Changes in gallbladder volume in healthy dogs after food was withheld for 12 hours followed by ingestion of a meal or a meal containing erythromycin

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Objective—To assess the influence of meal ingestion and orally administered erythromycin on gallbladder volume in dogs.

Animals—22 healthy dogs.

Procedures—Ultrasonographically determined gallbladder dimensions in unsedated dogs were used to calculate volume. Measurements were recorded after food was withheld for 12 hours (time 0) and 15, 30, 45, 60, 90, and 120 minutes after a 100-g meal without (n = 22) or with erythromycin (1.0 mg/kg [7], 2.5 mg/kg [7], and both dosages [8]). Gallbladder ejection fraction represented the percentage of volume change from time 0. Intraday and interday coefficients of variation determined operator repeatability and physiologic variation.

Results—We did not detect significant differences in gallbladder volume per unit of body weight between treatments at time 0 or in ejection fraction percentage within or between treatments. Median time 0 gallbladder volume was 0.6 mL/kg (range, 0.4 to 1.9) but was > 1.0 mL/kg in 3 of 22 (14%) dogs and ≤ 1.0 mL/kg in 19 of 22 (86%) dogs. Twenty dogs achieved an ejection fraction ≥ 25% with at least 1 treatment, but 2 dogs with a gallbladder volume ≤ 1.0 mL/kg at time 0 did not. Intraday and interday coefficients of variation were 18% and 25%, respectively.

Conclusions and Clinical Relevance—Gallbladder volume ≤ 1.0 mL/kg at time 0 and ejection fraction ≥ 25% were typical. No treatment consistently induced greater gallbladder contraction. Dogs with a gallbladder volume > 1.0 mL/kg and ejection fraction < 25% may require a combined meal and erythromycin protocol. (Am J Vet Res 2008;69:647–651)
veterinary practice, and we suspect that disturbances in gallbladder emptying may play a permissive or causal role by promoting bile and mucin accumulation as well as resorption of bile fluids. This idea is not novel because it has been proposed that altered gallbladder motility (biliary dyskinesia) precedes formation of gallstones and gallbladder mucocele in some humans. However, biliary dyskinesia has been relatively unexplored in companion animals. Interestingly, minimal to no contrac tion of the gallbladder was identified during sequential postprandial ultrasonographic imaging in 3 dogs prior to progressive accumulation of gallbladder sludge and mucocele formation. The study reported here was conducted to develop a practical ultrasonographic method for assessing changes in gallbladder volume in dogs following ingestion of a meal with or without the motilin-receptor agonist erythromycin. Ultimately, this method may allow regimented prospective investigation of dogs with suspected gallbladder disease.

Materials and Methods

Animals—Twenty-two healthy dogs (3 sexually intact males, 5 neutered males, 6 sexually intact females, and 8 neutered females) belonging to faculty or staff of the College of Veterinary Medicine at Cornell University were used in the study. Breeds represented were Shih Tzu (n = 7), Border Terrier (3), Labrador Retriever (3), Australian Cattle Dog (2), Tibetan Spaniel (1), Beagle (1), Pekingese (1), Dalmatian (1), and Shetland Sheepdog (1). In addition, there were 2 mixed-breed (German Shepherd Dog crossbred) dogs. Median age of dogs was 8 years (range, 2 to 12 years), and median body weight was 11.7 kg (range, 4.3 to 26.9 kg). Health status of each dog was determined on the basis of physical examination, clinical history (no history of inappetence, vomiting, diarrhea, exercise intolerance, coughing, or unexplained weight loss or gain), lack of chronic health problems requiring medical treatment, and determination that PCV and total protein concentration were within reference ranges (38% to 54% and 5.9 to 7.8 g/dL, respectively). All owners provided written consent for inclusion of their dogs in the study, and all dogs were handled in compliance with institutional animal care and use protocols of Cornell University.

Procedures—Food was withheld from dogs for 12 hours. Ingestion of a meal without and with erythromycin was used to induce gallbladder contraction. Because ingestion of a full-sized meal can interfere with sequential postprandial ultrasonographic imaging in dogs prior to progressive accumulation of gallbladder sludge and mucocele formation, ingestion of a meal without and with erythromycin was used to induce gallbladder contraction. Beagle (1), Pekingese (1), Dalmatian (1), and Shetland Sheepdog (1). In addition, there were 2 mixed-breed (German Shepherd Dog crossbred) dogs. Median age of dogs was 8 years (range, 2 to 12 years), and median body weight was 11.7 kg (range, 4.3 to 26.9 kg). Health status of each dog was determined on the basis of physical examination, clinical history (no history of inappetence, vomiting, diarrhea, exercise intolerance, coughing, or unexplained weight loss or gain), lack of chronic health problems requiring medical treatment, and determination that PCV and total protein concentration were within reference ranges (38% to 54% and 5.9 to 7.8 g/dL, respectively). All owners provided written consent for inclusion of their dogs in the study, and all dogs were handled in compliance with institutional animal care and use protocols of Cornell University.

