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Bacterial Culture Results from Liver, Gallbladder, or Bile in 248 Dogs and Cats Evaluated for Hepatobiliary Disease: 1998–2003

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Background: Information is lacking on the prevalence and susceptibility patterns of bacterial isolates in dogs and cats with suspected hepatobiliary disease.

Objectives: To characterize the prevalence, identity, and antimicrobial susceptibility of common hepatobiliary isolates from such patients.

Animals: Dogs and cats presented to the University of Wisconsin-Madison Veterinary Medical Teaching Hospital for which samples of bile, gallbladder, or liver were submitted for culture from 1998 to 2003, including 190 dogs (192 culture episodes) and 58 cats (61 culture episodes).

Methods: Cases were identified from the microbiology laboratory database. Data from patient medical records were extracted, including the history of antimicrobial administration, the presence of fever, the results of CBC and serum biochemistry, the presence of biliary obstruction or hepatobiliary inflammation, and the results of aerobic and anaerobic bacterial cultures and aerobic antimicrobial susceptibilities.

Results: Biliary cultures yielded a significantly higher percentage of positive results overall (30% [18 of 60]) than did hepatic cultures (7% [15 of 215]). In patients with cholecystitis, 62% (8 of 13) had positive biliary cultures. In patients with hepatic inflammation, 23% (7 of 30) had positive bile cultures, whereas only 6% (6 of 103) had positive hepatic cultures. *Escherichia coli, Enterococcus* spp., *Bacteroides* spp., *Streptococcus* spp., and *Clostridium* spp. were the most common true-positive isolates. More than 80% of Enterobacteriaceae were susceptible to ciprofloxacin or aminoglycosides, with only 30–67% susceptible to first-generation aminopenicillins and cephalosporins. Liver samples obtained by surgery or laparoscopy were more likely to yield positive cultures than those obtained by percutaneous needle biopsy.

Key words: Aerobic; Anaerobic; Biliary; Canine; Hepatic; Microbiology.

Bacterial infections are thought to contribute to various canine and feline hepatobiliary diseases.[1,2] In veterinary patients, bacteria have been isolated from cases of suppurative cholangitis, hepatic abscesses, cholecystitis, choledochitis, and choleliths.[3–13] In humans with biliary disease, certain conditions have been associated with a higher incidence of infection, such as cholelithiasis, cholecystitis, previous biliary-tract surgery, or other causes of obstructive jaundice.[14–18] In these studies, the most commonly identified biliary pathogens in human patients are enteric bacteria (*Escherichia coli, Enterococcus* spp., *Klebsiella* spp., *Bacteroides* spp., and *Clostridium* spp). A similar list of bacterial isolates has been compiled from cats and dogs diagnosed with a broader range of hepatobiliary diseases.[3–13] However, the overall prevalence and antimicrobial susceptibility of the different bacterial species cultured has not been evaluated in a large group of cases. Therefore, the purpose of this retrospective study was to characterize the results of biliary and liver cultures from dogs and cats evaluated for hepatobiliary disease at a veterinary teaching hospital, including the prevalence of positive cultures, the identity and antimicrobial susceptibility of common isolates, and clinical predictors of positive bacterial culture results.

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Materials and Methods

Criteria for Case Inclusion

The results of all bacterial cultures performed on canine and feline liver tissue, gallbladder tissue, and bile samples over a 5-year period, from September 1998 to June 2003, were compiled from the database of the microbiology laboratory at the University of Wisconsin Veterinary Medical Teaching Hospital (UW-VMTH). Only patients of the UW-VMTH with available and complete medical records were included in the study. Data for each patient, recorded at the time the culture was performed, were collected, including signalment, presenting complaint, presence or absence of fever (defined as a rectal temperature persistently >102.5°F on at least 2 measurements during hospitalization, before culture and biopsy procedures), a history of antimicrobial administration in the week before culture, results of CBC and serum biochemistry, presence of biliary obstruction (based on ultrasonographic, surgical, or postmortem evaluation); aerobic and anaerobic bacterial culture and aerobic antimicrobial susceptibility results; and histopathologic findings of liver and gallbladder biopsies. Samples for histopathology were obtained by laparotomy, laparoscopy, or needle biopsy. All needle biopsies of the liver were obtained by using a Bard biopsy gun with a 14- or 18-gauge needle, under ultrasound guidance, and 2 to 4 samples were obtained from each patient. Hepatobiliary histopathology was categorized broadly as hepatic inflammation (cholangitis, chronic hepatitis, reactive hepatitis), biliary inflammation (cholecystitis, with or without biliary obstruction), degenerative (vacuolar hepatopathy, hepatic lipidosis), cirrhotic (without inflammatory changes noted), malignant neoplasia, benign neoplasia (eg, hepatoma, cystadenoma), or vascular anomaly (consistent with portosystemic shunt or portal vein hypoplasia), based on the initial pathologists' reports.

