Journal of Veterinary Internal Medicine

Open Access

Standard Article
J Vet Intern Med 2017;31:1451–1458

Association between Gallbladder Ultrasound Findings and Bacterial Culture of Bile in 70 Cats and 202 Dogs

R. Policelli Smith, J.L. Gookin, W. Smolski, M.F. Di Cicco, M. Correa, and G.S. Seiler

Background: Bacterial cholecystitis often is diagnosed by combination of gallbladder ultrasound (US) findings and positive results of bile culture. The value of gallbladder US in determining the likelihood of bile bacterial infection in cats and dogs with suspected biliary disease is unknown.

Hypothesis/Objectives: To determine the value of gallbladder US in predicting bile bacterial culture results, identify most common bacterial isolates from bile, and describe complications after cholecystocentesis in cats and dogs with suspected hepatobiliary disease.

Animals: Cats (70) and dogs (202) that underwent an abdominal US and submission of bile for culture were included in the study.

Methods: A cross-sectional study design was used to determine the association of gallbladder US abnormalities and the results of bile cultures, and complications of cholecystocentesis.

Results: Abnormal gallbladder US had high sensitivity (96%) but low specificity (49%) in cats with positive and negative results of bile bacterial culture, respectively. Cats with normal gallbladder US findings were unlikely to have positive bile bacterial culture (negative predictive value of 96%). Gallbladder US had lower sensitivity (81%), specificity (31%), positive predictive value (20%), and negative predictive value (88%) in dogs. The most common bacterial isolates were of enteric origin, the prevalence being higher in cats. Incidence of complications after cholecystocentesis was 3.4%.

Conclusions and clinical importance: Gallbladder US has a high negative predictive value for bile culture results in cats. This modality is less predictive of infection in dogs. Percutaneous US-guided cholecystocentesis has a low complication rate.

Key words: Bile culture; Biliary; Cholecystocentesis; Ultrasonography.

Bacterial cholangitis is a relatively common cause of hepatobiliary disease in cats and dogs. Clinicians often rely on the US appearance of the gallbladder, in combination with clinical pathologic data, when prioritizing bacterial cholangitis as a differential diagnosis and when deciding whether or not to collect bile for bacterial culture. Recent studies have reported the prevalence and susceptibility patterns of the most common bacterial species isolated from the gallbladder.[1,2] In these studies, the most common etiologic agents of bacterial cholangitis were enteric bacteria, with the gallbladder yielding higher numbers of positive bacterial cultures compared to samples taken from liver parenchyma.[1,2] Other studies have sought to identify associations between bacterial infection of the gallbladder and results of bile cytology or hematologic and serum biochemistry data.[3,4] However, few studies have examined the potential association between bile bacterial infection and the US appearance of the gallbladder in either cats or dogs.[4] Studies have suggested that the presence of bacteria in the bile of cats is pathogenic.[1,5] In dogs, the clinical relevance of bacteria in bile remains controversial.[1,6–9]

Abbreviations:

PUC	percutaneous ultrasound-guided cholecystocentesis
US	ultrasound

In cats and dogs with suspected bacterial cholangitis, percutaneous US-guided cholecystocentesis (PUC) can be performed to obtain bile samples for cytologic examination and bacterial culture. Cholecystocentesis is minimally invasive, can be performed on an outpatient basis, and is relatively inexpensive. Therefore, it can be an expedient means to establish a diagnosis of bacterial cholangitis before performing more invasive laparoscopic or surgical liver biopsy procedures. It is, however, associated with possible adverse effects and should be reserved for patients with a reasonable expectation of a positive result. The prevalence of gallbladder US abnormalities in patients with suspected hepatobiliary disease has been described.[8,10–14] However, usefulness of the US appearance of the gallbladder for predicting the outcome of bile bacterial culture is unknown. Therefore, the purpose of our retrospective study was to determine the sensitivity, specificity, and positive and negative predictive value of gallbladder US in cats and dogs with suspected hepatobiliary disease that also had bile sampled for bacterial culture. Additional goals included describing the most common bacterial species isolated from bile samples obtained from cats and dogs and the incidence of complications associated with PUC in this patient population.

From the Departments of Molecular Biomedical Sciences, Clinical Sciences, and Population Health and Pathobiology, North Carolina State University, Raleigh, NC (Policelli Smith, Gookin, Smolski, Di Cicco, Correa, Seiler).

Corresponding author: G.S. Seiler, Department of Molecular Biomedical Sciences, North Carolina State University, 1060 William Moore Drive, Raleigh, NC 27607; e-mail: gsseiler@ncsu.edu.

Submitted February 13, 2017; Revised May 5, 2017; Accepted June 22, 2017.

