisposed to urinary tract infections. *E. coli* is a common cause of canine urinary tract infection. Current treatment emphasizes eradication of established infection rather than infection prevention, but increased antibiotic resistance necessitates strategies to prevent infection. We hypothesized that purified cranberry extract (CE) inhibits bacterial adhesion to canine uroepithelial cells. The results of our study show that CE supplementation can reduce adhesion of uropathogenic *E. coli* to canine uroepithelium and suggests one mechanism by which CE might improve urinary tract health.

Table of Contents

List of Figures	vii
List of Tables	viii
Acknowledgements	ix
Chapter 1 - Review of Urinary Tract Infections in Small Animals	1
Etiology and Pathogenesis	1
Incidence and prevalence	1
Asymptomatic bacteriuria	1
Inflammation versus infection	2
Microbial isolates	3
Antimicrobial resistance	3
Routes of infection	4
Normal host defense mechanisms	4
Normal urine storage and voiding	5
Anatomic structures	6
Mucosal defense barriers	6
Antimicrobial properties of urine	8
Localization	8
Complicating factors	10
Complicated versus uncomplicated UTI	10
Systemic disease	10
Conclusion	11
Chapter 2 - Review of Urinary Tract Infections in Small Animals	12
Diagnosis, Treatment and Complications	12
Clinical findings	12
History	12
Physical exam	12
Localization	13
Diagnosis	14
Urinalysis	14

Urine culture	15
Contamination.....	16
Antimicrobial susceptibility testing	17
Kirby-Bauer technique.....	17
Minimum Inhibitory Concentration	18
Result interpretation.....	21
Classifying Urinary Tract Infections	22
Recurrent UTI: Relapse versus reinfection.....	22
Superinfection	22
Hospital-acquired UTI	22
Treatment	23
Antimicrobial Therapy.....	24
Antimicrobial selection.....	24
Empirical antimicrobial therapy for uncomplicated UTI.....	25
Short course/high dose protocols	26
Complicated UTI	26
Asymptomatic bacteriuria.....	27
Treatment Failures	27
Antimicrobial resistance	28
Ancillary Prevention Therapies	29
Prophylactic antimicrobial treatment (low dose protocols)	29
Cranberry extract	30
Urinary antiseptics	30
Miscellaneous treatments.....	31
Complications of Urinary Tract Infections.....	31
Polypoid cystitis.....	31
Emphysematous cystitis and pyelonephritis	32
Magnesium ammonium phosphate (struvite) urolithiasis.....	33
Pyelonephritis	34
Prostatitis.....	34
Prognosis.....	35

Chapter 3 - Cranberry Extract Reduces Adhesion of Uropathogenic <i>E. coli</i> to Canine	
Uroepithelial Cells	36
Introduction.....	36
Materials and Methods	37
Dogs	37
Urine samples.....	38
Madin-Darby Canine Kidney (MDCK) cells.....	38
Propagation of uropathogenic <i>E. coli</i> strain.....	38
Adhesion assay.....	39
Direct killing assay	40
Statistical Analysis.....	40
Results.....	41
Study dogs.....	41
Discussion.....	43
Conclusion	45
Chapter 4 - Effect of Storage Time and Temperature on Canine Urine Enzymes	47
Introduction.....	47
Materials and Methods	48
Samples	48
Laboratory Methods.....	48
Statistical Analysis.....	49
Results.....	49
Discussion.....	51
Conclusion	53
References.....	54

List of Figures

Figure 2.1 Involuted or “hooded” vulva noted on physical examination	13
Figure 2.2 Cystoscopic image of a dog with a paramesonephric remnant	13
Figure 2.3 Ultrasound image of a mass-like lesion in the apex of the bladder consistent with polypoid cystitis	32
Figure 2.4 Lateral abdominal radiograph of a newly diagnosed diabetic cat with emphysematous nephritis and cystitis secondary to an <i>E. coli</i> and <i>Streptococcus</i> sp. UTI.....	33
Figure 3.1 Effect of CE Supplement on Bacterial Adhesion in Urine-Individual Dogs.....	42
Figure 3.2 Effect of CE Supplement on Bacterial Adhesion in Urine-Combined Data	42
Figure 3.3 Direct Killing Assay Demonstrates Inhibitory Effect	43

List of Tables

Table 1.1 Commensal Bacterial Genera in the Urogenital Tract of Dogs	2
Table 1.2 Localization of Urinary Tract Infections	9
Table 2.1 Significance of Quantitative Urine Cultures in Dogs and Cats Based on Collection Method	17
Table 2.2 Mean concentration of select antimicrobial agents in canine urine.....	20
Table 2.3 Ideal steps to follow in the management of UTIs in dogs and cats	24
Table 2.4 Reasons for poor therapeutic response in dogs and cats with UTI.....	28
Table 4.1 Phase 1 & 2: Storage of GGT and NAG at 5 days refrigeration, 5 and 30 days frozen (-20 °C).....	50
Table 4.2 Phase 2: Storage of GGT and NAG at 5 and 30 days refrigeration, 5 and 30 days frozen (-20 °C) and 30 days frozen (-70°C).....	51

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Chapter 1 - Review of Urinary Tract Infections in Small Animals

Etiology and Pathogenesis

Incidence and prevalence

Bacterial urinary tract infection (UTI) is common; approximately 5-27% of dogs will experience infections of the urinary tract at some point during their lifetime.¹⁻³ Female dogs are more commonly affected than are males. A large retrospective study of canine urine cultures reported bacterial growth in 37% of submissions from female dogs and 29% of submissions from male dogs.⁴ In contrast to dogs, bacterial UTI are relatively rare in cats. In young, otherwise healthy cats with signs of lower urinary tract inflammation, bacterial UTI is rare (<2%).^{5,6} Incidence of bacterial UTI in cats increases with age and occurs more often in cats older than 10 years.⁷⁻⁹

Asymptomatic bacteriuria

Not all species of bacteria will induce clinical signs or cause disease. In some cases the bacteria present in animals with an asymptomatic bacteriuria may provide protection against colonization of the urinary tract with more pathogenic strains of bacteria (**Table 1.1**).¹⁰ This is important since some patients that are treated for an asymptomatic bacteriuria may subsequently develop infections of the urinary tract with pathogenic strains of bacteria.¹¹ Asymptomatic bacteriuria associated with UTI most often occur secondary to systemic disease or immunocompromise. Chapter two discusses treatment considerations for asymptomatic bacteriuria.

Table 1.1 Commensal Bacterial Genera in the Urogenital Tract of Dogs

Genus	Distal Urethra of Males	Prepuce	Vagina
<i>Acinetobacter</i>		+	+
<i>Bacteroides</i>			+
<i>Bacillus</i>		+	+
<i>Citrobacter</i>			+
<i>Corynebacterium</i>	+	+	+
<i>Enterococcus</i>			+
<i>Enterobacter</i>			+
<i>Escherichia</i>	+	+	+
<i>Flavobacterium</i>	+	+	+
<i>Haemophilus</i>	+	+	+
<i>Klebsiella</i>	+	+	+
<i>Micrococcus</i>			+
<i>Moraxella</i>		+	+
<i>Mycoplasma</i>	+	+	+
<i>Neisseria</i>			+
<i>Pasteurella</i>		+	+
<i>Proteus</i>		+	+
<i>Pseudomonas</i>			+
<i>Staphylococcus</i>	+	+	+
<i>Streptococcus</i>	+	+	+
<i>Ureaplasma</i>	+	+	+

From Barsanti JA: Genitourinary infections. In Greene CE, editor: Infectious diseases of the dog and cat, ed 3, St Louis, 2006, Saunders, pp 935-961.

Inflammation versus infection

Evidence of inflammation (e.g., pyuria) in the urine sediment is not synonymous with a UTI.

There are several nonbacterial diseases that can result in urinary tract inflammation and subsequent clinical signs may be similar to those of a UTI (e.g., dysuria, pollakiuria, hematuria). Examples include sterile urocystoliths, urethral obstruction, lower urinary tract neoplasia, lower urinary tract trauma, as well as sterile polypoid, irritant, and idiopathic cystitis. Bacteriuria with inflammation on sediment exam are diagnostic for a UTI from cystocentesis samples, however in some cases urine culture may be necessary for a diagnosis.

Microbial isolates

The most common isolate from the canine urine is *Escherichia coli* which accounts for one-third to half of all positive urine cultures.^{4,12} The next major group of uropathogens includes Gram-positive cocci, such as *Staphylococcus*, *Streptococcus*, and *Enterococcus*. Remaining uropathogens include *Proteus*, *Klebsiella*, *Pasteurella*, *Mycoplasma*, *Enterobacter* and *Pseudomonas*.^{4,12} These 10 genera accounted for 95% and 97% of all urinary isolates in male and female dogs, respectively.⁴ A study evaluating prevalence in an Australian cat population found *E. coli* to be the most common pathogen (37%), followed by *Enterococcus* (27%) and *Staphylococcus* (20%).^{13,14} A retrospective analysis of feline urine cultures submitted to a teaching hospital also found *E. coli* to be the most common pathogen (47%), followed by *Staphylococcus* (18%), *Streptococcus* (13%) and *Klebsiella* (4%).¹⁴ In both dogs and cats with bacterial UTI, 75% of the time there is a single pathogen; in 20% of cases there are two pathogens and $\leq 5\%$ of the time there are three species.¹² Multiple organism infections are more commonly observed in female dogs.⁴ Infections with bacteria such as *Pseudomonas* are more commonly associated with opportunistic infection secondary to immunocompromise or alterations in host defenses.¹⁵

Antimicrobial resistance

Intrinsic and acquired forms of antimicrobial resistance exist. Intrinsic resistance mechanisms are genetic based bacterial properties.¹⁶ For example, penicillinase producing strains of *Staphylococcus* have intrinsic resistance to penicillins. Although there are limited ways to address intrinsic resistance, veterinarians play a large role in acquired forms of resistance since bacterial populations respond to selection pressures brought about by use of antimicrobial agents. With increasing use of antimicrobials there is increased risk of resistance. This not only has the potential to affect our veterinary patients, but also the people that live with them.^{17,18}

Acquired resistance is created by alterations in bacterial DNA. Bacteria have numerous mechanisms for exchanging genetic material which can contribute to acquired antimicrobial resistance. The genetic information in most bacteria is in the form of plasmids, which are small, circular stands of DNA. Plasmids do not carry genes responsible for essential metabolic function, but can carry genes for virulence and resistance. Transposons are another type of

mobile genetic element which can contribute to resistance by excising themselves from the donor chromosome and inserting into recipient chromosomes or plasmids. Plasmids and transposons can be passed between bacterial strains and across species within a bacterial family by conjugation.¹ Finally, chromosomal mutations are also possible which can produce resistant modifications in the antibiotic target.

Routes of infection

Most UTI are associated with bacterial pathogens from the gastrointestinal tract or skin surrounding the vulva and prepuce that ascend via the urethra to the urinary bladder. Once bacteria gains entrance to the urinary tract, they adhere and colonize the urothelial surface. The ability to establish these colonies depends on the number and virulence of the ascending microbes versus the competence of the host defense mechanisms.

Ascension of bacteria from the lower urinary tract is the primary route of infection of the upper urinary tract (versus hematogenous or direct extension from surrounding tissues). The most common site of infection in the upper urinary tract is the renal pelvis but invasion into the renal parenchyma is possible.¹⁹ Ascension from the lower urinary tract may occur in association with vesicoureteral reflux and/or an obstructive uropathy. Obstruction to urine flow can decrease renal medullary blood flow via increased back pressure and cause decreased delivery of antibody, complement, and white blood cells to the medulla, increasing the risk of bacterial colonization of the renal pelvis. A small percentage of UTI are caused by bacteria that have entered the tract through a hematogenous route. While the development of a UTI via the hematogenous route is uncommon, it occurs most frequently in the kidney. In cases of bacteremia, the high renal blood flow and the extensive filtration surface within the glomeruli expose the kidney to bacteria.

Normal host defense mechanisms

Host defense mechanisms are comprised of a combination of anatomic and physiologic factors that have a major role in preventing a UTI. Beyond prevention, the status of host defense mechanisms is one of the most important determinants of the outcome of a UTI. Appropriate antibiotic treatment will, at best, sterilize the urinary tract during the time of administration but it is the host defense mechanisms that prevent recurrent UTI (reinfections) after antibiotic

withdrawal. Diagnosis of defense breaches can help identify possible reasons for treatment failures as well as patients at risk of acquiring a UTI.

Normal urine storage and voiding

Production of a normal amount of urine with frequent and complete voiding helps reduce the number of bacteria ascending through the urethra and adhering to the bladder uroepithelium. Research in rats infected with *E. coli* identified two phases of bacterial clearance during voiding. The primary phase occurred 0-4 hours after the introduction of bacteria and the secondary phase occurred 4-24 hours after the introduction of bacteria. Ninety nine percent of bacteria are cleared from the urinary bladder via micturition within 4 hours of introduction.²⁰

Any condition that decreases the frequency of voiding can predispose a patient to a UTI by providing more time for bacterial adherence. For example, dogs with upper motor neuron lesions often have urine retention associated with increased outflow resistance and subsequent bladder atony; incomplete bladder emptying in these patients increases the potential for infection. Similarly, retention of urine associated with decreased detrusor contractility (e.g., lower motor neuron lesions and dysautonomia) is also associated with decreased bacterial washout and increased predisposition to UTI.²¹ From a clinical management perspective, prolonged time between voiding opportunities (e.g., owner work schedules) may also predispose or make it more difficult to eradicate a UTI.

