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Validation of screening examinations of the ureteral orifices in dogs: comparison of ultrasonography with dissection

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Abstract

In dogs, ultrasonography is performed to locate the ureteral orifices in the urinary bladder, but reference values for their normal location using this technique are missing. In this study, the ureterovesical-vesicourethral and inter-ureterovesical distances were determined in 20 freshly euthanized medium size dogs by detecting artificially produced ureteral jets in color-flow Doppler ultrasonography at two different bladder volumes, and comparing them to manual measurements in the dissected bladder. All distances determined by ultrasonography were in agreement with values found by dissection \( (P > 0.100) \). With increasing bladder volume only the left ureterovesical-vesicourethral distance changed \( (P = 0.041) \). The right ureteral opening was more cranial than the left in 16 dogs. The inter-ureterovesical distances differed by gender \( (P = 0.016) \), but spay/neuter status had no influence \( (P > 0.847) \). In conclusion, ultrasonography is a reliable modality for screening ureteral orifices in medium size dogs and agrees with anatomical findings.

Keywords: dog, ectopic ureter, ureteral orifice, bladder, ultrasound, dissection

Introduction

Ectopic ureters (EU) are rare congenital malformations characterized by one or both ureteral orifices opening in a location other than the trigone of the urinary bladder (Owen, 1973a). The ureter normally passes through the layers of the dorsal bladder wall, and after a short intramucosal course, it opens into the bladder with a slit-shaped orifice, the Ostium ureteris. The two ureteral openings together with the internal urethral orifice form the Trigonum vesicae (Nickel et al., 2004). EU in dogs has previously been described as intravesicular or extravesicular, which
refer to ureteral orifices within the urinary bladder elsewhere than the trigone or outside the bladder in the caudal urogenital tract, respectively (North et al., 2010). In clinical cases of canine EU, ureteral orifices were most commonly found in the urethra or in the bladder neck (Canizzo et al., 2003; Samii et al., 2004; Reichler et al., 2012). EU in dogs is present as unilateral left or right side with equal frequency, or as bilateral (Holt and Moore, 1995; Canizzo et al., 2003). Currently, EUs are categorized into intramural and extramural depending on their course, with the intramural form being more common (Holt and Moore, 1995; Cannizzo et al., 2003; Davidson and Westropp, 2014). Intramural EUs contact the dorsal bladder wall at the appropriate site, but instead of opening into the lumen after a short submucosal course, they tunnel further caudally within the bladder wall and terminate with an orifice in the bladder neck or in the urethra. Extramural EUs completely by-pass the bladder and convey urine directly to more caudal parts of the urogenital tract.

In dogs, single system ectopic ureters are predominant (Holt and Moore, 1995), while ureteral duplication with ectopia, which is the most common form in humans (Berroca et al., 2002) and in those cases commonly associated with a duplex kidney, is extremely rare in the dog (Newman and Landon, 2014). A prevalence of 0.016% has been reported for canine ureteral ectopia (Smith et al., 1981; Dean et al., 1988), but certain breeds are known to have a higher risk e.g., the Siberian Husky, Newfoundland, West Highland White Terrier, Fox Terrier, Skye Terrier, Miniature and Toy Poodles, Labrador and Golden Retrievers (Hayes, 1984; Holt and Moore, 1995). In previous studies, two Swiss national breeds, the Entlebucher and Appenzeller Mountain Dogs were over-represented (Bitterli, 2011; Reichler et al., 2012). Due to the increased frequency of EU in certain breeds and families, a genetic coherence is suspected. Recently, the hereditary basis of the condition was demonstrated in the Entlebucher Mountain Dog in a large multicenter
study using complex segregation analysis, and the involvement of a major gene was suspected regarding the extravesicular EU phenotype (Fritsche et al., 2014).