DOI: 10.1111/jvim.14792

Materials and Methods

Criteria for Case Inclusion and Study Design

Canine and feline patients from which a gallbladder aspirate was obtained between August 2003 and August 2013 at the North Carolina State University Veterinary Hospital (NCSU-VH) were identified by retrospective review of the medical record database. For each patient, the medical record, digital US images, and bacterial culture results of aspirated bile were reviewed. Criteria for inclusion were availability of aerobic or anaerobic bile bacterial culture results or both and an abdominal US examination within 24 hours of bile collection with recorded digitized still images or video clips.

Data recorded from the medical record included signalment, body weight, bile sample collection technique (surgical or PUC), results of aerobic and anaerobic bacterial culture of bile, US findings, and reported complications of bile collection. Bile culture was performed at the request of the primary clinician. Specific indications for bile culture were not sought from the medical record.

Ultrasonographic Data

All original US examinations were performed by a board-certified veterinary radiologist or a veterinary radiology resident under the supervision of a board-certified veterinary radiologist. Ultrasound examinations were performed by an US system with transducer frequencies ranging from 5 to 18 MHz.[a] All still images, video clips, and written reports from each examination were reviewed by one of the authors (RPS). If discrepancies between the report and images were identified, the materials were reviewed again by a board-certified veterinary radiologist (GS) before reaching a consensus. Investigators were unaware of the results of bile culture at the time of review of the US findings.

A gallbladder abnormality was defined as the presence of a thickened gallbladder wall, gallbladder wall edema, mucosal hyperplasia, hyperechoic biliary gallbladder contents, choleliths, or a mucocele (Fig 1). The recorded observations were limited to the gallbladder. A thickened gallbladder wall was defined as >1 mm in cats and >2 mm in dogs.[15,16] Gallbladder wall edema was defined as a thickened gallbladder wall with a hypoechoic layer within the gallbladder wall. Mucosal hyperplasia was suspected if there was villous proliferation of the mucosal surface of the gallbladder wall.[13] The presence of any hyperechoic biliary contents was recorded in both dogs and cats, regardless of the presence of shadowing or gravity dependence. A cholelith was defined as a hyperechoic well-defined gravity-dependent structure with distal acoustic shadowing. A mucocele was defined as immobile bile with a striated or stellate pattern.

Bile Collection

Cholecystocentesis was performed in each patient by either US guidance or at the time of abdominal surgery or laparoscopy. Percutaneous US-guided cholecystocentesis was performed aseptically