Clinicopathologic Data

Leukocytosis was defined as a total white blood cell count higher than the established laboratory reference interval (for cats, $5–19 \times 10^3$ cells/μL; for dogs, $6–17 \times 10^3$ cells/μL). A left shift was defined by the presence of >300 bands/μL in either species; the presence or the absence of toxic neutrophils also was recorded. Total bilirubin concentration was considered abnormally high if it was >0.6 mg/dL in both dogs and cats, without evidence of hemolysis. A clinically relevant increase in alanine aminotransferase (ALT) activity was defined for the purposes of this study as ≥2 times the upper reference interval. For serum alkaline phosphatase (ALP) activity, a clinically relevant increase was defined for dogs as ≥2 times the upper reference interval; for cats, any ALP activity above the upper reference interval was considered abnormal. This arbitrary definition was based upon the higher specificity of ALP and its relatively short half-life in cats versus dogs,[19,20] which make even small increases in ALP clinically relevants in cats.

Microbiology Techniques

Specimen Collection. Specimens for culture included bile-fluid aspirates or swabs, gallbladder swabs, and liver tissue. Specimens were cultured both aerobically and anaerobically when an adequate specimen volume was obtained. Bile aspirates were submitted to the microbiology laboratory in a Luer lock capped syringe within 15 minutes of collection. If a swab was used, 2 swabs were collected and placed in anaerobic culture transport media,[a] and transported to the laboratory within 1–2 hours of collection. One swab was used for aerobic culture, and the second swab was used for anaerobic culture. Liver tissue was placed in a sterile container and cultured within 15 minutes of collection according to VMTH Clinical Pathology Laboratory protocol. Specimens that were not handled appropriately were not accepted for anaerobic culture by the laboratory.

Aerobic and Anaerobic Culture Conditions. Specimens were cultured aerobically on trypticase soy agar supplemented with 5% sheep blood, chocolate agar, Levine eosin methylene blue agar, and CNA agar.[a] An additional sample was placed in a thioglycollate broth enriched with vitamin K and hemin. Anaerobic specimens were cultured with the following prereduced anaerobically sterilized (PRAS) media: brucella blood agar with vitamin K and hemin, brucella laked blood kanamycin vancomycin agar, Bacteroides bile esculin agar, and phenylethyl alcohol blood agar.[b] An additional sample was placed in PRAS chopped meat carbohydrate broth (CMC).

Aerobic cultures were incubated in 5% CO_2 at 36°C and were examined daily for growth for 5 days. Anaerobic specimens were processed and incubated in an anaerobic chamber.[c] Anaerobic cultures were examined for growth daily for 7 days. Growth was subjectively scored as follows: growth in broth only, minimal growth, moderate growth, or heavy growth. Bacterial isolates were identified by using standard identification procedures.[21] An isolate was considered a contaminant based on bacterial identification, amount of growth, and length of time for growth detection. Specifically, isolates known to be low-grade pathogens or inhabitants of normal human, canine, or feline skin microbiota that grew in thioglycolate or CMC broth only; took several days before visible growth was detected in the broth; or both were considered probable contaminants. Bacterial isolates identified as clinically important and not considered contaminants are referred to as the "true-positive" population in this study. All culture results refer to the "true-positive" population unless specifically referred to as contaminants.

Antimicrobial Susceptibility Testing. Antimicrobial susceptibility testing for aerobic gram-negative rods and gram-positive cocci was performed by using an automated microbiology system,[d] according to manufacturer instructions. Interpretations of susceptible, intermediate, or resistant were made according to breakpoints assigned by the Clinical and Laboratory Standards Institute, formerly the National Committee for Clinical Laboratory Standards.[22,23] Anaerobic gram-negative rods were tested for beta-lactamase activity with Cefinase disks.[e] All microbiologic culture and antimicrobial susceptibility results included in this study were reviewed by 1 microbiologist (FH).

