NOTE *Internal Medicine*

Evaluation of gallbladder volume and contraction index with three-dimensional ultrasonography in healthy dogs

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ABSTRACT. Three-dimensional (3D) ultrasonography has been shown to be an accurate and appropriate tool for measurement of gallbladder volume in humans. Therefore, we applied this novel technique for the first time to study fasting and postprandial gallbladder volume in 10 healthy dogs and compared the results with those of 2-dimensional (2D) ultrasonography. Fasting gallbladder volumes determined by 3D ultrasonography were significantly higher than corresponding volumes determined by 2D ultrasonography ($P<0.01$). Additionally, gallbladder volumes were significantly decreased in the postprandial state compared with the fasting state using 3D ultrasonography ($P<0.001$), but 2D ultrasonography showed no significant difference ($P=0.189$). The Gallbladder contraction index was higher in 3D ultrasonography than 2D ultrasonography; however, it did not reach statistical significance ($P=0.25$). In conclusion, 3D ultrasonography was able to measure gallbladder volume in healthy dogs in this study. It is suggested that 3D ultrasonography can be used to accurately estimate gallbladder volume and contractility.

KEY WORDS: canine, gallbladder contraction index, gallbladder volume, ultrasonography

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Normally, mechanical cleansing of the gallbladder has an important protective effect against the accumulation of stagnant biliary debris [22]. At meal time, neurohumoral mechanisms stimulate the contraction of the gallbladder muscular wall that coordinates with relaxation of the sphincter of Oddi [19, 22]. Structural changes or inflammation of the gallbladder can reduce gallbladder motility and impair emptying, which result in the accumulation of bile in the lumen of the gallbladder [9, 18, 22]. Due to impairment of gallbladder emptying, the gallbladder epithelium is exposed to concentrated bile acids for a long time, which is believed to play a role in the pathophysiology of biliary tract diseases, such as gallbladder mucocele, cholecystitis and cholelithiasis [27, 32]. Since impaired gallbladder emptying may eventually lead to several gallbladder diseases, evaluation of gallbladder motility requires an accurate and appropriate methodology.

Among the methods that have been used to measure gallbladder volume, real-time ultrasonography is an accurate, reproducible, noninvasive, cheap, relatively easy; and widely available method for studying gallbladder volume variations [7, 12, 19–21]. In dogs, 2 dimensional (2D) ultrasonography has been used widely to determine gallbladder volumes, mostly by the ellipsoid method [1, 3, 8, 22, 23, 27]. However, 2D ultrasonography is operator dependent, and deviations of gallbladder shape could affect the results [25, 26]. Three-dimensional (3D) ultrasonography is an emerging technology that has been developed to estimate gallbladder volumes. This method has been shown to be an accurate and appropriate tool for the measurement of organ volume in both dogs and humans [2, 6, 13–16, 24, 29, 30]. A potential advantage of 3D ultrasonography for measuring gallbladder volume is that a sagittal section of the gallbladder at its maximal diameter is not required, which may be useful in patients with irregular gallbladder shapes. Thus, the 3D ultrasonography technique may better take into account variations in gallbladder shape [17, 25]. To the best of our knowledge, thus far there have been no reports about using 3D ultrasonography for measuring gallbladder volume in healthy dogs or dogs suffering from gallbladder diseases. Therefore, the aim of this study was to assess the effect of ingestion of full-fat milk on gallbladder volume and gallbladder contraction index (GBCI) using 2D and 3D ultrasonography in healthy dogs.

Ten healthy mixed-breed dogs (4 intact males and 6 intact females) were included in this study. The dogs' body weights ranged from 18 to 33 kg (mean 26.7 kg), and their ages ranged from 2 to 5 years (mean 2.7 years). They were fed a standard diet, had free access to water and were housed under optimum condition. Health status of each dog was determined based on history, physical examination, complete blood count, serum biochemistry and ultrasonography assessment. All dogs were handled in accordance with the Guidelines for the Care and Use of Laboratory Animals as adopted by the Ethics Committee of the Faculty of Veterinary Medicine of the University of Tehran.