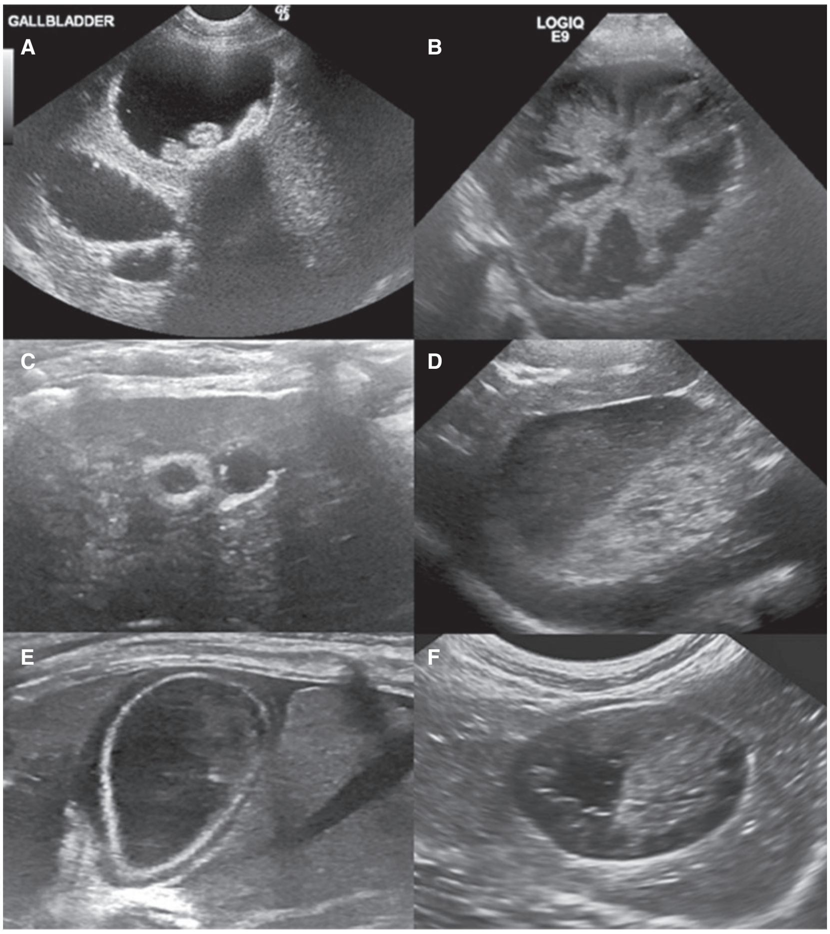

Fig 1. Examples of abnormal gallbladder ultrasound findings in patients included in the study. (**A**) Choleliths, (**B**) biliary mucocele, (**C**) thickened gallbladder wall in a cat with bilobed gallbladder, (**D**) gallbladder sludge, (**E**) gallbladder wall edema and free peritoneal fluid, and (**F**) mucosal hyperplasia and gallbladder sludge. [Color figure can be viewed at wileyonlinelibrary.com]

by a transhepatic approach whenever feasible, as described previously.[17] As much bile as possible was removed. For cholecystocenteses performed surgically, aspirates were obtained transhepatically during laparotomy or laparoscopy. All dogs that underwent PUC were re-evaluated by US immediately after the procedure. At clinician discretion, focal US re-evaluation was performed 15–30 minutes after the procedure to examine for complications.

Aerobic and Anaerobic Culture

Bile aspirates were submitted to the microbiology laboratory in a Luer lock-capped syringe immediately after collection. Bile samples for aerobic culture were plated on Columbia agar with 5% sheep blood[b] and MacConkey agar[b] and incubated at 36°C in 5% CO_2. Bile samples for anaerobic culture were processed in chopped meat broth and prereduced Brucella blood agar[c] and incubated in an anaerobic chamber. All cultures were examined for growth daily for 5 days. Growth was subjectively scored on a +1 to +4 scale depending on the number of quadrants of growth. Bacterial isolates were identified by standard identification procedures. At least 1 subculture was performed during the growth period. Contaminants were assessed as isolates known to be low-grade pathogens or normal human or canine skin microbial flora that grew in thioglycollate broth only.

Statistical Analysis

Descriptive statistics were calculated for the variables of interest. Comparisons of the prevalence of abnormal gallbladder US findings between cats and dogs with suspected hepatobiliary disease and concurrent bacterial culture of the bile were made using a chi-square test with a probability value ≤ 0.05. Univariate logistic regression was performed to determine associations between bile bacterial culture results in cats and dogs with abnormal results on gallbladder US examination. Odds ratios (OR) and 95% confidence intervals (CI) for the OR were estimated.[d] Sensitivity, specificity, and positive and negative predictive values and the corresponding 95% CI intervals (Clopper-Pearson) were estimated using a diagnostic test evaluation calculator[e] to determine the diagnostic value of gallbladder US using bile culture as the gold standard.

Results

Population Description

The NCSU-VH database identified 70 cats and 202 dogs that met the inclusion criteria. Two cats met the inclusion criteria on 2 separate visits. Three dogs met the inclusion criteria on 2, 3, and 4 separate visits, respectively. For these 5 patients with repeat visits, only the initial visit was included for determination of statistical associations between US findings and results of bile bacterial culture.

The median age of the 70 cats was 10.8 years (range, 1.4 years to 18.4 years). Thirty-seven cats were spayed females, 32 were neutered males, and 1 cat was an intact male. Breeds represented included 48 domestic shorthaired, 9 domestic longhaired, 7 Siamese, 2 domestic medium-haired, and 1 each of the following breeds: Devon Rex, Himalayan, Maine Coon, and Persian.

The median age of the 202 dogs was 9.0 years (range, 6 months to 16.2 years). There were 103 spayed females, 84 neutered males, 11 intact males, and 4 intact females. Common breeds represented included 20 Labrador Retrievers, 13 Miniature Schnauzers, 8 Cocker Spaniels, 8 Dachshunds, 8 Scottish Terriers, and 8 Shih Tzus. The remainder included mixed-breed dogs and other purebred dogs (breed not specified).

Bile Sample Collection

A total of 280 bile samples obtained from the 70 cats (72 samples) and 202 dogs (208 samples) were included in the study. Percutaneous fine needle aspiration with US guidance was used for collection of 7 of 72 (9.7%) samples of bile obtained from cats and 45 of 208 (21.6%) samples of bile obtained from dogs. The remaining samples were collected at the time of surgery or laparoscopy.

