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STANDARD ARTICLE

Gall bladder mucoceles in Border terriers

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Background: Gall bladder mucoceles (GBM) are a leading cause of biliary disease in dogs with several breeds, including the Shetland Sheepdog, American Cocker Spaniel, Chihuahua, Pomeranian, and Miniature Schnauzer apparently predisposed.

Objective: To determine risk factors, clinical features, and response to treatment of GBM in Border terriers (BT).

Animals: Medical records of 99 dogs (including 51 BT) with an ultrasonographic (±histopathologic) diagnosis of GBM from three referral centers in the United Kingdom were collected. A control group of 87 similar-aged BT with no ultrasonographic evidence of gall bladder disease was selected for comparison.

Method: Retrospective case-control study. Odds ratios were calculated to establish breed predisposition. Signalment, presence of endocrine disease, clinicopathologic results, and outcome were compared between the BT, other breeds, and control BTs.

Results: The odds of identifying a GBM in a BT in this hospital population was 85 times that of all other breeds (95% confidence interval 56.9-126.8). BT had similar clinical signs and clinicopathologic changes to other breeds with GBM. There was no evidence that endocrinopathies were associated with GBM in BT.

Clinical Significance: A robust breed predisposition to GBM is established for the BT.

KEYWORDS

biliary disease, hypercholesterolemia, cholecystectomy, liver, extrahepatic biliary tract obstruction

1 | INTRODUCTION

Gall bladder mucoceles (GBM) are a leading cause of biliary disease in the dog.[1] A GBM is an accumulation of green-black, tenacious, and immobile bile and mucus that provokes varying degrees of gall bladder distension and biliary duct obstruction. Recent reports of GBM in dogs reflect an increased awareness of the condition and greater availability of abdominal ultrasound[2] but also suggests that this might be an emergent phenomenon.[3]

There is breed predisposition to GBM for the Shetland Sheepdog, American Cocker Spaniel, Chihuahua, Pomeranian, and Miniature Schnauzer.[2,4] The over-representation of particular purebred dogs suggests a genetic contribution to GBM.[5] An insertion mutation in the ABCB4 gene was initially linked to GBM formation in Shetland Sheepdogs[6] but the association was not supported by a later study.[7]

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BT, Border terrier; cBT, Border terrier belonging to control group; GBM, gall bladder mucocele; GGT, gamma glutamyltranspeptidase; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; non-BT, other non-border terrier breeds; OR, odds ratio.

The absence of a comparable condition in humans limits our understanding of the pathogenesis of GBMs in dogs. Recognized risk factors for GBM formation include hyperadrenocorticism, hypothyroidism,[8] hyperlipidemia,[4] neonicotinoids,[9] and recently, increased serum leptin.[10] These conditions could act by altering the composition of bile and mucin[11] or through an effect on gall bladder motility.[12] It is likely that multiple mechanisms are involved in GBM development with both genetic and epigenetic factors affecting each predisposed breed.[13]

It is our perception that over recent years an increasing number of dogs presenting with GBM have been Border terriers (BT). The number of registrations of BT puppies doubled between 2000 and 2004 and the breed is now established in the top 10 most popular breeds in the United Kingdom. Epileptoid cramping syndrome[14] and a spongiform leukoencephalomyelopathy[15] are in the breed but the BT is not included among predisposed breeds for hypothyroidism,[16] hyperadrenocorticism,[17] diabetes mellitus,[18] or hyperlipidemia.[19]

The primary objective of this multicenter, retrospective study was to establish whether there is a breed predisposition for GBM in BT. A second objective was to identify potential breed-specific characteristics relating to risk factors, clinical features, and outcome for the BT by comparing this group to non-BT breeds with GBM. Finally, the incidence of endocrine disease and clinicopathologic abnormalities in BTs was compared directly to a cohort of BTs unaffected by GBM in order to explore potential GBM triggers in the breed.

2 | MATERIALS AND METHODS

Ethical approval was obtained from the institutional scientific and ethical review committees for each of the participating centers.

Retrospective evaluation of medical records was performed to identify all dogs with an ultrasonographic diagnosis of GBM presenting to Center 1 between January 2010 and September 2017, Center 2 between January 2014 and September 2017 and Center 3 between January 2011 and September 2017. Enrollment criteria included an ultrasonographic diagnosis of GBM determined by specialists in diagnostic imaging according to described criteria (briefly: hypoechoic/anechoic gall bladder rim and echoic, immobile core of biliary sludge with striated/stellate bands radiating out toward the periphery).[20]

Control Border terriers (cBTs) of similar ages to the BTs were sought from each center and were included in the study if they had a complete abdominal ultrasonographic examination with no evidence of GBM. BTs with a description of an early or developing GBM were excluded from the GBM and control groups.

