The Veterinary Journal 287 (2022) 105881

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

High frequency of cholecystitis in dogs with gallbladder mucocoele in Hong Kong

F.I. Hill [a,*], J.P. Speelman [b], K.K.L. Hui [b], O. Nekouei [c], P. Beczkowski [d], V.R. Barrs [d]

[a] CityU Veterinary Diagnostic Laboratory, City University of Hong Kong, Kowloon, Hong Kong
[b] CityU Veterinary Medical Centre, City University of Hong Kong, Sham Shui Po, Hong Kong
[c] Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Kowloon, Hong Kong
[d] Department of Veterinary Clinical Sciences, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Kowloon, Hong Kong

ARTICLE INFO

Keywords:
Canine
Cholecystectomy
Histopathology
Microbiology
Survival

ABSTRACT

The aims of this retrospective study were to characterise the epidemiological, clinical, histopathological, and microbiological findings as well as surgical outcomes in dogs admitted to a specialist veterinary hospital in Hong Kong for surgical management of gallbladder mucocoele (GBM). Inclusion criteria were cases with histopathological diagnosis of GBM and accompanying abdominal imaging, serum biochemistry, bile culture, and liver biopsy histology results. Fifty-six cases met the inclusion criteria. The median age at diagnosis was 12 years (range, 5–16 years). Miniature or toy pure-breed dogs were most commonly affected, including Poodles, Pomeranians, Schnauzers, Bichon frises and Chihuahuas. However, no breed was over-represented compared with their expected proportions among annual hospital admissions. Histological evidence of cholecystitis was present in 84% of cases, including acute cholecystitis in 18%, chronic cholecystitis in 37.5%, acute on chronic cholecystitis in 28% and acute with necrosis in 6%. The most common liver lesions were cholestasis in 64%, along with portal fibrosis in 55%, oedema in 50% and bile duct hyperplasia in 50%. Bile culture was positive in 29.6% of cases. *Escherichia coli* and *Enterobacter* species were most commonly isolated. *Stentrophomonas maltophili* was cultured from one case. Of the 16 cases where bacteria were isolated from bile culture, 94% had evidence of chronic cholecystitis and 81% had evidence of cholangiohepatitis. Fifty dogs (89.3%) survived to discharge including 5/5 dogs with ruptured gallbladders. Of 34 dogs with follow-up data, 21/34 (61.8%) were still alive 12 months later. Gallbladder mucocoeles were frequently associated with both acute and chronic inflammation. High survival rates to discharge were achieved.

Introduction

Gallbladder mucocoele (GBM) includes cystic mucinous hypertrophy of the mucosa (Kovatch et al., 1965) coupled with extreme mucin production and distension of the gallbladder (van den Ingh et al., 2006). Abnormal accumulation of mucous and bile precipitates (Smalle et al., 2015), can lead to biliary stasis or even gallbladder rupture.

Different studies have reported a higher incidence of GBM formation in certain breeds of dogs including Shetland sheepdogs (Aguirre et al., 2007), Miniature Schnauzers (Worley et al.,. 2004), Border terriers (Allerton et al., 2018), Cocker spaniels, Pomeranians, and Chihuahuas (Nagahara et al., 2018).

Excess bile duct epithelium secretion of mucin forming into a gel with abnormal properties is also implicated as an instigating factor for GBM formation in dogs (Kesimer et al., 2015). Decreased bile flow and decreased gallbladder motility precedes the development of GBM (Mizutani et al., 2017). Metabolomic analysis of dogs with a GBM suggests there may be significant metabolic changes in dogs forming mucocoeles (Gookin et al.,. 2018). In support of this, metabolic diseases associated with GBM in dogs include hyperadrenocorticism (Melián et al.,. 2006) and hypothyroidism (Mesich et al.,. 2009; Aicher et al., 2019). In some studies, prednisolone administration (Nagahara et al., 2018), imidacloprid use in Shetland sheepdogs (Aguirre et al., 2007), leptin concentrations (Lee et al.,. 2019), and hyperlipidaemia (Kutsunai et al., 2014) have also shown associations with GBM.

Histopathological examination of significant numbers of gallbladders with mucocoeles has been recommended as an important step in elucidating the pathophysiology of gallbladder mucocoeles (Mizutani

* Corresponding author.
E-mail address: fraser.hill@cityu.edu.hk (F.I. Hill).

https://doi.org/10.1016/j.tvjl.2022.105881
Accepted 7 August 2022
Available online 9 August 2022

et al., 2017).

The aims of this retrospective study were to characterise the clinical, epidemiological, histopathological, microbiological findings and surgical outcomes of an Asian population of dogs referred to a specialist veterinary medical centre for diagnosis and treatment of GBM.

Materials and methods

The laboratory database of the City University Veterinary Diagnostic Laboratory was searched for cases where gallbladder had been submitted for histopathology by veterinarians at City University Veterinary Medical Centre (VMC), from August 2018 to March 2021. Criteria for inclusion were cases with a histopathological diagnosis of a GBM characterised by mucosal hyperplasia and excess mucous production and where abdominal imaging, serum biochemistry, bile culture and histopathology of liver biopsies had also been performed.

Data were collected from the medical records including signalment (age, breed, sex, and desexing status), presenting signs, concurrent endocrinological disease diagnoses, serum biochemical parameters, results of bacterial culture of bile collected aseptically from the gallbladder at surgery, histopathological findings of resected gallbladder and liver biopsies, survival to discharge and to date of last follow-up.