The dogs were fasted, but had free access to water for 12 hr (overnight) prior to the experiment. Each of the dogs was placed in dorsal recumbency, and the hair was clipped from the cranioventral abdomen; between the xiphisternum and the umbilicus and extending several centimeters on each side of the midline. Ingestion of full-fat milk (3% fat, 3.3%

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protein; and 4.6% carbohydrate, 62 kcal per 100 m*l*) was used to induce gallbladder contraction. None of our dogs had a history of lactose intolerance. In addition, they did not show any symptoms of lactose intolerance after ingestion of full-fat milk within 24 hr of follow-up. Once the fasting (baseline) gallbladder volume was measured using 2D and 3D ultrasonography, the dogs were fed full-fat milk (10 m*l*/kg body weight). The amount of full-fat milk was determined based on the previously proposed method; however, instead of solid meal, we used liquid meal [22, 27]. Postprandial gallbladder volume was recorded 20 min after ingestion of full-fat milk using 2D and 3D ultrasonography [4, 28].

Two-dimensional ultrasonographic images of the gallbladder were acquired using an ultrasound unit (Voluson 730-Pro, General Electric, Kretztechnik, Zipf, Austria) equipped with a mechanical linear transducer with a frequency range of 5–12 MHz. The ellipsoid method was used to estimate fasting and postprandial gallbladder volumes with the following formula: 0.523 × length × width × height [3, 8, 22, 27]. In brief, dogs were placed in dorsal recumbency, and ultrasonography images of the gallbladder were obtained in longitudinal and transverse planes. The gallbladder contraction index (GBCI) at 20 min after feeding was calculated as follows: GBCI=([gallbladder volume in fasting state − gallbladder volume 20 min after ingestion of full-fat milk]/gallbladder volume in fasting state) × 100. GBCI is defined as the percentage change of the gallbladder volume after feeding relative to the fasting volume.

For 3D ultrasonography, the same ultrasound unit and 5–12 MHz 3D mechanical linear trapezoid transducer were used. Gallbladder volume by the 3D ultrasonography method was calculated from the 3D data sets. During real-time scanning, the transducer was placed over the gallbladder area to visualize the longitudinal plane of the gallbladder. The sector angle and penetration depth were then optimized to the abdominal size. The frame rate, lateral resolution and axial resolution were further adjusted by adjusting the level of magnification. The volume box was then adjusted for the region of interest. For obtaining the maximum diameter of the gallbladder, the volume sweep angle was set between 30 to 40 degrees. The fastest scan duration (quality low) was adjusted to have the fastest scanning protocol. In sectional plane mode, 3 orthogonal planes (longitudinal, transverse and coronal) were simultaneously displayed on the screen (Fig. 1). Once the scan was completed, the volume data were stored on an external hard disc. The saved images were then retrieved, and the virtual organ computer-aided analysis (VOCAL) mode introduced in the 4D View 5.1 software (General Electric, Kretztechnik) was used to assess the gallbladder volume. A 30° rotational angle was used, and the gallbladder contours in the longitudinal plane, as a reference plane, were manually traced (Fig. 1). Six tracings were performed to obtain one calculation of the gallbladder's volume. From the 6 tracings of the gallbladder's border, the 4D View program produced a 3D image of the gallbladder and subsequently determined its volume.

All data are expressed as the mean ± SEM. The normality of the distributions was tested with the Shapiro-Wilk test. The correlation between gallbladder volume and body weight for measurements obtained in the fasting state was investigated by use of the Pearson's correlation coefficient and linear regression analysis. Differences in mean values between 3D and 2D ultrasonography for both the fasting and postprandial states were tested by repeated measures analysis with post hoc Bonferroni's correction. The independent Student's *t*-test was performed to determine whether there were significant differences in GBCI between males and females. Bland-Altman analysis was used to compare the GBCI (%) determined by 3D ultrasonography with that determined by 2D ultrasonography. Data are presented as the mean difference and 95% confidence limits of agreement (mean ± 95% confidence limits of agreement). Confidence limits of agreement represent lower and upper limits of 1.96 SD of mean differences. This analysis was done using MedCalc software version 13.3.3 (MedCalc Software bvba, Ostend, Belgium). All other statistical analyses were performed using the analytical software package SPSS® 16.0 for Windows® (SPSS Inc., Chicago, IL, U.S.A.). For all analyses, values of $P<0.05$ were considered significant.

