Passive ureteral dilation secondary to ureteral stenting and biofilm evaluation formed on ureteral stents

by

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ABSTRACT

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In children, passive ureteral dilation occurs after placement of a ureteral stent. The objectives of the thesis were to determine if passive ureteral dilation occurs in dogs after stent placement, and if ureteroscopy is feasible. The second part of the thesis evaluated biofilm production.

In five healthy beagles, bilateral stent were placed. One stent was removed after two weeks (group 1) and the contralateral stent was removed after 6 weeks (group 2). A CT excretory urography was performed every 2 weeks for 10 weeks to measure the ureteral diameter. Ureteroscopy was attempted at the time of stent removal. Urinalysis and urine culture were performed every two weeks. The stent removed aseptically were evaluated for adhered bacteria using sonication and scanning electron microscopy. Also, seventy enterococci clinical isolates previously isolated were studied in vitro using microtitre plate assay. A repeated measures ANOVA as well as descriptive statistics were performed.

Passive ureteral dilation occurred. Median ureteral diameter was 1.65 mm (range 1.3-2.7 mm) prior to stenting in both groups. At stent removal, ureteral diameter was 2.9 mm (2.4-3.1 mm, p<0.0001) in group 1 and 2.7 mm (2.1-3.4 mm, p=0.0016) in group 2. Ureteroscopy was successfully performed up to the renal pelvis in all dogs without any evidence of complications.

Pyuria and positive urine culture were detected in 3 of 30 (10%) and 5 of 30 (17%) samples collected, respectively. None of the dogs had signs of lower urinary tract infection. After sonication, there was no growth noted from any stents at week 2 but bacteria were identified at week 6. Scanning electron microscopy failed to identify bacteria.
Of the seventy enterocci isolates tested, 42/70 (53%) produced biofilm; 20/23 (87%) were \textit{E. faecalis} and 16/47 (34%) were \textit{E. faecium}. Biofilm production was common, especially for the \textit{E. faecalis} isolates.

In conclusion, passive ureteral dilation occurs within 2 weeks of ureteral stent placement. Ureteroscopy can be performed at stent removal. Bacteriuria may occur within few weeks following stent placement. Lastly, enterococcal isolates produced biofilm, especially \textit{Enterococcus faecalis}.

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**Introduction**

Ureteral obstructions are challenging conditions to treat in veterinary medicine given the small ureteral diameters in cats (0.3-0.4 mm) and dogs (1-2 mm). Medical management has been associated with low success rates and 12 month survival times. Surgical interventions have been associated with high morbidity and mortality in both species. Common complications following surgery include uroabdomen, persistent ureteral obstruction and persistent azotemia in cats, and dogs. Safe and effective treatments for these species are important for the patients, their owners and the veterinary practitioners entrusted to care for them.

To date, ureteral stenting has been used as a palliative treatment for obstruction in children and veterinary patients. In veterinary patients, ureteral stents can remain in place long-term provided that they are not associated with complications, however, many complications have been reported for dogs and cats. Ureteral stents used in both humans and animals provide, following insertion, immediate renal drainage and decompression by allowing urine outflow. In children, ureteroscopy is challenging because of the small ureteral diameter, a situation similar to that seen in veterinary patients. Ureteral stents placed in children have lead to passive ureteral dilation (PUD). Ureteral stenting for 2 weeks in children has been shown to induce PUD that allows ureteroscopy to be performed after stent removal. To our knowledge, PUD secondary to ureteral stenting has not been evaluated in dogs. Being able to pass a ureteroscope after removal of the ureteral stent would be advantageous (as would eventual stone removal and biopsy, as necessary).

In humans, ureteral stents may be used before or after many diagnostic and therapeutic urologic procedures. These stents, however, have been associated with urinary tract infections (UTIs) and encrustations. A potential complicating factor in the elimination of infections is bacterial biofilm production. It is estimated that over 65% of hospital-acquired infections and 80% of all microbial infections are biofilm-related. Bacterial colonization and biofilm formation on ureteral stents is regularly observed in human medicine.
human patients, adherent pathogens were present in 90% of ureteral stents (indwelling time: 5-128 days) inserted after extracorporeal shock wave lithotripsy (ESWL), but urine cultures were positive in only 27% of these patients. Bacteria in the urine of dogs can lead to biofilm formation and this is of possible clinical relevance for long-term ureteral stenting in dogs. Enterococci are particularly important pathogens in human and veterinary medicine, especially for hospitalized or otherwise compromised individuals. Little is known about the biofilm-forming ability of important enterococcal species derived from canine infections.

References:


CHAPTER I
LITERATURE REVIEW, SUMMARY, OBJECTIVES AND REFERENCES
**Literature review**

This literature review covers topics relevant to upper urinary tract obstructions and the use of ureteral stents in veterinary medicine, particularly in dogs, and to biofilms in this medical contexts. As with many procedures in veterinary medicine, there are precedents in human medicine so germane human data are presented here also. First, anatomical details of the normal canine ureter are provided. Descriptions of aspects of upper urinary tract obstructions in dogs follow: etiology, pathophysiology, history and reasons for presentation, diagnostic procedures, and treatment indications and options. The preferred treatment for upper urinary tract obstructions in humans, both adults and children, is described: ureteral stenting (stent properties and biomaterials, utilization and indications, complications and pre-stenting) and ureteroscopy (techniques, indications, successes and complications). Because infections and biofilm formation are such important complications of human ureteral stenting, urinary tract infections in dogs (not to be confused with bacturiuria that is not associated with clinical signs) are then described with respect to factors and pathogens involved in their development, clinical signs and diagnostic tests, and the condition of subclinical bacteriuria. Urinary tract infections in humans and dogs arising specifically from catheters and ureteral stents are then described. Finally, and again because of the serious complications of bacterial colonization and biofilm-related infections in human patients with ureteral stents, a detailed description of biofilm formation, its incidence in the urinary tract, its assessment and the bacterial species (and antimicrobial resistance) most often implicated in human infections follows.
1.0 Anatomy of normal ureters in dogs

Each ureter is a fibromuscular tubular structure that is continuous with the renal pelvis of the ipsilateral kidney and carries urine from the kidney to the bladder via peristaltic activity. The length of canine ureter is poorly documented, but it averages between 12-16 cm in average-size dogs (35 pounds/7.5 kg). Due to the more cranial position of the right kidney, the right ureter is slightly longer compared to the left ureter. The normal diameter for a canine ureter ranges from 1.33 to 2.72 mm depending on the dog's size.

A general guideline for the normal luminal diameter of the proximal canine ureter is 0.07 times the length of the body of vertebra L2.

The abdominal ureter, as mentioned above, receives urine from the renal pelvis. It then runs caudoventrally and medially toward the urinary bladder. The pelvic portion of the ureter reaches the dorsolateral surface of the bladder, connecting cranially to the bladder neck. On ureterographic studies, a “J shape” curve is described as a normal finding and is situated just cranial to the urinary vesicular junction (UVJ).

2.0 Upper urinary tract obstructions in dogs

2.1 Etiology

Ureteral obstruction in dogs can be categorized into intraluminal, mural, and extraluminal diseases.

Intraluminal causes of ureteral obstruction include urolithiasis and blood clots. Urolithiasis is the most common cause of ureteral obstruction in dogs. Struvite and calcium oxalate calculi have been removed from ureters in dogs and cats. Calcium oxalate accounts for 30-60% of ureteral calculi in dogs. Struvites or mixed struvite ureteroliths are also commonly observed in dogs. In dogs with ureteroliths in which calculi were analyzed, struvite-containing calculi represented 7 of 13 dogs in one study and 1 of 3 dogs in another study. Calcium-based calculi (phosphate or oxalate) comprised the remainder of the minerals.
found in the ureteroliths. Urate mixed with struvite was noted in one dog. Cysteine and urate calculi may also be noted in the upper urinary tract. Mural causes of ureteral obstruction include ureteral stenosis, fibrosis, ureteroceles, proliferative ureteritis, ureteral fibroepithelial polyps, and ureteral neoplasia. Ureteral strictures are well described in the literature as a mural cause of ureteral obstruction and are a common complication of ureteral surgeries in small animals.

Extraluminal causes of obstruction include neoplasia of the upper or lower urinary tract (e.g., pelvic masses, bladder or prostatic neoplasia). Local tumor invasion progressively obstructs the ureters or the urethra, or both. Iatrogenic extramural ureteral obstruction can occur secondary to accidental ureteral ligation during ovariohysterectomy. Regardless of the cause of the ureteral obstruction, the outcome can be substantial renal damage.

2.2 Pathophysiology
Obstruction of the urine flow is usually unilateral, but if bilateral, it may lead to anuria. Based on the underlying pathology, the degree of ureteral obstruction, and the duration of obstruction, urine accumulates above the obstruction and distends the ureter as proximally as the renal pelvis. If a significant amount of urine accumulates, pressure starts to rise at different levels of the upper urogenital tract (ureters, renal pelvis, and calyces). Ultimately, if the obstruction remains, the increased renal pressure causes tubulointerstitial injury due to increased intratubular pressure: renal tubular cell damage and apoptosis occur. Renal tubular injury results from mechanical stretching, hypoxia, and exposure to reactive oxygen radicals that are the consequences of the increased hydrostatic pressure, and reduced blood flow (discussed below). Tubular injury starts as early as 5 minutes following the increase in tubular pressure. With chronicity, there is progression to tubulointerstitial injury that leads to chronic renal damage characterized primarily by renal tubular atrophy, inflammatory cell infiltration, and interstitial fibrosis. If
obstruction persists, some renal function will be lost permanently.\textsuperscript{Wilson 1977, Coroneos 1997}

The kidney’s initial response to obstruction is due to the release of vasodilator substances that cause an increase in renal blood flow. However, as obstruction persists, thromboxane A2 is released. This vasoconstrictive agent causes a decrease in renal blood flow, which eventually cause tubular cell damage and release of numerous chemicals that mediate inflammation and fibrosis.\textsuperscript{Coroneos 1997}

Ureteral obstruction also affects glomerular filtration rate (GFR). Glomerular filtration rate is driven by a balance of hydrostatic and colloid osmotic forces acting across the capillary membrane in each nephron. By adjustment of the resistance in the afferent and efferent arterioles, the kidneys regulate the hydrostatic pressure in both glomerular and peritubular capillaries, and thereby adapt the rate of glomerular filtration, tubular reabsorption, or both in response to body homeostatic demands. However, during obstructive uropathy, the glomerular hydrostatic pressure, the colloid osmotic pressure, and the pressure in the Bowman’s capsule can be affected. As mentioned above, shortly after obstruction, renal blood flow increases due to vasodilation and the GFR increases, thus increasing urine production and ureteral pressures within 2 hours. Approximately four hours following obstruction, ureteral pressure continues to rise while renal blood flow declines.\textsuperscript{Palmieri 2002} Then, the increase in intrarenal pressure causes activation of the renin-angiotensin system. The release of angiotensin II causes vasoconstriction and decreased renal blood flow. When ureteral obstruction occurs in normal dogs, renal blood flow can decrease to 40% of normal within the first 24 hours of the obstruction.\textsuperscript{Wilson 1977, Coroneos 1997} After two weeks of obstruction, the renal blood flow can decrease to 20% of normal.\textsuperscript{Wilson 1977, Coroneos 1997} The obstructive nephropathy is then exacerbated by angiotensin II as it promotes production of TGF-\(\beta\). Nitric oxide normally has the positive effect of protecting the kidneys against macrophage infiltration into the interstitium and decreasing the production of protein interstitial matrix, both of which ultimately reduce the progression of renal fibrosis. As a vasodilator, it also protects against excessive vasoconstriction caused by the activation of renin-angiotensin activation. Obstructive uropathy promotes the degradation of nitric oxide which leads to
increased renal vascular resistance and decreased GFR. In normal dogs glomerular filtration decreases permanently by 35% and 54% after one and two weeks of ureteral obstruction, respectively.\textsuperscript{Wilson 1977} If the contralateral kidney is free of obstruction, a compensatory increase in glomerular filtration from that kidney can be noted.

The physiologic response to ureteral obstruction is complex. Following ureteral obstruction, pressure increases in the renal pelvis and eventually is transmitted to the tubulointerstitial area that occupies the majority of the total kidney volume. During obstructive uropathy, the process of degradation/production of interstitial matrix is disrupted. First, there is excess production of protein in the interstitial matrix and too few mediators available for its destruction. The net effect is an overproduction of interstitial matrix causing tubular atrophy and a reduction in the number of functioning peritubular capillaries. Second, the increase in interstitial matrix causes fibroblast infiltration into the interstitium. Further, macrophages produce cytokines (interleukin (IL)-2, IL-6, transforming growth factor-\( \beta \) (TGF-\( \beta \)), clusterin) that promote inflammation.\textsuperscript{Hewitson 2009} TGF-\( \beta \) has a key role in the development of interstitial fibrosis. Transforming growth factor-\( \beta \), released by the macrophages in response to inflammation, has a primordial role in promoting interstitial fibrosis, acts as a regulator of production and degradation of the interstitial matrix, and attracts fibroblasts. It is also the primary up-regulator of the activation of the renin-angiotensin system during the process of fibrosis. Transforming growth factor-\( \beta \) also promotes the production of nuclear factor kappa-B (NF-KB) which is involved in tissue inflammation, angiotensin II production and the release of tubule cell chemoattractants. Tubular epidermal growth factor is noted to decrease in obstructive uropathy and involved in promoting fibrosis.\textsuperscript{Grandaliano 2000, Harris 1993}

Immediate assessment and treatment of obstructive ureteral diseases is warranted since delay in treatment may cause irreversible kidney damage.\textsuperscript{Wilson 1977, Coroneos 1997} Following relief of the obstruction, kidney damage may cause a permanent or temporary inability of the proximal tubules, loop of Henle and distal tubules to regulate water reabsorption.\textsuperscript{Coroneos 1997} This is even more pronounced in overhydrated patients.\textsuperscript{Yarger}
This post-obstructive diuresis is suspected to be due to tubular damage during the period of obstruction.

2.3 History and reasons for presentation

Clinical signs seen with ureteral diseases are typically associated with abnormalities in urination including dysuria, stranguria, hematuria, pollakiuria, polyuria (if chronic kidney failure is present) or anuria (if bilateral renal obstruction is present). Recurrent urinary tract infections, abdominal pain or signs of systemic illness (e.g., lethargy, vomiting, lethargy, anorexia, fever) may also be noted. In a study of 16 dogs with ureteral calculi, the most common clinical sign was lethargy (n=14), followed by vomiting (n=12) and anorexia (n=8). Stranguria and hematuria were noted in 3 and 2 dogs, respectively. The median duration of clinical signs in this study was 4 days. In another study in which obstructive pyonephrosis was noted in 13 dogs, the most common clinical signs were anorexia and vomiting (n=11), followed by lethargy (n=8), diarrhea (n=8), urinary incontinence (n=2), polyuria (n=2), polydipsia (n=2) and shaking (n=2). Abdominal pain and stranguria were noted in one dog each.

2.4 Diagnostic procedures

2.4.1 Clinical and laboratory abnormalities

On physical examination, findings may vary depending on the severity of the patient’s condition. The physical examination may also be unremarkable. If obstruction is complete and bilateral, patients show signs consistent with severe azotemia and anuria. Abdominal pain or renal colic may be noted, especially if there is renal capsular inflammation or pyelonephritis. Other findings may include pyrexia, dehydration, and tachycardia.

Complete blood counts performed on dogs with ureteral obstructions have shown moderate to severe neutrophilia if the obstruction was associated with concurrent pyelonephritis. Leukocytosis, neutrophilia and thrombocytopenia are seen in the majority of patients with ureteral diseases. Forty four percent of dogs with
obstructive ureterolithiasis are thrombocytopenic. Thrombocytopenia may be severe and is suspected to be due to concurrent immune-mediated processes or sepsis. Serum biochemical analyses have revealed azotemia in a majority (50-61.5%) of patients at the time of diagnosis. Renal azotemia could be secondary to bilateral pyelonephritis, chronic kidney disease, and/or ureteral obstruction. Other findings have included hypercalcemia, hyperkalemia, hyperphosphatemia and hypoalbuminemia. Coagulation profiles may also be abnormal. On urinalysis, several abnormalities may be noted based on the underlying cause of the ureteral obstruction. With struvite or calcium oxalate crystals, pyuria and hematuria are noted in a majority of dogs with ureterolithiasis. Positive urine or stone culture is fairly common (53-77%) in these dogs. The most frequently identified microorganisms include urease-producing bacteria (e.g., Staphylococcus spp, Proteus spp) and Escherichia coli. Neoplastic cells are rarely observed if ureteral obstruction is due to an underlying neoplastic process.

2.4.2 Imaging modalities
Many imaging modalities are available for the diagnosis of ureteral diseases including plain abdominal radiographs, urogenital ultrasounds, excretory urography, ureteropyelograms (antegrade or retrograde), and computed tomography (CT)

2.4.2.1 Radiography
The benefits of plain radiographs include the possibility of documenting changes of the size, shape and radioopacity of the kidneys, ureters and urinary bladder. If severely dilated, ureters may be seen on plain radiographs. They would appear as tortuous and tubular structures in the retroperitoneal space when followed from the kidney to the dorsal aspect of the bladder trigone. The location, size and number of radiopaque uroliths may also be documented. Orthogonal radiographic projections should be obtained in order to allow for thorough evaluation. Compression techniques or oblique projections may be used in order to better visualize ureteral calculi. Plain radiography is minimally invasive and readily available.
However, neither small uroliths or the ureter(s) may be visible due to their small size and larger soft tissue superimposition. Non-radiopaque calculi, such as cystine and urate cannot be seen. An enema may be helpful in improving visualization of the renal pelvis and ureters. On abdominal radiographs it is not possible to distinguish hydronephrosis from an overall enlarged kidney and dilated ureter, so urogenital ultrasonography is recommended for patients presenting in uremic crisis.

2.4.2.2 Urogenital ultrasonography
One advantage of ultrasonography is its capacity to detect renal mineralization, due to nephroliths or dystrophic mineralization. Renal mineralization is characterized by a strong echogenic surface and a distal acoustic shadow. The benefits of urogenital ultrasonography include the possibility of evaluating the shape, size, location and architecture of the kidneys and the renal pelvis. Also, the kidney outflow tract can be assessed for any evidence of dilatation. In normal dogs, dilations of 2 mm and 2.5 mm may be visualized due to fluids. In dogs with pyelonephritis, the dilatation is usually more pronounced (3 mm). Other observable abnormalities may include dilatation of the renal pelvis, termed pyelectasia or hydronephrosis depending on the degree of dilatation of the pelvis (i.e. <13 mm or >13 mm, respectively). When outflow tract obstruction is suspected, the severity of pelvic dilatation may aid with diagnosis in dogs: dilatation of >15 mm in width is suggestive of outflow obstruction. Furthermore, ultrasonographic evaluation may help identify obstructive complications associated with subcutaneous ureteral bypass (SUB). The degree of pelvic dilatation may help in evaluating for obstructive complications associated with SUB devices. A renal pelvis diameter of ≥ 4mm in the short and long term may predict obstruction regardless of the renal pelvis size prior to SUB placement.

Therefore, a combination of radiography and ultrasonography is preferred when evaluating a patient for potential ureteral obstruction. The sensitivity for detecting ureteral calculi is 88% for radiography and 100% for ultrasonography. If, despite ultrasonography, an obvious cause of obstruction can’t be documented, the renal resistive index of the kidney may be measured in order to help differentiate
obstructive from non-obstructive causes of ureteral dilation. It is calculated using the peak systolic and minimum diastolic frequency shifts. The normal intrarenal resistive index in uncedated dogs is 0.55-0.72 (0.70 being moderately accurate for diagnosis of ureteral obstruction; sensitivity and specificity of 74% and 77%, respectively). \textsuperscript{Dodd 1991, Nyland 1993} The renal resistive index has been used in human patients on occasions when specific conditions preclude the use of the usual diagnostic imaging procedures. \textsuperscript{McAleer 2004} When there are safety concerns in pregnant women, for example, the ultrasound and renal resistive index represent the first line studies for the diagnosis of urolithiasis. \textsuperscript{McAleer 2004, Bariol 2005}

\textbf{2.4.2.3 Excretory urography}

Due to the widespread availability of ultrasonography, the use of excretory urography has decreased over the past few years. Excretory urography is performed on a fasted and sedated/anesthetized patient following a cleansing enema ideally completed several hours prior to the study. An iodinated contrast agent is administered intravenously (600-880 mgI/kg). \textsuperscript{Berent 2015b} Ventrodorsal and right lateral radiographs are obtained following the administration of the contrast agent. Radiographs are repeated after 5, 20 and 40 minutes. Oblique projections may be obtained if necessary; they allow better visualization of the ureters. Excretory urography allows visualization of two phases: the nephrogram and the pyelogram. Normal findings include opacification of the renal arteries 5-7 seconds after injection of the contrast agent. Nephograms start between 10 seconds and 2 minutes after injection of contrast and are most radiopaque within 7 to 30 seconds. \textsuperscript{Biery 1978} The nephrogram decreases progressively and then the pyelogram starts. Two hours after contrast injection, approximately 25\% of normal dogs still have a positive nephrogram. \textsuperscript{Feeney 1980} Anatomically speaking, a nephrogram starts when the contrast agent travels into the glomerular vessels and continues as contrast moves into the nephrons, leading ultimately to opacification of the renal parenchyma before the contrast starts to fade. There is no specific time frame for the pyelogram phase, but it typically occurs within 5 minutes of contrast administration. Under normal conditions the ureter opacifies concurrently with the pyelogram phase. A delay in the pyelogram phase may be due to ureteral obstruction, but it can also be due to primary renal disease, so it
is not a conclusive measure. The pyelogram phase consists of the contrast agent reaching the renal tubules and eventually, the renal pelvis and ureters. In normal dogs with adequate kidney function, the renal collecting system is more radiopaque than the renal parenchyma. Also in normal dogs, the width of the renal pelvis should not exceed 2 mm and neither the renal pelvis nor the diverticula should be blunted or rounded. Due to ureteral peristalsis, ureters may not fill uniformly: their width should not exceed 2-3 mm in healthy dogs. Excretory urography and the opacification of the kidney are influenced by several factors that are related to the patients themselves (e.g., hydration status, renal perfusion) and their kidney function (e.g., GFR, tubular reabsorption).

