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A multicenter retrospective study assessing progression of biliary sludge in dogs using ultrasonography

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Abstract

Background: Biliary sludge (BS) frequently is identified on ultrasonographic examination and is described as incidental. It is hypothesized that biliary stasis and hypersecretion play a role in both BS and gallbladder mucocele (GBM) formation. Recent studies have documented similarities in composition of BS and GBM, and there are several examples of progression from BS to GBM in the veterinary literature.

Objectives: To assess the relationship between the presence of BS and later development of GBM in dogs, over time periods >12 months.

Animals: A total of 154 dogs with BS and ultrasonographic follow-up >12 months.

Methods: Medical records were retrospectively collected from 9 UK-based referral centers for all available time points. A semiobjective scoring system was used to track volume of BS within the gall bladder (GB) over time.

Results: Twenty dogs developed GBM during the study period. Shetland Sheepdogs (odds ratio [OR], 40.99; 95% confidence interval [CI], 3.61-465.95; $P = .003$) and Border Terriers (OR, 11.66; 95% CI, 3.28-46.63; $P < .001$) were independent risk factors for the development of GBM. Non–gravity-dependent BS (NDBS) was noted to form before GBM development in 9/20 dogs, and breeds at-risk for GBM were more likely to have NDBS. Odds for the development of GBM increased with BS score.

Conclusions and Clinical Importance: Dogs with NDBS may be at risk for the development of GBM and a stratified BS scoring system could allow for semiobjective monitoring over time, particularly in at-risk breeds.

KEYWORDS
gallbladder mucocele, nondependent biliary sludge, observational retrospective, risk factors

1 | INTRODUCTION

Biliary sludge (BS) frequently is identified during abdominal ultrasonography, with prevalence in healthy dogs ranging from 35% to 67%.[1-3] It is composed of precipitated crystals (primarily cholesterol

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BS, biliary sludge; CI, confidence interval; DBS, dependent biliary sludge; GBM, gallbladder mucocele; GGT, gamma glutamyltranspeptidase; HAC, hyperadrenocorticism; NDBS, nondependent biliary sludge; OR, odds ratio; RR, reference range; UDCA, ursodeoxycholic acid.

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monohydrate and calcium salts), glycoproteins, cellular debris, and mucins within the gallbladder (GB).[2] It has been described as incidental in dogs,[4] but in humans it is considered abnormal, associated with cholelith formation,[5] and complications include biliary colic, cholecystitis, and pancreatitis.[6] It is hypothesized that biliary stasis and mucus hypersecretion play a role in BS formation[1,7,8] and although not reportedly associated with hepatobiliary disease in dogs,[4] a previous study suggested that dogs with larger volumes of BS were more likely to have hepatobiliary disease than those with minimal GB contents,[3] although the diagnosis of hepatobiliary disease was not definitively established in all cases.

Gallbladder mucocele (GBM) is characterized as accumulation of inspissated bile within the GB, causing pressure necrosis, extrahepatic biliary duct obstruction, and GB rupture leading to bile peritonitis.[9] Biliary stasis and mucosal hypersecretion have been hypothesized as potential primary factors in GBM formation, in addition to BS formation.[10] Suggested risk factors include hyperadrenocorticism (HAC),[11-13] hypothyroidism,[11,13] dyslipidemias,[14] increased serum leptin concentration,[15] and neonicotinoid administration.[13] Known breed predispositions include Shetland Sheepdogs[13,16] and Border Terriers[17] with Miniature Schnauzers, Chihuahuas, Pomeranians, and Cocker Spaniels overrepresented in some studies.[10,13,14,18]

Infrared spectroscopy analyses found both BS and GBM contents are primarily composed of mucins, with similar constituents and concluded there may be a shared pathogenesis.[9,19] Impaired GB emptying occurs with both BS and GBM,[3] with the most marked change seen with GBM compared to controls,[7] and it has been linked to non–gravity-dependent BS.[20] The veterinary literature contains examples of progression of BS to GBM,[19,21] and it has been hypothesized that the presence of BS may be a risk factor for the development of GBM. Biliary sludge has been prospectively assessed using ultrasonography over 12 months in 45 dogs, with persistent BS in 88% cases, and 24% developing nondependent BS but no GBM.[1] To our knowledge, no studies have assessed changes in BS over time periods >12 months.

Ursodeoxycholic acid (UDCA) is used in the medical management of GBM and BS in dogs.[18,21,22] It has hydrophilic properties that draw water into bile, helping increase fluidity, and relieve biliary congestion, while displacing cytotoxic hydrophobic bile acids.[23,24] Mucins serve as a mucoprotectant within the biliary tract in humans,[25] and presumably in dogs, and hypersecretion occurs in vitro in response to excessive hydrophobic bile acid concentrations.[26] We hypothesized that UDCA might decrease mucin hypersecretion, and thus volume of BS, by displacement of hydrophobic bile acids.

Our multicenter retrospective study aimed to assess the presence of a relationship between BS and later development of GBM, over time periods >12 months. A secondary aim was to assess the effectiveness of UDCA in decreasing the volume of BS in dogs.