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Ultrasoundographic measurements—Two-dimensional ultrasonography was used by a single investigator (KLR) to determine the height, length, and width of the gallbladder of each dog. Dogs were restrained unsedated on an examination table in dorsal recumbency; all ultrasonographic examinations were video recorded to allow review of images and measurements. Gallbladder volume was calculated by use of the prolate ellipsoid equation (volume = length × width × height × 0.53). After determination of gallbladder volume, the ejection fraction (as a percentage) was calculated by use of the following equation:

\[
Ejection \ fraction = \frac{[gallbladder \ volume \ at \ time \ 0] - [gallbladder \ volume \ at \ specified \ time \ point]}{gallbladder \ volume \ at \ time \ 0} \times 100
\]

This value represented the change in gallbladder volume from initial measurements recorded after the 12-hour withholding of food. In 2 dogs from which food was withheld for 12 hours, the interday and intraday coefficients of variation were determined from serial measurements (6 and 10 measurements, respectively) to estimate the influence of operator repeatability and physiologic variability on recorded gallbladder dimensions.

Statistical analysis—The relationship between gallbladder volume and body weight for measurements obtained at time 0 was investigated by use of the Spearman rank correlation and linear regression analysis. Gallbladder volume at time 0 was adjusted on the basis of body weight to identify (by visual inspection of data) an expected cutoff value for healthy dogs. Box-whisker plots and histograms were used to evaluate the ejection fraction for Gaussian distribution. Because the data had a non-Gaussian distribution, analysis was performed by use of nonparametric methods (α = 0.05; 2-tailed P value), with a Bonferroni correction to account for multiple comparisons. The Wilcoxon signed rank test was used for paired measurements within a treatment and among treatments in a dog. The Wilcoxon rank sum test was used for comparisons between the various erythromycin doses. The Fisher exact test
was conducted by use of 2 × 2 tables to compare the number of dogs achieving gallbladder contraction (any magnitude) at each postprandial time point within and among treatments and to compare the number of dogs achieving maximal gallbladder contraction at each postprandial time point within and among treatments. The Spearman rank correlation was used to identify significant associations between body weight and fixed meal size and the maximal value for ejection fraction.

Results

The median gallbladder volume at time 0 (ie, after food was withheld for 12 hours) was 0.6 mL/kg (range, 0.4 to 1.9 mL/kg; n = 52 measurements [3 each for 8 dogs and 2 each for 14 dogs]). Most dogs (19/22 [86%]) had a gallbladder volume ≤1.0 mL/kg at time 0. The remaining 3 dogs had a median gallbladder volume of 1.5 mL/kg (range, 1.1 to 1.9 mL/kg; n = 7 measurements [3 for 1 dog and 2 each for 2 dogs]) at time 0. The coefficients of variation for gallbladder volume at time 0 within and among days were 18% and 25%, respectively. Gallbladder volume at time 0 was significantly correlated (r = 0.81; P < 0.001) with body weight; linear regression analysis revealed the relationship (r² = 0.66) described by the following equation: gallbladder volume = 3.006 + (0.425 X body weight).

Wide variation was detected among dogs for gallbladder ejection fraction within and among treatments (Table 1). There was a progressive increase in the proportion of dogs that achieved gallbladder contraction over time for each treatment (Figure 1); maximal values were attained at the later postprandial time points (data not shown). The maximal ejection fraction for a meal containing erythromycin exceeded that achieved for a meal without erythromycin in 10 of 22 (45%) dogs, was equivalent in 3 of 22 (14%) dogs, and was lower in 9 of 22 (41%) dogs. There were no significant (all P > 0.2) differences in ejection fraction percentage at time points within or among treatments. Considering cutoff values of 15%, 20%, and 25% for the ejection fraction at any time point, there were no significant differences among treatments, except that significantly (P = 0.03) more dogs achieved an ejection fraction ≥20% (7/15 [47%]) or ≥25% (6/15 [40%]) at the 15-minute measurement when fed a meal containing the higher dose of erythromycin, compared with the number of dogs achieving those ejection fractions when fed the lower dose of erythromycin (1/15 [7%] and 1/15 [7%], respectively). Comparatively, 7 of 22 (32%) and 4 of 22 (18%) dogs achieved an ejection fraction ≥20% or ≥25%, respectively, for the meal without erythromycin at the 15-minute interval. However, 4 of 6 dogs failing to achieve an ejection fraction ≥25% when fed a meal containing erythromycin did achieve an ejection fraction ≥25% when fed a meal without erythromycin. In addition, 4 of 6 dogs failing to achieve an ejection fraction ≥25% when fed a meal without erythromycin did achieve an ejection fraction ≥25% when fed a meal containing erythromycin.

