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Bacterial Cholangitis, Cholecystitis, or both in Dogs

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Background: Bacterial cholangitis and cholecystitis are rarely reported, poorly characterized diseases in the dog.

Objectives: To characterize the clinical features of these conditions.

Animals: Twenty-seven client-owned dogs with bacterial cholangitis, cholecystitis, or both.

Methods: Multicenter, retrospective cases series of dogs with bacterial cholangitis, cholecystitis, or both, presenting January 2000 to June 2011 to 4 Veterinary Schools in Ireland/United Kingdom. Interrogation of hospital databases identified all cases with the inclusion criteria; histopathologically confirmed cholangitis or cholecystitis and bile culture/cytology results supporting a bacterial etiology.

Results: Twenty-seven dogs met the inclusion criteria with approximately 460 hepatitis cases documented over the same study period. Typical clinical pathology findings were increases in liver enzyme activities (25/26), hyperbilirubinemia (20/26), and an inflammatory leukogram (21/24). Ultrasound findings, although nonspecific, aided decision-making in 25/26 cases. The most frequent hepatobiliary bacterial isolates were *Escherichia coli* (n = 17; 16 cases), *Enterococcus* spp. (n = 8; 6 cases), and *Clostridium* spp. (n = 5; 5 cases). Antimicrobial resistance was an important feature of aerobic isolates; 10/16 *E. coli* isolates resistant to 3 or more antimicrobial classes. Biliary tract rupture complicated nearly one third of cases, associated with significant mortality (4/8). Discharged dogs had a guarded to fair prognosis; 17/18 alive at 2 months, although 5/10 re-evaluated had persistent liver enzyme elevation 2–12 months later.

Conclusion and Clinical Significance: Bacterial cholangitis and cholecystitis occur more frequently than suggested by current literature and should be considered in dogs presenting with jaundice and fever, abdominal pain, or an inflammatory leukogram or with ultrasonographic evidence of gallbladder abnormalities.

Key words: Canine; Cholangiohepatitis; Hepatitis; Liver disease.

Bacterial cholangitis is reported rarely in the dog, with previous reports comprising a few case reports and one small case series.[1–6] Reports of bacterial cholecystitis in dogs are also not common, but this disease has been described more frequently than cholangitis.[7–10] The pathogenesis of these conditions is poorly understood with little information available to determine the relationship, if any, between them. There are also few data available about the clinical implications of and rate of occurrence of bactibilia. A retrospective study of biliary culture results from both dogs and cats demonstrated that (13/46) bile samples from dogs yielded positive bacterial cultures.[11] However, the clinical consequences of positive bile cultures were not evaluated.[11] A more recent study looked at the occurrence of bactibilia and cholecystitis in a retrospective case series, showing (10/40) bile samples were positive and that (6/6) dogs evaluated had cholecystitis.[10]

It has been suggested that canine bacterial cholangitis might occur with a greater frequency than is currently reflected in the literature.[3,10,12] The aim of this study was to conduct a large multicenter retrospective survey to characterize both the frequency of presentation in dogs with hepatitis and the clinical characteristics of bacterial cholangitis and cholecystitis in dogs.

Abbreviations:

ALP	Serum alkaline phosphatase
ALT	Serum alanine transaminase
HE	Hematoxylin and Eosin
IgA	Immunoglobulin A
SIRS	Systemic inflammatory response syndrome

Materials and Methods

Case records of all dogs presented to 4 veterinary college referral hospitals in the United Kingdom and Ireland (University College Dublin Veterinary Hospital, Cambridge Veterinary School Queen's Veterinary Hospital, University of Bristol Small Animal Hospital, and the Royal Veterinary College Queen Mother Hospital) between January 2000 and June 2011 were reviewed retrospectively. The inclusion criteria for cases of bacterial biliary tract disease were the combination of a positive bile/gallbladder wall culture or a bile cytology/Gram stain, demonstrating bactibilia, with contemporaneous histopathological evidence of cholangitis,

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cholecystitis, or both. Cholangitis was defined by the demonstration of neutrophilic infiltration of portal areas, with or without extension into the hepatic parenchyma.[13] Cholecystitis was defined as infiltration of neutrophils into the gallbladder wall. The data collected from each case meeting the inclusion criteria included signalment, clinical history, information on previous treatments, physical examination findings, hematology and biochemistry results (pre- and postreferral if available), abdominal ultrasound findings, bacteriology results, bile cytology or Gram stain results, where available, along with treatment and outcome information. In addition, the clinical/histopathology databases (as appropriate for each location) were interrogated at each university to quantify the number of histopathologically confirmed cases of hepatitis presented at each institution during the same time period for comparison. The study received ethical approval from the University College Dublin, Animal Research Ethics Committee.