2 | MATERIALS AND METHODS

Cases were recruited from 9 tertiary-level veterinary hospitals in the United Kingdom (Queen's Veterinary School Hospital, Davies Veterinary Specialists, Langford Small Animal Referral Hospital, Anderson Moores Veterinary Specialists, Willows Veterinary Centre and Referral Service, Pride Veterinary Centre, University of Glasgow Small Animal Hospital, Southern Counties Veterinary Specialists, and North Downs Specialist Referrals). Retrospective evaluation of medical records was performed, with the primary inclusion criterion being the presence of BS confirmed on ultrasonographic examination by a board-certified radiologist, or resident under supervision of a board-certified radiologist, with ultrasonographic follow-up examination at least 12 months after the primary scan. For each case, data that were retrospectively collected included breed, age, sex, neuter status, clinical signs, presence of concurrent disease, serum biochemistry results at first diagnosis and subsequent examinations, current, or past medications (including administration of UDCA), duration of follow-up, and number of repeat ultrasonographic examinations and their dates. Exclusion criteria were incomplete medical history or lack of ultrasonographic follow-up >12 months. A semiobjective scoring system was utilized, using still ultrasonographic images of the GB where available, to estimate the percentage of GB volume filled with BS. Images were scored 0 for no BS, 1 for BS occupying <25% of GB area, 2 for 26% to 50% of GB area, 3 for 51% to 75% of GB area, 4 for >75% GB area, and 5 for GBM, extrapolated from a similar system used previously.[1,3] A diagnosis of GBM was made when confirmed on the radiology report for each case. Change in sludge score was calculated as the net overall change in sludge score for each individual dog across all time points recorded.

Biliary sludge was defined as persistent (when present at all recorded time points), resolved, or recurrent (initial resolution and then recurrence at a subsequent time point). Biliary sludge was noted as gravity dependent (DBS) or nongravity dependent (NDBS) based on the radiology report for each time point where data had been recorded.

Serum biochemistry analyses were recorded from all available time points; variables included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma glutamyltranspeptidase (GGT) activities, and total bilirubin, resting bile acids, cholesterol, and triglyceride concentrations.

2.1 | Statistical analysis

Statistical analysis was performed using commercial software (IBM SPSS Statistics for Mac, version 26, IBM Corp, Armonk, New York). All categorical variables were summarized as frequencies.

Shapiro-Wilk ($n < 50$) or Kolmogorov-Smirnov ($n \geq 50$) tests were used to evaluate the normality of data.

Continuous variables with non-normal distribution were summarized by median values and range. Cases were grouped by sludge score at BS diagnosis[1-4] and by development of GBM compared to cases without the evidence of GBM for statistical analysis and comparisons. Cases were split into DBS and NDBS groups for analysis. Statistical significance was accepted at $P < .05$ (with a 95% confidence interval [CI]).

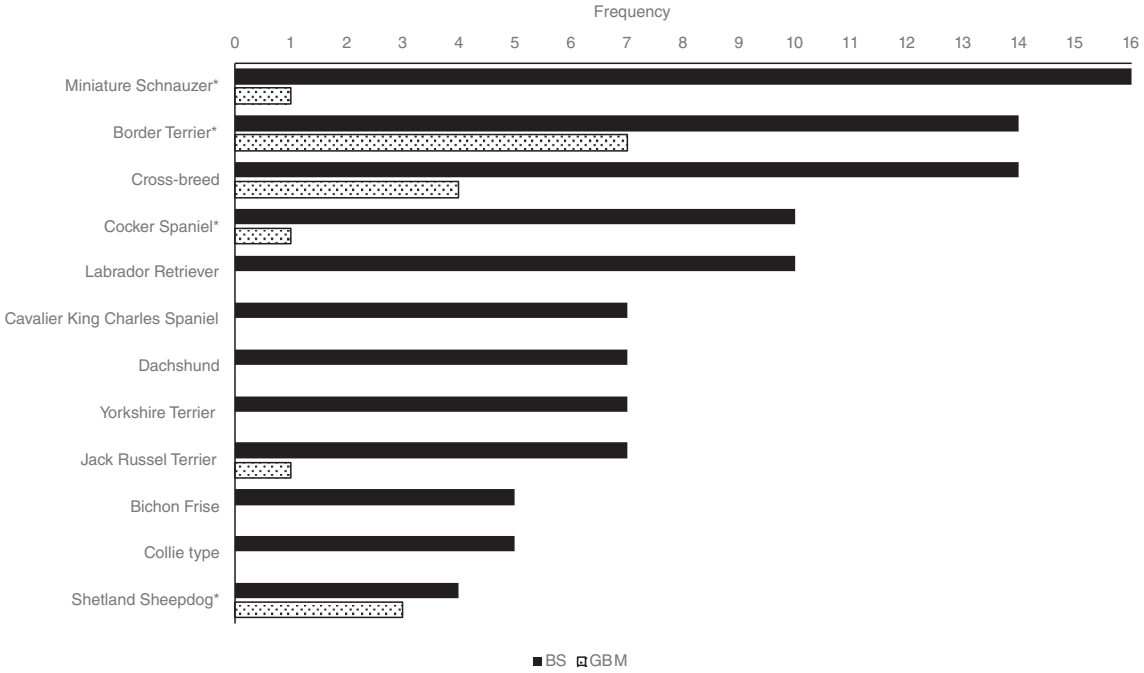

FIGURE 1 Clustered bar chart showing the frequency of the 12 most commonly encountered dog breeds with biliary sludge (BS; solid bars) alongside the frequency of progression to gallbladder mucocele (GBM) within those breeds (dotted bars). Breeds with a recognized predisposition for GBM are marked with *