For 17 of 19 (89%) dogs with a gallbladder volume ≤1.0 mL/kg at time 0, a maximal ejection fraction ≥25% was achieved with at least 1 treatment; the remaining 2 dogs did not achieve a maximal ejection fraction ≥25% after ingestion of any meal (maximum of 23% for a meal containing erythromycin [both doses] for 1 dog and maximum of 20% for the meal without erythromycin for the other dog). All 3 dogs with a gallbladder volume >1.0 mL/kg achieved a maximal ejection fraction ≥25% after a meal with or without erythromycin.

Table 1—Median (range) of the percentage change in gallbladder volume in healthy dogs after food was withheld for 12 hours and the dogs were then allowed to ingest a 100-g meal without erythromycin, a meal containing a low dose of erythromycin (1 mg/kg), or a meal containing a high dose of erythromycin (2.5 mg/kg).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>15 (min)</th>
<th>30 (min)</th>
<th>45 (min)</th>
<th>60 (min)</th>
<th>90 (min)</th>
<th>120 (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal (n = 22)</td>
<td>–12</td>
<td>–19</td>
<td>–15</td>
<td>–22</td>
<td>–27</td>
<td>–25</td>
</tr>
<tr>
<td></td>
<td>(–59 to 62)</td>
<td>(–62 to 58)</td>
<td>(–55 to 64)</td>
<td>(–51 to 33)</td>
<td>(–44 to –3)</td>
<td>(–56 to 28)</td>
</tr>
<tr>
<td>Meal containing erythromycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 mg/kg (n = 15)*</td>
<td>–15</td>
<td>–22</td>
<td>–27</td>
<td>–25</td>
<td>ND</td>
<td>–21</td>
</tr>
<tr>
<td></td>
<td>(–55 to 64)</td>
<td>(–51 to 33)</td>
<td>(–44 to –3)</td>
<td>(–56 to 28)</td>
<td>ND</td>
<td>–21</td>
</tr>
<tr>
<td>2.5 mg/kg (n = 15)*</td>
<td>–15</td>
<td>–26</td>
<td>ND</td>
<td>–21</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(–47 to 5)</td>
<td>(–60 to 45)</td>
<td>ND</td>
<td>–21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For the 2 meals that contained erythromycin, 7 dogs were fed a meal containing 1.0 mg/kg, 7 dogs were fed a meal containing 2.5 mg/kg, and 8 dogs were fed meals containing both doses of erythromycin. Time at which the meal was ingested was designated as time 0.

Figure 1.—Percentage of dogs that had measurable gallbladder contraction at various time points after food was withheld for 12 hours and were then allowed to ingest a 100-g meal without erythromycin (n = 22 [diagonal-striped bars]), a meal containing a low dose of erythromycin (1 mg/kg; 15 [black bars]), or a meal containing a high dose of erythromycin (2.5 mg/kg; 15 [horizontal-striped bars]). For the 2 meals that contained erythromycin, 7 dogs were fed a meal containing 1.0 mg/kg, 7 were fed a meal containing 2.5 mg/kg, and 8 dogs were fed meals containing both doses of erythromycin. Time at which the meal was ingested was designated as time 0.
Although no treatment or time point was consistently superior, 20 of 22 (91%) dogs achieved gallbladder contraction (maximal ejection fraction ≥ 25%) after ingestion of at least 1 treatment. There were no significant correlations between body weight and maximal ejection fraction for any treatment.

### Discussion

Control of gallbladder motor function involves a dynamic interaction between stimulatory and inhibitory hormones and neurotransmitters. Although conventional ultrasonographic imaging of the gallbladder can disclose information about static anatomic features, the technique described here offers a feasible means for investigating contractile changes in gallbladder volume in dogs.

Use of ultrasonography to estimate gallbladder contraction is dependent on the duration of the evaluation period and the frequency of evaluations. Furthermore, estimation of gallbladder volume can be complicated by the inherent limitation of methods that indirectly measure volume as well as by physiologic variability and operator accuracy. Several mathematic equations have been investigated for estimating gallbladder volume of dogs. Although the prolate ellipsoid equation is less precise than several other more complicated methods, it can nevertheless adequately estimate relative volume changes. For the study reported here, it is important to recognize that correction of estimated volumes was unnecessary because change in volume was the critical objective. In humans, wide variability in fasting gallbladder volume and lack of intra-individual reproducibility in fasting gallbladder volume have been attributed to variability in activity of the intestinal migrating motor complex. In healthy dogs, investigators of gallbladder motility also have confirmed an interdigestive bellows-like contraction.

