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Gallbladder Mucocele: Variables Associated with Outcome and the Utility of Ultrasonography to Identify Gallbladder Rupture in 219 Dogs (2007–2016)

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Background: Gallbladder mucocele (GBM) is an increasingly recognized extrahepatic biliary disease in dogs.

Objectives: To investigate cases of GBM and identify variables associated with survival and the sensitivity and specificity of ultrasonography to identify gallbladder rupture.

Animals: Two hundred and nineteen client-owned dogs with GBM.

Methods: Multicenter, retrospective study of dogs with GBM, presented from January 2007 to November 2016 to 6 academic veterinary hospitals in the United States. Interrogation of hospital databases identified all cases with the inclusion criteria of a gross and histopathologic diagnosis of GBM after cholecystectomy and intraoperative bacteriologic cultures of at least 1 of the following: gallbladder wall, gallbladder contents, or abdominal effusion.

Results: Two hundred and nineteen dogs fulfilled the inclusion criteria. Dogs with GBM and gallbladder rupture with bile peritonitis at the time of surgery were 2.7 times more likely to die than dogs without gallbladder rupture and bile peritonitis ($P = 0.001$; 95% confidence interval [CI], 1.50–4.68; n = 41). No significant associations were identified between survival and positive bacteriologic cultures, antibiotic administration, or time (days) from ultrasonographic identification of GBM to the time of surgery. The sensitivity, specificity, positive, and negative likelihood ratios for ultrasonographic identification of gallbladder rupture were 56.1% (95% CI, 39.9–71.2), 91.7% (95% CI, 85.3–95.6), 6.74, and 0.44, respectively.

Conclusion and Clinical Importance: Dogs in our study with GBM and intraoperative evidence of gallbladder rupture and bile peritonitis had a significantly higher risk of death. Additionally, abdominal ultrasonography had low sensitivity for identification of gallbladder rupture.

Key words: Bile peritonitis; Cholecystectomy; Cystic mucosal hyperplasia; Gallbladder perforation.

Gallbladder mucocele (GBM) is an increasingly recognized extrahepatic biliary disease in dogs, but the pathogenesis remains unclear. Anecdotally, the clinical importance of GBM is thought to be primarily related to risks of gallbladder rupture, infection, extrahepatic biliary duct obstruction, and systemic inflammatory response syndrome that can occur secondary to gallbladder necrosis or infarction. Gallbladder rupture has been identified in 60.9 (14/23),[1] 23.3 (10/43),[2] or 22.7% (5/22)[3] of dogs with GBM. However, preoperative identification of rupture is often challenging. This is evidenced by the imperfection of abdominal ultrasonography to detect gallbladder rupture preoperatively. The sensitivity of high-resolution ultrasound scanners used to identify gallbladder wall defects in people is 70%[4] and as high as 85.7%[1] in dogs with GBM.

Abbreviations:

CT	computed tomography
GBM	gallbladder mucocele

The incidence of positive bacteriologic culture of gallbladder wall or contents in dogs with GBM at the time of surgery is inconsistent and approximately 22%.[1,2,5,6] A lack of sensitive diagnostic tests to identify gallbladder rupture and concern for septic bile peritonitis, extrahepatic biliary duct obstruction, and systemic inflammatory response syndrome secondary to gallbladder inflammation, necrosis, and thrombosis have led to anecdotal recommendations of earlier, elective cholecystectomy in dogs with GBM to avoid complications associated with bile or septic peritonitis.[1,3,6]

Considering the limited and variable information pertaining to GBM, the few definitive criteria for determining the need for surgical intervention and perioperative mortality rates of approximately 27%,[1–3,5] larger observational studies are needed to describe prognostic indicators. The primary aim of our study was to determine whether antibiotic administration, timing of surgery, bacteriologic culture positivity, or gallbladder rupture with bile peritonitis were associated with survival after

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cholecystectomy. A secondary objective was to investigate the sensitivity and specificity of abdominal ultrasonography to identify gallbladder rupture.