Although diuresis and increased frequency of voiding in humans has been found to decrease the bacterial colony count in patients diagnosed with a UTI, reduced frequency of voiding in the face of dilute urine (e.g., at night when the patient is asleep) can result in increased urine bacterial counts.²² Dilute urine with low concentrations of urea may predispose dogs and cats to the development of UTI because bacteria have increased ability to grow in these conditions compared with the harsher environment of highly concentrated urine.^{23, 24} The importance of bladder washout versus antibacterial properties of concentrated urine has not been investigated in dogs; however it is likely both have clinical significance. In my opinion, in dogs and cats with decreased urine concentrating ability urine culture should be part of longitudinal monitoring recommendations.

Anatomic structures

There are several anatomic features of the urethra, ureters and prostate that help prevent infection and/or ascension of bacteria. First, the high pressure zone of the urethral sphincter impedes migration of bacteria. In contrast to urine retention, urethral sphincter mechanism incompetence (USMI) and lower urethral closure pressures may also compromise host defenses by allowing a greater number of bacteria to more easily ascend through the urethra to the bladder.

Secondly, ureteral peristalsis inhibits further ascension of bacteria above the level of the bladder. Finally, in male dogs, prostatic secretions that contain zinc and are bacteriostatic, along with increased urethral length, are important defense mechanisms that make UTI relatively rare.^{25,26}

Congenital and/or acquired anatomic abnormalities of the urogenital system frequently predispose patients to UTI. Vaginal strictures resulting in urine pooling or urine retention can increase the time for bacterial adherence/colonization of the lower urinary tract. Ectopic ureters lack the normal vesicoureteral valve; therefore bacteria from the lower tract may more easily ascend to the upper tract. A recessed vulva and excessive perivulvar skin folds often result in a localized moist bacterial dermatitis and increased bacterial ascension through the urethra.

Mucosal defense barriers

The uroepithelium lines the lumen of the urinary tract from the renal pelvis to the urethra and prevents the passage of water, ions, solutes and macromolecules from the plasma and interstitium into the urinary tract lumen.²⁷ A healthy uroepithelium also prevents adherence of bacteria. For example in a small study, perineal urethrostomy surgery did not increase the risk of UTI in cats with a healthy uroepithelium. However persistent uroepithelial inflammation, combined with surgical alteration in anatomic and functional barriers to ascending UTI is associated with a higher incidence of UTI in cats.²⁸

Within the bladder, the uroepithelium is composed of three layers: basal cells, intermediate polygonal cells, and the most superficial umbrella cells. The umbrella cells form a single layer, are separated by cellular tight junctions, and can alter their shape depending on the degree of bladder distention.²⁷ The apical membranes of the umbrella cells are composed of plaques and hinges that facilitate stretching and bladder filling. The plaques are made up of inner and outer leaflets that form an asymmetric unit membrane (AUM). The AUM is composed of

transmembrane proteins called uroplakins. One of the main functions of these uroplakins is to form a barrier to solute and water flow across the apical membrane.²⁷ If ascending bacteria bind to these uroplakins, uroepithelial apoptosis can be initiated resulting in washout of the infected cells.

In some cases, the adherence of bacteria to the uroepithelium can lead to the cellular internalization of bacteria. Bacteria within uroepithelial cells are protected from the host's immune response and have the ability to serve as a reservoir for recurrent infections.

Glycosaminoglycans (GAG) and proteoglycans are produced by umbrella cells and are part of the bladder surface mucus layer. Glycosaminoglycans are hydrophilic and bind water to the apical membrane of the transitional cell. This water layer decreases the ability of bacteria and crystals to adhere to the uroepithelium and contributes to the impermeability of the bladder wall.²⁹ With chemical (e.g., cyclophosphamide administration) or mechanical damage (e.g., presence of uroliths, neoplasia, or over-insertion of a urethral catheter), the GAG layer can be disrupted resulting in increased adherence of bacteria to the bladder mucosa.³⁰ Disruption of the GAG layer also increases the permeability of the bladder wall allowing irritating substances to pass through the uroepithelium and cause submucosal inflammation.³¹

Secretory IgA (sIgA) is the primary immunoglobulin in mucous secretions from the urogenital tract. It differs from serum IgA by being bound to a secretory protein component.³² The secretory component complexes with polymeric IgA released by the plasma cells in the lamina propria of mucous membranes allowing transport of IgA across the epithelial barrier and into the lumen.³³ Widening of the interstitial space between uroepithelial cells associated with inflammation may facilitate secretion of IgA.³⁴ Secretory IgA inhibits attachment of *E. coli* to human uroepithelial cells by binding to bacteria that enter the mucous layer of the uroepithelium.³⁵ Once a bacterium is coated with this secretory antibody, its ability to adhere to the uroepithelium is compromised.³⁴

Another mucosal defense mechanism involves normal flora occupying distal uroepithelium receptor sites. Normal flora of the urogenital tract compete with potential pathogens for nutrients and epithelial receptor sites. Bacteriocins are natural antibiotics produced by almost all bacteria.³⁶ Normal flora may produce bacteriocins that are inhibitory and/or bactericidal to

potential pathogens.³⁷ The antibiotic properties of these bacteriocins have a relatively narrow spectrum and are only toxic to closely related strains. Colicin is the most commonly evaluated bacteriocin in *E. coli* infections.³⁶ Urinary catheters coated with a colicin-producing strain of *E. coli* have been used to prevent UTI in people.³⁸ Currently there are no studies evaluating use of these catheters in veterinary medicine.

Antimicrobial properties of urine

The antibacterial properties of urine contribute to host defenses via bacteriostatic and possibly bactericidal effects depending on urine composition. Potential mechanisms for these antibacterial properties include low pH, high concentrations of urea and weak organic acids, and high urine specific gravity (USG). In children with recurrent UTI, inhibition of bacterial growth was correlated with increasing urine specific gravity (USG).³⁹ Urine osmolality and USG have been proposed as a reason why healthy cats have relatively few UTI. However, since high USG does not always correlate with antimicrobial activity, there are likely other factors or substances in concentrated urine that inhibit bacterial growth.^{23, 24} Maintaining a patient's urine pH between 5.0-6.5 helps inhibit the growth of bacteria in people.⁴⁰ However, a study looking at antibacterial properties of feline urine found no correlation with pH and inhibition of UTI.²³ In acidic urine in canine patients, the inhibition of bacterial growth may be more pronounced for bacteria like *Proteus*, that tend to grow better in an alkaline environment.

Localization

Urinary tract infections most commonly colonize the uroepithelial cells of the bladder but involvement of the kidneys, prostate, and uterus is also possible. If the male dog is intact, colonization of the prostate should be expected. The location of the UTI can be determined in most cases based on the history, physical examination findings, laboratory parameters and imaging results (**Table 1.2**).

Table 1.2 Localization of Urinary Tract Infections

Site of Infection	Compatible History	Potential Physical Examination Findings	Laboratory Findings	Diagnostic Imaging Findings
Lower urinary tract-uncomplicated	Dysuria, pollakiuria, periuria, hematuria, cloudy urine with abnormal odor, usually no systemic signs	Small, painful, thickened bladder	CBC: WNL Urinalysis: Pyuria, hematuria, proteinuria, bacteriuria Urine culture: Significant bacteriuria †	Often unremarkable
Lower urinary tract-complicated	Dysuria, pollakiuria, periuria, hematuria, cloudy urine with abnormal odor, usually no systemic signs	Small, painful, thickened bladder, palpable masses in urethra or bladder, flaccid bladder wall, large residual volume, +/- palpation of uroliths	CBC: WNL Urinalysis: Pyuria, hematuria, proteinuria, bacteriuria CHEM: consistent with underlying endocrinopathies Urine culture: Significant bacteriuria †	Possible changes in renal size and shape (with CKD), urinary tract masses, +/- uroliths, +/- thickening of bladder wall, +/- changes consistent with endocrine disease
Acute pyelonephritis	+/-polyuria, polydipsia, +/- possible lethargy, depression, anorexia, +/- renal failure	Fever, depression, lethargy, lumbar pain that would not be expected with a simple cystitis.	CBC: +/- Leukocytosis/inflammatory leukogram Urinalysis: pyuria, hematuria, proteinuria, bacteriuria, WBC or granular casts, impaired urine concentration, +/- azotemia	Renomegaly, +/- abnormal kidney shape, +/- nephroliths, ureteroliths, +/- dilated renal pelvis, dilated pelvic diverticulae, +/- evidence of outflow obstruction.
Acute prostatitis	Urethral discharge independent of micturition, +/- reluctance to urinate or defecate	Fever, depression, lethargy, painful prostate or abdomen, rectal exam showing asymmetric or enlarged prostate	CBC: +/- Leukocytosis/inflammatory leukogram Urinalysis: pyuria, hematuria, proteinuria, bacteriuria	+/- indistinct prostate borders, +/- prostatomegaly, +/- prostatic cysts
Chronic pyelonephritis	+/- polyuria, polydipsia, +/- signs of systemic infections, +/- renal failure	+/-abdominal pain, kidneys normal or decreased in size	CBC: +/- Leukocytosis/inflammatory leukogram Urinalysis: pyuria, hematuria, proteinuria, bacteriuria, WBC or granular casts, impaired concentration, +/- azotemia or worsening azotemia	+/- abnormal kidney shape, +/- nephroliths, ureteroliths, +/- dilated renal pelvis, dilated pelvic diverticulae, +/- evidence of outflow obstruction
Chronic prostatitis	recurrent UTI, urethral discharge independent of urination, +/- dysuria	Often no detectable abnormalities, +/- prostatomegaly or asymmetric prostate	CBC: WNL, Urinalysis: pyuria, hematuria, proteinuria, bacteriuria	+/- prostatomegaly, +/- prostatic cysts, +/- prostatic mineralization

† Significant bacteriuria defined based on collection method and bacterial quantification (see Table 2.1) Adapted from Pressler B, Bartges JW: Urinary Tract Infections. In Ettinger, editor: Textbook of Veterinary Internal Medicine, ed 7, St Louis, 2010, Saunders, pp 2040.

Complicating factors

Complicated versus uncomplicated UTI

Uncomplicated UTI occur in patients where no underlying structural, neurologic, or functional abnormalities exist. A UTI becomes complicated when host defense mechanisms are compromised either due to structural abnormalities altering urothelium (e.g., cystolithiasis), anatomic abnormalities (e.g., USMI) changes to the urine such as glucosuria, decreased urine osmolality, or decreased neutrophilic chemotaxis (e.g., diabetes mellitus) or suppression of the inflammatory or immune response secondary to hypercortisolemia (e.g., hyperadrenocorticism). In addition, UTIs are often considered complicated if they occur in cats, male dogs, and intact female dogs, or if they involve the kidneys or prostate.^{24,41} The distinction between uncomplicated and complicated UTI is helpful for determining prognosis, risk for recurrence, duration of treatment, and follow up recommendations for recheck evaluations.

Systemic disease

Urinary tract infections are common in dogs and cats with systemic diseases that may compromise normal antimicrobial defenses. In a retrospective study of 101 dogs with diabetes mellitus or hyperadrenocorticism or both, 42 (41.6%) had a UTI.⁴² Dogs receiving long-term glucocorticoids are also at risk with a reported incidence of 18-39%.^{43,44} Similarly, systemic disease can predispose cats to UTI. In retrospective studies evaluating known predisposing conditions in cats, positive urine cultures were found in 17-30 % of cats with CKD, 12-13% of cats with DM, and 12-22% of cats with hyperthyroidism.^{41,45,46} In one of these studies decreasing USG was not associated with positive urine culture; however, pyuria and hematuria were associated with positive urine cultures.⁴⁵ In addition, Persian breed, females, increasing age, and decreasing body weight were associated with positive cultures.⁴⁵ Urinary tract infections that occur secondary to immunocompromising systemic disease may be clinically silent due to decreased white blood cell chemotaxis to the bladder and may be difficult to detect if a low USG is present.

Conclusion

In conclusion, bacterial UTI are common in female dogs and older cats with concurrent systemic disease. Bacteria usually gain entrance to the urinary tract via ascension through the urethra. The ability of bacterial pathogens to adhere and colonize the uroepithelium is determined by the interplay of bacterial virulence factors and normal host defense mechanisms. Understanding these factors should improve treatment and prevention of UTI.

