transitional cell carcinoma of the urinary bladder is estimated to affect between 20,000 and 30,000 dogs each year in the United States. This is the most common form of urinary tract cancer and accounts for approximately 2% of all cancers in dogs. Treatment of urinary bladder cancer may involve surgical excision, radiation therapy, chemotherapy, or a combination of methods. Methods currently used to evaluate the response of bladder tumors to treatment include DCC, conventional 2-D ultrasonography, CT, and potentially MRI; however, each of these imaging modalities has disadvantages or limitations. Computed tomography is expensive, and DCC is time-consuming and can be inaccurate because of variability in positioning and distension of the bladder during imaging. Both of these methods require sedation or general anesthesia as well as urinary catheterization and bladder distension, which are associated with risk of urinary tract rupture in dogs with TCC. Cystoscopy is often used to inspect and map lesions of the urethra and bladder and obtain a biopsy sample; however, measurements of tumor size cannot be made and anesthesia is typically needed. Advantages of conventional 2-D ultrasonography include a shorter examination time than that required for DCC or CT and more readily available equipment, compared with CT. Sedation or anesthesia is rarely needed in 2-D ultrasonography, but to provide acceptable data, 2-D ultrasonography requires that the same operator perform every examination, that the patient be positioned in the same way for each evaluation, and that bladder distension be consistent during multiple examination.

Accuracy of three-dimensional and two-dimensional ultrasonography for measurement of tumor volume in dogs with transitional cell carcinoma of the urinary bladder

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Objective—To determine the accuracy of 3-D and 2-D ultrasonography for quantification of tumor volume in dogs with transitional cell carcinoma (TCC) of the urinary bladder.

Animals—10 dogs with biopsy-confirmed TCC.

Procedures—The urinary bladder of each dog was distended with saline (0.9% NaCl) solution (5.0 mL/kg), and masses were measured via 3-D and 2-D ultrasonography. Masses were also measured via 3-D ultrasonography after bladders were distended with 2.5 and 1.0 mL of saline solution/kg. Subsequently, the bladder was deflated and distended with CO₂ (5.0 mL/kg); CT was performed after IV contrast medium administration. Tumor volumes were calculated via 3-D ultrasonography, 2-D ultrasonography, and CT (reference method) and compared via ANOVA, Deming regression, and Bland-Altman plots. Repeated-measures ANOVA was used to assess effects of bladder distension on 3-D tumor volume measurements. Repeatability of measurements was estimated via the coefficient of variation for each method.

Results—Repeatability was considered good for all 3 methods. There was no significant difference in tumor volume measurements obtained via 3-D ultrasonography at different degrees of urinary bladder distension. Results of Deming regression and Bland-Altman plots indicated excellent agreement between tumor volume measurement with 3-D ultrasonography and CT, but not between 2-D ultrasonography and CT.

Conclusions and Clinical Relevance—Tumor volume in dogs with TCC of the urinary bladder was accurately measured via 3-D ultrasonography. Use of 3-D ultrasonography can provide a less expensive and more practical method for monitoring response to treatment than CT and was more accurate than 2-D ultrasonography. (Am J Vet Res 2012;73:1919–1924)
procedures. Therefore, some variability exists in the measurement of tumors to determine the response to treatment even under controlled circumstances. Three-dimensional ultrasonography has been used in humans to stage urinary bladder tumors and measure bladder volume. We hypothesized that 3-D ultrasonography would provide an accurate, noninvasive, and clinically practical method for measuring tumor volume in dogs with TCC of the bladder. Therefore, the primary objective of the study reported here was to determine the accuracy of 3-D ultrasonography for quantification of tumor volume in the urinary bladder of dogs with TCC, compared with the reference method of contrast-enhanced CT. A secondary objective was to determine the accuracy of 2-D ultrasonography for this same purpose.

Materials and Methods

Animals—Ten mature dogs with TCC of the urinary bladder were prospectively enrolled in the study from the population of clinical patients admitted to the Purdue University Veterinary Teaching Hospital between February 1, 2010, and January 31, 2012. Owner consent, a diagnosis of TCC confirmed via histologic examination of a biopsy sample by a board-certified veterinary pathologist, and intraluminal tumor location were required for study inclusion. Dogs with tumors of urethral or prostatic origin were excluded. The bladder was imaged in dorsal recumbency and the hind limbs extended toward the gantry. All fluid was removed from the bladder, which was subsequently insufflated with CO2 (5 mL/kg, administered through the urinary catheter) to maximize contrast between the tumor and surrounding bladder wall or residual urine. The caudal aspect of the abdomen was then scanned from approximately the caudal border of the left kidney to the pelvic urethra in 1.25-mm slices immediately following IV contrast medium administration (iopromide [2 mL/kg]). Computed tomographic data were stored for later analysis. Carbon dioxide was then aspirated from the bladder lumen, the catheter was removed, and the dog was monitored during recovery from anesthesia.