In each center, board-certified specialists in veterinary diagnostic imaging oversaw ultrasound examinations. The ultrasound reports were evaluated (where available); however, they were not systematically reviewed for predefined criteria by 1 ultrasonographer. Gallbladder wall thickening was defined by a thickness of ≥3 mm and bile duct distension by a diameter of >3 mm.[14–16]

Liver and extrahepatic biliary system biopsy samples were obtained by ultrasound-guided needle biopsy technique or during a laparotomy. After fixation in 10% neutral buffered formalin, biopsy samples were embedded in paraffin wax, sectioned (4 μm), and stained with Gill®-2 Hematoxylin and Eosin (HE). Biopsy samples from each case were evaluated by 1 pathologist (Jahns) using specific predetermined criteria.[3] Inflammation in the adventitia of the portal tracts was graded using a semiquantative score[17]: mild = few inflammatory cells with inconsistent involvement of portal triads, moderate = intermediate numbers of inflammatory cells affecting the majority of portal triads, severe = large numbers of inflammatory infiltrate with diffuse involvement of portal triads. Bile samples were obtained by cholecystocentesis, either by percutaneous ultrasound-guided sampling or directly during the course of a laparotomy. For all bacterial culture samples, both standard anaerobic and aerobic techniques were performed. If a positive culture was found, antibiotic susceptibility testing was then performed in the majority of cases. Cytology was performed on a proportion of the samples of bile which were stained with Giemsa and evaluated by a boarded clinical pathologist.

Results

Twenty-seven dogs fulfilled the inclusion criteria including 2 previously reported cases.[3,6] These cases comprised 12 males (6 neutered) and 15 females (14 neutered) with a mean age of 8.9 years (range 0.5–14 years). The median body weight was 15.4 kg (2.3–38.6 kg); weight was not recorded in 8 cases. The number of cases of hepatitis identified from databases during the study period was 378. However, the number of cases of hepatitis from Cambridge was only available between January 2007 and June 2011. Hence, the true overall hepatitis case figure for comparison is likely to be closer to 460 (based on extrapolation from 4.5 to 11.5 years for Cambridge). This would equate to an approximate prevalence of 6% for confirmed cases of bacterial cholangitis or cholecystitis within the hepatitis group.

The most frequent presenting complaints and physical examination findings (referring veterinarian history or at the time of referral) were vomiting (24/27), anorexia (19/27), lethargy (18/27), jaundice (15/27; described in history or noted in addition where hyperbilirubinemia [≥25 μmol/L] was recorded in the history), abdominal discomfort/pain (15/27), diarrhea (12/27), and pyrexia (9/27). Additional signs such as ascites (5 identified clinically and 9 ultrasonographically/27), weight loss, and polyuria/polydipsia were also reported in several cases. The duration of clinical signs prior to referral ranged from 1 day to several months. Nineteen dogs presented with an acute history (< 3 weeks) and 8 with chronic waxing and waning signs. Within the group with an apparently acute presentation, 4 cases had histories suggestive of an acute flare-up on a background of chronic disease. Approximately 2/3 of dogs had received antimicrobial treatment prior to referral. Summarized signalment, clinical presentation and clinical pathology findings are available in Table S1 presented as supporting information not appearing in the parent article.

The most frequently reported clinical pathology abnormalities, either prior to referral or at the time of referral, were increases in serum ALT (25/26 cases) and ALP activities (25/26 cases), hyperbilirubinemia (20/26 cases), hypercholesterolemia (13/22 cases), and an inflammatory leukogram (21/24 cases; neutrophilia 19 cases, band/toxic changes in 7 cases, and monocytosis in 8 cases). Concurrent band neutrophils/toxic changes and neutrophilia were apparent in 7 cases and monocytosis and neutrophilia in 7 cases. Globulin was increased in 4 cases; 2 of which had an inflammatory leukogram. Prothrombin time and activated partial thromboplastin time were within the reference intervals in 16 of the 19 cases evaluated.

The 19 cases with an acute presentation had either hyperbilirubinemia (15/18) or signs suggestive of an acute abdomen (8/19) or in a few cases, both abnormalities (4/18). Abdominal pain and pyrexia were commonly observed and were present in all except 4 of the acutely presenting cases. Neutrophilia was recorded in 15/19 of these acute cases, hyperglobulinemia in 4/19 of the cases, and in 2 cases both abnormalities were present. When considering the 8 cases with a chronic presentation, hyperbilirubinemia was documented in 5/8 cases, neutrophilia in 4/8 cases, and 3/8 cases presented with both abnormalities, leaving 2/8 cases presenting with neither change. All except 3 of the chronic cases had either abdominal pain or pyrexia.

Abdominal ultrasound reports were available in 26/27 cases. One case immediately underwent surgery having been referred with known gallbladder rupture. No abnormalities were found in 1 case. The most frequent findings were distended common bile duct (10/26), thickened gallbladder wall (9/26; Fig 1), distended gallbladder (9/26), gallbladder sludge (9/26), free abdominal fluid (8/26), heterogeneous hepatic parenchyma (7/26), hyperechoic hepatic parenchyma (6/26), and gallbladder mucocele (6/26). In each case where sludge was noted, there was at least one other abnormal finding reported. Choleliths (4/26), enlarged liver (3/26), gas in the gallbladder (3/26), thickened common bile duct wall (2/26), and ruptured gallbladder (2/26) were less commonly reported. In 3 cases, changes in echogenicity or size of portions of the pancreas were noted; however, reports of pancreatic findings were not available in the majority

of cases, so it was not possible to comment on the frequency of these abnormalities overall. Summarized ultrasound findings and clinical follow-up data are available in Table S2 presented as supporting information not appearing in the parent article.