Surgery was performed via an open celiotomy in all cases. The biliary tree was isolated where possible from other abdominal organs. Once satisfied that the common bile duct was patent, the gallbladder was carefully dissected away from the hepatic fossa using a combination of blunt dissection and mono and bipolar cautery. Dissection was continued up to the point where hepatic ducts co-joined the cystic duct. Right-angled forceps were placed across the cystic duct, and the gallbladder was excised en bloc. The common bile duct was then repeatedly flushed anterograde and in some cases retrograde to ensure no solid biliary material remained within the common bile duct (Putterman et al., 2021).

Antimicrobials were not routinely administered prior to collection of liver biopsies and bile samples. After these samples were collected, prophylactic perioperative cefazolin sodium (Stazolin, Standard Chemical and Pharmaceutical Co.; 22 mg/kg) was administered IV.

Entire gallbladders and liver biopsies were received fixed in 10% formalin with the bile duct ligated unless the gallbladder had ruptured. Once completely fixed, the gallbladders were typically transected at the cranial and caudal thirds, then cut longitudinally to prepare at least three representative sections of the gallbladder wall. Liver biopsies were cut along the longest access.

Tissue samples were processed overnight by standard histopathology methods, embedded in paraffin wax before cutting at 4 μm thick and staining with haematoxylin and eosin, along with Gram staining of gallbladder sections. Specialist veterinary pathologists reviewed all stained slides.

Cholecystitis was categorised into five subgroups; acute, chronic, acute on chronic, acute with necrosis and no inflammation based on previous published criteria (Rogers et al., 2020). The pathological definitions included acute cholecystitis (haemorrhage, neutrophil dominant infiltrates, or fibrin precipitates), chronic cholecystitis (infiltration of the submucosa and/or muscularis mucosa by lymphocytes, plasma cells, and/or macrophages), acute on chronic cholecystitis (haemorrhage, neutrophil infiltrates, or fibrin precipitates superimposed on chronic inflammation), cholecystitis with necrosis, and no inflammation.

Histopathological examination of the liver also assessed the type and degree of biliary and hepatic inflammation, portal fibrosis, oedema, cholestasis and/or bile duct hyperplasia. Cases were classified according to standardised histopathological criteria as acute or chronic neutrophilic cholangitis (van den Ingh et al., 2006). Acute neutrophilic cholangiohepatitis included a component of oedema and/or neutrophilic infiltrates in portal areas. Chronic neutrophilic cholangiohepatitis included a mixed portal inflammatory infiltrate comprising neutrophils, plasma cells and lymphocytes, with variable presence of bile ductular proliferation and fibrosis. Reactive hepatitis was defined as reactive to diseases in the gallbladder and was characterised by inflammatory cells in hepatic sinusoids or periportal tissue without hepatocyte apoptosis or necrosis and no significant fibrosis. Non-reactive hepatitis was characterised as primary liver pathology.

Bile samples were cultured aerobically and anaerobically. For aerobic culture, bile was plated directly onto blood agar, MacConkey agar, chocolate agar, and cooked meat medium. The inoculated media were then incubated at 37 °C before evaluation for bacterial growth at 48 h and 72 h. For anaerobic culture, bile was directly plated onto commercial anaerobic media before incubating at 37 °C for 4–7 days in an anaerobic environment produced by gas generation sachets (AnaeroGen 2.5 L Atmosphere Generation Systems, Thermo Fisher Diagnostics). Identification of cultured bacteria was performed by Matrix-Assisted Laser Desorption Ionisation-Time of Flight (Randall et al., 2015; MALDI-ToF system, Biotyper, Bruker Daltronics).

Antimicrobial susceptibility testing (AST)

AST was undertaken on any bacteria grown using the Kirby Bauer disc diffusion method (21 antimicrobials; Supplementary Table S1) in six cases and by broth microdilution techniques in 10 cases. Broth microdilution testing used commercially available 96-well Gram-positive and Gram-negative plates (Sensititre Thermo Fisher Diagnostics; 26 antimicrobials; Supplementary Table S1). The microtiter plates were inoculated and incubated for 18–24 h at 34–36°C, then analysed using automated fluorescence technology (Sensititre and OptiRead Thermo Fisher Diagnostics) and associated software (Sensititre SWIN Thermo Fisher Diagnostics,) to determine minimum inhibitory concentration of each antimicrobial tested. These categories were evaluated based on the veterinary breakpoints for each antibiotic determined by the Clinical and Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2020).

Statistical methods

To indirectly assess the potential association between breed and GBM, the proportion of the most common breeds of dogs in our dataset ($n \geq 5/56$) were compared with the expected proportion of these breeds among all dogs admitted to VMC per year, using the one-sample test of proportion. The annual, expected admission proportions of common breeds were estimated by averaging the total number of admissions in 2018 and 2019. In addition, the association between serum bilirubin with perioperative outcome of study cases (survival to discharge or not) was evaluated using an exact logistic regression with the survival as an outcome and bilirubin as the predictor. Statistical analyses were conducted using Stata v17 (StataCorp).

