Assessment of Bacteriuria and Surgical Site Infections in Dogs with Cranial Cruciate Ligament Disease

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Academic Abstract

Objective: The aims of this prospective clinical cohort study were to determine the prevalence of asymptomatic bacteriuria in dogs with cranial cruciate ligament disease and to determine which clinical parameters and clinicopathologic data were associated with asymptomatic bacteriuria. Additionally, another aim was to determine the incidence of surgical site infections after cranial cruciate ligament surgery in dogs with and without asymptomatic bacteriuria.

Results: In 156 dogs with cruciate ligament disease, the prevalence of asymptomatic bacteriuria was 7.1%. Furthermore, the prevalence was 12.4% in female dogs and 0% in male dogs. The most common bacterial isolate was *Escherichia coli.* Patient sex, urine white blood cells/ high-powered field, and microscopic bacteriuria were significantly different between dogs with and without asymptomatic bacteriuria. Only 60% of dogs with microscopic bacteriuria had growth on urine aerobic culture. No significant difference was found in age, body weight, body condition score, duration of lameness, limb affected, or other urinalysis values between dogs with and without asymptomatic bacteriuria. Of the dogs that had 8-week repeat cultures, 2/3 dogs with asymptomatic bacteriuria had negative urine cultures and 3/43 without asymptomatic bacteriuria had positive urine cultures. Of the 46 dogs available for 12-month follow-up, 4 dogs developed a surgical site infection and 11 dogs developed a surgical site infection prior to 12 months. All of these surgical site infections were in dogs without AB. The incidence of surgical site infection in this population was 26.3% (15/57).

Conclusions: Prevalence of asymptomatic bacteriuria in dogs presenting with cranial cruciate ligament disease was similar to previously reported values in male and female dogs. This suggests that dogs with cranial cruciate ligament disease are not more prone to asymptomatic bacteriuria than dogs in previously studied populations. Preliminary data suggests that asymptomatic bacteriuria does not predispose dogs to surgical site infection however further research and continued data collection is warranted.

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Public Abstract

Asymptomatic bacteriuria is defined as having bacteria in the urine without signs of lower urinary tract disease. The aim of this study was to determine the prevalence asymptomatic bacteriuria in dogs with cranial cruciate ligament disease. Additionally, another aim was to determine the incidence of surgical site infections after cranial cruciate ligament surgery in dogs with and without asymptomatic bacteriuria.

Prevalence of asymptomatic bacteriuria in dogs presenting with cranial cruciate ligament disease was found to be similar to previously reported values in male and female dogs. This suggests that dogs with cranial cruciate ligament disease are not more prone to asymptomatic bacteriuria than dogs in previously studied populations. Preliminary data suggests that dogs with bacteria in the urine does not predispose dogs to SSI however further research and continued data collection is warranted.

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LIST OF ABBREVIATIONS

AB – asymptomatic bacteriuria

CBLO – CORA (center of rotation of angulation)- based leveling osteotomy

CCL – cranial cruciate ligament

CFU – colony-forming unit

CWO – cranial tibial closing wedge osteotomy

HPF – high-powered field

IgA – immunoglobulin A

MDR – multidrug resistant

RBC – red blood cell

SSA – sulfosalicylic acid

SSI – surgical site infection

TPLO – tibial plateau leveling osteotomy

TTA – tibial tuberosity advancement

TTO – triple tibial osteotomy

UPEC – uropathogenic *E. coli*

UTI – urinary tract infection

WBC – white blood cells

Chapter I: Literature Review

1) Bacterial Urinary Tract Infections in Dogs

a. Pathophysiology of Disease

Bacterial urinary tract infections (UTIs) are common in dogs. Roughly 5-27% of dogs acquire a bacterial UTI at some point during their lifespan [1-5]. The causes of UTIs are multifactorial, but an ascending commensal bacterial infection from the genitourinary and gastrointestinal tract remains to be the most common cause. Normal anatomic and mechanical factors of the genitourinary tract are paramount in order to avoid opportunistic bacteria [2, 6]. Breach in these factors and immunity coupled with bacterial virulence factors allow bacteria to colonize and rapidly divide, leading to urinary tract inflammation and infection.

i. Anatomic and Host Factors

Though once considered sterile, urine in the normal dog contains a resident bacterial flora. Burton et al. identified a urinary microbiome in dogs without lower urinary tract disease signs [7]. DNA was extracted from 20 urine samples, collected via cystocentesis from male and female dogs with negative urine cultures. All samples revealed a urinary microbiome and four predominant taxa were found including: *Pseudomonas* sp, *Acinetobacter* sp, *Sphingobium* sp, and *Bradyrhizobiaceae*. This bacterial flora, likely, plays a role in preventing infection by competing with potential pathogens for nutrients and distal epithelial receptor

sites [2]. Additional defense mechanisms exist to prevent pathogenic bacteria from colonizing and establishing urinary infections. These defense mechanisms can be divided into anatomic factors, mechanical factors, urine antimicrobial properties and systemic immunity [8, 9].

Anatomic structures of the urinary tract that have physiologic properties to help prevent infection from ascending bacteria include the ureters, urethra, bladder and prostate. If bacteria enter the bladder, the ureters use peristalsis to move urine into the lower urinary tract as a way to prevent continued bacterial migration. In addition, the ureterovesical flap creates a valve effect to prevent ureterovesicular reflux. There is a high-pressure zone of the proximal urethral sphincter that minimizes bacterial migration [2]. The urethral pressure profile is contributed by elasticity of the smooth muscle and sympathetic components [10]. In males, not only does the increased urethral length help decrease the likelihood of bacterial infection, but the prostate secretes zinc into the ejaculate, which has bacteriostatic properties [2, 6]. Changes in these features including anatomic abnormalities and changes in sphincter pressures, genitourinary secretions, ureteral peristalsis, and voiding will predispose patients to urinary tract infections [2, 6, 8, 11].

Along with anatomic structures, mechanical factors such as normal micturition, including adequate urine volume, frequent voiding, and complete voiding, play a role in natural host defenses of the urinary tract. Micturition is

under the control of the autonomic nervous system (both sympathetic and parasympathetic components) and the voluntary somatic nervous system. During the storage phase of micturition, signals are sent to the preganglionic neurons and to the caudal mesenteric ganglion to synapse the postganglionic neurons that form the hypogastric nerve. This causes detrusor muscle relaxation and increased tone of the bladder neck. Norepinephrine release and activation of beta-adrenergic receptors in the bladder permits relaxation. Simultaneously, norepinephrine is also responsible for the constriction of the urethral smooth muscle to prevent urine leakage via alpha-adrenergic receptors, at the level of the internal urethral sphincter and the bladder neck. Urine storage is also achieved by somatic-mediated contraction of the striated periurethral muscle or external urethral sphincter, via the pudendal nerve. The voiding phase of micturition is initiated when stretch receptors in the bladder wall lead to parasympathetic pelvic nerve stimulation, sending signals to the lumbar spinal cord to the micturition center of the brain. Ascending spinal tracts include the reticulospinal tract and spinothalamic tract located in the ventral and lateral funiculus, respectively. At the micturition center, signals from the cerebral cortex and hypothalamus are processed. The descending pathways include the corticospinal tract in the lateral funiculus and the reticulospinal tracts. To initiate urination, signals are sent down the pelvic nerve and acetylcholine is released. Acetylcholine binds to muscarinic receptors and

stimulates detrusor muscle contraction. At the same time, the parasympathetic nervous system inhibits the sympathetic and somatic stimulation of the hypogastric and pudendal nerve, respectively, to relax the internal urethral sphincter and striated periurethral muscle, in order to allow emptying of the bladder [12-14].

Conditions that increase urine retention can predispose a patient to a urinary tract infection by allowing more time for bacterial adherence. For example, dogs with spinal cord lesions cranial to L3 have urinary retention with increase outflow resistance. A study reported that 35 out of 47 dogs, or 75%, that experienced chronic limb paralysis had at least one positive urinary culture, with $>10^5$ colony forming units/ml. This study demonstrated that hind limb paralysis is a risk factor for urinary tract infections, secondary to neurologic injury to the upper and lower motor neurons of the bladder and urethra causing urinary retention [15]. Furthermore, the prevalence of urinary tract infections in 92 dogs surgically treated for type 1 thoracolumbar intervertebral disc extrusion was 27% [16]. An increased prevalence of urinary tract infections has been found in females, dogs that cannot ambulate or voluntarily urinate, dogs not administered perioperative cefazolin and dogs whose body temperature dropped below 35 degrees Celsius during anesthesia [16]. Additionally, in dogs with acute thoracolumbar intervertebral disc herniation and urinary bladder dysfunction managed by manual expression, indwelling catheterization or intermittent catheterization, the duration of required urinary

bladder management was another risk factor for urinary tract infections; each additional day of treatment increased the risk by 1.5 times [17]. Lower motor neuron lesions and dysautonomia were characterized by decreased detrusor contractility and were associated with urine retention, decreased bacterial washout and predisposed to urinary tract infection [2].

Intrinsic factors of the urinary system include mucosal defense barriers and antimicrobial properties of urine. Examples of mucosal defense barriers include antibody production, surface layer of glycosaminoglycans, mucosal antimicrobial properties and exfoliation of urothelial cells. The urinary tract is lined with urothelium from the renal pelvis to the urethra, which functions to block passage of water, ions, solutes and macromolecules to and from the plasma and interstitium into the lumen of the urinary tract. The urothelium is composed of a basal cell layer, polygonal cell layer and a superficial umbrella cell layer. Umbrella cells contain plaque and hinges that facilitate bladder stretching and filling and on its surface glycosaminoglycans and proteoglycans are present. Plaques are made up of transmembrane proteins, called uroplakins, which form a barrier to the flow of solutes and water. Healthy urothelium prevents bacterial and crystal adherence by the hydrophilic properties of glycosaminoglycans, making the bladder impermeable. Chemical and mechanical damage to the urothelium, caused by drugs, toxins, uroliths, neoplasia, or traumatic catheterization, disrupt the

glycosaminoglycan layer and urothelium leading to bacterial adherence and colonization [2, 18-20].

The properties of normal urine that have antimicrobial effects include extreme high and low pH, hyperosmolality, high urea concentration, and presence of weak organic acids, low molecular weight carbohydrates, and mucoproteins [8, 11]. Urine pH can influence the growth of certain bacterial species. For example, *Proteus* species tend to grow better in alkaline environments [2]. Dilute urine with a low urea concentration can predispose patients to a urinary tract infection by allowing pathogens to grow in this condition easier when compared to harsher environments of concentrated urine [2].

While these local host defenses are first line in preventing ascending infection, systemic host defenses primarily prevent hematogenous spread of pathogens traveling to and from the urinary tract. Immunoglobulin A, or IgA, is a primary antibody that plays an important role in immune function of mucous membranes. Inflammation of the urinary tract causes a widening of the interstitial space between urothelial cells. This space facilitates the secretion of IgA, which binds to a secretory protein component, coats the pathogens, and inhibits them from binding to the urothelium [2].