Univariate analysis was performed using the χ^2 test (categorical variables) or Mann-Whitney *U* test (continuous variables) to compare differences between dogs that did and did not develop GBM, dogs with DBS and those with NDBS, and those that did or did not receive UDCA treatment. Kruskal-Wallis comparison was used to test differences between different sludge score groups, and Wilcoxon signed-rank was used to compare biochemical markers before and at the time of GBM diagnosis. Correlations between serum biochemistry results and sludge score at diagnosis were assessed using Spearman's rank. Odds ratios (ORs) were calculated using binary logistic regression analysis, and Mantel-Haenszel test of trends was used to assess for linear associations in ordinal variables (ie, for sludge score at diagnosis). Multivariate analysis was performed for all variables with significance ($P < .05$) for the development of GBM, using backward stepwise logistic regression.

2.2 | Ethical approval

The study protocol was approved by the University of Cambridge, Department of Veterinary Medicine Ethics and Welfare Committee (CR421).

3 | RESULTS

A total of 154 dogs met the inclusion criteria. One-hundred thirty-four dogs (87%; 95% CI, 80-92%) had BS only at all investigations. Twenty dogs (13%; 95% CI, 8-19%) developed GBM. Median age was 8 years (range, 0.5-13 years). There were 78 males (60 neutered, 18 intact) and 76 females (69 spayed, 7 intact). Median weight was 11.2 kg (range, 2.0-52.4 kg). Median number of follow-up ultrasound examinations was 2 (range, 1-10). Median length of follow-up was 19 months (range, 12-90 months). Represented breeds included Miniature Schnauzer (n = 16), Border Terrier (n = 14), crossbreed (n = 14), Cocker Spaniel (n = 10), and Labrador Retriever (n = 10) (Figure 1). Out of 154 dogs, 49 (32%) were from breeds considered at risk of development of GBM. A total of 124 (80.5%) cases had concurrent disease diagnosed either at or after BS diagnosis, and many cases without a concurrent disease diagnosis (19.5%) had been referred for evaluation of increases in liver enzyme activity or investigation of BS. Most diagnosed concurrent diseases are presented in Table 1. Thirty-one dogs (20%) had hepatobiliary disease and 29 (18.8%) had a concurrent endocrinopathy. Most encountered clinical signs at presentation were anorexia or lethargy (n = 72, 46.8%), vomiting (n = 59, 38.3%), and polyuria and polydipsia (n = 28, 18.2%; Table 2). Forty dogs (26%) had no clinical signs attributable to hepatobiliary or systemic disease. At BS diagnosis, no clinical sign was statistically more prevalent in the GBM group compared to dogs with BS only. Dogs with NDBS (31%) were significantly more likely to have polyuria and polydipsia compared to those with DBS (15%; $\chi^2 = 3.967$; $P = .05$). No other clinical sign was more prevalent in dogs with NDBS compared to the DBS group at BS diagnosis.

3.1 | Sludge score

Sludge scores were available for 137/154 (89%) cases. Median sludge score at BS diagnosis was 2 (range, 1-4); 56 dogs (41%) scored

TABLE 1 Frequencies of concurrent diseases for all cases, biliary sludge (BS) only cases, and cases that developed gallbladder mucocele (GBM).

	Total		BS only (/134)		Later developed GBM (/20)		
Concurrent disease	**#**	**%**	**#**	**%**	**#**	**%**	**OR for the development of GBM (95% CI)**
No concurrent disease	30	19.5	26	26.1	4	20	0.96 (0.30-3.12)
Nonhepatic neoplasia	35	22.7	33	24.6	2	10	0.34 (0.08-1.54)
Urogenital disease	23	14.9	21	15.7	2	10	0.60 (0.13-2.77)
Pancreatitis	23	14.9	22	16.4	1	5	0.27 (0.03-2.11)
Hyperadrenocorticism	18	11.7	14	10.4	4	20	2.14 (0.63-7.31)
Chronic enteropathy	18	11.7	14	10	4	20	2.14 (0.63-7.31)
Primary hyperlipaemia	12	7.8	11	8.2	1	5	0.59 (0.07-4.82)
Diabetes mellitus	11	8.2	10	7.5	1	5	0.65 (0.08-5.34)
Cholangiohepatitis	11	7.1	10	7.5	1	5	0.65 (0.08-5.34)
Cardiac disease	10	6.5	9	6.7	1	5	0.73 (0.09-6.10)
CNS disease	8	5.2	6	4.5	2	10	2.37 (0.44-12.65)
Hepatic neoplasia	6	3.9	5	3.7	1	5	1.36 (0.15-1.50)
Chronic hepatitis	5	3.2	3	2.2	2	10	4.86 (0.76-31.04)
Hypothyroidism	5	3.2	5	3.7	0	0	
Extrahepatic biliary obstruction (secondary to pancreatitis)	3	1.9	2	1.5	1	5	3.47 (0.30-40.12)
Endocrinopathy	29	18.8	25	18.7	4	20	1.1 (0.3-3.5)
Hepatobiliary disease	31	20.1	27	20.1	4	20	1.0 (0.3-3.2)