Results

Clinical data

Fifty-six cases met the inclusion criteria. There were 32 males (15 entire, 17 desexed) and 24 females (six entire, 18 desexed). The median age at diagnosis was 12 years (Q1 range, 7.8, 13.0; Q3 range, 5.0–16.0 years). The most common breeds affected were Miniature or Toy Poodles ($n = 16$), Pomeranians ($n = 6$), Miniature Schnauzers ($n = 6$), and Chihuahuas ($n = 5$; Table 1). Other breeds included Bichon frise ($n = 4$), Terrier breeds ($n = 4$), Crossbreeds ($n = 4$), Shetland sheepdogs ($n = 3$), Pugs (n = 2), and one each of the following - Beagle, Border collie, Collie, Corgi, Pekingese, and Shih tzu. Despite Poodles representing 29% of cases, this was not significantly different to the expected proportion of hospital admissions of this breed based on hospital admission records (Table 1). Abdominal ultrasound examination identified findings consistent with GBM in all dogs. The most common clinical signs at

Table 1
Comparison of the observed proportions of breeds most frequently diagnosed with gallbladder mucocoeles in this study compared to breed percentage of admissions to the City University Veterinary Medical Centre.

Breed	*n* (%)	Expected percentage[a]	*P*
Poodle (Miniature or Toy)	16 (28.6%)	21.9%	0.227
Pomeranian	6 (10.7%)	9.7%	0.797
Miniature Schnauzer	6 (10.7%)	6.4%	0.187
Chihuahua	5 (8.9%)	5.1%	0.192

[a] Proportion of admitted breeds to VMC, based on 2018 and 2019 weighted average data.

presentation were emesis in 41% ($n = 23$), diarrhoea in 14% ($n = 8$), anorexia in 13% ($n = 7$), and jaundice in 2% ($n = 1$). Abnormalities in hepatic biomarkers included elevations in alkaline transaminase in 88% ($n = 49$), alkaline phosphatase in 86% ($n = 48$), gamma-glutamyl transferase in 64% ($n = 36$), bilirubin in 52% ($n = 29$) and cholesterol in 38% ($n = 21$; Supplementary Table S2). Twenty-one dogs underwent endocrine testing due to clinical suspicion of an underlying endocrinopathy. Concurrent endocrinopathies were diagnosed in 10 dogs, including diabetes mellitus ($n = 3$), central diabetes insipidus ($n = 1$), hypothyroidism ($n = 4$), hyperadrenocorticism ($n = 1$), and atypical hypoadrenocorticism ($n = 1$). Other recorded co-morbidities including hypertriglyceridaemia ($n = 1$), cystic urolithiasis ($n = 10$; struvite-calcium phosphate-ammonium urate [$n = 1$]; calcium-oxalate [$n = 6$]; uncharacterised [$n = 3$]), renolithiasis ($n = 10$), pancreatitis ($n = 13$), and neoplasia ($n = 8$; Sertoli cell tumour [$n = 1$]; hepatocellular carcinoma [$n = 2$]; hepatic adenoma [$n = 2$]; hepatic lymphoma [$n = 1$]; duodenal gastrointestinal stromal tumour [$n = 1$]; and duodenal adenocarcinoma [$n = 1$]).

Pathology and histopathology

Grossly, the excised gallbladders were often turgid and firm with visible haemorrhage (Fig. 1). Five gallbladders (9%) were ruptured.

On sectioning, gallbladders were expanded by firm, grey material with radial striations associated with granular bile precipitates (Fig. 2). In addition to mucosal hyperplasia and evidence of excess mucous production, other typical histological features included tendrils of hyperplastic mucosa forming cysts filled with lightly basophilic, precipitated mucoid material and a precipitate of bile debris among the mucous (Fig. 3). There was evidence of cholecystitis in 84% (n = 47) of cases, including 20% (n = 11) with acute cholecystitis, 38% (n = 21) with chronic cholecystitis, 29% (n = 16) with acute on chronic cholecystitis, and 18% (n = 10) with necrosis (Table 3). The latter included mucosal

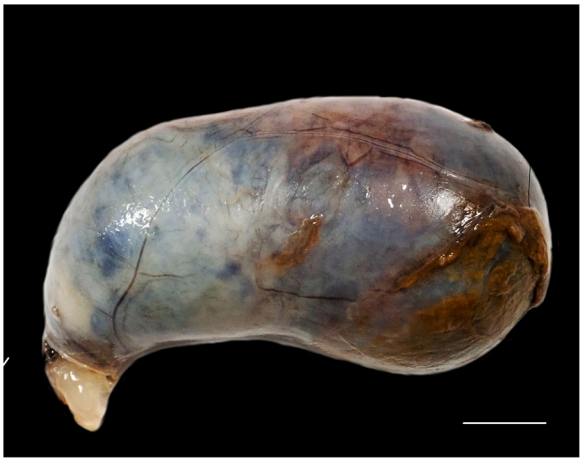

Fig. 1. Gallbladder surgically removed from a dog presenting with a history of vomiting and increased liver and biliary enzyme activities. Bar = 10 mm.

Fig. 2. Cut surface of a gallbladder mucocoele showing a firm precipitate of white to brown staining mucoid precipitate dilating the gallbladder, with radial striations, and centrally located, black, granular material consistent with bile precipitates. Bar = 10 mm.