In the past, ureteral ectopia was regarded as a condition predominantly affecting bitches (Hayes, 1984; Holt and Moore, 1995). Since then, EU is also increasingly described in males in the veterinary literature (Lamb and Gregory, 1998; Reichler et al., 2012). The most prevalent clinical symptom of EU is urinary incontinence (UI). In bitches, incontinence most often manifests itself already in puppyhood, while in some males UI may occur at a more advanced age (Holt and Moore, 1995; Reichler et al., 2012). The urethra in male dogs is longer and the closing pressure is usually high enough to maintain continence. In cases of ectopic ureteral openings located in the prostatic urethra, urine flow may be retrograde back into the bladder and the animal becomes incontinent only when urethral tone declines with age (Holt and Moore, 1995).

Several modalities are available for the diagnostic work-up of EU in dogs. Contrast radiography of the urinary tract including intravenous excretory urography (IVU) and retrograde urography (RU) are seldom used nowadays. IVU allows assessment of the size of the renal pelvis and the size and course of the ureters. However, in one study, a definite diagnosis of EU was obtained only in 70% of the cases when IVU was used alone (Samii et al., 2004). Dilution of the contrast medium given as an intravenous drip improves visibility of the ureters and prevents renal shutdown (Owen, 1973b). During RU, concentrated contrast medium is instilled directly into the caudal urethra and bladder, but retrograde filling of the ureters is unlikely unless they are abnormally dilated. Consequently, this modality catches only a fraction (47%) of affected ureters (Samii et al., 2004). In both methods, diagnostic specificity is restricted due to insufficient
accumulation of the contrast medium and/or superimposition in the pelvic region (McLoughlin and Chew, 2000).

Ultrasonography is a non-invasive and commonly used method to assess the anatomical integrity of the urogenital tract. It allows accurate examination of the distal ureters and ureterovesical junctions, however, the proximal part of the ureters may be difficult to visualize except when abnormally dilated (e.g., hydroureter) (Lamb and Gregory, 1994 and 1998; Elliot and Grauer, 2007). The ureteral orifices are identified by urine jets entering the bladder (Lamb and Gregory, 1994). It is challenging to see them in grey-scale ultrasound images and therefore the color-flow Doppler mode is often used to enhance accuracy. Furthermore, intravenous infusion of a crystalloid solution or the administration of diuretics (Lamb and Gregory, 1998) may allow the production of more dilute urine and facilitate visibility of ureteral jets. Advantages of ultrasonography compared to other diagnostic modalities are its simplicity, rapidity, repeatability, lack of need for general anaesthesia, and as a result, lower costs.

Helical computed tomography (CT) excretory urography represents one of the most precise, complex and extensive modalities. It gives an accurate overview of the structures and the course of the entire urogenital tract without superimposition (Rozear and Tidwell, 2003; Samii et al., 2004). CT is reported to have 94% diagnostic accuracy and is recommended especially for preoperative surgery planning (Samii et al., 2004). Magnetic resonance imaging is a useful thin slice imaging technique for small and delicate structures such as the ureters. Its superior soft tissue contrast makes it even more accurate than CT (Arora et al., 2007). However, CT and MRI require general anesthesia, they are time-consuming and are expensive.

Cystoscopy of the lower urinary tract is a useful aid in the clinical work-up of EU, but its accuracy is only 75% and there are no standardized, objective measurements to diagnose ureteral ectopia with this method (Samii et al., 2004). Cystoscopy allows close inspection of the lower
urogenital tract and assessment of the severity of existing abnormalities (Canizzo et al., 2003). However, the structures of interest are evaluated subjectively, which requires a great deal of expertise from the clinician.

Ultrasonographic examinations are performed regularly in dogs suspected with EU during diagnostic work-up, or in individuals of certain breeds designated for breeding, where a genetic predisposition is presumed (Bitterli, 2011; Fritsche et al., 2014). There are no studies in the veterinary literature that assessed the normal distances between the ureteral openings and the vesicourethral junction by ultrasonography in medium size dogs. Furthermore, data evaluating the impact of bladder distension are scarce (Rozear and Tidwell, 2003). The aim of the present study was to determine the location of ureteral orifices in freshly euthanized medium size dogs with artificially produced ureteral jets in color-flow Doppler ultrasonography, and to investigate the influence of bladder volume on the ureterovesical-vesicourethral and inter-ureterovesical distances in two different filling stages. As a control and to evaluate the precision of ultrasonography, measurements obtained during scanning were compared to those taken manually after dissection of the bladder.