Materials and Methods

Criteria for Selection of Cases

Ours was a multi-institutional, retrospective study. Medical records of dogs examined at The Ohio State University Veterinary Medical Center, University of Georgia College of Veterinary Medicine, University of Florida College of Veterinary Medicine, University of Missouri Veterinary Health Center, Iowa State University College of Veterinary Medicine, and Oklahoma State University Center for Veterinary Health Sciences between January 2007 and November 2016 were searched for a diagnosis of GBM. Inclusion criteria included a gross and histopathologic diagnosis of GBM after cholecystectomy and intraoperative bacteriologic cultures performed on at least 1 of the following: gallbladder wall, gallbladder contents, or abdominal fluid.

Procedures

Data retrieved from medical records included: signalment; histologic description of gallbladder; abdominal ultrasonographic findings; the presence of gallbladder rupture and bile peritonitis at the time of surgery; antibiotic administration within 48 hours before surgery (preoperative) or intraoperatively; bacteriologic growth from gallbladder wall, bile, or abdominal fluid obtained at the time of surgery; cultured bacterial populations; outcome (ie, survival to discharge); and cause of death. Cases were identified at each institution by searching both histopathology and surgery logs for "GBM" and "cholecystectomy," respectively. Diagnosis of GBM was made based on gross appearance of a distended gallbladder lumen with an abnormal accumulation of thick amorphous material in conjunction with histologic evidence of mucosal proliferation as well as formation of mucus-filled cysts.[1,7] All interpretations were made by board-certified veterinary pathologists. Abdominal ultrasonographic studies either were completed or reviewed by a board-certified veterinary radiologist. Abdominal-focused assessment with sonography for trauma studies was not considered for this study. The ultrasonographic diagnosis of GBM was made based on the interpretation of a boarded-certified veterinary radiologist. The ultrasonographic diagnosis of gallbladder rupture was made based on modification of previously described criteria including if a defect within the wall of the gallbladder was identified with confluence of gallbladder content into the cranial peritoneum or if incongruity of the gallbladder wall was identified combined with pericholecystic effusion, cranial abdominal peritonitis, or steatitis.[1,8] Furthermore, gallbladder rupture was considered in the absence of gallbladder visualization with the presence of abdominal effusion. Identification of gallbladder rupture and bile peritonitis were based on intraoperative presence of gross appearance of gallbladder wall perforation, bile leakage, and peritonitis.[2,9]

Statistical Analysis

Statistical analysis was performed using commercial software.[a] All categorical variables were summarized as frequencies. A Shapiro-Wilk test was used to assess normality of numerical variables. Continuous variables that were not normally distributed were summarized by quartiles, median values, and range. For survival information, death or euthanasia before discharge because GBM was considered as a complete event. To explore the association between prognostic variables and survival, univariate analysis by means of the chi-square test was performed comparing dogs that survived to those that died. Significant variables on univariate analysis were evaluated using relative risk and 95 percent confidence intervals (CI). The Spearman correlation coefficient was used to determine the correlation between survival and time from ultrasonographic identification of GBM to surgery. For analysis of the time from ultrasonographic identification of GBM to the time of surgery, dogs having surgery within 24 hours of diagnosis were categorized as 1 day. Sensitivity and specificity and positive and negative likelihood ratios for identification of gallbladder wall rupture with ultrasonography were calculated; intraoperative identification of gallbladder rupture was the reference standard. The level of significance was set at $P < 0.05$.

