Journal of Veterinary Internal Medicine
Open Access

Standard Article

J Vet Intern Med 2018;32:172–180

Cholangitis and Cholangiohepatitis in Dogs: A Descriptive Study of 54 Cases Based on Histopathologic Diagnosis (2004–2014)

J.L. Harrison, B.J. Turek, D.C. Brown, C. Bradley, and J. Callahan Clark

Background: Cholangitis in dogs appears to be more common than previously thought, but understanding of the disease remains incomplete.

Objective: To describe a population of dogs with cholangitis or cholangiohepatitis.

Animals: Fifty-four client-owned dogs with cholangitis or cholangiohepatitis.

Methods: Medical records of dogs with cholangitis or cholangiohepatitis confirmed by histopathology between January 2004 and December 2014 were identified using a computer-based search and retrospectively reviewed.

Results: Clinical signs included vomiting (72.2%), lethargy (70.4%), and inappetence (64.8%). Most dogs (49/50) had increased liver enzyme activities, hyperbilirubinemia (32/50), and hypercholesterolemia (24/43). Ultrasonographic abnormalities of the hepatobiliary system were seen in 84% of cases. On histopathology, 53 of 54 affected dogs had neutrophilic cholangitis (NC) or cholangiohepatitis, whereas 1 dog had lymphocytic cholangitis. Most cases (42/54) were chronic. Evidence of concurrent biliary disease (46.2%) and biliary tract obstruction (42.6%) was common. Seventeen of 36 biliary and 11 of 25 liver cultures were positive for bacterial growth; *Escherichia coli* and *Enterococcus spp*. were most common. Median patient survival was 671 days (95% confidence interval [CI]: 114–1,426). On Cox regression, dogs that did not have a cholecystectomy performed had a 2.1 greater hazard for death ($P = 0.037$; 95% CI: 1.0–4.3) compared to cholecystectomized dogs. Dogs >13 years old had a 5.0 greater hazard for death ($P = 0.001$; 95% CI: 1.9–13.2) compared to younger dogs.

Conclusions and Clinical Significance: Chronic NC or cholangiohepatitis was most common. Cholecystitis and biliary tract obstruction often occurred in conjunction with cholangitis. Cholecystectomized dogs had decreased risk of death; thus, cholecystectomy may improve patient outcome.

Key words: Biopsy; Culture; Gallbladder; Liver.

The incidence of cholangitis in dogs appears to be more common than previously thought.[1,2] In contrast to cholangitis in cats, the clinical findings, diagnosis, and treatment of cholangitis in dogs are not well documented.[3–5] The World Small Animal Veterinary Association (WSAVA) established guidelines for the clinical and histologic diagnosis of canine and feline liver disease and classified cholangitis into 4 groups: neutrophilic cholangitis (NC), lymphocytic cholangitis (LC), destructive cholangitis, and chronic cholangitis associated with liver fluke infestation.[6] In this classification scheme, the term cholangitis is used in preference to cholangiohepatitis because involvement of the hepatic parenchyma is not a consistent feature, and if present, it is usually an extension of primary cholangitis. Neutrophilic cholecystitis also has been described, associated with bacterial infection,[6] and may occur in combination with NC or as a solitary process.[1,7–9] Recently, reports characterizing the treatment and outcome of neutrophilic cholecystitis have been published.[7,8,10] However, the pathogenesis and relationship between NC and cholecystitis remain unclear.

Before publication of the WSAVA guidelines, few case reports of cholangitis in dogs existed, including 1 case series describing 4 dogs with cholangitis.[8,11–13] The histopathology described mixed inflammatory cell populations, with a predominance of neutrophils.[8,11–13] Based on the WSAVA guidelines, the findings are most consistent with NC.[6] *Escherichia coli* and *Enterococcus* species were most commonly isolated from bile and liver cultures.[8,11–13] Few case reports describe destructive cholangitis[14–16] in dogs, but to our knowledge, no reports describe dogs with LC or chronic (fluke-associated) cholangitis.

More recently, larger studies investigating cholangitis, cholecystitis, and bactibilia in dogs have been published.

Abbreviations:

WSAVA	World Small Animal Veterinary Association
NC	neutrophilic cholangitis
LC	lymphocytic cholangitis
SD	standard deviation
CBC	complete blood count
HCT	hematocrit
RI	reference interval
CI	confidence interval

From the Department of Clinical Studies, (Harrison, Brown, Callahan Clark); and the Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA (Turek, Bradley).

Present addresses: Antech Diagnostics, Irvine, CA (Turek); Elanco, Indianapolis, IN (Brown); Tufts Veterinary Emergency Treatment and Specialties, Walpole, MA (Callahan Clark).

This work was conducted at the University of Pennsylvania School of Veterinary Medicine.

This study was not supported by a grant.

Corresponding author: J. L. Harrison, University of Pennsylvania School of Veterinary Medicine, 3900 Delancey Street, PA 19104; e-mail: jenn.harrisn@gmail.com.

Submitted April 9, 2017; Revised August 10, 2017; Accepted October 11, 2017.