TABLE 2 Frequencies of clinical signs across all groups and odds ratios (ORs) for the development of gallbladder mucocele (GBM) with 95% confidence interval (CI). Frequency of clinical signs was not significantly different for any sign across biliary sludge (BS) and GBM groups via chi-square test. Bold typeface indicates ORs with a 95% CI that does not cross 1

	Total		BS only		Later development of GBM		
Clinical sign	**#**	**%**	**#**	**%**	**#**	**%**	**OR for the development of GBM (95% CI)**
None	40	26	35	87.5	5	12.5	1.1 (0.4-3.1)
Vomiting	59	38.3	50	37.3	9	45	1.3 (0.5-3.5)
Anorexia/lethargy	72	46.8	60	44.8	12	60	1.9 (0.7-4.8)
Polyuria/polydipsia	28	18.2	23	17.2	5	25	1.6 (0.5-4.9)
Pyrexia	13	8.4	12	9.0	1	5	0.5 (0.1-4.4)
Diarrhea	24	15.6	19	14.2	5	25	2.0 (0.7-6.2)
Weight loss	19	12.3	17	12.7	2	10	0.8 (0.2-3.6)
Abdominal pain	14	9.1	14	10.4	0	0	
Other	27	17.5	23	17.2	4	20	1.2 (0.4-3.9)

1, 51 dogs (37%) scored 2, 16 dogs (12%) scored 3, and 14 dogs (10%) scored 4. Biliary sludge was persistent in 134 dogs (87%), resolved in 15 dogs (9.7%), and recurrent in 5 dogs (3.3%). Three cases were recorded in the imaging reports as having persistent BS without image availability for sludge scoring. Fifty-six dogs (40.3%) had no net change in sludge score over the study period. Fifty-seven dogs (41.0%) had increased sludge score, and 26 dogs (18.7%) had decreased sludge score. Sludge score at diagnosis was significantly positively correlated with serum GGT activity ($P = .03$); no significant correlation of sludge score was found with any other serum biochemistry result.

Thirty-three dogs (21.4%) received corticosteroid treatment during the study period. No difference in change in sludge score was found between dogs that did and did not receive corticosteroids. No difference in change in sludge score was found between dogs with and without concurrent endocrinopathies, between dogs considered to be an at-risk breeds and those without known predispositions, or between dogs with or without hepatobiliary disease.

3.2 | Biochemistry

Increases in ≥1 hepatobiliary marker were found in 120 (77.9%) dogs. No significant difference was found in any biochemical marker across different sludge scores at BS diagnosis. At the time of BS diagnosis, no significant difference was found between dogs that progressed to GBM and those that did not, in any of the measured biochemical markers (Table 3). For dogs later diagnosed with GBM, ALT, ALP, and GGT activities, and cholesterol and total bilirubin concentrations were significantly higher at diagnosis of GBM than results measured at BS diagnosis ($P \leq .03$). Activity of GGT was significantly higher in dogs without concurrent disease compared to those with concurrent disease ($P = .001$), otherwise no significant difference was found between dogs that did or did not have concurrent disease in any other measured biochemical marker at the time of BS diagnosis.

3.3 | Gallbladder mucocele

Twenty dogs (13%; 95% CI, 8-19%) developed GBM during follow-up. These consisted of 9 male neutered, 2 male intact, and 9 female spayed dogs. Median age was 9 years old (range, 4-13 years) with median weight of 11.9 kg (range, 3.3-27 kg). No significant difference was found in age or weight between dogs that did and did not develop GBM. Median time from initial BS diagnosis to GBM development was 19 months (range, 7-36 months). Median time from last BS diagnosis to GBM development was 12 months (range, 3-36 months). Duration of follow-up was not associated with GBM development. Most common breeds in this group were Border Terrier (n = 7), crossbreed (n = 4), and Shetland Sheepdog (n = 3) with 1 case each of Miniature Schnauzer, Jack Russell Terrier, Chihuahua, Cocker Spaniel, Shiba Inu, and Nova Scotia Duck Tolling Retriever (Table 4). Shetland Sheepdogs were at increased risk of development of GBM (OR, 23.47; 95% CI, 2.31-238.54) as were Border Terriers (OR, 9.77; 95% CI, 2.96-32.21).