Results

Animal Population

Two hundred and nineteen dogs fulfilled the inclusion criteria of the study from January 2007 to November 2016; 51 from The Ohio State University Veterinary Medical Center, 50 from University of Georgia College of Veterinary Medicine, 40 from University of Florida College of Veterinary Medicine, 36 from University of Missouri Veterinary Health Center, 23 from Iowa State University College of Veterinary Medicine, and 19 from Oklahoma State University Center for Veterinary Health Sciences. No dogs were excluded. Not all data could be obtained for every dog because diagnostic tests and therapies were performed at the discretion of the primary clinician at the time of evaluation. In addition, data extraction was limited by completeness of medical records. A total of 179 purebred dogs and 40 mixed breed dogs were included. Breeds were Shetland Sheepdog (n = 20), Cocker Spaniel (17), Bichon frise (12), Shih Tzu (10), Chihuahua (10), Pomeranian (9), Beagle (9), Miniature Schnauzer (9), Jack Russell Terrier (8), Pug (8), Toy Poodle (6), Miniature Poodle (4), Golden Retriever (4), West Highland White Terrier (4), Labrador Retriever (4), Yorkshire Terrier (4), Cairn terrier (3), Dachshund (3), Miniature Dachshund (3), Border collie (2) Collie (2), Maltese (2), Miniature Pincher (2), and 1 each of American Eskimo, Australian Shepherd, Basset hound, Boxer, Brittany spaniel, Catahoula Leopard dog, Chow Chow, Rough Collie, Doberman, German Shepherd, Havanese, Italian Greyhound, Keeshond, Lhasa Apso, Miniature Australian Shepherd, Norwegian Elkhound, Standard Poodle, Standard Schnauzer, Rat Terrier, Shiba Inu, Silky Terrier, and Wheaten Terrier. The median age at presentation was 10 years (Q1, Q3, range; 8.5, 12, 1.5–17). There were 127 spayed females, 85 neutered males, 3 intact females, and 4 intact males.

Medical records detailing antibiotic administration in the 48 hours before surgery were available for 153 of 219 dogs. Seventy-one of 153 (46.4%) dogs did not receive antibiotics 48 hours before surgery. Anesthesia records of intraoperative antibiotic usage were available for 214 of 219 dogs. Twenty-four (11.2%) dogs were not given antibiotics intraoperatively. A total of 190 of 214 (88.8%) dogs were given antibiotics in the 48 hours before surgery or intraoperatively. A total of 152 dogs had medical records that indicated whether pre- and

intraoperative antibiotics were administered. Of those 152 dogs, 7 dogs did not receive pre- or intraoperative antibiotics.

Description of the surgical procedure and intra-abdominal findings were available in 198 of 219 cases. Gallbladder rupture was noted in 42 of 198 (21.2%) dogs, the remainder had an intact gallbladder. Bacterial culture and susceptibility testing were performed on 255 specimens including gallbladder contents alone (57/255 [22.4%]), gall bladder tissue alone (127/255 [49.8%]), abdominal effusion alone (3/255 [1.2%]), gallbladder tissue and gallbladder contents (14/255 [5.5%]), abdominal effusion and gallbladder contents (2/255 [0.8%]), gallbladder tissue and liver (5/255 [2.0%]), abdominal effusion and liver (2/255 [0.8%]), gall bladder contents and liver (5/255 [2.0%]), or a combination of 3 sample types (4/255 [1.6%]).

A total of 188/219 (85.8%) of the dogs had no growth on culture. Of the dogs that had cultures performed that yielded no growth, 166/188 (88.2%) had antibiotics administered at some point in the 48 hours before procurement of material for culture. Results of bacterial cultures were positive for 31 of 219 (14.2%) dogs, 28 of which had received antibiotics before procuring material for culture. Three of the 7 dogs (42.9%) that received neither pre- nor intraoperative antibiotics had positive cultures.

Culture yielded 1 bacterial species in 21 dogs, 2 bacterial species in 7 dogs, 4 and 5 bacterial species in 1 dog each. The isolated aerobic species included *Escherichia coli* (5), *Enterococcus* sp (5), *Staphylococcus epidermidis* (5), *Enterococcus faecium* (3), *Staphylococcus nonhemolytic* (2), Coagulase-negative group (2), *Enterococcus avium* (1), *Pseudomonas aeruginosa* (1), *Staphylococcus hemolyticus* (1), *Staphylococcus* sp, *Streptococcus* sp (1), *Streptococcus salivarius* (1), *Streptococcus peroris* (1), Gram-positive cocci (1), *Staphylococcus* coagulase-negative (1), *Acinetobacter lwoffi* (1), *Bacillus pumilis* (1), *Bacillus* spp (1), and *Micrococcus* spp (1). The only anaerobic bacterium cultured was *Clostridium perfringens* (5). The isolated facultative anaerobic species were *Lactobacillus* sp (1) and *Proteus* sp (1).