Opinions exist that some dogs may have an increased risk of urinary tract infections such as females with perivulvar dermatitis and males with perineal

urethrostomies [8]. A study states that in cats undergoing perineal urethrostomies inflammation and surgery altering the anatomic and functional barriers increased the incidence of urinary tract infections [21]. On the other hand, studies report that vulvoplasty or episioplasty procedures reduce the incidences of urinary tract infections, vaginitis and perivulvar dermatitis [22, 23]. No data currently exists documenting occurrence of UTI in dogs with dermatitis. Studies exist relating the frequency of urinary tract infections in dogs with allergic dermatitis treated with either cyclosporine or long-term glucocorticoids [24, 25]. These studies conclude that treatment with immunosuppressives increases the frequency of urinary tract infection but not the presence of dermatitis.

ii. Bacteria and Virulence Factors

Breach in the above host factors can lead to inflammation of the urinary tract. Inflammation is not synonymous with infection; there are many noninfectious causes of urinary disease that may cause local inflammation. Examples of non-infectious inflammatory conditions include sterile urocystolithiasis, urethral obstruction, neoplasia, trauma and sterile, polypoid, irritant and idiopathic cystitis. It is important to distinguish inflammation from infection for diagnostic and therapeutic reasons. Infection can occur from ascending infection via the urethra from the skin surrounding the vulva or prepuce

to the urinary bladder, from the gastrointestinal tract, or rarely a hematogenous route [2].

Escherichia coli, or *E. coli,* is the most common uropathogen in humans, dogs, and cats. Gram-positive cocci, such as *Staphylococcus* spp., *Streptococcus* spp and *Enterococcus* spp., are the second major group of uropathogens [2]. The remaining pathogens include *Proteus, Klebsiella, Pasteurella, Pseudomonas, Enterobacter, Corynebacterium and Mycoplasma* spp. Bacteria that are nonpathogenic in a healthy animal can be pathogenic in an animal with altered host defenses as previously discussed. In general, approximately 75% of bacterial urinary tract infections are caused by one bacterial species, 20% are caused by two, and 5% are caused by three bacterial isolates [8]. *E. coli*, specifically, has been isolated in 35-50% of cultures and its significant strains include commensal, intestinal pathogenic and extraintestinal pathogenic strains. Urinary tract infections involving uropathogenic *E. coli*, or UPEC, can be intracellular or extracellular. Those that are intracellular are long-lived and shielded from host immunologic defenses and antimicrobial exposure [26].

E. coli, the most studied isolate in urinary tract infections, has many intrinsic virulence factors. *E. coli*'s virulence factors include somatic (O) antigens, capsular (K) antigens, adhesive fimbriae, hemolysin, cytotoxic necrosis factor, aerobactin, R-plasmids, resistance to serum bactericidal activity and short generation time in

urine. Certain O antigens form part of an outer polysaccharide portion of the bacterial envelope and certain K antigens form a capsule surrounding the bacterium, both inhibiting host phagocytosis and complement-mediated bactericidal activity. The adhesive fimbriae are proteinaceous filamentous organelles, which enhance the adherence of the bacterium to the urothelium. For instance, UPEC strains have a tip adhesion, called Fim H, which bind to mannose molecules within the urothelium. In addition, *E. coli* secretes hemolysin, which increases the amount of free iron necessary for bacterial growth and tissue damage. Aerobactin, a bacterial iron-chelating agent, is produced by *E. coli* to utilize host nutrients to facilitate bacterial growth by sequestering iron in iron-poor environments, such as the urinary tract. R-plasmids are extrachromosomal structures that self-replicate and carry genes to promote resistance to antimicrobials [8, 26].

Other bacterial species have factors that enhance their uropathogenicity. *Proteus, Staphylococcus* and some species of *Klebsiella* have adherence factors. They are also notorious urease producers that hydrolyze urea to ammonia. This ammonia causes direct injury to the urothelium to promote continued bacterial adherence. *Pseudomonas* species can evade the host and cause injury due to its unique heavy mucoid polysaccharide capsule that prevents host antibody coating and transfer of R-plasmids [8].

iii. Terminology of Urinary Tract Infection

A urinary tract infection is defined as the presence of an infectious agent adhering, multiplying, and persisting in the urogenital system and is associated with clinical signs of lower urinary tract disease [8]. Several classifications of UTI exist, however, the most commonly accepted classifications include: simple uncomplicated, complicated and subclinical [2, 27]. The difference between a simple uncomplicated and a complicated UTI is the presence of underlying comorbidities or compromised host defense mechanisms, which can lead to persistent or recurrent infections. Recurrent UTIs can be further defined as either relapses or re-infection. A relapse is an infection caused by the same bacteria as the original culture results. Relapses generally occur within several days of stopping previous treatment. They are most frequently associated with ineffective antimicrobial treatment (inappropriate drug selection, dose, frequency or duration of therapy). Re-infections occur when successful treatment of a previous UTI, as documented with a negative culture, is followed by a recurrence of clinical signs and positive bacterial culture within 6 months. A superinfection is when new bacteria colonize the urinary tract during antimicrobial treatment of either a UTI or other infection [2, 27]. Lastly, subclinical UTI indicates a stage where absent clinical signs may develop later [28].

b. Clinical Signs and Diagnosis of Urinary Tract Infections

The manifestation of clinical signs associated with UTI in a patient is dependent on the number and virulence of the bacteria, the presence or absence of predisposing causes (such as diabetes mellitus, hyperadrenocorticism, chronic kidney disease, cystolithiasis or bladder neoplasia), the body's compensatory response, the infection duration, and the location of the infection [2, 8]. In lower urinary tract infection, dysuria, pollakiuria, stranguria, and inappropriate urinations may be observed. However, patients with upper urinary tract disease may exhibit pain localized to the kidneys, or general malaise (nausea, vomiting, lethargy, inappetence, anorexia) associated with septicemia or renal failure.

Diagnosis of a UTI combines history, physical examination findings, complete urinalysis, and urine culture. Multiple diagnostic tests have been described to diagnose a UTI, such as urinalyses, rapid dipsticks, urine gram stains, urine sediment examinations and urine cultures. Urinalyses are helpful in differentiating a possible urinary tract infection from other lower urinary tract disorders, and identifying some predisposing conditions with the presence of glucosuria and crystalluria. Urine sediments can assist in identifying pyuria and bacteriuria but if the urine is dilute, or there is low urine specific gravity, it can negatively affect detection of cells and bacteria [29]. Furthermore, pyuria in the urine sediment does not appropriately diagnose an active urinary tract infection, as

there are several non-bacterial diseases that can result in inflammation of the urinary tract, as previously mentioned. A urine dipstick paddle was evaluated, in 207 urine specimens, as an alternative method for diagnosis and identification of UTIs. They concluded that the dipstick system was sensitive for screening however; identification was inconsistent at 75.8% [30]. A more recent study set out to determine the accuracy of a rapid immunoassay for UTI diagnosis. This test had a sensitivity and specificity of 97.4% and 98.8%, respectively, in detecting UTI with $\geq$ 1,000 CFU/ml [31]. When comparing wet mount, Wright-Giemsa and gram stained urine sediment for predicting bacteriuria, a study found that Wright-Giemsa and gram stained sediments were superior [32]. A urine gram stain has been proven to be more sensitive (96%) and specific (100%) in detecting bacteriuria when compared to a routine urinalysis, including sediment examination (76%, 77%; sensitivity and specificity, respectively) [33]. Compartmented bacteriologic culture and antimicrobial susceptibility testing plate was compared to standard culture and found an 81% sensitivity and 99% specificity, leading to accurate exclusion of dogs without UTI [34]. Even with multiple methods in detecting UTI, quantitative bacterial urine culture remains the gold standard in diagnosing bacteriuria, as it provides bacterial identification, number of organisms per ml of urine and allows for antimicrobial susceptibility testing.

Bacteriuria is defined as the presence of any growth of bacteria via urinary culture. Bartges further defines bacteriuria as significant when there is greater than 1,000 colony-forming units (CFU)/ ml of urine in dogs with samples obtained by cystocentesis and 10,000 CFU/ml for male dogs with samples collected by urinary catheterization to distinguish it from contaminants [8, 35]. Therefore, dogs with significant bacteriuria without clinical signs have AB. Those dogs with significant bacteriuria with clinical signs have a UTI. A study evaluated urinalyses and urine cultures of 50 clinically normal dogs via antepubic cystocentesis, catheterization and midstream voided samples [36]. All cystocentesis samples were sterile and bacterial growth was present in 26% and 85% of catheterized and voided specimens, respectively. While they concluded that cystocentesis is the method of choice for interpreting urine cultures, they determined that $\geq 10^5$ CFU/ml of urine indicated a clinical infection for catheterized specimens. Significant bacterial numbers have been documented in order to distinguish between infectious agents and contaminants, as well as guidance for treatment [8, 9, 27, 29, 35, 36]. Typically when there are low numbers of minimally pathogenic commensal bacteria, contamination of sample is present [35, 36].

c. Treatment of Urinary Tract Infections

Empiric treatment of UTIs is most commonly instituted, particularly as culture and sensitivity results are pending. Empirical antimicrobials for common uropathogens, including amoxicillin clavulanate, third-generation cephalosporins, and enrofloxacin, have been used [13]. However, treatment guidelines must reflect UTI classifications, location of infection, comorbidities and prior poor response to treatment of our small animal patients.

Based on the Working Group of the International Society for Companion Animal Infectious Diseases, treatment of simple uncomplicated UTIs generally require a 7-14 day course of antimicrobials [27]. Strong evidence for this treatment course recommendation is lacking so there is a possibility that a shorter treatment time may also be effective. Based on recent literature in human and veterinary medicine, shorter durations of therapy with appropriate antimicrobial selection at higher doses may be just as efficacious [29, 37-40]. A prospective study evaluated client owned dogs with simple uncomplicated UTI split into two treatment groups [39]. Group 1 received a high dose, short duration therapy of enrofloxacin (18-20mg/kg PO every 24 hours for 3 days) while group 2 received the conventional protocol of amoxicillin- clavulanic acid (13.75-25mg/kg PO every 12 hours for 14 days). They found that the high dose, short duration therapy was not inferior to the conventional protocol with a microbiologic cure of 77.1% and 81.2%, respectively,

as well as a clinical cure rate of 88.6% and 87.9%, respectively. Another similar study compared dogs with uncomplicated bacterial cystitis treated with trimethoprim-sulfamethoxazole (15mg/kg PO every 12 hours) for 3 days to those treated with cephalexin (20 mg/kg PO every 12 hours) for 10 days [40]. They also did not identify a difference between high dose, short duration therapy and conventional therapy. Expected resolution of clinical signs for simple uncomplicated urinary tract infections is within 48 hours of initiation with appropriate treatment. Evidence of inflammation in the urine sediment should resolve in 3-5 days after starting therapy. Treatment recommendations are generally first generation penicillins and cephalosporins. Potentiated sulfa drugs are also suggested for initial therapy in uncomplicated cases [29]. For complicated UTI's, prolonged treatment has been proposed for at least 4 weeks with an antimicrobial selected based on urine culture and sensitivity results. Contrary to simple uncomplicated UTIs, re-evaluation of urine sediment and urine culture is strongly recommended for complicated UTIs to evaluate response to treatment [27, 29]. Based on Weese et al., urine culture should be performed 5-7 days after starting treatment, especially in patients with a history of recurrent infections [27]. In all cases, culture is recommended 7 days after completion of antimicrobial course to ensure infection has resolved. Based on these international recommendations for UTIs, proper diagnosis and therapy are essential to avoid

failure to resolve the infection, costs of repeated or prolonged treatment, and antimicrobial resistance.