In one of these studies, it was clearly established that ultrasonographic-determined basal gallbladder volume fluctuates by approximately 17%, similar to the intra-assay coefficient of variation for our study. Our novel finding that most (86%) healthy dogs had a gallbladder volume ≤ 1.0 mL/kg after withholding of food for 12 hours may prove to be clinically useful. In fact, we substantiated that most (86%) healthy dogs had a gallbladder volume 

Regardless, because of the weak linear relationship between body weight and gallbladder volume at time 0, we do not recommend use of this equation. Instead, on the basis of the collective data from our study and from another study, we propose that gallbladder volume in healthy dogs after a period of food withholding is typically ≤ 1.0 mL/kg. Because in the study reported here we specifically measured gallbladder volume after food was withheld for 12 hours and at specific postprandial time points, it remains unclear whether a longer duration of food withholding would substantially alter gallbladder volume.

Ultrasonographic assessments of gallbladder contractility in dogs have been used to characterize the response to complicated provocative treatments (eg, IV administration of cholecystokinin, caerulein, or oleandomycin and gavage administration of magnesium sulfate) or after ingestion of a meal. In other studies in dogs, investigators have reported meal-induced gallbladder contraction of up to 60% within 60 minutes after ingestion and an estimated time for 50% gallbladder evacuation of 32 minutes. Radioactive tracers were used in a small number of dogs to determine that the gallbladder empties most rapidly during the first 30 minutes after meal ingestion and maximally contracts within 120 minutes. Our results yielded a similar physiologic response because we detected a progressive increase in gallbladder contraction over time with maximal values attained at the later postprandial time points (Figure 1). Because there were no significant correlations between body weight and maximal ejection fraction for any treatment, it is unlikely that meal size in relation to body weight influenced our results. We also detected no difference between large (≥ 15 kg) and small (< 15 kg) dogs with regard to the magnitude of gallbladder evacuation in response to any treatment.

Rather than imposing gavage treatments (ie, magnesium sulfate) or IV injections of uncommonly used medications (eg, cholecystokinin), we sought to develop a method that could be easily and safely used in most veterinary practices. Erythromycin, a macrolide antimicrobial, acts as a motilin-receptor agonist to stimulate gastrointestinal motility in dogs from which food has been withheld. Oral administration of erythromycin at doses of 5 to 10 mg/kg in lasting humans has a prokinetic action on the gallbladder within 15 minutes after administration and achieves an ejection fraction of 32% by 30 minutes. Although there is a dose-dependent effect of erythromycin on gallbladder contraction in humans, we encountered adverse effects on the gastrointestinal tract with erythromycin doses ≥ 5 mg/kg in healthy dogs. Even though a lower dose was used, meals that contained erythromycin stimulated gallbladder evacuation in 4 of 6 dogs that had failed to have adequate gallbladder contraction (ejection fraction ≥ 25%) after ingestion of a meal without erythromycin.

Analysis of our results revealed wide variation among dogs in their response to ingestion of a meal with or without erythromycin. Nevertheless, analysis of the data indicated that > 90% of healthy dogs achieved...
an ejection fraction ≥ 25% for at least 1 treatment. The 2 dogs with gallbladder volume < 1.0 mL/kg at time 0 that failed to achieve an ejection fraction ≥ 25% likely represented individual or physiologic variation in gallbladder contraction after food withholding (eg, gallbladder maximally contracted) because their basal gallbladder size was among the smallest in the study. Such variation in baseline gallbladder volume obviously would complicate discrimination between normal and abnormal gallbladder emptying if the proposed cutoff value (≤ 1.0 mL/kg) for expected baseline volume were disregarded. None of the treatments consistently elicited a time-linked gallbladder evacuation. However, on the basis of our results and a review of the literature on canine gallbladder responses to chologogues or food, we propose a technique for dogs with suspected gallbladder dysmotility. First, a simple post-prandial ultrasonographic evaluation is recommended for the initial assessment, with a reasonable expectation of an ejection fraction ≥ 25%. Second, for dogs that fail to achieve this standard, consideration of the gallbladder size (after food has been withheld) in relation to body weight is in order because a small baseline volume may obviate further gallbladder contraction. Third, with consideration for the aforementioned exception, ingestion of a meal containing erythromycin may prove diagnostically useful. Clearly, additional studies are necessary to elucidate the use of these techniques to evaluate the gallbladder in clinically ill dogs and dogs with suspected gallbladder dysfunction.

a. Erythromycin estolate (230 mg/mL cherry-flavored syrup), Alpharma Pharma, Baltimore, Md.

b. ATL 3000 Ultrasound System, convex 8- to 5-MHz 14-mm-radius transducer, Phillips Medical Systems, Bothell, Wash.

References