Material and methods

Canine cadavers of different breeds with a body weight (BW) of 15 to 36 kg were included in the study. Intact and spayed/neutered dogs of both genders were represented. Euthanasia was carried out in the Small Animal Hospital of the Vetsuisse Faculty for reasons unrelated to the study. Dogs with urinary tract diseases were excluded. All examinations and dissections were performed immediately after euthanasia and before the onset of rigor mortis.
**Urethral catheterization**

The cadaver was placed in dorsal recumbency and a Foley catheter (8 Fr – 12 Fr depending on gender and anatomy) was introduced into the urethra ensuring easy input of water to the bladder and avoiding loss through the urethra (Fig. 1). After the bladder was emptied of all remaining urine, the tip of the catheter including the balloon was positioned in the cranial urethra to avoid interference with ultrasonography, and water was instilled into the balloon to prevent displacement.

**Coeliotomy and catheterization of the ureters**

The abdominal cavity was opened by standard coeliotomy. The bladder was lifted caudally and the ureters and their course in the surrounding adipose tissue were visualized. The cranial parts of the ureters were separated from the fat for easier handling, and after a clean transverse cut, a Jackson catheter (3 Fr) for cats was inserted into the lumen of each ureter and fixed with stay sutures (Fig. 2).

**Ultrasonography measurements at different bladder distensions**

The empty urinary bladder was filled with 5mL/kg BW normal tap water through the Foley catheter. This condition was defined as *moderately filled bladder*. Ultrasonography was performed with a LOGIQ e GE Healthcare machine using a linear 8L-RS trapezoidal probe (4.0-12.0 MHz) placed directly onto the caudoventral portion of the urinary bladder without pressure (Fig. 3). By injecting water into the Jackson catheter, urine jets were simulated artificially, and the ureteral orifices were visualized in two-dimensional color-flow Doppler-mode. In the longitudinal view, the reference point indicating the beginning of the urethra was the cranial pole of the prostate in males, and the first point distal to the bladder from which the urethral walls ran
parallel in females (Rozear and Tidwell, 2003). The distances from the left and right ureteral orifices to the vesicourethral junction were measured in the longitudinal plane (Fig. 4A).

For the inter-ureterovesical distances, both ureteral orifices were clearly visualized in the transverse plane, and separate images of each orifice in the exact same position of the probe were created (Fig. 4B). The two images were compared and the distance between the left and right ureterovesical junction was measured on the screen. After capturing at least three measurements of each distance, the bladder was filled with an additional 5mL/kg BW tap water, adding up to a total of 10mL/kg. This bladder condition was defined as full bladder. Ultrasonography measurements were repeated as described above.

Measurements in the dissected bladder

A ventromedial incision was performed through the layers of the bladder wall and further expanded cranially and caudally with scissors. After identification of the ureteral orifices, measurements on the right and left side from the ureterovesical to the vesicourethral junction as well as between the left and right ureteral orifices were made manually in the opened bladder with a spacer ruler (Fig. 5).

After completion of ultrasonography and manual measurements, the abdomen of the cadavers was closed with adequate sutures.

Data collection and analysis

Microsoft Excel 2010® was used to record data, and statistical analyses were performed using StatView 5.0® (SAS Institute Inc., Cary, NC, USA). Age and body weight were analyzed as continuous variables, and gender and spay/neuter status as nominal or categorical variables.
Correlations between measurements and age or body weight were calculated. The distribution of gender and spay/neuter status was analyzed by Chi-square test. Age and weight by gender and by spay/neuter status were compared by t-test. ANOVA repeated measures was used to analyze the effect of the method (ultrasonography at two bladder distensions and dissection) on the ureterovesical-vesicourethral distance on the right and the left sides and on the inter-ureterovesical distance. Gender and spay/neuter status were considered as the main between-subject factors. Upon detecting a significant main effect, data were further analyzed with the Bonferroni/Dunn post-hoc test. The difference between measurements by gender and spay/neuter status were evaluated by the Mann-Whitney U test. Values are presented as median and 95% confidence interval (CI). A P-value ≤ 0.05 was considered significant.