In those patients with recurrent UTIs, or cases of relapses, identifying and treating comorbidities or predisposing conditions will often achieve successful treatment. Meanwhile, for patients where these causes cannot be identified or treated, additional therapies are available to prevent recurrent UTIs.

Ancillary prevention therapies include daily prophylactic low dose antimicrobial protocols, cranberry extract, urinary antiseptics, probiotics, and induction of bacteriuria. Cranberry extract contains proanthocyanidins, which have anti-adhesion effects similar to polysulfated glycosaminoglycans. Probiotics work to restore the genitourinary flora to lactobacillus and remove pathogenic bacteria. Urinary antiseptics, specifically methenamine hippurate, convert to formaldehyde, which is bactericidal, and ammonia. Lastly, induction of AB with less pathogenic strains of *E.* coli has been experimentally performed as potential treatment or prevention of UTIs, working by competing with pathogenic bacteria [26, 29, 41].

The risks of failing to appropriately treat urinary tract infections include polypoid cystitis, emphysematous cystitis, pyelonephritis, urolithiasis, and prostatitis [29]. Currently, veterinary recommendations are lacking for patients exhibiting asymptomatic bacteriuria. Overall, proper diagnosis, classification and

treatment is crucial to avoid further injury and for the prevention of antimicrobial resistance in the small animal population.

i. Theory of Antimicrobial Resistance

There are two forms of antimicrobial resistance, intrinsic and acquired. The intrinsic form is when bacterial properties are inherited, such as penicillinase-producing bacterial strains. The acquired form, which veterinarians play a larger role in, occurs when bacterial populations are forced to respond to selection pressures in order to survive following the use of antimicrobials. This latter form of antimicrobial resistance occurs in numerous ways involving alterations in bacterial DNA through genomic DNA and plasmids, exchanging genetic material via both transposons and plasmids by conjugation, and chromosomal mutations [2].

Multidrug resistant, or MDR, bacterial isolates are those that lack susceptibility of 3 or more classes of antimicrobials. Multidrug resistant risk factors include immunosuppression, prolonged hospitalization, and surgical intervention. MDR *E. coli* strains usually have extended spectrum of beta-lactamases [29]. The most common extraintestinal source of multidrug resistance are *E. coli* and *Enterobacter*, found in 62% and 58%, respectively, of urine samples in one study of 37 dogs [42].

Eighty-seven percent of dogs in a study had their antimicrobial susceptibility patterns investigated [4]. This study focused on 1,028 incidences of infections in dogs with simple UTIs (35.3%) and complicated UTIs (64.7%), including pyelonephritis; they found that the most common isolates were *E. coli* (52.5%), *Staphylococcus* spp. (13.6%), and *Enterococcus* spp. (13.3%). There was large variability in treatment in this study, however, none of the bacteria isolated achieved greater than 90% susceptibility to any oral antimicrobial option. Over the 51-month study period, they found no change in resistance rate against commonly prescribed antimicrobials, amoxicillin/ clavulanic acid and cephalexin, compared to a significantly higher resistance rate with dogs that received amoxicillin, doxycycline, and enrofloxacin. Additionally, a higher level of resistance from isolates obtained from dogs with complicated UTIs compared to dogs with simple UTIs was determined [4]. Other studies have corroborated these results [3, 37, 38, 43].

Antimicrobial resistance is a consideration when deciding on the antimicrobial used to treat urinary tract infections. In a study where 5,923 urine samples were submitted from 4,530 dogs over a 10-year period, there was a reduced effectiveness of enrofloxacin, cephalexin and oxytetracycline in treatment of urinary tract infections. The authors hypothesize, that since these drugs are being used as first line antimicrobials for patients with suspected UTIs, it led to the

development of resistance, given that over this time period the prevalence of the bacterial species did not change [3]. Due to this resistance, our veterinary approach to diagnosis and treatment must be modified.

2) Asymptomatic Bacteriuria

a. Human Medical Literature

i. Definition

Asymptomatic bacteriuria (AB), in humans, is documented to be a benign process and incidental finding [44]. Asymptomatic bacteriuria is common among patients over the age of 60, especially women, and patients with uncontrolled diabetes mellitus [45-48]. In elderly patients, a number of physiological, cognitive, and behavioral reasons can impair bladder emptying and necessitate catheterization. In women, AB may be common due to the close proximity of the urethral opening to the perianal area or even due to lack of estrogen in postmenopausal women which increases vaginal and urethral pH, reduces lactobacillus levels and increases bacterial susceptibility [48-50].

Diagnostics and Treatment Recommendations

Diagnosis of AB involves the absence of lower urinary tract symptoms and the isolation of the same organism in two consecutive voided urine specimens, in quantitative count $\geq$ 100,000 CFUs/ml, contrary to $\geq$ 1,000 CFUs/ml obtained by a

single cystocentesis sample used in veterinary medicine [51]. Two consecutive voided samples are required for diagnosis of AB in women, while only a single sample is needed for men [44].

The Infectious Diseases Society of America has recommended against screening for and treating AB with antimicrobials unless patients are undergoing invasive genitourinary procedures or renal transplant, or in patients that are pregnant [51, 52]. For example, AB in pregnant women can lead to pyelonephritis, low birth weight, and preterm labor [44, 52]. They, otherwise, recommend against treatment because treatment may lead to antimicrobial resistance, adverse drug reactions and increased costs [51]. Further evidence against screening or treatment of AB was established in human orthopedic surgery.

Although the American Academy of Orthopedic Surgeons recommends urine sampling only in patients with a history of lower urinary tract disease, patients presenting for elective orthopedic surgery are frequently screened for bacteriuria even if asymptomatic [53]. Out of 510 patients presenting for elective total joint (knee and hip) replacement, 182 (36%) had pre-operative AB based on bacterial culture results. This was not statistically different from the post-operative AB culture rate (41%) obtained 3-5 days post-operatively [54]. Perioperative antimicrobial prophylaxis failed to statistically change post-operative positive bacterial cultures rates. Through an 11-month period, in this study, only 25 patients

(5%) developed a symptomatic urinary tract infection despite lack of antimicrobial therapy in all AB patients. Two-thirds of organisms cultured from these individuals were different to those found on previous cultures. These patients were treated with targeted oral antimicrobials and no difference in implant-associated infection was found over the 3-month surveillance period [54]. The authors concluded that a 5% development of symptomatic UTI was within the realm of disease occurrence in the general population. This study concluded that checking urine samples pre- and post-operatively is unnecessary in asymptomatic patients. It may also lead to increased costs and inappropriate use of antimicrobials. No proven benefit has been shown in screening and treating AB patients undergoing elective orthopedic procedures.

Other human studies have supported these results. Specifically, the prevalence of AB in knee arthroplasty patients and risk for prosthesis infection was studied [55]. Asymptomatic bacteriuria was diagnosed in 5.1% (11/215 patients) based on urine culture. While 4/11 AB patients were treated with specific antimicrobials (Group A), the remaining 7/11 were not (Group B). One patient in group A, the treatment group, suffered a prosthesis infection within the first 3 months after surgery. The bacteria cultured from the surgical site were different to those identified in the urine culture. No patients in group B developed a prosthetic infection over the minimum follow up period of 48 months [55]. This study

hypothesized that pre-operative AB may not be responsible for spread of bacteria to the surgical site.

Likewise, in a multicenter cohort study with 2497 patients undergoing prosthetic joint implantation, the prevalence of AB was 12.1% (303/2497), and the overall prosthetic joint infection rate was low (1.7%) [56]. The surgical infection rate was significantly higher in the asymptomatic bacteriuria group (4.3%) than in the symptomatic bacteriuria group (1.4%), however, within the asymptomatic bacteriuria group, there was no significant difference in surgical infection rate between antimicrobial treated (3.9%) and untreated (4.7%) AB patients [56]. The microorganisms isolated in urine cultures were always different from those in the prosthetic joint.

Furthermore, a Cochrane review of antimicrobials for AB, compiling 9 studies with a total of 1,614 participants, found that there wasn't a clinical benefit for treating AB [57]. Even though antimicrobials were effective at reaching a bacteriologic cure, antimicrobial therapy led to more adverse side effects [57]. Inappropriate treatment of AB can have profound effects on intestinal flora and can lead to the development of diarrhea, including *Clostridium difficile* infection and pseudomembranous colitis, drug allergies/adverse drug reactions, and antimicrobial resistance [44, 48, 56, 58].

In summary, the human literature concludes that antimicrobial treatment does not show any benefit for preventing implant-associated infection or joint infection in patients diagnosed with AB. Potential hematogenous spread of bacteria from the urinary tract to a joint or implant has not been documented with the same pathogen. Postponing surgery or treating for AB prior to surgery could not be recommended based on these studies [47, 48, 51, 54-58].

b. Veterinary Medicine Literature

i. *Definition*

Asymptomatic bacteriuria is defined in patients with a significant amount of bacteria present in the urine in the absence of clinical signs. Though once referred to as having a subclinical UTI, these patients are more appropriately termed as having AB. Asymptomatic bacteriuria is of unknown clinical significance in veterinary medicine [8].

ii. Patient Populations and Prevalence of AB

Although AB has been poorly studied in small animal patients, a small number of veterinary studies have been performed. Wan et al. determined the prevalence of AB and its clinical course over three months in 101 healthy client-owned female dogs [59]. In this study, patients were diagnosed with AB if two bacteriologic urine cultures were positive and if they remained asymptomatic for 3

months. Nine percent of the dogs enrolled in the study had AB. Bacteriuria was persistent in 4 dogs and transient in 4 dogs at 7-14 days and 3-months post screening. No dog experienced clinical signs within the 3-month study period. Contrary to the human literature, the prevalence of asymptomatic bacteriuria in female dogs did not increase with age in this study [59].

The prevalence of AB in a cohort of 140 client owned dogs presenting for various elective orthopedic (n=80) and soft tissue (n=60) surgeries [49] was 2.1% [60].

Obesity has been linked with comorbidities, including UTIs, in humans [61, 62]. Wynn et al. documented the prevalence of AB in morbidly obese dogs at 13% [63]. The percentage of AB patients was only identified in dogs with a body fat percentage greater than 45% based on dual energy absorptiometry scans [63]. Body condition score has been utilized to estimate body fat percentage, with an accepted optimal body fat percentage of 15-20% [64]. A study found that a body condition score of 5/9 was associated with a 19 +/- 8% body fat and further that the percent of body fat changes by 5% on a 9-point scale [65]. According to this, Wynn et al.'s findings of positive AB patients in those with a greater than 45% body fat corresponds to body condition score of greater than 8/9. A study has also documented that high body condition scores and obesity are risk factors for the development of orthopedic diseases [66]. Based on these initial reports, AB

appears to remain a static non-progressive condition prevalent in 2.1-13% healthy female dogs, morbidly obese dogs, and dogs presenting for elective soft tissue and orthopedic surgery.

iii. Treatment Recommendations

Treatment of AB in dogs is controversial. Some authors recommend treatment of AB if there is a high risk of ascending or systemic infection, as in immunocompromised patient or patients with underlying renal disease or in patients undergoing urogenital surgery [27, 29]. These recommendations have little to no research. It has been hypothesized that the bacteria present in asymptomatic patients may actually provide protection against colonization of more pathogenic strains of bacteria within the urinary tract. Treatment may cause harm to the patient as they subsequently develop clinical signs associated with more pathogenic strains of bacteria [2, 27, 29, 42, 41].