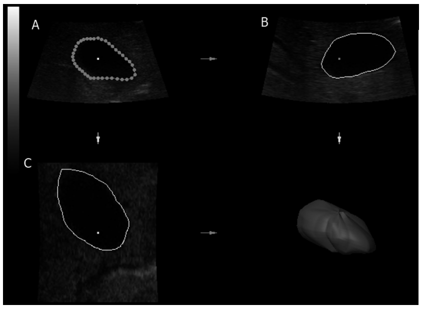

Fig. 1. Representative 3D ultrasound image of the gallbladder. The upper left image represents the longitudinal plane (the A plane), the upper right image represents the transverse plane (the B plane), and the lower left image represents the coronal plane (the C plane). A 30° rotational angle was used, and the gallbladder contours in the longitudinal plane, as a reference plane, were manually traced. Then, the VOCAL software constructed a 3D image of the gallbladder (lower right image) and automatically measured the gallbladder volume.

Fasting gallbladder volumes determined by 3D ultrasonography were significantly higher than corresponding volumes by 2D ultrasonography (1.11 ± 0.07 vs. 0.77 ± 0.06 m*l*/kg, $P<0.01$) (Fig. 2). However, there was no statistically significant difference in postprandial gallbladder volume between 3D and 2D ultrasonography (0.81 ± 0.08 vs. 0.61 ± 0.04 m*l*/kg, respectively, $P=0.117$). In addition, gallbladder volumes were significantly decreased in the postprandial state compared with the fasting state using 3D ultrasonography (1.11 ± 0.07 vs. 0.81 ± 0.08 m*l*/kg, $P<0.001$), but 2D ultrasonography showed no significant difference (0.77 ± 0.06 vs. 0.61 ± 0.04, $P=0.189$) (Fig. 2). In both 3D and 2D ultrasonography, the fasting gallbladder volume had a strong positive correlation with body weight (r=0.75 and 0.87, respectively; $P<0.05$). In addition, linear regression analysis revealed the relationship (r2=0.642) described by the following equation: fasting gallbladder volume=1.476 × body weight − 9.496 for 3D ultrasonography. For 2D ultra-

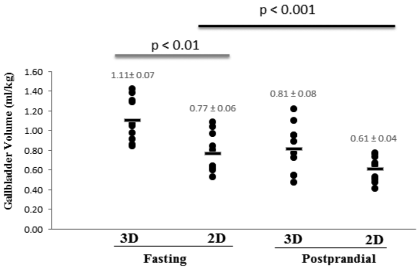

Fig. 2. Dot plot representing the fasting and 20-min postprandial gallbladder volumes of healthy dogs determined by 2D and 3D ultrasonography. The horizontal line represents the mean gallbladder volume.

sonography, linear regression analysis revealed the relationship (r2=0.56) described by the following equation: fasting gallbladder volume=1.075 × body weight − 7.707.

GBCI (%) was determined 20 min after ingestion of full-fat milk using both 3D and 2D ultrasonography. GBCI (%) was higher in 3D ultrasonography compared with 2D ultrasonography (27.28 ± 4.08 vs. 17.35 ± 6.99), however, this did not reach statistical significance (*P*=0.25). Furthermore, there was no statistically significant difference in GBCI between males and females after ingestion of full-fat milk in both 3D and 2D ultrasonography (30.28 ± 3.54 vs. 25.29 ± 6.53 and 9.14 ± 14.47 vs. 22.83 ± 6.91, respectively, *P*>0.05). In Fig. 3, the Bland-Altman plot shows a comparison of GBCI (%) by 3D and 2D ultrasonography. The bias (±SD) of the 3D ultrasonography was 9.9% (±25.5), indicating that 3D ultrasonography resulted in a higher GBCI than 2D ultrasonography. The 95% limits of agreement between 3D and 2D ultrasonography were −40.1% to 60% (Fig. 3).

Following assessment of the canine adrenal gland, thyroid gland and eyes volumes using 3D ultrasonography by our research group [2, 29, 30] and also use of this novel technique to measure gallbladder volume in humans [6, 17, 25, 26], we applied this novel technique for the first time to study fasting and postprandial gallbladder volume in healthy dogs. In the present study, we assessed the feasibility of using 3D ultrasonography for determining gallbladder volumes and compared the results to those of 2D ultrasonography with the ellipsoid method in healthy dogs.