DOI: 10.1111/jvim.14866

A recent retrospective study evaluating 27 dogs with bacterial cholangitis and NC found that these diseases occur more frequently than has been thought.[7] Both NC and cholecystitis were seen concurrently in the majority of cases suggesting a link between bacterial cholangitis and gallbladder disease. A smaller case series reported the presence of bactibilia and cholecystitis in dogs with signs of nonspecific hepatobiliary disease that had immobile biliary sludge on ultrasound examination.[10] Both medically and surgically managed cases had good outcomes.[10] In both of these reports, resistant bacterial infections were identified.[7,10] A third study of bile cytology and culture in cats and dogs with suspected hepatobiliary disease found that in 30% of canine cases, infectious agents were involved. *E. coli* and *Enterococcus* species were most common, as in prior reports.[17] Only 4 of 6 dogs had gallbladder histopathology confirming a diagnosis of cholecystitis, whereas 3 dogs had cholangiohepatitis. Concurrent diseases identified in dogs with biliary inflammation or infection included hepatopathies, pancreatic disease, and inflammatory bowel disease.[17]

Despite these recent additions to the literature, cholangitis in dogs remains insufficiently characterized. Therefore, the aim of our study was to describe a population of dogs with cholangitis or cholangiohepatitis confirmed by histopathology, in terms of clinicopathologic findings, microbial culture, prevalence of concurrent disease, and overall outcome. Histopathologic changes were classified according to the WSAVA guidelines, and severity and evidence of obstruction, infection, or both were noted.

Materials and Methods

The surgical biopsy service database at the investigators' institution was searched from January 1, 2004 to December 31, 2014 for dogs with a histopathologic diagnosis of cholangitis or cholangiohepatitis. Fifty-four cases met the inclusion criteria. Cases with incomplete medical records were excluded. All hematoxylin and eosin-stained archived slides were reviewed by a board-certified veterinary pathologist and pathology resident and classified according to the WSAVA guidelines.[6] Cholangitis was defined as an inflammatory infiltrate confined to the portal region of the liver with either infiltration into the bile duct epithelium or within the ductal lumen. Cholangiohepatitis was diagnosed in cases in which cholangitis, as described above, extended beyond the limiting plate into the adjacent periportal region. Severity of inflammation was defined as the approximate number of inflammatory cell layers within the portal region, between the bile ductule and limiting plate: mild = 1–2, moderate = 3–5, and severe >5. Features including the presence and extent of fibrosis or fibroplasia, bile duct hyperplasia, and cellular composition of the inflammatory infiltration were used to identify chronicity. A Masson's trichrome stain was used to identify the presence of fibrous connective tissue. The extent of fibrosis was subjectively scored as absent, mild, moderate, and severe as has been previously reported.[18] A rubeanic acid staining method to assess hepatocellular copper accumulation also was performed.

Results of bile or liver cultures or both collected within the same hospitalization as the liver biopsy were recorded. Bile cytology was not performed in the majority of cases and therefore was not described in this population. For aerobic cultures, bile samples were plated directly to blood agar, MacConkey agar, and Columbia colistin-nalidixic acid agar and enriched in thioglycollate.[a] Liver tissue was macerated by aseptic technique before inoculation into thioglycollate broth. Media was incubated at 37°C and evaluated for growth at 24 and 48 hours. Subsequent subculture was performed as needed to obtain pure cultures for identification and susceptibility testing performed using appropriate panels with a commercial automated system.[b] Anaerobic culture was performed with the anaeropack system[c] according to manufacturer guidelines. Tissue and bile were incubated in thioglycollate supplemented with hemin and vitamin K.[a] Cultures were incubated at 37°C for 5 days and subculture performed on Brucella and anaerobic blood phenylethyl alcohol agars.[a] Identification was performed with a commercially available kit.[d] Multidrug-resistant bacteria were named as such based on the Dutch guideline for preventing nosocomial transmission of highly resistant organisms.[19] All *Staphylococcus* species except *Staphylococcus pseudintermedius* were considered likely contaminants.

The medical records were reviewed, and signalment, clinical signs, physical examination findings, and known comorbidities were recorded. Complete blood count (CBC), serum biochemistry profile, and abdominal ultrasound examination findings at the time of initial presentation were recorded when available. Abdominal ultrasound examination findings were recorded if performed by, or under the supervision of, a board-certified radiologist at the time of the patient's initial presentation.

Medical records and surgical reports were reviewed to document performance of cholecystectomy and evidence of cholelithiasis, biliary obstruction, or both. Treatments utilized, duration of hospitalization, survival to discharge, and overall survival times were recorded. Date of death was determined from a combination of medical records and owner phone communication.

Descriptive statistics were calculated for all dogs. For each continuous variable, a test of skewness and kurtosis was performed to assess normality of the data. Mean and standard deviation (SD) were reported for normally distributed variables, whereas median and range were reported for non-normally distributed data. Categorical data are presented as frequencies and percentages. Median survival time was determined by use of the Kaplan–Meier product limit method. Cox proportional hazards modeling was used to determine which variables were associated with overall survival time. The proportional hazards assumption was tested using Schoenfeld residuals. Two-way interactions among the main effects were investigated. Variables with <10% missing data were considered in the multivariate model if $P < 0.20$ on univariate analysis. The absence of confounding was based on a variable changing model coefficients by <15%. Variables were kept in the model based on a significant statistic at $P < 0.05$. All analyses were performed in Stata version 13.[e]