3) Cranial Cruciate Disease in Dogs

a. Anatomy and Function of the Cranial Cruciate Ligament

In the normal canine stifle, an intact cranial cruciate ligament (CCL) is composed of a thin craniomedial and a thick, short caudolateral band that twist on each other [67, 68]. The CCL attaches to the femur on the caudomedial part of lateral condyle and caudolateral part of the intercondyloid fossa. It crosses the joint

and attaches to the cranial intercondylar area of the tibia. The cranial cruciate ligament is considered intra-articular, but extra-synovial. It is composed of collagen fibrils and fibroblasts, and further covered by an epiligamentous region composed of synovial intima and underlying loose connective tissue. This region is absent where the two bands wrap around each other. The cranial cruciate ligament is rich in mechanoreceptors and proprioceptors that aid in its function [67].

In the stifle, which is a diarthrodial joint, an intact cranial cruciate ligament works to oppose hyperextension, cranial translation and internal rotation of the tibia. Stauffer et al. define cranial tibial thrust as "an internally generated force that causes cranial translation of the tibia during weight bearing. This force is a function of both tibial compression and the slope of the tibial plateau" [72]. Cranial translation is not present in healthy animals due to the intact cranial cruciate ligament and caudal pole of the medial meniscus, along with the counteracting pull of the hamstring muscles and pes anserinus. The pes anserinus is an aponeurosis of the muscles of the gracilis, semitendinosis, and caudal belly of the sartorius. Components involved in stifle joint stability and load sharing, in all directions, not only include cranial and caudal cruciate ligaments but also the patellar ligament and quadriceps mechanism, the medial and lateral menisci, and the joint capsule. A protective mechanism of the CCL exists when increased load is placed on the joint, or ground reaction forces, during weight bearing. Contraction of the caudal thigh

muscles decreases strain on the CCL, which is counteracted by relaxation of the quadriceps muscles that increases strain on the CCL. Within the joint, when the stifle is extended, both bands of the cranial cruciate ligament are taut but when the stifle is flexed, the craniomedial band is taut while the caudolateral band is lax. Additionally, the two bands of the CCL twisting on each other limits internal rotation but neither band has been found to limit external rotation [68-71].

b. Pathophysiology of Disease

In veterinary medicine, cranial cruciate ligament disease is the most common hind limb orthopedic abnormality. A degenerative process most commonly causes cranial cruciate ligament tear, but traumatic injuries have also been reported. Avulsion of a small piece of bone occurs in skeletally immature animals where the attachment of the ligament to the bone is stronger than the bone itself. Although rare, acute traumatic tear of the cranial cruciate ligament results from excessive loading, traumatic hyperextension, and/or excessive internal tibial rotation [67].

The pathophysiology of cranial cruciate disease is not fully understood. Histologically, the ligament has been found to involve loss and metaplasia of ligamentocytes, failure of the ligament to maintain its collagen fibers, and a decrease in fibroblast numbers [67]. These changes result in inflammation from the release of inflammatory mediators leading to cartilage breakdown and degenerative

joint disease progression [72]. It is believed that CCL disease is a gradual degeneration of the ligament, resulting in a partial or complete ligament tear, where cranial tibial thrust exceeds the tensile strength of the CCL [69, 70]. There have been other proposed mechanisms. One mechanism is that stress is caused by contact of the cranial cruciate ligament with the caudal cruciate ligament and the lateral edge of the medial femoral intercondylar notch during internal rotation. This contact may lead to weakness and eventual tear of the cranial cruciate ligament. Another mechanism involves immunologic reactivity, where dogs with CCL disease have antibodies to collagen type I and II [67, 68].

Along with these theories, several risk factors for CCL disease have been evaluated. Steep tibial plateau angle, abnormal conformation and gait were thought to be involved, from repetitive strain leading to mechanical failure, but were not found to have a causative relationship with dogs with CCL disease. In addition, the majority of dogs with CCL disease are overweight [73, 74]. Other risk factors such as breed and neutering have been shown to be associated with CCL disease [67, 68].

c. Diagnosis of Cranial Cruciate Ligament Disease

Diagnosis of CCL tear is a physical examination finding. Cranial drawer and cranial tibial thrust test the integrity of the CCL by putting the stifle through range of motion [71]. Cranial translation of the proximal tibia relative to the femur

results from contraction of the extensor muscles of the stifle and hock joints during weight bearing. Abnormal movement of the tibia relative to the femur is considered a positive test for CCL disease. The cranial drawer test is performed by applying a force to the tibia, while holding the femur stable. The tibia is firmly manipulated in a caudal and cranial direction and sagittal plane motion is monitored. Any motion in an adult animal is considered abnormal. In cases of partial tears, where the craniomedial band is torn but the caudolateral band is intact, cranial drawer will only be present in flexion. If only the caudolateral band is torn, no cranial drawer will be appreciated. Other supportive findings of CCL disease include the presence of joint effusion, variable degrees of crepitus, pain on stifle hyperextension, and/or positive 'sit test', where the affected leg projects out to the side as the dogs sits, rather than sitting squarely. In chronic cases, quadriceps muscle atrophy and medial periarticular fibrosis, known as medial buttress, may be appreciated. Radiographic evidence of degenerative joint disease with concurrent joint effusion is also supportive of CCL disease [67, 75]. Advanced diagnostics, such as ultrasonography, CT and MRI have been used, though not regularly, to help in diagnosis of CCL tear, especially in cases of partial tears without stifle instability [67].

d. Treatment Options

Surgical treatment is preferred over conservative management and has been recommended for early return to function. Conservative management, including activity restriction for 3-8 weeks, weight loss, and analgesics, has been evaluated. Pond and Campbell found that the majority of small breed dogs recovered to satisfactory function with two months of enforced rest while large breed dogs and working dogs required surgical treatment methods for improved outcomes [76]. The success in conservative management is hypothesized and explained by an increase in fibrous tissue and joint capsule thickening over time that provided natural re-stabilization and reduced cranial tibial displacement [76]. Vasseur similarly found that about 85% of dogs weighing 15 kg or less were normal or had reduced lameness after conservative treatment, after a follow-up period of 36.6 months [77]. Contrary, only 19% of dogs greater than 15kg were normal or had improved lameness after conservative management. Another method of conservative management is the use of a custom stifle orthotic, or brace. A retrospective study in dogs with unilateral CCL disease treated with an orthotic found that total pressure index percentage improved by 5.1% at 90 days or greater when compared to baseline [78]. Additionally, total pressure index percentage improved by 3% in the affected limb with the orthotic off when compared to the unaffected contralateral limb. Importantly, even though some dogs showed improved lameness with conservative treatment, all dogs developed degenerative

joint disease. Because of this, surgical recommendations are aimed at early intervention and restoration of normal joint stability regardless of body weight.

Other studies in dogs with CCL disease support the need for surgical treatment. Objective measures found severe and persistent lameness in the absence of surgical treatment in dogs weighing 28.7 kg or greater [79, 80]. A kinematic study revealed that no improvement was found in female dogs, weighing 20-30 kg, with CCL tear over a period of 2 years when no surgical treatment was performed [81]. Additionally, this study found worsening in peak anterior tibial translation of the CCL deficient stifle at 6-12 months. Providing immediate joint stabilization through surgery is hoped to slow down the progression of degenerative joint disease and provide early return to function.

Surgical treatments can be divided into 3 broad groups: intracapsular, extracapsular, and osteotomy procedures. The goal of these procedures is to achieve functional joint stability, but they do so in different ways [82-90]. Intracapsular and extracapsular techniques stabilize the joint by mimicking the passive constraint provided by the cranial cruciate ligament. Osteotomy procedures change the active constraints to achieve joint stability, which involve muscles and forces acted on the stifle during weight bearing.

i. Intracapsular and Extracapsular Procedures

Intracapsular techniques with ligament reconstruction and grafting has been used primarily for research purposes in dogs, such as cadaveric studies, and clinical and comparative studies are deficient in the veterinary literature. The goal with ligament reconstruction, or ligamentization, is to gradually replace the graft over time with fibrous connective tissue. Allografts, autografts, bovine or porcine xenografts, and synthetic grafts have all be used in attempts to reconstruct or replace the cranial cruciate ligament [67, 91, 92]. Autografts and allografts used include the patellar tendon, hamstring tendon, quadriceps muscle tendon, and Achilles tendon. Synthetic grafts include permanent replacements or prostheses, augmentation devices, and scaffolds. These procedures are far more common in human medicine, where trauma is the primary cause of ligament injury. In contrast, dogs with a degenerative ligament have not tolerated reconstruction techniques, likely due to lack of compliance in postoperative rehabilitation or failure of appropriate ligamentization to achieve required function [67].

Extracapsular procedures have been designed to stabilize cranial cruciate ligament deficient stifles by mimicking the anatomy and function of the cranial cruciate ligament. Many extracapsular techniques have been described and ultimately rely on periarticular fibrosis for long-term stability [82, 93-96]. The most common extracapsular technique is the lateral fabellotibial suture secured via

knots or crimps. Many modifications of this procedure exist, but have been based on ideal isometric points of the femur and tibia, where distance and tension between the two points do not change during joint motion [67]. Overall extracapsular procedures are technically easier to perform, require shorter surgical and anesthetic times, and fewer complications than osteotomy procedures [82].

ii. Osteotomy Procedures

Osteotomy procedures have been the forefront of surgical treatment of the canine cranial cruciate ligament deficient stifle. The main emphasis behind osteotomy procedures is changing the biomechanics of the joint to neutralize cranial tibial subluxation during weight bearing. These osteotomy procedures do not prevent internal tibial rotation or hyperextension [67]. The most commonly performed osteotomy procedures include tibial plateau leveling osteotomy (TPLO) and tibial tuberosity advancement (TTA). Other procedures include CORA-based leveling osteotomy (CBLO), cranial tibial closing wedge osteotomy (CWO), and triple tibial osteotomy (TTO).

The TPLO changes the tibial plateau angle to meet the ground reaction force. The goal is to decrease the tibial plateau angle to approximately six degrees by rotation of the proximal tibial fragment [67, 70, 97]. This adjustment neutralizes

cranial tibial thrust and increases the effectiveness of the hamstring and biceps femoris muscles.

The TTA aligns the patellar ligament so that it is perpendicular to the tibial plateau by cranial advancement of the tibial tuberosity. This advancement neutralizes the tibiofemoral shear forces [98].