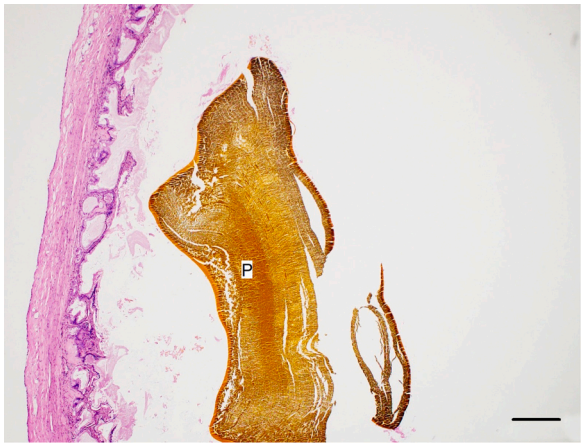

Fig. 3. Histopathology of a gallbladder surgically removed from a dog with a diagnosis of gallbladder mucocoele but no inflammation: hyperplastic tendrils of mucosa forming cysts along with a dense golden-brown, laminated bile precipitate (P) within the gallbladder lumen. No inflammation is present in the mucosa and submucosa. Haematoxylin and eosin. Bar = 400 μm.

necrosis and ulceration or erosion associated with haemorrhage, fibrin precipitates, and dense neutrophil infiltrates into the mucosa and submucosa (Fig. 4). Sometimes inflammation also involved the muscle layers and serosa. Evidence of chronic cholecystitis included infiltration of the submucosa and sometimes the muscularis layers by lymphocytes, plasma cells, and/or macrophages (Fig. 5).

Nine gallbladders (16%) had GBM but no inflammation on histopathology. Dogs with this diagnosis included one presenting with vomiting, three with increased liver enzymes, one with diarrhoea, and five where the GBM was an incidental finding on abdominal ultrasound.

Reactive histopathological lesions in the liver in response to extrahepatic cholestasis of the GBM included acute neutrophilic cholangiohepatitis in 5% (n = 3), chronic neutrophilic cholangiohepatitis in 84% (n = 47), periportal fibrosis in 55% (n = 32), oedema in 50% (n = 29), bile duct hyperplasia in 50% (n = 29), and bile duct or canaliculi cholestasis in 64% (n = 36). Non-reactive primary histopathology lesions in the liver included hepatocellular carcinoma (n = 2),

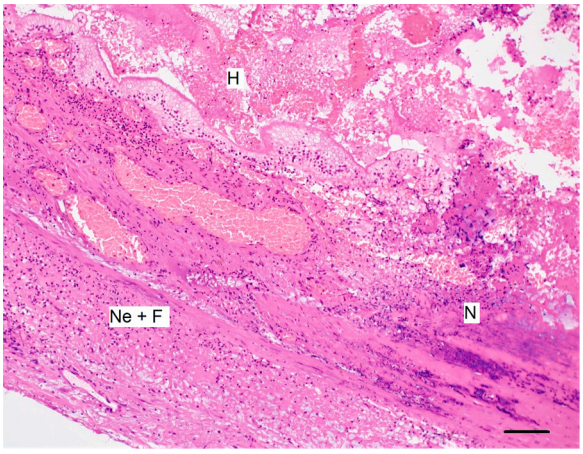

Fig. 4. Histopathology of acute inflammation in a gallbladder surgically removed from dog with a diagnosis of gallbladder mucocoele and inflammation (including haemorrhage (H) and necrosis (N) in the mucosa, along with neutrophil infiltrates and fibrin precipitates (Ne + F) in the submucosa, muscularis mucosa and serosa). Haematoxylin and eosin. Bar = 100 μm.

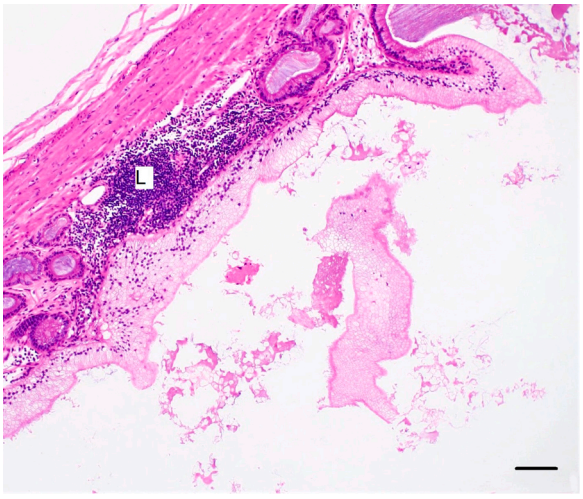

Fig. 5. Histopathology of chronic inflammation in a gallbladder surgically removed from dog with a diagnosis of gallbladder mucocoele and inflammation associated with a dense focal infiltrate of lymphocytes (L), plasma cells, and macrophages into the submucosa. Haematoxylin and eosin. Bar = 100 μm.

hepatic adenoma ($n = 2$), hepatic lymphoma ($n = 1$), metastatic duodenal gastrointestinal stromal tumour ($n = 1$), hepatic abscess ($n = 1$), excess copper accumulation ($n = 1$) and portal plate abnormality ($n = 1$). Bile culture was positive in 30% (n = 16) of cases; while in the 50 cases with acute or chronic cholangiohepatitis, bacteria were cultured 13 (26%).