Thirty-eight of 219 (17.4%) dogs included in this study either died or were euthanized during hospitalization postcholecystectomy. The cause of death was euthanasia because of poor prognosis and declining clinical condition (18), septic peritonitis and sepsis (5), bile peritonitis (4), unknown cause (4), acute respiratory distress syndrome (3), multiple organ dysfunctions (2), and 1 each of the following: disseminated intravascular coagulopathy and hypersensitivity reaction to canine albumin.

Sensitivity and Specificity of Abdominal Ultrasonography to Identify Gallbladder Rupture

A total of 218 of 219 dogs had transabdominal ultrasound examination performed before surgery; 1 dog did not. GBM was identified by ultrasound examination in 174 of 218 (79.8%) dogs. All dogs with intraoperative identification of gallbladder rupture had an ultrasonographic diagnosis of GBM before surgery. Of 174 dogs with evidence of GBM on ultrasound examination, 100% had identification of GBM on histopathology. A total of 44 of 218 (20.2%) dogs with GBM did not have evidence of GBM or immobile echogenic material on ultrasound examination. However, 39/44 (88.6%) of the aforementioned dogs had evidence of gravity-dependent echogenic material (eg, debris, sediment, sludge). In these cases, cholecystectomy was performed at the discretion of the surgeon intraoperatively. The indications for surgery in the aforementioned 44 dogs included worsening of serum liver enzyme activities, increased serum total bilirubin concentrations despite medical treatment or both (12), surgical intervention for a solitary liver mass (6), common bile duct obstruction related to cholecystolithiasis (6), splenectomy (4), cystotomy (4), diagnostic liver biopsy (2), adrenalectomy (2), management of emphysematous cholecystitis (3), other problems (4), and splenectomy and adrenalectomy (1). The median time (days) from ultrasonographic identification of GBM to surgery was 1 day (Q1, Q3, range; 1, 3, 1–570 days). Thirty-four dogs were reported to have gallbladder rupture ultrasonographically. The median time (days) from ultrasonographic identification of GBM rupture to surgery was 1 day (Q1, Q3, range; 1, 1, 1–4 days). Of the dogs reported to have gallbladder rupture ultrasonographically, 23 had evidence of gallbladder rupture and 11 had intact gallbladder walls at the time of surgery. A total of 140 dogs had intact gallbladder walls ultrasonographically. Of these, 18 dogs had evidence of gallbladder rupture and 122 had intact gallbladder walls at the time of surgery. The sensitivity and specificity for ultrasonographic identification of gallbladder rupture were 56.1% (95% CI, 39.9–71.2) and 91.7% (95% CI, 85.3–95.6), respectively. The positive and negative likelihood ratios for ultrasonographic identification of gallbladder rupture were 6.74 and 0.44, respectively.

Variables Associated with Survival

Dogs with GBM and gallbladder rupture with bile peritonitis at the time of surgery were 2.7 times more likely to die than dogs without gallbladder rupture and bile peritonitis (Table 1). One dog was excluded from statistical analysis of the association between GBM rupture and bile peritonitis with survival because bile peritonitis was not identified concurrently with rupture. No significant association was found between survival and positive bacteriologic culture or antibiotic administration (Table 1). Spearman rank correlation was used to identify whether associations existed between survival and time (days) from ultrasonographic identification of GBM or ultrasonographic identification of GBM rupture to the time of surgery. Five of the 174 dogs with an ultrasonographic identification of a GBM were excluded from analysis because this information was not available. No significant association was found between survival and time (days) from ultrasonographic identification of GBM to the time of surgery ($P = 0.844$; $r_s = -0.0145$; $n = 185$). Furthermore, a