The CBLO procedure is based on the principles of deformity correction and center of rotation of angulation (CORA). The tibia has normal procurvatum. This means that the proximal and distal anatomic axes, or middle of the bone in relation to the joint, do not match. The point at which the proximal anatomic axis and distal anatomic axis intersect is the CORA. Its magnitude represents the angle of correction needed to achieve the desired postoperative tibial plateau angle, generally 9-12 degrees [67].

In summary, the osteotomy procedures achieve the same goal but in different ways. Their main advantage is to promote early postoperative weight bearing [99]. Importantly, these procedures do not work to eliminate the cranial drawer sign but instead are aimed to manage underlying anatomical and conformational abnormalities.

e. Surgical Outcomes and Prognosis

There are various studies looking at surgical outcomes and prognosis for dogs undergoing these procedures through subjective evaluations, such as owner

assessments and pain scales, and objective measurements, primarily force plate analysis. As previously mentioned, intracapsular techniques are largely used in research, however some clinical studies have been reported. A 1980 study by Hulse et al. reported good short-term clinical function but it did not correlate with joint stability [100]. A more recent study by Geels et al. looked at fascia lata grafts and evaluated outcomes subjectively and objectively [101]. The fascia lata graft yielded good clinical results in 85-93% of dogs subjectively. At 17.5 months, dogs bore less weight and had significantly less peak vertical forces and vertical impulses in operated limbs compared to unoperated limbs. Further clinical studies in dogs with intracapsular techniques and long-term functional outcomes are warranted.

Looking more closely at extracapsular techniques, a study found that in 18 healthy dogs, weighing 28.7 +/- 3.9 kg, that underwent force-plate gait analysis, had a significant decrease in peak vertical forces and vertical impulses after CCL tear [79]. By 20 weeks post-modified retinacular imbrication technique, an extracapsular technique, they were the same as preoperative values. This study suggests that extracapsular techniques are good options for CCL repair, but the extracapsular techniques do not compare to the outcome of osteotomy procedures. A more recent study finds a strikingly different result in patients that underwent extracapsular repairs. This prospective clinical trial evaluated long-term functional

outcome with force plate analysis in TTA, TPLO and extracapsular repair procedures [90]. Comparing the three methods, TTA patients achieved normal function by 12 months at a walk, TPLO patients achieved normal function by 6-12 months at a walk and trot, and extracapsular repair was found to not achieve normal function. This study found that TPLO patients performed better or similar long-term than TTA patients, and those with extracapsular technique did not perform well.

There are good outcomes after osteotomy procedures. One study reported an excellent return to function in 73%, good function in 21%, and fair result in 3% of 394 cases, based on orthopedic examination and history, using both the cranial closing wedge technique and tibial plateau leveling osteotomy [70]. Similarly, a more recent retrospective study by Gatineau et al., using orthopedic examination and owner assessment, recorded an excellent or good clinical outcome in 93.7% of 476 TPLOs [102]. Subjectively, dogs' activity levels were documented in Priddy et al. in 151 of 193 TPLO cases with completed owner assessments [103]. Specific results include that 5.3% of owners thought their dogs were continuously lame, 15.9% were intermittently lame, and 78.8% were not lame. Additionally, 93% of owners were satisfied with the outcome of the procedure.

TTA procedures were evaluated in the following subjective and objective manner. In a retrospective study, looking at 65 canine stifles that underwent a TTA

procedure, they found that owners reported an excellent outcome in 75% and good to excellent in 90% [104]. A prospective clinical trial objectively assessed the functional outcome in 37 TTA patients using force plate analysis and found that even though vertical ground reaction forces were significantly higher at follow-up (4 to 16 months) they were still significantly lower than unoperated control dogs [105].

Seemingly TPLO and TTA osteotomy procedures function similarly in the short-term period. Ferreira et al. looked at comparing outcome of TTA and TPLO procedures using kinetic analysis by way of computerized baropodometry and found that limb function in 27 dogs had improved significantly following either a TTA or TPLO procedure [106]. Both procedures were found equally effective in promoting weight bearing.

In another objective study, results of 19 patients undergoing an intracapsular technique, 7 patients undergoing an extracapsular technique, 9 TPLOs, 7 TTAs, and 5 TTO procedures showed that ground reaction force measurements, alone, were insufficient in determining long-term outcome [107]. There was no significant difference found in average ground reaction forces between surgically treated and control dog limbs. However, in surgically treated limbs 30% had decrease in weight bearing when symmetry was evaluated and 40-50% had limited active range of motion. Additionally, lower extension and higher flexion angles in

these limbs were found when compared to the unaffected contralateral joint. This study found that patients with an intracapsular technique performed, peak vertical force was lower than those treated with an osteotomy technique and vertical impulse was lower than the contralateral limb.

A comparative retrospective case series evaluating 253 dogs that underwent CCL repair (including intracapsular, extracapsular, and osteotomy techniques) has been studied, using the Helsinki chronic pain index (range of 0-24) [108]. Based on owner assessment, 54% had excellent outcomes, 42.9% had good outcomes, and approximately 30% of dogs after CCL repair had long-term chronic pain. This study also reported that postoperative lameness duration was shorter after osteotomy procedures compared with lameness after intracapsular procedures.

Unfortunately, there isn't a comparative study to date including CBLO procedures with other repair techniques. However, when looking at patients that underwent a CBLO procedure, a retrospective study found that out of 70 dogs, 77% had full function, 19% had acceptable function, and 4% had unacceptable function [82]. Overall, at the time of mid to long-term follow-up (mean of 107 days), most had returned to full function. These findings are similar to studies involving TTA and TPLO surgeries.

In summary, regardless of method of stifle stabilization, about 85% of dogs show improvement but many will have intermittent pain or lameness [71]. Through

subjective and objective measures, all methods, with the exception of intracapsular techniques, have been found to be successful in providing a good prognosis for these patients. Long-term, these patients may experience not only lameness but also chronic pain, likely due to the presence or progression of osteoarthritis.

f. Surgical Complications of Osteotomy Procedures

Osteotomy procedures not only share similar surgical outcomes, but also similar complications. Complications for osteotomy procedures include incisional complications (seroma, dehiscence, infection), anesthetic complications, implant failure, implant infection, tibial fractures, patellar desmitis, patellar luxation, subsequent meniscal injury (SMI) and pivot shift. Even when surgical repair goes without complications, the possibility of contralateral injury is prominent with CCL disease. Up to 60% of dogs with unilateral CCL tear ultimately tear the contralateral CCL within 14 months from initial surgery [109].

The overall complication rate was reported as 20.6% in a study of 253 TPLOs [103]. Complications included osteomyelitis, fracture of fibular neck, broken drill bit retained in the patient, avulsion of tibial tuberosity and incisional infection. A more detailed study looked at intraoperative, acute postoperative (within 14 days), and chronic postoperative (greater than 14 days) complications in 346 dogs that underwent a TPLO [110]. Out of a total of 136 complications,

intraoperative complications, including tibial fractures, intra-articular screw placement and hemorrhage, occurred in 5%, acute postoperative complications occurred in 46%, and chronic postoperative complications in 49%. This study had a total complication rate of 28% out of 397 TPLO procedures, similar to other studies, and a reoperative rate of 5%.

Long-term complications have further been evaluated in another study [72]. Tibial tuberosity fractures were found to be the most common complication, though only occurring in four percent of 696 TPLOs within 2-6 weeks postoperatively. Swelling of patellar tendon occurred in three percent of dogs, 5 to 8 weeks postoperatively, and implant complications occurred in one percent of dogs, diagnosed radiographically at the 8-week recheck or upon palpation. A more recent study reports a 6% complication rate including seroma, postoperative infection and tibial tuberosity fracture in dogs that underwent a TPLO procedure [111]. Additionally, chronic lameness was reported in 17/130 cases (13%).

TTA procedures had reported a 25% postoperative complication rate, comparable to TPLOs [105]. These complications included implant or fixation failure, fissure fracture, surgical site infections (SSI), and unsatisfactory limb function. Twelve and a half percent of these cases required revision. Additional complications associated with TTA procedures include patellar luxation and subsequent meniscal injury. In a retrospective case series, 1.2% developed patellar

luxation, 6.9% superficial SSI (based on cardinal signs of inflammation), 1.1% deep SSI (described by at least partial incisional dehiscence, requiring wound treatment), 1% implant failure, and 0.7% fractures out of 1613 patients and were considered major complications thus requiring further treatment [98]. Further complications were reported in dogs with TTAs and included joint pain and effusion in 10%, subsequent meniscal injury in 5%, and joint crepitus in 4% all occurring after 2 weeks, while superficial SSI was found in 5% of cases within 2 weeks [104].

In essence, osteotomy procedures have an overall complication rate ranging from 16-28% with the majority of these complications involving the surgical site or incision [84, 103, 105]. Surgical site inflammation and infection will be discussed hereinafter in detail.

4) Surgical Site Infections in Small Animal Surgery

a. Definitions and Classifications

Many studies involving SSI surveillance in dogs lack clarity and consistency in definitions for infection in the immediate and long-term post-operative period [112]. They also lack consistency in follow up results and length of follow up. In an attempt to clarify terminology when discussing SSI, Turk et al. used the CDC

SSI Classification System described in three categories—superficial, deep and organ/space surgical site infection [112].

Another study has defined SSI as either acute or chronic. Acute infections were those that were present as early as 2-weeks post-operatively. Authors speculated that infection could be attributed to pre-existing infections prior to surgery including UTIs, pyodermas or from direct surgical contamination [113]. Chronic infections included SSIs that occurred years after the osteotomy site had healed, which can include infections due to hematogenous spread from a distant source, potentially dental disease and endocarditis, for example [113]. Again, proof of a causative relationship between these hypothesized factors and SSI has not been scientifically proven.

Inconsistency also exists when describing SSIs versus incisional inflammation. Many studies do not include the WHO and CDC guidelines for classification of SSI and inappropriately conclude that their incisional complication rates are all infectious. Often times true infection was not present. In these papers, the authors distinguish inflammation from infection when looking at outcomes [112, 114, 115]. Inflammation was defined as an incision with ≥ 1 of the following prior to or at the time of suture removal: redness, swelling, pain, or heat. Infection was defined as an incision with > 1 of the following: a positive culture result, drainage > 48 hours after the end of surgery, abscess, fistula, or dehiscence [115].

In an additional study, Frey et al. defined a wound as “infected-inflamed” when purulent drainage, abscessation, or fistulation were detected or when ≥ 3 of the following were evident simultaneously: redness, swelling, pain, heat, serous wound drainage or dehiscence [114]. Discrepancies in definitions of inflammation and infection are essential to note and establishing standard definitions are critical. Turk et al. stated that published rates for dogs and cats may be inaccurate due to the failure of using standard definitions for SSIs and failure to differentiate infection from inflammation [112].

Additionally, consistent follow-up for SSIs in the veterinary literature was lacking. In one study after implant placement, all SSIs developed within 30 days [112]. In contrast, other retrospective studies detected SSI following TPLO within the first year after surgery, with most dogs developing an infection within six weeks post operatively [102]. In addition, infection requiring TPLO plate removal due to surgical site discharge, loosening of implants or screws, or chronic lameness, has been reported to occur past 6 months [116]. This latter study discussed plate removal in patients with discharge from the surgical site but lacks discussion on the number of patients or how SSI were defined and diagnosed [116].