Excretory urography is indicated when ureteral tear, ectopic ureter or ureteral obstruction are suspected. It can reveal ureteral obstruction associated with a filling defect and lack of progression of the contrast medium distally to the filling defect. Excretory urography, however, has limitations. In light of a ureteral obstruction, ureteral filling is often poor due to decreased renal function and increased interstitial pressure, making this technique less suitable for diagnosis of ureteral obstruction. Another limitation of excretory urography is its lack of continuous evaluation unlike antegrade and retrograde ureteropyelograms. Contraindications of excretory urography include acute kidney injury, dehydration, hypotension and known hypersensitivity. Nausea, vomiting, hypotension and renal failure have been seen following the use of iodinated contrast medium. Nonionic contrast medium is associated with fewer side effects due to its lower osmolality. Because of these potential side effects, excretory urography is rarely considered a first-line imaging modality.

2.4.2.4 Antegrade and retrograde ureteropyelography
The upper urinary tract can be imaged using a contrast agent injected directly into the urinary tract and followed with fluoroscopy as the agent travels through the ureters and renal pelvis. The contrast agent can be injected into the renal pelvis (antegrade ureteropyelogram) or into the distal ureter via the UVJ (retrograde ureteropyelogram).
Antegrade pyelography can be performed percutaneously using ultrasound guidance. The technique consists of inserting a 22-G IV catheter into the renal pelvis through the renal greater curvature under ultrasound guidance. Contrast agent is then injected into the renal pelvis. Pelvic and ureteral filling is observed under fluoroscopic guidance. Antegrade pyelography has been successful in dogs and with no reported complications.

Rivers 1997

Retrograde ureteropyelography consists of injecting contrast agent through the UVJ in order to fill the ureter and the renal pelvis. It can be performed under cystoscopic and fluoroscopic guidance in female dogs and cats and in male dogs through a transurethral approach. The technique consists of cannulating the ureteral papilla with a hydrophilic guidewire via urethrocystoscopy. A ureteral catheter is then advanced over the wire into the ureter under fluoroscopic guidance to the level of the ureteropelvic junction. After removal of the wire, contrast agent is injected in the catheter to obtain a pyelogram. As the catheter is pulled back, contrast agent fills the ureter. Under fluoroscopic guidance, a ureteropyelogram can be used to help determine if there is obstruction by a calculi, mass or stricture. Performing a retrograde ureteropyelogram has the advantage of being less invasive than the antegrade technique, limiting the risk of urine leakage and bleeding from the renal parenchyma. However, performing a retrograde ureteropyelogram requires special equipment and skills (i.e. cystoscopy and ureteral catheterization). Using retrograde pyelography is safer than IV urography as there is no injection of contrast agent intravenously.

2.4.2.5 Computed tomography and excretory urography (CTEU)

The use of CTEU is more sensitive than radiography as structural superimposition is not an issue. CTEU also provides more information about renal anatomy than abdominal radiographs and facilitates the visualization of all ureteral segments throughout the entire length of the ureter. Using a contrast agent can help identify a partial or complete obstruction as well as the presence of a stone or stricture. A filling defect or the lack of progression of the contrast agent distal to the filling defect confirms the diagnosis of ureteral obstruction. Administration of furosemide (4 mg/kg) prior to
scanning helps with filling of the ureters and is safe in non azotemic dogs.\textsuperscript{Secrest 2013} Drawbacks associated with CTEU include the need for deep sedation or general anesthesia as well as the use of intravenous contrast agent with the above mentioned risks.

Computed tomography is also used in human medicine and there are data from studies not yet replicated in veterinary medicine. Non-contrast helical computerized tomography (NCCT) has been shown to have sensitivity and specificity approaching 100\% for diagnosis of acute flank pain in humans.\textsuperscript{Bariol 2005} It provides information regarding the urologic and non-urologic sources of flank pain.\textsuperscript{Hamm 2001, Chen 1999} For diagnosis of upper urinary tract calculi, NCCT is considered the modality of choice in humans.\textsuperscript{Bariol 2005} It allows precise measurement of stone diameters; the transverse measurement is more precise than the cranio-caudal measurement.\textsuperscript{Katz 2003, Van Appledom 2003, Olcott 1997, Bariol 2005} Also, NCCT has the advantage of allowing the differentiation of calcium oxalate and uric acid calculi.\textsuperscript{Nakada 2000} Using the gray-scale values, differences between struvite, calcium phosphate/oxalate and calcium mono/di-hydate calculi have been described.\textsuperscript{Oehlschlager 2003} Recently demonstrated, but not widely used, CT may help predict the likelihood of stone fragmentation prior to extra-corporeal shock wave lithotripsy (ESWL).\textsuperscript{Pareek 2003} Non-contrast helical computerized tomography offers several advantages, but the use of contrast-CT and reconstruction provide further anatomical details which are of particular importance in detecting ureteral obstruction.

2.5 Indications for treatment in dogs

Ureterolith(s) removal is recommended: (1) if there is partial or complete ureteral obstruction causing progressive hydronephrosis and uremia, (2) when accompanied by severe hematuria, (3) if there is discomfort or pain associated with the presence of the stone, or (4) if there are recurrent urinary tract infections despite appropriate treatment.\textsuperscript{Berent 2015b}

Once partial or complete ureteral obstruction is confirmed, treatment is recommended. Immediate assessment and treatment of ureteral obstruction is warranted to avoid
patient deterioration and irreversible kidney damage. Wilson 1977, Coroneos 1997 Beneficial effects of renal decompression include: (1) correction/improvement of renal azotemia, (2) correction/improvement of electrolyte abnormalities, (3) prevention of further damage/loss of kidney function, and (4) limited signs of ureteral colic due to the obstruction.

2.6 Treatment options

Ureteral obstructions have always been challenging to manage in veterinary medicine. Treatment strategies include medical/supportive approaches, expulsive therapy, surgical management, and a number of procedures deemed minimally invasive: ureteral stents, SUB and ESWL, all with advantages and drawbacks.

2.6.1 Medical/supportive treatments

In cases of ureteral calculi or strictures, medical management is required for patient stabilization but generally will not result in alleviation of the obstruction.

2.6.2 Expulsive therapy

Expulsive therapy is indicated in cases of partial ureteral obstruction. It consists of aggressive diuresis and administration of antispasmodic and osmotic diuretics to induce ureteral stone movement towards the bladder. Fluids are usually administered to promote rehydration but also maintenance fluids in order to promote diuresis (Plasmalyte or lactated Ringer solution, and 0.45% saline mixed with 2.5% dextrose). Berent 2015b Some antispasmodic medications such as $\alpha$-adrenergic antagonists, calcium channel blockers, glucagon and osmotic diuretics can be administered to help stone passage by decreasing ureteral spasm. Canine and feline studies confirm the presence of $\alpha$-adrenergic receptors and $\beta$-adrenergic receptors in the ureters with a predominance of $\alpha$-1 receptors. Ettinger 2010 The $\alpha$-1 receptor antagonists inhibit basal tone and also decrease the amplitude and frequency of peristaltic movement. This helps reduce intraperitoneal pressure and allows better fluid transport within the ureters. Prazocin, an $\alpha$-adrenergic antagonist, seems to have little effect and is suspected at higher doses to enhance contractions. Wanajo 2005 Tamsulosin, a potent $\alpha$-1 adrenergic antagonist, acts as
a potent spasmolytic and is considered to be the treatment of choice for expulsion of small (<5 mm) ureteral calculi in humans, but not yet investigated in veterinary medicine. \textsuperscript{Dellabella 2003}

Calcium channel blockers could potentially help decrease ureteral spasm as they open voltage-gated potassium channels and act as a potent urinary smooth muscle relaxer. Amytryptilline has been used successfully in cats to cause urinary smooth muscle relaxation and relieve urethral obstructions, but no studies have evaluated its clinical effect in dogs. \textsuperscript{Achar 2003}

In one study of canine ureteral obstruction, glucagon administered to dogs revealed some evidence of reduced ureteral pressure and peristaltic rate but duration of effect was short and suspected to be of limited clinical significance. \textsuperscript{Stower 1986}

Osmotic diuresis increases urine flow in the ureter and is used to help stone passage. A bolus of mannitol (0.25-0.5 mg/kg over 20-30 minutes) followed by a continuous rate infusion (CRI) (1 mg/kg/min) can be administered for 24 hours as long as the cardiac function is normal. If after 24-48 hours of mannitol CRI there is no improvement observed on ultrasonography or bloodwork, CRI should be discontinued and more aggressive management should be considered. Overall, the success rate of expulsive therapy is low based on one study of cats: only 8-17% of cats had movement of ureteral calculi from the ureters into the bladder. \textsuperscript{Kyles 2005}

If expulsive therapy is not an option or fails, surgical intervention has traditionally been used in small animals to manage ureteral disease.

2.6.3 Surgical management
Possible interventions include ureterotomy, ureteral re-implantation, ureteral resection-anastomosis, ureteronephrectomy, or renal transplantation. \textsuperscript{Kyles 2005, Snyder 2005, Dupre 1990,} Surgery for ureteral diseases has been associated with high morbidity (31%: 27/88) and mortality (18%: 16/88) in cats as well as in dogs where the morbidity rate was 35%
(6/16) and the mortality rate 25% (4/16). Depending on the type of obstruction and if calculi are present, the type of surgical procedure may vary.

Ureterotomy and ureteral re-implantation are the two most commonly performed surgical procedures in dogs and cats for ureteral obstruction. If calculi are very proximal, nephrocystopexy and ureterotomy +/- nephrostomy tube may also be considered. If calculi are more distal, ureteral re-implantation may be considered. In case of mural or extraluminal obstructions, ureteral re-implantation and ureteral resection and anastomosis can be performed in an attempt to preserve renal function.

Ureteronephrectomy consists of removing the kidney and the ipsilateral ureter. Provided that the contralateral kidney has normal GFR, complications of ureteronephrectomy are the same as for celiotomy. However, 38% of dogs remain azotemic following traditional surgical intervention to relieve the ureteral obstruction, which implies that the contralateral kidney is already damaged. Therefore this procedure should be performed as a last resort, only in non-azotemic patients with a normal contralateral kidney. One study documented that, following surgery, a second ureteral obstruction occurred in 40% (14/35) of cats. Furthermore, as contralateral ureteral obstruction is frequent, this is another argument in favor of keeping both kidneys, as this may increase the life expectancy of the patient in the long term.

Complications associated with traditional surgical management of ureteral obstructions in cats and dogs have been reported. In one report, two dogs received ureterotomy for the treatment of ureterolithiasis. Both dogs underwent the surgical procedure with no complication. In a large population of dogs, Snyder et al (2005) reported the outcome of dogs with ureteral calculi following ureterotomy (+/- pyelotomy or cystotomy) and ureteronephrectomy. In this population, additional surgery was performed in 15% of dogs within 30 days due to reobstruction (ie: stricture or calculi). While 63% had elevation in urea and/or creatinine on admission, 50% of these dogs remained azotemic following the surgical procedure.
Reported surgical procedures performed in cats include ureterotomy, partial ureterectomy or ureteral re-implantation. Post-operative complication rate may be as high as 31%, and the mortality rate in the perioperative was 18% in one study, and 21% in another.\textsuperscript{Scott 2011, Kyles 2005} Uroabdomen is a common complication, occurring in 6%-16%, and 15% following ureterotomy, and ureteral re-implantation, respectively.\textsuperscript{Scott 2011, Kyles 2005} A second surgical procedure was necessary in more than 60% of these cats.\textsuperscript{Kyles 2005, Scott 2011} Recurrence of ureteral obstruction can be due to remaining calculi, re-obstruction associated with new calculi formation or migration of concurrent nephrolith, and stricture secondary to the surgical procedure. In cats undergoing ureterotomy, 2.9% had evidence of reobstruction.\textsuperscript{Kyles 2005} Among cats with ureteral re-implantation, 11% had reobstruction, and required a second procedure.\textsuperscript{Kyles 2005} Improved but persistent azotemia is noted in approximately 40% of cats following surgical intervention.\textsuperscript{Kyles 2005}

Other major complications reported in the post-operative period include pulmonary edema, post-operative cardiac arrest, pancreatitis, and pyelonephritis.\textsuperscript{Scott 2011, Kyles 2005, Snyder 2004} Those complications may not be related to the surgical procedure itself, but they highlight the severity and the intensive care associated with cats with ureteral obstructions. The high mortality rate and the potential of significant complications in the post-operative period is to be considered on a case by case basis if surgical intervention is to be considered. Despite, significant morbidity and mortality associated with surgical procedures, outcome is still considered superior to medical management alone.\textsuperscript{Kyles 2005} With the development of minimally invasive procedures, surgical procedures are now uncommonly performed in cats.

2.6.4 Minimally invasive procedures
Considering the low success rate of medical management and the high morbidity and mortality associated with surgical procedures, three minimally invasive options have been developed in the past few years for small animals. These include ureteral stenting, SUB and ESWL.
2.6.4.1 Ureteral stents and placement techniques

Placement of ureteral stents was first described in 1967 with cystoscopy guidance in humans and via percutaneous/laparotomy access in dogs in 2011. Zimskind 1967, Berent 2011c

Ureteral stenting is now performed in cats and dogs with malignant obstructions, ureterolithiasis, obstructive pyonephrosis and following ESWL and ureteral trauma or surgery. Stents have been designed for use in humans and have been adapted for use in animals. In humans, self-retaining ureteral stents are made of synthetic polymeric compounds (e.g., silicone, polyurethane, Silitek®, C-flex®, Percuflex®, Tecoflex®), all with advantages and disadvantages. The first synthetic polymeric ureteral stent was made of polyethylene. It was stiff enough to be used to manage ureteral strictures. However, it promoted protein deposition, increasing the likelihood of encrustation and infection. Mardis 1982

Silicone is non-irritating and resistant to encrustation. Silicone stents are very flexible, however, they are associated with poor mechanical strength and migrate easily. They also have a high friction coefficient and therefore are hard to manipulate in strictured or tortuous ureters. Lam 2004 Polyurethane combines polyethylene’s stiffness and silicone’s flexibility, is highly versatile, and is not an expensive biomaterial. Lam 2004 However, it is associated with epithelial ulceration/erosions and has limited durability. Cormio 1995 C-flex®, with its modified silicone, is less likely to encrust and is softer compared to polyurethane. Metallic stents and biodegradable stents have also been developed. Lingeman 2003, Kulkarni 2001

Metallic, superalloy stents have a prolonged dwell time and are commonly used for malignant obstructions and ureteral strictures. After their implantation and over time, they gradually become covered by the urothelium; this prevents encrustation and infection. However, they are associated with collagenous ingrowth, fibrosis, and ureteral narrowing that can lead to obstruction. Pauer 1999

Ureteral stents used in small animals are typically a soft, hollow, polymeric, multifenestrated catheter with a double pigtail end. Several stents are available for cats and dogs. Stents come in different diameters: 2, 2.5, 3.7, 4.7 and 6 Fr. To be adapted to a lot of different breeds of cats and dogs, each diameter is available in different lengths: the 2 Fr stent is available in 6, 10, 12, 14 and 16 cm; the 2.5 Fr stent is available in 12, 14,
16 cm, either soft or stiff; the 3.7 Fr stent is available 12, 15, and 18 cm; the 4.7 Fr stent is available in 16, 18, and 20 cm; and the 6 Fr stent is available in 17, 20 and 23 cm. In cats, the 2 and 2.5 Fr stents are typically used. The 2.5 Fr stent is available in a soft or stiff option. Although a soft stent is preferred, if the ureteral obstruction is difficult to bypass or in order to facilitate passage through the ureterovesical junction, a stiff stent may be used. In humans, the use of softer stents was associated with less urinary symptoms (ie.: pain) and those stents had less negative impact on daily activity (ie: physical activities, impact on work, etc) in one study. Park 2015

The 3.7 and 4.7 Fr stents are usually used in medium sized dogs. The 6 Fr stents are used in large breed dogs. The distal end of this stent is not multi-fenestrated, which decreases the risk of tumor ingrowth at the UVJ. Therefore this stent is used in neoplastic ureteral obstructions secondary to neoplastic infiltration. Infinity medical 2016

Stents designed for human use may also be used in dogs. Those stents are also available in different sizes and lengths, and may be multi-length, allowing the stent to be cut to size in order to accommodate the length of the ureter. Diameters available are 4.7, 6, 7, and 8 Fr and those are all available in 14, 20, 22, 24, 26, 28 cm as well as a multilength 22-32 cm. Bard /Medical 2016

When placed in the ureter, the stent allows urine passage from the kidney to the bladder, bypassing the ureteral obstruction. Berent 2015b One pigtail is located in the renal pelvis and the other in the bladder, decreasing the risk of migration proximal or distal. In small animal medicine, ureteral stents are mainly used as a long-term palliative treatment for renal or ureteral urolithiasis. They have been placed with a high success rate in dogs and cats (>94%). Berent 2011d, Berent 2015b

Stents are placed either cystoscopically or surgically in small animals. In some cases retrograde placement is not possible because of urethral or ureteral papilla obstruction (e.g., transitional cell carcinoma). In this situation, an antegrade technique can be performed. This can be done percutaneously or surgically similar to nephrostomy tube placement.
### 2.6.4.1.1 Retrograde placement

**In female dogs**

The animal is placed in dorsal recumbency with the feet/hind end hanging at the end of the table. The surrounding skin is clipped and prepared for an aseptic procedure. The patient is draped. A rigid cystoscope is advanced in order to allow visualization of the UVJ. Through the working channel of the cystoscope, an angled hydrophilic guide wire is advanced through the ureteral opening and then up to the ureter. An open-ended ureteral catheter is then advanced over the guide wire until it is at the level of mid ureter and then the wire is removed. Through the ureteral catheter, urine can be collected for further analysis (e.g., urinalysis, urine culture) if needed. A retrograde ureteropyelogram is performed by injecting a 50:50 mixture of sterile saline and contrast agent through the catheter. It allows visualization of the ureter, the renal pelvis and also any other abnormalities (e.g., ureteral lesions, obstruction, calculi, filling defects). The wire is then replaced and advanced up the ureter towards the renal pelvis until it curls and forms one loop. The catheter is then removed from the level of the ureteropelvic junction (UPJ) and then withdrawn down to the level of the UVJ under fluoroscopic guidance to measure the ureteral length from the UPJ to the UVJ. While maintaining one loop of the wire in the renal pelvis, the ureteral catheter is removed and an appropriately-sized ureteral stent is advanced over the wire. The cystoscope is then slowly removed while the pusher catheter is advanced into the bladder, pushing the distal loop of the ureteral stent into the urinary bladder.