Table 1. Categorical variables associated with survival based on chi-square test and relative risk

Variable	Survivor	Nonsurvivor	*P* Value	RR	95% CI
Gallbladder rupture and bile peritonitis	26/161 (16.1%)	15/36 (41.7%)	0.001	2.7	1.50, 4.68
Pre- and intraoperative antibiotic administration	118/124 (95.1%)	27/28 (96.4%)	0.834		
Intraoperative antibiotic administration	160/179 (89.4%)	30/35 (85.7%)	0.736		
Bacteriologic culture positivity	22/181 (12.2%)	9/29 (31.0%)	0.11		

RR, relative risk; CI, confidence interval.

significant association between survival and time (days) from ultrasonographic identification of gallbladder rupture to the time of surgery was not identified ($P = 0.784$; $r_s = -0.0483$; n = 34).

Discussion

Dogs with GBM and gallbladder wall rupture and bile peritonitis identified at the time of surgery had a significantly higher risk of death than those that did not in our study. No association was found between survival and bacteriologic culture or antibiotic administration nor was an association found between survival and time (days) from ultrasonographic identification of GBM gallbladder to the time of surgery. Abdominal ultrasonography had poor sensitivity (56.1%) but good specificity (91.7%) for identification of gallbladder rupture in our study.

Gallbladder perforation is a life-threatening complication of nontraumatic acute cholecystitis in people, with survival as low as 58% and incidence as high as 11%.[10–13] The incidence of gallbladder wall rupture associated with GBM in dogs is not known, owing to a limited number of reports, small study populations, and short evaluation periods. However, the prevalence ranges from 22.7 (5/22)[3] to 60.9% (14/23).[1] The prevalence of gallbladder wall rupture identified intraoperatively in our study was 21.4%.

We found that 21.2% of dogs had gallbladder rupture and bile peritonitis, and the risk of death in these dogs was significantly higher than in dogs without rupture and bile peritonitis. This finding is in contrast to previous reports in which no significant difference in survival was found in dogs with or without intraoperative identification of gallbladder rupture.[2,3] One reason for this difference is that the aforementioned reports may not have been sufficiently powered to detect a difference if 1 had been present. The frequency of gallbladder wall rupture confirmed at the time of surgery in those reports was 22.7 (5/22)[3] and 60.8% (14/23).[2] Another variable that may have influenced survival is the time from initial onset of clinical signs to surgical intervention. In people with acute cholecystitis, morbidity and mortality are significantly increased when surgical intervention occurs >72 hours after the onset of clinical signs.[14] The association between time from onset of clinical signs in dogs with GBM and surgical intervention on morbidity and mortality in dogs has not been evaluated. However, it is reasonable to extrapolate the pathophysiologic consequences of prolonged bile peritonitis secondary to gallbladder perforation from people. Bile constituents are toxic to tissues, causing alterations in permeability and necrosis.[15] Altered vascular permeability promotes transudation of fluid and translocation of endogenous anaerobic bacteria from the liver, intestines, and blood into the peritoneum.[15] Furthermore, bile in the peritoneal cavity impairs host defense mechanisms against bacteria.[16] These sequelae likely augment the systemic inflammatory response as well increase susceptibility to sepsis and multiple organ dysfunctions.