Ascites was identified at presentation in 9 cases, either detected by clinical examination or by ultrasonography. In 5/9 cases, it was confirmed to be bilious. A further 3 cases were recorded as having an exudate, although bilirubin had not been measured in the fluid. In one of these 3 cases, an orange fluid was described at postmortem examination, and in another case, a small perforation was noted in the gallbladder at surgery and this dog developed postsurgical bile peritonitis. One of the 9 cases had previously undergone cholecystectomy for a mucocele at the referring veterinary practice and then subsequently developed ascites. At the time of referral, surgery revealed multiple acquired shunts and portal hypertension with common bile obstruction caused by stricture of the duodenal papilla.

Overall, there were 23 bile cultures (22/23 positive), 9 gallbladder wall cultures (9/9 positive), and 10 liver cultures (3/10 positive). Antimicrobial sensitivity testing was available in 24 cases. In 3 cases, bile was resampled, in 2 cases on 1 occasion and in 1 case on 2 occasions. The second samples were taken 3, 4, and 6 weeks after the first sample and antimicrobial treatment and were all positive (3/3 positive, n = 2 recultured *Enterococcus* spp., n = 1 cultured a different bacterium). The second resample culture, performed after 8 weeks of treatment, was negative. Concurrent cultures from different sites were available in a proportion of cases, as follows: bile and gallbladder wall cultures (n = 5, all the same isolates), gallbladder wall and liver culture (n = 1, same isolate), liver and bile cultures (n = 8, all bile culture positive and n = 2 liver cultures positive, same isolates), and liver, bile, and gallbladder wall cultures (n = 1, only gallbladder wall positive). Bile was obtained percutaneously using ultrasound guidance in 5 cases with no reported complications associated with this procedure. One additional case had bactibilia evidenced by a Gram stain (performed on histological sections taken from the common bile duct), but unconfirmed on culture. Cytological examination of the bile was performed in 4 cases, all of which had positive culture results. In 3 of these cases, the cytological examination confirmed bactibilia; 2 cases had rods (Fig 2) and one had cocci identified on the smear. In 1 case, there was no bactibilia on cytology. None of the samples had cytological evidence of inflammation.

Overall, there were 40 separate bacterial isolates from 26 dogs. The most frequent isolates from bile, gallbladder wall, or liver were *Escherichia coli* (n = 17 isolates in 16 cases), *Enterococcus* spp. (n = 8 isolates in 6 cases of which 4 were specifically speciated as *Enterococcus faecalis*), and *Clostridium* spp. (n = 5 in 5 cases of which 4 were specifically speciated as *Clostridium perfringens*). Other isolates included untyped coliforms (4), *Enterobacter cloacae* (1), *Klebsiella* sp. (1), *Proteus* sp. (1), *Bacteroides* sp. (1), a Gram-negative bacillus, and an untyped anaerobe. More than one bacterial species was isolated in 7/27 cases. In 10 of the cases from which *E. coli* was isolated, this was the sole bacterial isolate. In 31/32 aerobic isolates tested, antimicrobial resistance was identified (Table 1). Sixteen of these isolates were *E. coli*, of which 10/16 showed resistance to 3 or more classes of antimicrobials. All of the *Enterococcus* spp. isolates showed resistance to several agents. Resistance to amoxicillin clavulanate was noted in 2 *Enterococcus* spp. isolates. Four of 6 first isolates showed sensitivity to fluoroquinolones; although in 2 of these cases, a repeat culture 4–6 weeks later grew an isolate with enrofloxacin resistance.

After referral, 21 animals underwent surgery with cholecystectomy performed in 18 of these dogs. One additional dog had a cholecystectomy 3 weeks after a Tru-cut liver biopsy due to confirmation of cholelithiasis following a previously equivocal ultrasound examination. Overall, the indications for cholecystectomy were various: mucocele (8/19), gallbladder rupture (7/19), suspected cholecystitis due to gallbladder wall thickening, irregularity or gas in the bile duct/

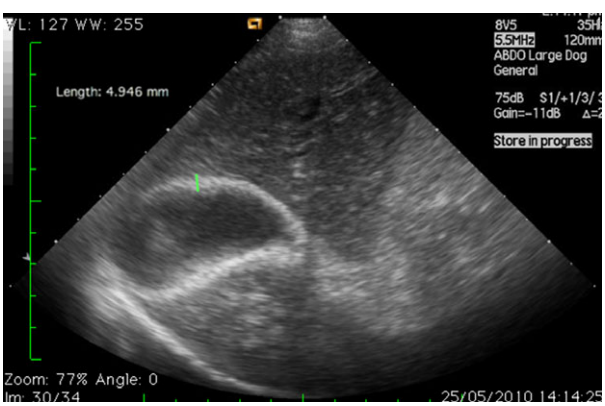

Fig 1. Ultrasound of gall bladder. This picture shows a thickened gall bladder wall, defined as ≥3 mm[13–15]. This was a common finding (9/26) in the study.