**In male dogs**

In male dogs, a flexible ureteroscope can be used if the patient weighs more than 6 kg. However, the working channel of the ureteroscope is much smaller compared to a rigid cystoscope and therefore, a ureteral catheter cannot be introduced through it. In this situation, a stiffened, angle-tipped, hydrophilic guide wire is advanced through the working channel of the ureteroscope, passed through the UVJ, up to the renal pelvis. Once in place, the endoscope is removed. The catheter is advanced under fluoroscopic guidance rather than both endoscopic and fluoroscopic guidance. The remainder of the procedure is performed as mentioned above. In female dogs, ureteral stents are placed
in a retrograde manner under cystoscopic and fluoroscopic guidance. In male dogs, a perineal urethrotomy approach may be required to place a stent in a retrograde manner. For rigid cystoscopy using percutaneous perineal approach, the diameter of the pelvic urethra should be large enough to accommodate a 14-16 Fr peel-away sheath (+/- 5-6 mm), therefore, patients smaller than 10 kg may not be candidates for this approach. The patient is placed in dorsal recumbency with the hindlimbs pulled cranially. The anus is temporarily closed using a purse string suture to prevent contamination. The urinary bladder is catheterized and the bladder is filled with an Iohexol mixture (50% saline). The catheter is then pulled and placed into the penile urethra. Contrast is injected in order to visualize the ischial urethra using fluoroscopy. A skin incision (+/- 4-5 mm) is performed at the level of the ischium. A renal puncture needle (18 gauge) is introduced into the perineum and advanced into the pelvic urethra under fluoroscopic guidance. Once urine is identified, the stylet is removed. A 0.035” angled hydrophilic guide wire is introduced into the needle and advanced into the urinary bladder. An 18 gauge catheter is then advanced over the wire into the prostatic urethra. The hydrophilic wire is then exchanged for a 0.035” Amplatz Super Stiff™ guidewire. The catheter is removed. The tract is dilated using dilators (4 to 16 Fr) in order to accept a 14 or 16 Fr peel away sheath. Once introduced up to the level of the mid pelvic urethra, the dilator is removed and the access sheath secured to the skin with suture material. Rigid cystoscopy is then used through the sheath and urethrocystoscopy is performed. The ureteral stent is placed as mentioned above. Following successful placement, the sheath is removed. A catheter is placed in the urinary bladder for 12 hours or less, if deemed necessary. The perineal incision does not need to be closed. An adhesive bandage dressing can be placed over the incision.

2.6.4.1.2 Antegrade placement
This procedure can be done percutaneously or be surgically assisted. In cats, surgery is typically performed. Using a renal access needle or an over-the-needle intravenous catheter, the renal pelvis is accessed. Urine is collected for analysis. An antegrade pyelogram is performed. Through the needle, a wire is advanced and passed down the renal pelvis, the ureter (negotiating around the ureteral obstruction), and finally down
into the urinary bladder in order to obtain a through-and-through access through a small cystotomy. Rarely necessary in dogs, more commonly performed in cats, a dilator is passed over the wire from kidney to bladder. The ureteral length is measured using a ruler if the ureteral stent is placed surgically. Otherwise, the ureteral catheter is used if the stent is placed percutaneously. Once the appropriately-sized ureteral stent is chosen, it is passed over the wire (up to the back of the previously placed ureteral dilator if present). The stent is passed down the ureter to the urinary bladder until the distal end of the ureteral stent curls into the urinary bladder after it has passed the UVJ. Once the distal end of the stent is placed into the urinary bladder and the stent is through-and-through and with the proximal end no longer located in the renal parenchyma (but in the renal pelvis), the guide wire (and ureteral dilator if present) are removed.

2.6.4.1.3 Complications associated with ureteral stents
The complication rate associated with ureteral stenting in dogs is low. Possible perioperative complications are associated with urine leakage and subsequent ureteral perforation, but has been observed, each in less than 1% of dogs. Berent 2015b The post-operative mortality rate in dogs was less than 2%. Pavia 2014 Shortly after the procedure, occasionally dogs may have dysuria (<3%) which is usually glucocorticoid-responsive, hematuria (<5%), or persistent stent occlusion (<2%). Long-term complications can be seen but are usually minor and in most cases, treatable with minor procedures or medical treatment. Tissue proliferation at the UVJ has been reported in 5 to 25% of cases. Pavia 2014, Kuntz 2015 Stent encrustation and migration, stent occlusion, hematuria, and urinary tract infection after ureteral stent placement were observed in 2%, 5%, 10%, 6% and 10-60% or less, respectively, in dogs. Kuntz 2015, Pavia 2014 If recurrent infection is observed or if stent obstruction is suspected, removal or replacement of the stent could be necessary. Following ureteral stent placement, urinalysis and urine culture every 3-6 months for the next 1-2 years are recommended. Berent 2015b

2.6.4.2 Sub-cutaneous ureteral bypass (SUB)
Subcutaneous ureteral bypass consists of palliative option to bypass urine from the kidney to the urinary bladder. A locking-loop nephrostomy tube combined with a
cystotomy tube, are connected together with a shunting port implanted sub-cutaneously. The subcutaneous port allows for urine sampling and flushing of the system. SUB is indicated in cats with ureteral obstruction at any level. It is also indicated in dogs with ureteral obstruction or stricture/trauma that can’t be repaired or stented. For example, in one dog with malignant ureteral obstruction due to extensive transitional cell carcinoma, in which a ureteral stent could not be placed, total cystectomy was performed in association with bilateral partial ureterectomies and urethrectomy and the placement of a SUB device was reported.\textsuperscript{Weisse 2014} A similar procedure in a cat with transitional cell carcinoma has recently been described.\textsuperscript{Takayuki 2016}

Currently, SUB is the first choice for feline ureteral obstruction because of potentially fewer short- and long-term complications. In dogs, ureteral stents can be placed endoscopically and are better tolerated than in cats. Ureteral stents may be associated with lower morbidity and mortality than SUBs in dogs, so stents are the preferred method in dogs.\textsuperscript{Berent 2015b} However, if stent placement fails (e.g., with a stricture), an SUB may be a valuable treatment option for a dog.\textsuperscript{Berent 2015b}

Flushing the SUB device every 3 months and performing urinalysis and urine culture every 3-6 months for the first 1-2 years, and every 6 months thereafter if there are no complications, is recommended. More frequent flushing may be considered in patients who are stone formers.

The use of SUB devices has increased in popularity since their development due to the low rate of long-term complications. In cats, peri-operative or postoperative complications include dysuria (2-8.2%), urine leakage (3.4-5%), kinking of the device (5%), occlusion due to blood clot (2-18%), and urinary tract infections (15%).\textsuperscript{Steinhaus 2015, Berent 2011b, Berent 2015b} Catheter mineralization may also be noted in the long term (median 364 days) with some cases (13%) requiring exchange of the device due to obstruction. Similar data are not available for dogs.
2.6.4.3 Extra-corporeal shock wave lithotripsy (ESWL)
Extra-corporeal shock wave lithotripsy consists of fragmenting the uroliths (nephroliths and ureteroliths) using external shock waves transmitted through a water medium and the soft tissues of the body wall. The goal of this treatment is to achieve sufficient fragmentation of the urolith(s) such that they are small enough to pass down the ureter and eventually be voided spontaneously. Treatment of small animals is performed under fluoroscopic guidance and requires general anesthesia to avoid damage to the surrounding tissues.

Both nephroliths and ureteroliths are successfully fragmented in dogs using this technique. One study reports success rates of 80% and 85% with calcium oxalate nephroliths and ureteroliths, respectively. However, dogs may require retreatment in about 30% and 50% of cases for nephroliths and ureteroliths, respectively. Other authors suggest that approximately 15-30% of dogs require more than one treatment for complete fragmentation. For canine ureteroliths <5 mm, ESWL can be performed safely. For larger calculi, a ureteral stent should be placed prior to performing ESWL in order to facilitate the passage of stone debris.

The most commonly observed complication associated with ESWL is further ureteral obstruction that occurs in 10% of dogs treated for nephroliths or ureteroliths. If the stone has obstructed the ureter for more than 24-48 hours, a ureteral stent should be placed in order to avoid kidney damage and loss of kidney function over time. Other complications are asymptomatic elevation of lipase and amylase, and canine pancreatic lipase following ESWL treatment of the right kidney. Approximately 2% of dogs develop clinical pancreatitis. Even less common (<0.5%) complications are perirenal hematoma and shock-wave induced arrhythmias. Feline uroliths seem to be more resistant to fragmentation by ESWL than canine uroliths. In one study, ESWL was performed on five cats with ureteroliths and nephroliths: only one cat had successful fragmentation of ureteroliths,
and nephrolith fragmentation had little success. Use of ESWL in cats is associated with major complications including worsening uremia, hemorrhage, and life-threatening hematuria.

2.6.4.4 Ureteroscopy

Ureteroscopic procedures have been described in large breed dogs only as part of the treatment of renal hematuria. The median body weight of dogs included in the study was 42.3 kg and ureteroscopy was performed successfully in one dog. To the best of our knowledge, this is the only report of the use of flexible ureteroscopy in dogs. Ureteroscopy has never been described in smaller dogs. Inserting the ureteroscope in smaller dogs may require active dilation of the UVJ (see section ureteroscopy in humans below). If made feasible in dogs of smaller size, the use of ureteroscopy could potentially change our approach of ureteral obstruction by allowing the clinician to treat the primary cause such as removing a stone for example instead of bypassing it with a ureteral stent or a SUB.

3.0 Minimally invasive treatment for upper urinary tract obstructions in humans

Urolithiasis is one of the most common reasons for presentation of human patients to urology practices. The incidence of urolithiasis has increased over the past few years. The prevalence is 1-13% depending on geographic region, with a higher prevalence in the United States and a lower prevalence in Asia. Stone composition and localization vary among countries. Current treatment modalities for ureteral calculi are observation with supportive care (analgesia) with or without medical expulsion therapy, ESWL, ureteroscopy, percutaneous nephrolithotomy, and open surgery. This literature review will focus only on ureteral stenting and ureteroscopy as they relate to this project.
3.1. Ureteral stenting

3.1.1 Ureteral stent utilization and indications

Ureteral stents are used in humans before and after diagnostic and therapeutic urologic procedures. They are used to relieve obstruction from a stone, tumor or a stricture, or to prevent intrinsic or extrinsic renal and ureteral obstructions after ESWL, percutaneous nephrolithotomy, ureteroscopy, endopyelotomy, open/laparoscopic ureteral surgery, ureteral injury and renal transplantation. However, ureteral stents are associated with urinary tract infection, encrustation, and discomfort that decrease the patient’s quality of life. For these reasons, ureteral stents are not placed routinely in people.

As opposed to dogs and cats in which ureteral stents are used as a long-term palliative option for the treatment of ureteral obstructions, ureteral stents are left in place for a maximum of 3 months in human patients. If the patient still needs a ureteral stent after this period, then a replacement recommendation is made to decrease the risk of encrustation and subsequent infection. A safe and optimal duration of ureteral stenting hasn’t been determined in dogs and cats.

3.1.2 Ureteral stenting complications

Ureteral stenting can be associated with early and late complications in humans. Early complications such as increased frequency and urgency of urination, dysuria, flank pain, suprapubic pain and hematuria are noted in up to 85-90% of patients. Proximal or distal stent migration occurs in 1-4% of patients and may cause urinary incontinence if the stent migrates in the urethra. Poor patient compliance may lead to a retained ureteral stent and significant late complications. Stents remaining indwelling for a prolonged period of time may be associated with severe encrustation, large stone formation, recurrent urinary tract infection and hematuria. Infections associated with chronic ureteral stenting vary from asymptomatic bacteriuria to symptomatic urinary tract infections. Symptomatic infections are 2.3 times more likely than asymptomatic bacteriuria.
Risk factors for urinary tract infections include female gender, being an immune-compromised patient, and having more than one month of dwell time. Colonization of the ureteral stent, biofilm formation, and deposition of calcium-based deposits may induce stent encrustation. The longer the ureteral stent is left in place, the more encrustation forms. Encrustation happens in 9-27% of cases six weeks post-implantation and in 76% of cases after twelve weeks. Significant encrustation and secondary stone formation may lead to stent obstruction and difficult removal. Retained stents producing hydronephrosis have been reported by several groups. Stent fracture is a rare complication with a prevalence of 0.3% to 10%. Patients with stent fragmentation usually present with hemorrhage, pain or signs compatible with sepsis. Rapid disintegration associated with urinary tract infections and spontaneous excretion of fragments have also been reported.

3.1.3 Pre-stenting
There are still controversies regarding the benefits of pre-stenting patients to avoid future complications. Placement of ureteral stents is commonly used in children to facilitate future ureteroscopy. The presence of a ureteral stent for several weeks has been shown to induce passive ureteral dilation that facilitates introduction of the endoscope in the ureteral papilla in children.

Ureteral stenting is also used in humans to avoid ureteral obstruction, stricture or ureteral colic after ureteroscopy or ESWL, especially if ureteral trauma, residual stone fragments, or ureteral perforation is suspected. However, several randomized, prospective studies reveal no significant difference in post-operative complications between stented and non-stented patients and therefore, ureteral stents are not routinely placed prophylactically at this time. Several studies also document significant cost-savings if ureteral stents are not placed after uncomplicated ureteroscopies. Similarly, routine ureteral stenting is not recommended in uncomplicated ESWL for renal and ureteral calculi. Routine placement of a ureteral stent to promote stone passage is no
longer recommended as it does not decrease the rate of complications such as steinstrasse (i.e. accumulation of calculi in the ureter), hematuria, fever, infection, pain, nausea, vomiting, and the need for analgesia or auxiliary treatments. Moreover, the presence of the ureteral stent is associated with lower urinary tract clinical signs that impair quality of life. The use of ureteral stents in kidney transplant patients can reduce ureteral complications (e.g., stenosis or kinking of the kidney transplant ispsilateral ureter, necrosis), macrohematuria and duration of hospitalization.

Musa 2008

3.2 Ureteroscopy

3.2.1 Ureteroscopy techniques (children and adults)

In children, in order to avoid patient movement, ureteroscopy is almost always performed under general anesthesia. Antibiotics are administered pre-operatively. Starting with cystoscopy using an appropriately-sized cystoscope (7.5-18 Fr), a guidewire is placed in the distal ureter. An open-ended ureteral catheter is advanced over the wire and a retrograde pyelogram is performed allowing visualization of the anatomy and any filling defect. The ureteral catheter is removed and the guidewire advanced in the renal pelvis under fluoroscopic guidance. The rigid cystoscope is replaced by a semirigid (4.5 Fr or 7.5 Fr) or a flexible (6.9 Fr) ureteroscope. The ureteroscope is advanced over the wire. If the ureteral orifice cannot accommodate the ureteroscope, active dilation is performed using an 8/10 Fr coaxial ureteral dilator. Passive ureteral dilation by ureteral stent placement can facilitate ureteroscopy. However, this requires that ureteral stenting is performed first and followed by removal of the stent and ureteroscopy. Several studies describe the use of ureteral stents preoperatively: they are used in a little less than half of the reported pediatric cases (range: 0-100%).


In adults, passive ureteral dilation may be performed using a ureteral stent, but this is rarely necessary. It may facilitate ureteroscopy and stone management as well as reduce complications and improve the stone-free rate. Ureteral access sheaths that facilitate access may be used to decrease potential ureteral damage, decrease operative time and maintain low renal pelvis pressures.

Vanlangendonck 2004
Furthermore, access sheaths minimize difficulties associated with ureteroscopic procedures by improving irrigant flow and therefore facilitate the clinician's endoscopic visualization while minimizing the elevation of intrarenal pressure. They may be used in specific circumstances (e.g., difficult access, presence of ureteral calculi, complicated anatomy, use of basket for stone removal). However, if too large for the ureteral size, the sheath may induce ureteral ischemia and ureteral stricture formation.

Once the stone is visualized, basket extraction may be attempted. If the stone is too large, lithotripsy can be used to fragment it. Ultrasonic or electrohydraulic lithotripters may be valuable options, but Ho-YAG lithotripsy is the safest and most efficient at fragmenting calculi. Post-operative ureteral stents are used in more than two-thirds of patients (range: 31-100%).

Usually, the placement of a ureteral stent is recommended post-operatively if there is significant ureteral edema/injury, stone impaction, stone infection, or a solitary kidney involved. The ideal duration of stenting hasn’t been determined but most urologists recommend 1-2 weeks.

### 3.2.2 Ureteroscopy in adults

#### 3.2.2.1 Indications and success rate

With rapid technological advances, the use of ureteroscopy, with or without lithotripsy or basket retrieval for treatment of urolithiasis has increased over the past 30 years. Ureteroscopy is indicated for the treatment of renal and ureteral calculi.

For treatment of ureteral calculi, ureteroscopy is considered a valuable option for calculi of up to 10 mm in diameter. A large prospective study of 11,885 patients reveals that ureteroscopy is successful in 81-97% cases for the treatment of ureteral calculi, depending on their size and location: the highest calculi-free rate is for distal ureter calculi smaller than 10 mm. Another prospective study demonstrates that the calculi-free rate following ureteroscopy is 84.5%, 89.4% and 94.2% for calculi located in the proximal, mid and distal ureter, respectively. Regardless of calculi
location, a significantly lower calculi-free rate was noted for calculi larger than 10 mm. The need for repeat treatment is higher in patients with calculi in multiple locations. Castro 2014

Aside from risk related to general anesthesia, there is no specific contra-indication for ureteroscopy. Turna et al. (2008) have reported a mean procedure time of 50 minutes and a stone free rate of 96% for a mean kidney stone size of 10 mm (11.1 +/- 6.6 mm). Hussain et al. (2011) have reported stone-free rates of 96.5% for calculi less than 20 mm and 58.3% for calculi more than 20 mm. Another study reports a mean operative time of 82.5 minutes and a stone-free rate of 93.7% for a mean stone size of 25 mm. Hussain 2011 The average procedure number was 1.6 procedures per patient. Aboumarzouk 2012 Ureteroscopy is considered the second-line treatment, after percutaneous nephrolithotomy, for large nephroliths (>20 mm). Turk 2016

3.2.2.2 Complications of ureteroscopy

The most common complications associated with ureteroscopy are sepsis (2-7.8%), ureteral stricture (1-2%), ureteral injury (3-6%), urinary tract infections (2-4%), bleeding (<2%), and re-admission for various reasons (13.8%; e.g., ureteral obstruction, sepsis, discomfort associated with a ureteral stent(s), repeat treatment). Breda 2008, Bach 2011, Traxer 2013, Hyams 2010, Geavlete 2014, Preminger 2007, Castro 2014, Turk 2016 Complications vary depending on stone location with the highest complication rate in patients with calculi in multiple locations. Castro 2014

3.2.3 Ureteroscopy in children

3.2.3.1 Ureteral dilation

As it is in veterinary patients, the small ureteral diameter in children offers a significant challenge to urologists. With smaller endoscopes, ureteroscopy is now increasingly available but the access to the ureteral opening remains a challenge compared to adults. Therefore, in children, and whenever deemed necessary in adults, the ureteral opening may be dilated with serial rigid dilation or balloon dilation. Thomas 1993, Schuster 2002, Al Busaidy 1997, Jaya nthi 1999, Bassiri 2006 However, the use of active dilation has been associated with some complications, including vesicoureteral reflux, and ureterovesical stricture. Al Busaidy 1997, Thomas 1993, Schuster 2002
Therefore, other options have been investigated. Hubert et al. (2005) demonstrated that following placement of a ureteral stent, passive ureteral dilation is induced and ureteroscopy, using a semi-rigid or flexible ureteroscope, can be safely and successfully performed in children following removal of the ureteral stent. No complications were reported with the procedure and none of the patients required active dilation.

3.2.3.2 Indication and success rate
With the advances in optics and equipment as well as miniaturization of equipment, ureteroscopy of the upper urinary tract has become an increasingly popular approach in children. Several studies have documented the safety and efficacy of this technique and it is now being used as the first-line approach in many centres. Based on several studies, stone-free rates after ureteroscopy of renal and ureteral calculi in children vary between 84-100%. It is particularly helpful for cystine and calcium oxalate monohydrate calculi as they are particularly resistant to ESWL. The stone-free rate after ureteroscopy in children is similar to that seen in adults and varies between 77.8-100% for ureteral calculi and 58.0-93% for renal calculi. The stone-free rate after ureteroscopy is affected by stone location and stone size but not patient age or gender.

3.2.3.3 Complications of ureteroscopy
The complication rate in children is low (10.5%). Most common complications are similar to those in adults which include ureteral perforation (up to 14.3%), hematuria (up to 17%), urinary tract infection (up to 13%) and, less commonly, renal colic, pyelonephritis, fever, vomiting/nausea, urinoma, ureteral stricture and mucosal damage. The complication rate (24% vs. 7.1%) and treatment failure rate (4.4% vs. 1.7%) are higher in patients <6 years old. However, the stone-free rate of the younger children is
higher (91.7% vs. 85.8%). Spontaneous passage of calculi may be easier in younger patients.\cite{Ishii2015}

Ureteroscopy is contra-indicated in cases of staghorn calculi, aberrant anatomy complicating retrograde access, previous bladder neck reconstruction or closure, previous cross-trigonal re-implantation, and previous endoscopic failure.\cite{Gupta2014}

4.0 Urinary tract infections in dogs
4.1 Definitions and incidence
A urinary tract infection (UTI) is defined as a temporary or permanent defect in a host’s defense mechanisms that eventually allows microbes to invade, multiply and persist within the urine or the urinary tract.\cite{Greene2012} Between 5-27\% of dogs will experience one urinary tract infection in their lifetime.\cite{Ling1984, Bush1976, Kivisto1977} In small animals, most urinary tract infections are caused by bacteria, but fungi, viruses, mycoplasma, and parasites may also be involved. There are multiple infection routes. Ascending infection is the most common type of infection route and is usually caused by gastrointestinal tract or skin bacteria.\cite{Smee2013, Greene2012} Pyelonephritis may also be associated with bacterial cystitis. Other routes of infection include hematogenous spread as well as direct extension from surrounding tissues.\cite{Smee2013} Multiple factors are necessary for the development of a UTI including disruption of the local environment (physiology, anatomy and immunocompetence) and the number and virulence of the pathogen.