Chapter 2 - Review of Urinary Tract Infections in Small Animals

Diagnosis, Treatment and Complications

Clinical findings

History

Clinical signs associated with a urinary tract infection (UTI) depend on bacterial virulence, status of the host immune system, duration of the infection, and the site or sites of infection. The most common clinical signs associated with lower UTI include pollakuria, stranguria, dysuria, hematuria and inappropriate urination or periuria. If a UTI occurs secondary to a micturition disorder, such as an ectopic ureter, urethral sphincter mechanism incompetence (USMI), or urine retention, clinical signs associated with the primary condition may predominate. Similarly, in dogs and cats with systemic disease and compromised host defense mechanisms, clinical signs associated with the underlying disease may predominate and the UTI may be relatively asymptomatic.

Physical exam

Dogs with uncomplicated UTI often have unremarkable physical examination findings. Predisposing causes such as vulvar involution (**Figure 2.1**), severe perivulvular dermatitis, vaginal stenosis, cystic or urethral calculi, or urethral thickening may be identified in patients with complicated UTI. Whenever possible, female dogs that present with a UTI should have a digital vaginal examination performed in order to identify an anatomical abnormality that may alter normal host defense mechanisms. For example, palpation of a persistent paramesonephric remnant (**Figure 2.2**) may prompt further investigation for ectopic ureters and palpation of vaginal strictures may prompt further investigation for urine retention. Rectal examination (male and female dogs and cats) may also identify abnormalities such as pelvic trauma, urethral stones, or urethral thickening associated with neoplasia or granulomatous urethritis. These findings can help the clinician determine the extent of the work-up required and may help with treatment recommendations by aiding in the identification of factors that may complicate treatment.

Figure 2.1 Involved or “hooded” vulva noted on physical examination



Figure 2.2 Cystoscopic image of a dog with a paramesonephric remnant



Localization

Acute bacterial pyelonephritis, prostatitis, and metritis are often associated with systemic signs such as fever, an inflammatory leukogram, lethargy, and anorexia. In contrast, patients with chronic prostatitis and pyelonephritis may not exhibit systemic signs and therefore it may be more difficult to localize the infection. Localization of the UTI is important to optimize antimicrobial choices and duration of therapy (**Chapter one, Table 1.2 Localization of Urinary Tract Infections**).

Diagnosis

Diagnosis of a UTI is made based on information obtained from history, physical examination, urinalysis (including method of collection), and ideally urine culture and sensitivity.

Cystocentesis is the method of choice for collection of urine samples, especially if a urine culture is anticipated. Patients presenting with lower urinary tract signs (e.g., pollakiuria and stranguria) may make collection by cystocentesis difficult due to the small size of the urinary bladder.

Alternative methods of urine collection may be acceptable if cystocentesis cannot be performed or is contraindicated (e.g., suspected transitional cell carcinoma of the urinary bladder or pyoderma of the ventral abdomen). Urethral catheterization provides a superior sample compared with a free-catch voided sample, but requires more technical skill, especially in female patients. Care should be taken during catheterization to prevent contamination from external structures by clipping surrounding hair and cleansing the external genitalia prior to the procedure. Based on current recommendations, free catch urine samples are not acceptable for culture.⁴⁷ Urinalysis and urine culture results should always be interpreted in light of the urine collection method.⁴⁸⁻⁵⁰

Urinalysis

Urinalysis may be helpful in differentiating a urinary tract infection (UTI) from other disorders causing lower urinary tract signs (e.g., bladder neoplasia), making initial antibiotic recommendations, and in some cases identifying potential predisposing disorders (e.g., glucosuria and crystalluria). Urine specific gravity can be variable in patients with a UTI. Dilute or minimally concentrated urine may be observed in patients with concurrent disease predisposing to UTI (e.g., diabetes mellitus or hyperadrenocorticism), or may be observed if the infection involves the upper urinary tract. With a UTI, hematuria and proteinuria are frequently observed on dipstick analysis. The dipstick analyses for nitrite (bacteria) and leukocyte esterase (WBCs) are designed for human urine and are not reliable tests for canine and feline patients.^{51,52}

A urine sediment examination should be performed to identify pyuria and bacteriuria. If urine is dilute or the patient is immunocompromised (e.g., suffers from hyperadrenocorticism or is receiving exogenous corticosteroids), it may be difficult to identify WBCs or bacteria on urine sediment examination. Air-dried, stained sediment evaluations were more accurate than wet, unstained mounts for identification of bacteria when urine culture results were used as the gold

standard (sensitivity 82.8% and specificity 98.6%).⁵³ This air-dried, stained technique is easily performed in practice by placing one drop of sediment on a glass slide, allowing it to air dry (spreading may be necessary if sediment is thick), and then staining the sample with a commercially available modified Wright's stain (Diff Quik®). It is important to remember, however, that a culture is required for definitive diagnosis, and will provide additional information (e.g., bacterial identification, number of organisms/ml of urine, and antimicrobial susceptibility results).

Urine culture

Although clinical signs and urinalysis findings may increase the index of suspicion for a UTI, a urine culture is the definitive diagnostic test. Ideally, the urine sample for culture should be obtained prior to starting treatment. In patients already receiving antimicrobial therapy, it may be necessary to discontinue treatment for 3-5 days before collection of urine culture. It is also important to consider storage and transport of samples for culture in practices where immediate culture processing is not possible.^{54,55} Sterile containers that do not contain additives or preservatives should be used. Commercially available urine collection kits may be acceptable for up to 72 hours if sample processing is delayed (Urotube Roche and Becton Dickinson Urine C&S Transport kit).^{56,57} Bacterial counts can increase in urine stored at room temperature within a few hours. In refrigerated samples, quantitative bacterial counts differed after 6 hours of storage; however this was not associated with a change in interpretation of the clinical significance. It is recommended that urine samples be cultured immediately; however, it is acceptable for urine to be refrigerated in a closed container for up to 6 hours prior to culture.⁵⁴

Alternatives to sending a urine sample to a commercial laboratory for culture include using a calibrated bacterial loop (0.01 or 0.001 ml volume) to inoculate blood or MacConkey's agar plates in house. Culture plates are then incubated for 24 hours at 37°C and if a significant numbers of colonies are produced in light of the urine collection method, the plate can be sealed and shipped to a microbiology laboratory for bacterial identification and antimicrobial sensitivity testing. In most cases a quantitative urine culture will aid interpretation of results. In addition to bacterial numbers/ml of urine, knowledge of the normal flora may also be helpful in determining contamination versus infection when methods other than cystocentesis are used (**Chapter one, Table 1.1 Commensal bacterial genera in the urogenital tract of dogs**).

Contamination

Bacteriuria is expected in patients with UTI, however bacteriuria may also be observed in healthy dogs and cats if the urine sample has been contaminated with normal flora and/or pathogens from the distal urethra/urogenital tract or if the urine sample contacts a contaminated surface. Certain organisms are known to be commensal within the lower urinary tract and therefore knowledge of the organism and urine collection method may help determine contamination versus UTI. There is however overlap between normal flora and potential pathogens. Bacterial contamination of urine usually occurs when collecting a voided sample, but can also occur during urethral catheterization or be associated with collection of urine from a contaminated surface (e.g., cage floor, examination table). It is important to differentiate urine contamination from a UTI since the former does not need to be treated. Contamination can be ruled out by repeating a urinalysis with urine obtained via cystocentesis or by performing a quantitative urine culture on the voided or catheterized sample. Depending on the method of urine collection, the number of bacterial colony-forming units (cfu)/ml of urine will vary between contamination and UTI (**Table 2.1**). As stated previously, free catch urine samples are not recommended for culture and sensitivity and should only be performed when cystocentesis is not possible or recommended (e.g., transitional cell carcinoma or patients with bleeding disorders).

Table 2.1 Significance of Quantitative Urine Cultures in Dogs and Cats Based on Collection Method*

METHOD OF COLLECTION	Significant		Borderline		Contaminant	
	Dogs	Cats	Dogs	Cats	Dogs	Cats
Cystocentesis	≥1000	≥1000	100-1000	100-1000	≤100	≤100
Catheterization	≥10,000	≥1000	1000-10,000	100-1000	≤1000	≤100
Midstream voiding †	≥100,000	≥10,000	10,000-90,000	1000-10,000	≤10,000	≤1000
Manual expression †	≥100,000	≥10,000	10,000-90,000	1000-10,000	≤10,000	≤1000

*Values are given in colony-forming units per milliliter of urine (cfu/mL). Data represent generalities. Occasionally bacterial UTI may be detected with fewer organisms (i.e., false-negative results).

† For midstream voiding and manual expression, the contamination level may be 10,000 cfu/mL or higher (i.e., false-positive result). These samples should not be used for routine diagnostic culture.

From Pressler B, Bartges JW: Urinary Tract Infections. In Ettinger, editor: Textbook of Veterinary Internal Medicine, ed 7, St Louis, 2010, Saunders, pp 2037.

Antimicrobial susceptibility testing

Ideally, antimicrobial administration should be based on results from culture and susceptibility. Unnecessary or inappropriate usage of antibiotics can lead to antimicrobial resistance or delay the diagnosis of a non-infectious cause of lower urinary tract signs.⁴⁷ In addition to susceptibility results, the route and ease of administration, potential adverse effects, cost, and concentration in the urine (or other targeted tissues e.g., prostate, kidney) should be considered when choosing an antibiotic.

Kirby-Bauer technique

Agar disk diffusion (Kirby-Bauer) is a form of antimicrobial susceptibility testing. This test requires inoculation of a Mueller-Hinton agar plate with a standardized suspension of a bacterial isolate. Paper disks impregnated with antibiotics are then placed on the plate and the plate is

allowed to incubate. Bacterial growth is inhibited around the disk, and the zone of inhibition is compared to a standard identified for that antibiotic. Results of the disk susceptibilities are usually listed as susceptible, resistant, and intermediate; varying degrees of susceptibility or resistance are not described. Agar disk diffusion has been replaced by the microwell dilution technique described below. Testing and analysis should be performed in accordance with recommendations from the Clinical and Laboratories Standards Institute (CLSI).

Minimum Inhibitory Concentration

Determining the minimum inhibitory concentration (MIC) uses serial dilutions of an antibiotic to determine the lowest concentration that will inhibit bacterial growth. This concentration is the MIC and although results are reported as susceptible, intermediate, and resistant, they usually also include the actual MIC followed by the breakpoints used to determine susceptibility. The goal of the breakpoint is to predict clinical outcome for an individual patient. Breakpoints are determined by a combination of information including the species, bacteria, disease, antibiotic, dose, route and frequency. It is important to keep in mind that there are limited data available for veterinary medicine; therefore breakpoints are often determined by using information available from human breakpoints.

A result reported as "susceptible" indicates a high likelihood of treatment success (>80%) with most antibiotics; "intermediate" indicates possible success in treatment with potential alterations in normal dosing, and a "resistant" result indicates that a clinical cure is unlikely to occur with that antimicrobial. The result is considered susceptible if the MIC is in the breakpoint range or below, intermediate if it is at the high end of the breakpoint, and resistant if it is above the breakpoint.

Urine antibiotic concentrations are usually more important than plasma concentrations when treating a UTI. Minimum inhibitory antibiotic concentrations and the anticipated urine antibiotic concentration may be used to guide treatment choices. Clinical efficacy is expected if urine concentration is maintained at greater than four times the MIC of the pathogen between doses (**Table 2.2**).⁵⁸ For example, the MIC of ampicillin for a *Staphylococcus* organism is approximately 10 µg/ml. The expected serum and tissue concentrations of ampicillin are 1-2 µg/ml whereas the expected urine concentration of ampicillin is > 300 µg/ml. Since the expected urine concentration is > four times the MIC, it is reasonable to expect the UTI will resolve with

ampicillin therapy. Minimum inhibitory antibiotic concentration testing based on anticipated urine concentrations is not appropriate for deeper tissue infections (e.g., pyelonephritis, thickened bladder wall) where serum and tissue antibiotic concentrations are expected.