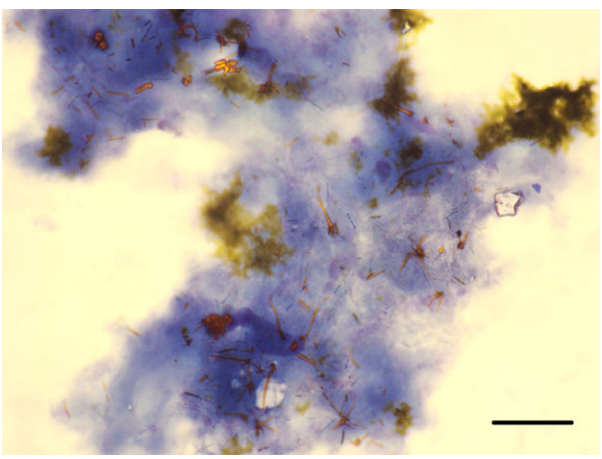

Fig 2. Cytology of the bile. This picture shows rod-shaped bacteria, as identified in 2 cases. Bar 10 μm.

Table 1. Antimicrobial sensitivity testing in bacterial isolates from 27 dogs with concurrent bactibilia and cholangitis, cholecystitis, or both.

		Penicillin				Cephalosporin						
Isolate Type	Number	N	A	+C	Carb	1st	2nd	3rd	Fluoroquinolone	Aminoglycoside	Tetracycline	TMP
E. coli	17	7/7 R	13/15 R	10/16 R + 1 I	2/2 R	12/16 R	8/11 R	4/9 R	3/16 R	3/5 R (1 S Amikacin R Gentamicin)	9/16 R	6/16 R
Other Coliforms/ *Proteus* sp.	6	2/3 R	4/6 R	1/6 R	1/1 R	1/6 R	1/4 R	0/2 R	2/6 R	0/2 R (1 I Streptomycin)	5/6 R	1/6 R
Enterococcus spp.	8	4/5 R	4/8 R	2/8 R	–	7/8 R	1/3 R	2/2 R	4/8 R	1/1 R	6/8 R	5/8 R

This table shows the proportion of those isolates tested that was resistant to given antimicrobial classes.

In total, there were 40 bacterial isolates in 26 cases. One additional case was diagnosed on a Gram stain with no culture.

Seven dogs had multiple isolates and 3 cases had positive culture results on a second sample taken after antimicrobial treatment.

Antimicrobial sensitivity testing was performed in 36 isolates using the Kirby-Bauer method according to the standard procedures of individual laboratories. All anaerobes tested (4/4) were sensitive to metronidazole; *Clostridium* spp. isolates (2), *Bacteroides* sp. (1), and an untyped anaerobe (1). The sensitivity results from 31 aerobic isolates are summarized in the table, 1 additional case (not included in the table) was reported as a profuse growth of a Gram-negative bacillus, sensitive to aminopenicillin and clavulanic acid with no further sensitivity testing reported. 3 *Clostridium* spp. isolates and 1 Coliform isolate were not sensitivity tested.

Treatment with antimicrobials prior to referral was reported in 18 cases, and no antimicrobial medication was recorded in 7 cases and was unknown in 2 cases. The most commonly prescribed antimicrobial prior to referral was amoxicillin clavulanate.

Key: R, resistant; S, sensitive; I, intermediate sensitivity; N, natural penicillin; A, aminopenicillin; +C, aminopenicillin +clavulanic acid; Carb, carboxypenicillin; 1^{st}, 2^{nd}, 3^{rd}, generation cephalosporin; TMP, trimethoprim sulfonamide.

gallbladder (or combination of these) (5/19), cholelithiasis (3/19), and 1 case with a distended gallbladder and solid-appearing contents at ultrasound (1/19). In 6 cases, the gallbladder rupture occurred concurrently with lithiasis, a mucocele, or emphysematous cholecystitis. Surgery was performed in the other 3 noncholecystectomy cases for duodenal stenosis at a previous surgical site (n = 1), surgical liver biopsies (n = 1) and persistent jaundice, distended common bile duct, and ascites in a dog with a history of previous mucocele excision (n = 1).

Liver histopathology was available in 26/27 cases (20 surgical, 5 Tru-cut biopsies, and 1 postmortem) and gallbladder histopathology in 20/27 cases, including 1 case that did not have liver histopathology (Table 2). All liver biopsy samples revealed cholangitis (Fig 3). This was mild in 11/26 cases, moderate in 14/26 cases, and severe in 1 case. Histopathological examination of the gallbladders (Fig 4) showed cholecystitis in 14/20 cases, gallbladder infarction in 5/20, and mucocele in 2/20 cases, although a greater number were noted on examination at surgery, giving a total number of 8. In 1 case, both cholecystitis and infarction were noted.

Overall, 21/27 dogs were discharged. Five dogs died or were euthanased perioperatively, and 1 additional dog was euthanased without treatment. Three of these dogs had bile peritonitis, 1 developed SIRS postoperatively, and the other died perioperatively following choledochotomy and stenting of the common bile duct. Four of these 6 patients that died presented with signs of an acute abdomen and were confirmed to have gallbladder rupture. The culture of multiple isolates on biliary culture did not correlate with a poorer prognosis. Short-term outcome (1–2 months) was available in 18 of the discharged cases: 10 cases were clinically well, 5 showed persistent clinical signs, in 2 dogs the serum activities of liver enzymes remained elevated but no other clinical information is available, and 1 dog died. Long-term outcome (1–3 years) was available in 11 cases with 6 alive at 3 years. Treatment information was available for 19/21 discharged dogs, although this was incomplete in several cases. The overall duration of treatment was difficult to discern clearly due to the retrospective nature of the study, but ranged from 4 to 12 weeks for antibiotic administration. Overall, 10 dogs received ursodeoxycholic acid, 13 received amoxicillin clavulanate, 7 received fluoroquinolones (typically enrofloxacin), 4 received metronidazole, and 3 received other antibiotics.