4.2 Factors involved in the development of urinary tract infections
Several physiological and anatomical host factors are involved in the prevention of a UTI.

The resident microflora of the vagina, vestibule, prepuce, and distal urethra are of clinical importance as they compete for nutrients with pathogenic bacteria and by interfering with their adhesion.\cite{Greene2012} As an example, lactobacilli which have been isolated from the vagina of dogs have been shown to have antimicrobial activities
against three common uropathogens: *Escherichia coli*, *Proteus mirabilis*, and *Staphylococcus aureus*.

Normal physiological mechanisms play an important role in UTI prevention. Aspects of the urethra, ureters and prostate are also significant factors in the prevention of infections. The urethral sphincter, with its high tone, acts as a physical barrier to bacteria. Peristaltism that occurs in the ureters prevents migration of the bacteria that may otherwise progress beyond the bladder to access the ureters and kidneys. In male dogs, prostatic secretions that contain zinc and a bacteriostatic constituent help prevent colonization. For these reasons, but also due to their longer urethra, male dogs are less at risk of developing UTIs than female dogs. Female dogs are also more commonly infected with more than one bacterium. The bladder epithelium has a glycosaminoglycan coating that reduces the ability of bacteria to adhere to its wall. Incomplete voiding or decreased frequency of urination increase the duration of contact between bacteria and bladder, potentially increasing the risk of bacterial adhesion and infection.

Congenital and acquired anatomic abnormalities may impair the host’s ability to eliminate contaminants. A recessed vulva may promote dermatitis and an increased bacterial count which increases the risk of bacterial ascension to the urinary tract.

4.3 Uropathogens

Despite the above mechanisms of defense, some dogs develop UTIs. More than 70-90% of UTIs are caused by a single organism. The most common pathogen identified is *Escherichia coli*, accounting for a third to half of the positive urine cultures. Other commonly cultured pathogens are gram positive bacteria (e.g., *Staphylococcus* spp., *Streptococcus* spp. and *Enterococcus* spp.). Pathogens occasionally identified include *Proteus* spp., *Klebsiella* spp., *Pasteurella* spp., *Mycoplasma* spp., *Enterobacter* spp., and *Pseudomonas* spp. All
together, these pathogens represent 95% and 97% of the UTIs in male and female dogs, respectively.\textsuperscript{Ling 2001}

4.4 Clinical signs and diagnostic tests

Clinical signs associated with the development of a UTI include pollakiuria, dysuria or stranguria, and microscopic or macroscopic hematuria. Diagnosis is based on the history, clinical signs and the results of urinalysis, urine cytology and culture. The urinalysis and microscopic sediment analysis changes compatible with a UTI include hematuria and proteinuria on the urine dipstick, and bacteriuria, pyuria, and hematuria in the sediment.\textsuperscript{Greene 2012} However, while bacteriuria is considered the most specific finding for diagnosis of UTI, high concentrations of bacteria may be noted despite absence of clinical signs. Furthermore, infection may occur without hematuria, and therefore hematuria is a poor indicator of UTI.\textsuperscript{Greene 2012} This highlights the complexity associated with diagnosis of UTI. Differentiating contamination of a urine sample as opposed to bacterial infection is still controversial. Table 1 describes criteria that may be used for differentiation of bacterial infection as opposed to contamination of the urine sample.\textsuperscript{Greene 2012} However, there is lack of objective studies to support those data. Urine sediment interpretation may be complicated if the urine is dilute (i.e. low urine specific gravity can be associated with false negative results) or if the patient is immune-compromised or unable to mount an inflammatory response (e.g., because of exogenous or endogenous corticosteroids).

<table>
<thead>
<tr>
<th>Collection method</th>
<th>Contaminant Level (bacteria/ml)</th>
<th>Infection Level (bacteria/ml)</th>
<th>Pyuria Level (leukocytes/HPF\textsuperscript{*})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystocentesis</td>
<td>&lt;1000</td>
<td>$&gt;10^{3}$</td>
<td>&gt;3</td>
</tr>
<tr>
<td>Catheterization</td>
<td>Cats and male dogs: $&lt;10^{3}$</td>
<td>Cats: $&gt;10^{3}$</td>
<td>&gt;8</td>
</tr>
<tr>
<td></td>
<td>Female dogs: any</td>
<td>Male dogs: $&gt;10^{4}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female dogs: not recommended</td>
<td></td>
</tr>
</tbody>
</table>
Another important factor to consider when evaluating urinalysis results is to differentiate between infection and inflammation (**Table 1). They can be difficult to differentiate between the two as they may be associated with similar clinical signs: hematuria, pollakiuria and dysuria. The presence of pyuria does not necessarily mean infection as pyuria may also be seen with sterile inflammation. Other causes of sterile inflammation may include sterile uroliths, urethral obstruction, malignant neoplasia, polypoid cystitis, idiopathic cystitis, and irritation from metabolites (e.g., hemorrhagic cystitis associated with administration of cyclophosphamide). Therefore, the presence of white blood cells in the urine should not be interpreted as infection but rather as inflammation, and a urine culture should be performed in order to confirm the presence of bacteria.

Definitive diagnosis of UTI is obtained from a positive urine culture from a properly handled and collected urine sample in the presence of clinical evidence of lower urinary tract disease that does not have another apparent cause. Cystocentesis is the ideal method of collection as it avoids contamination from the lower urinary tract provided that there is no contamination through accidental bowel penetration or from the skin. Over the site of puncture, skin should be clipped and cleaned. Possible complications from this technique include urine leakage and hemorrhage from inadvertent puncture of a blood vessel. Buckley 2009 If cystocentesis is not successful or if the bladder can’t be palpated, ultrasound guidance can facilitate urine collection by cystocentesis. In female dogs and unsedated cats, if cystocentesis is not possible, subcutaneous fluids or diuretic administration may be used to fill the bladder. Greene 2012 An alternative is urinary catheterization. In male dogs, the prepuce should be cleaned and dried. A sterile urinary catheter is introduced aseptically using sterile gloves. This technique is not recommended for female dogs as large numbers of bacteria ($10^5$/ml) may be introduced
despite aseptic technique\textsuperscript{Comer 1981} and catheterization may be challenging. A midstream, voided urine sample is an easy method of collection and not invasive. However, it should not be used for urine culture as large numbers of bacteria (sometimes $>10^5$/ml) may be introduced.\textsuperscript{Comer 1981, Weese 2011}

Collecting a urine culture helps to identify the underlying pathogen involved in the UTI. Bacterial antibiotic susceptibility panels help in determining which antibiotics are able to inhibit the bacteria \textit{in vitro}. Most UTIs involve a single agent and high counts ($>100,000$ CFU/ml).\textsuperscript{Ling 1995, Greene 2012} If more than one pathogen is identified, or if the count is low ($<1000$/ml), artefactual contamination should be considered.\textsuperscript{Greene 2012} For the best results, a fresh urine sample should be submitted to the laboratory as soon as possible. The sample should be refrigerated and cultured within 6 hours.\textsuperscript{Padilla 1981}

4.5 Bacteriuria without evidence of clinical signs
It is important to differentiate the presence of bacteria in the urine from the presence of clinical infection. Bacteriuria in absence of clinical signs, occasionally referred as asymptomatic bacteriuria in the litterature, refers to the presence of bacteria in the urine when there are neither clinical signs nor urine cytologic abnormalities. It can be an incidental finding. It can be associated with hospital-acquired infections in patients that are critically ill, the presence of foreign material (e.g., indwelling catheters), or with diseases that suppress the inflammatory response (e.g., hyperadrenocorticism). \textit{Escherichia coli} are the most common bacteria identified in bacteriuria without clinical signs.\textsuperscript{Litster 2009} Antibiotic treatment of patients free of clinical signs in which bacteriuria is identified is still controversial. In humans, treatment of bacteriuria when not associated with clinical signs doesn’t decrease the frequency of symptomatic infections or the prevalence of bacteria in the urine, and has been associated with increased risk of subsequent UTI, bacterial resistance and bacteriuria.\textsuperscript{Cai 2012 and 2015} It is also suspected to contribute to antibiotic resistance.\textsuperscript{Greene 2012, Gibson 2008}

4.6 Catheter-associated UTIs in humans and dogs
Catheter-associated UTIs (CAUTI) are considered iatrogenic. Development of CAUTI is
a important cause of morbidity in humans. Yearly, healthcare-associated UTIs cause
13,000 human deaths in the United States of America: a majority of these infections are
associated with the presence of a urinary catheter. Furthermore, 1/27 hospital
patients with CAUTI develop bacteriemia. Bloodstream infections due to UTIs
are associated with a mortality rate of 12.7% in humans. Catheter-associated UTIs are also associated with significant morbidity in dogs even
though some patients remain free of clinical signs. A single catheterization can cause a UTI in a female dog, however, the risk is
greater if the catheter remains indwelling. In one report, Escherichia coli was the most frequently identified bacteria causing catheter-
associated UTIs: the infections were suspected to be secondary to fecal contamination
of the urinary catheters. In CAUTIs, bacteria ascend through and around the
catheter within 24-48 hours of placement. In general, once bacteria gain access to the
urinary tract, they multiply rapidly and faster than in healthy, non-catheterized
patients. The risk of developing a CAUTI increases after three days of
catheterization and by 27% for each day that the catheter remains indwelling in
dogs. The risk also increases by 20% per year of dog’s age. Prophylactic antimicrobial treatment while the patient is catheterized
increases the risk of developing a urinary tract infection in dogs by 454%.

4.7 Ureteral stent-associated UTIs in humans and dogs
Ureteral stent-associated bacteriuria, with or without development of UTIs, is reported in
several human studies. Although it is an area of active investigation, clinical data are not as detailed as
for CAUTIs. The most common organisms isolated from stents or urine in people are
Escherichia coli, Pseudomonas spp, Enterococcus spp, Enterobacter spp,
Staphylococcus spp, and Candida spp. Following ureteral stenting, colonization of the ureteral stent occurs. The longer the stent remains
indwelling, the higher the rate of colonization and the greater the chance of developing
bacteriuria. Bacteriuria occurs as quickly as 24-48 hours following ureteral stent placement.
Diagnosis of stent colonization requires stent analysis and culture as usually there is a significant discrepancy between urine and stent culture results. Positive urine cultures are associated with stent colonization in only 27-69% of cases. In humans, the colonization rate may be as high as 71.4% six weeks after ureteral stenting. Some reports reveal a higher colonization rate in women, but another report states similar rates for gender. Despite colonization of the ureteral stents (42.6%) and presence of bacteriuria, the rate of clinical UTI is low (4.6%). According to Kehinde (2002), the presence of systemic diseases (e.g., diabetes mellitus, chronic renal failure, and diabetic nephropathy) is another risk factor for the development of bacteriuria and stent colonization. To our knowledge there are no studies evaluating stent-associated UTIs in veterinary medicine.

5.0 **Bacterial biofilm**

5.1 Bacterial biofilm formation

Biofilm is composed of a community of microorganisms encapsulated within a self-produced matrix that forms on an inert or living surface. Currently, it is estimated that over 65% of hospital-acquired infections in people and 80% of all microbial infections are biofilm-related. Bacterial colonization and biofilm formation on ureteral stents is regularly observed in human medicine.

Bacteria within a biofilm have different characteristics compared to those of planktonic bacteria. In biofilms, microorganisms are embedded into a self-produced matrix of extracellular polymeric substances (EPS). This matrix represents about 90% of the biomass and bacteria within EPS exhibits difference in phenotype (e.g. growth rate) and gene transcription compared to planktonic bacteria. This can be associated with differences in expressed surface molecules, virulence factors, and metabolic states. This transition allows bacteria to acquire properties to survive even in unfavorable conditions, and enhance resistance to the immune response and antimicrobial pressure.
Biofilm formation is divided into five steps. First, planktonic bacteria attach reversibly to surfaces. The initial attachment is influenced by the repelling force of the microenvironment of the site (e.g., nutrient levels, pH, and temperature) and inherent adhesion properties of the bacteria. This step is followed by an irreversible attachment phase that appears to be species specific. For example, for *E. coli*, this step is mediated by type 1 pili, curli fibres, and antigen 43, while an ATP-binding cassette that is encoded by the *lap* genes is required for adhesion of *P. fluroescens*. For *P. aeruginosa*, BfiSR, a two-component regulatory system, and the SadB protein are required to irreversibly attach to a surface. The irreversible attachment step is followed by the synthesis of a layer of biomolecules and the secretion of EPS; they become part of the external matrix. The production of polysaccharides by biofilm-producing strains facilitate aggregation, adherence and surface tolerance, all of which allows better surface colonization. *E. coli*’s matrix is formed of cellulose, polyglucosamine, and colonic acid. Other constituents may be found in the matrix including nucleic acids, proteins, surfactants, lipids, glycolipids, membrane vesicles, and ions. The fourth step in biofilm formation is called maturation. It consists of the development of the three-dimensional structure. Depending on the species, the biofilm structural unit is composed of 10-25% of cells with the rest represented by the exopolysaccharide matrix (EPS, adhesins, amyloid-forming proteins). Nutrients, water, and waste products can be found within the biofilm structure and they play a crucial role in individual cell metabolism. The last step in biofilm formation is dispersion. It occurs when the biofilm is fully mature. Dispersion allows individual cells to gain back their planktonic state, potentially resulting in disseminated active infection or formation of biofilm elsewhere in the host. It is suspected that this mechanism of detachment may be initiated by the cell itself, either by active (biofilm matrix enzymatic degradation) and passive processes (external forces), or by environment sensing (nutrient and oxygen depletion). An organized biofilm is, therefore, constructed of three sections: (1) a linking film at the surface of the inert or living surface, (2) a base film containing a high concentration of microorganisms and (3) a surface film containing the planktonic bacteria that spread on the surface and may be released in the environment.
5.2 Bacterial biofilm in the urinary tract

Biofilm may be found on the urothelium. It may be noted in the kidney and induce pyelonephritis, invade the prostate and cause recurrent bacterial prostatitis, or invade the bladder and induce an UTI. Nickel 1985, Soto 2007, Kravchick 2004. Biofilm formation on catheters and stents offers significant challenges to urologists. In human patients, adherent pathogens were present on 90% of ureteral stents (indwelling time: 5-128 days) inserted after ESWL; interestingly, urine cultures were positive only in 27% of these patients. Reid 1992

Biofilm development on a ureteral stent or a catheter involves the formation of a conditioning film composed of urinary components. Tamm-Horsfall glycoproteins, ions, polysaccharides and other urinary components accumulate at the surface of the implant within minutes after stent placement and this favors biofilm formation. Tenke 2006. These components provide receptors for bacterial adhesins that facilitate the attachment process of planktonic bacteria. This conditioning film is of primary importance for many microorganisms that may not have the capacity to adhere directly to the implant surface. Following development of the conditioning film, bacteria attach. By releasing protons and other signaling proteins, planktonic bacteria have the ability to "sense" the environment and attach to the indwelling device and eventually biofilm forms (as described above).

In humans, the strains most commonly isolated from urinary catheters are Enterococcus faecalis, Pseudomonas aeruginosa, and Escherichia coli. Hola 2010. Proteus mirabilis, E. faecalis, Candida tropicalis, and Staphylococcus aureus have the strongest biofilm-producing ability but are found less commonly. Hola 2010. Biofilm can form on the inner and outer surfaces of urinary catheters and stents. Tenke 2004, Tenke 2012. The development of mature biofilm on ureteral stents may be associated with some complications especially when urease-producing bacteria are involved. Proteus mirabilis and Staphylococcus spp. have the ability to hydrolyze urea into ammonium ions, resulting in a raised urine pH. This promotes formation of hydroxyapatite and struvite crystals while biofilm continues to form and therefore promotes bacterial persistence. Progression of this
process allows for subsequent encrustation of the ureteral stent which decreases the efficacy of antimicrobial coatings aiming to prevent bacterial attachment on the ureteral stent and eventual blockage of the stent lumen.  

Biofilm-associated bacteria display specific characteristics not seen as frequently in planktonic bacteria: resistance to voiding during urination, resistance to eradication by the immune system, and resistance to antimicrobials.  

The latter is due to several mechanisms. First, antimicrobials are unable to fully penetrate the biofilm matrix. Second, microorganisms present in the biofilm grow slowly which makes them more resistant to antibiotics; antibiotics require active growth to be efficient. Further, microorganisms in biofilms poorly express antimicrobial binding proteins and they activate genes associated with alteration of the cell envelope, molecular targets and antimicrobial susceptibility.

Prevention of biofilm formation and bacterial colonization on ureteral stents has been studied in vivo and in vitro.  Some studies in humans have shown promising results: fluoroquinolone administration prior to stent placement may prevent biofilm formation. However, pre-formed biofilm could not be eradicated by levofloxacin and ulifloxacin.  Other methods of preventing biofilm formation have been tried with various degrees of success including modification of the biomaterial surface, use of antimicrobial impregnated catheters, and the use of various catheter coatings (e.g., silver, heparin, hydrophilic, gendine, triclosan). An ideal method for the prevention of biofilm has not been discovered yet.

5.3 Bacterial biofilm assessment
Biofilm formation has been extensively studied. Different techniques are available including, but not limited to, microtiter plate, electron microscopy, and sonication/culture. This literature review will focus only on the techniques that relate to this project.
The microtitre plate assay technique is one of the most frequently used methods for biofilm assessment. Cultured in broth, bacteria form biofilm on the walls and bottom of the wells of the microtiter plate or on the surface of a coupon that is placed in the well itself. Biofilm is subsequently stained with crystal violet and its optical density is measured, providing a quantitative assessment. This technique offers several advantages: plates are inexpensive, easy to use, and allow evaluation of a large number of isolates simultaneously. They are used to evaluate the activity of different substances on biofilm including antibiotics, chemicals, disinfectants, and plant extracts. The technique also allows for the evaluation of biofilm under various conditions of temperature and humidity, in different growth media, and in the presence or absence of O₂ and CO₂. A downside of this technique is the lack of differentiation between matrix, living cells and dead cells. Also, the environment is not reflective of the in vivo environment or the complex interactions with oxygen and nutrients present in the setting of an infection.

Electron microscopy techniques use an electron beam (short-wavelength/high-energy radiation) to image the surfaces of microcolonies and biofilms at high resolution. Samples are prepared in ultra-thin slices to allow visualization of bacteria and EPS. Downsides of this technique involve the need for sample preparation and specialized equipment making this technique expensive and time consuming. Scanning electron microscopy allow the visualisation of three dimensional structure of microbial biofilm at high resolution. It allows measurement and quantification of the presence of organisms and the associated polysaccharide matrix, which appears as amorphous material. Assessment of biofilm thickness may be important as biofilm thickness may be of significant importance when studying the diffusion rate of antibiotics or nutrients. The overall appearance of the biofilm structure is therefore evaluated (e.g. bacterial colonies, individual cells, polysaccharide matrix).

Because biofilm-associated bacteria are sessile and adhered to surfaces, direct culture
techniques have limited utility. Dispersion of biofilm is required to allow for successful culture. Sonication allows quantitation of biofilm production by dispersing adherent bacteria. During sonication, bacteria are dislodged but not killed. Subsequent culture of the sonicated fluid allows rapid and quantitative assessment of the biofilm. Studies have show that sonication improves the detection rate of bacteria on human prostheses and is associated with a high predictive value for catheter-associated bacteriuria.\textsuperscript{Trampuz 2007, Tunney 1998, Esteban 2008} A major downside of this technique is the lack of standardization of the technique, which makes cross-study comparisons difficult as different methodologies may have some impact on the organism tested. For example, at a predetermined frequency, time of sonication had a significant effect on percentage of bacterial killing. Increase duration of sonication was associated with increase bactericidal effect.\textsuperscript{Scherba, 1991} Furthermore, survival of bacteria is influenced by the type of microorganism, the temperature of the buffer as well as the material of tube used for sonication.\textsuperscript{Monsen 2009} In light of those findings, sonication is a valuable tool to recover organisms associated with biofilm, however, its sensitivity may be affected by the methodology used.