Table 2.2 Mean concentration of select antimicrobial agents in canine urine

Drug	Dosage †	Typical Antimicrobial Activity	Steady State Mean Urine Concentration
Amikacin	5 mg/kg, SQ	<i>Staphylococci</i> , some <i>Streptococci</i> , some enterococci, <i>E. coli</i> , <i>Proteus</i> spp., <i>Klebsiella</i> spp., <i>Pseudomonas</i> spp., <i>Enterobacter</i> spp.	342 µg/mL
Amoxicillin	11 mg/kg, PO q 8 hrs	<i>Staphylococci</i> , <i>Streptococci</i> , enterococci, <i>Proteus</i> spp.	202 µg/mL
Ampicillin	26 mg/kg, PO, q 8 hrs	<i>Staphylococci</i> , <i>Streptococci</i> , enterococci, <i>Proteus</i> spp.	309 µg/mL
Amoxicillin/clavulanic acid	12.5 mg/kg, PO, q 8 hrs	<i>Staphylococci</i> , <i>Streptococci</i> , enterococci, <i>Proteus</i> spp., <i>E. coli</i> , <i>Klebsiella</i> spp.	201 µg/mL
Cephalexin	30 mg/kg, PO, q 8 hrs	<i>Staphylococci</i> , <i>Streptococci</i> , <i>Proteus</i> spp., <i>E. coli</i> , <i>Klebsiella</i> spp.	225 µg/mL
Chloramphenicol	33 mg/kg, PO	<i>Staphylococci</i> , <i>Streptococci</i> , enterococci, <i>E. coli</i> , <i>Klebsiella</i> spp.	124 µg/mL
Enrofloxacin	5 mg/kg, PO, q 24	<i>Staphylococci</i> , some <i>Streptococci</i> , some enterococci, <i>E. coli</i> , <i>Proteus</i> spp., <i>Klebsiella</i> spp., <i>Pseudomonas</i> spp., <i>Enterobacter</i> spp.	40 µg/mL
Gentamicin	4-6 mg/kg, SQ, q 24 hrs	<i>Staphylococci</i> , some <i>Streptococci</i> , some enterococci, <i>E. coli</i> , <i>Proteus</i> spp., <i>Klebsiella</i> spp., <i>Pseudomonas</i> spp., <i>Enterobacter</i> spp.	107 µg/mL
Penicillin G	36,700 U/kg, PO	<i>Staphylococci</i> , <i>Streptococci</i> , <i>Proteus</i> spp.	295 µg/mL
Tetracycline	18 mg/kg, PO, q 8 hrs	<i>Streptococci</i> , some <i>Staphylococci</i> and Gram negatives at high urine concentrations	300 µg/mL
Trimethoprim/sulfadiazine	15 mg/kg, PO, q 12 hrs	<i>Staphylococci</i> , <i>Streptococci</i> , <i>E. coli</i> , <i>Proteus</i> spp., some activity against enterococci and <i>Klebsiella</i> spp.	55 µg/mL

† Doses listed were used in the studies calculating mean urine concentrations and may not be consistent with current guidelines for treatment recommendations.

Adapted from Ling GV: Therapeutic strategies involving antimicrobial treatment of the canine urinary tract. *J Am Vet Med Assoc* 185 (10): 1162-1164, 1984 and Dowling PM: Antimicrobial therapy of urinary tract infections. *Can Vet J* 37 (7): 428-41, 1996.

PO, Per os ; SD, standard deviation and SQ, subcutaneous.

Result interpretation

Clinical and Laboratory Standards Institute (CLSI) is a committee which provides recommendations that are considered the gold standard for breakpoint determination. To determine breakpoints, the committee examines pharmacokinetics, microbiology, pharmacodynamics, bacterial population, MIC distribution, and clinical trial results. Veterinary laboratories (or a human laboratories with appropriate susceptibility panels for commonly used veterinary antibiotics) that follow CLSI guidelines should be used for culture and susceptibility testing.

Depending on the laboratory, comparing antibiotics with similar susceptibility (e.g., cephalexin and cefazolin) may be necessary but requires making assumptions. For example, when comparing cefazolin with cephalexin, cefazolin is typically administered intravenously every 8 hours whereas cephalexin is typically administered orally every 8-12 hours. It is also important to remember that *in vitro* culture and susceptibility results may not correlate with *in vivo* efficacy. For example, regardless of the susceptibility results for *Enterococcus*, certain antibiotics including cephalosporins, trimethoprim-sulfamethoxazole, clindamycin, and aminoglycosides should always be considered resistant since these are not effective clinically and do not correlate with *in vitro* results.⁵⁹

In many cases, antibiotic susceptibility testing results provide a clear direction for treatment with an antibiotic that is orally administered, has few potential adverse effects, and reasonable cost. In some cases, susceptibility results demonstrate a high degree of bacterial resistance and route of administration, high cost, or potential adverse effects of the potentially effective antibiotic may be a concern. Although MIC may be used as a guideline for antibiotic selection, veterinary breakpoints for most antibiotics in the urinary tract of dogs and cats have not been determined. When intermediate results are obtained, it may be possible to achieve a cure if no alternative antimicrobial exists. This would most likely be achieved by using a higher dose of an antimicrobial that is excreted in the urine. In resistant infections, requesting an expanded antimicrobial susceptibility profile (e.g., ceftazidime, carbenicillin, nitrofurantoin) may suggest alternative treatment regimes. Most commercial bacteriology laboratories have a second and third tier of antibiotics for expanded susceptibility testing; however, high cost and parenteral administration may be trade-offs.

Classifying Urinary Tract Infections

Recurrent UTI: Relapse versus reinfection

The appropriate classification of a recurrent UTI can help the clinician modify therapy, identify predisposing factors, identify reasons for treatment failures, and identify the need for prophylactic therapies. Recurrent UTIs can be defined as either relapses or reinfections. A relapse is an infection caused by the same bacteria after clearance of the infection (confirmed by negative culture) that caused the original infection, and usually occurs within several days of cessation of treatment. Relapses are most frequently associated with ineffective antimicrobial treatment. This may be due to improper antibiotic usage (inappropriate selection, dose, duration, or poor owner compliance), emergence of drug-resistant pathogens, or failure to eliminate predisposing causes that alter normal host defense mechanisms and allow the persistence of bacteria. Relapse UTIs may be associated with a higher degree of antimicrobial resistance compared to the original infection.

The other type of recurrent UTI is a reinfection. Reinfections occur when the initial infection was effectively treated (documented by negative culture after therapy and resolution of clinical signs) and repeat culture confirms another infection with a different bacterial species or strain. Typically the time between reinfections is greater than the time between relapses. Reinfections usually indicate that host defense mechanisms are compromised.

Superinfection

Superinfections occur when new bacteria colonizes the urinary tract during the course of antimicrobial treatment for a UTI or another infection. These can be associated with urine diversion procedures (e.g., cystostomy, urethrostomy, and indwelling urinary catheters) and are not common.

Hospital-acquired UTI

Urinary catheterization is often useful in the management of patients with urethral outflow obstructive disease, for monitoring urine production, or for patient cleanliness. Several patient risk factors need to be considered when weighing the risk and benefit of placement of an

indwelling urinary catheter. The risk of hospital-acquired UTI in canine patients increases by 27% for every day an indwelling urinary catheter is in place.⁴ In addition, risk increases by 20% with every year of age, and increases in patients receiving antibiotics by 450%.⁶⁰ Gender did not correlate with increased risk of UTI for indwelling catheterization; however male dogs may be at decreased risk following a single catheterization.^{60,61}

The overall risk of nosocomial bacteriuria in catheterized patients is 10%.^{62,63} Attempts should be made to minimize iatrogenic UTI by avoiding indiscriminate use of urinary catheters, and using indwelling urinary catheters cautiously when pets are undergoing diuresis, are receiving immunosuppressive therapy, or are immunosuppressed. In addition, appropriately using antimicrobials and using diagnostic and therapeutic techniques that minimize trauma and microbial contamination of the urinary tract can help minimize the chance of infection.⁶⁴ Whenever possible, intermittent catheterization is preferred to indwelling catheterization especially in patients that are immunosuppressed and/or already receiving antibiotic treatment. Previously used IV fluid bags that did not contain dextrose and are <7 days old can be used as part of a closed urinary system if sterile bags are not available since they are unlikely to be sources of bacterial contamination.⁶⁵

Treatment

Treatment recommendations for bacterial UTI are dependent on several factors and should be tailored to each individual patient. Factors to consider include classification of the UTI (e.g., complicated/uncomplicated, relapse, reinfection, superinfection), location of infection (e.g., bladder, kidney, prostate), and concurrent complicating factors (e.g., systemic disease causing predisposition or poor response to therapy, underlying disease affecting antimicrobial selection). These factors affect both the duration of therapy and antimicrobial selection. Additional considerations include side effects, cost and compliance. Steps to follow for the ideal management of UTIs are listed in **Table 2.3**.

Table 2.3 Ideal steps to follow in the management of UTIs in dogs and cats

1. Diagnosis should be determined on the basis of history, urine sediment, and ideally, urine culture and susceptibility results. Rule out contamination in voided or catheterized samples with quantitative culture. Cystocentesis is the ideal way to collect urine for culture.
2. Select an antimicrobial agent based on culture and susceptibility. Without culture and susceptibility results use air-dried sediment findings and Gram stain to guide antibiotic choice (e.g., cocci vs. rods).
3. Re-culture urine (or at least examine the urine sediment) 3 to 5 days after antibiotic administration has begun. Urine culture should be negative and/or there should be no WBCs in the urine sediment.
4. Examine urine sediment or culture 3 to 4 days before discontinuing antibiotic treatment.
5. Repeat urinalysis and culture 5-7 days after cessation of antibiotic therapy. (Steps 4 and 5 are especially important for recurrent UTI.)
6. Patients with recurrent UTIs should undergo imaging studies (e.g., plain and/or contrast-enhanced radiography and/or ultrasonography), a complete blood count, and serum biochemistry profile to determine whether they have local or systemic underlying predisposing factors.
7. In frequent reinfections, low-dose nighttime antibiotic regimes or cranberry extract may be considered after the initial inflammation has been cleared up in response to standard-dose antibiotic treatment.

Antimicrobial Therapy

Antimicrobial selection

Inappropriate antibiotic dosages or unnecessary usage can lead to resistant organisms impacting not only the individual patient, but potentially bacterial resistance in other veterinary patients and owners.¹⁶ Antimicrobials that are excreted in the urine are the mainstay of UTI treatment. Initial antibiotic considerations can vary somewhat based on opinion. There is variation among recommendations and no consensus has been reached; therefore I recommend amoxicillin and cephalexin as initial treatment options. Trimethoprim-sulfadiazine has been suggested as well, however based on potential side effects (e.g., hypersensitivity, immune-mediated effects and keratoconjunctivitis sicca) should be chosen with careful patient selection and owner education. When host defense mechanisms are compromised, previous or current susceptibility results indicate potential resistance, or when the infection involves the prostate or kidneys, second tier

antibiotic choices may be considered with selection on an individual basis. Possible options include potentiated β -lactams (e.g., amoxicillin-clavulanic acid), fluoroquinolones, or extended release cephalosporins (e.g., cefovecin).^{66,67} Second tier antibiotics are not recommended as a first line treatment for an uncomplicated UTI, without appropriate culture and susceptibility results. However, these may be considered when rapid treatment is necessary to return to function, prevent systemic spread of bacteria, or when penetration into a specific tissue, such as the prostate is needed. For penetration into prostatic tissue, antibiotics should be lipid soluble, not highly protein bound, and ionize at the pH of the prostatic tissue. Good prostate gland penetration can usually be achieved with fluoroquinolones, trimethoprim-sulfa, and chloramphenicol.

Empirical antimicrobial therapy for uncomplicated UTI

Dogs and cats with uncomplicated UTI are typically treated with a 7-14 day course of antibiotics. It is reasonable to assume that shorter durations of appropriate antibiotics (≤ 7 days) may be effective; however clinical trials are needed to further evaluate shorter duration treatments.⁴⁷ It is always ideal to treat on the basis of urine culture and susceptibility results; however, economic constraints may preclude this in first time patients with suspected uncomplicated UTI. In these cases, antimicrobial selection should be based on bacterial characteristics observed in the urine sediment (e.g., Gram positive vs. Gram negative, cocci vs. rods). For example, if a Gram negative rod or bacillus is seen (e.g., *E. coli*, *Enterobacter*, *Klebsiella* or *Proteus*), cephalosporins would be a good option. With Gram positive cocci (e.g., *Staphylococcus* or *Streptococcus*), amoxicillin and cephalosporin would be options. There are disadvantages to consider with this technique. For example, identification of Gram positive cocci could also be consistent with *Enterococcus*, and would be resistant to a cephalosporin. Clinical signs should resolve within 48 hours with appropriate treatment. Along with resolution of clinical signs, evidence of inflammation in urine sediment should also resolve in 3-5 days of antibiotic treatment.

When empiric antibiotic treatment is used, instructions to owners regarding what to expect as well as follow-up plans are critical. If presenting clinical signs do not resolve quickly, owners should be aware of the need for a follow-up urinalysis and culture and susceptibility. Owners should also be aware of the importance of completing the entire antibiotic regime, even if signs

resolve early in the course of treatment. Finally, owners should be instructed to observe their pets closely for signs of UTI recurrence after the treatment regime is complete. Clear communication and understanding of the “game-plan” will result in 1) improved treatment outcome and 2) increased client willingness to return in cases of empiric treatment failures.

Short course/high dose protocols

Due to concerns with owner compliance during longer treatment protocols, short course/high dose protocols have been proposed for the treatment of uncomplicated UTI in dogs. In a recent study in dogs, a short course, high-dose of enrofloxacin (18-20 mg/kg once daily for three days) was as effective as traditional dose amoxicillin-clavulanate (13.75-25 mg/kg twice daily for 14 days) in treating uncomplicated UTI.⁶⁸ Preliminary evidence suggests this may be an effective option; however, additional research may be necessary to incorporate this into routine clinical practice.