Twenty organisms were cultured, with 14 samples yielded one isolate, one sample yielded two isolates (*Escherichia coli* and *Enterococcus faecalis*), and one sample yielded three isolates (*E. coli, Klebsiella pneumonia,* and *Bacteroides fragilis*; Table 2). Of the culture positive cases, two dogs had evidence of acute cholecystitis, eight dogs had chronic cholecystitis, 11 dogs had chronic cholangiohepatitis, and one dog had no evidence of cholangiohepatitis or cholecystitis (Table 3). Gram-stained histological sections revealed the presence of Gram-negative bacteria in only one gallbladder, from which case *E. coli* was isolated from bile. No bacteria were identified in Gram-stained sections of gallbladder from the remaining 55 cases.

Antimicrobial resistance was pronounced among the seven isolates of *E. coli* with six resistant to beta-lactams and five resistant to sulphonamides (Supplementary Table S1).

Table 2
Bacteria culture results from bile samples collected at surgery from 56 dogs with gallbladder mucocoeles.

Culture result	*n*
No growth	40
Aerobic bacteria isolated	19
Escherichia coli	7
Enterococcus faecalis	2
Klebsiella pneumoniae	2
Enterococcus hirae	2
Actinomyces spp.	1
Enterococcus cloacae	1
Enterococcus spp.	1
Micrococcus luteus	1
Pseudomonas aeruginosa	1
Stentrophomonas maltophilia	1
Anaerobic bacteria isolated	1
Bacteroides fragilis	1

Treatment and outcome

All dogs survived surgery. There was no significant association between presence or absence of a ruptured gallbladder or serum bilirubin concentration and perioperative survival, respectively. Fifty dogs (89%) survived to discharge including all five dogs with ruptured gallbladders diagnosed by abdominal ultrasonography pre-operatively. Six dogs died peri-operatively including three that died the day after surgery and three that were euthanised from two to six days after surgery. Two of the euthanised dogs had concurrent neoplasia (one each of hepatic lymphoma and gastrointestinal stromal tumour). Of the 34 dogs for which at least 12-months follow-up data were available, 21 dogs (62%) were alive one year after surgery.

Discussion

The majority (84%) of dogs with GBM in this population had histopathological evidence of acute or chronic cholecystitis. By contrast, other studies have reported cholecystitis associated with GBM much less frequently in 28.8% (Rogers et al., 2020), 16.6% (Pike et al.,. 2004), and 9.9% (Malek et al., 2013) of cases. One study reported higher rates of necrosis (25.6% cf. 16% in this study) in the gallbladder wall but a much higher rate of perforation or rupture (23% cf. 9% in this study; Malek et al., 2013), perhaps reflecting earlier diagnosis of cases in our study.

The frequency of positive bile culture (30%) in our study is the highest reported to date among cases of GBM. In previous reviews, 3% (Malek et al., 2013), 13.5% (Smalle et al., 2015), 13.9% (Harrison et al.,. 2018), 14.2% (Rogers et al., 2020), 14.3% (Mizutani et al., 2017), and 22% (Jaffey et al., 2018) of GBM had evidence of concurrent bacterial colonisation. The high rate of positive cultures in our study could be due to the microbiological sampling technique used, where no antimicrobials were administered until after the gallbladder had been removed at surgery and the use of a comprehensive array of culture media to optimise the conditions for bacterial growth.

The use of Gram stains on formalin fixed paraffin embedded (FFPE) preparations of gallbladder was ineffective at demonstrating the presence of bacteria, since bacteria were identified in only one of the 16 cases with positive bile culture in a case where *E. coli* was isolated. Based on the results of this study, Gram staining of FFPE is insensitive as an effective test for identifying the presence of bacteria and only bacterial culture would be recommended.

Bacteria can reach the gallbladder by translocation from the duodenum or by bloodborne entry into the liver followed by bile secretion into the gallbladder (Grigor'eva and Romanova, 2020). Previous studies in humans have shown the presence of the biofilm-forming bacteria *Pseudomonas aeruginosa, E. coli, K. pneumoniae, Enterococcus* spp., and

Table 3
Bacterial culture results from bile and histopathological characteristics of the gallbladder and liver in 56 cases of canine gallbladder mucocoele.