Results

Sample Population and Clinical Data

Fifty-four dogs met the specified inclusion criteria. Mean age at the time of presentation was 9.72 years (SD 3.0) with a median weight of 10.8 kg (range, 1.6–50.5 kg). The population included 31 spayed females (57.4%), 18 castrated males (33.3%), 3 intact females (5.6%), and 2 intact males (3.7%). Most common breeds included mixed breeds (10), Cocker spaniels (5), Bichon Frise (4), Labrador retrievers (3), Pomeranians (3), Miniature Dachshunds (2), Pugs (2), Golden retrievers (2), and Airedale terriers (2). The median duration of clinical signs was 3 days (range, 0–56 days) with the most common presenting signs including

vomiting (39/54, 72.2%), lethargy (38/54, 70.4%), inappetence (35/54, 64.8%), and diarrhea (17/54, 31.5%). Physical examination findings at presentation included increased body temperature (>103°F, 16/54, 29.6%), dull mentation (14/54, 25.9%), icterus (14/54, 25.9%), abdominal pain (12/54, 22.2%), hepatomegaly (6/54, 11.1%), and ascites (2/54, 3.7%).

A wide range of concurrent diseases was reported at the time of presentation including osteoarthritis (6/54, 11.1%), diabetes mellitus (5/54, 9.3%), idiopathic epilepsy (5/54, 9.3%), allergic skin disease (4/54, 7.4%), pancreatitis (3/54, 5.6%), hypothyroidism (3/54, 5.6%), and urinary calculi (3/54, 5.6%).

Laboratory Findings

Most patients (49/50) had increased liver enzyme activities, often in combination with hypercholesterolemia and hyperbilirubinemia at presentation (Table 1). Increases in liver enzyme activities were not seen in 1 dog diagnosed with both mild acute NC and a minimal-to-mild centrilobular hepatitis (see histopathology below). A CBC was performed in 48 of 54 patients at presentation. Leukocytosis was present in 16 of 48 (33.3%) and band neutrophils were seen in 16 of 48 (33.3%). Anemia (hematocrit [HCT] <41%) was identified in 21 of 48 (43.8%) (median HCT, 41.2%; range, 15–54%; reference interval [RI], 40.3–60.3%).

Abdominal Ultrasound Findings

A complete abdominal ultrasound examination was performed by a board-certified radiologist or a resident under the supervision of a board-certified radiologist in 43 of 54 (79.6%) patients, whereas a focal hepatobiliary ultrasound examination was performed in 2 of 54 (3.7%) patients at presentation (Table 2). Liver and gallbladder abnormalities were present in 38 of 45 (84.4%), and ascites was seen in 21 of 43 (48.8%) of patients. Pancreatic and gastrointestinal abnormalities were present in 16 of 43 (37.2%).

Table 1. Serum liver enzyme activity and total bilirubin and cholesterol concentrations.

Parameter	Reference Interval (RI)	Median	Range	% above RI
ALT (n = 49)	16–91 U/L	596	25–3,254	87.8
AST (n = 39)	23–65 U/L	162	38–1,123	79.3
ALP (n = 48)	20–155 U/L	1,506.6	59–8,103	97.9
GGT (n = 29)	7–24 U/L	33	0–392	51.7
TBILI (n = 50)	0.1–0.5 mg/dL	2.5	0.1–81	64.0
CHOL (n = 43)	128–317 mg/dL	339	114–1,402	55.8

Summary of biochemical data available at presentation for all 54 cases.

Bacterial Culture Results

Most dogs (45/54, 83.3%) had bacterial cultures performed, either during surgery (43/45, 95.6%) or by percutaneous ultrasound-guided cholecystocentesis (2/45, 4.4%) Bile cultures were performed on 36 of 45 (80.0%) dogs with 33 of 36 (91.7%) having anaerobic and aerobic cultures and 3 of 36 (8.3%) having aerobic cultures only. Liver cultures were performed on 25 of 45 (55.6%) dogs, in which both anaerobic and aerobic cultures were obtained from all but 1 dog. Most bile and liver cultures were negative for bacterial growth. Fifteen dogs had concurrent aerobic and anaerobic liver and gallbladder cultures performed. Of those, 11 of 15 (73.3%) had concordant results; in 4 cases, the bile and liver culture results differed. In 3 cases, the bile grew a bacterium or additional bacteria that were not cultured from the liver. In 1 case, the liver culture was negative. In another case, the bile culture was negative and the liver grew *E. coli*. Forty-two of 45 dogs (93.3%) cultured had been treated with antibiotics at some time before or at the time of sample collection (including perioperative antibiotics), and 30 of 42 (71.4%) were treated with antibiotics for at least 1 day before sample collection. Antibiotics were initiated perioperatively in 12 of 42 (28.6%) cases. Of the 3 dogs that did not receive antibiotic treatment before or during sample collection, all had liver cultures only performed and 2 were positive for bacterial growth whereas the third was negative.

Escherichia and *Enterococcus* spp were the most common isolates (Table 3). Of the bile cultures, 5 of 36 (13.9%) grew mixed bacterial populations, and 3 of 5 (26.6%) included *Enterococcus* spp. and *E. coli*. Of the liver cultures, 1 of 25 (4.0%) grew multiple bacterial species, which included an *Enterococcus* spp. and *E. coli*.

Three dogs on antimicrobial treatment ranging from 1 to 4 days in duration had multidrug-resistant bacteria cultured, all *E. coli*, with 1 isolated from a liver culture, 1 from a bile culture, and the third from both a liver and bile culture. A single methicillin-resistant

Table 2. Hepatobiliary, gastrointestinal, and pancreatic ultrasonographic abnormalities.