Concurrent conditions/relevant drug exposures identified in the cases included gallbladder mucocele in 8 dogs, cholelithiasis in 5 dogs, endogenous or exogenous glucocorticoid exposure in 4 dogs, the latter including treatment for immune-mediated thrombocytopenia, lymphoplasmacytic gastritis (also treated with Azathioprine), and long-term skin disease. Two dogs had previous surgery that could have predisposed them to biliary infection; one had duodenal stenosis and the other stricture of the duodenal papilla following previous cholecystectomy for a gallbladder mucocele. One additional dog had mild lymphoplasmacytic enteritis, 1 dog had a calcified gallbladder wall (which could have been an incidental finding), and 3 dogs had ultrasonographic signs suggestive of pancreatitis.

Discussion

This study presents data from a large number of confirmed cases of bacterial cholangitis, cholecystitis, or both in dogs and suggests that this disease might be

Table 2. Summarized histopathology findings and final diagnosis for 27 dogs with concurrent bactibilia and cholangitis, cholecystitis, or both.

		Liver Histopathology				Gallbladder Histopathology			
Groups of Cases	Number of Cases	A/C	+	++	+++	Cholecystitis	Infarction	Mucocele	Final Diagnosis and Potential Predisposing Factors
Acute presentation	11 Clinical cases 11 Liver histopathology 7 Gallbladder histopathology	8/11 Acute 3/11 Chronic	3/11	8/11	0/11	5/7	2/7	3/7	11/11 Cholangitis 5 Confirmed cholecystitis (incl. 2 with cholelithiasis and 1 with mucocele) 2 Thickened gallbladder wall (ultrasound) 3 Gallbladder mucocele (incl. 1 with Hypothyroidism) 2 Cholelithiasis 1 Receiving corticosteroids 1 Previous surgery (altered anatomy)
Acute presentation with signs of acute abdomen	8 Clinical cases 7 Liver histopathology 8 Gallbladder histopathology	7/7 Acute	3/7	3/7	1/7	6/8	3/8	2/8	7/7 Cholangitis 6/8 Cholecystitis (incl. 2 with cholelithiasis and 1 with mucocele) 2 Gallbladder mucocele 3 Cholelithiasis
Chronic presentation	8 Clinical cases 8 Liver histopathology 5 Gallbladder histopathology	6/8 Acute 2/8 Chronic	6/8	2/8	0/8	3/5	0/5	3/5 (+1 Mucocele previously removed)	8/8 Cholangitis 3/5 Confirmed cholecystitis (incl. 1 with mucocele, 1 partial calcification of gallbladder wall) 3 Gallbladder mucocele (incl. 1 with IBD receiving corticosteroids) 1 Previous surgery (altered anatomy—duodenal papilla stricture and previous mucocele removed) 2 Receiving corticosteroids 1 Hyperadrenocorticism 3 Inflammatory bowel disease

This table summarizes the liver and gall bladder histopathology findings of the 27 cases meeting the inclusion criteria. Cases are divided within the table into those that presented acutely and those that presented more chronically (>3 weeks history). The acute cases are further divided, with those in the shaded section all having presented with signs of an acute abdomen (acute presentation and free abdominal fluid).

Cholangitis was subjectively defined as +: mild, ++: moderate, +++: severe. A denotes acute and C denotes more chronic classification of the lesion.

IBD, inflammatory bowel disease; Incl., Including.

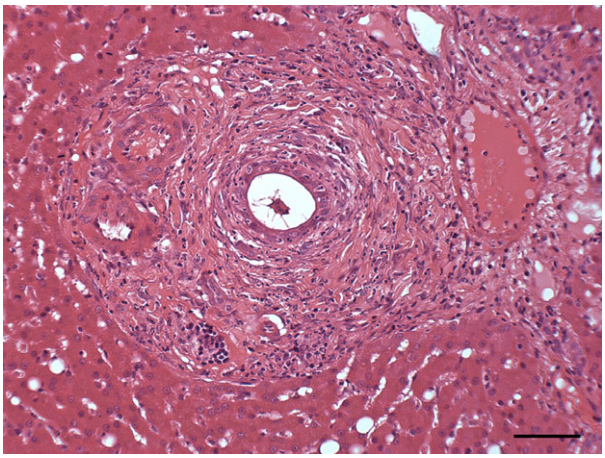

Fig 3. Liver, portal triad histopathology. Chronic active cholangitis; The portal area including bile duct epithelium (centre) are infiltrated by mainly neutrophils with some plasma cells, lymphocytes and macrophages. There is marked fibroplasia of the portal tract. Haematoxylin and Eosin (H&E) stain; Bar, 100μm