Statistical Analysis

Chi-square analysis was performed to compare the prevalence of positive culture results between different groups (eg, cats versus dogs, liver versus biliary sources), as well as the association between various clinical pathologic variables and positive culture results. Chi-square analysis also was used to evaluate the relation between positive culture results and antimicrobial use within the week before culture; the liver biopsy method (surgical or laparoscopic versus percutaneous needle biopsy); the presence of choleliths, extrahepatic biliary obstruction, or gallbladder rupture; and the presence of hepatic or biliary inflammation on histopathology. Median total leukocyte counts, magnitude of increases in ALT and ALP, and patient age also were compared for patients with positive and negative culture results by using the Mann-Whitney U and Wilcoxon rank sum test. Statistical calculations were performed with a commercial software package,[f] with $P < .05$ considered significant.

Results

The UW Veterinary Microbiology Laboratory database yielded 226 hepatic and 60 biliary bacterial cultures performed on dogs and cats at the UW-VMTH between September 1998 and June 2003. Of these, medical records were available for 215 hepatic cultures and 60 biliary cultures, representing 58 cats (61 visits) and 190 dogs (192 visits). This population included 3 cats and 2 dogs who were evaluated on 2 separate occasions each, with different clinical presentations at each visit. All cases had clinical findings consistent with primary or secondary hepatobiliary disease, including increased liver enzyme activities, decreased serum albumin concentration, hyperbilirubinemia, increased serum bile acid concentrations, abnormal hepatobiliary findings on ultrasound examination, or suspicion of hepatic involvement of systemic neoplasia.

Signalment and Clinical Presentation

The 58 cats included in the study ranged in age from 11 months to 19.5 years (median, 11.2 years). There were 30 spayed female cats and 28 male cats (27 neutered). Thirty-seven cats were Domestic Shorthairs; 9 were Domestic Longhairs; 3 were Domestic Medium Hair; 4 were Siamese; and 1 each were Maine Coon, Burmese, Devon Rex, Scottish Fold, and Persian breeds. The 190 dogs ranged in age from 4 months to 16 years (median, 8.5 years). There were 98 spayed females, 9 intact females, 71 neutered males, and 12 intact males. Common breeds represented included Labrador Retrievers (6.8%), Golden Retrievers (6.3%), Cocker Spaniels

Table 1. Clinicopathologic findings in dogs and cats with suspected hepatobiliary disease for which biliary or liver cultures were submitted at the University of Wisconsin-Madison Veterinary Medical Teaching Hospital (September 1998 to June 2003).

Clinical Finding	% Cats	% Dogs
Leukocytosis	37 (21 of 57)	36 (63 of 174)
Leukopenia	7 (4 of 57)	2 (3 of 174)
Left shift	14 (8 of 57)	20 (35 of 174)
Toxic neutrophils	5 (3 of 57)	13 (23 of 174)
Hyperbilirubinemia[a]	67 (41 of 61)	40 (75 of 188)
Increased ALT[b]	75 (46 of 61)	77 (145 of 188)
Increased ALP[c]	52 (32 of 61)	78 (147 of 188)

ALP, serum alkaline phosphatase; ALT, alanine aminotransferase.

[a] Hyperbilirubinemia was defined as >0.6 mg/dL for both species.

[b] Increased ALT was defined as ≥2 times the upper reference interval.

[c] Increased ALP was defined as ≥2 times the upper reference interval in dogs and as outside the normal reference interval in cats.

Table 2. Methods used for obtaining and submitting biliary and hepatic cultures in dogs and cats suspected of hepatobiliary disease.

	Cats (%)	Dogs (%)
Liver	n = 49	n = 166
Method		
Needle biopsy	80	80
Surgical biopsy	20	17
Laparoscopic biopsy	0	3
Culture type submitted		
Aerobic only	24	15
Aerobic and anaerobic	76	85
Gallbladder or bile	n = 14	n = 46
Method		
Percutaneous aspirate	14	22
Surgical aspirate or swab	86	78
Culture type submitted		
Aerobic only	7	15
Aerobic and anaerobic	93	85

(5.3%), Doberman Pinschers (4.7%), and Miniature Schnauzers (4.7%). The remainder was composed of mixed-breed dogs (15.8%) and of other purebreds. Approximately half of the patients (51% of cats and 48% of dogs) had received antimicrobial treatment within 1 week before culture. Fever was recorded at presentation in 20% of patients overall (11% of cat visits and 23% of dog visits) before culture.