Culture Results

Among the 280 bile samples collected for bacterial culture, 233 (83%) samples were cultured under both aerobic and anaerobic conditions. The remaining 47 (17%) bile samples were cultured under aerobic conditions only. Identities of the bacteria cultured under aerobic or anaerobic conditions from the bile of both cats and dogs are reported in Table 1. In cats, 35% (25/72) of cultures from 33% (23/70) of cats were positive for bacterial growth. In dogs, 19% (40/208) of the cultures from 18% (36/202) of dogs were positive for bacterial growth. Growth of bacteria in cultures of sampled bile was significantly more common in cats than in dogs (χ^2 $P = 0.006$). In both cats and dogs, positive cultures yielded a single bacterial isolate 75% of the time. *Escherichia coli* and *Enterococcus* spp. were the most common bacterial species identified by bile culture in both cats and dogs. Among the mixed bacterial cultures, approximately half (9/16 or 56%) could be attributed to coinfection by both *Escherichia coli* and *Enterococcus* spp. There was no statistically significant difference in the prevalence of mixed versus pure cultures in cats versus dogs that had positive culture results. Similarly, there was no statistical significant difference in the isolation of aerobic versus anaerobic bacteria between cats and dogs. Contaminants were rare (<0.1%). In the 2 cats and 3 dogs from which multiple samples of bile were collected, all but 1 dog had repeatedly positive bile culture results, and in each case, a change in the identity of the bacteria over time was observed.

Ultrasonographic Findings

One or more abnormal findings pertaining to the gallbladder were documented in 46 of 70 (66%) of US examinations performed on cats and 143 of 202 (71%) of US examinations performed on dogs (Table 2). The highest proportion of US abnormalities observed in cats was gallbladder wall thickening. Gallbladder wall thickening was more common in cats than in dogs. In dogs, the highest proportion of gallbladder abnormalities was the presence of sludge. There was no statistically significant difference in the proportion of sludge, mucosal

Table 1. Identities and prevalence of bacteria isolated by culture of bile from dogs and cats with suspected bacterial cholangitis.

	Number (%) of Bacterial Cultures				
Bacterial Identity	Total	Canine	Feline	Pure	Mixed
Positive bile culture	65/280 (23%)	40/208 (19%)	25/72 (35%)	49/65 (75%)	16/65 (25%)
Escherichia coli	32/65 (49%)	18/40 (45%)	14/25 (56%)	19/32 (59%)	13/32 (41%)
Enterococcus spp.	25/65 (38%)	15/40 (37.5%)	10/25 (40%)	13/25 (52%)	12/25 (48%)
E. faecium	13	8	5	7	6
E. faecalis	6	5	1	4	2
E. casseliflavus	2	0	2	1	1
E. gallinarum	2	0	2	0	2
Enterococcus sp.	2	2	0	1	1
Streptococcus spp.	6/65 (9%)	4/40 (10%)	2/25 (8%)	4/6 (67%)	2/6 (33%)
Strep. sp. *(Group G)*	3	1	2	1	2
Strep. anginosus	1	1	0	1	0
Strep. bovis	1	1	0	1	0
Strep. mutans	1	1	0	1	0
Staphylococcus spp.	5/65 (8%)	4/40 (10%)	1/25 (4%)	5/5 (100%)	0/5 (0%)
Staph. epidermidis	2	1	1	2	0
Staphylococcus sp.	3	3	0	3	0
Clostridium sp.	3/65 (5%)	2/40 (5%)	1/25 (4%)	3/3	0/3
Corynebacterium sp.	2/65	2/40	0/25	2/2	0/2
Klebsiella pneumoniae	2/65	2/40	0/25	0/2	2/2
Bacillus sp.	2/65 (3%)	1/40 (2.5%)	1/25	2/2	0/2
Bacteriodes sp.	1/65 (1.5%)	1/40	0/25	0/1	1/1
Lactococcus lactis	1/65	1/40	0/25	0/1	1/1
Proteus mirabilis	1/65	1/40	0/25	0/1	1/1
Pseudomonas aeruginosa	1/65	1/40	0/25	0/1	1/1
Enterobacter cloacae	1/65	0/40	1/25	0/1	1/1

hyperplasia, or wall edema between dogs and cats. Dogs' proportion of choleliths was higher than the proportion in cats (Table 2).

Ultrasonographic Predictors of Positive Bacterial Culture of Bile

Cats—Cats having ≥1 abnormal gallbladder US findings were 21 times more likely to have positive results of bacterial culture of bile when compared to cats having normal gallbladder US findings. Specific US abnormalities associated with positive bacterial culture results in cats were the presence of a thickened gallbladder wall or sludge (Table 3). Odds of positive bacterial culture results of bile were greatest for observation of a thickened gallbladder wall and were not increased by combining presence of a thickened gallbladder with the presence of gallbladder sludge (OR, 6.3; 95% CI, 1.6–24.1, $P = 0.007$). The sensitivity (ie, the probability that US will indicate disease among cats with positive bile culture) of the gallbladder US compared to the bile culture was 96% (95% CI, 78–99.9%). The specificity of gallbladder US (ie, the probability that US will indicate no disease among cats with negative bile culture) was 49% (95% CI, 34.1–63.9%). At a prevalence of 33%, the positive predictive value (PPV) was 48% (95% CI, 41–55%), whereas the negative predictive value was 96% (95% CI, 77–99; Table 4). The PPV and NPV represent the probability that animals with a positive or negative test results will or will not have the disease, correspondingly.