Volume calculations—Tumor volume was determined via 2-D ultrasonography by drawing a perimeter outline defining the largest cross-sectional area in the sagittal plane (Figure 1). Volume was then estimated by multiplying the largest cross-sectional area in the sagittal plane by the maximum medial-to-lateral dimension in the transverse plane; this method effectively models the tumor as a cylinder for volume determination. Volume measurements were determined 3 times for each tumor to provide an estimate of repeatability.

Three-dimensional ultrasonographic tumor volumes captured via ultrasonographic equipment were imported into a proprietary software program. This program allowed visualization of the mass in 3 planes (dorsal, sagittal, and transverse) in which the tumor was divided into various numbers of segments along the long axis for each plane. Initially, regardless of overall tumor size, each mass was divided into 8 segments for volume calculation (Figure 2). A region of interest was drawn in each of the 8 segments via a freehand technique to define the perimeter of the mass. The software program computed the tumor volume after the perim-
eters of all 8 segments were defined. All tumors were measured via this standard procedure. Each tumor was also divided into 4 and 12 segments to determine the potential value of a fine segmentation matrix (12 segments), which was presumed to increase measurement accuracy but required more time for analysis, compared with a coarse segmentation matrix (4 segments), which was presumed to decrease measurement accuracy but required less time for analysis. Volume measurements were determined 3 times for each tumor at each level of segmentation (4, 8, and 12) to provide an estimate of repeatability.

Tumor volumes measured via CT were calculated with a dedicated workstation (Figure 3). A region of interest was drawn to indicate the perimeter of the tumor on each postcontrast 1.25-mm slice. The area of each tumor slice was then calculated by the software program via a proprietary algorithm and multiplied by the slice thickness (1.25 mm) to determine the volume of each tumor slice. The slice volumes were then summed to determine the tumor volume. Volume measurements were determined 3 times for each tumor to provide an estimate of repeatability.

Statistical analysis—Data were expressed as mean ± SD, and values of P < 0.05 were considered significant. Kurtosis and skewness were calculated, and normal distribution of the data was confirmed. Measurement repeatability was estimated by calculating the mean and range CV (%) for each of the 3 methods of tumor volume measurement. Tumor volumes measured via 3-D ultrasonography, 2-D ultrasonography, and CT of the urinary bladder distended with 5 mL of saline solution/kg were compared by means of ANOVA. Tumor volumes measured via 3-D ultrasonography of the bladder distended with 3 different volumes of saline solution (1.0, 2.5, and 5.0 mL/kg) were compared by means of repeated-measures ANOVA. Estimated tumor volumes for the 10 dogs were compared by calculating the Pearson correlation coefficient (r) and via linear regression, Deming regression, and Bland-Altman plots with CT volume as the reference method; Bland-Altman plots were constructed to determine bias (mean difference) and 95% limits of agreement (±1.96 × SD) between 2-D or 3-D ultrasonography and CT.

Results

Dogs weighed between 8.6 and 48.9 kg (mean weight, 23.9 kg) and ranged in age between 10 and 13 years (mean age, 11.7 years). There were 5 spayed females, 4 neutered males, and 1 sexually intact male. Breeds represented were 1 each of Labrador Retriever, Golden Retriever, Scottish Terrier, Shih Tzu, Border...
Collie, and Shetland Sheepdog and 4 mixed-breed dogs. Six dogs each had a single urinary bladder mass (5 at the trigone and 1 at the dorsal aspect of the bladder wall), and 4 had multiple masses throughout the bladder (including a mass at the trigone in 2/4 dogs).

Mean CVs for triplicate measurements determined with postacquisition data for urinary bladders distended with 5 mL of saline solution/kg were 3.0% (range, 1.2% to 8.1%), 6.9% (range, 0.3% to 15.8%), and 3.0% (range, 0.4% to 5.2%) for 2-D ultrasonography, 3-D ultrasonography, and CT, respectively. Repeatability was considered good for all 3 methods.

Measurements of tumor volume obtained via 2-D ultrasonography and CT were moderately correlated (r = 0.70; Figure 4). Results of Deming regression analysis indicated that the relationship between 2-D ultrasonography and CT tumor volume fit the line of identity (slope, 3.33; SE, 4.99; intercept, –3.19; SE, 2.12), but confidence intervals for the estimate were very large. Estimates for bias (1.63 cm³) and 95% limits of agreement (–1.65 to 4.91 cm³) obtained via Bland-Altman analysis indicated substantial variability in the agreement between 2-D ultrasonography and CT for estimates of tumor volume.