Clinicopathologic Findings

Clinicopathologic findings for the patients included in this study are summarized in Table 1. CBC were performed at 174 of 192 visits for dogs and at 57 of 61 visits for cats. Serum biochemistry testing was performed at all visits for cats and at 188 of 192 visits for dogs before culture. Sixty-seven percent of cats and 40% of dogs had increased total bilirubin concentrations before culture. Seventy-five percent of cats had increases in ALT activity before culture, with a mean increase of 5.6 times above the upper limit of the reference range, whereas 77% of dogs had increases in ALT activity, with a mean increase of 8.5 times the upper limit of the reference range. Fifty-two percent of cats had increased ALP activity, with a mean increase of 2.3 times the upper limit of the reference range, whereas 78% of dogs had increased ALP activity, with a mean increase of 13.0 times the upper limit of the reference range.

Samples Obtained

From dogs, 166 liver samples and 46 bile or gallbladder samples were submitted for culture. From cats, 49 liver samples and 14 biliary samples were submitted for culture (Table 2). Twenty dogs and 2 cats had both liver and biliary cultures performed simultaneously. Overall, 80% of liver samples in both species were obtained with ultrasound guidance. Laparoscopic samples were obtained in 5 dogs, and the remaining liver samples were obtained at surgery (Table 2). Because of the relatively small number of samples obtained by laparoscopy, data for surgical and laparoscopic samples were pooled for subsequent analyses. Bile samples were obtained by percutaneous fine needle aspiration with ultrasound guidance in 14% of cats (2 of 14) and 22% of dogs (10 of 46) who had bile cultured. The remaining bile or gallbladder samples were obtained at surgery either by needle aspirate or gallbladder mucosal swab.

Of 215 canine and feline liver samples cultured, 178 (83%) had both aerobic and anaerobic cultures performed, and the remaining 37 samples (17%) were submitted only for aerobic culture. Of 60 bile and gallbladder samples cultured, 52 (87%) had both aerobic and anaerobic cultures performed, and the remaining 8 (13%) had only aerobic cultures performed (Table 2).

Surgical and Histopathologic Findings

Hepatic histopathology was identified as inflammatory in 46% of cats (28 of 61 visits) and 53% of dogs (101 of 192 visits). Other histopathologic diagnoses included cholecystitis or biliary obstruction, without hepatic inflammation, hepatic lipidosis, vacuolar hepatopathy, cirrhosis, hepatoma, cystadenoma, lymphoma, biliary adenocarcinoma, and vascular anomalies. No patients had hepatic abscessation. Biliary obstruction was definitively identified at surgery or postmortem examination in 6 of 61 cat visits (10%), and in 16 of 192 dog visits (8%). Gallbladder rupture was identified in 1 cat and 3 dogs. Cholelithiasis was identified by surgical or ultrasonographic evaluation in only 2 dogs and in no cats.

Culture Results

Results of aerobic and anaerobic bacterial cultures for both cats and dogs, excluding cultures that grew contaminants, are reported in Table 3. In the cats, 14% of hepatic cultures and 36% of biliary cultures were

Table 3. Culture results from 248 dogs and cats evaluated for hepatobiliary infection at the University of Wisconsin-Madison Veterinary Medical Teaching Hospital from 1998 to 2003.

Outcome	% Cats	% Dogs	% All Cases
Positive bacterial growth			
Liver	14[a] (7 of 49)	5[a] (8 of 166)	7[b] (15 of 215)
Bile	36 (5 of 14)	28 (13 of 46)	30[b] (18 of 60)
Multiple bacterial species from liver or bile	17 (2 of 12)	52 (11 of 21)	39 (13 of 33)
Single bacterial species from liver or bile	83[c] (10 of 12)	48[c] (10 of 21)	61 (20 of 33)

Data with identical superscripts are significantly different. [a] $P = .022$; [b] $P < .0001$; [c] $P = .04$.