Dogs—No US abnormalities of the gallbladder were associated with positive bacterial culture results of bile

Table 2. A comparison of the prevalence of abnormal gallbladder ultrasonographic findings between cats and dogs with suspected hepatobiliary disease that had concurrent bacterial culture of bile.

Clinical Finding	Number (%) of Cats	Number (%) of Dogs	χ^2 P Value
Gallbladder ultrasound performed	70/70 (100%)	202/202 (100%)	
Abnormal gallbladder ultrasound	46/70 (66%)	143/202 (71%)	0.522
Sludge	25/46 (54%)	96/143 (67%)	0.111
Thickened wall	31/46 (67%)***	48/143 (33%)	<0.0001
Cholelith	4/46 (9%)	34/143 (24%)*	<0.028
Mucosal hyperplasia	4/46 (9%)	23/143 (16%)	0.239
Wall edema	6/46 (13%)	14/143 (10%)	0.568
Mucocele	0/46 (0%)	6/143 (4%)	0.169

*$P < 0.05$, ***$P < 0.001$.

Table 3. Univariate logistic regression analysis for positive results of bile bacterial culture in cats and dogs with abnormal results of gallbladder ultrasound.

	Cats			Dogs		
Clinical Finding	Odds Ratio for Positive Bile Culture	95% CI (OR)	*P* Value	Odds Ratio for Positive Bile Culture	95% CI (OR)	*P* Value
Abnormal gallbladder ultrasound	21.0	2.6–170	<0.001***	1.9	0.8–4.6	0.223
Thickened wall	6.7	2.2–20.5	0.001***	0.6	0.2–1.5	0.375
Sludge	3.2	1.1–9.3	0.050*	1.9	0.9–4.1	0.106
Wall edema	1.1	0.2–6.5	1.00	1.3	0.3–4.8	0.997
Cholelith	7.4	0.7–75.9	0.089	1.5	0.6–3.7	0.479
Mucosal hyperplasia	2.3	0.3–17.5	0.585	0.7	0.2–2.4	0.729
Mucocele[a]	–	–	–	–	–	–

[a]No cats were identified as having a mucocele and no dogs with a mucocele had positive results of bile bacterial culture.
$*P \leq 0.05$, $***P \leq 0.001$.

Table 4. Sensitivity, specificity, positive predictive value, and negative predictive value of gallbladder ultrasound results in cats with suspected hepatobiliary disease in reference to results of concurrent bile culture as gold standard.

Condition		Bile Culture: Positive	Negative	Disease Prevalence % (95% CI)	Positive Predictive Value % (95% CI)	Negative Predictive Value % (95% CI)
Abdominal ultrasound	Abnormal	22	24		48 (41–55)	
	Normal	1	23			96 (77–99)
	Total	23	47	33 (22–45)		
	Sensitivity % (95% CI)	96 (78–99.9)				
	Specificity % (95% CI)		49 (34.1–63.9)			

in dogs (Table 3). The sensitivity and specificity for gallbladder US were 81% and 31% (95% CI, 64–92% and 95% CI, 24–39%, respectively). At 18% prevalence, the PPV and NPV were 20% and 88% (95% CI, 17–24 and 95% CI, 79–94%, respectively; Table 5).

Complications Associated with Cholecystocentesis

Complications occurring immediately after aspiration of the gallbladder were reported in 7 of 208 (3.4%) cholecystocenteses in dogs and in 2 of 72 (2.8%) cholecystocenteses in cats. Two of the complications were associated with aspirates obtained at the time of surgery; the remainder were associated with PUC. The most common complication reported was a small amount of presumed peritoneal hemorrhage immediately after aspiration in 1 cat and 5 dogs. The remaining complications consisted of gallbladder wall edema immediately after aspiration (1 dog), gallbladder collapse with needle pressure (1 dog), and intraluminal gallbladder hemorrhage (1 cat). In the latter case, the patient was reported to have moved during the PUC procedure. Immediately after aspiration, hemorrhage was noted to occur and fill the gallbladder lumen. This patient was reexamined by means of US periodically for 3 days after aspiration and was noted to have resolution of the hemorrhage and no clinical deterioration. No significant association was found between reporting of complications and the presence of abnormal gallbladder US findings or sample technique. There was no significant difference in prevalence of complications between dogs versus cats.