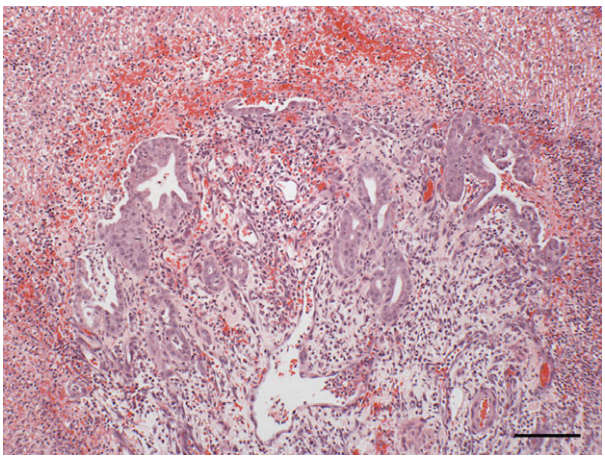

Fig 4. Gall bladder wall histopathology. There is marked epithelial hyperplasia with multifocal ulceration and necrosis. The lumen contains a thick exudate consisting of neutrophils, fibrin, and erythrocytes. The submucosa is edematous and markedly infiltrated by neutrophils, few plasma cells, and lymphocytes. Hematoxylin and eosin (H&E) stain; bar 100 μm.

more common in this species than previously believed. Twenty-seven cases met the inclusion criteria for this study, against a background of approximately 460 cases of hepatitis diagnosed in the same institutions over the equivalent time period. These findings are in agreement with the suggestion made that bacterial cholangitis, with or without concurrent cholecystitis, occurs more frequently than has been suggested by the literature to date.[3] Overall, bacterial biliary disease presented in 26 dogs as cholangitis of varying severity and 14 of these cases had confirmed concurrent cholecystitis. One additional dog had cholecystitis with no liver histopathology performed on this case.

The most frequent presenting complaints and physical examination findings in this study were vomiting, anorexia, lethargy, jaundice, abdominal discomfort, and diarrhea. In addition, pyrexia was noted in 9/27 of the dogs, with 20/27 cases presenting with either pyrexia or abdominal pain. These are reported to be common findings in animals with inflammatory or obstructive biliary tract disease.[18,19] The duration of clinical signs prior to presentation was variable, 19 cases presented acutely (signs present <3 weeks), and 8 dogs had a more chronic time course, typically with signs waxing and waning over this period. At least 18 cases had received antibiotic treatment prior to referral. In several cases, the clinical signs were reported to have improved with this treatment, although the data available from referring veterinarians were incomplete in many cases. In view of this, when figures were reported for the number of cases with clinical, such as jaundice or vomiting, they included cases that had the signs reported either in the referral history or at presentation, as it was thought to be more representative of the data, particularly when the signs wax and wane.

The main clinical pathology abnormalities noted in the present case series were liver enzyme elevation, hyperbilirubinemia, hypercholesterolemia, an inflammatory leukogram, and in a few cases, hyperglobulinemia, findings consistent with cholestasis and an inflammatory process.[18] These findings are broadly similar to those reported in previous cases; however, 10/10 cases in previous reports had been jaundiced and 8/8 had an inflammatory leukogram (2 others not reported) at some point in their history.[1–6] When the cases in this study are considered based on their presentation, 15/18 of the acute cases presented with hyperbilirubinemia (one additional acutely presenting case had no serum biochemistry performed) and 17/19 had at least one of neutrophilia, monocytosis, or hyperglobulinemia. This suggests that finding increased bilirubin and an inflammatory leukogram should increase the index of suspicion for cholangitis and/or cholecystitis. The cases presenting with acute signs in this report presented with either hyperbilirubinemia or an acute abdomen with biliary tract rupture, with a few cases showing both signs. The 3 cases without hyperbilirubinemia in this group of acute cases all had gallbladder rupture when investigated further. In the cases with chronic presentation, 5/8 dogs presented with hyperbilirubinemia and 4 had a mature neutrophilia with/without a concurrent monocytosis.

The ultrasound findings in the cases were variable and nonspecific, with almost all cases having abnormalities identified. Abnormalities relating to the biliary tract, such as gallbladder wall thickening and distension of the common bile duct, were identified commonly and liver parenchymal abnormalities in approximately 40% of cases. More specific abnormalities identified included choleliths, mucoceles, and free abdominal fluid. Gallbladder sludge has been reported to occur in normal animals, although its clinical significance is debated.[10,20] In addition, distension of the gallbladder is a subjective finding that was not clearly quantified. In view of this,

these latter 2 findings alone were not considered sufficient to warrant the description of an abnormal ultrasound. In each case, when present, the change was in addition to at least one other abnormal finding. One exception with respect the gallbladder size was that of a small gallbladder, noted in 2 cases, which were then found to have gallbladder rupture.

Although overall many of the ultrasound findings were nonspecific, they were generally helpful in case management. In 19 cases, abnormalities indicated a potential requirement for surgical intervention; these included free abdominal fluid and suspected ruptured gallbladder, choleliths, gas in the gallbladder, and biliary mucoceles. In 4 additional cases, the presence of thickening, irregularity, or abnormal echogenicity of the gallbladder wall raised suspicion of cholecystitis and directed bile sampling and culture. In 1 case, the presence of a gallbladder mass/cholelith and ascites led to the decision for euthanasia. In 1 case, increasing quantities of gallbladder sludge with persistent liver enzyme elevation led to a decision to resample bile. In only 1 case was the ultrasound not directly instrumental in the management decision. However, at this point, it is worth acknowledging the inherent bias of a study such as this; dogs with ultrasonographic abnormalities of the gallbladder, gallbladder wall, or bile duct are more likely to have gallbladder aspirates, culture, and/or histology and be included in this study, than dogs with normal ultrasound findings. It is not possible to determine the sensitivity of ultrasound for the confirmation of the presence of mucocele in these cases, as in several cases known to have a mucocele at surgery, the full ultrasound report was not available.