Complicated UTI

Antibiotic treatment of complicated UTIs should be prolonged (typically 4 weeks) and always based on urine culture and susceptibility results. It is possible that shorter duration of therapy may be effective in some or all clinical situations, however further studies are needed to provide more specific recommendations.⁴⁷ One week into treatment and one week prior to discontinuing antimicrobials, re-evaluation of the urine sediment (+/- culture) can help evaluate response to therapy. When effective, no bacteria or white cells should be observed on sediment exam. Recheck urine culture is again recommended 5-7 days after completion of therapy to confirm successful resolution of the UTI. Prolonged treatment of a complicated UTI may be necessary to sterilize the urinary tract, but “buying time” to allow for correction of host defense mechanism abnormalities is another important consideration. If host defense mechanism abnormalities are not corrected, it may be difficult to clear the current infection or, more likely, the patient will experience reinfections.

Asymptomatic bacteriuria

Identification of bacteria in the urine, with an absence of clinical signs is defined as asymptomatic bacteriuria. Asymptomatic bacteriuria may be hospital-acquired in critically ill patients or patients with indwelling catheters, may be an incidental finding on culture, or may be associated with a disease that suppresses the normal inflammatory response. *E. coli* is most commonly isolated in patients with asymptomatic bacteriuria. Uropathogenic isolates from asymptomatic patients have been shown to express fewer virulence factors than those identified from patients with symptomatic UTI.⁶⁹ In my opinion, asymptomatic bacteriuria should be treated when quantitative culture results suggest an actual infection rather than contamination (**See Table 2.1**). Treatment of asymptomatic bacteriuria associated with lower numbers of bacteria in urine (i.e., numbers associated with contamination) may contribute to antibiotic resistance and colonization of the bladder with more pathogenic strains of bacteria.¹¹ Experimental urinary tract colonization of less pathogenic strains of *E. coli* have been evaluated in dogs as a potential treatment option for recurrent UTI, further suggesting that asymptomatic bacteriuria does not necessarily require treatment.¹⁰

Treatment Failures

Treatment failures may either involve an inability to eradicate the current infection (persistent infection), relapses or reinfections. Relapses and persistent infection are most commonly associated with antibiotic treatment failures whereas reinfections are most commonly associated with compromised host defense mechanisms (**Table 2.4**).

Table 2.4 Reasons for poor therapeutic response in dogs and cats with UTI

1. Use of ineffective drugs or ineffective duration of therapy
2. Failure of owner to administer prescribed dose at proper intervals
3. Gastrointestinal tract disease, decreased bioavailability of a drug, conditions resulting in decreased drug absorption, or impaired renal concentrating ability resulting in decreased antibiotic concentrations in the urine
4. Impaired action of drugs, either because bacteria are not multiplying or because they are sequestered in an inaccessible site (e.g., prostate, neoplasia, or uroliths)
5. Failure to recognize and eliminate predisposing causes
6. Presence of mixed bacterial infections in which only one of the pathogens is eradicated by antimicrobial therapy
7. Iatrogenic reinfection caused by catheterization
8. Development of drug resistance in bacteria

Antimicrobial resistance

Multi-drug resistance (MDR) is becoming more commonly recognized in veterinary medicine. The urinary tract was the most common extra-intestinal source of MDR *E. coli* and *Enterobacter* in dogs accounting for 62 and 58% of the isolates, respectively.¹¹ Twenty-seven percent of dogs with UTI had a urinary catheter and all but one was given antimicrobials while a urinary catheter was in place.¹¹ Risk factors identified were underlying illness contributing to immunosuppression (97%), hospitalization for ≥ 3 days (82%), and surgical intervention (57%).¹¹ Several canine retrospective studies evaluating UTI isolates and resistance are available. One study found a significant increase in recurrent *E. coli* infections and increased resistance with all antimicrobials tested with the exception of tetracycline and trimethoprim-sulfamethoxazole.⁷⁰ Another study found an increase in fluoroquinolone resistance.⁶⁶ Evaluation of *Staphylococcus* resistance found 100% of isolates to be resistant to at least one drug and 77% showed MDR.⁷¹ Increased incidence of multidrug-resistant *Enterococcus* from the urinary tract has also been identified.⁷²

Inability to eradicate and/or recognize underlying causes

Compromised local or systemic host defense mechanisms increase the risk of UTI and make treatment more difficult. Although a transient breach in host defenses may result in a simple uncomplicated UTI, it is of utmost importance to identify and resolve potential underlying causes whenever possible. Common systemic disorders known to predispose to UTI include chronic kidney disease, endogenous or exogenous glucocorticoid excess, diabetes mellitus, and hyperthyroidism.^{24, 41, 42, 44} Although some of these disorders may not be correctible, control of the underlying disease is often helpful. Local disorders of the lower urinary tract also increase the risk of UTI. Examples include anatomic abnormalities like a “hooded” or recessed vulva, ectopic ureter and urolithiasis.⁷³ Systemic and local defense mechanism abnormalities are often ruled out with a complete minimum data base (e.g., complete blood count, chemistry, and urinalysis) and imaging, respectively.

Ancillary Prevention Therapies

Ancillary therapies designed to prevent recurrent UTI are considered in patients where breaches in host defenses are present but are not correctable or in cases where an underlying cause for reinfection is not identified. Care should be taken to treat any underlying infection prior to starting any prevention measures. Owners should watch closely for clinical signs and be advised that frequent rechecks will be necessary to detect and treat any break through infections.

Prophylactic antimicrobial treatment (low dose protocols)

Prophylactic antimicrobial treatment involves long-term daily administration of low-dose antimicrobials in order to inhibit or minimize uropathogen growth, thus decreasing the opportunity for bacterial adhesion and colonization of the uroepithelium. Unfortunately there are no studies in dogs that have evaluated the efficacy and adverse effects of these protocols. Due to risk of selecting for resistant organisms, prophylactic, low-dose antibiotic administration should be reserved for refractory cases and then only after all attempts to resolve correctable problems have been exhausted.

Prophylactic, low-dose antibiotic treatment should only be initiated after standard dose antibiotic treatment has been successful (negative urine culture). Best results are expected with drugs that are excreted in the urine. Other considerations should include potential side effects of the drug

and previous culture and susceptibility results. Commonly used protocols include fluoroquinolone, cephalosporins, or β -lactam antimicrobials. If *E. coli* or enterococci is previously cultured, nitrofurantoin is another option. The dose used should be one-half to one-third the therapeutic daily dose administered immediately after the last voiding before bedtime. This protocol is typically recommended for a minimum of 6 months and urinalysis and culture should be performed every 4-8 weeks. If at any point during the treatment a UTI occurs, it is treated as a complicated UTI. Prophylactic, low-dose therapy can be re-started after the reinfection has been resolved.¹⁵

Cranberry extract

Proanthocyanidins, specifically A-type isoforms, found in cranberries inhibit *E. coli* attachment by blocking interaction of bacterial P-fimbriae with the surface of uroepithelial cells.^{74,75} Canine studies evaluating the efficacy of cranberry extract are limited; however, some *in vitro* data show promising results. One study demonstrated that *E. coli* in urine from dogs receiving oral cranberry extract (CE) had decreased ability to agglutinate to human red blood cells (Nutramax Laboratories, Inc. Crananidin™).⁷⁵ Similarly a second study (see **Chapter 3**) demonstrated that in urine from dogs receiving oral CE (Vetquinol, Paxon™), *E. coli* had decreased ability to adhere to Madin-Darby Canine Kidney (MDCK) cells.⁷⁶ Based on these *in vitro* studies, oral administration of cranberry extract may help reduce *E. coli* reinfections in patients with compromised host defense mechanisms. Due to large variations of the active compound in over-the-counter products, it may be of benefit to use the products evaluated for canine use (Crananidin™ or Paxon™) dosed according to manufacturer recommendations. Chapter 3 will further discuss the study performed as part of this thesis on the use of CE (Vetquinol, Paxon™).

Urinary antiseptics

The most commonly used urinary antiseptic is methenamine which is converted to formalin in an acidic environment. Methenamine may be considered in patients with recurrent infections or patients with potentially untreatable or unresolved breaches in immunity. Just as with the use of cranberry extract, this prophylactic treatment is not recommended as a sole treatment for UTIs. Methenamine should only be used in conjunction with antibiotics for the treatment of current infections or as a preventative once the previous UTI has been cleared. Usage should be avoided

in patients on treatment with sulfonamides or medications that cause alkaline urine. Urine acidifiers added to methenamine cause acidic urine that can also decrease activity of fluoroquinolone and aminoglycoside antibiotics. High doses can cause irritation to bladder mucosa; however this is more common in people than dogs.

Miscellaneous treatments

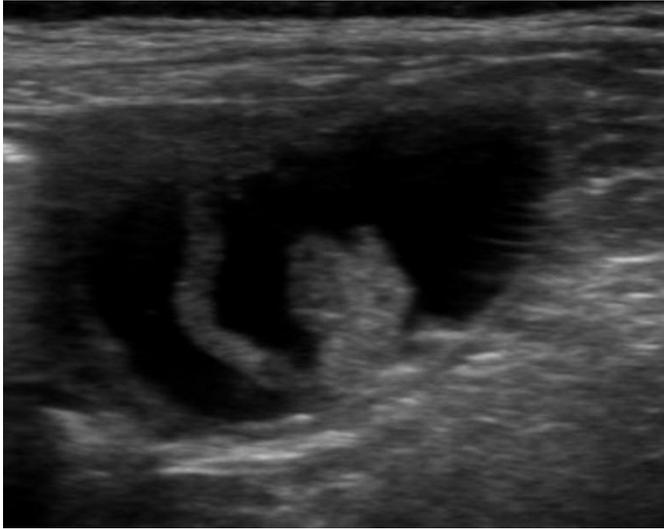
Urinary acidifiers are not recommended as adjunctive treatment of UTI, since dogs and cats usually have an acidic pH and many factors (e.g., diet, concurrent disease, and certain bacteria) can cause alkaline urine and prevent activation. A possible exception is concurrent use with methenamine. Similarly, antimicrobial agents instilled directly into the urinary bladder are not recommended as they have not been shown to be effective. In addition, potential complications could include bladder rupture and absorption of toxic concentrations of drugs through an inflamed bladder wall. Antibiotic administration via a urinary catheter would only be of use in patients with urine retention disorders that require intermittent urethral catheterization to empty the bladder (e.g., upper motor neuron bladder disorders).

Complications of Urinary Tract Infections

Polypoid cystitis

Polypoid cystitis occurs when uroepithelial proliferation is severe; resulting in mass-like lesions or diffuse thickening of the bladder wall associated with intramural accumulation of inflammatory cells.^{77,78} Typically these lesions are identified by ultrasound or contrast radiographs during evaluation for predisposing factors (**Figure 2.3**). If bladder wall thickening is present in the apex of the bladder and the lesion does not have prominent vascular flow on Doppler, polypoid cystitis is likely; however histopathology is required for definitive diagnosis. *Proteus* is commonly associated with the development of polypoid cystitis.^{77,78} Polypoid lesions often contribute to patient discomfort but may also serve as a nidus for relapse. Medical management involving treatment of the UTI with appropriate antimicrobials may resolve the lesions. Surgical excision and biopsy of localized lesions should be considered to decrease duration of medical therapy and to rule out TCC as the possible etiology.⁷⁷ If cystoscopy is available, it may provide a less invasive option to collect samples for histopathology and culture.

Figure 2.3 Ultrasound image of a mass-like lesion in the apex of the bladder consistent with polypoid cystitis



Emphysematous cystitis and pyelonephritis

Gas can be produced in the bladder wall or lumen and/or renal pelvis as a consequence of bacterial infection and most commonly occurs in dogs and cats with diabetes mellitus since the presence of glucosuria provides a fermentable substrate (**Figure 2.4**).⁷⁹ Emphysematous cystitis is usually caused by *E. coli* infection, but *Proteus*, *Clostridium*, and *Aerobacter aerogenes* infections have also been reported.^{79,80} Therapy should be similar to treatment for a complicated UTI with additional treatment to resolve glucosuria if present. Emphysematous pyelonephritis can occur for the same reasons as emphysematous cystitis, and appears to be a rare complication in veterinary medicine.