Table 5. Sensitivity, specificity, positive predictive value, and negative predictive value of gallbladder ultrasound results in dogs with suspected hepatobiliary disease in reference to results of concurrent bile culture as gold standard.

Condition		Bile Culture: Positive	Negative	Disease Prevalence % (95% CI)	Positive Predictive Value % (95% CI)	Negative Predictive Value % (95% CI)
Abdominal ultrasound	Abnormal	29	114		20 (17–24)	
	Normal	7	52			88 (79–94)
	Total	36	166	18 (13–24)		
	Sensitivity % (95% CI)	81 (64–92)				
	Specificity % (95% CI)		31 (24–39)			

Discussion

In the population of patients in our study, positive results of bile culture were more common in cats compared to dogs (33% versus 18%). This finding is similar to a previous study in which 36% of cats and 28% of dogs with suspected hepatobiliary disease were reported to be culture-positive for bacterial infection of bile.[1] Another study reported positive culture results in 14% of cats.[18] The prevalence of positive culture results in the cats and dogs in our study is likely higher than an average population because in each case clinical suspicion of infection likely influenced the decision to sample and culture bile. Moreover, it is likely that clinical suspicion was influenced to an unknown extent by the gallbladder US findings in our patients. Although it would be of interest to better understand the clinical rationale for bile sampling in our patients, this rationale would be difficult to accurately ascertain from a retrospective examination of medical records. Therefore, the results of our study may not be applicable to a different patient population.

One or more US abnormalities of the gallbladder also were common in cats and dogs in our study, which likely reflects inclusion criteria that required concurrent collection of bile for culture. Nearly all cats with positive results of bile bacterial culture had ≥ 1 abnormal results on gallbladder US examination. This observation is interesting from the standpoint that previous studies suggest that the presence of bacteria in the bile of cats is pathologic.[1,5] Bacterial pathogenicity could explain why the presence of infection was significantly associated with abnormal gallbladder US findings in these cats. Gallbladder US findings in cats that were significantly associated with increased odds of positive results of bile culture were the presence of a thickened gallbladder wall or observation of sludge. Gallbladder wall thickening previously was reported to be 87% sensitive and 90% specific for a histopathologic diagnosis of both infectious and noninfectious cholangitis in cats.[19] However, abnormal results of gallbladder US lacked specificity with respect to results of bile bacterial culture. It is possible that the low specificity of abnormal gallbladder US findings was related to an unknown history of antibiotics, failure to grow fastidious bacteria, or bacteriostatic effects of bile resulting in false-negative bile culture results. On the other hand, there also are many noninfectious causes for abnormal gallbladder US findings. For example, a thickened gallbladder wall can be secondary to a variety of noninfectious causes including hypoproteinemia, right-sided congestive heart failure, and the presence of contiguous peritonitis, making this finding nonspecific for diagnosis of bacterial cholangitis.[15,20] Previous studies have identified an association between gallbladder sludge and increased liver enzyme activities and serum bilirubin concentration in cats. Echogenic gallbladder content in cats also has been associated with cholecystitis and other US findings such as a large and hyperechoic liver, bile duct dilatation, and changes suggestive of pancreatitis.[11,14] Although the mechanism of sludge formation in cats is unknown, ours and others' observations suggest that this finding may be pathologic in this species.[1,11] The fact that a higher percentage of cats in our study had gallbladder sludge (54%) compared to a previous report (14%) likely reflects our inclusion of only cats that had their bile cultured, which may have been prompted by the presence of sludge.[11] The high negative predictive value (96%) of gallbladder US findings in cats suggests that bile bacterial culture results are unlikely to be positive if performed in cats with a normal gallbladder US findings (low false-positive rates).

In dogs, results of gallbladder US were less sensitive and lacked specificity with respect to results of bile bacterial culture. No individual or collective gallbladder US findings were identified to be significantly associated with increased odds of positive results of bile culture. Sludge was the most common US finding of the gallbladder observed in dogs in our study and generally is considered to be normal or associated with a decreased gallbladder emptying.[21,22] In contrast to cats, previous studies in dogs have not found an association between gallbladder sludge and biochemical markers of biliary disease.[21] Based on lack of a significant association between results of abdominal US and results of bile bacterial culture in dogs, further studies to evaluate the clinical relevance of bactibilia in this species appear to be warranted.