Of the 20 dogs that developed GBM, 15 (75%; 95% CI, 51-91%) had ≥1 previously described predisposing factors for development of GBM. Four dogs (20%; 95% CI, 6-44%) had concurrent endocrinopathies (3 dogs with HAC, 1 dog with concurrent HAC and diabetes mellitus), 13 dogs (65%; 95% CI, 41-85%) were considered at-risk breeds (Border Terrier, Shetland Sheepdog, Miniature Schnauzer, Chihuahua, and Cocker Spaniel[10,14,16,17]), and 2 dogs (10%; 95% CI, 1-31%) being treated with exogenous corticosteroids. Four dogs had hepatopathy (cholangiohepatitis [n = 1], extrahepatic portosystemic shunt and hepatitis [n = 1], biliary obstruction secondary to pancreatitis [n = 1], and vacuolar hepatopathy [n = 1]). The presence of concurrent disease, including hepatobiliary disease or corticosteroid treatment, was not found to be a risk factor for development of GBM.

Median sludge score at BS diagnosis was 2 (range, 1-4). No significant difference in sludge score at BS diagnosis was found between cases that did and did not develop GBM. Binary logistic regression analysis showed increasing OR for development of GBM

TABLE 3 Biochemical marker levels at the time of biliary sludge diagnosis for dogs that did and did not develop gallbladder mucocele (GBM). Figures are presented as medians and interquartile ranges because of non-normal distribution. There was no statistically significant difference in any value between dogs that did and did not develop GBM via Mann-Whitney *U* analysis ($P = .05$). (H) denotes a reading above reference range (RR) and (L) denotes a reading below RR

		All cases			Dogs that later developed GBM			Dogs without GBM		
Biochemical marker (units)	Reference range (RR)	Number available case data (/154)	Median (and interquartile range)	Dogs outside RR (%)	Number available case data (/20)	Median (and interquartile range)	Dogs outside RR	Number available case data (/134)	Median (and interquartile range)	Dogs outside RR
ALT (U/L)	14-67	152	76 (47-175)	85/152 (H)	20	67 (40-112)	10/20 (H)	132	76 (49-183)	75/132 (H)
AST (U/L)	12-49	93	38 (30-50)	24/93 (H) 1.93 (L)	11	39 (20-43)	2/11 (H) 1/11 (L)	82	38 (30-51)	22/82 (H)
ALP (U/L)	26-107	152	198 (77-720)	101/152 (H) 3/152 (L)	20	141 (52-874)	12/20 (H)	132	221 (80-720)	89/132 (H) 3/132 (L)
GGT (U/L)	0-10	117	4 (0.9-10)	27/117 (H)	15	4.1 (3.0-11.0)	4/15 (H)	102	3.9 (0.08-9.6)	23/102 (H)
Cholesterol (mmol/L)	3.3-6.5	133	6.36 (4.82-8.88)	61/133 (H) 10/133 (L)	17	6.33 (5.14-9.54)	7/17 (H) 1/17 (L)	115	6.40 (4.80-8.80)	54/116 (H) 9/115 (L)
Total bilirubin (μmol/L)	0-12	132	3.00 (2.00-6.00)	10/132 (H)	18	3.30 (1.75-6.5)	1/18 (H)	114	3.00 (2.00-6.03)	9/114 (H)
Triglycerides (mmol/L)	0.4-1.3	79	1.19 (0.80-3.10)	36/79 (H) 1/79 (L)	8	1.48 (0.67-4.16)	4/8 (H)	71	1.19 (0.80-2.90)	32/71 (H) 1/71 (L)

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyltranspeptidase.

TABLE 4 Odds ratio (OR) for each breed developing gallbladder mucocele (GBM) with 95% confidence interval (CI). Bold typeface indicates breeds where 95% CI does not cross 1.0 and are at increased risk. OR for single cases were not run

Breed	GBM cases/ total in breed included in study	Odds ratio (95% CI)
Border Terrier	7/14	**9.77 (2.96-32.21)**
Crossbreed	4/14	3.10 (0.87-11.05)
Shetland Sheepdog	3/4	**23.47 (2.31-238.54)**
Miniature Schnauzer	1/16	0.49 (0.06-3.96)
Jack Russel Terrier	1/7	1.12 (0.13-9.85)
Chihuahua	1/2	7.00 (0.42-116.64)
Cocker Spaniel	1/10	0.73 (0.09-6.10)
Shiba Inu	1/1	
Nova Scotia Duck Tolling Retriever	1/1	

TABLE 5 Binary logistic regression analysis of sludge score against the development of gallbladder mucocele (GBM). Significance was set at $P = .05$. Seventeen cases (including 1 case that developed GBM) did not have sludge score recorded and were excluded from this analysis

Sludge score at BS diagnosis	Development of GBM/total cases per group	% cases developed GBM	Sig.	OR (95% CI)
1	5/56	8.9	.29	
2	7/51	13.7	.44	1.62 (0.48-5.48)
3	3/16	18.8	.28	2.35 (0.50-11.15)
4	4/14	28.6	.06	4.080 (0.93-17.91)

Abbreviations: BS, biliary sludge; CI, confidence interval; OR, odds ratio.

as sludge score at diagnosis increased (Table 5), with a linear association between increasing sludge score at BS diagnosis and likelihood of development of GBM ($P = .05$).