positive for bacterial growth. In the dogs, only 5% of hepatic cultures but 28% of biliary cultures were positive for bacterial growth. The prevalence of bacterial growth in hepatic cultures was significantly higher in cats than in dogs ($P = .022$). Biliary cultures overall yielded a significantly higher percentage of positive results in both species (30%) than did hepatic cultures (7%; $P < .0001$). In 22 patients who had both biliary and hepatic cultures obtained concurrently, biliary cultures were positive in 7 patients (32%) compared with hepatic cultures, which were only positive in 1 patient (5%; $P = .15$). The only 1 of these patients that had a positive liver culture also had a positive biliary culture, with the same organism isolated from both tissues. In cats, most positive cultures yielded a single bacterial isolate (83% of positive feline cultures), whereas, in dogs, less than half of the positive cultures (48%) yielded a single bacterial species. This difference between dogs and cats was statistically significant (Table 3).

Aerobic bacteria comprised 74% of true-positive isolates (43 of 58), and anaerobes 26% (15 of 58) (Table 4). The most common aerobic bacteria identified in all samples were *E coli* (19% of all true-positive isolates), *Enterococcus* spp. (16%), and *Streptococcus* spp. (7%) (Table 4). The most common anaerobic bacteria identified in all samples were *Bacteroides* spp. (12% of all true-positive isolates) and *Clostridium* spp. (7%). Staphylococci were the most common contaminants, comprising 28% of all isolates (24 of 87 total isolates) and 83% of all contaminants (24 of 29 contaminants). Bacteria identified as contaminants comprised 9% of all biliary isolates (4 of 45) and 44% of all hepatic isolates (26 of 59), and were not included in the true-positive population (Table 4).

Variable antimicrobial susceptibility to first-generation aminopenicillins or cephalosporins was observed for gram-negative enteric bacteria. For example, 45% of *E coli* isolates were susceptible to ampicillin, 67% to amoxicillin/clavulanate, and 64% to cephalothin (Table 5A). However, >80% of gram-negative enterics were susceptible to ciprofloxacin, amikacin, or gentamicin. All isolates of enterococci tested were susceptible to penicillin, and 86% of these isolates were susceptible to ciprofloxacin (Table 5B). Susceptibility profiles were not

Table 4. Bacterial isolates cultured from the hepatobiliary system in dogs and cats from 1998 to 2003 at the University of Wisconsin-Madison Veterinary Medical Teaching Hospital.

Cultured Bacteria	From Liver Only (no.)	From Bile Only (no.)	From Both Liver and Bile (no.)	% All True-Positive Organisms (no. positive)
Gram-negative aerobes	12	10	5	47 (27 of 58)
Escherichia coli	4	4	3	19 (11)
Pseudomonas	2	—	1	5 (3)
Serratia marcescens	2	1	—	5 (3)
Klebsiella	1	1	1	5 (3)
Others	3	4	—	12 (3)
Gram-positive aerobes	2	8	6	28 (16 of 58)
Enterococcus spp.	—	5	4	16 (9)
Streptococcus spp.	1	1	2	7 (4)
Staphylococcus spp.	0	2	—	3 (2)
Others	1	0	—	2 (1)
Anaerobes	3	7	5	26 (15 of 58)
Bacteroides	1	4	2	12 (7)
Clostridium	1	1	2	7 (4)
Others	1	2	1	7 (4)
Contaminants	25	3	1	(29 of 87 total isolates)
Staphylococcus spp.	21	2	1	(24)
Propionibacterium	3	1	—	(4)
Bacillus	1	0	—	(1)

Table 5A. Antimicrobial susceptibility of gram-negative microorganisms cultured from the hepatobiliary system in 248 dogs and cats.