Abdominal ultrasonography had a sensitivity of 56% to identify gallbladder wall rupture before surgery in our study. This result is lower than the sensitivity (70%) of high-resolution ultrasound scanners used to identify gallbladder wall defects in people[4] and lower than previously reported sensitivities (75–85.7%)[1,8,17] of conventional ultrasonography to identify gallbladder rupture in dogs with GBM. In contrast to its poor sensitivity, ultrasonography had good specificity (91.7%) for identification of gallbladder rupture before surgery in our study. Our findings are similar to previously reported specificities (81–100%).[1,8,17] The high specificity for ultrasonography to detect gallbladder rupture highlights its clinical utility in guiding therapeutic intervention in dogs with GBM. If ultrasonography indicates the presence of gallbladder rupture, the probability of a false-positive result is low (0–19%).[1,8,17] Likelihood ratios allow quantitation of probability of disease for an individual patient with a positive or negative test result and are independent of prevalence.[18] The farther the likelihood ratio is from 1.0, the greater its effect is on the probability that the target disorder is present.[18] Our study yielded positive and negative likelihood ratios of 6.74 and 0.44, respectively. These findings indicate that ultrasonographic identification of gallbladder rupture is 6.74 times more likely in dogs with GBM and gallbladder rupture than in dogs without rupture. Likewise, a lack of ultrasonographic evidence of gallbladder rupture is 0.44 as likely in a dog with GBM and gallbladder rupture than in a dog without.

Because of the common occurrence of gallbladder rupture in dogs with GBM and its significant association with survival, a more sensitive imaging modality is needed. Recently, the effectiveness of contrast-enhanced ultrasonography to identify gallbladder wall necrosis or rupture in dogs was evaluated.[8] This diagnostic modality was 100% sensitive and specific in the identification gallbladder wall necrosis or rupture in dogs.[8] Computed tomography (CT) is superior to ultrasonography in identifying gallbladder wall defects in people.[19] Furthermore, when advanced imaging is inconclusive,

evaluation of bile acid concentrations in peritoneal fluid is useful for identifying gallbladder rupture when effusion is present.[20] Future studies evaluating the use of CT for identification of gallbladder wall rupture are needed to assess whether this imaging modality is superior to dogs. Earlier identification of gallbladder wall defects in dogs with GBMs might decrease morbidity and mortality.

An unexpected finding of our study was that 20.2% of dogs with gross and histopathologic diagnoses of GBM were not identified ultrasonographically before surgery. Classically, ultrasonographic characteristics of GBM included gravity-independent bile sludge and echogenic bile in a stellate pattern, often referred to as a kiwi fruit pattern. Variations in ultrasonographic characteristics from the previously accepted generalized appearance of GBM have been proposed previously.[5] However, it was only recently that these subjective evaluations were modified to describe the ultrasonographic characteristics of 6 types of GBM.[17] It is therefore possible that GBM were not identified ultrasonographically before surgery in our study because the criteria defining the 6 types of GBMs were not available during the majority of the study period. However, it is also possible that GBM was not identified before surgery because of confounding variables including equipment, operator skill, and cooperation of the dogs being imaged.

There were 31 dogs (14.2%) in our study with positive bacterial cultures of the bile, gallbladder, or abdominal effusion at the time of surgery. An additional 2 dogs were noted to have intralesional bacteria within the gallbladder wall on histologic evaluation, but no organism grew in culture. We did not identify a significant association between survival and culture positivity or antibiotic administration. The frequency of positive intraoperative bacterial cultures in dogs with GBM is controversial but reported to be approximately 22%.[1,2,5,6] A confounding variable in the aforementioned studies as well as in our own study is the heterogeneity and number of tissue specimens from each dog submitted for bacteriologic evaluation. It is currently unclear what type of tissue has the highest probability of bacteriologic positivity in dogs undergoing cholecystectomy for GBM. Moreover, standard bacteriologic techniques may not be sensitive enough to identify the presence of bacteria in GBM. Fluorescence *in situ* hybridization recently was found to be more sensitive than bacterial culture for detection of bacteria.[b] Additional prospective studies are needed to investigate the optimal type and number of tissue specimens submitted for standard bacteriologic cultures. In addition, future studies utilizing fluorescence *in situ* hybridization might indicate the actual incidence of bacteriologic positivity in dogs in GBM.