In our study, choleliths were significantly more common in dogs than in cats and not associated with increased odds of positive bile culture results in either species. Choleliths are reportedly associated with bacterial infection of the bile in both cats and dogs.[23,24] Too few cats (4/46 or 9%) had choleliths in our study to perform any meaningful analysis of this data. For dogs, it is likely that identification of this association would require a larger sample size. Mucocele formation was identified in a small subset of dogs in our study, none of which had positive results of bacterial culture of bile. Any association of bile bacterial infection with gallbladder mucocele formation in dogs is inconsistently observed,[13,25,26] and the role of infection in disease pathogenesis remains unknown. A low sample size of mucoceles in our study (6/143 or 4%) limited any meaningful comparison of culture results reported here to those of other studies.

Important limitations of our retrospective study are a lack of information regarding potential historical episodes of hepatobiliary infection or use of antibiotics that could confound the US appearance of the gallbladder or results of bile bacterial culture in our patients. Furthermore, it is unknown whether our patients continued to exhibit these US abnormalities after treatment for their underlying hepatobiliary disease. We chose to focus on analysis of the association between the US appearance of the gallbladder and concurrent results of bile culture. Accordingly, we did not attempt to establish any other potential clinical predictors of positive bacterial culture results or to define the presence of any concurrent disease. Previous studies already have examined a variety of associations among cholecystitis, bile cytology, bile culture, hematologic and serum biochemistry data,[3,4] and other abdominal US findings.[11,14] Retrospective review of the medical records of these 272 patients in attempt to determine concurrent diagnoses was beyond the scope of our study.

Percutaneous US-guided cholecystocentesis to obtain bile for culture was associated with few immediate complications in cats and dogs. The most common complication was minor abdominal hemorrhage after aspiration. This finding corroborates those of previous smaller studies in cats.[2,17] A higher complication rate was reported (17%) in a recent study, but was suspected to be associated with ancillary procedures performed at the same time as percutaneous cholecystocentesis.[27] Bacteria identified by bile culture were similar to previous reports and were predominantly aerobic enteric pathogens, most notably *E. coli* and *Enterococcus* species.[1,2,13,18,27] Our study may have underestimated the prevalence of anaerobic infections because not all animals had cultures performed under anaerobic conditions. Bacterial infections involving ≥2 or more species of bacteria were observed in 25% of patients, and frequently were characterized by coinfection with both *E. coli* and *Enterococcus* species. Unlike results from a previous study, we observed no difference between cats and dogs in the prevalence of single versus multiple bacterial species cultured from bile.[1]

In summary, in our population of patients with suspected hepatobiliary disease, absence of abnormalities on gallbladder US had high negative predictive value for results of bile bacterial culture in cats. Nearly all cats with positive results of bile bacterial culture had abnormal results of gallbladder US (96% sensitivity). The presence of a thickened gallbladder wall or gallbladder sludge was statistically associated with greater odds of positive bile culture in cats. However, abnormal results of gallbladder US were poorly specific (49%) because of their common occurrence in cats with negative results of bile culture (high false-negative rate). The prevalence of biliary infection in our study population likely is higher than in a general population, because the decision to perform bile culture presumably was based on a clinical or US suspicion of hepatobiliary disease. Accordingly, these data reflect a higher positive predictive value and lower negative predictive value than would be expected if applied to cats and dogs for which there were less suspicion of hepatobiliary disease. In a clinical setting, cholecystocentesis is unlikely to be considered without suspicion for hepatobiliary disease, and therefore, our results directly apply to the circumstances under which such decisions are made.

Footnotes

[a] GE Logiq e9; GE Medical Systems, Milwaukee, WI
[b] Remel; Lenexa, KS 66215
[c] [B]BE/LKV agar; Anaerobe systems, Morgan Hill, CA 95037
[d] SAS 9.4, Copyright©2002–2013, SAS Institute Inc., Cary, NC
[e] MedCalc Version 16.8, Copyright©1993-2016, MedCalc Software bvba, Belgium https://www.medcalc.org