Abnormality	# of Dogs and %
Hyperechoic liver	14/45 (31.1)
Mixed echogenicity liver	14/45 (31.1)
Liver enlargement	16/45 (35.6)
Increased gallbladder sediment	27/45 (60.0)
Distended gallbladder	24/45 (53.3)
Dilated bile ducts	24/45 (53.3)
Evidence of pancreatic inflammation	13/43 (30.2)
Evidence of enteritis[a]	6/43 (14.0)
Gastric wall thickening	5/43 (11.6)
Ileus	4/43 (9.3)

Summary of hepatobiliary, gastrointestinal, and pancreatic ultrasound data of 45 of 54 cases that had abdominal ultrasounds performed at the time of presentation.

[a]Enteritis as reported by the ultrasonographer, predominantly refers to small intestinal wall thickening.

Table 3. Hepatobiliary culture results.

Culture Result	Liver (n = 25)	Bile (n = 36)
Negative	14	19
Escherichia coli	6	10
Enterococcus faecium	3	4
Enterococcus faecalis	1	2
Enterococcus raffinosus	0	1
Enterococcus durans/hirae	1	0
Citrobacter freundii	0	2
Clostridium sordelli	0	1
Klebsiella pneumoniae	0	1
Proteus mirabilis	0	1
Staphylococcus pseudintermedius	1	1

Summary of bacterial cultures in 45 of 54 cases.

S. pseudintermedius was isolated from the liver, and that dog had only received perioperative antibiotics at the time of biopsy and culture collection.

Hepatic and Gallbladder Histopathology

On hepatic histopathology, NC or cholangiohepatitis was observed in the majority of cases (53/54, 98.1%), with a single case of LC (1/54, 1.9%). Most cases were classified as cholangitis (45/54, 83.3%) rather than cholangiohepatitis (9/54, 16.7%; Fig 1). The severity of inflammation varied with most categorized as mild (23/54, 42.6%), whereas 20 of 54 (37.0%) were moderate, and 11 of 54 (20.4%) were severe. Most cases had evidence of chronicity (42/54, 77.8%; Fig 2) with 12 of 54 (22.2%) cases categorized as acute. Most cases had some degree of predominately portal fibrosis (46/54, 85.2%), and 23 of 54 (42.6%) had evidence of intrahepatic biliary obstruction on histopathology with concentric periductular fibrosis (Fig 3). The extent of fibrosis often was mild to moderate (19/54 and 17/54, respectively). Six cases had severe fibrosis, with bridging between portal regions.

Rubeanic acid staining for copper identified hepatocellular copper accumulation in 3 cases. Associated hepatocellular inflammation was not identified in these cases. Copper quantitation by mass spectrometry was not performed. Comorbidities in these cases included diabetes mellitus in 2 dogs and neoplasia in 2 dogs (hepatocellular adenoma distant from the biopsy site diagnosed as cholangitis and a poorly differentiated splenic sarcoma).

Bile ductule hyperplasia was noted in 43 of 54 (79.6%) cases. The major changes to the hepatic parenchyma were noted as lipogranulomas, pigment granulomas or both (19/54, 35.2%), hepatocellular swelling and clearing compatible with glycogen accumulation (8/54, 14.8%), hepatic lipidosis (8/54, 14.8%), extramedullary hematopoesis (7/54, 13.0%), neoplasia (3/54, 5.6%), and concurrent hepatic inflammation (centrilobular hepatitis; 1/54, 1.9%).

Thirty-one dogs (57.4%) had concurrent gallbladder biopsies, gastrointestinal biopsies, or both collected with disease documented in 30 of 31 (96.8%) on histopathology. Twenty-five dogs had gallbladder disease including cholecystitis (8/25, 32.0%), infarcts (6/25, 24.0%), mucocele (2/25, 8.0%), and neoplasia (neuroendocrine carcinoma; 1/25, 4.0%). The remaining 8 of 25 (32.0%) dogs had multiple diseases including concurrent gallbladder mucoceles and infarcts (4/8, 50.0%), cholecystitis and enteritis (2/8, 25.0%), gallbladder infarction and rupture (1/8, 12.5%), and cholecystitis and rupture (1/8, 12.5%). Five dogs had gastrointestinal disease alone including enteritis (2/5, 40.0%), gastric neoplasia (2/5, 40.0%), and gastritis (1/5, 20.0%).

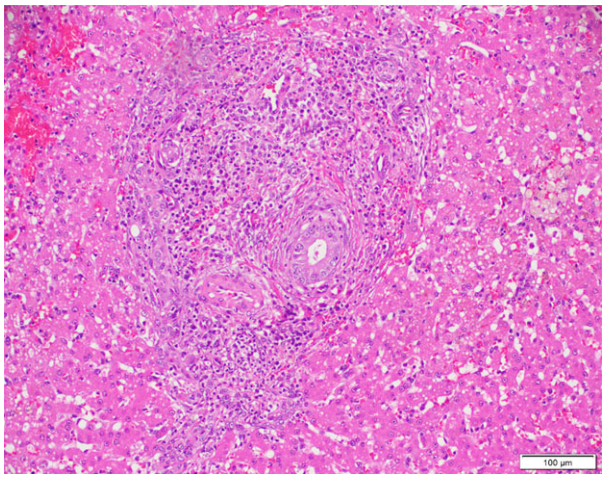

Fig 1. Liver Histopathology—Cholangiohepatitis. Severe neutrophilic cholangiohepatitis with inflammation extending from the portal region beyond the limiting plate into the periportal parenchyma. Hematoxylin and eosin (H&E) stain.