It is anecdotally recommended that intraoperative antibiotics be given to dogs undergoing gallbladder surgery to avoid complications secondary to leakage of bile from dogs with bacterial cholangitis because septic bile peritonitis has survival rates ranging from 27[9] to 45%.[21] Consequently, most dogs evaluated retrospectively were treated with antibiotics perioperatively, making it difficult to draw definitive conclusions about the association between culture and survival. Furthermore, the study with the highest incidence of bacteriologic positivity was the first study to investigate this variable in dogs with GBM.[5] This inaugural study set a precedent for antibiotic administration in dogs with GBM, which has been perpetuated since that time. Although an association between survival and antibiotic administration was not identified in our study, approximately 43% (3/7) of dogs not treated with antibiotics yielded at least 1 bacterial isolate on culture. Given the association between gallbladder rupture and the risk for septic bile peritonitis, these results reinforce the recommendation to use antimicrobial drugs prophylactically in dogs with GBM.

Our analysis did not identify an association between survival and time (days) from ultrasonographic diagnosis of GBM or gallbladder rupture to surgery. These variables have not yet been evaluated as prognostic factors in dogs with GBM. The lack of association should be interpreted with caution because in clinical practice many dogs already have been clinically ill when an ultrasonographic diagnosis of GBM with or without rupture was made. In people with acute cholecystitis, morbidity and mortality are significantly decreased when surgical intervention occurs within 72 hours after the onset of clinical signs.[14] The average time from the onset of clinical signs to surgery is 7.8 days in dogs with GBM.[3] Additional studies are needed to evaluate whether an association exists between survival and the time from onset of clinical signs in dogs with GBM to surgery. If a relationship exists, it would support the possibility that the high rate of mortality (approximately 27%) associated with cholecystectomy in dogs with GBM might be decreased with earlier surgical intervention.

The main limitations of our study are related to its retrospective nature. Not all medical records were complete, potentially influencing interpretations about the sensitivity and specificity of abdominal ultrasonography to correctly identify gallbladder rupture, and associations between pre- and intraoperative antibiotic administration and survival. Another limitation is that outcome was defined as survival to discharge. This outcome may have underestimated the overall mortality in dogs with GBM in our study. The inclusion criteria of gross and histopathologically confirmed GBM with intraoperative bacteriologic culture were intentionally rigid to accurately investigate our objectives. This approach may have excluded severe cases that died intra- or postoperatively that then had bacteriologic culture analysis withdrawn. Furthermore, this approach would have excluded dogs in which gallbladder rupture was suspected and surgery was not performed. The types and number of tissues cultured were not consistent among the dogs in our study. The lack of conformity in these variables may have resulted in an underestimation of positive bacteriologic cultures in dogs with GBM. In addition, most dogs in our study were treated with antibiotics pre- and intraoperatively, likely affecting our ability to accurately detect positive

bacteriologic cultures. Another limitation is that a single radiologist did not retrospectively review ultrasonographic videos or images from the dogs included in our study. Furthermore, confounding variables inherent to our study design including numerous ultrasonographers, evolving criteria to ultrasonographically identify GBM and gallbladder rupture, as well as differences in technology over time, represent limitations. GBMs were diagnosed ultrasonographically based on the opinion of board-certified veterinary radiologists. This lack of conformity in the criteria used to diagnose GBM ultrasonographically in our study may have affected the sensitivity for their identification. However, the heterogeneity of ultrasonographers from various different institutions also represents a strength of our study because it decreases regional bias and reflects the variability expected in clinical practice.

Our findings indicate that dogs GBM and evidence of gallbladder wall rupture and bile peritonitis at the time of surgery have higher risk of death. Additionally, abdominal ultrasonography had a poor sensitivity (56.1%) but good specificity (91.7%) for the identification of gallbladder rupture in our study.

Footnotes

[a] SigmaPlot, Systate Software, San Jose, CA, USA

[b] Wennogle S, Twedt D, Simpson K. Fluorescence *in situ* hybridization identifies occult bacterial infection in gallbladder mucoceles, in *Proceedings*. ACVIM Forum 2016;762

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