Case	Bacteria isolated from bile	Cholecystitis					Hepatitis		Cholangiohepatitis	
		Acute	Chronic	Acute on chronic	With necrosis	None	Reactive	Non- reactive/ other pathology	Acute neutrophilic	Chronic neutrophilic
1	*Micrococcus luteus*	–	+	–	–	–	+	–	–	+
2	–	+	–	–	+	–	+	–	–	+
3	–	–	+	–	–	–	+	–	–	+
4	*Escherichia coli*	–	+	–	–	–	+	–	–	+
5	*E. coli*	–	–	+	–	–	+	–	–	+
6	–	–	+	–	–	–	+	–	–	+
7	–	–	+	–	–	–	+	–	–	+
8	–	–	+	–	–	–	+	–	–	+
9	–	–	+	–	–	–	+	–	–	+
10	–	–	+	–	–	–	+	–	–	+
11	–	–	+	–	–	–	+	–	–	+
12	*Stentrophomonas maltophilia*	+	–	–	+	–	+	–	–	+
13	*E. coli*	–	+	–	–	–	+	–	–	+
14	–	+	–	–	+	–	+	–	–	+
15	–	–	–	–	–	+	+	–	–	+
16	*Enterococcus faecalis*	–	–	+	–	–	–	HCC	–	–
17	*E. coli, Klebsiella pneumoniae, and Bacteroides fragilis*	–	–	+	+	–	+	–	–	+
18	–	+	–	–	+	–	+	–	–	+
19	–	–	–	+	–	–	–	HCC	–	–
20	*Enterococcus* spp.	–	–	–	–	+	–	Lipidosis	–	–
21	–	+	–	–	+	–	+	–	–	+
22	*Enterobacter cloacae*	–	–	+	–	–	+	–	–	+
23	–	+	–	–	–	–	+	–	–	+
24	–	–	–	+	–	–	+	–	+	–
25	–	+	–	–	–	–	+	–	–	+
26	*E. coli, K. pneumoniae*	–	+	–	–	–	+	–	–	+
27	–	–	–	–	–	+	+	–	+	–
28	–	–	–	–	–	+	+	–	–	+
29	*E. coli*	–	+	–	–	–	+	–	–	+
30	–	–	–	+	+	–	+	–	+	–
31	–	–	–	+	–	–	+	–	–	+
32	–	–	+	–	–	–	+	–	–	+
33	*E. coli, E. faecalis*	–	+	–	–	–	+	–	–	+
34	*Pseudomonas aeruginosa*	+	–	–	+	–	+	–	–	+
35	–	+	–	–	+	–	–	Lymphoma	–	–
36	–	–	–	+	–	–	+	–	–	+
37	–	–	+	–	–	–	+	–	–	+
38	–	–	–	+	–	–	–	HAD	–	–
39	–	–	–	–	–	+	+	–	–	+
40	–	–	–	–	–	+	+	–	–	+
41	*Enterococcus hirae*	–	–	+	–	–	+	–	–	+
42	*Actinomyces* spp.	–	+	–	–	–	–	Copper hepatopathy	–	+
43	–	–	–	+	–	–	+	HAD	–	+
44	–	–	–	–	–	+	+	–	–	+
45	–	–	+	–	–	–	+	–	–	+
46	–	–	–	–	–	+	–	–	–	–
47	–	–	–	+	–	–	+	GITAC	–	+
48	–	–	–	–	–	+	+	–	–	+
49	–	–	+	–	–	–	+	GIST	–	+
50	–	–	+	–	–	–	+	–	–	+
51	–	–	–	+	–	–	+	–	–	+
52	*Enterococcus hirae*	–	+	–	–	–	+	–	–	+
53	–	–	+	–	–	–	–	Abscess	–	+
54	–	–	–	+	–	–	+	–	–	+
55	–	+	–	–	+	–	+	–	–	+
56	–	+	–	+	–	–	+	–	–	+

HCC, hepatocellular carcinoma. HAD, hepatic adenoma. GITAC, gastrointestinal adenocarcinoma. GIST, gastrointestinal stromal tumour.

Acinetobacter spp. in bile and gallbladders (Tajeddin et al., 2016). However, the finding of bacteria in bile in isolation is not indicative of a pathogenic role. Bile from healthy dogs was previously thought to be sterile, but bile collected aseptically and percutaneously has been found to contain bacteria (Kook et al., 2010) and the gallbladder has a complex microbiota even without pathology (Grigor'eva and Romanova, 2020). Despite healthy bile not being sterile, in 14/16 cases in this study with bacteria cultured from bile, there was histological evidence of neutrophilic cholecystitis and/or cholangiohepatitis, suggesting their presence was clinically significant.

Micrococcus luteus was cultured from one isolate and was likely a contaminant, as it is commonly found on human skin. *Stentrophomonas maltophili,* an environmental bacterial species, has emerged as a nosocomial pathogen, often associated with respiratory infection in humans in conjunction with *P. aeruginosa,* and has also been isolated from patients with biliary sepsis (Brooke, 2012). It may have been a primary pathogen here, as it was associated with acute cholangiohepatitis.

Only one anaerobe was cultured, identified as *Bacteroides fragilis*. This organism is a normal constituent of the colonic flora (Hedberg and Nord, 2003) and most likely translocated from the intestine into the gallbladder. The dog from which *B. fragilis* was isolated presented with emesis and lethargy, had evidence of acute cholecystitis and

cholangiohepatitis, survived surgery and was discharged.

There were epidemiological differences in the cohort studied here, compared to other investigations of GBM. The median age at the time of surgery of 12 years, was older than previous studies where the median age at diagnosis was between 9 and 10 years (Pike et al., 2004; Cornejo and Webster, 2005; Malek et al., 2013; Smalle et al., 2015; Mizutani et al., 2017; Harrison et al., 2018; Rogers et al., 2020). However, the age range of dogs in our study (5–16 years) was similar to other studies (Pike et al., 2004; Cornejo and Webster, 2005).

Slightly more males (59%) than females (41%) were presented for surgery for GBM, a sex ratio of 1.43. In other studies, a wide variation in the ratio of females to males affected has been reported, ranging from 0.82 to 1.7, and there is no evidence that sex is related to the development of GBM (Aguirre et al., 2007; Malek et al., 2013; Kutsunai et al., 2014; Gookin et al., 2015; Harrison et al., 2018).

Nearly a third of all cases in our study were Poodles but this breed is also the most popular canine breed in Hong Kong and no breed predisposition was identified. Similarly, although Pomeranians, Miniature Schnauzers, and Chihuahuas were over-represented in other studies of GBM and were among the most common breeds affected in our study, they were not over-represented compared to annual hospital admissions.