It is generally accepted that the human gallbladder and bile are normally sterile,[21] but whether this is the case in dogs and cats is unclear. Older studies suggested that organisms could readily be isolated from the normal canine liver.[22] More recently, it has been proposed that bile from dogs and cats is sterile in the absence of biliary tree pathology,[23–26] although intermittent bacterial isolation from the gallbladders of healthy dogs[27] and the culture of a variety of bacteria from liver biopsies of normal animals have been described.[28] There is known to be hepatobiliary-enteric circulation of bacteria whereby enteric bacteria gain entry into the portal circulation are delivered to the liver for extraction/killing and any remaining organisms are excreted in bile.[29] This mechanism can be overwhelmed experimentally by the administration of portal vein innocula,[25] a situation exacerbated by chronic biliary stasis. The cases presented in this report all showed histopathological evidence of neutrophilic cholangitis and/or cholecystitis, suggesting that the bacteria present in the bile were of clinical importance. One of the inclusion criteria for selection into the study was the presence of these conditions making it difficult to comment on the occurrence of bactibilia in the absence of inflammation. Further prospective studies of the normal biliary microbial flora of dogs and cats are necessary to address this area more thoroughly.

The literature available regarding bile or gallbladder wall culture in dogs with cholangitis is limited,[2,3,5,6] with more information available for cholecystitis[7–10] and cholelithiasis[29]in this species. There has been 1 large retrospective study looking at the prevalence and identity of bacterial isolates in canine and feline hepatobiliary cultures,[11] and 1 smaller case series looking at cholecystitis and bactibilia,[10] but the current report describes the largest reported series of positive bile/gallbladder wall cultures in the dog to date and is the only report to examine liver histopathology concurrently in the majority of cases. Overall, the results are in broad agreement with the findings from the previous reports; the predominant bacterial isolates from the hepatobiliary system were enteric isolates. *E. coli* was the most frequent bacterium isolated with *Enterococcus* spp. and *Clostridium* spp. also frequently cultured. In just under one third of dogs, more than 1 bacterial isolate was cultured; a lower figure than previously reported; 11/21 dogs in 1 previous study[11] and 6/10 in another.[10] In agreement with previous reports,[10,11] anaerobic organisms were cultured less frequently than aerobic organisms, but still represented an important group (7 obligate anaerobes and 1 facultative anaerobe) emphasizing the importance of anaerobic cultures in these cases.

An important finding in this report was the frequency with which antimicrobial resistance occurred. Resistance occurred frequently in *E. coli* isolates, with just under two thirds of resistant isolates showing resistance to 3 or more classes of antimicrobials. Two thirds of the *E. coli* isolates were resistant to amoxicillin clavulanate and 3 of these resistant isolates were also resistant to fluoroquinolones and 1st-generation cephalosporins. As would be expected, all of the *Enterococcus* spp. isolates showed resistance to several antimicrobial agents; however, an important finding was the development of fluoroquinolone resistance over time in the 2 cases that were resampled. Overall, the bacteriology and antimicrobial sensitivity results underline the importance of culture and sensitivity testing, particularly in view of the number of animals that had been referred with ongoing disease despite antibiotic treatment. They would also suggest that repeat sampling is important both to confirm or refute resolution of bactibilia, as has been previously suggested,[3,10] and to ensure there is continued antimicrobial sensitivity.

There is little known about the etiopathogenesis of bacterial cholangitis in dogs. The 2 main routes by which bacteria can invade the biliary tract are by ascending infection from the duodenum or hematogenously via the hepatic portal venous blood.[21] Normal biliary defense mechanisms comprise a number of elements: a mechanical barrier provided in part by the sphincter of Oddi, the flushing action of bile and bacteriostatic action of bile salts, and potent local immunological defense mechanisms (Kupffer cells, secretory IgA and mucus), aiding prevention of bacterial adhesion and colonization.[21] Factors that predispose to biliary infection are likely to impair these natural defense mechanisms, for example, biliary stasis and increased

biliary pressure,[21,22] or overwhelm the hepatobiliary-enteric circulation of bacteria, for example, large portal innocula or cholestasis.[25] Concurrent clinical conditions or exposure to immunosuppressant agents that could have predisposed to biliary infection were identified in 22/27 of the cases in this report. These conditions included gallbladder abnormalities, such as mucoceles, cholelithiasis, and a calcified gallbladder wall, which could cause cholestasis or provide a nidus for infection; surgery-induced anatomical abnormalities, such as duodenal stenosis and stricture of the duodenal papilla, which could affect biliary pressure and the potential for reflux into the biliary tree; and finally pancreatitis and lymphoplasmacytic enteropathy, which could again affect biliary pressure, biliary defenses, and in addition the gut microflora and the enterohepatic circulation of bacteria.