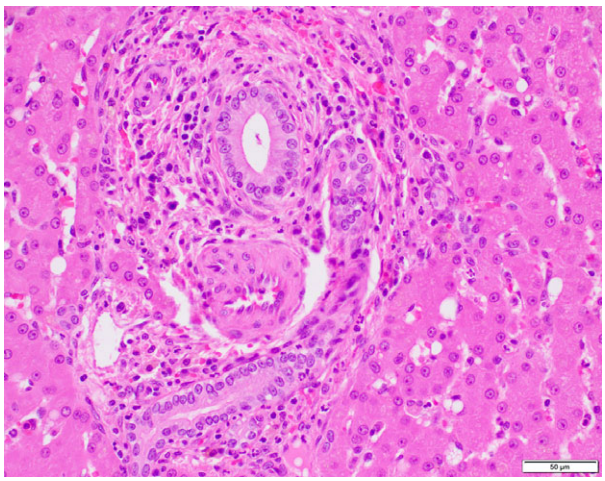

Fig 2. Liver Histopathology—Chronic Cholangitis. Severe chronic neutrophilic cholangitis. Hematoxylin and eosin (H&E) stain.

Medical and Surgical Intervention

The majority of the dogs (47/54, 87.0%) had liver biopsies collected by laparotomy whereas 5 of 54 (9.3%) had ultrasound-guided needle biopsies

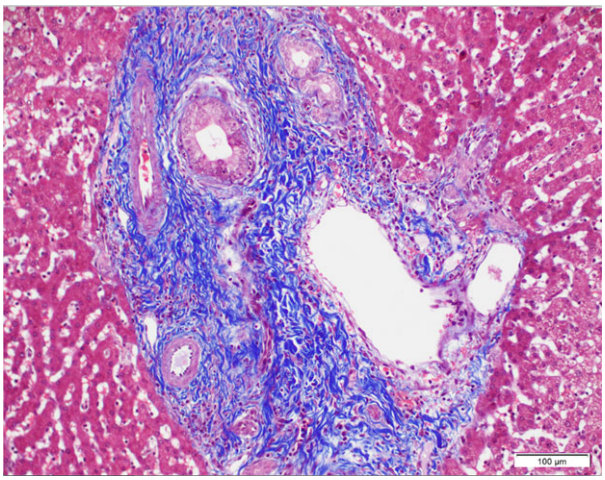

Fig 3. Liver Histopathology—Severe Fibrosis. Moderate chronic neutrophilic cholangitis with severe fibrosis. Masson's trichome stain.

performed, and the remainder (2/54, 3.7%) had laparoscopically collected hepatic biopsies. Reasons for biopsy or surgery included suspicion of primary liver disease (14/54, 25.9%), ultrasonographically suspected biliary obstruction, rupture or both (32/54, 59.3%), concern for neoplasia (5/54, 11.1%), or an incidental abnormality identified during exploratory surgery (3/54, 5.6%). Five of the 47 cases (10.6%) had laparotomy and hepatic biopsy performed for reasons other than hepatobiliary disease. These included grossly abnormal livers in cases with gastrointestinal foreign material (1), pyometra (1), pyloric duodenal mass with an ill-defined hepatic mass (1) and 2 cases with splenic masses with concern for hepatic metastasis. A biliary obstruction was noted during laparotomy in just over half of the cases (27/47, 57.4%), and 9 of 27 (33.3%) had obstructive choleliths (Fig 4). One additional case had a nonobstructive cholelith.

Thirty of the 47 cases (63.8%) that underwent laparotomy had cholecystectomy performed, including 21 of 27 (77.8%) cases of bile duct obstruction. The remaining obstructed cases underwent either biliary stent placement (4/6, 66.7%), choledocholithotomy (1/6, 16.7%), or a choledochal stent placement after removal of a choledocholith (1/6, 16.7%).

A variety of antibiotics at different dosages and durations were used. Before biopsy, the median duration of antibiotic administration was 1 day (range, 0–49 days) with 4 dogs receiving antibiotics for an unknown duration. The majority of dogs were treated with ≥1 of the following antibiotics: metronidazole (34/54, 63.0%), enrofloxacin (30/54, 55.6%), ampicillin (25/54, 46.3%),

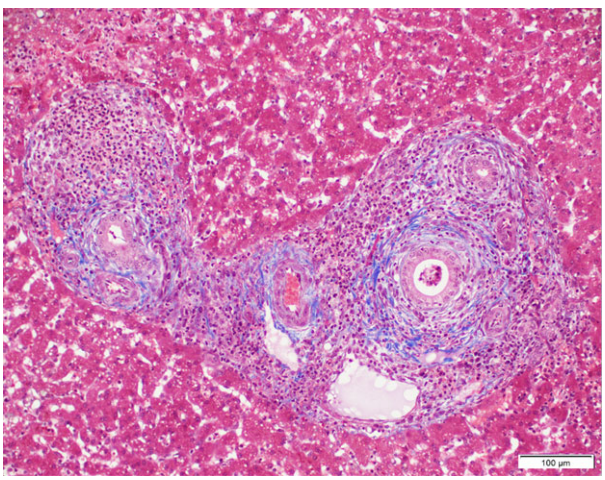

Fig 4. Liver Histopathology—Severe Neutrophilic Cholangitis. Severe neutrophilic cholangitis and focal cholangiohepatitis with moderate fibrosis. Note the periductal concentric fibrosis indicative of obstructive biliary disease. Masson's trichrome stain.