Results

Distribution of body weight, age, breed and spay/neuter status

Twenty dog cadavers with a median BW of 28 kg (95% CI 24-31 kg) were included in the study, from December 2011 until January 2013. The median age of the dogs at the time of euthanasia was 11 years (95% CI 9-13 years). Most breeds were represented by one individual except for Golden and Labrador Retrievers (n=4 and n=3, respectively).

Six of the eleven females were spayed, and four of the nine males were neutered. Spay/neuter status was similar within gender (P = 0.653). Age and BW did not differ by gender (P = 0.732 and P = 0.398, respectively) or by spay/neuter status (P = 0.455 and P = 0.646, respectively).
Ureterovesical-vesicourethral and inter-ureterovesical distances at different bladder distensions and after bladder dissection

All ureterovesical-vesicourethral distances on the right and on the left sides were within the range of 2.15 to 4.84 cm (median 3.26 cm, 95% CI 3.09 to 3.45 cm) and 1.13 to 4.34 cm (median 3.07 cm, 95% CI 2.78 to 3.16 cm), respectively. Individual values are shown in Fig. 6. The right ureterovesical-vesicourethral length was similar in all three measurement (P = 0.28; Fig. 7). By ultrasonography, the median distance from the right ureteral orifice to the vesicourethral junction was 3.26 cm (95% CI 2.89 to 3.52 cm) in the moderately filled bladder, 3.23 cm (95% CI 2.92 to 3.61 cm) at full distension and 3.30 cm (95% CI 3.05 to 3.65 cm) by manual measurement in the dissected bladder.

The left ureterovesical-vesicourethral distance differed between the measurements (P = 0.041; Fig. 8). The length between the left ureterovesical and vesicourethral junctions in the dissected bladder (median 3.10 cm, 95% CI 2.64 to 3.26 cm) was similar to the values obtained by ultrasonography in the moderately filled and full bladder (P = 0.368 and P = 0.100, respectively), but it was shorter (P = 0.013) in the moderately filled bladder (median 2.89 cm, 95% CI 2.57 to 3.19 cm) than in the fully distended one (median 3.09 cm, 95% CI 2.69 to 3.47 cm).

The inter-ureterovesical distances ranged from 0.27 to 3.4 cm (median 1.74 cm, 95% CI 1.54 to 1.92 cm) and did not differ between measurements (P = 0.307).

Comparison of the right and left ureterovesical-vesicourethral distances

The location of the two ureteral orifices in the bladder was not at the same level in any of the dogs (P = 0.034). A clear difference between the right and left ureterovesical-vesicourethral distance was detected in the dissected bladder (P=0.011), but not by ultrasonography in the moderately filled (P = 0.091) or full bladder (P = 0.345). The ureteral orifice on the right side was
further cranial from the internal urethral orifice than on the left side in 16 of 20 dogs. When the
difference between the left and right ureterovesical-vesicourethral distance obtained by the three
measurements (full and moderately filled bladder, bladder dissection) was averaged for each of
these 16 dogs, the right ureteral opening was located 0.40 cm more cranially than the left (95%
CI 0.28 to 0.63 cm).

Influence of gender, spay/neuter status and body weight on the ureterovesical-
vesicourethral and inter-ureterovesical distances

The distances from the right and from the left ureteral orifices to the vesicourethral junction were
similar between males and females (P = 0.736 and P = 0.454, respectively). In contrast, the inter-
ureterovesical distance differed by gender (P = 0.016), and was longer in females than in males
when measured by ultrasonography in the moderately filled and full bladder (P = 0.021 and P =
0.015, respectively), but not in the dissected bladder (P = 0.074; Fig. 9).

Spay/neuter status and body weight had no effect on any of the measurements (P ≥ 0.847 and P ≥
0.272, respectively).