Attempts have been made to have consistency in defining SSI and time frames necessary for case follow-up. Therefore, the authors of the present proposed

study chose to follow the CDC SSI definitions (See Appendix A). Superficial SSI is defined as a surgical site abnormality involving the skin or subcutaneous tissues that develops within 30 days of the procedure with the presence of one or more of: purulent discharge, isolation of clinically relevant bacteria from an aseptically collected sample, or at least one sign of infection in a wound that is deliberately opened in response to these signs. Deep SSI is defined as an abnormality involving the deep tissues of the incision, and that develops within 90 days, based on the presence of an implant. Deep SSI must include one or more of: purulent discharge from the deep incision site; spontaneous dehiscence along with fever or localized pain; or abscess or other evidence of infection identified using imaging or histopathology. Organ/space SSI is similar to deep SSI but involves a location other than the incision that was manipulated during surgery (ie. joint space). (See Appendix A) This classification follows the national standards of the World Health Organization and the U.S. Centers for Disease Control and Prevention Criteria [117-119].

b. Pathophysiology of Infection

i. Bacterial Isolates

In several veterinary studies, the main pathogen isolated from SSIs in dogs is *S. pseudintermedius* [109, 112, 113, 120-122]. For clean surgical procedures, such

as orthopedic surgeries, this pathogen has been reported in up to 60% of cases [119]. This pathogen is different than the gram negative *E. coli* isolated in the urinary tract of AB patients in the aforementioned studies. Less commonly, *Escherichia coli*, *Pseudomonas*, *Proteus* and *Klebsiella* species are some gram-negative organisms that have been described in SSIs as well [119].

ii. Biofilm formation

A biofilm is described as a "microbially derived sessile community" where bacteria attach to each other, attach to a substrate, embed in a matrix and exhibit phenotypic alterations [123]. Biofilm has three components: the microbe, its glycocalyx and the surface of the biomaterial. Its formation consists of four stages—reversible attachment where microbes adhere to a conditioning film, irreversible attachment through production of glycocalyx, growth and differentiation where bacteria reproduce and form colonies, and dissemination [123]. Infections involving an orthopedic implant can be difficult to treat since a biofilm coats and protects the bacteria. This protective coat limits host immune response and penetration of antimicrobials to that site [113]. There are three mechanisms by which biofilm's microbes are highly resistant to systemic antimicrobials. The first mechanism is that the glycocalyx produced by the microbes inhibits perfusion of the antimicrobials to the cellular targets. Second, the

biofilm microbes are slow growing and many antimicrobials, which act to interfere with growth and reproduction of microbes, are therefore ineffective. Lastly, the microenvironment of the biofilm affects antimicrobial activity due to low pH, high partial pressure of carbon dioxide, and low partial pressure of oxygen [123]. This combination of bacterial properties and biofilm formation makes deep SSI in patients with orthopedic implants concerning.

iii. Osteomyelitis and Implant Associated Infection in Veterinary Medicine

Osteomyelitis, which falls under CDC classification of organ/space SSI, ensues when bone ischemia and vascular compromise are combined with contamination of previously mentioned bacterial isolates [123]. Pathogens gain access to bone via hematogenous spread to the metaphyseal region of bone, spread from focal soft tissue infection, or spread from posttraumatic causes, such as surgery or penetrating wounds [123].

c. Surgical Site Infections after Treatment for Cranial Cruciate Ligament Disease

Clean elective surgical procedures in veterinary medicine carry an infection rate of 2-4.8% [121, 124, 125]. Risk factors of SSI include duration of surgery and anesthesia, surgical site preparation methods, wound closure methods,

antimicrobial prophylaxis, presence of an implant, and comorbidities [123, 126, 127].

Though elective orthopedic procedures are classified as clean surgeries, surgical infection rates following a TPLO range from 0.8% to 25.9% [72, 102, 103, 109, 110, 112-114, 116, 121, 122]. This is over twice the documented rate of SSIs for clean surgical procedures. Likewise, SSI prevalence in 224 stifles that underwent a TTA was 5.3% (12/224), using CDC definitions (See Appendix A) and included 3 superficial infections and 9 deep incisional infections [127]. Seven surgical site cultures isolated *Staphylococcus* species and 3/12 isolated *Streptococcus* species. Additionally, the authors found that the use of antimicrobial therapy postoperatively was not significantly associated with lower SSI rates.

While one study found a SSI rate of 8.4% in TTA cases, a retrospective study looking at complications in 54 TPLO versus 91 TTA procedures found that there was a SSI rate of 25.9% and 15.4%, for TPLO and TTA respectively [98, 128]. These SSI rate in both TPLO and TTA procedures are higher that previous studies, which may be due to the smaller sample size and the use of non-locking plates. The bacterial isolate found in these cases were, also, *Staphylococcus* species.

When evaluating predictive variables for complications after TPLO in 1000 consecutive dogs, 66/148 (44.6%) of complications were defined as SSI, 36 were

based on positive bacterial culture and 30 were based on clinical signs using CDC definitions. Implants were removed in 30.3% (20/66) of dogs with infection [109]. Based on the reduction of heat, pain, swelling or lameness resolution, the median duration of antibacterial therapy for dogs requiring plate removal was statistically higher (90 days) compared with those not requiring plate removal (54 days). Heavier dogs and intact males were linked with greater infection rates while the administration of postoperative antimicrobials was associated with lower infection rates, both statistically significant. Additionally, no difference in SSI frequency was identified for dogs bandaged with a padded dressing versus a light adhesive dressing. There was also no statistical association between SSI and duration of anesthesia or duration of surgery in this study [109].

In contrast, a prospective clinical study found that total anesthetic time and use of oral antimicrobials were the only statistically significant factors [129]. Each minute increase of anesthesia time, increased infection by 2%. Infection was reduced by 84% in those given post-operative oral antimicrobials. This study looked at 76 corrective osteotomies, 12 fractures and 5 arthrodeses that involved a stainless steel implant. Surgical and anesthesia time have been reported as risk factors for SSI [126, 127, 130, 131].

Various factors, increasing SSI risk, have been evaluated in dogs undergoing orthopedic stifle surgery and found to have a higher rate than those undergoing

other clean surgeries. These factors may include thermal injury from the blade used to perform the osteotomy, excessive soft tissue and periosteal dissection, prolonged anesthesia and surgery times, presence of an implant and associated periosteal compression, minimal soft tissue coverage over the proximal tibia, and increasing prevalence of opportunistic pathogens resistant to antimicrobials used for perioperative prophylaxis [72, 102, 103, 109, 110, 112, 113, 114, 116, 122]. A study that found a SSI rate of 7.5%, also found that dogs with implanted medical devices were 5.6 times more at risk for SSIs than dogs that did not have implants surgically placed [112]. They proposed that this increase in risk is likely due to the fact that even with aseptic technique, implants can become colonized with bacteria and act as substrates for biofilm formation.

The effect of using different suture in TPLO procedures and the rate of SSIs has also been evaluated. Triclosan-impregnated, or antimicrobial coated, suture used for incisional closure has not been shown to decrease the rate of SSI or inflammation [115].

Additionally, dogs with distant infections have been thought to be at an increased risk of SSIs. However, dogs with UTIs and pyodermas, in one study, were not detected to have an increase rate of infection and it was speculated that it might be due to the administration of postoperative antimicrobials [114].

Based on human surgical practice, it is recommended for perioperative antimicrobial prophylaxis to begin 60 minutes prior to making the incision and every 90 minutes after that. A study in dogs undergoing TPLOs, showed that there is an earlier increase in SSI occurrence in dogs that did not receive intraoperative antimicrobials compared to dogs that did [122]. A further increase in SSI occurrence was seen when the first dose was given greater than 60 minutes before surgery.

A randomized prospective clinical study looked at 2 antimicrobial regimes on SSI rate in 400 dogs undergoing an orthopedic procedure involving a metal implant [132]. Group 1 had perioperative antimicrobials only (Cefuroxime at 22mg/kg), while Group 2 had perioperative antimicrobials (Cefuroxime at 22mg/kg IV) and 5 days of postoperative antimicrobials (Cephalexin 20mg/kg BID PO). They found that 5.24% in group 1 developed SSI within 6 weeks compared to 3.54% in group 2. After 6 weeks, 7.22% in group 1 and 8.24% in group 2 developed an infection. Overall, SSI rates were not significantly different between the two groups at any time point, nor when TPLO was considered independently of other orthopedic procedures.

Many risk factors have been evaluated in dogs undergoing orthopedic procedures and their association with SSI. A recent review aids in prevention and surveillance of surgical site infections to optimize patient care [133]. However,

there is no data present specifically in orthopedic patients that present with asymptomatic bacteriuria and whether or not they are predisposed to SSIs.

d. Economic Impact

Surgical site infections not only affect the patient but also have an economic impact to the client. The severity of SSI can vary, but addressing SSI may involve prolonged antimicrobial therapy, repeated diagnostic imaging and surgical procedures, laboratory testing and other procedures that increase costs [134]. In one study, signalment, weight, breed, gender, initial surgical cost, number of post-operative visits, time from surgery to final case closure, and total post-operative cost were recorded in TPLO patients [134]. The mean cost of SSI diagnosis and treatment approached 50% of the cost of the original surgery, and in some dogs exceeded it. Considering that another study found that clients spent $1.32 billion treating CCL tear in 2003 in dogs, Nicoll et al. further estimates that about 93,000 procedures were performed by ACVS surgeons, using overall cost and per procedure cost estimates [134, 135]. Given this information and by estimating that 75% of the surgical procedures were TPLOs with SSIs ranging from 6.6 to 11%, it would suggest a total cost of $9.6-15.9 million in the United States per year. This retrospective study showed that post-operative costs, post-operative recheck visits, and time until final case closure were higher for dogs that developed SSIs than for

controls [134]. Post-operative costs for SSI management after TPLO ranged from $145 to $5,022. It is crucial to emphasize the importance of SSI and the corresponding need for good infection control practices [133].

5) Conclusions

In summary, classification and definition of lower urinary tract diseases must be consistent throughout the veterinary literature. More specifically, the role AB plays in veterinary medicine remains largely unknown. The prevalence of AB in the general canine population, including female dogs, dogs undergoing elective surgery, and morbidly obese dogs is documented. Though prevalent, management of AB with antimicrobials is controversial especially in orthopedic patients undergoing surgery.

The purpose of the following study is to prospectively determine AB in both male and female dogs presenting with cranial cruciate ligament disease. Specifically, our objective is to determine an association between the presence of AB and post-operative SSIs in dogs that have undergone repair for cranial cruciate ligament disease. Our first hypothesis is that the prevalence of AB is higher in dogs with cranial cruciate ligament disease than historical controls. Furthermore, we hypothesize there will not be a significant difference in SSI rates in dogs with or without AB. The results of this study can be used to make objective

determinations regarding the need for pre-operative screening, antimicrobial therapy, and timing of surgery.