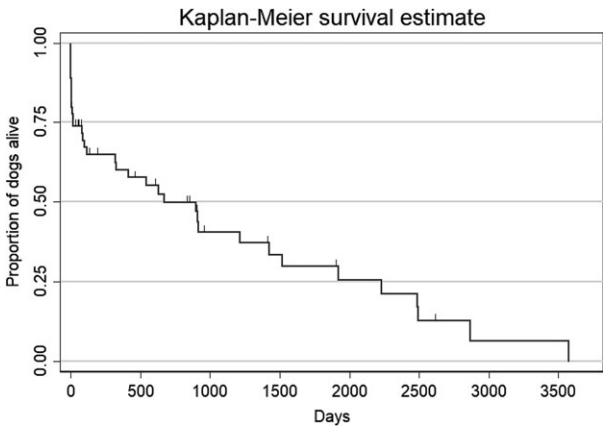

Fig 5. Kaplan–Meier Survival estimate for Dogs with Cholangitis. The median survival time was 671 for the 54 dogs included in the study. Each hash mark along the survival curve represents the last point at which a dog that was lost to follow-up was known to be alive (ie, censored).

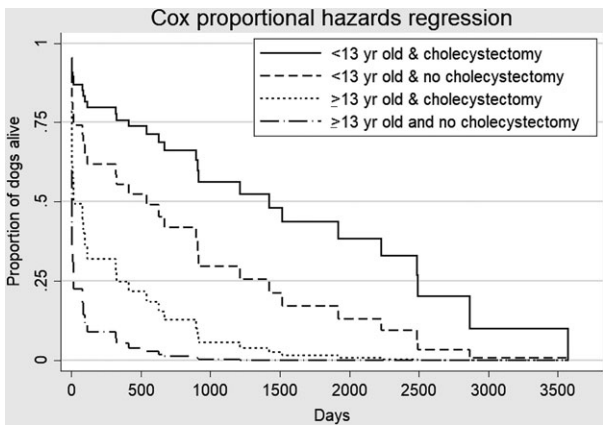

Fig 6. Survival Curves Base on Cox Proportional Hazards Regression. Dog age and surgical treatment with cholecystectomy were significantly associated with survival. Dogs <13 years old and dogs that had a cholecystectomy had improved survival compared to dogs that were older or did not have surgical removal of the gallbladder.

amoxicillin trihydrate/clavulanate potassium (16/54, 29.7%), and amoxicillin (6/54, 11.1%). Other antibiotics administered included doxycycline, cefpodoxime proxetil, clindamycin, cefazolin, marbofloxacin, cefoxitin, meropenem, amikacin, chloramphenicol, cefotaxime, and cefovecin.

Half of the cases were treated with liver supportive medications (27/54, 50.0%) including ursodiol (22/54, 40.7%), Denosyl[f] (12/54, 22.2%), Denamarin[g] (5/54, 9.3%), and Marin[h] (4/54, 7.4%) with the majority of dogs being given some of these products in combination. Additional nutriceutical treatments included milk thistle, vitamin E, and Hepatosupport.[i] Liver supportive medications were initiated before biopsy in 14 of 27 (51.9%) dogs with the remainder receiving liver supportive medications postbiopsy. Only 9 of 54 (16.7%) patients received corticosteroid treatment, including prednisone (4/54, 7.4%), dexamethasone (4/54, 7.4%), or budesonide (1/54, 1.9%).

Overall Survival

Forty-four of 54 cases (81.5%) were discharged from the hospital. Median time to discharge was 4 days (range, 1–10 days). Nine of the 10 dogs (90.0%) not surviving to discharge had postoperative complications, whereas the remaining dog was euthanized immediately after a needle liver biopsy because of severe hemorrhage. Of the 10 dogs that did not survive to discharge, 2 were taken to surgery for unrelated diseases. One dog had immune-mediated hemolytic anemia and splenic thrombosis, whereas the other had a pyloric and duodenal mass. Postoperative complications varied and included acute kidney injury, pancreatitis, and pneumonia. In 1 instance, the patient was persistently mentally inappropriate and painful postoperatively.

As of December 1, 2015, 8 of 45 (17.8%) patients were still alive whereas 28 of 45 (62.2%) were dead, and 9 of 37 were lost to follow-up. Median patient survival was 671 days (95% CI: 114–1,426; Fig 5). Cox proportional hazards modeling identified 2 variables, age, and the performance of cholecystectomy that were significantly associated with overall survival time. Controlling for age, dogs that did not have cholecystectomy performed had a 2.1 greater hazard for death ($P = 0.037$; 95% CI: 1.0–4.3) compared to dogs that had cholecystectomy performed (Fig 6). Additionally, controlling for cholecystectomy, dogs ≥13 years old had a 5.0 greater hazard for death ($P = 0.001$; 95% CI: 1.9–13.2) compared to dogs <13 years old (Fig 6).