Discussion

Common modalities for imaging the ureteral orifices in dogs are excretory urography in CT as
the gold standard, and transurethral cystoscopy (Canizzo et al., 2003; Rozear and Tidwell, 2003;
Samii et al., 2004). Both methods were used in clinical cases of EU and validated by surgical
findings (Canizzo et al., 2003; Rozear and Tidwell, 2003; Samii et al., 2004). They are usually
well accepted by owners of clinically affected dogs when performed during diagnostic work-up.
To facilitate owner compliance in the screening examinations of progeny testing for breeding
purposes, less invasive methods without the need for general anesthesia are preferred. Doppler
ultrasonography was described as a useful diagnostic tool in clinical settings, and recently it has also been successfully used for EU testing of future breeding dogs in certain breeds (Bitterli, 2011; Fritsche et al., 2014). However, this modality has not yet been validated, and the physiological range of the distances from the ureterovesical to the vesicourethral junction as well as the inter-ureterovesical length in normal, unaffected dogs has not been determined.

For clinical screenings of the ureteral openings, it is important to know if the filling stage of the bladder has any influence on the distance measurements. If this were the case, screening examinations should include emptying the bladder and then refilling it with a known amount of fluid. Fortunately, the ureterovesical-vesicourethral and inter-ureterovesical distances did not differ considerably between measurements at the two bladder distensions. Furthermore, ultrasonographic measurements were also similar to those in the dissected bladder. Therefore, localization of ureteral orifices by ultrasonography is representative of the actual anatomical situation of the animal. Moreover, these results also confirm the diagnostic utility of color-flow Doppler-ultrasonography for screening EU in dogs.

We found a wide range of ureterovesical-vesicourethral and inter-ureterovesical distances within the same weight category of dogs, which may be considered normal, as only animals without urinary tract diseases were included. Part of this variation may be due to the difficulty in defining the beginning of the urethra. In our study, we used the attachment of the prostate to the urethra in males, and the first point from where the walls of the urethra ran parallel in females as reference points to increase the reliability of the measurements. However, it is still a subjective assessment, although the variations do not seem to be of clinical relevance. There are only a few standard values described in the veterinary literature for normal lengths between the ureteral orifices and
the internal urethral orifice, or between the two ureteral openings. Very similar ranges were
found by Rozear and Tidwell (2003) regarding the distance from the ureterovesical junction to
the internal urethral orifice using helical computed tomographic excretory urography in seven
healthy dogs between 20 and 30 kg. The consistency of these findings confirms that
ultrasonography is a valid modality for the screening of EU. The ranges for the ureterovesical-
vesicourethral distances reported here can be used as reference values for screening examinations
of dogs between 15 and 36 kg within progeny testing. On the other hand, there seems to be a
discrepancy in the inter-ureterovesical distances obtained by helical computed tomographic
excretory urography and our ultrasonography and manual measurements, as the previously
described values (Rozear and Tidwell, 2003) were higher and their range lower compared to our
findings. Therefore, the reliability of the inter-ureterovesical measurements in the present study
may be questioned. However, this parameter has little or no clinical relevance, because the wide
physiological range does not allow differentiation between normal and ectopic ureteral openings.
Furthermore, long inter-ureterovesical distances may occur with unilateral ectopia, and short
distances with bilateral ectopia.

It has previously been reported that the two ureterovesical junctions are not at the same level in
most dogs (Rozear and Tidwell, 2003). We also showed the more cranial opening of the right
ureter within the bladder, as the right ureterovesical-vesicourethral distance was longer than the
left. This is not surprising considering the more cranial position of the right kidney due to its
embryologic development. The right ureter and its junction at the bladder wall are therefore also
situated further cranially.
An increase in the ureterovesical-vesicourethral distance depending on bladder volume was seen by ultrasonography for the left but not for the right side. This volume dependency disagrees with the results of a study assessing ureteral openings and bladder volume in six dogs using helical computed tomographic excretory urography (Rozear and Tidwell, 2003). However, measuring ureterovesical-vesicourethral distances for ureteral ectopia screenings without exact standardization of bladder volume in dogs that did not urinate shortly before presentation still seems to be reliable, because the difference between the two bladder filling phases was less than one tenth of the total distance.