Figure 2.4 Lateral abdominal radiograph of a newly diagnosed diabetic cat with emphysematous nephritis and cystitis secondary to an *E. coli* and *Streptococcus* sp. UTI



Magnesium ammonium phosphate (struvite) urolithiasis

Urolithiasis may damage the uroepithelium and predispose dogs and cats to UTI, but infections may also result in urolith formation. Struvite urolith formation most frequently occurs secondary to an infection involving a urease producing bacteria such as *Staphylococcus*, *Proteus*, and less commonly *Corynebacterium*, *Klebsiella*, and *Ureaplasma*. Urease hydrolyzes urea to ammonia, which buffers urine hydrogen ions, forming ammonium ions and increasing urine pH leading to an increased amount of dissolved ionic phosphate. The increased concentration of ammonia and phosphate along with increased urine pH sets the stage for struvite crystal formation, and inflammatory debris associated with the UTI may serve as a nidus for urolith formation. Differences exist between canine and feline patients with regard to struvite urolith pathophysiology. In canine patients, > 90% of struvite uroliths occur secondary to a UTI, however in feline patients, struvite uroliths are more likely to be sterile. If medical dissolution therapy is not an option for struvite urolithiasis or if other complicating factors exist (such as other underlying host defense compromise or urinary obstruction), surgical removal may be indicated and the uroliths and a piece of bladder mucosa should be analyzed and cultured.^{81,82}

Pyelonephritis

Most commonly pyelonephritis (infection of the renal pelvis) occurs secondary to an ascending UTI. The kidneys are typically protected from ascending bacterial infection by long ureters which keep urine flow mostly one-way with peristalsis, the valve-like nature of the vesicoureteral junction that prevents reflux of urine during voiding, and a relatively hypoxic environment present in the renal medulla. Risk factors for pyelonephritis, similar to those for a lower UTI, include ectopic ureters, renoliths, obstructive uropathies, urine retention disorders, and systemic immunocompromise. In comparison to a typical lower UTI presentation, acute pyelonephritis may be associated with signs of systemic illness (lethargy, anorexia, fever, inflammatory leukogram, lumbar pain). In addition, renal azotemia and urine concentrating deficits may exist with bilateral pyelonephritis. If present, pyelectasia is commonly observed on ultrasound and a skilled ultrasonographer may be able to perform pyelocentesis to obtain samples for cytology and culture.⁸³ If pyelonephritis is suspected or confirmed, the UTI should be treated as a complicated UTI with a minimum of 6-8 weeks of antibiotics. Regular ultrasound monitoring should be performed to evaluate for resolution of pyelectasia, monitor for progression, or identification of other underlying causes of pyelectasia (e.g., obstructive ureterolith) that may require additional antibiotic therapy or alternative therapies.¹⁹ It has also been suggested that treatment duration of 4-6 weeks may also be adequate.⁴⁷ As with other suggested protocols, further research may help determine if a shorter duration may also be effective.

Prostatitis

The prostate is exposed to commensal microorganisms from the distal urethra and with this constant exposure comes risk of infection. Bacterial prostatitis can occur as a diffuse infection or as a prostatic abscess. A healthy prostate gland has local protective mechanisms that typically prevent colonization and infection and it is likely that bacterial prostatitis occurs due to a breakdown in the normal host defenses or secondary to other prostatic pathology (e.g., prostatic neoplasia, or benign prostatic hyperplasia).^{25, 26} In patients with suspected prostatic disease, catheterization for retrieval of prostatic fluid or washings with prostatic massage, ejaculate or prostatic aspirates may be considered in addition to cystocentesis for culture evaluation.⁸⁴ When treating chronic prostatitis, it is necessary to treat with antibiotics that penetrate the blood-

prostate barrier such as fluoroquinolones, trimethoprim-sulfa combinations, and chloramphenicol. Treatment should be continued initially for 4 weeks; however extended therapy (6 weeks) may be needed.^{47,85} In patients with acute prostatitis, disruption of the prostatic capsule should allow any antibiotic concentrated in the urine to penetrate into the prostatic tissue; however, as the inflammation subsides with treatment, antibiotic penetration may become an issue. In patients with abscessation of the prostate, surgery or ultrasound guided drainage should be considered.^{86,87} In intact male dogs, castration may be beneficial in resolving chronic bacterial prostatitis.⁸⁸ Recurrence is possible, especially if underlying conditions are present.

Prognosis

Most UTIs are uncomplicated and appropriate antibiotic treatment rapidly clears the infection resulting in a good prognosis. However, the prognosis for a complicated UTI may be guarded. It is of utmost importance to correct underlying defects in host defenses whenever possible to achieve the best outcome with a complicated UTI. Following protocols for ideal UTI management and monitoring will improve treatment success and decrease the incidence of recurrent UTI.

Chapter 3 - Cranberry Extract Reduces Adhesion of Uropathogenic *E. coli* to Canine Uroepithelial Cells

Introduction

Urinary tract infection (UTI) caused by *Escherichia coli* (*E. coli*) species is common in pet dogs. *E. coli* organisms caused ~ 45% of all UTI infections in a general referral population of dogs,⁴ however in dogs with illnesses that convey a higher risk of UTI, such as diabetes or hyperadrenocorticism, the prevalence of *E. coli* UTI is greater still and approached 70%.⁴² In dogs presenting to a referral institution for persistent or reinfection UTI, 71% were found to have an underlying disorder thought to be associated with the recurrent UTI.⁸⁹ In this study, clinical resolution of the UTI was only achievable in 35% of these cases.⁸⁹ This data suggests there are patients “at risk” who may benefit from UTI prevention.

One potential strategy to address UTI prevention in at-risk patients involves the use of cranberry products to reduce UTI incidence. Cranberry fruit juice and cranberry-derived products have been reported to have antibacterial properties, although the basis for the actions of cranberry is not completely understood. Oral administration of cranberry extract (CE) alters urine pH and increase urinary excretion of hippuric acid.⁹⁰ These alterations had previously been postulated as mechanisms of action for CE; however more recent research has questioned these mechanisms.⁹⁰ Currently proanthocyanidins (PACs), specifically A-type isoforms, contained in cranberry juice are proposed to inhibit *E. coli* attachment by blocking interaction of bacterial fimbriae with the surface of uroepithelial cells, effectively preventing bacterial adherence and reducing colonization of the urinary epithelium.^{74,90} Cranberry extract (CE) is a complex organic mixture, the composition of which varies with the methods used to extract and concentrate the cranberry products. Other compounds found in cranberry extracts including 1-*O*-methylgalactose, prunin, and phloidyin also have demonstrated anti-adherence activity *in vitro*.⁹¹ Drinking cranberry juice was shown to effectively reduce the incidence of bacteriuria in human subjects.⁹² A study of a commercially available cranberry powder used in an *in vitro* study found inhibition of *E. coli* adherence to bladder or vaginal epithelial cells.⁹⁰

Research evaluating CE efficacy in veterinary medicine is limited. A study of urine from dogs fed a CE product using a modified MIC method, found no significant inhibition of bacterial

adherence to canine uroepithelial cells.⁹³ These results are difficult to evaluate as the method used to determine bacterial adherence was not detailed and it was not clear whether the urine was analyzed for A-type proanthocyanidins and other bioactive compounds contained in CE.

Previous human studies have evaluated cultured bladder, vaginal epithelial, and urothelial cells for adhesion studies.^{90,91} Madin-Darby Canine Kidney (MDCK) cells are a well-characterized canine uroepithelial cell model. MDCK cells were established in 1958 from the kidney of a normal dog and have become a mainstay model for epithelial cell research.⁹⁴ Earlier work used the MDCK cell model to study infection by uropathogenic bacteria, including *E. coli*.^{95,96}

Current treatment strategies focus on eradication of established infections and rely almost exclusively on the use of antibiotics to establish a cure. Evidence for antibiotic resistance among common bacterial organisms does exist. This underscores the potential for therapeutic failure and persistent infection. Further, an expert panel has expressed concerns about the broader dangers posed by injudicious and improper use of antibiotics to treat canine infections.¹⁶ Given these concerns and the limitations of antibiotic therapy, new strategies for treatment and prevention of urinary tract infections in dogs need to be developed.

Effective alternatives to antibiotics may offer a partial solution to the problem of antibiotic resistance, and use of compounds, such as CE, are likely to play an increasingly important role in UTI prevention in dogs. This study assessed the efficacy of a commercial CE product (Vetoquinol, Paxon™) to inhibit bacterial adhesion to canine uroepithelial cells. The hypothesis was that purified CE would inhibit bacterial adhesion to canine uroepithelial cells.

Materials and Methods

Dogs

Five client-owned female dogs, (three spayed and two intact) were enrolled in the study. At enrollment, all dogs were determined to be healthy based on medical history and a complete physical examination. The dogs also had no previous significant medical history, including no signs of lower urinary tract disease. Each dog received an oral CE supplement (Vetquinol, Paxon™) daily for 30 days. The CE supplement was administered once daily by mouth at the dose specified on the product label (10-20 mg/kg). For all dogs, voided urine samples were

collected before (PRE) and 4 weeks (30-DAY) after beginning CE supplementation. The protocol was approved by the Kansas State University Institutional Animal Care and Use Committee. Dog owners were dispensed a 30-day supply of a supplement that contained 100 mg CE/tablet.

Urine samples

Urine samples were collected at-home by dogs' owners and refrigerated until submitted to the laboratory for processing. Upon receipt in the laboratory, urine samples were prepared for storage by centrifugation at 3500 rpm for 5 min to sediment particulate matter. The supernatant was drawn off using a sterile Pasteur pipette and vacuum filtered using a commercial filtration unit with a 0.22 µm polyethersulphone (PES) filter (Steriflip Filter Unit, Millipore). The filtered urine was collected in a sterile 50 ml conical tube and frozen at -20°C until used in experiments.

Madin-Darby Canine Kidney (MDCK) cells

Madin-Darby Canine Kidney (MDCK) cells were maintained in Eagle's minimum essential medium (MEM) plus 10% (v/v) heat inactivated fetal bovine serum and supplemented with 1 mM sodium pyruvate and 1% (v/v) antibiotic solution (penicillin-streptomycin-amphotericin B). Stock cultures of cells were propagated in 75 cm² plastic flasks at 37° C in a humidified 95% O₂/5% CO₂ atmosphere and passaged as needed.

Propagation of uropathogenic E. coli strain

An UPEC *E. coli* strain isolated from a human clinical UTI was used for adhesion experiments. The *E. coli* strain was grown on blood agar plates at 35°C for 24-48 hours. After the appearance of distinct bacterial colonies, plates were sealed and stored at 4°C until needed for experiments. For experiments, an *E. coli* colony was picked, streaked, and incubated at 35°C overnight on colony forming agar (CFA) to promote fimbrial expression.⁹¹ Colony forming agar was prepared with casamino acids (10g/L), yeast extract (1.5 g/L), MgSo4 (0.05 g/L), MnCl2 (0.005 g/L) and 2% (v/v) agar per liter water, as previously described.⁹²

Adhesion assay

The efficacy of CE to inhibit bacterial adherence to MDCK uroepithelial cells was evaluated using an *in vitro* adhesion assay modified from Turner et al.⁹¹ Adhesion assays were performed as follows.

Preparation of bacterial suspension

E. coli colonies grown overnight on CFA agar were picked and suspended in 200 µl saline to a standard bacterial concentration of $\sim 3 \times 10^6$ CFU/ml, which was determined by absorption spectroscopy (between 0.075 and 0.085 AU at 650 nm).

Preparation of MDCK cells

The MDCK cells that had been grown to confluence at 37°C were prepared in 96-well plastic plates. Immediately before being used in the adhesion assay, MDCK cells were fixed with methanol. For fixing, culture media was discarded and each well washed with 100 µl phosphate buffered saline. The wash was discarded and the plate tapped dry on absorbent paper. Methanol (100 µl) was added to each well and allowed to remain for 2 minutes. The methanol was discarded and the plate tapped dry on absorbent paper. The plate was dried in a laminar flow hood for 10 minutes.

Preparation of test samples

To prepare samples for testing, an aliquot of the bacterial suspension was mixed 1:10 with urine samples (either PRE or 30-DAY). To block mannose-dependent Type 1 fimbriae, a 96-well plastic plate was prepared by adding 50 µl of the test sample (urine + bacteria) to 150 µl modified MEM media supplemented with 2.5% methyl-mannose (final volume 200 µl/well) and the plate incubated at 25°C for 30 min.

Adhesion phase

After incubation with mannose for 30 minutes, a 100 µl aliquot of the test sample containing *E. coli* was added to 96-well microplates containing methanol-fixed MDCK cells. The microplate was centrifuged at 500 rpm for 2 min to collect fluid into the bottom of the well and the plate incubated for 60 min at 35°C to permit bacterial attachment. After 60 minutes, non-adhered

bacteria and media were removed by aspiration and wells were rinsed three times with 200 μ l PBS. After the wash step, 200 μ l fresh RPMI + 5% heat inactivated FBS were added to each well and the plates incubated at 35°C for 4 hours to grow attached bacteria to detection level. A standard curve was prepared for each plate by making serial dilutions of a suspension containing 1×10^7 bacteria. Absorbance at 650 nm was determined for each well at 0 hr (baseline value) and 4 hr using a plate reader. The difference in absorbance between 0 and 4 hr represented growth of bacteria that had been adhered at 0 hr. The initial concentration of adhered bacteria in each well was determined using known concentrations obtained from standard curve information. All samples were analyzed in duplicate.

Direct killing assay

Cranberry extract was assessed for bacteriostatic or bactericidal effects by exposing urine to bacteria in the absence of MDCK cells. The direct killing assay was performed similarly to the adhesion assay. Preparation of the bacterial suspension and the test samples was performed as described. To assess direct effects on bacterial growth or viability, the plate assay was performed as described for the adhesion assay except that the plates did not contain MDCK cells and the aspiration step was eliminated. After incubation with mannose for 30 minutes, a 100 μ l aliquot of the test sample containing *E. coli* was added to 96-well microplates. The microplate was centrifuged at 500 rpm for 2 min to collect fluid into the bottom of the well. A standard curve was prepared for each plate by making serial dilutions of a suspension containing 1×10^7 bacteria. The plate incubated for 60 min at 35°C to permit bacterial growth. Absorbance at 650 nm was determined for each well at 0 hr (baseline value) and 4 hr using a plate reader. The difference in absorbance between 0 and 4 hr represented growth of bacteria. PRE and 30-DAY samples from each dog were pooled for use in the direct killing assay. In addition to PRE and 30-DAY urine samples, a control (bacteria in phosphate-buffered saline) was evaluated.