5.4 Enterococcal species

Enterococci are facultative, anaerobic, catalase negative, gram-positive cocci. Enterococci are organized in pairs or short chains. It is not possible to differentiate them morphologically from streptococci, so in the past they were considered members of the Lancefield group D streptococci. However, DNA mapping has revealed no homology with streptococci and as such genus \textit{Enterococcus} was then created.\textsuperscript{Greene, 2006}

Enterococci are well adapted to different environments. They are capable of cellular respiration in environments with poor or rich oxygen concentrations and tolerate extreme temperatures and pH as well as high sodium chloride concentrations. Enterococci are intrinsically resistant to some antibiotics commonly used in veterinary medicine today (e.g. \textit{β}-lactams, cephalosporins and aminoglycosides).\textsuperscript{Hollenbeck 2012, Linden 2007}

In humans, 20 species of \textit{Enterococcus} have been identified with \textit{Enterococcus faecalis} being the most commonly identified (80-90%) and \textit{Enterococcus faecium} being second
most commonly identified (10-15%) in clinical isolates. Enterococcus was initially considered a commensal microorganism of the gastro-intestinal tract and to be of limited clinical importance. More recently, thinking about enterococci has evolved. It is now the third most commonly isolated bacteria involved in nosocomial infections (e.g., endocarditis, UTIs, bacteremia, cholecystitis, meningitis, secondary and tertiary peritonitis, wound infection).

A recent study showed that of all of the urine specimens with significantly defined colony forming units/ml, Enterococcus faecalis was the only enterococci isolated from cats and predominated (77.4%) in dogs followed by E. faecium (12.9%), E. durans (3.2%). And other Enterococcus spp. (6.5%). The majority of specimens with significant enterococcal growth resulted in complicated urinary tract infections in 83.9% of dogs and 81.8% of cats. Multidrug resistance was identified in 7 of 31 dogs and 6 of 11 cats, and E. faecium isolates from dogs were 4.5 times more likely to be multidrug resistant than E. faecalis.

5.4.1 Enterococcal species’ biofilm production

In clinical settings, biofilm is now recognized as an etiologic agent participating in chronic infections. The adherence and biofilm production of enterococcal species have been described in numerous reports. The production of biofilm by E. faecalis and E. faecium, the two most commonly isolated enterococcal species in humans, has been demonstrated on different biomaterials. Medical devices like ureteral stents, vascular catheters, biliary stents, ocular lenses and gastrostomy devices have been infected by enterococci able to produce biofilm.

5.4.1.1 Epidemiology of E. faecalis and E. faecium biofilm production

The prevalence of enterococcal strains able to produce biofilm varies worldwide. Table 2 shows the results of studies investigating the biofilm-producing ability of E. faecalis
and *E. faecium* in different human clinical situations and environments. Collectively, data suggest that *E. faecalis* produces biofilm more often than *E. faecium*.

Table 2. Results of Human Studies Evaluating the Biofilm-producing Ability of *Enterococcus faecalis* and *Enterococcus faecium* Strains in Samples from Different Clinical and Environmental situations.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th><em>E. faecalis</em> isolates</th>
<th><em>E. faecium</em> isolates</th>
<th>Sample source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandoe <em>et al.</em>, 2003</td>
<td>United Kingdom</td>
<td>100%</td>
<td>42%</td>
<td>Clinical (bloodstream)</td>
</tr>
<tr>
<td>Seno <em>et al.</em>, 2005</td>
<td>Japan</td>
<td>100%</td>
<td>Not studied</td>
<td>Clinical (urinary tract)</td>
</tr>
<tr>
<td>Baldassarri <em>et al.</em>, 2006</td>
<td>Italy</td>
<td>96%</td>
<td>Not studied</td>
<td>Clinical</td>
</tr>
<tr>
<td>Di Rosa <em>et al.</em>, 2006</td>
<td>Italy</td>
<td>95%</td>
<td>29%</td>
<td>Clinical and environmental</td>
</tr>
<tr>
<td>Mohamed <em>et al.</em>, 2004</td>
<td>United States and elsewhere</td>
<td>93%</td>
<td>Not studied</td>
<td>Clinical</td>
</tr>
<tr>
<td>Dupre <em>et al.</em>, 2003</td>
<td>Italy</td>
<td>87%</td>
<td>16%</td>
<td>Clinical</td>
</tr>
<tr>
<td>Baldassarri <em>et al.</em>, 2001</td>
<td>Italy</td>
<td>80%</td>
<td>48%</td>
<td>Clinical</td>
</tr>
<tr>
<td>Toledo-Arana <em>et al.</em>, 2001</td>
<td>Spain</td>
<td>57%</td>
<td>Not studied</td>
<td>Clinical</td>
</tr>
<tr>
<td>Prakash, 2005</td>
<td>India</td>
<td>26%</td>
<td>0%</td>
<td>Clinical</td>
</tr>
<tr>
<td>Almohamad <em>et al.</em>, 2014</td>
<td>Argentina, Belgium, China, United States</td>
<td>Not studied</td>
<td>67.5%</td>
<td>Clinical</td>
</tr>
</tbody>
</table>
5.4.1.2 Factors influencing biofilm production

Several factors are known to influence biofilm production by enterococcal species. Regulation of biofilm production is partly understood, but it is multifactorial and still an area of active investigation.

Various nutrients contained in growth media have been shown to influence the ability to produce biofilm. Supplementation of the growth medium with 1% glucose has been associated with increased biofilm production. Carbon dioxide, iron, pH, osmolarity, temperature and addition of serum may influence biofilm production. High osmolality (2-3% sodium chloride) has been shown to affect the ability to produce a biofilm despite bacterial growth.

Genetics can also play a role in the different biofilm formation steps and the evaluation of potentially relevant genes such as esp, gelE, fsr, epa, atn, icaA and bopD has been reported for human isolates.

6.0 Research Rationale

Accessing the ureters of dogs to treat calculi and strictures is tricky because of their small size. Currently available surgical treatments are associated with high morbidity and mortality and palliative treatment consisting of long-term ureteral stenting is associated with long-term complications of infection, possible encrustation, and compromised quality of life. For these reasons, the aim of this research was to find a way to access ureteral disease with an endoscope. In children, after two weeks of stent placement, PUD was seen. Immediately after stent removal and during the same general anesthesia, physicians were able to pass the ureteroscope because of the dilated ureteral size. Our primary research goal was to replicate this procedure successfully in dogs. A secondary goal was to evaluate both for the presence of biofilm on the ureteral stent removed following an indwelling time of 2 and 6 weeks, as well as evaluating enterococcal bacteria for their ability to produce biofilm in vitro; these bacteria are common in stented dogs without inducing clinical signs.
7.0 Objectives
In response to the need for safe and effective ways to access ureteral calculi in dogs and to investigate the potentially complicating issue of biofilm production, the primary objectives of this thesis were:

1. To investigate the viability of non-palliative ureteral stenting and immediate ureteroscopy in dogs (Chapter 2):
   a. To determine if passive ureteral dilation occurs after an indwelling ureteral stent has been in place for 2 or 6 weeks,
   b. To determine if ureteroscopy is possible after passive ureteral dilation occurs, and
   c. To determine if passive ureteral dilation is reversible after ureteral stent removal.

2. To evaluate indwelling ureteral stents for the presence of bacterial biofilm following removal (chapter 3)

3. To evaluate the in vitro biofilm-producing ability of enterococci isolated from clinical infections in dogs. (Chapter 4)

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CHAPTER II

PASSIVE URETERAL DILATION AND URETEROSCOPY AFTER URETERAL STENT PLACEMENT IN 5 HEALTHY BEAGLES

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2.1 Abbreviations
CBC complete blood count
CTEU computed-tomographic excretory urography
PUD passive ureteral dilation
RBC red blood cell
RUPG retrograde ureteropyelogram
UA urinalysis
UD ureteral diameter
UPJ ureteropelvic junction
UVJ ureterovesical junction

2.2 Introduction
Ureteral obstructions are challenging conditions to treat in veterinary medicine given the small ureteral diameter (UD) in cats (0.3-0.4 mm) and dogs (1-2 mm). Medical management (e.g., intravenous fluid management, osmotic diuresis, analgesia, and/or alpha-adrenergic blockade) has been associated with a low success rate (10%) and a 12 month survival time of 66%. Surgical interventions such as ureterotomy or ureteronephrotomy have been associated with high morbidity (21% in cats, 35% in dogs) and mortality (31% in cats, 25% in dogs) rates. Common complications following surgery include uroabdomen (16% in cats), persistent ureteral obstruction (6% in cats) and persistent azotemia (52% in cats, 43% in dogs). The long-term survival in dogs following surgery was 904 days whereas in cats the 12-month survival time was 91%.

To date, ureteral stenting has been used as a palliative treatment for obstruction in children and veterinary patients. Double pigtail ureteral stents used in both humans and animals are soft, multi-fenestrated, hollow polymeric tubes that provide, following
insertion, immediate renal drainage and decompression by allowing urine outflow through their lumen. In children, ureteral stent placement leads to passive ureteral dilation (PUD). In veterinary medicine, ureteral stents can remain in place long-term provided that they are not associated with complications. Many complications have been reported for dogs and cats. Short term (<1 month) complications in canine patients include hematuria (20%), dysuria (<5%), obstruction (~2%), migration (<2%) and mortality (<2%). The most common long term (>1 month) complications include urinary tract infection (10-60%), recurrent obstruction (10%), tissue proliferation at the ureteropelvic junction (UPJ) (5-25%) and less commonly (<6% each) hematuria, migration, encrustation and dysuria are reported. In feline patients, ureteral stents are associated with more significant perioperative complications (i.e. fluid overload 17%, mortality 7.5%, pancreatitis 6%) as well as short-term (i.e. temporary inappetence 25%, dysuria <10%, stent migration 3%) and long-term (i.e. dysuria 38%, hematuria 18%, obstruction 19-26%, urinary tract infection 13%) complications. Given the significant rate of complication associated with ureteral stents in cats, a subcutaneous ureteral bypass (SUB) device was developed and is now commonly used in cats. It consists of a locking-loop nephrostomy tube and a cystostomy locking-loop tube, connected together to a shunting port placed sub-cutaneously. With this device, the incidence of dysuria in the short and long term has markedly decreased (<2%), but similar incidences of short-term (i.e. temporary inappetence 25%, fluid overload <5%, device obstruction 2%) and long-term (i.e. hematuria 18%, obstruction 18%, urinary tract infection 15%) complications have been reported. In humans, ureteral obstructions caused by calculi are managed by extracorporeal lithotripsy or intracorporeal laser lithotripsy using ureteroscopy. Ureteroscopy is often used in humans to biopsy lesions, investigate hematuria and superficial ureteral neoplasia, and manage nephrolithiasis. Following these procedures, a stent is often placed for a short time to avoid future ureteral edema and associated obstruction. Ureteral stents are also used in order to
alleviate symptoms associated with acute renal colic due to ureteral obstruction\textsuperscript{2015}. In humans, it is generally recommended that a ureteral stent be removed or replaced after 3 months to avoid encrustation, infection and patient discomfort\textsuperscript{2004}. In children, ureteroscopy is challenging because of the small ureteral diameter, a situation similar to that seen in veterinary patients. Ureteral stenting for 2 weeks in children has been shown to induce PUD that allows ureteroscopy to be performed after stent removal\textsuperscript{2005}. To our knowledge, PUD secondary to ureteral stenting has not been evaluated in dogs. Canine ureteroscopy has been reported in one dog as part of another study evaluating the use of sclerotherapy for the treatment of canine essential hematuria\textsuperscript{2013}.

The purpose of this prospective pilot study was 3-fold: (1) to evaluate if PUD occurs in healthy dogs after ureteral stent placement for 2 and 6 weeks, (2) to evaluate if ureteroscopy can be performed safely at stent removal, and (3) to see if PUD is reversible over time. Our hypotheses were that (1) the presence of a ureteral stent for 2 and 6 weeks in dogs will induce PUD, (2) the presence of a ureteral stent will induce sufficient dilation for a 2.9 mm ureteroscope to pass through the ureter, and (3) PUD will be reversible after removal of the ureteral stent.

\section*{2.3 Materials and Methods}
This study was conducted at the Ontario Veterinary College, University of Guelph and was approved by the university’s Animal Care Committee. Five, 3-year old female purpose bred beagles\textsuperscript{b} were enrolled and deemed healthy based on history, normal physical examination, prothrombin time/partial thromboplastin time, complete blood count (CBC), biochemistry, urinalysis (UA), urine culture and urogenital ultrasound examination.

\textit{Timeline}
Following enrolment and collection of baseline parameters for the CBC, biochemistry, UA, urine culture and ultrasonography, dogs were anesthetized in order to collect baseline ureteral measurements by computed-tomographic excretory urography (CTEU)
and for ureteral stent placement under cystoscopic and fluoroscopic guidance. Every 2 weeks until week 10, blood and urine were collected for analysis, a urogenital ultrasonographic examination was performed and dogs were routinely anesthetized in order to repeat the CTEU to measure ureteral diameters. For each dog, the first ureteral stent was removed at week 2 and the contralateral stent was removed at week 6. Ureteroscopy was attempted immediately following stent removal.

Physical examination
Physical examinations were performed once or twice daily by the same observer (co-author CV), including pain assessment using the Colorado Pain Scale. Other daily observers included a veterinary technician (twice daily) and a dog walker (once daily for 30 minutes). Cages and walking space were inspected for presence of hematuria.

Clinicopathologic data
At weeks 0, 2, 4, 6, 8 and 10, CBC, renal profile, UA and urine culture were obtained. For UA, any sample with more than 3 white blood cells per high power field was classified as pyuria. Di Bartola 2010

Urogenital ultrasonography
At weeks 0, 2, 4, 6, 8 and 10, urogenital ultrasonography was performed to evaluate the urinary tract and to measure the maximum pelvic width (excluding the proximal ureter) in a transverse plane.

Cystoscopy
At week 0 and after the CTEU, dogs were transferred to the surgery suite and placed in dorsal recumbency with the limbs and tail hanging over the end of the table. An area from the pubis to the dorsal aspect of the vulva was clipped and aseptically prepared and cepha-zolin (22 mg/kg [10 mg/lb]) was administered intravenously (IV). A 14.5 Fr rigid cystoscope with a 30 degree lens was advanced into the bladder through the urethra and both ureteral papillae were identified.
Ureteral stent placement

Ureteral stents were placed as described elsewhere. A 0.025-inch (150 cm long) or 0.035-inch (180 cm long) diameter angle-tipped hydrophilic guide wire as well as a 4 or 5 French ureteral catheter were used for ureteral stent placement. Under cystoscopic and fluoroscopic guidance, the guide wire was advanced through the ureteral papilla into the ureter up to the ureteropelvic junction (UPJ). The ureteral catheter was advanced over the guidewire and pyelogram performed. Ioxehol was the contrast material (diluted 1:1 with sterile 0.9% saline) used for retrograde ureteropyelogram (RUPG). The guide wire was reintroduced and the ureteral catheter removed. Appropriately-sized, double pigtail ureteral stents were placed bilaterally, with one pigtail loop of the stent placed in the renal pelvis and the other one in the urinary bladder. Bupivacaine (0.3 mg/kg [0.14 mg/lb]) diluted in 3 ml of 0.9% saline was infused into the urethral lumen while the cystoscope was removed. Procedure time was recorded. Enrofloxacin was administered intramuscularly (10 mg/kg [4.55 mg/lb]) at recovery followed by once a day oral administration (mean 10.76 mg/kg [4.89 mg/lb]; range 8.55-9.90 mg/kg [3.89-4.50 mg/lb]; PO q24h) for 17 days (i.e. discontinued 3 days after removal of the stent at week 2). Intravenous fluids (lactated Ringer’s solution) were administered for 12 hours (40 ml/hr) postoperatively. Buprenorphine was administered (0.02 mg/kg [0.01 mg/lb]; IV q8h) twice (with a third dose if deemed necessary based on pain assessment) followed by 3 days of oral tramadol (mean 4.65 mg/kg [2.11 mg/lb]; range 4.27-4.95 mg/kg [1.94-2.25 mg/lb]; PO q8h).

Ureteral stent removal and ureteroscopy

Two weeks after the stent placement, a ureteral stent (side randomized) was removed; this is group 1 data. Six weeks following stent placement the contralateral ureteral stent was removed; this is group 2 data. The anesthetic protocol, surgical site preparation, analgesia, and perioperative antibiotic treatment were identical for ureteral stent placement and removal. For ureteral stent removal, routine transurethral cystoscopy was performed. The distal loop of the ureteral stent was grasped with endoscopic forceps and pulled to the level of the vulva with the cystoscope. Under fluoroscopic
guidance, an appropriately-sized (0.035 inch for a 4.7Fr ureteral stent and 0.025 inch for a 3.7Fr ureteral stent) angle-tipped stiff hydrophilic guide wire\textsuperscript{n-o} was passed through the ureteral stent and advanced into the ureter to the level of the renal pelvis to confirm stent patency. The ureteral stent was removed over the wire and the wire was left in place in the ureter. The time required to remove the stent was recorded from the time of insertion of the cystoscope until complete stent removal from the urethra. Immediately following stent removal, a 2.9 mm flexible ureteroscope\textsuperscript{p} was advanced over the guide wire under fluoroscopic guidance to the level of the mid-ureter under constant irrigation with 0.9% saline. The guide wire was then removed and the ureteroscope was advanced to the level of the UPJ where the renal pelvis was visualized and then the ureteroscope was pulled back to the level of the ureterovesical junction (UVJ). The ureteroscopy procedure time was recorded as the time between introduction of the ureteroscope into the ureter at the UVJ until its removal from the UVJ.

\textit{Computed-tomographic excretory urography}

At baseline and every 2 weeks thereafter, dogs were sedated with butorphanol (0.2 mg/kg, 0.09mg/lb) and acepromazine (0.05mg/kg, 0.23mg/lb) intramuscularly. A 5 ml/kg (2.27 ml/lb) intravenous fluid bolus (lactated Ringer’s solution) was administered followed by 10 ml/kg/h continuous rate infusion. With the animal under general anesthesia induced by IV propofol (4 mg/kg, 1.81 mg/lb) and maintained by inhalation isoflurane in oxygen, a CTEU was performed.\textsuperscript{Secrest 2013} Ureteral diameter measurements were taken at 3 locations: cranially at the level of the renal pelvis, at the level of the L4 vertebra (called “mid”) and caudally at the UVJ.

\textit{Statistical analyses}

Each parameter for CBC, renal profile and UA was averaged for each week prior to analysis. A repeated measures ANOVA was used to evaluate the effect of time on ureteral dilation. Time and side were included as potential interaction effects for ureteral dilation. Descriptive statistics were calculated for the mean ureteral and pelvic dilations as well as for the total duration of ureteroscopy and stent removal. The Shapiro-Wilk test was used to ensure normal data distribution for all statistical analyses and log
transformation was performed when deemed necessary. The mean UD measurements for each ureter group were compared over time. A commercial software package was used for statistical analyses.\(^9\) P < 0.05 was considered statistically significant.

### 2.4 Results
Five female Beagles (1 intact, 4 spayed) were enrolled in this study. The median age of the dogs was 3 years (+/- 0.08) while the median weight was 10.8 kg (10.1 to 11.7 kg). At enrolment, prothrombin time/partial thromboplastin time, CBC, biochemistry, UA, urine culture and urogenital ultrasound were normal in all five dogs.

**Physical examination**
All dogs had good appetite, were normal on physical examination and had soft and comfortable abdominal palpation throughout the study. Dog 5 was in heat at week 2 for 7 days.

**Clinicopathologic data**
The CBC and biochemistry parameters remained within reference intervals throughout the study. While creatinine levels remained within the reference intervals throughout the study, comparisons revealed that creatinine at week 2 (p=0.0011) and week 6 (p=0.0094) were significantly higher compared to week 8. On UA, the red blood cell (RBC) and white blood cell count varied significantly throughout the study period. A significant hematuria was noted at week 2 compared to week 0 (p=0.0319). At week 2, the RBC was at its maximum compared to the other time points studied. Compared to week 2, RBC was significantly decreased at week 8 (p=0.0002) and week 10 (p=0.0012). Compared to week 4, the RBC was decreased significantly at week 8 (p=0.0012). Compared to week 6, the RBC was decreased significantly at week 8 (p=0.0054) and week 10 (p=0.0048). Pyuria was detected in 3 of 30 samples collected during the study and among those, 2 had positive urine cultures. Two of the 27 samples without pyuria also had a positive urine culture. A significant increase in mean white blood cell counts in all dogs was noted at week 2 (p<0.0001), week 4 (p=0.0007), week 6 (p=0.0003), week 8 (p=0.0058) and week 10 (p=0.0039) compared to week 0.
white blood cell count was at its maximum at week 2, and significant decreases were noted at week 8 (p=0.0335) and week 10 (p=0.0473). During the study, dog 4 had positive urine cultures at week 4 (>10^5 cfu/mL), week 6 (>10^5 cfu/mL) and week 8 (1.3x10^4 cfu/mL). Urine culture was negative at week 10 for dog 4. The urine culture was positive (>10^5 cfu/mL) at week 6 for dog 5, but negative at week 8 and week 10 for the same dog. The microorganisms identified were *Enterococcus faecium* and *Streptococcus canis* for dogs 4 and 5, respectively.