We found that the inter-ureterovesical distance was significantly longer in females than in males when measured by ultrasonography, but not in the dissected bladder. During dissection, the bladder is emptied, which possibly reduces pressure and distension of the wall, hence no difference was detected. Longer inter-ureterovesical distance due to increased bladder volume was described before, but no influence of gender was reported (Rozear and Tidwell, 2003). The shorter length in males in our study may be explained by less flexible Trigonum vesicae, because the prostate holds the internal urethral orifice within firm limits. Interestingly, we found no difference in the inter-ureterovesical distance between neutered and intact males despite the fact that the size of the prostate is reduced after castration (Smith, 2008).

All dogs were relatively old at the time of euthanasia. Fourteen animals were over ten years of age, and only one was younger than five, which might be a limitation of our study. However, it is very unlikely that the anatomical location of the ureteral orifices changes with age, and furthermore, age was not identified as an influencing factor in any of the measurements.
The use of cadavers allowed direct inspection of the anatomy of the urinary bladder and clear visualization of the ureteral orifices. Additionally, experimentation on live animals was also avoided. Impairment of muscle flexibility appears with *rigor mortis*, which represents a state when all adenosine triphosphate is depleted, and consequently muscles become rigid (Paredi et al., 2012). To prevent methodical bias by using dog cadavers, all examinations were done immediately after euthanasia and before the onset of *rigor mortis*. Although *post-mortem* processes may have somewhat impaired tissue quality, all measurements would have been affected to the same degree and thus comparisons are still valid.

## Conclusion

Color-flow Doppler-ultrasonography is a feasible diagnostic modality for localization of the ureteral openings. In medium size dogs, the ureterovesical-vesicourethral distances may normally range between 2.15 to 4.84 cm on the right side and 1.13 to 4.34 cm on the left side, and can be used as reference values. Taking these measurements as absolute standards with millimeter precision should, however, be avoided.

## Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

## Acknowledgements

We are very grateful for the kind help of Jeanne Peter with the illustrations.
References


**Figure captions**

**Fig. 1.** A Foley catheter size 10 Fr placed in the urethra of a male neutered dog

**Fig. 2.** Jackson catheters (3 Fr) placed in the ureters of a dog cadaver

**Fig. 3.** A linear 8L-RS trapezoidal probe is placed on the caudoventral portion of the urinary bladder while creating artificial urine jets through the Jackson catheter within the left ureter

**Fig. 4.** Ultrasonographic images of the ureteral orifices by simulated urine jets:

A: The left ureteral orifice is visualized by a jet in the longitudinal plane of two-dimensional color-flow Doppler mode in the moderately filled bladder in a female dog cadaver; B: Left and right ureteral orifices in transverse view in two-dimensional color-flow Doppler mode, and measurement of the inter-ureterovesical distance; ureter links: left ureter, ureter rechts: right ureter

**Fig. 5.** Manual measurement of the inter-ureterovesical distance with a caliper in the dissected urinary bladder

**Fig. 6.** Simplified illustration of the bladder and ureteral orifices (n = 20). Dots of the same color in each image represent the ureteral openings of one animal within the respective groups: A - intact females, B - spayed females, C - intact males, D - neutered males
Fig. 7. Distance between the right ureteral orifice and the vesicourethral junction in the moderately filled and full bladder and after bladder dissection (n=20). The horizontal line in the box represents the median, the bottom and top of the box are the first and third quartiles, respectively, and whiskers show the maximum and minimum values. MFB: moderately filled bladder (5mL/kg), FB: full bladder (10mL/kg), BD: bladder dissection.

Fig. 8. Distance between the left ureteral orifice and the vesicourethral junction in the moderately filled and full bladder and after bladder dissection (n=20). The horizontal line in the box represents the median, the bottom and top of the box are the first and third quartiles, respectively, and whiskers show the maximum and minimum values. MFB: moderately filled bladder (5mL/kg), FB: full bladder (10mL/kg), BD: bladder dissection.

Fig. 9. Inter-ureterovesical distances in the moderately filled and full bladder and after bladder dissection in male (n = 9) and female (n = 11) dogs. The horizontal line in the box represents the median, the bottom and top of the box are the first and third quartiles, respectively, and whiskers show the maximum and minimum values. MFB: moderately filled bladder (5mL/kg), FB: full bladder (10mL/kg), BD: bladder dissection.