Chapter II: Assessment and Prevalence of Asymptomatic Bacteriuria in Dogs with Cranial Cruciate Ligament Disease

6) Introduction

Asymptomatic bacteriuria (AB), defined as bacteriuria in patients lacking clinical signs of urinary tract disease, occurs in 2.1-13% of healthy female dogs, morbidly obese dogs, and dogs presenting for elective soft tissue and orthopedic surgery [59, 60, 63]. Asymptomatic bacteriuria is not a urinary tract infection, which involves clinical signs and the presence of an infectious agent in the urogenital system [2, 27, 28]. Furthermore, AB is not synonymous with subclinical urinary tract infection, which indicates a stage where absent clinical signs may develop later [28]. Though once considered sterile, urine in the normal host contains a resident bacterial flora. This characterized canine urinary microbiome likely prevents infection by competing with potential pathogens for nutrients and receptor sites [7, 27]. Similarly, it is hypothesized that bacteria present in AB patients may provide protection against colonization by more pathogenic bacterial strains within the urinary tract [27].

Asymptomatic bacteriuria has been poorly defined in small animals; however, few veterinary studies document AB in dogs. McGhie et al. reported an AB prevalence of 2.1% in 140 dogs presenting for elective orthopedic (80) and soft tissue (60) surgery [60]. Wan et al. found a 9% prevalence in 101 healthy female

dogs, and Wynn et al. found a 13% prevalence in a group of morbidly obese dogs [59, 63]. These studies defined AB as a positive aerobic urine bacterial culture with the absence of clinical signs of lower urinary tract disease.

Dogs with cranial cruciate ligament disease may have difficulty posturing, which may cause urinary retention leading to bacteriuria. In addition, the majority of dogs with CCL disease are overweight [73, 74]. This combination may predispose this population to AB. Some veterinarians theorize that distant bacterial sources, such as pyoderma and bacteriuria, can lead to surgical site infections.

Our objective was to determine the prevalence of and factors associated with AB in dogs with CCL disease. Our second objective was to determine the incidence of surgical site infections after CCL surgery in dogs with and without AB. We hypothesized there is a higher AB prevalence in dogs presenting for CCL disease compared to the historical prevalence of 2.1-13% in healthy female dogs, morbidly obese dogs, and dogs presenting for elective soft tissue and orthopedic surgery [59, 60, 63]. Additionally, we hypothesized that dogs with AB are not more predisposed to SSIs.

7) Materials and Methods

a. Animals

One hundred and fifty-six dogs that presented to the Virginia- Maryland College of Veterinary Medicine for evaluation of CCL disease were enrolled from 22 June 2016 through 11 January 2017. Dogs were included following confirmed CCL disease (based on orthopedic examination), absence of clinical lower urinary tract disease (pollakiuria, stranguria, micturition dribbling, hematuria, and/or painful urination), absence of predisposing conditions (diabetes mellitus, spinal disease, urinary sphincter mechanism incontinence, and hyperadrenocorticism), and lack of antimicrobial or immunosuppressive therapy. Patient history and available blood work determined patient inclusion.

b. Study design

A prospective, clinical cohort study was used. Enrolled dogs had a urinalysis, sediment examination, and aerobic urine culture performed. Age, breed, sex, body weight, body condition score, clinical history, and physical examination findings were recorded. Asymptomatic bacteriuria was defined as a positive urine culture. Follow-up urine culture results were recorded for dogs that presented for their 8-week recheck, after undergoing an osteotomy procedure for CCL disease. All dogs that received surgery received cefazolin (West-Ward Pharmaceutical Corp,

Eatontown, NJ) at 22 mg/kg IV perioperatively at induction, every 90 minutes, and discontinued after extubation. Dogs were excluded from an 8-week urinary culture recheck if antibiotics were given for any reason after extubation. Additional phone call follow-up was performed at 2 week, 4 week, 6 months, and 1 year to assess urination habits, incision and limb use. An owner questionnaire (see Appendix B) was used at each time point. Surgical site infections were classified based on CDC definitions (See Appendix A).

c. Urine sample collection and analysis

Urine (3-5ml) was collected via antepubic cystocentesis, submitted for quantitative bacteriologic culture and antimicrobial susceptibility testing. Trained staff reviewed the urinalysis and sediment on wet mount and with Wright Giemsa staining. Abnormal RBC/HPF and WBC/HPF, averaged from 10 or more fields under 10x magnification, were defined as > 5. Crystals and casts were examined similarly under 40x magnification and recorded as present or absent. The quantitative cultures, plating a minimum of 100 μL of urine, were evaluated for growth at 24 and 48 hours and quantitated as the number of colony-forming units (CFU) per milliliter of urine. In accordance with human and veterinary studies, a positive urine culture was defined as any bacterial colony growth [1, 33, 59, 60]. Minimal inhibitory concentrations were determined by broth dilution for all

clinically significant isolates. Methods and antimicrobial susceptibility breakpoint interpretations were performed in accordance with Clinical Laboratory Standards Institute guidelines [136, 137].

d. Statistical analysis

The data analysis was generated using SAS software (SAS version 9.4, SAS Institute Inc., Cary, NC, USA). The overall prevalence of AB and incidence of SSI were calculated as a percentage. Dogs with AB were compared to dogs without AB considering normally distributed variables (age, body weight, and urine specific gravity), skewed variables (body condition score, lameness duration, and urine pH), and categorical data (sex status, limb affected, clinicopathologic values, and culture results). Normally distributed variables were analyzed using a 2-sample test and reported as a mean and standard deviation. Skewed variables were compared using the Wilcoxon rank sum test and numerical data reported as median and range. Categorical data were compared and percentages were calculated using contingency tables. All methods were used to evaluate potential risk factors between dogs with and without AB. P values of < 0.05 were considered significant.

8) Results

One hundred and fifty-six dogs were enrolled and included 89 female dogs (80 spayed and 9 intact) and 67 male dogs (61 neutered and 6 intact). Breeds represented were mixed-breed (72), Labrador Retrievers (19), 7 of each Boxers, German Shepherds, and Bulldogs, and numerous other breeds. Body weights ranged from 3.6 kg to 84 kg (mean of 32.3 kg). The overall median body condition score was 6/9. Ages ranged from 1 to 12.8 years (mean: 5.7 years). Lameness duration ranged from 0-60 months (median: 3 months).

The prevalence of AB was 7.1% (11/156 dogs). The most common bacterial isolate was *Escherichia coli* (6/11 dogs). Other isolates found included *Streptococcus* spp. (3/11 dogs), *Enterococcus faecalis* (2/11 dogs), and *Lactobacillus murinus* (1/11 dogs). *E. coli and E. faecalis* were both isolated in 1 dog. Associated colony counts are listed in Table 1.

In comparing dogs with and without AB, no significant difference was found in age, body weight, body condition score, lameness duration, or limb affected. Additionally, no significant difference was found in urine specific gravity, pH, glucose, SSA Bumin test, RBC/HPF, casts, or crystals between each group. Clinical parameters and urine clinicopathologic data for each group are listed in Tables 2 and 3, respectively.

Patient sex, urine WBC/HPF, and microscopic bacteriuria were significantly different between dogs with AB and dogs without AB. No male dogs had AB. Of the 89 female dogs, prevalence of AB was 12.4% (11/89). All three dogs with abnormal urine WBC/HPF had AB. Microscopic bacteriuria had only a 60% positive predictive value for predicting positive urine culture. In contrast, lack of microscopic bacteriuria had a negative predictive value of 98.6%.

One hundred and twenty-one dogs out of 156 dogs had a corrective osteotomy procedure for CCL disease. Owners elected conservative management in 29 dogs and an extracapsular repair in 6 dogs. Out of the 121 dogs with a corrective osteotomy procedure, 75 were excluded from follow-up urine cultures for the following reasons: 35 were lost to follow-up, 22 received IV antibiotics after extubation due to surgeon preference, and 18 received oral postoperative antibiotics. Out of these 18 dogs that received oral postoperative antibiotics, 11 were for SSI confirmed by veterinary examination and/or culture. An 8-week urine culture was available for 46 dogs (3 AB dogs and 43 dogs without AB). Of the three AB dogs that had repeat culture, two had negative results. Their initial culture result revealed *Enterococcus* and *Lactobacillus* species, respectively. In one dog with AB, *Streptococcus spp* was initially cultured and follow-up culture revealed three different bacterial isolates, *E. faecium, E. coli, and L. murinus*. Three female

dogs out of 43 dogs without AB developed AB at the 8-week follow-up. All three cultures revealed *E. coli*.

Of the 46 dogs available for 12-month follow-up and the 11 dogs that developed a SSI prior to 12 months, 14 developed a superficial infection and 1 deep infection. One patient had a SSI that progressed to organ/space. All of these SSIs were in dogs without AB. Incidence of SSI in this population was 26.3% (15/57). Surgical site infections in these patients were diagnosed between 5 and 22 days with a mean of 12 days. Only five dogs had confirmed SSI by veterinary examination and culture swabs; the remaining 10 dogs were diagnosed with SSI by veterinary examination only. The organisms that were isolated included Staphylococcus spp (2), Klebsiella spp. (1), E. coli (1), Pasteurella spp (1), Steptococcus spp (2) and Bacillus spp (1).

9) Discussion

In our study, the prevalence of AB in dogs with CCL disease was 7.1%, which was within the previously reported prevalence of 2.1-13% in healthy female dogs, morbidly obese dogs, and dogs presenting for elective soft tissue and orthopedic surgery [59, 60, 63]. When comparing AB prevalence rates to a sub-cohort of dogs from McGhie et al that underwent orthopedics surgeries (2.5%), the prevalence in the current study was more than two-fold [60].

Lameness duration was not significantly different between dogs with AB and those without, suggesting that lameness with difficulty and pain in posturing, leading to urinary retention, may not play a role in the mechanism of AB. Additionally, body condition score was not associated with AB. A study documented the prevalence of AB as 13% in morbidly obese dogs, defined as greater than 45% body fat [63]. While body fat percentage was not calculated in our study, the median body condition score of 7/9 in AB dogs correlated to 30% body fat [65]. Therefore this canine population did not appear at risk for AB.

The majority of values on routine urinalysis were not significantly different between dogs with AB and those without. Abnormal urine RBC/HPF, found in 36.4% (4/11) of AB dogs, was likely due to the cystocentesis procedure. Increased urine WBC/HPF and microscopic bacteriuria were the only factors significantly different in dogs with AB, compared to dogs without AB. These two parameters can suggest bacteriuria. Only 3 of 11 culture positive urine samples had increased WBC/HPF. Furthermore, only 9/15 had microscopic bacteriuria. A urine sample could have microscopic bacteriuria but a negative urine culture by having an inadequate amount of bacteria or involve anaerobes that do not grow in the culture media used. Additionally, cases involving intracellular bacteria will not have a positive urine culture based on a urine sample alone. A previous report of dogs

without lower urinary tract disease signs also found a poor agreement between bacteria present on urinalyses and positive urine cultures [60].