Acknowledgments

Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

1. Wagner KA, Hartmann FA, Trepanier LA. Bacterial culture results from liver, gallbladder, or bile in 248 dogs and cats evaluated for hepatobiliary disease: 1998–2003. J Vet Intern Med 2007;21:417–424.
2. Brain PH, Barrs VR, Martin P, et al. Feline cholecystitis and acute neutrophilic cholangitis: Clinical findings, bacterial isolates and response to treatment in six cases. J Feline Med Surg 2006;8:91–103.
3. Peters LM, Glanemann B, Garden OA, et al. Cytological findings of 140 bile samples from dogs and cats and associated clinical pathological data. J Vet Intern Med 2016;30:123–131.
4. Lawrence YA, Ruaux CG, Nemanic S, et al. Characterization, treatment, and outcome of bacterial cholecystitis and bactibilia in dogs. J Am Vet Med Assoc 2015;246:982–989.
5. Otte CM, Gutierrez OP, Favier RP, et al. Detection of bacterial DNA in bile of cats with lymphocytic cholangitis. Vet Microbiol 2012;156:217–221.
6. Kook PH, Schellenberg S, Grest P, et al. Microbiologic evaluation of gallbladder bile of healthy dogs and dogs with iatrogenic hypercortisolism: A pilot study. J Vet Intern Med 2010;24: 224–228.
7. O'Neill EJ, Day MJ, Hall EJ, et al. Bacterial cholangitis/cholangiohepatitis with or without concurrent cholecystitis in four dogs. J Small Anim Pract 2006;47:325–335.
8. Secchi P, Poppl AG, Ilha A, et al. Prevalence, risk factors, and biochemical markers in dogs with ultrasound-diagnosed biliary sludge. Res Vet Sci 2012;93:1185–1189.
9. Bromel C, Barthez PY, Leveille R, et al. Prevalence of gallbladder sludge in dogs as assessed by ultrasonography. Vet Radiol Ultrasound 1998;39:206–210.
10. Uno T, Okamoto K, Onaka T, et al. Correlation between ultrasonographic imaging of the gallbladder and gallbladder content in eleven cholecystectomised dogs and their prognoses. J Vet Med Sci 2009;71:1295–1300.
11. Harran N, d'Anjou MA, Dunn M, et al. Gallbladder sludge on ultrasound is predictive of increased liver enzymes and total bilirubin in cats. Can Vet J 2011;52:999–1003.
12. Choi J, Kim A, Keh S, et al. Comparison between ultrasonographic and clinical findings in 43 dogs with gallbladder mucoceles. Vet Radiol Ultrasound 2014;55:202–207.
13. Besso JG, Wrigley RH, Gliatto JM, et al. Ultrasonographic appearance and clinical findings in 14 dogs with gallbladder mucocele. Vet Radiol Ultrasound 2000;41:261–271.
14. Marolf AJ, Leach L, Gibbons DS, et al. Ultrasonographic findings of feline cholangitis. J Am Anim Hosp Assoc 2012;48:36–42.
15. Hittmair KM, Vielgrader HD, Loupal G. Ultrasonographic evaluation of gallbladder wall thickness in cats. Vet Radiol Ultrasound 2001;42:149–155.
16. Nyland TG, Hager DA. Sonography of the liver, gallbladder, and spleen. Vet Clin North Am Small Anim Pract 1985;15:26.
17. Savary-Bataille KC, Bunch SE, Spaulding KA, et al. Percutaneous ultrasound-guided cholecystocentesis in healthy cats. J Vet Intern Med 2003;17:298–303.
18. Byfield VL, Callahan Clark JE, Turek CJ, et al. Percutaneous cholecystocentesis in cats with suspected hepatobiliary disease. J Feline Med Surg 2017; https://doi.org/10.1177/1098612X16689335

19. Newell SM, Selcer BA, Girard E, et al. Correlations between ultrasonographic findings and specific hepatic diseases in cats: 72 cases (1985-1997). J Am Vet Med Assoc 1998;213:94–98.

20. Spaulding KA. Ultrasound corner: Gallbladder wall thickness. Vet Radiol Ultrasound 1993;34:3.

21. Cook AK, Jambhekar AV, Dylewski AM. Gallbladder sludge in dogs: Ultrasonographic and clinical findings in 200 patients. J Am Anim Hosp Assoc 2016;52:125–131.

22. Tsukagoshi T, Ohno K, Tsukamoto A, et al. Decreased gallbladder emptying in dogs with biliary sludge or gallbladder mucocele. Vet Radiol Ultrasound 2012;53:84–91.

23. Hirsch VM, Doige CE. Suppurative cholangitis in cats. J Am Vet Med Assoc 1983;182:1223–1226.

24. Kirpensteijn J, Fingland RB, Ulrich T, et al. Cholelithiasis in dogs: 29 cases (1980-1990). J Am Vet Med Assoc 1993;202: 1137–1142.

25. Pike FS, Berg J, King NW, et al. Gallbladder mucocele in dogs: 30 cases (2000–2002). J Am Vet Med Assoc 2004;224: 1615–1622.

26. Crews LJ, Feeney DA, Jessen CR, et al. Clinical, ultrasonographic, and laboratory findings associated with gallbladder disease and rupture in dogs: 45 cases (1997–2007). J Am Vet Med Assoc 2009;234:359–366.

27. Tamborini A, Jahns H, McAllister H, et al. Bacterial cholangitis, cholecystitis, or both in dogs. J Vet Intern Med 2016;30:1046–1055.