A previous study found that increases of serum bilirubin greater than 4.3 times the upper limit of the reference range was associated with increased odds of death (Jaffey et al., 2019). In our study, the proportion of dogs not surviving to discharge was not associated with serum bilirubin concentration. One explanation for this finding could be the limitation of our data due to the low number of only six deaths among a total of 56 cases.

Elective cholecystectomy is recommended as the best treatment option for canine GBM and should be undertaken in dogs with early signs of gallbladder disease to avoid greater mortality associated with gallbladder rupture (Youn et al., 2018). In contrast to a previous study where gallbladder rupture found at surgery was related to a 2.7 times increase in the risk of death and 41.7% of dogs with ruptured gallbladders died (Jaffey et al., 2018), none of the five dogs with a ruptured gallbladder in our study died. The survival rate to discharge in this study was 89% and compares favourably to other studies where survival rates vary from 59% to 91% (Harrison et al., 2018; Youn et al., 2018; Jaffey et al., 2019).

Conclusions

Cholecystitis was present in the majority of the gallbladders in this study with reactive lesions of cholangiohepatitis also common. Hepatic pathology unrelated to GBM was present in 16% of cases, highlighting the importance of hepatic biopsy at surgery. Gallbladder rupture was not associated with peri-operative mortality.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgements

We thank our contributing veterinary surgeons; Drs. Almendros, Ho, Kam, Lam, Saulnier-Troff, and Woodbridge for providing biopsies for analysis, Drs. Tse and Sandy for reading some of the histopathology, and Dr Bhardwaj for provided the microbiology methods. Thanks to the microbiology and histology technologists at CityU VDL for processing the samples.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tvjl.2022.105881.

References

Aguirre, A.L., Center, S.A., Randolph, J.E., Yeager, A.E., Keegan, A.M., Harvey, H.J., Erb, H.N., 2007. Gallbladder disease in Shetland sheepdogs: 38 Cases (1995-2005). Journal of the American Veterinary Medical Association 231, 79–88.

Aicher, K.M., Cullen, J.M., Seiler, G.S., Lunn, K.F., Mathews, K.G., Gookin, J.L., 2019. Investigation of adrenal and thyroid gland dysfunction in dogs with ultrasonographic diagnosis of gallbladder mucocele formation. PLOS ONE 14, 1–33.

Allerton, F., Swinbourne, F., Barker, L., Black, V., Kathrani, A., Tivers, M., Henriques, T., Kisielewicz, C., Dunning, M., Kent, A., 2018. Gall bladder mucoceles in Border terriers. Journal of Veterinary Internal Medicine 32, 1618–1628.

Brooke, J.S., 2012. *Stenotrophomonas maltophilia*: an emerging global opportunistic pathogen. Clinical Microbiology Reviews 25, 2–41.

Clinical and Laboratory Standards Institute, 2020. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Test for Bacteria Isolated from Animals, fifth ed. Clinical and Laboratory Institute, Wayne, PA. CLSI Supplement VET01S,.

Cornejo, L., Webster, C.R.L., 2005. Canine gallbladder mucoceles. Compendium on Continuing Education for the Practicing Veterinarian 27, 912–928.

Gookin, J.L., Correa, M.T., Peters, A., Malueg, A., Mathews, K.G., Cullen, J., Seiler, G., 2015. Association of gallbladder mucocele histologic diagnosis with selected drug use in dogs: a matched case-control study. Journal of Veterinary Internal Medicine 29, 1464–1472.

Gookin, J.L., Mathews, K.G., Cullen, J., Seiler, G., 2018. Qualitative metabolomics profiling of serum and bile from dogs with gallbladder mucocele formation. PLOS ONE 13, 1–31.

Grigor'eva, I.N., Romanova, T.I., 2020. Gallstone disease and microbiome. Microorganisms 8, 835–851.

Harrison, J.L., Turek, B.J., Brown, D.C., Bradley, C., Callahan Clark, J., 2018. Cholangitis and cholangiohepatitis in dogs: a descriptive study of 54 cases based on histopathologic diagnosis (2004–2014). Journal of Veterinary Internal Medicine 32, 172–180.

Hedberg, M., Nord, C.E., 2003. Antimicrobial susceptibility of *Bacteriodes fragilis* group isolates in Europe. Clinical Microbiology and Infection 9, 475–488.

van den Ingh, T.S., Cullen, J.M., Twedt, D.C., Van Winkle, T., Desmet, V.J., Rothuizen, J., 2006. Morphological classification of biliary disorders of the canine and feline liver. WSAVA Standards for Clinical and Histological Diagnosis of Canine and Feline Liver Diseases. Saunders Elsevier, St. Louis, MO, USA, pp. 61–77.

Jaffey, J.A., Graham, A., VanEerde, E., Hostnik, E., Alvarez, W., Arango, J., Jacobs, C., DeClue, A.E., 2018. Gallbladder mucocele: Variables associated with outcome and the utility of ultrasonography to identify gallbladder rupture in 219 dogs (2007–2016). Journal of Veterinary Internal Medicine 32, 195–200.

Jaffey, J.A., Pavlick, M., Webster, C.R., Moore, G.E., McDaniel, K.A., Blois, S.L., Brand, E. M., Reich, C.F., Motschenbacher, L., Hostnik, E.T., et al., 2019. Effect of clinical signs, endocrinopathies, timing of surgery, hyperlipidemia, and hyperbilirubinemia on outcome in dogs with gallbladder mucocele. The Veterinary Journal 251, 105350.