Discussion

In this population of dogs with cholangitis or cholangiohepatitis confirmed by biopsy, most had NC according to WSAVA guidelines.[6] Just under half (28/61) of the included hepatobiliary cultures were positive for bacterial growth with *E. coli* and *Enterococcus* spp. cultured most often. Most dogs had chronic disease identified on histopathology as well as concurrent gallbladder disease. Overall, median survival was 671 days. Survival was negatively impacted by lack of cholecystectomy and age >13 years. Given that the majority of cases had NC and associated gallbladder disease, dogs suspected to have cholangitis should have hepatobiliary culture performed as part of their diagnostic evaluation. Furthermore, our results provide prognostic information that may help guide safe and effective treatment in similar cases.

Cholangitis in dogs appears to largely affect middle-aged, medium-sized dogs with no clear breed predominance. Females may be slightly overrepresented compared to males, but statistical significance was not demonstrated in our study or in a prior study.[7] Duration of clinical signs was variable, ranging from 0 to 56 days in our study, which likely reflects the various concurrent disease processes encountered in this population. A more acute onset would be expected in a dog with a bacterial infection compared to a progressive inflammatory process such as formation of a mucocele, which may account for the greater frequency of acute cases in the prior report of dogs with bacterial cholangitis or cholecystitis.[7] As previously reported, the most common presenting clinical signs included nonspecific gastrointestinal signs and lethargy, whereas fever, icterus, dull mentation, and abdominal pain were the most common physical examination abnormalities.[7,8,11,12]

The majority of dogs had increased liver enzyme activities with increases in alkaline phosphatase activity identified most often. Hyperbilirubinemia was present in 64% of cases, which is slightly less than the 77% reported in a group of dogs with bacterial biliary tract disease.[7] Our results reaffirm that the presence of hyperbilirubinemia in combination with an inflammatory leukogram, fever, or abdominal pain increases the clinical suspicion for biliary tract disease. Similar to previous reports,[7] ultrasonographic hepatic and biliary abnormalities were relatively common in our study. The most common abnormality encountered was an increase in gallbladder sediment, but the sediment was not retrospectively scored as in a prior report which found that immobile biliary sludge was both sensitive and specific for bactibilia.[10] Further investigation into the clinical relevance of increased gallbladder sediment is warranted, ideally in a prospective manner.

Several concurrent diseases were present in our study population with no particular disease occurring often. An association with hyperadrenocorticism, hypothyroidism, and biliary mucoceles previously has been demonstrated.[20] No link between hyperadrenocorticism or hypothyroidism was identified in our study.

Bile has been shown to be largely sterile in healthy dogs,[21] but in our study, just under half of bile and liver cultures were positive for bacterial growth. Therefore, based on our data, it is unclear whether bactibilia is the main trigger for NC in dogs. Although most cases of cholangitis are neutrophilic, a proportion may be sterile, emphasizing the need for culture to guide treatment. Alternative inciting sources of cholangitis may include noninfectious biliary tract or gastrointestinal disease, as was seen in our population of dogs. Unfortunately, most cases (42/45) were being treated with

antibiotics at the time of bacterial culture, which could have led to a lower number of cases that were negative for bacterial growth. Alternatively, false-positive culture results cannot be ruled out given that the majority of cases did not have concurrent bile cytology performed to document an inflammatory response typical of clinically relevant infection.

A higher frequency of positive bile cultures compared to liver cultures was not identified in our study.[7,22] All liver cultures were collected surgically, which may be more sensitive than cultures of liver aspirates.[22] *E. coli* was the most frequently identified bacterial species in both liver and bile cultures with *Enterococcus* species (especially *Enterococcus faecium* and *Enterococcus faecalis*) seen with the second highest frequency, consistent with prior studies.[7,8,10–13,22] Overall, anaerobes were only isolated in 3 cases, all of which were detected in bile. Unlike prior reports, *Clostridium* was only identified in a single case, and *Bacteroides* was not isolated.[7,8,10,22] The isolation of common gastrointestinal bacteria supports the potential for ascending biliary infection or translocation.[23]

Enterococcus faecium was the predominant *Enterococcus* species isolated in our study, consistent with a previous 2 case series.[13] Another recent study that evaluated dogs with bacterial cholangitis reported *E. faecalis* as the most common *Enterococcus* species detected.[7] The slightly higher prevalence of *E. faecium* is contrary to the pattern seen in urinary tract infections in which *E. faecalis* is most common,[24] and the clinical relevance of this observation is unclear. *Enterococcus* species have shown an increased rate of antimicrobial resistance. The higher incidence of *E. faecium* may arise because it is a species that is especially known for its antimicrobial resistance, but this was not appreciated in our current study.[25] Comparatively few multidrug-resistant bacteria were identified, all of which were *E. coli*.[7,8,10,13] The relatively lower number of resistant bacteria compared to prior studies may be due to the different guidelines for antimicrobial resistance followed by the study institution's microbiology laboratory.[19]

Hepatic histopathology almost exclusively identified NC in dogs with only 1 case of LC. Most prior reports of cholangitis in dogs have described NC with rare descriptions of destructive cholangitis, and there have been no prior reports of LC based on current WSAVA guidelines. Chronic changes on histopathology were common (42/54) with the remainder of cases classified as acute. This finding differs from the only other larger study on cholangitis in dogs containing hepatic histopathology and descriptive case data, which reported a higher incidence of acute histopathologic changes (21/27).[7] The reason for this difference is unclear, but may reflect the inclusion criteria of cases with bactibilia rather than a diagnosis of cholangitis. The severity of histopathologic changes also differed between our study and the prior study, with our study finding mild change to be most common (23/54) followed by moderate changes (17/54) versus moderate (14/26) followed by mild (11/26) changes in the previous study.[7] The prior study did not specifically define severity guidelines, which makes direct comparison between studies difficult.