AB in humans is a benign and incidental finding, found more frequently in older women [44, 47-48]. Our study did not find an association between age and presence of AB, but did find AB only in female dogs, with AB present in eight spayed and three intact dogs. Since adequate numbers are lacking, we hypothesize that the majority of dogs with AB were spayed due to low estrogen levels, which was found in women. Low estrogen led to increased vaginal and urethral pH, lowered levels of lactobacillus and increased bacterial susceptibility. No clinical benefit has been found in treating AB in humans. Instead, it has led to adverse side effects, such as diarrhea, drug allergies, adverse drug reaction, and antibiotic resistance [29, 42, 48, 57].

Notably, in our study, bacteriuria was no longer present in two out of three AB dogs and developed in three out of 43 dogs at 8 weeks postoperatively. None of the 3/11 AB dogs in the current study developed a clinical urinary tract infection based on owner follow-up and questionnaire. These results corroborate a study documenting 3-month follow-up of AB in 101 healthy female dogs [59]. AB was identified in eight dogs, and was either persistent (4) or transient (4), but none developed clinical signs.

Understanding AB and its role in veterinary patients can help guide diagnostics and treatment. Anecdotally, there is concern for bacteriuria predisposing orthopedic patients to SSI via hematogenous spread. Human literature concludes that AB does not cause detrimental effects on long-term outcomes of healthy patients undergoing elective surgical procedures, even those involving orthopedic implants [54-56, 58]. Veterinary research has not evaluated treatment of AB.

Preliminary data found an overall SSI incidence of 26.3% in a total of 57 dogs, diagnosed within 30 days postoperatively. Our results were similar to previously reported SSI rates in dogs with osteotomy procedures, which ranged from 0.8% to 25.9% [72, 102, 103, 109, 110, 112-114, 116, 121, 122]. Further studies are recommended for long-term follow-up on dogs with AB undergoing orthopedic surgery to determine if they are at a higher SSI risk. Our preliminary results do not show an increased SSI risk in AB patients however; only three AB patients were available for long-term follow-up.

A limitation of this study is that we were reliant on owner's memory for patient information, such as lameness duration. Additionally, during follow-up phone calls, we were reliant on owner's perception on how patients were doing at home. Another limitation was that degree of lameness was not evaluated, neither subjectively nor objectively with force plate gait analysis. Degree of lameness

could be important in determining if severity of clinical signs could predispose patients to urinary retention and therefore AB. Lastly, in this study there were a large number of patients that were lost to follow-up and therefore a small number of AB patients included for follow-up. Based on our power analysis, we would need 70 dogs with AB and 70 dogs without to determine if AB predisposes dogs to SSI. Therefore, interpretation of these results should be made cautiously.

In summary, the prevalence of AB in canines presenting with CCL disease was 12.4% and 0% in female and male dogs, respectively. Further research is warranted to determine whether AB predisposes patients to SSIs but our preliminary data suggests that it does not. Continued follow-up data in dogs with AB and without will determine the role of AB and SSI risk in dogs presenting for CCL disease.

Chapter III: Conclusions

This study found that the prevalence of AB in dogs presenting with CCL disease was 7.1%. In comparing dogs with and without AB, female dogs, greater than 5 WBC/HPF and presence of microscopic bacteriuria were all found to be significantly different. The prevalence calculated in this study, was similar to other studies looking at healthy female dogs and female dogs undergoing orthopedic surgery. The prevalence in this study was more than two-fold when compared to a sub-cohort of dogs from McGhie et al. that underwent orthopedics surgeries [60]. This could be because the current study's population involved dogs with hind limb lameness, while McGhie et al. included dogs with other orthopedic issues, such as those affecting the forelimb that would not lead to urinary retention.

The clinical significance of AB remains unknown, especially in relation to SSI. Further investigation is warranted for our second objective in determining if dogs with AB are predisposed to SSI. Our preliminary studies suggest that dogs with AB are not predisposed to SSI but adequate numbers are required to show statistical significance.

This study had financial limitations and therefore patient enrollment was only performed for one year. This limitation restricted adequate patient numbers for our second objective. Additionally, a longer enrollment period would be

needed to acquire adequate numbers of both dogs with AB and dogs without, in order to establish AB significance in SSI.

Even though adequate numbers were not obtained in this study for our second objective, the study design was well planned. An additional clinical parameter that I would investigate further would be lameness severity, as it could influence prevalence of AB. Overall, more data is required to determine the clinical significance of AB in dogs undergoing CCL surgery. However, results are promising in suggesting that dogs with AB are not predisposed to SSI.

Analgesic protocol could have had an effect on postoperative urinary retention. Epidurals, intravenous, and intramuscular opioid administration have similar effects leading to urinary retention. Opioids act on mu receptors in the spinal cord and inhibit release of acetylcholine from the parasympathetic nerves of the sacrum [104]. In the current study, analgesic or anesthetic protocols were not standardized and could have affects results.

Given the results of this study, I would recommend urine screening in dogs presenting for surgery for CCL disease to ensure the patient is otherwise healthy. However, I would not treat AB in these dogs since no predisposition of SSI in dogs with AB was found in this study.

Table 1. Urinary bacterial isolates	
Bacterial isolate	**Colony forming units/mL**
Escherichia coli	100 (1) and >100,000 (5)
Streptococcus spp.	>100,000 (3)
Enterococcus faecalis	200 (1) and >100,000 (1)
Lactobacillus murinus	35,000 (1)

Table 2. Clinical parameters of dogs with and without asymptomatic bacteriuria			
Variable	**AB dogs (n=11)**	**Dogs without AB (n=145)**	**P-value**
Mean age in years[a]	6.2 +/- 2.8	5.6 +/- 2.5	0.498
Mean body weight in kg[a]	29.8 +/- 4.6	32.5 +/- 12.9	0.503
Median body condition score (1-9)[b]	7 (4-8)	6 (4-9)	0.166
Median lameness duration in months[b]	3 (0-48)	3 (0-60)	0.588
	Number (Percentage)		
Sex			0.002
Spayed female	8 (72.7)	72 (49.7)	
Intact female	3 (27.3)	6 (4.1)	
Neutered male	0 (0.0)	61 (42.1)	
Intact male	0 (0.0)	6 (4.1)	
Limb affected			1.000
Unilateral	11 (100.0)	140 (96.6)	
Bilateral	0 (0.0)	5 (3.4)	

[a]Data reported as mean $\pm$ standard error

[b] Data reported as median (range)

AB: asymptomatic bacteriuria

Table 3. Urinary clinicopathologic data in dogs with and without asymptomatic bacteriuria			
Variable	**AB dogs (n=11)**	**Dogs without AB (n=145)**	**P-value**
Urine specific gravity (mean)	1.030	1.030	0.276
pH (median)	7.5	7.5	0.557
	Number (Percentage)		
Glucose			1.000
Negative	11 (100.0)	143 (98.6)	
1+	0 (0.0)	2 (1.4)	
SSA Bumin test			0.326
Negative	8 (72.7)	122 (84.1)	
1+	1 (9.1)	12 (8.3)	
2+	1 (9.1)	6 (4.1)	
3+	1 (9.1)	3 (2.1)	
4+	0 (0.0)	1 (0.7)	
5+	0 (0.0)	1 (0.7)	
RBC/ HPF			0.068
Normal (0-5)	7 (63.6)	125 (86.2)	
Abnormal	4 (36.4)	20 (13.8)	
WBC/ HPF			<0.001
Normal (0-5)	8 (72.7)	145 (100.0)	
Abnormal	3 (27.3)	0 (0.0)	
Casts			1.000
Absent	11 (100.0)	137 (94.5)	
Present	0 (0.0)	8 (5.5)	
Crystals			0.088
Absent	7 (63.6)	123 (84.8)	
Present	4 (36.4)	22 (15.2)	
Bacteria			<0.001
Absent	2 (18.2)	139 (95.9)	
Present	9 (81.8)	6 (4.1)	

AB: asymptomatic bacteriuria; HPF: high-powered field; RBC: red blood cells;

SSA: sulfosalicylic acid; WBC: white blood cells

Appendix A

U.S. Centers for Disease Control and Prevention Criteria for Surgical Site Infections			
	Superficial SSI	Deep SSI	Organ/space SSI
Timing	Within 30 days	Within 30 days (90 days for some procedures)	Within 30 days (90 days for some procedures)
Location	Skin and subcutaneous tissues only	Deep soft tissues (fascial and muscle layers)	Any area other than the incision which was opened or manipulated during surgery

Aspects	• Purulent discharge
	• Organisms isolated from an aseptically collected sample of fluid or tissue
	• One or more of the following:
	  ○ Pain or tenderness
	  ○ Localized swelling
	  ○ Redness
	  ○ Heat
	  ○ Incision is deliberately opened by a surgeon *unless* culture negative
		• Deep purulent drainage—not in organ/space
		• Organisms isolated from an aseptically collected sample of fluid or tissue
		• One or more of the following:
		  ○ Pus
		  ○ Spontaneous dehiscence of deeper incision
		  ○ Incision is deliberately opened by a surgeon when patient has fever, localized pain or tenderness *unless* culture negative
		  ○ Abscess or other evidence of infection on histopathology or diagnostic imaging
			• Involving any part of the body, excluding skin incision, fascia and muscle that is opened or manipulated during the operation.
			• Organisms isolated from an aseptically collected sample of fluid or tissue
			• One or more of the following:
			  ○ Pus
			  ○ Bacteria
			  ○ Abscess or other evidence of infection on histopathology or diagnostic imaging, exam or re-operation

Appendix B

Owner Questionnaire

1. Did you notice any problems with your pet's surgical site incision (ie. oozing, redness, tenderness, pain)?
   a. YES or NO (circle)
      - If YES—
        - When did you first notice the problem(s)?
        - What specifically did you see?
        - Which veterinarian did you see and when?
        - Were any treatments prescribed?
          - If so, what was given and for how long (drug name and dose)?
          - What was pet's response to therapy?
        - Was your pet admitted to a veterinary clinic for treatment?
        - Was there a need for additional surgery?
        - Has the problem resolved?
        - Currently, are there any problems with the surgical site?
          - If so, please described…
      - Was your pet wearing the provided E-collar after surgery and until suture removal?
      - Did you see your pet licking or rubbing the surgical site before you noticed any abnormalities?

2. Did you notice any problems with the use of your pet's surgical leg?
      - Did you follow the outlined instructions for exercise restriction?
      - What is your pet's current activity level?

3. Did your pet experience any other problems after surgery such as diarrhea, coughing or decreased appetite?
   a. YES or NO (circle)
      - If YES—
        - What problems were encountered? Please describe
        - Were any treatments prescribed?
          - If so, what was given and for how long (drug name and dose)?
          - What was pet's response to therapy?
        - Was your pet admitted to a veterinary clinic for treatment?
        - Was there a need for additional surgery?
        - Has the problem resolved?

4. Did your pet experience any other problems after surgery such as difficulty urinating, blood in the urine, foul smelling urine, increased urgency to urinate or any inappropriate urination (urinating in house or other locations not customary, multiple small amounts)?
   a. YES or NO (circle)
      - If YES—
        - What problems were encountered? Please describe
        - Were any treatments prescribed?
        - If so, what was given and for how long (drug name and dose)?
        - What was pet's response to therapy?
        - Was your pet admitted to a veterinary clinic for treatment?
        - Was there a need for additional surgery?
        - Has the problem resolved?

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