Kesimer, M., Cullen, J.M., Cao, R., Radicioni, G., Mathews, K.G., Seiler, G., Gookin, J.L., 2015. Excess secretion of gel-forming mucins and associated innate defense proteins with defective mucin un-packaging underpin gallbladder mucocele formation in dogs. PLOS ONE 10, 1–20.

Kook, P.H., Schellenberg, S., Grest, P., Reusch, C.E., Corboz, L., Glaus, T.M., 2010. Microbiological evaluation of gallbladder bile of healthy dogs and dogs with iatrogenic hypercortisolism: a pilot study. Journal of Veterinary Internal Medicine 24, 224–228.

Kovatch, R.M., Hildebrandt, P.K., Marcus, L.C., 1965. Cystic mucinous hypertrophy of the mucosa of the gall bladder in the dog. Veterinary Pathology 2, 574–584.

Kutsunai, M., Kanemoto, H., Fukushima, K., Fujino, Y., Ohno, K., Tsujimoto, H., 2014. The association between gall bladder mucoceles and hyperlipidaemia in dogs: a retrospective case control study. The Veterinary Journal 199, 76–79.

Lee, S., Lee, A., Kweon, O.K., Kim, W.H., 2019. Changes in pre- and postoperative serum leptin concentrations in dogs with gallbladder mucocele and cholelithiasis. BMC Veterinary Research 15, 1–9.

Malek, S., Sinclair, E., Hosgood, G., Moens, N.M.M., Baily, T., Boston, S.E., 2013. Clinical findings and prognostic factors for dogs undergoing cholecystectomy for gall bladder mucocele. Veterinary Surgery 42, 418–426.

Melián, C., Morales, M., Perez-Alenza, M.D., Corbera, J.A., Diez Bru, N., 2006. Gallbladder mucocele in two dogs with pituitary-dependent hyperadrenocorticism: a case report. Journal of Applied Animal Research 30, 117–120.

Mesich, M.L.L., Mayhew, P.D., Paek, M., Holt, D.E., Brown, D.C., 2009. Gall bladder mucoceles and their association with endocrinopathies in dogs: a retrospective case-control study. Journal of Small Animal Practice 50, 630–635.

Mizutani, S., Torisu, S., Kaneko, Y., Yamamoto, S., Fujimoto, S., Ong, B.H., Naganobu, K., 2017. Retrospective analysis of canine gallbladder contents in biliary sludge and gallbladder mucoceles. Journal of Veterinary Medical Science 79, 366–374.

Nagahara, T., Ohno, K., Kanemoto, H., Kakimoto, T., Fukushima, K., Goto-Koshino, Y., Tsujimoto, H., 2018. Effect of prednisolone administration on gallbladder emptying rate and gallbladder bile composition in dogs. American Journal of Veterinary Research 79, 1050–1056.

Pike, F.S., Berg, J., King, N.W., Penninck, D.G., Webster, C.R.L., 2004. Gallbladder mucocele in dogs: 30 Cases (2000-2002). Journal of the American Veterinary Medical Association 224, 1615–1622.

Putterman, A.B., Selmic, L.E., Kindra, C., Duffy, D.J., Risselada, M., Phillips, H., 2021. Influence of normograde versus retrograde catheterization of bile ducts in dogs treated for gallbladder mucocele. Veterinary Surgery 50, 784–793.

Randall, L.P., Lemma, F., Koylass, M., Rogers, J., Ayling, R.D., Worth, D., Klita, M., Steventon, A., Line, K., Wragg, P., et al., 2015. Evaluation of MALDI-ToF as a method

for the identification of bacteria in the veterinary diagnostic laboratory. Research in Veterinary Science 101, 42–49.
Rogers, E., Jaffey, J.A., Graham, A., Hostnik, E.T., Jacobs, C., Fox-Alvarez, W., Van Eerde, E., Arango, J., Williamns, F., DeClue, A.E., 2020. Prevalence and impact of cholecystitis on outcome in dogs with gallbladder mucocele. Journal of Veterinary Emergency and Critical Care 30, 97–101.
Smalle, T.M., Cahalane, A.K., Koster, L.S., 2015. Gallbladder mucocoele: a review. Journal of the South African Veterinary Association 86, 13–18.
Tajeddin, E., Sherafat, S.J., Majidi, M.R.S., Alebouyeh, M., Alizadeh, A.H.M., Zali, M.R., 2016. Association of diverse bacterial communities in human bile samples with biliary tract disorders: a survey using culture and polymerase chain reaction-denaturing gradient gel electrophoresis methods. European Journal of Clinical Microbiology and Infectious Diseases 35, 1331–1339.
Worley, D.R., Hottinger, H.A., Lawrence, H.J., 2004. Surgical management of gallbladder mucoceles in dogs: 22 Cases (1999-2003). Journal of the American Veterinary Medical Association 225, 1418–1422.
Youn, G., Waschak, M., Kunkel, K., Gerard, P., 2018. Outcome of elective cholecystectomy for the treatment of gallbadder disease in dogs. Journal of the American Veterinary Medical Association 252 (8), 970–975. https://doi.org/10.2460/javma.252.8.970.