Twenty-nine dogs (18.8%; 95% CI, 13-26%) had NDBS at BS diagnosis. Seven of 29 (24%; 95% CI, 10-44%) dogs later developed GBM and were significantly more likely to develop GBM than were dogs with DBS (10%; 95% CI 5-16%) at diagnosis ($\chi^2 = 3.931$; $P = .05$), with OR 2.74 (95% CI, 0.98-7.65). Nondependent BS was noted to develop before GBM in 9/20 dogs. Dogs from breeds at risk of GBM were significantly more likely to have NDBS than those not considered at risk ($\chi^2 = 4.626$; $P = .03$) with OR 2.46 (95% CI, 1.07-5.69). In addition, 23 dogs (14.9%) with DBS progressed to NDBS during the study period.

3.4 | Ursodeoxycholic acid

Sixty-two cases (40.9%; 95% CI, 32-48%) were treated with UDCA at median dosage of 12.2 mg/kg (range, 4.8-21.7 mg/kg) with median course duration of 17 months (range, 1.0-84.0 months). Dogs were more likely to be treated with UDCA at sludge score 2 compared to other scores ($\chi^2 = 7.830$; $P = .05$). No significant difference in change in sludge volume between the UDCA treatment group and non-treatment group was found, with both DBS and NDBS. Dogs that were treated with UDCA were at increased risk of development of GBM compared to untreated dogs (OR 3.12; 95% CI, 1.17-8.34).

3.5 | Multivariate analysis

Initial univariate analysis documented significant differences in the odds of developing GBM for the variables "DBS vs NDBS," "UDCA treatment," "Border Terrier," and "Shetland Sheepdog," as well as an association between increasing sludge score at diagnosis and increasing OR for the development of GBM. Multivariate logistic regression analysis indicated that at-risk breeds, the Shetland Sheepdog (OR, 40.99; 95% CI, 3.61-465.95; $P = .003$) and Border Terrier (OR, 11.66; 95% CI, 3.28-46.63; $P < .001$), were independent risk factors for the development of GBM. No confounding factors were identified.

4 | DISCUSSION

Previous studies have suggested that BS and GBM may represent a disease continuum rather than separate entities.[1,7,19] Our multicenter retrospective observational study assessed risk factors for progression from BS to GBM over time periods >12 months. We documented that NDBS might be a risk factor for the development of GBM, although future prospective studies are needed. Breed predisposition was the predominant risk factor for development of GBM in our study.

Twenty dogs (13% cases) developed GBM in our study. Although true prevalence data on GBM within the general canine population is lacking, we consider this number to be a higher-than-expected proportion of cases with BS, with 1 previous study assessing BS in 200 patients finding evidence of GBM in just 2% of cases.[3] In our study, median time from BS to GBM diagnosis was 19 months. Previous prospective research into the progression of BS has been limited to time periods up to 12 months, which may have led to underestimation of the number of dogs that developed GBM.[1]

Age and weight of dogs included in our study were consistent with previous studies suggesting that GBM is more prevalent in small- to middle-sized, older dogs.[10,11,16,18] No significant differences in age or weight were found between dogs that did and did not develop GBM, which could help to support the hypothesis that BS and GBM exist as part of a spectrum of disease. Interestingly, of the 12 most encountered breeds in our study (Figure 1), all except the Labrador Retriever (n = 10) and Collie-type dogs (n = 5) were small- to medium-sized breeds. Median weight for crossbreed dogs in our study was 13.3 kg (range, 5-34.5 kg), again showing similarities in weights of dogs with BS and those that developed GBM. However, breed popularity in the United Kingdom creates an important bias in these data, and thus these results must be interpreted with caution.

Of the 5 most common breeds in our study, 3 are considered at risk for the development of GBM (Miniature Schnauzer,[10,14] Border Terrier,[17] and Cocker Spaniel[10,14]). In addition, 32% (49/154) of dogs in the study population are from breeds considered either at increased risk of GBM or overrepresented in some studies (Border Terrier, Shetland Sheepdog,[13,14,16] Miniature Schnauzer, Chihuahua,[14] and Cocker Spaniel). United Kingdom breed prevalence data for the Border Terrier found an annual birth proportion of 0.78% of all dogs,[27] whereas the Border Terrier makes up 9% of our study population. This finding suggests that these breeds also have a higher incidence of BS and could imply a link between the 2 conditions. It is possible, however, that breeds with a known predisposition for GBM that are diagnosed with BS are monitored more frequently, creating potential selection bias, in addition to the popularity of many of these breeds in the UK population. A study of the incidence of BS did not record the breed prevalence of dogs in the study,[4] and further research is needed to assess the prevalence of BS within these breeds.