Microorganism	% Susceptible (no. isolates tested)							
	A	A/C	CIP	CR	CZ	AN	GM	TE
Escherichia coli	45 (11)	67 (6)	82 (11)	64 (11)	64 (11)	100 (11)	91 (11)	45 (11)
Citrobacter freundii	50 (2)	0 (1)	100 (2)	0 (2)	0 (2)	100 (2)	100 (2)	100 (2)
Enterobacter cloacae	0 (2)	0 (1)	100 (2)	0 (2)	0 (2)	100 (2)	100 (2)	100 (2)
Klebsiella pneumoniae	0 (3)	100 (2)	100 (3)	67 (3)	100 (3)	100 (3)	100 (3)	67 (3)
Serratia marcescens	0 (3)	0 (3)	100 (3)	0 (3)	0 (3)	100 (3)	100 (3)	0 (3)
Pseudomonas aeruginosa	NT	NT	100 (3)	NT	NT	100 (3)	100 (3)	NT
Pasteurella multocida	100 (2)	100 (1)	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)

A, ampicillin; A/C, amoxicillin/clavulanate; AN, amikacin; CIP, ciprofloxacin; CR, cephalothin; CZ, cefazolin; GM, gentamicin; NT, not tested; TE, tetracycline.

generated for anaerobic bacterial isolates, but 6 of 7 *Bacteroides* spp. tested were positive for beta lactamase.

Clinical Predictors of Positive Cultures

Positive bacterial culture results were not significantly associated with any of the following clinical abnormalities: fever, leukocytosis or leukopenia, left shift, toxic change in neutrophils, hyperbilirubinemia, increased ALT or ALP activity, or the presence of biliary obstruction. However, the number of patients in some of these categories (eg, leukopenia, biliary obstruction) was small. Because some patients did not have laboratory work repeated just before hepatobiliary culture (eg, in 8 of 61 cats visits and in 32 of 192 dogs visits), these statistical analyses were repeated only for those patients with CBC and serum biochemistry performed within 24 hours of culture, but no significant associations were found.

Factors that may have predisposed to infection were noted in a small number of patients. Ten dogs had a clinical diagnosis of hyperadrenocorticism, but none of these dogs had positive hepatobiliary cultures. Twenty dogs had a recent history of glucocorticoid administration, and only 1 of these had a positive culture, whereas 12 cats had a history of recent glucocorticoids, with 2 positive cultures. Of 5 patients with diabetes mellitus, only 1 had a positive hepatobiliary culture. Two dogs had received chemotherapy at that time of sampling, and neither had positive cultures.

We could not demonstrate a significant difference in the prevalence of positive hepatic cultures in dogs and cats with inflammatory hepatic histopathology (6% [6 of 103]), compared with those with noninflammatory disease (Table 6), but, this finding should be interpreted with caution because of the small numbers of patients in some categories and the lack of uniform histopathologic review. With regard to biliary cultures, patients with cholecystitis or biliary obstruction had a significantly higher prevalence of positive results (62% [8 of 13]), compared with patients with hepatic inflammation (23% [7 of 30], $P = .03$). These results, however, were not controlled for bias regarding which samples (hepatic, biliary, or both) were chosen for submission for culture. Samples obtained by surgery or laparoscopy were more likely to yield true-positive culture results (17% [7 of 42]) than those obtained by percutaneous needle biopsy (4% [7 of 172], $P = .003$).

Discussion

The purpose of this study was to characterize the results of hepatic and biliary cultures in dogs and cats suspected of having hepatobiliary disease and who presented to a referral veterinary medical teaching hospital over a 5-year period. Our goals were to determine the most common bacterial pathogens and their antimicrobial susceptibility patterns, and to identify possible clinical risk factors associated with positive culture results.

The prevalence of bacterial infection in hepatic cultures from cats (14%) was significantly higher than in hepatic cultures from dogs (5%). In addition, cultures from cats were more likely to yield a single bacterial

Table 5B. Antimicrobial susceptibility of gram-positive microorganisms cultured from the hepatobiliary system in 248 dogs and cats.

Microorganism	% Susceptible (no. isolates tested)							
	P	A	A/C	CIP	CZ	CC	GM	TE
Staphylococcus spp. coagulase-negative	50 (2)	50 (2)	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)	100 (2)
Enterococcus spp.	100 (9)	100 (9)	100 (9)	86 (7)	NT	NT	NT	67 (9)
Streptococcus spp.	100 (2)	100 (2)	NT	NT	50 (1)	100 (2)	NT	100 (2)

A, ampicillin; A/C, amoxicillin/clavulanate; CC, clindamycin; CIP, ciprofloxacin; CZ, cefazolin; GM, gentamicin; NT, not tested; P, penicillin; TE, tetracycline.

Table 6. Distribution of positive culture results based upon category of histopathologic findings on liver biopsy in dogs and cats suspected of hepatobiliary disease.