Concurrent gallbladder disease in our study and prior studies suggests the possibility of gallbladder disease as a primary cause of intrahepatic cholangitis. Most cases had liver biopsies performed because of concern for concurrent diseases, with gallbladder disease being most common. Cholecystitis was present in nearly half of the cases in which gallbladder biopsies were performed. Gallbladder infarcts and mucoceles also occurred frequently with cholangitis. Because gallbladder histopathology was only performed in dogs that had cholecystectomies, it is possible that cholecystic disease had an even higher prevalence in our population. Unfortunately, the retrospective nature of our study resulted in a biased sample population, making it difficult to determine the true incidence of concurrent gallbladder disease. It is unclear as to whether intrahepatic cholangitis is a sole (primary) disease process or mainly a sequela, especially of gallbladder disease in dogs. This question would be best addressed in a prospective study.

Dogs with cholangitis that undergo cholecystectomy had decreased risk of death. Therefore, performing cholecystectomy may improve patient outcomes. Prior studies evaluating dogs with biliary disease that underwent cholecystectomy reported perioperative mortality ranging from 0 to 41%.[1,9,26–29] A recent study of 20 dogs focused on laparoscopic cholecystectomy for uncomplicated biliary disease and found a low complication rate and no perioperative deaths.[29] Complicated biliary surgery performed because of extrahepatic biliary duct obstruction, severe biliary inflammation (necrotizing cholecystitis), or biliary tract rupture resulted in higher perioperative mortality rates that correlated with certain risk factors including postoperative hypotension and biliary diversion surgeries (21.7–41%) in other studies.[1,9,26–28] Patients surviving the immediate postoperative period had an excellent prognosis.[1,9,26–28] Thus, when determining whether a patient should undergo cholecystectomy, factors such as evidence of concurrent biliary obstruction as well as the need for biliary diversion surgery should be considered when assessing overall patient risk. Because ours was a retrospective study, bias may have selected for a healthier population of dogs undergoing cholecystectomy, overall leading to the perception of improved outcome because of cholecystectomy itself. Prospective evaluation of hepatobiliary disease after cholecystectomy therefore is warranted.

Based on our study, age does not appear to be a negative risk factor for outcome in patients with cholangitis until the age of 13 years. This information would be especially beneficial when determining potential risk for an older patient before considering cholecystectomy. Before age 13, age itself should not be a contraindication for cholecystectomy. However, we also recognize the possibility that older age in itself is likely a risk factor for death. One retrospective study evaluating risk factors in dogs undergoing biliary surgery found age to be a significant risk factor for perioperative mortality, but a particular age for increased risk of death was not

specified.[9] This risk has not been demonstrated consistently in prior studies.[1,26–28]

Our study had several limitations, largely because of its retrospective nature. This design caused a portion of cases to become lost to follow-up, preventing a full assessment of survival data and limiting assessment of the efficacy of different medical treatments. Because some cases initially were evaluated at a primary care hospital or had ultrasound examinations performed by nonboard-certified radiologists, some sets of data were incomplete, which limited multivariate data analysis on several variables. Retrospective design also could result in missing information such as a complete list of concurrent systemic conditions. Ideally, further evaluation of larger populations of dogs with cholangitis should be carried out in a prospective manner to confirm whether the findings of our study are repeatable and also explore the relationship between gallbladder disease and cholangitis.

Our study described cholangitis or cholangiohepatitis in dogs in terms of clinicopathologic data, abdominal ultrasound findings, bacterial cultures, surgical findings, histopathology, and overall outcome. Chronic NC or cholangiohepatitis is the most common type of cholangitis in dogs. Increased liver enzyme activities as well as ultrasonographic biliary and liver abnormalities were frequent. Despite prior antibiotics in the majority of cases, nearly half of acquired cultures were positive, most frequently for *E. coli* and *Enterococcus* spp. Gallbladder disease, specifically cholecystitis and biliary tract obstruction, occurred often in conjunction with cholangitis. Dogs with cholangitis >13 years of age may be at increased risk of death. Dogs with cholangitis and cholecystic disease that underwent cholecystectomy had decreased risk of death, thus cholecystectomy may improve patient outcome. Prospective studies are needed to further evaluate whether canine intrahepatic cholangitis is a primary disease process or a sequela of ascending biliary disease.

Footnotes

[a] Remel, Lenexa, KS
[b] MicroScan Walkaway 40s system with PC20 and NC31 panels, Brea, CA
[c] Mitsubishi Gas Chemical Company, New York, NY
[d] RapID ANA II test kit, Lenexa, KS
[e] StataCorp, College Station, TX
[f] S-Adenosylmethionine, Nutramax, Lancaster, SC
[g] S-Adenosylmethionine and Silybin, Nutramax, Lancaster, SC
[h] Silbin and vitamin E, Nutramax, Lancaster, SC
[i] Rx Vitamins Inc., Elmsford, NY

Acknowledgments

The authors thank Stephen Cole from the University of Pennsylvania for providing assistance with the interpretation of microbial culture results.

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

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

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