Hyperadrenocorticism has been linked to both BS and GBM.[3,11,13] Biliary sludge was diagnosed with concurrent HAC in 11.7% cases in our study, with 20% of cases that later developed GBM also having concurrent HAC. However, dogs with HAC did not have increased OR for the development of GBM in our study, compared to a 29 times increased risk in a previous study.[11] Twice daily, hydrocortisone administration in dogs has been shown to cause a reversible shift in bile acid composition toward hydrophobic unconjugated bile acids.[28] These cytotoxic bile acids can cause mucin hypersecretion[26,29] and GBM.[29] The presence of high rates of HAC in our study could support the hypothesis that BS and GBM represent a continuum of a single pathologic entity, although our results should be interpreted with caution. Only 18 dogs in our study had HAC, and these results could reflect type II statistical error. Iatrogenic hypercortisolism did not lead to an increased prevalence of BS compared to controls in 1 study,[12] and corticosteroid treatment was not seen to have an effect on sludge score in our study. In fact, no significant difference was found in sludge score between dogs that did and did not have any recognized predisposing factors for GBM.

The wide variety of concurrent diseases identified in our study makes the interpretation of clinical signs difficult. The most common clinical signs were nonspecific (anorexia, lethargy, vomiting, polyuria, and polydipsia) and therefore it is difficult to assess their clinical relevance. However, 40 dogs with BS had no clinical signs attributable to hepatobiliary or systemic disease, making it less likely that BS was the cause of clinical signs in the other cases, consistent with most previous research suggesting BS is often subclinical.[1] This observation is in contrast to BS in humans, which often is linked to biliary colic, being the cause of unexplained biliary pain in 83% of cases in a recent systematic review.[6] Interestingly, 1 recent study assessing NDBS noted clinical signs in 14/16 dogs included in the study.[20] These signs were also nonspecific (primarily diurnal inappetence and exercise intolerance). In that study, no clinical sign was associated with NDBS, compared to an association with polyuria and polydipsia in our study, although small group size may have limited the power of those analyses. The cause of polyuria and polydipsia in these cases remains unclear, with undiagnosed HAC as a possible explanation.

Gamma-glutamyl transpeptidase is a liver enzyme activity induced by cholestasis and is frequently measured as part of routine serum biochemical analysis.[30] In our study, sludge score was significantly positively correlated with serum GGT activity, which suggests that BS is not an entirely benign condition and supports previous research suggesting BS is associated with GB dysmotility.[7,10] This finding is in contrast to a previous study[1] that did not find an association between GGT activity and increasing BS. However, that study had a smaller population and thus could have been underpowered to detect statistical differences. In addition, GGT activity was significantly higher in dogs without concurrent disease and the authors proposed that further research would be required to ascertain the potential of GGT activity as a biochemical monitoring tool for progression of BS.

Nondependent BS has been hypothesized as an intermediate stage in progression of DBS to GBM.[1,7,31] In a prospective study, DBS progressed to NDBS in 24% dogs over 12 months,[1] although no dog developed GBM. To our knowledge, no studies have reported direct progression of NDBS to GBM. In our study, of 20 dogs that later developed GBM, 9 were noted to have NDBS before GBM formation, at an intermediate ultrasonographic examination. In addition, breeds predisposed to GBM were found to be twice as likely as other breeds to have NDBS (OR, 2.46; 95% CI, 1.07-5.69). These findings add credence to the argument that NDBS could represent an intermediate stage in the development of GBM. Limitations in group size limited the power of these analyses, and these authors suggested that further prospective research is required to ascertain the risk of GBM development in these cases. However, these findings suggest that dogs with NDBS should be monitored closely. Prospective research with serial ultrasonographic examination of the GB may allow for more accurate assessment of the natural course of this progression. In recent studies, a grading system has been proposed and utilized for the classification of GBM.[10,31] Type I GBM, using this system, is defined as "echogenic immobile bile occupying the GB" and thus allows for the early identification of "pre" or "forming" GBM.[32] Increasing severity of GBM type (I-VI) was associated with increased mortality in 1 study,[32] and thus early identification is important for prognosis and management. Utilizing that system, NDBS noted in our study could be classified as an early mucocele, although this system does not account for the proportion of GB filled with BS. There is, therefore, a potential equivocal zone between dogs with NDBS taking up <100% of the GB and dogs with type I GBM. For the purposes of our study, we believe the classification system used here is more appropriate, because semiquantitative assessment is more useful for tracking the progression of BS over time. However, if evidence grows to support the hypothesis that BS is a precursor to GBM, these classification systems will require combination, although this consideration is beyond the scope of our study.

Several breeds have been noted to be at increased risk for GBM; these include the Border Terrier,[17] Shetland Sheepdog,[16] and Miniature Schnauzer,[10] although in the case of the Miniature Schnauzer, this risk may be related to the increased risk of hyperlipemia in this breed, a known predisposing factor for GBM.[14] In our study, after multivariate analysis, both Shetland Sheepdogs (OR, 40.99; $P = .003$; 95% C,I 3.61-465.95) and Border Terriers (OR, 11.66; $P < .001$; 95%

CI, 3.28-46.63) were at increased risk of development of GBM, consistent with previous findings in the veterinary literature. Given the predisposition of these breeds to the development of GBM as documented here and in other studies,[14,16,17] future, prospective study should include breed-matched controls, to decrease the risk of confounding factors.