Histopathologic Pattern	Positive Liver Cultures (215 cultures submitted)	Positive Biliary Cultures (60 cultures submitted)
Hepatic inflammation	6 of 103 (6%)	7 of 30 (23%)
Cholecystitis or biliary obstruction without hepatic inflammation	1 of 5	8 of 13 (62%)[a]
Malignant neoplasia	3 of 14	1 of 4
Degenerative	4 of 53 (8%)	1 of 9
Vascular anomaly	1 of 10	None cultured
Benign neoplasia	0 of 8	None cultured
Cirrhosis	0 of 6	0 of 1
Normal histopathology	0 of 12	0 of 0
Nondiagnostic or report not available	0 of 4	0 of 3

[a] Significantly higher than either hepatic inflammation or noninflammatory conditions as a group ($P = .03$).

isolate (83%) compared with cultures from dogs, which were equally likely to yield either multiple bacterial species or a single isolate. The higher overall positive culture rate in cats, along with a higher prevalence of single isolates, suggests that hepatobiliary infections may be more common in cats compared with dogs. In a recent study of healthy dogs, positive liver cultures were obtained in 12 of 20 dogs who underwent elective abdominal surgery.[24] Isolates were polymicrobial in about half of these positive cultures. Therefore, it is possible that the positive hepatic cultures obtained in some of the dogs in our study represented resident flora and were not directly related to hepatic pathology.

Biliary cultures for both dogs and cats produced a significantly higher percentage of positive results (30%) than did hepatic cultures (7%). This difference suggests that the biliary system may be more susceptible to bacterial infection or may be a more sensitive site to document hepatobiliary infection. Interestingly, in the 22 patients in whom both liver and biliary samples were submitted concurrently, none had a positive liver culture without a positive biliary culture. In the patients in our study, however, biliary cultures were submitted more commonly in dogs and cats taken to surgery than in those who underwent ultrasound-guided procedures. This procedural difference could have introduced sampling bias in which grossly abnormal biliary tissue was more likely to be submitted for culture. Therefore, a larger number of cases with concurrent hepatic and biliary cultures should be evaluated to determine the relative sensitivity of each culture site for yielding positive results.

We could not demonstrate a significant association between a positive liver culture and the presence of hepatic inflammation in this heterogeneous population. Biliary cultures were positive, however, in 23% of patients with hepatic inflammation and in 62% of patients with cholecystitis or gallbladder obstruction. This latter finding is similar to 1 survey of 60 dogs who underwent extrahepatic biliary surgery, which reported a 50% incidence of positive biliary cultures.[11] One important limitation of our study is that a single pathologist did not review all biopsy results. In addition, most patients did not have both a liver and a biliary culture, and the results, therefore, are susceptible to selection bias on the part of the clinicians and surgeons. Therefore, conclusions about the relation between histopathology and culture results are somewhat limited by this study design.

This study also attempted to identify clinical, CBC, or serum biochemical findings that were associated with positive cultures. Variables evaluated included those predictive of biliary infection in humans: patient age, presence of fever, prior antimicrobial administration, leukocytosis, left shift, hyperbilirubinemia, and extrahepatic biliary obstruction.[18,25–30] Additional variables that were examined in our study included toxic change in neutrophils and increases in liver enzyme activities (ALT, ALP). However, none of these factors was found to be significantly associated with positive bacterial cultures in either dogs or cats.

True associations with some of these factors could have existed in these patients in our study, and patient numbers may have been insufficient to identify these relations. For example, cholelithiasis, which is strongly associated with bacterial infection in both human and veterinary patients,[7,8,17,27,28,30,31] was found in only 2 animals in our study. Both had positive biliary cultures, but the number of patients was too small to evaluate this factor for predictive significance. Another limitation in establishing clinical predictors is the retrospective nature of this study, which did not allow standardization of the time between blood collection for clinical laboratory evaluation and collection of culture samples. In addition, some of our measured variables lacked specificity. For example, leukocytosis can result from stress alone, without infection, and ALP activity in dogs can be induced by glucocorticoids or hyperadrenocorticism or as a result of bone growth or remodeling.