Statistical Analysis

A repeated measures ANOVA was used to evaluate the effect of oral cranberry extract supplementation on bacterial adhesion. The model outcome was the difference in bacterial adhesion count between pre (PRE) and post (30-DAY) treatment samples. The analysis accounted for the effects of dog and replicate. A commercial software program was used (Stata

12) was used for all analyses (StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP).

Results

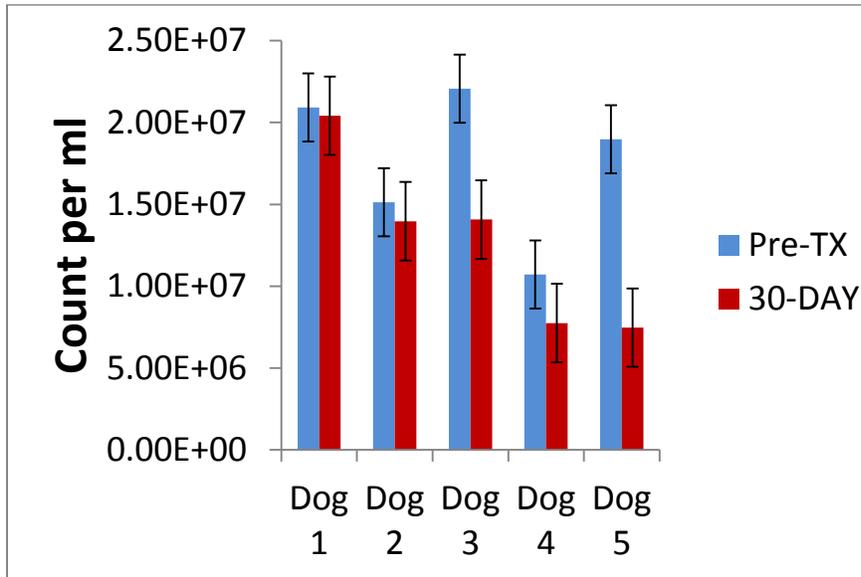
Study dogs

Five female dogs [Labrador Retriever (n=2), Shetland Sheepdog (n=2), and Boston Terrier (n=1)] completed the 30-day study. PRE and 30-DAY urine samples were obtained from all enrolled dogs. The mean weight was 16.2 kg (35.6 lbs) with a range from 5.9 – 29.5 kg (13 – 65 lbs). The mean age was 9 years with a range from 1-14 years. No adverse effects attributable to CE supplementation were reported and all dogs remained healthy throughout the study period. Owner compliance with CE supplementation was 100% with no missed doses reported. All 5 dogs readily consumed the tablets containing the CE supplement.

Bacterial adhesion assay

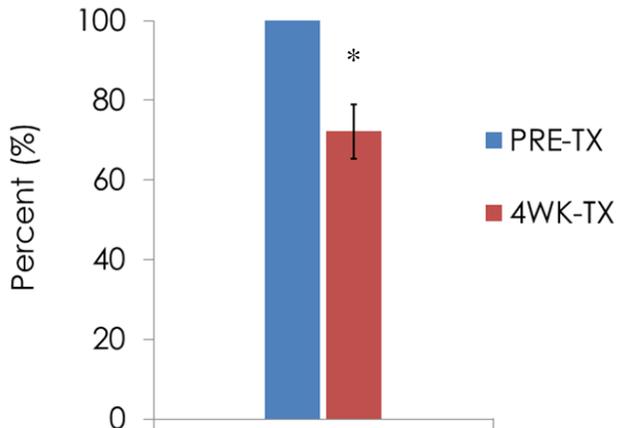
PRE and 30-DAY urine samples from each dog were assayed in duplicate in 4 separate assays. Compared with PRE urine, bacterial adhesion was reduced in 30-DAY urine in each of the 5 dogs. The magnitude of reduction ranged from 2.4 – 61% (mean 27%) across individual dogs (**Figure 3.1**). When PRE and 30-DAY urine samples were compared, the combined results for all dogs (n=4 separate assays) showed that bacterial adhesion was significantly reduced in 30-DAY samples (p=0.015) (**Figure 3.2**).

Figure 3.1 Effect of CE Supplement on Bacterial Adhesion in Urine-Individual Dogs



This figure represents N=4 separate assays, for each of the five dogs. The PRE is compared to 30-DAY for each dog. The bars represent the standard deviation.

Figure 3.2 Effect of CE Supplement on Bacterial Adhesion in Urine-Combined Data

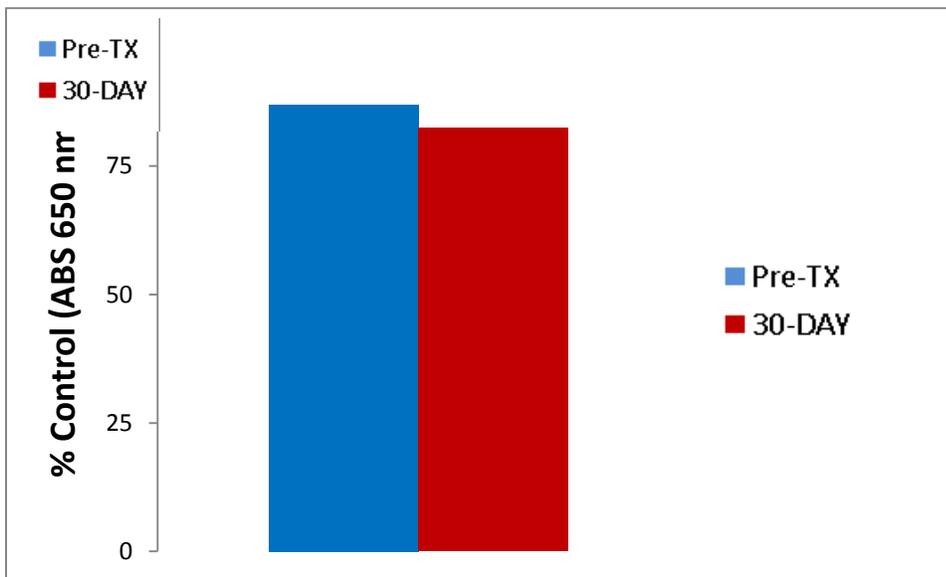


This figure shows the overall inhibition of bacterial adherence observed in urine samples from all dogs. Compared with pre-treatment urine, urine for dogs that received CE supplementation for 30 days had 30% overall reduction in bacterial adhesion. * Denotes statistical significance from PRE-TX (P = 0.015).

Direct killing assay

The results of the direct killing assay demonstrated an inhibitory effect of urine on bacterial growth. Bacterial growth was reduced by 13% and 18%, respectively, in PRE and 30-DAY urine samples compared with saline controls. However, there was no difference in bacterial survival and growth when PRE and 30-DAY urine samples were compared (**Figure 3.3**).

Figure 3.3 Direct Killing Assay Demonstrates Inhibitory Effect



This figure shows the inhibitory effect of urine on bacterial growth. Compared with the saline control bacterial growth is reduced in pooled urine samples from dogs that received CE supplementation. There was no significant difference between bacterial growth in PRE (n=3) and 30-DAY (n=4) samples.

Discussion

Urinary tract infection by uropathogenic *E. coli* (UPEC) involves a complex process of bacterial adhesion and invasion. Adhesion is mediated in part via structures, such as mannose-sensitive Type 1 fimbriae and non-Type 1 fimbriae such as Types P, S, and F1C.^{97,98} It is reported in people that over 90% of pathogenic and non-pathogenic *E coli* strains express Type 1 fimbriae.⁹⁷ In people, non-Type 1 fimbriae are associated with UPEC strains that exhibit tropisms within the urinary tract; Type P fimbriae, for example, are often expressed by UPEC strains that cause pyelonephritis and acute prostatitis.⁹⁹ CFA agar in the present study was used because *E. coli*

grown under these conditions to promote P fimbriae expression by *E. coli* colonies.⁹¹ Polymerase chain reaction testing of *E. coli* grown on CFA agar would have been needed to confirm P fimbriae gene expression but was not performed.⁹¹ To further ensure that any observed bacterial adherence was mediated via non-Type 1 fimbriae typically expressed by UPEC, bacteria were incubated with mannose to block Type 1 and mannose-sensitive adhesion mechanisms. Adhesion observed in the presence of mannose indicates that adhesion involved mannose-insensitive mechanisms, which typically mediate UPEC infection.

In the present study, the goal was to investigate an overall effect of oral CE supplementation on bacterial adhesion in canine urine. The major finding was that *E. coli* adhesion to canine uroepithelial cells was reduced on average by 27 % in urine from dogs that had received the CE supplement. The adhesion assay detects *E. coli* bacteria that remained adhered to MDCK cells despite mannose-blockade and extensive washings. Bacterial persistence under such conditions is likely the result of pathological attachment mechanisms, rather than non-specific interaction with the MDCK cell surface. It is possible that bacterial growth in the last step of the adhesion assay could be impaired if CE exerted a bacteriocidal or bacteriostatic effect. This possibility was addressed in the direct killing assay, which showed a minor bacteriocidal effect of urine but no direct bacterial killing by CE. The anti-bacterial effects of urine on the UPEC strain used in these experiments is explained by the fact that normal urine has biological and physiochemical characteristics that prevent bacterial growth.²³ However, although bacterial growth was suppressed in PRE and 30-DAY urine samples compared with saline controls, there was no difference in bacterial growth when PRE and 30-DAY samples were compared.

A previous canine study found that a CE inhibited bacterial adherence to primary cultures of uroepithelial cells, but that urine from dogs given an oral CE preparation did not have a similar effect.⁹³ Dogs fed the CE preparation were only treated for 7 days prior to analysis, and it is possible that a longer period of administration was needed for an effect to become apparent. However, it was reported in a recent abstract that *E. coli* in urine from dogs receiving oral CE had decreased ability to agglutinate human red blood cells as early as 7 days after CE supplementation.⁷⁵ In contrast, in a human study, reduction of bacteriuria after ingestion of cranberry juice was not evident until 1-2 months of treatment.⁹² While the duration of

supplementation may have some role in determining the onset of the CE effect, it is also likely that other factors are involved.

The concentration of PAC equivalents is a factor that influences the anti-bacterial effects of CE supplements. A human study evaluating dose effect of ingested cranberry powder in urine on a human epithelial cell line found a clear dose-dependent inhibition of *E. coli*; the effect increased with the amount of PAC equivalents consumed.¹⁰⁰ An *in vitro* study found *E. coli* adherence to uroepithelial cells to be inhibited in a linear, dose-dependent manner over PAC concentration range of 5 to 75µl/ml.⁹⁰ Ideally the concentration of PAC's would have been measured in 30-DAY urine samples and the CE tablet in the present study to determine optimal CE doses. Comparison between the current and previous studies cannot be made easily since PAC's were not measured in the CE product or in the urine samples tested. However, the dosing protocol used in the current study was sufficient to induce a CE effect in all 5 dogs studied. After the 30 day treatment period, urine from all dogs studied presumably contained enough PAC equivalents to exert an inhibitory effect on bacterial adhesion.

The current study was not designed to be a clinical study, and thus it was not the intention to detect differences between individual dogs. However, it is interesting to note that there were obvious differences in the CE effect between individual dogs. The reason(s) for such pronounced individual differences in the CE effect are not known. Some of the difference between treated individuals could be related to the ingested dose of CE. The CE was administered using product label guidelines and tablets were not divided. Individual dogs received between 10-20 mg/kg, with the dose being rounded to the nearest tablet size. Differences in gastrointestinal absorption, CE metabolism and renal excretion could also contribute to the individual differences observed. Additional studies would be needed to investigate the physiological and pharmacological basis for these findings. Future studies could further evaluate the clinical efficacy of CE supplementation to reduce *E. coli* UTI as well as identify individuals most likely to benefit from treatment.

Conclusion

The current *in vitro* study shows that urine from dogs that received an oral dose of CE for 30 days dramatically reduces bacterial adhesion to canine uroepithelial cells. These results suggest that oral CE supplementation may have a beneficial action to reduce bacterial UTI in dogs. Prospective clinical trials, including normal dogs as well as “at risk” dogs, are needed to assess the full effect of CE supplementation on urinary tract health.

Chapter 4 - Effect of Storage Time and Temperature on Canine Urine Enzymes

Introduction

Acute kidney injury (AKI) in dogs can occur secondary to administration of potentially nephrotoxic drugs (e.g., antibiotics, antifungals, chemotherapeutics, and NSAIDs) and has a high mortality rate.¹⁰¹ Even in cases with reversible renal lesions, hospital-acquired AKI often leads to more serious consequences than the underlying disease process that required the initial hospitalization.¹⁰¹ Early recognition of kidney injury is important since extensive injury can occur before changes in routine blood work are detected. In addition, continued administration of nephrotoxic medication after initial kidney injury occurs can lead to more serious injury. Early detection of AKI may result in adjustment of therapy, including discontinuing potentially nephrotoxic therapeutics, or when that is not possible, dose adjustments (e.g., either increasing time between doses or dose reduction), or initiating supportive measures (e.g., fluid therapy).