**Urogenital ultrasonography**
Renal pelvis dilation was only noted in 2 dogs and it was seen 2 weeks after stent placement. For those cases, pelvic dilation was minimal: 0.19 cm bilaterally for dog 1 and 0.18 cm unilaterally for dog 2. For both dogs, dilation had resolved on reevaluation at 4 weeks after stent placement.

**Cystoscopy**
Cystoscopic examination revealed mild bilateral intramural ectopic ureters in dog 2 with ureteral papillae located at the urethrovesical junction (Figure 4). No abnormalities were noted in the other dogs during cystoscopic examination.

**Ureteral stent placement**
Dogs 1, 2 and 3 had a 4.7 Fr, 20 cm stent placed bilaterally. Dog 4 had a 3.7 Fr, 18 cm stent placed in the left ureter and a 4.7 Fr, 20 cm stent placed in the right ureter. Dog 5 had a 4.7 Fr multi-length (22-32 cm) stent placed bilaterally. Mean procedure time for unilateral retrograde placement was 17.9 minutes (range 13-25 minutes).

**Ureteral stent removal and ureteroscopy**
Mean procedure time for ureteral stent removals were 13.0 minutes (range 5-24 minutes) and 8.6 minutes (range 6-15 minutes) at weeks 2 and 6, respectively (Figure 5). Cystoscopies were uneventful at weeks 0, 2 and 6. Ureteroscopy was successfully performed in all dogs with mean durations of 3.2 minutes (range 2-5 minutes) and 4.6 minutes (range 1-11 minutes) at weeks 2 and 6, respectively (Figure 6). The flexible
ureteroscope was advanced along the guide wire to the level of the UPJ. The morning after the procedure and until the end of the study, all dogs had a soft abdominal palpation and a good appetite. All dogs had intermittent macroscopic hematuria for a mean total of 9 days (range 2-13 days) in the first 38 days of the 48 day study. No hematuria was noted after the last stent removals and endoscopies at week 6.

**Computed-tomographic excretory urography:**
At week 0, CTEU didn’t reveal any significant abnormalities in any dogs. At week 2, CTEU revealed the distal loop of the left ureteral stent was within the urethra in dog 1 and that the ureteral stent was looped within the proximal left ureter in dog 5. In the latter dog, the ureteral loop persisted until removal at week 6 with evidence of focal dilation at week 4, but not at week 6. At weeks 8 and 10 in dog 5, the same ureter wall was thickened along its entire length (i.e. poor opacification). At week 4 in the same dog, the unstented ureter was poorly opacified along its entire length and the ureteral papilla was thickened. Another dog, dog 4, also showed a thickened right ureteral papilla at week 10 which was 4 weeks after the stent had been removed.

A total of 216 ureteral measurements were collected (Table 1, Figure 7). For the group 1 stents removed at week 2, the UD increased by 53% (2.16 to 3.32 mm), 81% (1.58 to 2.86 mm) and 65% (1.86 to 3.06 mm) in the cranial, mid and caudal ureters, respectively. For the group 2 stents removed at week 6, the UD increased by 21% (2.52 to 3.06 mm), 53% (1.82 to 2.8 mm) and 53% (1.86 to 2.86 mm) in the cranial, mid and caudal ureters, respectively. Ureteral diameter peaked at 2 weeks (cranial and mid) and 4 weeks (caudal) for group 1 whereas the UD peaked at 4 weeks for group 2 for the cranial, mid and caudal ureters (Figures 1, 2 and 3). Cranial, mid and caudal ureteral segment diameter measurements p-value comparisons at weeks 2, 4, 6, 8, and 10 for both groups compared to UD measurements obtained at weeks 0, 2 and 4 for group 1 and at weeks 0, 2, 4 and 6 for group 2 are seen in tables 2 and 3, respectively.

For group 1 (Table 2), UD-2, UD-4, UD-6 were all significantly higher than UD-0 at all levels of the ureter. UD-8 at all levels of the ureter and UD-10 at the cranial and mid
levels of the ureter were not significantly different from UD-0, but UD-10 was significantly different from UD-0 caudally. UD-8 and UD-10 were significantly decreased compared to UD-2 when measured at the mid and caudal levels of the ureter. UD-6, UD-8 and UD-10 were significantly decreased compared to UD-4 when measured caudally.

For group 2 (Table 3), cranial level UD-4, mid level UD-10 and mid and caudal levels UD-2, UD-4, UD-6 and UD-8 were significantly increased compared to UD-0. At all levels, UD-4, UD-6, and UD-8 were not significantly decreased compared to UD-2, UD-4 and UD-6, respectively. At mid and caudal levels, UD-10 was significantly decreased compared to UD-2 and UD-4. In dog 2 (a dog with mildly ectopic ureters), UD of the 2 week-stented ureter was similar to that of the other dogs. In dog 2 the 6 week-stented ureter was markedly dilated: 4.5 mm (mid and caudal) and 3.3 mm (mid and caudal) at weeks 4 and 6, respectively.

In order to ensure that dog 2 was not significantly affecting the results of the study, statistical analyses were reviewed. While 72% of all of dog 2’s ureteral diameters (i.e. all ureteral segments included) were equal or below the median of this group of dogs, analysis was repeated excluding that dog. For group 1, with dog 2 included in the study, all the ureteral segments were significantly dilated at weeks 2, 4, and 6 compared to baseline. When those data were excluded, dilation of the cranial ureteral segment remained significantly greater at weeks 2 (p=0.0389) and 4 (p=0.0499) (but not at week 6, p=0.2323) and dilation of the mid ureteral segment remained significant at weeks 2 (p=0.0003) and 6 (p=0.0043) (but not at week 4 (p=0.0942)). For the caudal ureteral segment, significant dilation was still noted for weeks 2 (p=0.0008), 4 (p=0.0017) and 6 (p=0.0446). For group 2, with dog 2 included in the study, the cranial ureteral segment was significantly dilated at week 2 only (p=0.0146), while after removal of that dog, there were no significant differences for the cranial segment at weeks 2 (p=0.2150), 4 (p=0.1262) or 6 (p=0.5903). For the mid ureteral segment, significant dilation was noted at weeks 2, 4 and 6 with dog 2 included in the study, and at weeks 2 (p=0.0005) and 6 (p=0.0401), but not week 4 (p=0.0997) with dog 2 excluded. For the caudal ureteral segment, significant dilation was noted at weeks 2, 4 and 6 with (p<0.0001, p<0.0001
and p=0.0011, respectively) or without (p=0.0009, p=0.0114 and p=0.0261, respectively) dog 2 included.

The intact female, dog 5, was noted to be in proestrus the day prior to removal of the ureteral stent at week 2. In order to ensure that dog 5 was not significantly affecting the results of the study, statistical analysis was also repeated without the ureteral diameter measurements of this dog. Results were significant with or without dog 5 in the study in regards to passive ureteral dilation occurring for all ureteral segments at week 2 (cranial: p=0.0010, mid: p=0.0002, caudal: p=0.0004), week 4 (cranial: p=0.0074, mid: p=0.0004, caudal: p=0.0007) and week 6 (cranial: p=0.0004, mid: p=0.0277, caudal: p=0.0014) compared to baseline for group 1. For group 2, results were still significant for mid and caudal segment at week 2 (cranial: p=0.1189, mid: p=0.0001, caudal: p=0.0005), for cranial, mid and caudal at week 4 (cranial: p=0.0157, mid: p=0.0002, caudal: p=0.0022) and for mid and caudal at week 6 (cranial: p=0.0941, mid: p=0.0017, caudal: p=0.0121).

Complications

Complications associated with the ureteral stent placement procedure included guide wire puncture of the renal parenchyma through a non-dilated calix on 3 occasions (dogs 2, 4 and 5) and one mild subcapsular extravasation of contrast due to this puncture (dog 4). No complications were seen with ureteroscopy. In dog 1, mild resistance was noted while trying to advance the flexible ureteroscope that resolved with manual irrigation. Continuous manual irrigation was performed subsequently for all dogs while advancing the scope and resistance was no longer noted. After stent removal, the safety wire previously in the ureter backed out of the ureter while the flexible ureteroscope was being advanced in 3 ureters of 2 dogs. This required regaining wire access to the ureter using fluoroscopic and cystoscopic guidance as described elsewhere.\textsuperscript{a}Berent 2011

2.5 Discussion

The results of this study confirm that PUD occurs in healthy dogs within 2 weeks after ureteral stenting and that ureteroscopy can be performed safely and successfully at the time of ureteral stent removal. In our study, UD increased by 21 to 81% within 2 to 4 weeks of an indwelling ureteral stent. Similarly, PUD was found to develop in 36
Yorkshire pigs stented for 10 weeks\textsuperscript{Hadaschik 2008}, in 3 female pigs stented for 7 days\textsuperscript{Natalin 2009}, and in 26 children stented for 14 days\textsuperscript{Hubert 2005}. In pigs, retrograde ureteropyelograms (RUPG) showed that there was little change in UD for the (control) non-stented ureters (<10mm), but that stented ureter UD increased to >20 mm\textsuperscript{Hadaschik 2008}.

Since the 1930’s an indwelling stent or catheter has been shown to cause reversible ureteral dilation, but the mechanism has not been elucidated\textsuperscript{Wiseman 1934}. Physiological relaxation or direct cytotoxic effects have being suggested\textsuperscript{Drake 1962}. Ureteral dilation is suspected to occur due to the presence of foreign material in the ureter, but it may also be related to an altered renal pelvis and ureteral peristalsis induced by the stent, thus slowing down urine transport\textsuperscript{Ryan 1994, Kinn 2002}. Ureteral dilation is also associated with ureteral wall inflammation in humans and pigs\textsuperscript{Natalin 2009, Venkatesh 2005}. Passive ureteral dilation may depend on the stent properties and design. Natalin et al. (2009) evaluated 11 female domestic pigs fitted with a flat-shaped stent, the “ribbon stent”, which has a small diameter tube and two flat wings affixed in opposition to each other\textsuperscript{Natalin 2009}. The ribbon stent showed little immediate interference on peristalsis and induced less PUD than a standard ureteral stent, probably reflecting its smaller contact surface with the ureteral wall\textsuperscript{Natalin 2009}. Given these results, the desire for PUD for future ureteral interventions and to aid in stone passage, a tubular-shaped stent was considered the best type of stent to induce PUD in dogs.

In our study PUD was reversible. This was seen in group 1, the group that had the stent removed after 2 weeks and that were followed for an additional 8 weeks. Because of the shorter follow-up time in group 2 (i.e. only 4 weeks after stent removal), we cannot confirm whether a longer period of stenting leads to a longer recovery to the initial UD. However, there was a tendency for the UD to reduce in size over time. A CTEU at 12 weeks may have answered this, but was not performed in our study. To our knowledge there is no study reporting the resolution of the ureteral dilation after ureteral stenting in pigs. Previously published studies were terminal and reported post-mortem examinations\textsuperscript{Hadaschik 2008, Natalin 2009}. 

In the study of Jones et al. (1990), ureteral stents in people were retained for periods ranging from 2 to 4 weeks, but the time for dilation to occur and thus improve the success rate of ureteroscopy was not fully established. In our study and for group 2 ureters, the maximum UD was reached at 4 weeks for all dogs even if the stent was left in place for an additional 2 weeks. The number of stented ureters (n=10) is too small to draw solid conclusions, but our results suggest that maximal PUD may be reached at 4 weeks and may plateau no matter how long the ureteral stent is retained. Clinically, if the goal of PUD is to perform future ureteroscopy, then this is only necessary for 4 weeks in order for this to be successfully accomplished. In our study, the UD stayed significantly increased compared to UD-0 at weeks 2, 4 and 6 for group 1 and at weeks 8 and 10 compared to UD-0 for group 2. Exactly how long it takes for the ureter to return to its original diameter is unknown. It may be possible to perform ureteroscopy beyond the initial stent removal time in some cases.

In recent years, the vast majority of human urolithiasis cases are managed by ureteroscopy. Ureteroscopy has supplanted surgical removal of stones and extracorporeal lithotripsy and has been associated with lower morbidity and mortality and better stone-free rates. Management of renal and ureteral calculi is challenging in canine patients. At this time, canine upper urinary tract stones are not usually removed, but rather bypassed using ureteral stents or subcutaneous ureteral bypass. Ureteroscopy would offer a minimally invasive therapeutic modality for dogs. It could allow the removal of renal or ureteral stones by use of baskets (diameter: 1.5-1.9F) or in conjunction with laser lithotripsy. These techniques could decrease surgical and implant-related complications including the risk of recurrent infection and stent-related clinical signs (e.g., dysuria, pollakiuria).

It is questionable whether dogs with ureteral pathology will undergo the same degree of ureteral dilation after ureteral stent placement. In rats, it has been demonstrated that fibrotic tissue slowly replaces the smooth muscle layer in obstructed ureters after 21 days of obstruction and that fibrotic tissue occupies 90% of the muscle layer after 42
The dilation capacity of fibrotic tissue is certainly less than healthy smooth muscle, and thus, it may have an impact on the capacity for PUD. Stents may have to remain indwelling for a longer period of time to achieve sufficient dilation, or dilation may or may not ultimately occur. If it does it would likely be to a lesser degree if significant fibrotic tissue develops. In a previous study where ureteral stents were placed in cats with ureteral strictures, PUD was not seen at the stricture site. A similar process has not been described in dogs, but is suspected to also occur. However, it is possible that feline ureters are more prone to stricture because of their small diameter.

In our study, dog 2 was incidentally diagnosed with bilateral ectopic ureters. Because of their different muscular composition compared to normal ureters and a larger ureteral orifice with higher outflow pressure during urination, it is possible that ectopic ureters are prone to exaggerated passive dilation secondary to ureteral stenting, but a solid conclusion cannot be made on a single case. However, ureteral openings in dog 2 were still noted in the bladder (at the urethrovesical junction) and no clinical abnormalities were detected. There was also no evidence of ureterocele nor ureteral dilation on ultrasound and CTEU at baseline. However, because of the concern about the anatomical abnormality present in dog 2, the impact of removing that dog from the analysis was evaluated. Despite removing dog 2 from the analysis (thereby reducing statistical power), the main results were still significant. Also, almost 75% of the measurements were equal or below the median of all the other dogs included in the study, further supporting the assumption that this dog’s anatomical abnormality did not bias the results and indicating retention of its data would be appropriate.

The intact female dog, dog 5, was in proestrus the day prior to removal of one ureteral stent at week 2 and so might have had an increased concentration of progesterone during the study period. The hormonal effect of progesterone on the ureters is controversial in the human literature. As reported by Swift (1993), hydronephrosis of pregnancy is well described in people, but there are still controversies as to whether the physiologic hydroureter is related to a mechanical obstruction due to
the pregnancy or hormonal secretion.\textsuperscript{Swift 1993} In rats progesterone is suspected to cause ureteral dilation due to stimulation of ureteral $\beta$-adrenegic receptors.\textsuperscript{Raz 1972} Similar studies have not been performed in dogs, so it is unclear if the increase in progesterone associated with the proestrus and estrus for dog 5 may have contributed to the ureteral dilation noted with CTEU. In order to ensure that dog 5 did not significantly affect the results of the study, the statistical analyses were repeated without the ureteral diameter measurements of this dog and results were significant with or without dog 5 in the study with respect to PUD.

In the literature, RUPG and gross measurements were used to assess pig ureters with ureteral stents.\textsuperscript{Hadaschik 2008, Natalin 2009} In our study we used CTEU as described by Secrest et al. (2013).\textsuperscript{Secrest 2013} Retrograde ureteropyelogram would likely have worked equally well or better in our study as it maximally distends the ureteral lumen and is not influenced by ureteral contractions during peristalsis which will occur on a CTEU. However, because RUPG requires ureteral catheterization and cystoscopy, it was not considered a practical approach for the in between measurements of the UD when cystoscopy was not being performed. It could also have increased the risks of bacterial contamination of the urinary tract and stents. Consistent methodology was considered ideal and more clinically relevant. In our study, we measured the outer UD since the inner diameter was frequently difficult to differentiate from the indwelling ureteral stent on CT. In one study performed in dogs where ureteral stents were placed for a 4-week period, persistent ureteral muscular hypertrophy and collagen deposition in the subepithelial ureteral wall were noted histologically 8 weeks after stent removal and to a lesser extent after 4 weeks.\textsuperscript{Ryan 1994} These changes may lead to overestimation of the ureteral diameter by compromising the distinction of the outer ureteral wall from the stent. To avoid such a situation, the wide window width was used on CT to facilitate clear definition of the ureteral wall without a bloom effect from the contrast media. This increased accuracy of measurement by differentiating the outer wall and the stent in the two dogs with thickened ureteral walls in our study.
We observed a discrepancy between the cranial UD measurements and the mid and caudal measurements. This discrepancy is likely due to the normal, flared shape of the ureter at the UPJ and the caudal curvature of the cranial ureter on viewing oblique planes.

In children, ureteral stenting for 2-8 weeks allowed successful ureteroscopy in 100% of patients (by passive dilation of the ureteral papilla) when a previous attempt to pass a ureteroscope into the ureteral orifice was unsuccessful. This eliminated the need for active dilation of the ureteral orifice. Other authors have reported an 84% success rate after ureteral stent placement compared to 45% for an unstented group when using ureteroscopy to treat ureterolithiasis.

An attempt to pass the ureteroscope before stenting was not made in our study to avoid iatrogenic damage such as mucosal abrasion, ureteral perforation, intussusception or avulsion which have all been reported in humans. On the basis of the measured UD on CTEU at baseline (week 0), it seems unlikely that ureteroscopy would have been successful without active dilation. In 30% of children and 10% of adults, the ureter appears resistant to active dilation which precludes ureteroscopy. Finally, pre-stenting induces PUD not only of the ureteral orifice, but also of the entire ureter including the other two known anatomical sites of ureteral narrowing in humans and in pigs: at the UPJ and at the crossing of the iliac vessels. This factor likely contributed to our ability to perform complete ureteroscopic evaluation of the entire ureter successfully.

Our study showed that ureteroscopy is safe in healthy beagles after 2 and 6 weeks of ureteral stenting. It is uncertain if the safety and morbidity associated with stenting followed by ureteroscopy will be as low as demonstrated in this study if performed on diseased canine ureters. Despite their proven efficacy and safety, the presence of ureteral stents result in morbidity in up to 80% of human patients, but this has not been the case in dogs. In humans, ureteral stent placement is associated with troublesome urinary symptoms such as low-grade fever, flank pain, hematuria, dysuria and urinary tract infection in 20% of patients.
Morbidity is minimal with indwelling polymeric stenting for up to 3 months, but longer stenting times are associated with increased frequency of encrustation, infection, secondary stone formation, and obstruction of the stented tract\textsuperscript{Damiano 2002} which is why the aim is typically for the shortest stenting period possible in humans.\textsuperscript{Hadaschik 2008, Ryan 1994, Joshi 2003, Duvdevani 2006, Singh 2001} The etiology of discomfort associated with symptoms is not completely understood. Some have suggested that high pressure transmitted to the renal pelvis during urination and trigonal irritation by the intravesical portion of the stent could be causal factors.\textsuperscript{Deliverliotis 2006} It has also been suggested that stent-related pain and urination frequency could be related to lower ureteral spasm or local trigone sensitivity.\textsuperscript{Deliverliotis 2006} These symptoms usually continue until removal of the ureteral stents. Early complications of a double pigtail stent usually appear during the first 4 weeks after stent insertion.\textsuperscript{Damiano 2002} A meta-analysis of 9 randomized, controlled trials of stenting following uncomplicated ureteroscopy in humans reported that the incidence of lower urinary tract symptoms was significantly higher in participants who had a stent inserted after ureteroscopy.\textsuperscript{Damiano 2002} This same meta-analysis suggested that ureteroscopy is well tolerated compared to ureteral stenting.\textsuperscript{Damiano 2002}

Clinical signs in dogs after ureteral stenting have been far different than those reported in people.\textsuperscript{Cheung 2003, Damiano 2004} A clinical report of ureteral stents in 44 dogs for benign ureteral obstructions did not show any signs of dysuria regardless of stent size, length, or pigtail location.\textsuperscript{a} Dysuria and pain is reported in less than 2\% of dogs.\textsuperscript{Berent 2011c, Kuntz 2015, Pavia 2014, Lam 2012} The explanation for the low rate of dysuria seen in dogs (<2\%) compared to humans (>80\%) and cats (<38\%) is not known, but is suspected to be associated with (1) the location of the UVJ (in cats it is in the proximal urethra) and (2) the quadraped stance which result in the pigtail location being at the bladder apex rather than the trigone as occurs in people.\textsuperscript{Berent 2014, Hao 2008, Lamb 2011} In our study all dogs had intermittent hematuria throughout the study which was not related to the cystoscopy or ureteroscopy and resolved after stent removal. These results suggest that hematuria may be related to the presence of the stent. In humans, a positive urine culture, crossing of the lower coil to the other side of the bladder, calyceal position of the upper coil, longer stents, larger stent diameter, and presence of Percuflex stents are significant
factors for development of stent-related symptoms. None of the dogs with positive urine culture in our study showed signs of discomfort. The dog with the longest ureteral stents had similar signs as the others. However, we may have underestimated the stent-related urinary symptoms because micturition could not be carefully assessed since the dogs were caged, research colony dogs untrained to urinate on walks. None of the dogs in our study had urinary disease prior to ureteral stent placement, so the external validity of our results to diseased dogs is unknown. No dog was noted to have any signs of stranguria, pollakiuria or lethargy, all of which are the classic signs in humans and cats of stent-associated discomfort.