There was no significant (P = 0.088) difference between mean tumor volumes measured via 8-segment 3-D ultrasonography (2.10 ± 0.85 cm³) and CT (1.99 ± 0.87 cm³); however, mean tumor volumes measured via 12-segment 3-D ultrasonography (2.20 ± 0.86 cm³) and 4-segment 3-D ultrasonography (2.29 ± 0.95 cm³) were significantly (P = 0.002 and P < 0.001, respectively) different from those measured via CT. There was no significant (P = 0.95) difference among mean tumor volumes measured via 3-D ultrasonography of the urinary bladder distended with different volumes of saline solution (1.0 mL/kg [2.10 ± 0.84 cm³], 2.5 mL/kg [2.12 ± 0.85 cm³], and 5.0 mL/kg [2.10 ± 0.85 cm³]). Measurements of tumor volume obtained via 3-D ultrasonography and CT were highly correlated (r = 0.92; Figure 5).
ing regression indicated that the relationship between 3-D ultrasonography and CT tumor volume fit the line of identity (slope, 1.00; SE, 0.09; intercept, –0.04; SE, 0.15). Estimates for bias (0.11 cm³) and 95% limits of agreement (−0.62 to 0.84 cm³) obtained from the Bland-Altman plot indicated strong agreement between measurements obtained via 3-D ultrasonography and CT.

Discussion

Results of the study reported here indicated that 3-D ultrasonography is likely to provide an accurate method for measuring the volume of TCC masses in the urinary bladder of dogs. Repeatability of measurements obtained via the 3-D ultrasonographic method was evidenced by the mean CV of 6.9%, which indicated that the images were of sufficient quality to allow differentiation of the tumor from the surrounding bladder wall and bladder contents. In some dogs, clear differentiation of the tumor from the normal tissue of the bladder wall at the invasion site was difficult because of the invasive nature of the tumor. In these situations, an extrapolation or best-guess approach was used to estimate the degree of invasion and thus the perimeter of the tumor at this site. Also, in dogs with tumors that were located in the neck of the bladder or extended into the proximal portion of the urethra, some difficulty was occasionally encountered in differentiating tumor edges from the adjacent normal bladder wall or urethra because the bladder tended to collapse around the tumor with differing amounts of transducer pressure.

The numerically lower CV of 2-D ultrasonography measurements of tumor volume (3.0%), compared with that of 3-D ultrasonography (6.9%), was attributed to the excellent quality of the 2-D ultrasonography images and the necessity for 1 straight line measurement and 1 perimeter measurement in a single plane. This should be compared with the use of 8 perimeter measurements for 3-D ultrasonography, in which some margins were difficult to discern, thereby increasing measurement variability. For this reason, we recommend the use of triplicate measurements when estimating tumor volume by 3-D ultrasonography. The low CV found in CT measurements of tumor volume (3.0%) was attributed to a clear definition of the contrast-enhanced mass surrounded by either gas or small volumes of nonenhanced fluid (residual bladder contents or urine produced during the CT procedure). In these scans, luminal edges of the tumors were usually easily seen, and in cases where the mural edge was not obvious, an extrapolation or best-guess technique could be applied with relative ease.

The excellent correlation between 3-D ultrasonography and CT volume measurements as well as the results of Deming regression and Bland-Altman plots suggests that these 2 imaging modalities can be used interchangeably to estimate TCC volume in the urinary bladder of dogs. This is beneficial because the typical cost of 3-D ultrasonography is substantially less than that of CT. Additionally, imaging expense is further reduced in 3-D ultrasonography because, although sedation is occasionally needed, anesthesia is usually unnecessary. Although time to perform the various procedures may vary among referral hospitals, at our institution, the imaging procedure for 3-D ultrasonography requires approximately 5 minutes to isolate the desired tumor, optimize ultrasound machine settings, and acquire the measurements for 3-D volume, compared with 30 to 45 minutes required to sedate or anesthetize the dog, accurately position the animal in the CT machine, administer contrast medium, and acquire sufficient images to calculate tumor volume. Other potential benefits include the possibility for increased patient throughput and decreased use of ionizing radiation. An interesting finding in the present study was the accuracy of 2-D ultrasonography in estimating tumor volume, in that 2-D ultrasonography was moderately correlated with CT, although not to the same extent as 3-D ultrasonography. Three of 10 tumor volumes measured with 2-D ultrasonography were markedly different from that measured with the reference method.

We found that measurement of 3-D volumes with the use of 8 segments was optimal. The finding that 4-segment volume determination was significantly different from CT volume was expected due to the coarse nature of the segmentation and additional extrapolation by the software. However, an unexpected finding was that the use of 12 segments, a finer and presumably more accurate process, also yielded volume measurements significantly different from those obtained with CT. We cannot provide a satisfactory explanation for this finding. Perhaps the edges on 12-segment image groups were hazy enough to preclude accurate perimeter definition. Additional studies are needed to determine the source of this discrepancy.