Measurement of enzyme activity in urine may aid diagnosis of early nephrotoxicant-induced AKI. γ -glutamyl transpeptidase (GGT) and N-acetyl- β -D-glucosaminidase (NAG) are two of the more commonly measured canine urine enzymes. GGT originates from the proximal tubule brush border and NAG is present in the proximal tubule lysosomes.¹⁰² Although both enzymes are also present in the plasma, they are too large to be freely filtered by the normal glomerular capillary wall. Studies have shown that measurement of these enzymes in the urine facilitates detection of early renal tubular damage prior to changes in serum biochemical parameters and urine specific gravity.^{103,104}

After acquisition of a urine sample, enzymuria determination may be delayed for sample batching and shipping to an outsourced laboratory. In addition, sometimes sequential tests may be performed on an individual and compared, for example, baseline urine samples may be saved and run with post-treatment urine samples collected at a later time. Therefore, it is important to know how storage of urine samples affects enzyme activity.

Previous studies of GGT stability in the urine of healthy human beings found no changes in enzyme activity after short term storage (24 h) at room temperature or at 4°C, but found only

partial preservation of enzyme activity in urine supernatant stored at -70°C .¹⁰⁵ Urine enzyme stability was not maintained even after a few days of freezing at -20°C .¹⁰⁵ A recent study evaluating urinary biomarkers in dogs with known hereditary renal disease assessed urine NAG stability and reported decreased enzyme activity in 2 of 4 urine samples stored at room temperature for 12-24 hours.¹⁰⁶ In addition, there was increased urine enzyme activity in 2 of 4 samples stored at 4°C .¹⁰⁶ Freezing at -20°C or -80°C for up to one year did not affect urine NAG activity.¹⁰⁶ To the authors' knowledge there have been no previous studies evaluating the stability of GGT in canine urine. The purpose of the present study was to determine how sample storage affects NAG and GGT activity in canine urine.

Materials and Methods

Samples

Sixty-six canine urine samples submitted to the Clinical Pathology Laboratory at Kansas State University were initially included. Samples were collected from clinical patients at the Veterinary Medical Teaching Hospital at Kansas State University that had a variety of medical or surgical disorders. Additionally, samples were selected based on the hours of operation of Clinical Pathology Laboratory to ensure timely sample analysis. A minimum urine sample volume of 10 ml was required.

Laboratory Methods

A complete urinalysis, including physical appearance, specific gravity via refractometer, chemical assessment via dip-stick, and microscopic evaluation of the sediment was performed on each sample. Samples were excluded ($n=25$) if the pH was ≥ 8 , there were > 10 white blood cells/hpf, > 50 red blood cells/hpf, or bacteriuria or gross pigmenturia. Analysis of NAG, GGT and creatinine was performed on a Cobas c501 chemistry analyzer (Roche Diagnostics). NAG activity in the fresh urine supernatant was assessed by colorimetric assay (Roche Diagnostics) in which 3-Cresolsulfonphthaleinyl-N-acetyl- β -D-glucosaminide is hydrolyzed by NAG which results in the release of 3-cresolsulfonphthalein (also referred to as 3-cresol purple). The amount of 3-cresol produced is measured by photometric analysis to determine the NAG activity in the sample. GGT activity in urine supernatant was measured by a colorimetric assay (Roche

Diagnostics) in which GGT transfers the γ -glutamyl group of L- γ -glutamyl-3-carboxy-4-nitroanilide to glycylglycine. The GGT activity in the sample is proportional to the amount of 5-amino-2-nitrobenzoate (measured photometrically) produced in the sample. For this methodology for both NAG and GGT, the amount of enzyme activity is determined by measuring the amount of product produced (i.e., the amount of product produced is directly proportional to the concentration of active enzyme). Creatinine concentrations were determined using the Jaffe reaction in which creatinine reacts with picrate in an alkaline solution to produce a yellow-red complex (Roche Diagnostics). The creatinine concentration in the sample is directly proportional to the color intensity of the yellow-red complex produced. In the first phase of the study aliquots of urine supernatant were refrigerated at 4°C for 5 days and frozen at -20°C for 5 and 30 days at which time enzyme activity was reassessed (n=41). In a second phase of the study, additional time and temperature points were added to the previous time points to include a 30 day refrigerated sample (4°C) and a 30 day frozen sample (-70°C). Thirty-two urine samples were initially evaluated for the second phase and 10 samples were excluded based on the previously described exclusion criteria. For time/temperature points included in both phases of the study, data were analyzed together (n=63). For comparison of the additional time-temperature points added in phase 2, data from samples from the second phase (n=22) were analyzed separately.

Statistical Analysis

Data were analyzed (STATA 11.2) by ANOVA accounting for repeated measures on samples. Differences in enzyme activity following storage were compared with activity in day 0 (fresh) samples using the ANOVA associated regression coefficients. The overall model effects of storage were assessed using the Greenhouse-Geisser epsilon adjustment for repeated measures.

Results

In analysis of the data points in the first phase, creatinine concentrations remained stable at all time and temperature points (data not shown). In the analysis of time and temperatures from the first phase, urine GGT activity was stable in samples refrigerated for 5 days but there were significant declines ($P < 0.01$) in GGT activity in urine samples frozen at -20°C for 5 and 30 days (**Table 4.1**). Similarly, urine NAG activity was stable in samples refrigerated for 5 days,

but there were significant declines ($P < 0.01$) in NAG activity in urine samples frozen at -20°C for 5 and 30 days (**Table 4.1**). In the analysis of time-temperatures from the second phase, creatinine concentrations remained stable at all-time/temperature points (data not shown). Urine GGT activity was stable in the second study in samples refrigerated for 5 and 30 days and frozen at -70°C for 30 days but there were significant declines ($P < 0.01$) in GGT activity in urine samples frozen at -20°C for both 5 and 30 days (**Table 4.2**). Similarly, urine NAG activity was stable in samples refrigerated for 5 and 30 days and frozen at -70°C for 30 days, but there were significant declines ($P < 0.01$) in NAG activity in urine samples frozen at -20°C for 5 and 30 days (**Table 4.2**). To summarize the results from both studies, enzyme activities for both NAG and GGT were stable after 5 days and 30 days of refrigeration (4°C) and after 30 days at -70°C , however there were significant ($P < 0.01$) declines in GGT and NAG activities when urine supernatants were frozen for 5 and 30 days at -20°C .

Table 4.1 Phase 1 & 2: Storage of GGT and NAG at 5 days refrigeration, 5 and 30 days frozen (-20°C)

Storage Temp	GGT-Mean (se)	NAG-Mean (se)
Fresh	55.98 (9.75) ^a	5.84 (1.24) ^a
5 days refrigeration	50.97 (6.20) ^a	4.62 (0.85) ^a
5 days frozen (-20°C)	10.35 (3.12) ^b	4.28 (0.83) ^b
30 days frozen (-20°C)	4.87 (1.14) ^b	4.35 (0.84) ^b

a,b-Results in column only compared to fresh measurement. Values in columns with b superscripts are different from fresh ($p < 0.05$). N = 63. se-Standard Error

Table 4.2 Phase 2: Storage of GGT and NAG at 5 and 30 days refrigeration, 5 and 30 days frozen (-20°C) and 30 days frozen (-70°C)

Storage Temp	GGT-Mean (se)	NAG-Mean (se)
Fresh	55.14 (15.66) ^a	3.24 (0.64) ^a
5 days refrigeration	53.27 (15.03) ^a	3.20 (0.70) ^a
30 days refrigeration	44.95 (13.22) ^a	3.60 (0.81) ^a
5 days frozen (-20°C)	5.93 (1.89) ^b	2.93 (0.66) ^b
30 days frozen (-20°C)	4.11 (2.08) ^b	2.95 (0.67) ^b
30 days frozen (-70°C)	44.48 (12.52) ^a	3.16 (0.69) ^a

a,b-Results in column only compared to fresh measurement. Values in columns with b superscripts are different from fresh ($p < 0.05$). N = 22. se-Standard Error

Discussion

Use of spot urine samples and urine enzyme/creatinine ratios (vs. timed urine collections) facilitates monitoring enzymuria in the clinical setting and correlates with 24-hour urinary enzyme excretion.¹⁰⁷ Previous studies have suggested that a 2-3 fold increase in urine GGT and NAG activity compared with baseline values is indicative of renal tubular cell damage.¹⁰⁴ The present study suggests that time and temperature do not affect urine creatinine concentrations but that storage temperature does affect urine GGT and NAG activity.

Previously published canine reference intervals for normal dogs for urine NAG/creatinine ratios (0.02 to 3.63 U/g) and for urine GGT/creatinine ratios (1.93 to 28.57 U/g) demonstrated wide variability.¹⁰⁸ In the current study, we made no comparison to gender since all comparisons were made to individual samples. However a study in age matched healthy beagle dogs found NAG activity to be double in males versus females.¹⁰⁹ Another study that evaluated GGT and NAG in healthy dogs found that GGT did not differ between sexes, however again there was a significant difference between male and female dogs and urine NAG.¹⁰⁸ Maddens et al found significant differences in mean NAG/creatinine ratio between healthy bitches (2.3 U/g) and dogs with pyometra (14.3 U/g). However, significant overlap existed between the two groups (range 1.4-

3.9 U/g in healthy bitches and 0.8-14.3 U/g in pyometra dogs).¹¹⁰ This variability and the wide reference intervals, underscores the importance of comparing pre and post-treatment urine enzyme activity from the same patient rather than comparing an individual post-treatment value with a published normal value.

Exclusion criteria for the urine samples in this study were selected based on knowledge that certain conditions in the urine (pH \geq 8, and the presence of gross pigmenturia, bacteria and/or pyuria) may affect accurate measurement of enzyme activity. Under alkaline conditions (pH of about 8), Morita et al found that NAG isoenzyme A, which is the major measured enzyme in normal urine, can become inactivated.¹¹¹ There were no changes in urine NAG activity when measured in acidic urine.¹¹¹ Morita et al suggested usage of an assay evaluating isoenzyme B if measurement should be performed on alkaline urine. Another study by Madnic et al found that a pH of 8 or greater was associated with significant decreases in total NAG activity.¹¹² Madnic et al used an alternative method of analysis by determining urine NAG after ultrafiltration, dialysis, and chromatographic separation on DEAE cellulose to isolate separate isoenzymes for analysis for more accurate results. Since these additional analytical techniques were not used in the present study, samples containing alkaline urine were excluded.

Samples containing gross pigmenturia were also excluded. The current study used a colorimetric assay to evaluate enzyme activity of both NAG and GGT. Therefore any sample that contained pigmenturia could lead to inaccurate results. In addition to pigmenturia, samples with hematuria (defined in this study as >50 rbc/hpf) were also excluded. The intention of measuring NAG and GGT in urine is to determine if there is increased release of enzymes as a result of renal tubular epithelial cell injury. However, since these enzymes are also present in the blood, any sample that contained hematuria was excluded.

Samples that contained any bacteriuria or pyuria (pyuria was defined as > 10 wbc/hpf in this study) were also excluded. A study evaluating NAG and GGT activity in clinically normal adult dogs, and with normal biochemical profile, found concentrations within reference intervals for patients with positive cultures (4/38 or 10% of the time).¹⁰⁸ However, since the enzyme to creatinine ratio may be affected by the presence of pyuria or infection, samples containing bacteriuria or pyuria were excluded.^{102,107}

Our results are similar to previous reports of GGT measurements in people finding decreased activity of GGT freezing at -20°C , however enzyme activity was maintained at -70°C .¹⁰⁵

Since pH has been previously shown to affect enzymuria measurement, it is also possible that changes in pH could also affect protein stability. In this study, no attempt was made to standardize or compare pH at different storage levels. However, comparing pH between individual samples, or standardization of pH prior to storage could be considered for future studies. It is also unclear if the same decrease in enzyme activity would occur if blood samples were analyzed, or if some other compound present in the urine contributes to the storage differences. Based on the assay performed, we are unsure if the concentration of the enzyme or the activity of the enzyme is responsible for the decline in measured product. Future studies evaluating the structure of the protein before and after storage in combination with measured results could provide more insight into reasons for the difference seen between freezing temperatures.

Conclusion

Measurement of urine NAG and GGT activity may aid early detection of AKI. To ensure accurate and precise results, it is important for the clinician to be aware of factors that affect urine enzyme activity measurement, including appropriate sample storage techniques. The interpretation of urine enzyme activity should always include a complete urinalysis as well as a creatinine concentration for determination of the enzyme/creatinine ratio. If possible, a baseline sample should be collected. Refrigeration or freezing of the urine supernatant sample at -70°C can provide accurate and reliable results for up to 30 days. The present study shows that urine GGT and NAG activity is decreased in urine supernatant frozen at -20°C .

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