Bacteriuria and recurrent urinary tract infections are complications of stents in humans. In humans it is well known that the longer a stent remains indwelling, the higher the rate of positive culture. Stents with the highest colonization rates (i.e. 75-100%) tend to be those that have been in place for more than 3 months. In another report, urinary tract infections were reported to be present in approximately 60% of stented dogs, but the same dogs were documented to have had an infection prior to stent placement. In our study two dogs had bacteriuria without clinical signs or pyuria. The same organisms were isolated from the stent cultures after removal, but the urine cultures of these dogs were negative after stent removal. Our study dogs might have developed clinical signs of urinary tract infections if the ureteral stents had been left for longer than 6 weeks. The low rate of infection in our study may be explained by the short period of stenting and the absence of concurrent or historical urinary tract disease. Also, enrofloxacin prescribed following ureteral stent placement likely contributed to low morbidity.

In our study, the Enterococcus faecium isolated was multi-drug resistant whereas the Streptococcus canis was sensitive to all drugs tested. While Enterococcus faecium often shows a multi-drug resistance pattern due to its intrinsic antibiotic resistance, it is unclear if the enrofloxacin administration contributed to this multi-drug resistance. Prophylactic antibiotic treatment is still controversial for many procedures in humans. Appropriate prophylactic regimens (i.e. what drug to use, if any) remain
untested in veterinary medicine. Enrofloxacin was used because of its good efficacy against the main uropathogens particularly *Escherichia Coli* and *Staphylococcus spp.* Amoxicillin/clavulanic acid or plain amoxicillin would be similarly effective, however it has been demonstrated that fluoroquinolones prevent biofilm formation on ureteral stents in humans. Minardi 2008, El-Feky 2009, Reid 2001 For this reason, we chose to use enrofloxacin.

Creatinine levels varied significantly after stent placement, but remained within the reference interval throughout the study. In contrast, creatinine remained stable in 36 pigs with ureteral stents for up to 7 weeks. Hadaschik 2008 Increased creatinine in our study could be associated with renal damage secondary to the presence of a stent Ryan 1994, Kinn 2002, a partial ureteral obstruction, or contrast agent toxicity. In our study the renal pelvis dilation was minor and not supportive of an obstruction, a finding in line with other studies on dogs and cats. D’Anjou 2011 Our CTEUs did not support that there were ureteral obstructions. For all of our dogs, the guide wire could be advanced up the lumen of the stent. This confirmed stent patency and the lack of a ureteral obstruction. Only two of our study dogs had a slightly dilated renal pelvis (i.e. <2mm). Pelvic dilation has been reported in dogs with normal renal function, in dogs receiving fluid therapy D’Anjou 2011 and in dogs with inflammation or infection Neuwirth 1993 Additionally the renal pelvis could passively dilate, as does the ureter, accommodating the presence of the stent.

Our study has some limitations. They include the small sample size of a pilot study, the short follow-up period of following stent removal of group 2 and the lack of a detailed dysuria evaluation. Questions remain regarding whether similar dilation can be expected in ectopic ureters, small ureters in small dog breeds and diseased ureters. It is questionable if the same level of PUD will be achieved in these circumstances. Also, by only measuring UD by CTEU rather than by RUPG, ureteral dilation seems to be underestimated since the ureteroscope was 2.9 mm in diameter and many ureters on CTEU measured smaller than 2.9 mm, and in all cases the endoscope easily passed up them. Without active ureteral dilation the true UD measurement is not possible.
To our knowledge this study is the first to evaluate the use of PUD for ureteroscopy by stenting canine ureters. Our study showed the technique to be straightforward, successful and fast. The need for dogs to undergo an additional anesthetic procedure for stenting prior to ureteroscopy may be considered a trade off, but the mean procedure time was 17 minutes making this a simple, outpatient task. It should be made clear that the ureteral stent procedures were performed by operators with lots of interventional experience, making the procedure more efficient than what would have been possible for a novice operator. The ureteral stent removal and ureteroscopy procedures were performed by operators with less interventional experience, yet these too were fast and efficient in our study. Further evaluation of PUD and ureteroscopy in dogs with ureteral diseases is the next obvious step. In conclusion, the results of this study suggest that significant PUD occurs within 2 weeks of ureteral stent placement, that it peaks at 4 weeks and is reversible over time in healthy beagles. Ureteroscopy can be safely performed at the time of ureteral stent removal.

2.6 Footnotes


b. Vivocore Inc, Toronto, ON

c. Canine acute pain scale, Colorado State University, Fort Collins, CO

d. Cystoscope Hopkins Rigit Cystoscope 2.7mm STORZ, Karl Storz Veterinary Endoscopy, Goleta, CA

e. Weasel Wire (0.025in.) Angled/regular taper 150cm standard hydrophilic, Infiniti Medical TM, Menlo Park, GA

f. Weasel Wire (0.035in.) Angled/regular taper 180cm standard hydrophilic, Infiniti Medical TM, Menlo Park, GA

g. Tigertail™ Flexible Tip Ureteral Catheter, diameter 4Fr, Length 70cm, Bard Polyurethane Ureteral Catheters, Bard, C.R. Bard, Inc., Covington, GA
h. Tigertail™ Flexible Tip Ureteral Catheter, diameter 5Fr, Length 70cm, Bard Polyurethane Ureteral Catheters, Bard, C.R. Bard, Inc., Covington, GA

i. Iohexol, Omnipaque, iohexol injection USP 65%, GE Healthcare Canada Inc, Mississauga, Ontario

j. Ureteral Stent 3.7 Frx18cm: Small dog kit, Infiniti Medical™, Menlo Park, CA

k. Ureteral Stent 4.7 Frx20cm: Large dog kit, Infiniti Medical™, Menlo Park, CA

l. Ureteral stent 4.7 Fr, 22-32cm, InLay Optima Multilength, Bard Inc, Covington, GA

m. Biopsy Forceps (1mm x 120cm), Karl Storz veterinary endoscopy. Goleta, CA USA

n. Weasel Wire (0.025in.) Angled/regular taper 180cm stiff hydrophilic, Infiniti Medical TM, Menlo Park, GA

o. Weasel Wire (0.035in.) Angled/regular taper 180cm stiff hydrophilic, Infiniti Medical TM, Menlo Park, GA

p. Ureteroscope Flex X2 Fiberscope (8.8 Fr, 2.9mmx100cm), STORZ, Goleta, CA, USA


2.7 References


Wiseman JL. Observation of the stimulating influence of temporary rubber splinting on regeneration following ureteral resection. Br J Urol. 1934;6;11-16.
2.8 Figure Legend

Figure 1
Mean ureteral diameters of the cranial ureteral segment as evaluated by computed tomographic excretory urography performed every other week.

Figure 2
Mean ureteral diameters of the mid ureteral segment as evaluated by computed tomographic excretory urography performed every other week.

Figure 3
Mean ureteral diameters of the caudal ureteral segment as evaluated by computed tomographic excretory urography performed every other week.

Figure 4
Cystoscopic image of urethro-bladder junction of dog 2. A wire is inserted in the right ureteral opening (black arrow). The left ureteral opening (white arrow) is visible just beside the right one.

Figure 5
Endoscopic image of the removal of the indwelling ureteral stent (arrow) with a forcep (F).

Figure 6
Endoscopic image of the ureteroscopy.

Figure 7
Transverse CT excretory urogram images (dog 4-right ureter part of group 1, left ureter part of group 2) of the cranial (A and B), mid (C and D) and caudal (E and F) ureters before (A, C, E) and 4 weeks following passive ureteral dilation of the right ureter (B, D, F).
2.9 Table Legend

Table 1
Summary of means (standard deviations) and ranges of ureteral diameters (mm) for each ureteral segment (cranial, mid and caudal) in 5 dogs with stents removed at weeks 2 (group 1) and 6 (group 2).

Table 2
Cranial, mid, and caudal ureteral segment diameter measurement p-value comparisons at weeks 2 to 10 for group 1 (ureteral stent removed at week 2) compared to ureteral diameter measurements obtained at weeks 0, 2 and 4. A p-value of < 0.05 is considered significant and marked with an *.

Table 3
Cranial, mid, and caudal ureteral segment diameter measurement p-value comparisons at weeks 2 to 10 for group 2 (ureteral stent removed at week 6) compared to ureteral diameter measurements obtained at weeks 0, 2, 4 and 6. A p-value of < 0.05 is considered significant and marked with an *. 
2.10 Figures

Figure 1

![Figure 1](image1)

**Mean ureteral diameter (mm)**

- **Group 1** (blue diamonds)
- **Group 2** (red squares)

**Time (Weeks)**

Figure 2

![Figure 2](image2)

**Mean ureteral diameter (mm)**

- **Group 1** (blue diamonds)
- **Group 2** (red squares)

**Time (Weeks)**
Figure 3

Mean ureteral diameter (mm) vs. Time (Weeks) for Group 1 and Group 2.

Figure 4

[Image of a medical procedure or observation with arrows pointing to specific areas]
## 2.11 Tables

Table 1

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<th>Stent removal Weeks</th>
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CHAPTER III

EVALUATION OF BIOFILM ON INDWELLING URETERAL STENTS
CHAPTER III
EVALUATION OF BIOFILM ON INDWELLING URETERAL STENTS

3.1 Introduction:
Indwelling ureteral catheters are used in veterinary medicine as well as in human medicine for a variety of reasons, including treatment of malignant or benign ureteral obstructions, prior to or after ureteroscopy, following extracorporeal shock wave lithotripsy, or ureteral surgeries. They are usually well tolerated in both human and veterinary patients. In veterinary patients, the complication rate is low and when noted, complications are usually minor and not life threatening. Minor procedures or medical treatment are usually sufficient to control complications such as dysuria, hematuria, pollakiuria, and urinary tract infection that occur. Dysuria, stent encrustation, migration, stent occlusion, hematuria and urinary tract infection after ureteral stent placement are observed in <2%, 2%, 5%, 10%, 6% and 10% or less, of cases respectively.

In humans, the presence of invasive devices such as ureteral stents has been reported to increase the risk of both urinary tract infection and bacteriuria and ureteral stent-associated bacteriuria (shedding of bacteria in urine). The longer the stent remains indwelling, the higher the rate of colonization and the greater the chance to develop bacteriuria. The colonization rate may be as high as 71.4% after 6 weeks of the stent placement. Bacteriuria may or may not lead to clinical urinary tract infection. In humans, symptomatic infection is more commonly observed than asymptomatic bacteriuria. In veterinary medicine, bacteriuria following placement of indwelling urinary devices has been documented. Catheter-associated urinary tract infection can occur as in humans, and may also be associated with significant morbidity in dogs, while some patients remain free of clinical signs.
Bacteria are thought to ascend through and around the catheter within 24-48 hours. Once bacteria gain access to the urinary tract, bacteriuria increases rapidly, and faster than in healthy, non-catheterized patients. The risk of developing clinical signs of urinary tract infection is greater with prolonged indwelling time, but a single catheterization can cause a urinary tract infection in female dogs. In two reports, _Escherichia coli_ was the most frequently identified bacteria causing catheter-associated urinary tract infection.

Bacterial colonization and biofilm formation on ureteral stents is regularly observed in human medicine. Biofilm is comprised of a community of microorganisms that are encapsulated in a self-produced matrix of polysaccharide. Biofilm is of particular concern on abiotic surfaces and biofilm-producing bacteria could become a complicating factor in the management of ureteral stent-associated bacteriuria or infection. Once biofilm has formed, the microorganisms attached to the ureteral stent may detach. In certain conditions (ie: overcrowding, lack of nutrients) or following cell growth and division or due to removal of biofilm aggregates, sessile organisms can detach from the biofilm. Presence of planktonic organisms may eventually be associated with bacteriuria, clinical infection and clinical signs of urinary tract disease. Despite colonisation of the ureteral stents and presence of bacteriuria, the rate of clinical urinary tract infection may be low, as low as 4.6% despite stent colonization in 42.9% in one study.

The purpose of this study was to evaluate bacteriuria, and changes indicative of clinical infection following stent placement in dogs and observe for any evidence of colonization or biofilm formation on ureteral stents _in vivo_.

### 3.2 Material and methods

This study was approved by the University of Guelph’s Animal Care Committee. Five, 3-year old female spayed (n=4) or intact (n=1) Beagles were enrolled and deemed healthy based on history, physical examination, prothrombin time/partial thromboplastin time
(PT/PTT), complete blood count, biochemistry, urinalysis, urine culture (from ultrasound-guided cystocentesis) and urogenital ultrasonography.

Every 2 weeks, dogs were sedated, an IV catheter was placed and general anesthesia induced in order to perform a computed-tomographic excretory urography (CTEU) for the purpose of another study. The CTEU was performed prior to any manipulation associated with ureteral stent placement/removal. At T0, after the CTEU, dogs were transferred to the surgery suite and placed in dorsal recumbency with the limbs and tail hanging over the end of the table. An area from the pubis to the dorsal aspect of the vulva was clipped and aseptically prepared and cephazolin (22 mg/kg) was administered IV. A 14.5 Fr rigid cystoscope with a 30 degree lens was advanced into the bladder through the urethra and both ureteral papillae were identified. A 0.025 or 0.035-inch diameter angle-tipped hydrophilic guide wire was advanced through the working channel of the cystoscope. Under cystoscopic guidance, and fluoroscopic guidance, the first side to be stented was randomly chosen. A ureteral catheter (4 or 5 Fr), appropriately sized to the patients ureteral orifice was advanced over the appropriately sized guide wire into the mid/distal ureter. A retrograde ureteropyelogram (RUPG) was performed to localize the renal pelvis using 1-2 mL of iohexol contrast material, diluted 1:1 with sterile 0.9% saline. The catheter markings were used to determine the length of the ureter and an appropriate sized double pigtail ureteral stent was placed. One pigtail loop was placed in the renal pelvis and the other was pushed into the urinary bladder. The procedure was repeated on the contralateral side.

Bupivacaine (0.3 mg/kg) diluted in 3ml of 0.9% saline was infused into the urethral lumen while removing the cystoscope. Enrofloxacin was administered (10 mg/kg, IM) at recovery followed by once a day oral administration (mean 9.42 mg/kg, range: 8.55-10.42 mg/kg), PO q24h) for 17 days. Intravenous fluids (lactated Ringer’s solution) were administered for 12 hours (40 ml/hr) postoperatively. Buprenorphine was administered (0.02 mg/kg IV q8h) two to three times post-procedure based on pain assessments followed by 3 days of oral tramadol (50 mg PO q8h). Two weeks after the stent placement, 1 ureteral stent (side randomized) was removed. Six weeks following stent placement the contralateral ureteral stent was removed. The anesthetic and CTEU protocols, surgical site preparation, analgesia, and perioperative antibiotic treatment
were identical for ureteral stent placement and removal. For ureteral stent removal, a routine transurethral cystoscopy was performed. The distal loop of the ureteral stent was grasped with endoscopic forceps and pulled to the level of the vulva with the cystoscope. Under fluoroscopic guidance, an appropriately sized (0.035 if ureteral stent 4.7Fr or 0.025 inch if ureteral stent 3.7Fr) angle-tipped stiff hydrophilic guide wire was passed through the ureteral stent and advanced into the ureter, to the level of the renal pelvis, confirming stent patency. The ureteral stent was removed over the wire, while the wire was left in place in the ureter. For the purpose of another study, immediately following stent removal, a 2.9 mm flexible ureteroscope was advanced over the guide wire under fluoroscopic guidance to the level of the mid-ureter while performing constant irrigation (saline 0.9%). The guide wire was then removed and the ureteroscope was advanced to the level of the UPJ where the renal pelvis was visualized, and then pulled back at the level of the UVJ. Enrofloxacin administration was discontinued 3 days following the first stent removal.

At weeks 2, 4, and 6, complete blood count and biochemistry were performed. Urine was collected at the same timepoints by cystocentesis for urinalysis and urine culture. Any sample with 3 white blood cells or more per high power field was qualified as pyuria.

Ureteral stents removed were aseptically manipulated and immediately cut into 4 even sections. The distal segment of the stent was discarded. Each section was subsequently divided again into 2 portions, and randomly assigned for assessment of the presence of biofilm with sonication or scanning electronic microscopy (SEM). Sonication was performed in order to quantify adhered bacteria based on the protocol of Slobbe et al (2009). Stent segments were rinsed three times in 9 mL of sterile phosphate buffered saline (PBS) to remove non-adherent bacteria. They were then placed in 10 ml of tryptone soy broth (TSB), and sonicated for 2 minutes at a frequency of 40 000Hz. Following sonication, broth was vortexed, and serial 10-fold dilutions were made in TSB. Three Colombia blood agar plates were inoculated with 100 ul, for each 10-fold dilution. Each plate was incubated at 35 degrees for 48 hours. If growth was noted,
bacteria were enumerated by counting colonies and multiplying by the dilution factor. Colonies were subcultured and pure growth was identified using matrix-assisted laser desorption/ionization (time-of-flight mass spectrometer).

For SEM, cut sections of the stent (n=3) were rinsed three times in sterile PBS. Sections were perfusion-fixed in 4.0 % glutaraldehyde in 0.07 M Sorensen’s buffer (pH 7.4) at room temperature. Subsequently, catheter sections were washed in phosphate buffered saline for 15 min before post-fixing in 1 % osmium tetroxide, prepared in Sorensen’s phosphate buffer, pH 6.8, for 1 h. A further 15 min wash 0.07 M buffer was carried out before samples were dehydrated in an ascending ethanol series (50%, 70%, 80%, 90%, 100 ethanol). Finally, sections were dried with a Critical Point Dryer through CO₂. The samples were gold/palladium sputter-coated prior to visualization using Hitachi S-570 scanning electron microscope at an accelerating voltage of 10 kV. The three sections of the ureteral stent were evaluated. For each section, at least twenty areas, randomly chosen, of the inner and the outer surfaces of the ureteral stent were evaluated. Areas were evaluated for the presence or absence of adhered organisms.

Descriptive statistics were performed to evaluate the presence of biofilm with sonication and SEM. Cohen’s Kappa statistic was performed in order evaluate the agreement between urine culture and sonication.

3.3 Results
The complete blood count and biochemistry parameters remained within reference intervals throughout the study. On urinalysis, pyuria was detected in 3 of 30 samples collected. Dog 4 had evidence of pyuria at week 6. Dog 5 had evidence of pyuria at weeks 2 and 6. Positive culture and pyuria were present concurrently in 2/3 (67%) of samples with pyuria, but only 2/27 (7%) samples without detectable pyuria. Two dogs were noted to have a positive urine culture at least once during the study period. Positive urine culture was noted on 5 of 30 (17%) samples collected during the study. Dog 4 had a positive urine culture on 3 occasions. *Enterococcus faecium* was isolated on each occasion, at week 4 (>10^5 cfu/ml), week 6 (>10^5 cfu/ml) and week 8 (1.3x10^4 cfu/ml).
Dog 5 had a positive urine culture on one occasion at week 6. *Streptococcus canis* was isolated ($>10^5$ cfu/ml). No clinical signs of lower urinary tract disease were identified in dogs with positive urine cultures.

After sonication, there was no growth noted from any stents at T2. However, at T6, bacteria were identified after sonication for 2 of 5 (40%) dogs: dogs 4 and 5, the same dogs for which urine cultures were positive at that timepoint. The concentrations of adherent bacteria liberated via sonication were $10^1$ CFU/mL for the distal part of the ureteral stent for dog 4 and $10^2$ CFU/mL, $10^1$ CFU/mL, $10^1$ CFU/mL for the cranial, mid and distal part of the ureteral stent, respectively, for dog 5. The same bacteria as were found in the corresponding urine samples, *Enterococcus faecium* (dog 4) and *Streptococcus canis* (dog 5), were identified. The Kappa coefficient was 0.76, indicating substantial agreement between urine culture results and sonication results.

Examination of the surface of the ureteral stents removed at T2 and T6 by SEM did not reveal any evidence of bacterial colonization or biofilm formation on the ureteral stent at any location (proximal, mid or distal) from any of the dogs.

### 3.4 Discussion

The results of this study indicate that bacteriuria occurs commonly in dogs with ureteral stents and that it may be associated with pyuria. It was expected that bacteriuria would be common in this study population; however, clinical evidence of disease was not identified at any time, highlighting the potential for subclinical (and potentially clinically irrelevant) colonization of both the ureteral stent and urine.

