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—Ultrasoundographically 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)

Normal gallbladder motility provides an important mechanical cleansing effect that protects against the accumulation of stagnant biliary debris. Several neurohumoral factors and alterations in biliary architecture can influence gallbladder motility. Cholecystokinin secreted from the duodenal mucosa functions as a potent cholagogue.1,2 Motilin secreted from the duodenum and proximal portion of the jejunum also stimulates gallbladder contraction, with vagal tone having a milder influence.1,2 Structural changes or inflammation involving the gallbladder, cystic duct, common bile duct, or sphincter of Oddi can modify gallbladder contraction and impair emptying.3-5

At meal time, neurohumoral mechanisms promote water and bicarbonate production by the biliary epithelium and stimulate an increase in bile flow that coordinates with gallbladder contraction and relaxation of the sphincter of Oddi. These events augment expulsion of bile into the intestinal lumen. Abnormalities thwarting efficient and coordinated gallbladder contraction may result in the accumulation of debris in the lumen of the gallbladder. Although bile is continuously produced in the biliary tree, a substantial amount is diverted into the intestines during the interdigestive interval (between meals) because of intermittent bellows-like gallbladder contractions.6-10 However, as much as 53% and 75% of newly produced hepatic bile accumulates in the gallbladder after a prolonged period of food withholding in dogs and fasting in humans, respectively.10,11 Following ingestion of a meal, approximately 31% and 75% of gallbladder bile is expelled into the intestines in dogs and humans, respectively, although there is wide variation among subjects.10,12

The gallbladder functions as an interdigestive storage reservoir for hepatic bile, but it also concentrates bile (by absorbing water and certain electrolytes) and produces mucin. Although mucin contributes important viscoelastic properties to bile, excessive accumulation of mucin appears integral to formation of gallbladder mucocele in dogs as determined on the basis of gross evaluation of affected gallbladders.13 Gallbladder mucocele in dogs has been increasingly recognized in

Received May 22, 2007.
Accepted October 18, 2007.
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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 motor activity (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 contraction 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 obtaining ultrasonographic images of the gallbladder because of gastric distention with food and gas, only a small meal was fed to each dog. The meal consisted of approximately 100 g of a prepared diet (1.41 kcal of metabolizable energy/g of diet; 7.4 g of protein/100 kcal and 6.1 g of fat/100 kcal). In a preliminary study, 3 dogs were used to determine the contractile response of the gallbladder after ingestion of a small meal containing various doses of erythromycin (1.0, 1.5, 2.5, 5.0, and 10.0 mg/kg). There was no difference between the doses of 1.0 and 1.5 mg/kg, and vomiting and signs of abdominal discomfort were observed with doses ≥ 5.0 mg/kg. On the basis of results for that preliminary study, we determined that the protocol should consist of feeding a small meal without erythromycin (to all 22 dogs) and also with erythromycin at 1.0 and 2.5 mg/kg (7 dogs were fed only the meal containing 1.0 mg/kg, 7 dogs were fed only the meal containing 2.5 mg/kg, and 8 dogs were fed meals containing 1.0 and 2.5 mg/kg).

Ultrasonographic assessment of gallbladder volume was completed after food was withheld for 12 hours (time 0) and at 15, 30, 45, 60, 90, and 120 minutes after ingestion of a meal. Data were not obtained at 90 minutes for dogs fed a meal containing erythromycin at 1.0 mg/kg. A washout period of at least 24 hours was allowed between subsequent treatments. Order of treatments was randomized such that 12 dogs were fed a meal containing erythromycin before they were fed the meal only. Of the 8 dogs that were fed meals containing both doses of erythromycin, 4 dogs received the lower dose initially, and 4 dogs received the higher dose initially.

Ultrasonographic 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 = ([gallbladder volume at time 0 – gallbladder volume at specified time point]/gallbladder volume at time 0) × 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-and-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

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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 × 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 with a meal without erythromycin for 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.

![Figure 1](image)

**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.

### Table 1—Median (range) of the percentage change in gallbladder volume in healthy dogs after food was withheld for 12 hours and 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>15</td>
<td>19</td>
<td>15</td>
<td>22</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>1.0 mg/kg (n = 15)*</td>
<td>2</td>
<td>19</td>
<td>15</td>
<td>26</td>
<td>ND</td>
<td>21</td>
</tr>
<tr>
<td>2.5 mg/kg (n = 15)*</td>
<td>17</td>
<td>27</td>
<td>25</td>
<td>29</td>
<td>27</td>
<td>7</td>
</tr>
<tr>
<td>Meal containing erythromycin</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>ND</td>
<td></td>
<td></td>
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</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.

ND = Not determined.
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 intraindividual reproducibility in fasting gallbladder volume have been attributed to variability in activity of the intestinal migrating motor complex.

In healthy dogs, investigations 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 consistency of this relationship of gallbladder volume per unit of body weight by the use of reported raw data derived from healthy dogs in another study.

Although results of the study reported here supported a linear relationship between body weight and gallbladder volume at time 0 (ie, after withholding food for 12 hours) determined from real-time ultrasonographic measurements in healthy dogs, this observation is in contrast with results of other investigators. The disparity may be explained by differences in food-withholding intervals before measurements as well as body size of dogs included in these studies. All dogs in the study reported here were not allowed access to food for 12 hours and weighed between 4.3 and 26.9 kg, whereas in that other study, intervals of food withholding were undeclared and most dogs (10/14) weighed > 40 kg.

Calculation of weight-predicted gallbladder volume after food withholding by use of the regression equation reported here would be valid only when applied to similar-sized dogs deprived of food for 12 hours. 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 clonobutin 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 would 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 fasting 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, 10,16–18,22–24 we propose a technique for dogs with suspected gallbladder dysmotility. First, a simple postprandial 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.

References