Our results support previous observations from smaller case series,[3,4,6–10,12,13] in which the predominant bacteria isolated from the hepatobiliary system of dogs and cats were of enteric origin. Bacteria that were identified as contaminants in this study primarily were those associated with the skin, mostly coagulase-negative staphylococci, and probably were the result of collection- or specimen-handling contamination. The bacteria identified in this study as true-positive isolates were pre-

dominantly aerobic bacteria but included a substantial number of anaerobes (26% of all true-positive isolates), emphasizing the likely important role of anaerobes in hepatobiliary infections. Our data my have underestimated the prevalence of anaerobic infections, because 24% of samples from cats and 15% of samples from dogs did not have anaerobic cultures performed. It is also possible, however, that patients clinically suspected of having an anaerobic infection actually were more likely to have had both types of cultures submitted.

The antimicrobial susceptibility patterns of the most common aerobic isolates in this study were consistent with those previously described,[5] based on data generated in humans with biliary disease. When considering the range of bacterial species isolated in this study, broad spectrum antimicrobials are indicated in hepatobiliary disease. Therapy should include coverage for anaerobes, which typically are sensitive to metronidazole, amoxicillin/clavulanate, or chloramphenicol.[32] Clindamycin also may be effective, but only 80–83% of veterinary isolates of *Bacteroides* or *Clostridium* are reported to be sensitive to clindamycin.[32] Coverage also should include gram-positive aerobes (which could be accomplished by the administration of amoxicillin/clavulanate, clindamycin, or tetracyclines) and gram-negative aerobes (typically susceptible to fluoroquinolones in this survey). Aminoglycosides also could be considered in place of fluoroquinolones, but nephro- and ototoxicity would limit long-term use in most cases. Broad-spectrum empirical coverage, therefore, could be achieved with a combination of a fluoroquinolone, penicillin, and metronidazole, or with a fluoroquinolone combined with amoxicillin/clavulanate or clindamycin. Because resistance can occur, culture and antimicrobial susceptibility ideally should be obtained for each patient.

Because sampling methods potentially can affect the accuracy of microbiologic diagnoses, we compared the frequency of positive culture results from surgical or laparoscopic biopsies with percutaneous liver biopsies. Surgical or laparoscopic liver biopsy samples were significantly more likely to yield positive cultures (17%) compared with percutaneous needle biopsies (4%). This finding likely is due to the larger sample sizes possible with surgery or laparoscopy, and the ability of the surgeon to select a visibly abnormal tissue. Patients with bacterial involvement also may have been more ill and, therefore, more likely to be taken to surgery. The higher prevalence of positive cultures from surgical liver biopsies did not appear to have influenced the higher prevalence of positive hepatic cultures in cats versus dogs, because the percentage of samples obtained by surgery or laparoscopy was comparable between species (35% in cats versus 29% in dogs).

In summary, the findings of this study further support the concept that the predominant pathogens involved in hepatobiliary infections in dogs and cats are enteric bacteria, and the most common organisms in this study were *E coli*, *Enterococcus* spp., *Bacteroides* spp., and *Clostridium* spp. Nearly all of the aerobic bacteria were susceptible to ciprofloxacin, and numerous isolates of *E coli* were resistant to amoxicillin/clavulanate and first-generation cephalosporins. All anaerobic gram-negative rods tested, primarily species of *Bacteroides*, were found to be beta lactamase positive. Therefore, empirical antimicrobial therapy should include extended coverage for aerobic gram-positive and gram-negative bacteria and anaerobes, and may require more than 1 antimicrobial agent. Hepatic cultures from cats were more commonly positive than those from dogs, and infections in cats usually involved a single bacterial species. Surgical or laparoscopic liver biopsies were more likely to yield positive cultures than were needle biopsies. Biliary cultures appear more likely to detect bacterial infection than liver cultures. Therefore, bile or gallbladder tissue should be cultured whenever possible when trying to identify bacterial hepatobiliary disease. Prospective evaluation of patients with suspected hepatobiliary infection is needed to identify potential risk factors for infection.

Footnotes

[a] Remel, Lenexa, KS
[b] All anaerobic culture materials were obtained from Anaerobe Systems, Morgan Hill, CA
[c] Bactron II anaerobic chamber, Anaerobe Systems, Morgan Hill, CA
[d] Vitek 32 automated microbiology system, BioMérieux, Inc, Durham, NC
[e] BBL, Microbiology Systems, Cockeysville, MD
[f] StatView, Abacus Concepts, Berkeley, CA

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