Biofilm formation on indwelling devices can be evaluated various ways. One approach is to assess adherent bacteria, based on the assumption that those are present within biofilm. After six weeks of indwelling time, sonication identified a small concentration of adherent bacteria on stents from two dogs. Those results were consistent with urine culture as demonstrated by the high coefficient of the Copen’s Kappa statistic result, indicating that urine culture is a reasonable indicator of the status of the stent.
Interestingly, and in contrast to these canine data, urine cultures were positive only in 27% of human patients with colonized stents, \textsuperscript{Reid 1992} likely because of low level or intermittent shedding of biofilm-associated bacteria. The commonness of bacterial colonization is consistent with human literature, where bacterial colonisation and biofilm formation on ureteral stents are regularly observed. \textsuperscript{Keane 1994, Bonkat 2011, Rahman 2010, Reid 1992, Minardi 2008}

For example, 90% of ureteral stents inserted for 5 to 128 days after extracorporeal shock wave lithotripsy had adherent pathogens observed on them in one study. \textsuperscript{Reid 1992} Results of the study reported here are consistent with one human study that reported stent colonization rate of 23.5 and 33% in ureteral stents remaining indwelling for less than 4 weeks and for 4-6 weeks, respectively. \textsuperscript{Rahman 2010}

Adherent bacteria were identified through sonication, yet neither adherent bacteria nor biofilm matrix were evident on SEM. This could suggest the presence of low levels of adherent bacteria or heterogenous colonization of the stents (with failure to visualize colonized segments). Sonication would detect biofilm formation on any part of the analysed catheter, as opposed to SEM, where focal areas of limited biofilm formation could potentially be overlooked. It is also possible that the bacteria identified after sonication were not truly adherent, but were not removed during rinsing, particularly bacteria within the stent lumen. It is also possible that adherent bacteria were removed during sample preparation for SEM.

While negative urine culture cannot rule out the presence of biofilm on a urologic device such as a stent, agreement between urine culture and sonication culture was high, with positive urine culture results from all samples where bacteria were identified via sonication. This might suggest that culture is a reasonable indicator of the likelihood of stent contamination or colonization.

Dogs were treated prophylactically with enrofloxacin during the initial study period, a commonly used (but unvalidated) approach. This could have accounted for the lack of evidence of colonization or biofilm after 2 weeks as fluoroquinolones have been
demonstrated to prevent biofilm formation on ureteral stents in humans. Minardi 2008, El-Feky 2009, Reid 2001

Limitations of this study include the short duration of ureteral stenting. Longer durations, similar to those used clinically, may have lead to more evidence of biofilm production on the ureteral stents because of a greater time for colonizing bacteria to form biofilm and a greater likelihood of colonization with a biofilm forming strain as well as greater time without antibiotic exposure. The small sample size also limited the conclusions that can be made about the prevalence of colonization and likelihood of biofilm formation.

In conclusion, subclinical bacteriuria may occur following ureteral stent placement in dogs, within a few weeks of stent placement. Biofilm was not visible by SEM during this time period but adherent bacteria were likely present, perhaps as a precursor to subsequent detectable biofilm.

3.5 Footnotes:

a. Cystoscope  Hopkins Rigit Cystoscope 2.7mm STORZ, Karl Storz Veterinary Endoscopy, Goleta, CA
b. Weasel Wire (0.025in.) Angled/regular taper 150cm standard hydrophilic, Infiniti Medical TM, Menlo Park, GA
c. Weasel Wire (0.035in.) Angled/regular taper 180cm standard hydrophilic, Infiniti Medical TM, Menlo Park, GA
d. Tigertail™ Flexible Tip Ureteral Catheter, diameter 4Fr, Length 70cm, Bard Polyurethane Ureteral Catheters, Basrd, C.R> Bard, Inc., Covington, GA
e. Tigertail™ Flexible Tip Ureteral Catheter, diameter 5Fr, Length 70cm, Bard Polyurethane Ureteral Catheters, Basrd, C.R> Bard, Inc., Covington, GA
f. Iohexol, Omnipaque, iohexol injection USP 65%, GE Healthcare Canada Inc, Mississauga, Ontario
g. Ureteral Stent 3.7 Frx18cm: Small dog kit, Infiniti Medical™, Menlo Park, CA
h. Ureteral Stent 4.7 Frx20cm: Large dog kit, Infiniti Medical™, Menlo Park, CA
i. Ureteral stent 4.7 Fr, 22-32cm, InLay Optima Multilength, Bard Inc, Covington, GA
j. Weasel Wire (0.025in.) Angled/regular taper 180cm stiff hydrophilic, Infiniti Medical TM, Menlo Park, GA
k. Weasel Wire (0.035in.) Angled/regular taper 180cm stiff hydrophilic, Infiniti Medical TM, Menlo Park, GA
l. Ureteroscope Flex X2 Fiberscope (8.8 Fr, 2.9mmx100cm), STORZ, Goleta, CA
m. Sterile phosphate buffered saline, Sigma-Aldrich, Oakville, ON, Canada
n. Tryptone soy broth, Oxoid, Nepean, ON, Canada
o. Ultrasonic cleaner, Branson 2510, Branson Ultrasonics Corp., Danbury, CT, USA
p. Fisher Vortex Genie 2, Fisher Scientific, Ottawa, ON, Canada
q. Colombia blood agar plates, Oxoid, Nepean, ON, Canada
r. Gold/palladium sputter-coated, Emitech K550 sputter-coater, Ashford, Kent, UK
s. Hitachi S-570 scanning electron microscope Hitachi High Technologies Inc. Tokyo, Japan

3.6 References:


CHAPTER IV

EVALUATION OF THE BIOFILM-PRODUCING ABILITY OF
ENTEROCOCCUS FAECIUM AND ENTEROCOCCUS FAECALIS ISOLATED FROM
DOGS
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DOGS

4.1 Introduction
Enterococci are facultative, anaerobic, catalase negative, gram-positive cocci that are
important opportunistic pathogens. They are leading causes of hospital-associated
infections in human medicine, with Enterococcus faecalis and Enterococcus faecium
most commonly involved. Enterococcal infections are also commonly identified in companion animals, particularly in hospital-
associated infections. Some infections can be difficult to treat, particularly those
associated with invasive devices such as urinary catheters and intravenous lines, and
surgical implants. One potential complicating factor in elimination of infections is bacterial biofilm production. Bacteria within biofilms are
relatively protected from the immune system and antibiotics, complicating elimination of
infection and creating a potential nidus for chronic infections. Biofilm-embedded bacteria
can tolerate extreme concentrations of some antimicrobials, 10 to 1000 times more than
planktonic bacteria would require to be killed.

Enterococcus faecalis is responsible for 80-90% of human enterococcal infections, with
E. faecium being involved with the majority of the remaining infections. The biofilm producing ability of E. faecalis and E. faecium isolates recovered from
human infections has been studied, and while results are somewhat variable, biofilm
production is commonly reported amongst both species, with higher rates for E.
faecalis.

Despite the importance of enterococcal infections and increasing awareness of the
potential role of biofilm in infections in dogs, little is known about the biofilm-forming
ability of important enterococcal species from canine infections. The objective of this
study was to evaluate the biofilm-producing ability of enterococci isolated from clinical infections in dogs.

### 4.2 Materials and methods

Seventy isolates of *E. faecalis* and *E. faecium* that were previously isolated from dogs were studied. Isolates were from animals with clinical infections and were epidemiologically unrelated. *In vitro* biofilm production was evaluated using a quantitative spectrophotometric microtitre plate assay. Isolates were sub-cultured onto Columbia blood agar overnight and pure growth was inoculated into tryptone soy broth (TSB) supplemented with 1% glucose to achieve a turbidity equivalent to a 0.5 McFarland standard (approximately $1.5 \times 10^8$ CFU/mL). Isolates were tested in triplicate. For each isolate, 200 uL of inoculated broth was added to 3 wells of a 96 well polystyrene microtitre plate and incubated overnight (35-37°C). Following incubation, well contents were discarded and wells were washed three times with phosphate buffered saline (pH 7.2) to remove planktonic cells. The adherent (biofilm embedded) cells were heat fixed at 60°C for 60 minutes, then dyed with 150uL of 0.1% crystal violet for 15 minutes at 22°C. Wells were rinsed with tap water to remove excess crystal violet and allowed to air-dry for 30 minutes at 35°C, then the stain was re-solubilized with 150 uL of 95% ethyl alcohol. The optical density (OD) of each well was measured at a wavelength of 570 nm (OD$_{570}$) with a spectrophotometer (BioTek ELx8000-BioTek Instruments, Inc.,Winooski, Vermont,USA). Three wells were used as negative controls, and contained only TSB plus 1% glucose. The net optical density of each isolate was obtained by subtracting the mean OD$_{570}$ of negative control from the mean OD$_{570}$ of the triplicates. Isolates were classified as non-, weak or strong biofilm producers if the net OD$_{570}$ was $< 0.120$, $0.120 < \text{OD}_{570} < 0.240$ or $\text{OD}_{570} > 0.240$, respectively.

Descriptive statistics were calculated. The mean biofilm absorbance values were run through a general linear mixed model. A Shapiro Wilk test and residual analysis were used to check for normality. Dependent on normality a Wilcoxon Mann Whitney test or a student’s t test was performed. Categorical comparisons were performed using Fisher’s
exact test. All analyses were performed using SAS OnlineDoc® 9.2 (SAS Institute Inc. 2007 Cary, NC: SAS Institute Inc). A p value < 0.05 was considered significant.

4.3 Results
Twenty three isolates of *E. faecalis* and 47 isolates of *E. faecium* were tested. A boxplot of the net optical density of both species is displayed (Figure 1). Overall, 36/70 (51%) isolates produced biofilm; 20/23 (87%) *E. faecalis* and 16/47 (30%) *E. faecium* ($P<0.0001$). When biofilm production was assessed using continuous data (OD values) there was also a significant difference between species, with the mean OD and standard deviation (SD) being 0.119 +/- 0.120 for *E. faecium* and 0.821 +/- 1.269 for *E. faecalis* ($P<0.0001$).

When only biofilm producers are considered, 14/20 (70%) biofilm-producing *E. faecalis* isolates were classified as strong biofilm producers compared to 6/16 (38%) *E. faecium* (Figures 2 and 3) ($P=0.09$). There was a significant difference in the degree of biofilm formation between species when OD values were evaluated with the mean OD and SD being 0.943 +/- 1.329 for *E. faecalis* and 0.238 +/- 0.141 for *E. faecium* ($P=0.04$). Four isolates were identified as producing very high levels of biofilm (OD$_{570}$ 1.156-3.874), with all four being *E. faecalis*.

4.4 Discussion
Biofilm production might be an important virulence factor in some types of infections, and *in vitro* biofilm production was common amongst this collection of clinical isolates from dogs. The majority of the *E. faecalis* isolates evaluated in this study produced biofilm, with the majority of those classified as strong biofilm producers. Included amongst those were four *E. faecalis* isolates that produced particularly high levels of biofilm.

These results are similar to studies of enterococci from humans, where the majority of *E. faecalis* isolates (57-100%) have been identified as producing biofilm, compared to a minority (16-48%) of *E. faecium* isolates. Baldassari 2001, Toledo-Arana 2001, Sandoe 2003, Dupre 2003
Another study of human isolates reported lower biofilm production rates, with biofilm production identified in only 26% of the *E. faecalis* and none of the *E. faecium* isolates. Various reasons could account for these differences, including differences in study populations and differences in methods. In particular, culture media (e.g. glucose concentration) and conditions likely play major roles.

This study only assessed *in vitro* biofilm formation, and questions always arise about the relevance of *in vitro* study to the more complex *in vivo* situation. This study does not indicate that these strains form biofilm during clinical infections; however, it demonstrates that they have the ability to form biofilm, at least under some conditions. While the clinical relevance of this is currently unclear, the potential that biofilm formation could be an important virulence factor in some enterococcal infections has been previously suggested.

Regulation of biofilm production by enterococci is poorly understood and is likely multifactorial. Various genes can play a role in different biofilm formation steps, and study of potentially relevant genes such as *esp*, *gelE*, *fsr*, *epa*, *atn*, *icaA* and *bopD* has been reported in human isolates. The enterococcal surface protein gene *esp* is reported to contribute and promote biofilm production; however, additional factors are involved as some studies have revealed that *esp*-negative isolates were able to produce biofilm while some *esp*-positive did not. Genetic manipulation studies revealed that gelatinase (*gelE*), an extracellular zinc metalloprotease, is determinant for biofilm formation. However, epidemiological studies failed to confirm a similar link between gelatinase and biofilm formation. The *fsr* locus in *E. faecalis*, containing *fsrA*, *fsrB*, *fsrC* genes has been noted to influence biofilm production while *fsr* mutants were noted to have a decrease biofilm production. Earlier studies revealed an interaction with gelatinase but later, it was shown that *fsr* has an effect on biofilm that is independent of gelatinase. This highlights the complexity of biofilm production and the need for further studies to better understand and characterise the complex interactions between environmental factors, as well as genetic factors and signals.
Biofilm formation is a common in vitro property of clinical enterococcal isolates from dogs, especially amongst E. faecalis. While the clinical relevance of this is currently unclear, the potential that biofilm formation could be an important virulence factor in some enterococcal infections must be considered.

4.5 References:


Di Rosa R, Creti R, Venditti M, et al. Relationship between biofilm formation, the enterococcal surface protein (Esp) and gelatinase in clinical isolates of Enterococcus faecalis and Enterococcus faecium. FEMS microbiol lett. 2006;256:145-150.


4.6 Figure legend

Figure 1
Boxplot of *Enterococcus faecium* (n=47) and *Enterococcus faecalis* (n=23) by net optical density. The bottom and top of the box represent first and third quartiles, respectively. The bar inside the box represent the second quartile/median. The bars outside the box, below and above the box represent the minimum and maximum, respectively.

Figure 2
Classification of *Enterococcus faecium* isolates based on biofilm-forming ability.

Figure 3
Classification of *Enterococcus faecalis* isolates based on biofilm-forming ability.

4.7 Figures

**Figure 1**

Boxplot of *Enterococcus faecium* and *Enterococcus faecalis* by net optical density.
Figure 2: Classification of Enterococcus faecium isolates based on biofilm-forming ability.
**Figure 3:** Classification of *Enterococcus faecalis* isolates based on biofilm-forming ability

- **61%** Strong biofilm producer
- **26%** Weak biofilm producer
- **13%** Non biofilm producer
CHAPTER V

SUMMARY AND CONCLUSIONS
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SUMMARY AND CONCLUSION

Ureteroscopy has become a first choice procedure in human patients for diagnosing and treating ureteral obstructive disease. Unfortunately, ureteroscopy is not commonly performed in veterinary medicine because of the small diameter of the canine and feline ureters. However, ureteral stenting, is commonly used as a minimally invasive option for the treatment of dogs with obstructive uropathies (e.g., urogenital malignancies, ureteral uroliths, and strictures), following extracorporeal shock wave lithotripsy, or following ureteral surgery. Ureteral stenting has similar indications in humans and is also used in children to induce passive ureteral dilation and facilitate future ureteroscopy. To our knowledge, passive ureteral dilation secondary to ureteral stent placement has never been confirmed in dogs. If ureteral stenting induces passive ureteral dilation, ureteroscopy could potentially be used to diagnose and treat ureteral diseases in dogs.

The first main objective of this research was to document if passive ureteral dilation occurs in dogs following ureteral stenting and if this would allow ureteroscopy without the need of further dilation. Our main finding was that passive ureteral dilation does occur in healthy dogs following at least 2 weeks of ureteral stenting. The dilation seems to persist for 4 weeks before the ureteral diameter starts to decrease over time. Furthermore, ureteroscopy performed at the time of stent removal was easily and successfully performed in (Beagle) dogs and without any complications.

These findings confirmed our hypotheses that: (1) passive ureteral dilation occurs after a short period of ureteral stenting; (2) passive ureteral dilation is reversible over time; and (3) ureteroscopy can be successfully and safely performed in healthy dogs after ureteral stenting. These results indicate that ureteral stenting is an efficient option to induce passive ureteral dilation to facilitate ureteroscopy in 10 kg dogs. The presence of a plateau of ureteral diameter following stent removal at week 2 was unexpected. It is unclear if this is due to the study population or if a plateau usually occurs following stent removal. This finding needs further investigation to clarify if there is such a physiologic
response following ureteral stent removal. If this is the case, it could have significant clinical implications. For example, persistence of the ureteral dilation would allow ureteroscopy to be performed at the time of stent removal but also several days following stent removal if necessary. It is unclear at this time if the persistence of ureteral dilation may be associated with complications, like predisposing patients to ureterovesical reflux, ascending infection in the upper urinary tract, etc.

Limitations of the above study include the small sample size and the use of healthy dogs. Morbidity may be higher in clinical patients and further studies are needed to better assess the behavior of diseased ureters and whether an obstructed ureter would dilate to the same degree as a normal ureter. Furthermore, it is unclear if a ureteroscope can be successfully passed in smaller dogs's ureters.

Bacterial colonization and biofilm formation are currently increasingly recognized as a playing roles in complications following placement of indwelling urinary devices. Bacterial biofilm may be associated with significant morbidity and it is difficult to eradicate once established; therefore biofilm formation on catheters and stents offer significant challenges to urologists. Limited studies have evaluated the development of bacterial biofilm in dogs following placement of indwelling devices and none have evaluated it following placement of ureteral stents.

In our study two dogs had subclinical bacteriuria characterized by positive urine cultures but without significant pyuria or clinical signs. The same organisms (multi-drug resistant Enterococcus faecium and Streptococcus canis that was susceptible to all drugs tested) were isolated from the stents after their removal, but the urine cultures of these dogs were negative after stent removal. Our study dogs might have developed clinical signs of urinary tract infections if the ureteral stents had been left in place for longer than 6 weeks; however, during the 6 weeks in which the stent remained indwelling, none of the dogs developed clinical signs associated with a urinary tract infection. The low morbidity in our study may be explained by the short period of stenting, the absence of concurrent
or historical urinary tract disease, the limited virulence of colonizing bacteria and the enrofloxacin administered following ureteral stent placement.

As partially described above, we assessed the ureteral stents of the research dogs both with and without bacteriuria for the presence of biofilm. Adherent bacteria were identified through sonication in the dogs with bacteriuria, yet neither adherent bacteria nor biofilm matrix were evident on SEM. This could suggest the presence of low levels of adherent bacteria or heterogenous colonization of the stents (with failure to visualize colonized segments). It is also possible that the bacteria identified after sonication were not truly adherent, but were not removed during rinsing. Therefore, a false positive result due to contamination and growth of planktonic bacteria cannot be ruled out as a potential cause for the presence of microorganisms after sonication. While a negative urine culture cannot rule out the presence of biofilm on a urologic device such as a stent, agreement between urine culture and sonication culture was high, with positive urine culture results from all samples in which bacteria were identified via sonication. This suggests that culture might be a reasonable indicator of the likelihood of stent contamination or colonization; however, the small sample size of our study must be taken into consideration as a limitation of this conclusion. Further studies are needed to better characterize the implications of ureteral stent colonization with uropathogens and the subsequent development of bacterial biofilm.

Enterococci are common pathogens isolated in hospital environments and their ability to produce biofilm has been evaluated in humans but little is known about their biofilm-producing ability in dogs. In order to discriminate between the presence of planktonic bacteria and the presence of biofilm, clinical isolates derived from dogs were evaluated for their ability to produce biofilms in vitro. This study addressed the third main objective of this thesis: to evaluate clinical, canine enterococcal isolates for their ability to form biofilm in vitro. We found that Enterococcus faecium and Enterococcus faecalis isolates, were often able to produce biofilm in vitro, with E. faecalis a stronger biofilm producer than E. faecium. The clinical relevance of this is currently unclear, but it is likely that a ability to produce biofilm would be present in clinical situations (i.e. in vivo).
Enterococcus species and their biofilm-producing ability should be considered as a potential virulence factor, especially in situations where a bacterial infection is difficult to eradicate.

In conclusion, passive ureteral dilation occurs in dogs following ureteral stenting, which acts as a minimally invasive tool to allow ureteroscopic access the to ureters in small dogs. Next, we showed that the ureteral stents remaining indwelling for prolonged period of time are at risk of being colonized by adherent bacteria. Lastly, we showed that Enterococcus faecalis, isolated from dogs, is a strong biofilm producer. Further studies are needed to better characterize the presence of bacterial biofilm on ureteral stents in dogs.

Future research would include evaluating the degree of passive ureteral dilation in dogs with ureteral diseases as well as in cats without ureteral diseases to see if similar degree of ureteral dilation are detected. Furthermore, as ureteroscopy was noted to be safe and successful, further research may focus on the ability to diagnose and treat ureteral diseases. For example, laser lithotripsy or basket removal of ureteral calculi may be attempted in the future as a definitive treatment for dogs with ureteral calculi. Also, some enterococcal isolates were noted to be strong biofilm producers. Further research investigating enterococcal biofilm formation would include evaluating their ability to produce biofilm on ureteral stents as well as evaluating genes involved in its production and their virulence factors.

References