The ellipsoid method was used in this study to measure the gallbladder volume in healthy dogs via 2D ultrasonography. Because of its practical application, this method has been used widely to determine gallbladder volumes in dogs [1, 3, 8, 22, 23, 27]. The sum of cylinders (SC) method is another technique for evaluation of gallbladder volume using 2D ultrasonography that provides a good approximation of the actual volume of the gallbladder. Even though this method has been reported to be more accurate than the ellipsoid method, it is cumbersome and time-consuming, and therefore, it is not suitable to use in clinical veterinary medicine [3, 6]. The ellipsoid and SC methods are based on a regular geometric gallbladder shape, and therefore, volume estimates in irregularly shaped gallbladders may become inaccurate [17]. Compared with the ellipsoid and SC methods, 3D ultrasonography has a better overall accuracy with a smaller systematic bias and a smaller measurement error and can measure gallbladder volume with higher accuracy and precision, particularly in irregularly configured gallbladders [6, 17].

In the current study, the majority (80%) of the healthy dogs had a 12-hr fasting gallbladder volume ≤1.00 m*l*/kg using 2D ultrasonography. Similar results have been reported by Ramsted *et al*. in healthy dogs [22], in which most (86%) healthy dogs had a gallbladder volume ≤1.0 m*l*/kg after withholding food for 12 hr. In both studies, the ellipsoid method was used to measure the gallbladder volume via 2D ultrasonography. In addition, mean fasting gallbladder volumes in healthy dogs have also been reported to be <1 m*l*/kg by other studies using 2D ultrasonography [5, 23, 27]. In our study, the postprandial gallbladder volume was decreased compared with that in the fasting state using both 3D and 2D ultrasonography; however, the difference reached statistical significance only in 3D ultrasonography. Similar to our results, Ramsted *et al*. reported lower postprandial gallbladder volumes after ingestion of solid fatty meal at different time points using 2D ultrasonography [22]. However, we used full-fat milk, not solid fatty meal, to induce gallbladder contraction in our study. Milk as a stimulus for gallbladder contraction has been used in several studies in humans [4, 11, 28]. Its ability to cause maximal gallbladder contraction faster than solid meals [11] and its availability and cheapness make it a useful stimulus for gallbladder contraction in dogs that are not lactose intolerant. In addition, in lactose intolerant dogs, lactose-free milk can be used for gallbladder contraction. In contrast to Ramsted *et al*. [22], we recorded postprandial gallbladder volume 20 min after ingestion of full-fat milk, instead of different time points. Since the ultrasound unit used in this study was also required to examine patients at the veterinary teaching hospital, postprandial gallbladder volume was estimated only 20 min after ingestion of full-fat milk. In some studies in humans, postprandial gallbladder volume has been estimated 20 min after ingestion of milk [4, 28]. In another study, around 90% of maximal gallbladder contraction was reached within 25 min after ingestion of milk with different percentages of fat (1.5 g, 3.5 g, 6.5 g and 10 g fat) in healthy human subjects [11]. Therefore, based on our study's limitation and reports from studies in humans, we decided to measure postprandial gallbladder volume 20 min after ingestion of full-fat milk as a first step of our study. However, we plan to evaluate the proper timing for estimating maximum gallbladder contraction after ingestion of full-fat milk in the dog in our future studies.

There was a strong positive correlation between fasting gallbladder volume and the body weight of the dogs using both 2D and 3D ultrasonography. Similar correlations have also been reported in healthy dogs [3, 22] and humans [10, 12], which suggests that the gallbladder volume increases with body weight. However, another study found no relationship between the gallbladder volume of healthy dogs and their body weights determined by an ellipsoid method [5]. This discrepancy may be due to differences in food-withholding intervals before measurements as well as the body weights of the dogs included in this study. In our study, food was withheld for 12 hr in all dogs, and also the weight of the dogs ranged between 18 and 33 kg; in the above mentioned study [5], however, the food withholding time was not declared and most dogs (71%) had a large body size (>40 kg). In the present study, a linear regression equation was formulated for both 2D and 3D ultrasonography to estimate the fasting gallbladder volume in healthy dogs based on their body weights. These formulas can be used for quick prediction of the gallbladder volume from the weight of the dogs; however, with the small sample size of the current study, these formulas need to be interpreted with caution.