The potential interrelationship between bacterial cholangitis and cholecystitis has not been clearly defined, although it would seem likely that their etiology is interrelated due to similar predisposing factors.[29] Concurrent cholangitis has been observed in several cases within case series of cholecystitis, although not evaluated systematically[7,9,30] and 3 of the 4 cases in a case series of bacterial cholangitis had evidence of cholecystitis.[3] The inclusion criteria for the study were deliberately designed to be wide, including cases of confirmed bactibilia with histopathological evidence of cholangitis, cholecystitis, or both. One hypothesis would be that when bacterial biliary disease occurs, inflammation and infection become established in the gallbladder and then may progress to involve the biliary tree and liver. The confounding problem with this approach is that mild cases of cholecystitis are likely to be excluded due to lack of histopathological confirmation as their management would not entail cholecystectomy. In this study, 26 dogs had confirmed cholangitis, 20 had confirmed gallbladder pathology, a further 3 had gallbladder changes suggested by ultrasound findings, and 1 case had previous removal of a gallbladder mucocele. This shows a very large proportion of the cases had concurrent liver and gallbladder pathology; indeed as gallbladder evaluation was not performed in all cases, it is difficult to exclude the possibility of an even greater proportion having gallbladder pathology, particularly as it is well recognized in humans that ultrasound is not 100% sensitive for the detection of cholecystitis.[31–34] This concurrent presentation would tend to support a link between bacterial cholangitis and gallbladder pathology in dogs.

Cholecystis was identified in 14 of the 20 cases in which gallbladder histopathology was performed, suggesting that cholecystitis and cholangitis share similar predisposing causes or that one predisposes to the other. The occurrence of noncholecystitis gallbladder pathology in the other 6 cases is potentially of more significance, as one interpretation would be that a variety of gallbladder pathologies might predispose to cholangitis or that these conditions are also predisposed to by similar factors. Five of these 6 cases had biliary mucocele and 1 dog had gallbladder infarction and cholelithiasis. The presence of gallbladder pathology might disrupt the hepatobiliary-enteric circulation of bacteria, with any abnormal tissue acting as a potential nidus of infection, a situation shown experimentally.[25] Once there is any cholestasis or further perturbation of biliary defense mechanisms, the dogs would be likely to become symptomatic for cholangitis, as seen in the experimentally manipulated cats.[25] This situation is likely to be further exacerbated should cholecystitis develop, as bacterial infection has been shown to result in proliferative activity in the bile duct epithelium.[35] In several of the chronic cases in this report, antibiotics only provided a temporary resolution of clinical signs, which then resolved with cholecystectomy suggesting a continued focus of infection. It is noteworthy that bile or gallbladder wall cultures yielded a far higher proportion of positive culture results than liver cultures, in agreement with Wagner et al.[11]

The overall management of the cases was variable reflecting the diverse clinical presentations, different participating institutions, and the retrospective nature of the study. This type of study is not an appropriate design to allow clear recommendations on the most appropriate management of these cases. However, treatment with a broad-spectrum antibiotic is indicated for bacterial hepatobiliary disease with coverage for Gram-positive and Gram-negative aerobes and anaerobes, as evidenced by the bacteriological culture results. These findings, along with the results of antimicrobial sensitivity testing, underscore the importance of this type of evaluation. While empirical coverage with either a fluoroquinolone and amoxicillin clavulanate or a fluoroquinolone, metronidazole, and an amino penicillin could be suggested based on the likely organisms involved, resistance remains a potential problem. Significant resistance to both amoxicillin clavulanate and fluoroquinolones among *E. coli* and *Enterococcus* spp. isolates, along with examples of changing resistance over time in isolates from individual cases, highlights that an empirical approach to antimicrobial treatment should be used with caution. This is particularly pertinent where a medical approach is taken with the gallbladder left in situ, as a clinical response does not always equate to resolution of bactibilia.[3,10] Ursodeoxycholic acid would seem an appropriate choice to promote choleresis once biliary obstruction is relieved, although again the evidence base for this approach is currently limited.[29]

The main limitations of this study reflect its retrospective nature. All clinical records were not complete, hindering the ability to make clear interpretations about the sensitivity of investigations such as ultrasound for predicting particular clinical abnormalities. The analysis of treatment and outcomes was also limited by this approach and the variation in treatments between institutions. The inclusion criteria of histopathologically proven cholangitis, cholecystitis, or both were deliberately strict as little is known about these conditions and only clearly defined cases were included. This type of approach is likely to bias toward the inclusion of more severe cases that had samples taken for histopathology. This is particularly the case for cholecystitis cases where

tissue samples were available following cholecystectomy. In addition, cases with a histopathological diagnosis of neutrophilic cholangitis and an unproven bacterial etiology did not meet the inclusion criteria, likely to result in an underestimate of the true case numbers due to some of these cases being culture negative following antimicrobial treatment. However, despite these limitations, by virtue of the number of cases presented against a sparse literature in this area, this study offers important information about the rate of occurrence, clinical presentation, and features of canine cholangitis and cholecystitis.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Summarized signalment, clinical presentation, and clinical pathology findings for 27 cases with concurrent bactibilia and cholangitis, cholecystitis, or both.

Table S2. Summarized ultrasound findings and clinical follow-up data for 27 cases with concurrent bactibilia and cholangitis, cholecystitis, or both.