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

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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. 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. Other studies have sought to identify associations between bacterial infection of the gallbladder and results of bile cytology or hematologic and serum biochemistry data. However, few studies have examined the potential association between bile bacterial infection and the US appearance of the gallbladder in either cats or dogs. Studies have suggested that the presence of bacteria in the bile of cats is pathogenic. In dogs, the clinical relevance of bacteria in bile remains controversial.

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. 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.

Abbreviations:

- PUC: percutaneous ultrasound-guided cholecystocentesis
- US: ultrasound
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. 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.
by a tranhepatic approach whenever feasible, as described previously. As much bile as possible was removed. For cholecysto-
toctentes performed surgically, aspires were obtained transhep-
tically 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 per-
formed 15–30 minutes after the procedure to examine for compli-
cations.

Aerobic and Anaerobic Culture

Bile aspirates were submitted to the microbiology laboratory in a
Luer lock-capped syringe immediately after collection. Bile sam-
ple for aerobic culture were plated on Columbia agar with 5% sheep
blood, and MacConkey agar and incubated at 36°C in 5% CO₂.
Bile samples for anaerobic culture were processed in chopped
meat broth and preduced Brucella blood agar 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 patho-
gens or normal human or canine skin microbial flora that grew in
thioglycolate broth only.

Statistical Analysis

Descriptive statistics were calculated for the variables of interest.
Comparisons of the prevalence of abnormal gallbladder US find-
ings 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. Sensitivity, specificity,
and positive and negative predictive values for the corresponding
95% CI intervals (Clopper-Pearson) were estimated using a diag-
nostic test evaluation calculator 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 domes-
tic 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 collected under both
aerobic and anaerobic conditions. The remaining 47
(17%) bile samples were cultured under anaerobic condi-
tions only. Identities of the bacteria cultured under aer-
obic 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
\( \chi^2 P = 0.006 \). In both cats and dogs, positive cultures
yielded a single bacterial isolate 75% of the time.

Statistical tests were used to determine if there was a
significant difference in the prevalence of mixed versus pure cul-
tures in cats versus dogs that had positive culture results.
Similarly, there was no statistically significant differ-
ence in the isolation of aerobic versus anaerobic bac-
teria 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 repeat-
edly 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 gall-
bladder 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 thick-
ening 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 signifi-
cant difference in the proportion of sludge, mucosal
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 1.** Identities and prevalence of bacteria isolated by culture of bile from dogs and cats with suspected bacterial cholangitis.

| 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 |
| *Corinebacterium sp.* | 2/65 | 2/40 | 0/25 | 2/2 | 0/2 |
| *Klebsiella pneumoniae* | 2/65 | 2/40 | 0/25 | 0/2 | 2/2 |
| *Bactillus 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 |

**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 | χ² P Value |
|------------------|-------------------|-------------------|-----------|
| Gallbladder ultrasound performed | 70/70 (100%) | 202/202 (100%) | 0.522 |
| Abnormal gallbladder ultrasound | 46/70 (66%) | 143/202 (71%) | 0.111 |
| Sludge | 25/46 (54%) | 96/143 (67%) | < 0.0001 |
| Thickened wall | 31/46 (67%)* | 48/143 (33%) | < 0.0028 |
| Cholelith | 4/46 (9%) | 34/143 (24%) | 0.239 |
| Mucocele hyperplasia | 4/46 (9%) | 23/143 (16%) | 0.568 |
| Wall edema | 6/46 (13%) | 14/143 (10%) | 0.169 |
| Mucocele | 0/46 (0%) | 6/143 (4%) | *P* < 0.05; ***P* < 0.001.
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 re-examined 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 3.** Univariate logistic regression analysis for positive results of bile bacterial culture in cats and dogs with abnormal results of gallbladder ultrasound.

| Clinical Finding                  | Cats Odds Ratio for Positive Bile Culture 95% CI (OR) P Value | Dogs 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*                        | – – – – – –                                                 | – – – – – –                                                 |

*aNo cats were identified as having a mucocele and no dogs with a mucocele had positive results of bile bacterial culture.

*P ≤ 0.05, ***P ≤ 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                  | Positive Disease Prevalence % (95% CI) | Positive Predictive Value % (95% CI) | Negative Predictive Value % (95% CI) |
|----------------------------|----------------------------------------|--------------------------------------|---------------------------------------|
| Abdominal ultrasound       | 48 (41–55)                             | 96 (77–99)                           |                                       |
| Normal                     | 1 23                                   |                                       |                                       |
| Abnormal                   | 22 24                                  |                                       |                                       |

**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                  | Positive Disease Prevalence % (95% CI) | Positive Predictive Value % (95% CI) | Negative Predictive Value % (95% CI) |
|----------------------------|----------------------------------------|--------------------------------------|---------------------------------------|
| Abdominal ultrasound       | 20 (17–24)                             | 88 (79–94)                           |                                       |
| Normal                     | 7 52                                   |                                       |                                       |
| Abnormal                   | 29 114                                 |                                       |                                       |
| 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. Another study reported positive culture results in 14% of cats. 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. 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. 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 bacterial culture results. 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. 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 described 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 US findings and results 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.
and other abdominal US findings.\textsuperscript{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.\textsuperscript{2,17} A higher complication rate (17\%) in a recent study, but was suspected to be associated with ancillary procedures performed at the same time as percutaneous cholecystocentesis.\textsuperscript{27} Bacteria identified by bile culture were similar to previous reports and notably \textit{E. coli} and \textit{Enterococcus} species.\textsuperscript{1,2,5,16,18,27} Our study may have underestimated the prevalence of anaerobic infections because not all animals had cultures performed under anaerobic conditions. Bacterial infections involving \textgeq{}2 or more species of bacteria were observed in 25\% of patients, and frequently were characterized by coinfection with both \textit{E. coli} and \textit{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.\textsuperscript{4}

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

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

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