The fasting and postprandial gallbladder volumes measured by 3D ultrasonography were larger than those measured by 2D ultrasonography; however, the difference was statistically significant only in the fasting state. In the study of Hashimoto *et al*., fasting and postprandial gallbladder volumes at different time points assessed by 3D ultrasonography tended to be larger than those assessed by 2D ultrasonography with the SC method; however, this difference did not to reach statistical significance in the fasting and postprandial states [6]. In the current study, the mean postprandial GBCI was higher in 3D ultrasonography (27.28%) than 2D ultrasonography (17.35%); however, it did not reach statistical significance. The possible reasons for this include the difficulties in determination of a large fasting gallbladder volume by 2D ultrasonography in large and deep-chested dogs and also the small number of dogs (n=10) involved in this study. Similar to our results, Hashimoto *et al*. [6] did not find a significant difference in gallbladder volume changes at different time points between 2D and 3D ultrasonography after ingestion of raw egg yolk in fourteen healthy human subjects. However, ultrasonographic measurement of gallbladder volume at different time points and use of different fatty meals to induce gallbladder contraction by various researchers in dogs [22, 27] and humans [11, 25, 28] make it difficult to compare our study's results with those of other studies. We did not find any difference in GBCI between males and females in both 2D and 3D ultrasonography. This finding is in agreement with previous studies in humans, which showed that gender does not significantly influence fat-induced gallbladder contraction [28, 31].

The Bland-Altman plot in this study showed that 3D ultrasonography estimates GBCI 9.9% higher than 2D ultrasonography. In the *in vitro* study of Hashimoto *et al.* using 10 balloons (5–68 m*l*), the overall accuracy errors for 3D ultrasonography and the SC method were 1.8% and 9.2%, respectively [6]. In addition, the mean differences between measured and the actual volumes (the systematic bias) and the limits of agreement were smaller with 3D ultrasonography (−0.56 [–2.46 to +1.34] m*l*) than with the SC method (−2.88 [–7.04 to +1.28] m*l*), indicating that 3D ultrasonography measured the balloon volume with higher accuracy and lower measurement error than the SC method [6]. However, they did not determine the bias and limits of agreement between 3D ultrasonography and the SC method in estimating gallbladder volume. In the current study, due to difficulties in determination of the true gallbladder volume noninvasively and lack of a gold standard like computed tomography (CT) and magnetic resonance imaging (MRI), we were not able to determine the accuracy of both 3D and 2D ultrasonography in estimating gallbladder volume. Therefore, the gallbladder volumes measured with 3D ultrasonography were compared with the results of 2D ultrasonography in our study. In addition, we did not perform a repeatability and reproducibility study for both ultrasonography techniques, which is another limitation of the current study. However, we plan to determine the accuracy, repeatability and reproducibility of 3D and 2D ultrasonography in measuring gallbladder volume in our future studies.

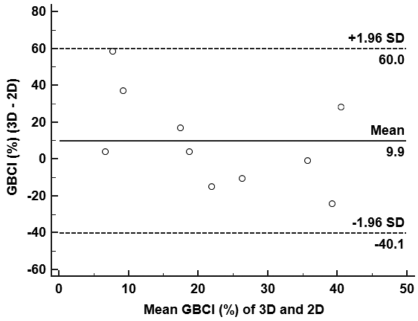

Fig. 3. Bland and Altman plot for the mean difference and 95% limits of agreement of the GBCI (%) 20 min after ingestion of full-fat milk as estimated by 3D and 2D ultrasonography.

In the current study, 2D ultrasonography was not able to catch the whole outline of the gallbladder in three dogs weighing ≥29 kg. Two-dimensional ultrasonography can only obtain longitudinal and transverse views of the gallbladder. Since these dogs had larger fasting gallbladder volumes, 2D ultrasonography was not able to obtain the whole longitudinal view of the gallbladder. Thus, an imaginary line was drawn to calculate the gallbladder volume. Three-dimensional ultrasonography was able to obtain a coronal view of the gallbladder in these dogs, in addition to the transverse and longitudinal views. Therefore, 3D ultrasonography added the coronal view of the gallbladder, enabling us to measure gallbladder volume, especially in deep-chested, large and extra-large dogs with an irregular and/or large gallbladder.

In conclusion, 3D ultrasonography was able to measure gallbladder volume in healthy dogs in this study. The fasting and postprandial gallbladder volumes of the healthy dogs obtained by 3D ultrasonography were higher than those obtained by the 2D ultrasonography method. It is suggested that 3D ultrasonography can be used to accurately estimate gallbladder volume and contractility. To determine the clinical value of 3D ultrasonography for gallbladder imaging, more research needs to be undertaken.

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