Although no significant difference was found in sludge score at BS diagnosis between dogs that did and did not develop GBM, logistic regression analysis found an increasing OR for the development of GBM with increasing sludge score, with a linear association (Table 5). Group sizes for sludge scores of 3 and 4 were small (16 and 14 cases, respectively), making strong conclusions difficult to draw. This is an area for further research in the future, with recruitment of higher score BS cases (with BS taking up >50% GB area) for long-term monitoring. These findings suggest that a consistent sludge grading system should be utilized in all routine ultrasonographic examinations, to enable more accurate tracking of BS over time, as well as facilitating decision-making on potential treatment of BS, and individual risk of progression to GBM.

A secondary aim of our study was to assess the effects of UDCA administration on the volume of BS within the GB over time. No significant change in sludge volume score was observed between dogs that were and were not treated with UDCA, with both DBS and NDBS. This observation supports the findings of a recent study in which there was no response to UDCA treatment in 16 dogs with NDBS.[20] These results suggest that UDCA may not be useful in the medical management of dogs with BS. However, many of these dogs were being managed for concurrent diseases along with their BS, and thus it is difficult to draw conclusions about the effectiveness of this treatment. There might also have been potential selection bias as to which cases were treated with UDCA (dogs with sludge score 2 were more likely to receive UDCA than dogs with scores of 3 or 4, and dogs treated with UDCA had an increased OR for progression to GBM), which may have affected results. Further prospective research, utilizing a randomized controlled trial format, would be required to assess the effectiveness of UDCA treatment in dogs with both DBS and NDBS. A recent study[33] documented differences in the composition of bile acids between dogs with GBM and NDBS vs dogs with DBS and healthy controls, with lower compositional ratios of taurodeoxycholic acid and taurolithocholic acid (both hydrophobic bile acids) in the control and DBS groups. It is plausible to hypothesize that BS is not responsive to UDCA, but GBM might be, because of differences in bile acid constituents and additional studies are needed to elucidate this possibility. In addition, UDCA dosage varied from 4.8 to 21.7 mg/kg, with highly variable course duration (1-84 months). This inconsistency, in addition to variability of other treatments used in dogs in our study, was a limitation of our study.

Our study had several other limitations. The multicenter nature meant that there were center-by-center variations in how ultrasonography reports were written, and images captured, because of examinations being performed by various operators, and laboratory testing was performed at different laboratories with minor differences in reference ranges (RRs). This situation led to 17 cases that were included in the study having to be excluded from sludge score analysis, decreasing the potential power of these calculations. The retrospective nature of the study meant it was not possible to verify if ultrasonographic GB images represented the maximal long axis view, thus limiting the assessment of gallbladder volume (GBV) and creating potential misinterpretation of percentage of actual GB area filled with BS, thus preventing evaluation of the effects of GBV on risk of GBM formation. Changes in GBV despite no change in the total volume of sludge could have led to an artificial increase in sludge score. Fasting status was unverified in many cases, preventing analysis, although we believe that, in a tertiary referral setting, standard procedure requires sedation for ultrasonography, necessitating fasting, which could not, however, be confirmed. In addition, all cases in our study were recruited from tertiary level facilities and thus these findings are likely to be subject to substantial selection bias, requiring cautious interpretation. Our study was retrospective, meaning there were occasional inconsistencies in the data collected, as well as an absence of consistent long-term follow-up. Consistent ultrasonographic follow-up at defined time points would have enabled more accurate tracking of sludge score and gravity dependency over time, which would have helped to determine any intermediate changes in BS before development of GBM in those cases. A further limitation was the lack of a control group. A control group of dogs without BS followed over time periods >12 months would have enabled direct comparison in the frequency of GBM development, and the absence of this group limits the ability of our study to accurately identify risk factors. Finally, a diagnosis of GBM was made in all cases using ultrasonography, without surgical or histopathological confirmation. However, the ultrasonographic appearance of GBM is diagnostic and highly specific[10,31,34] and histopathology was not considered essential to the findings of our study.

In conclusion, we assessed BS over time periods >12 months. Several interesting findings merit further prospective research. Based on our study, we suggest that a stratified sludge scoring system, similar to that used here and in other studies,[1,3] should be utilized as part of the routine ultrasonographic examination to allow more accurate monitoring of BS over time. This system might require modification in the future to incorporate both BS and GBM types. Our findings also suggest that BS taking up >50% of the GB area, as well as NDBS, even noted incidentally, should be monitored closely because these dogs may be at increased risk of the development of GBM. In addition, we hypothesize that NDBS may represent an intermediate stage in the progression from BS to GBM. We suggest future prospective research monitoring BS over time periods of at least 24 months to ascertain whether our findings are repeatable in a controlled setting. It is likely that GBM is a multifactorial disease, but our study suggests that BS is not as benign as previously thought.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the University of Cambridge, Department of Veterinary Medicine Ethics and Welfare Committee (CR421).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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