Our finding that the extent of urinary bladder distension did not significantly affect tumor volume measurements obtained via 3-D ultrasonography was unexpected because variability in bladder distension has been reported to profoundly affect 2-D ultrasonographic measurements in some cases. The results of our study suggest that TCC tumors can be accurately measured via 3-D ultrasonography in bladders with various degrees of distension. This would be clinically beneficial to some patients because it eliminates the need for catheterization, which is currently performed in many patients at our institution to distend the bladder with a consistent volume of fluid before obtaining 2-D ultrasonographic measurements. In dogs that cannot be catheterized, this finding eliminates the need for the dogs to be kept in the clinic until the bladder fills naturally, which may be uncomfortable for dogs with large or diffuse bladder tumors or impossible for those that have minimal or no control of voiding. Despite the encouraging findings of the present study, we remain cautious regarding the potential effects of bladder distension on results of 3-D ultrasonography in some dogs with TCC of the bladder. Caution is warranted because for purposes of our study, a single well-defined mass was measured in each dog and we recognize that it will be important to measure multiple lesions in dogs with TCC to evaluate variability in tumor responsiveness to treatment. We anticipate that the degree of bladder distension could influence 3-D ultrasonographic tumor volume measurements when TCC tumors include broad or diffuse attachment to the bladder wall because.

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the extent to which the bladder wall is folded into or around the mass can affect tumor appearance and measurement. Additional studies may be indicated to confirm that bladder distension does not affect 3-D ultrasonographic measurements of tumor volume, at least over the luminal pressure ranges induced by fluid volumes of 1 to 5 mL/kg.

Individual operator experience also plays a role in the ability to accurately measure tumor dimensions and volumes. Familiarity with equipment function, transducer capabilities, image optimization, and postprocessing becomes easier as the operator gains experience and has potential to affect the results of measurements.

In the study reported here, CT was used as the reference standard. For purposes of precise margin definition in this comparison study, the bladder was distended with CO2 to maximize contrast between the mass, bladder wall, and any residual or newly produced urine. Depending on the patient’s blood pressure, IV catheter diameter, rate of contrast medium delivery, and timing of the postcontrast scan, mass opacification was occasionally suboptimal. In these patients, the surrounding lucent CO2 gas made margin delineation somewhat more efficient. Only the urinary bladder and proximal urethra were imaged via ultrasonography and CT. At our institution, most patients with TCC undergo complete abdominal ultrasonographic examination as well as abdominal and thoracic radiography at the time of initial evaluation and at 8-week intervals throughout their course of treatment. Additionally, ultrasonography of the urinary tract (including the kidneys, ureters [when detectable], sublumbar lymph nodes, bladder, detectable portion of the urethra, and prostate, if present) is performed at 4-week intervals. The examination at 4-week intervals is abbreviated primarily because of owners’ financial constraints; although there are limitations to its accuracy, the authors consider 2-D ultrasonography currently to be the most efficient and cost-effective method of restaging bladder tumors. At our institution, research level fees for 2-D ultrasonography, 3-D ultrasonography, and contrast-enhanced CT of the urinary tract in early 2012 were at $40, $54, and $450, respectively, without anesthesia-related expense, with standard clinical charges being approximately 20% higher. Although fee schedules may differ, we consider it likely that the cost differences would be similar at other institutions.

To our knowledge, 2 academic institutions currently have 3-D ultrasonography capability but its availability in private practice is unknown. Total cost for the 3-D ultrasonographic equipment used in the present study was approximately $41,000, including the cost of transducers and software. This is a potential limiting factor in the current and future availability of the equipment in veterinary practice, making it likely that this procedure may be performed only at tertiary care practices in the foreseeable future. With respect to CT, there are no special software or equipment requirements for calculation of tumor volumes. Several readily available, public domain software options are available that may be used to calculate tumor size.

Our results indicate that 3-D ultrasonography has potential for clinical use in assessment of TCC tumor volumes. Efficiency of the 3-D ultrasonographic method was similar to that of conventional 2-D ultrasonography, with slightly more time required to perform measurements with individual images. Although postexamination processing of the information required more time than that needed with 2-D ultrasonography, the increased time investment was balanced by the increased accuracy of measurements. We estimate that only 10 additional minutes, including acquisition and postprocessing time, are needed for the 3-D ultrasonographic method versus conventional 2-D ultrasonography. Additional studies are needed to determine interoperator variability, confirm that urinary bladder distension does or does not affect measurements, and confirm the clinical utility of this approach to detect response to treatment during sequential visits.

References