For all GBM cases and cBTs, information recorded, where available, was: breed, age, sex, and neuter status, bodyweight; presence or absence of previously diagnosed endocrinopathies (hyperadrenocorticism, hypothyroidism, or diabetes mellitus), results of routine clinicopathologic testing (hematologic and serum biochemical profiles), and total thyroxine measurement.

For GBM cases additional data was also collected from the medical records including the presence or absence of specific clinical signs (vomiting, diarrhea, abdominal pain, inappetence, lethargy, hypersalivation, weight loss, and icterus), approach to case management (surgical or medical), medications prescribed, presence or absence of gall bladder rupture (as recorded in the ultrasound, surgery or histopathology reports), outcome (measured as number surviving beyond 7 days, 6 months and 1 year post surgery/diagnosis and total survival time), and cause of death (classified as related to GBM or unrelated).

Clinicopathologic results were only included if they had been performed within 72 hours of diagnosis of GBM (or in the case of cBTs, the ultrasound examination) and if the tests had been performed on an automated analyzer at a standard reference laboratory or using automated systems and reagents at one of the study centers. Hematologic variables included hematocrit, total RBC count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and total and differential leucocyte and platelet counts. Serum biochemical tests included concentrations of total protein, albumin, globulin, urea, creatinine, sodium, potassium, chloride, calcium, phosphate, glucose, cholesterol, triglyceride, and total bilirubin as well as activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma glutamyltranspeptidase (GGT) activities.

Histopathology reports were reviewed and classified according to World Small Animal Veterinary Association (WSAVA) guidelines;[21] the presence/absence of neutrophilic cholangitis (defined as a neutrophilic infiltration predominantly involving portal regions of the liver), cholangiohepatitis (extension of neutrophilic infiltration into the adjacent periportal region),[22] and cholecystitis (neutrophilic infiltration of the gall bladder wall)[21] was noted. Culture results for bile, liver, and gall bladder wall were also retrieved if available.

Referring veterinarians were contacted to provide follow-up information including up-to-date clinical records and, if relevant, date and cause of death. Owners were contacted to verify the dog's vital status if the dog had not been seen by the referring vet within the previous month.

For purposes of comparison, dogs were grouped as either BT or non-BT. Dogs were also categorized according to whether they underwent cholecystectomy or were managed medically. Dogs that were euthanized without specific treatment for GBM were excluded from this latter comparison.

2.1 | Calculations

Cohort selection for breed predisposition calculations was performed according to the method outlined by Aguirre and others.[2] Briefly, the number of dogs seen by a referral clinician (excluding dogs under 2 years of age) with and without GBM and the number of each specific breed (eg, Border terrier) with and without GBM during the time interval of this retrospective study were calculated. Odds ratio (OR) calculations including 95% confidence intervals and p values were performed using an online odds ratio calculator[1]. Breeds in which the 95% confidence interval did not overlap 1.0 were considered to be significantly predisposed to GBM (at the 5% significance level).

[1]Medcalc® statistical software: https://www.medcalc.org/calc/odds_ratio.php

TABLE 1 Number of gall bladder mucocele (GBM) cases per breed and total number of each breed presenting to each center (and combined totals) over the study period

Breed	GBM cases/ dogs in breed (Center 1)	GBM cases/ dogs in breed (Center 2)	GBM cases/ dogs in breed (Center 3)	Total GBM/total in breed	Odds ratio (all centers)	All center 95% confidence limits
Affenpinscher	**2/6**	**0/4**	**2/10**	**4/20**	**169.8**	**55.7–517.3**
Bichon Frise	**1/161**	**1/74**	**1/197**	**3/432**	**4.7**	**1.5–14.8**
BT	**15/208**	**18/232**	**18/408**	**51/848**	**85.0**	**56.9–126.8**
Cavalier King Charles Spaniel	2/532	1/434	0/1015	3/1981	1.0	0.3-3.1
Chihuahua	1/168	0/162	0/277	1/607	1.1	0.2-7.7
Cocker Spaniel	0/800	1/676	0/1568	1/3044	0.2	0.0-1.5
Cockapoo	0/35	0/3	1/142	1/180	3.7	0.5-26.5
English Bull Terrier	1/77	0/31	0/103	1/211	3.1	0.4–22.5
English Setter	0/22	0/29	1/41	1/92	7.2	0.1-5.2
Golden Retriever	0/447	0/249	1/791	1/1487	0.4	0.1–3.1
Jack Russell Terrier	3/712	0/413	0/956	2/2081	0.9	0.3-3.0
Labrador Retriever	0/269	1/1121	2/3427	3/4817	0.4	0.1-1.2
Miniature Schnauzer	**3/157**	**1/239**	**2/382**	**6/778**	**5.3**	**2.3–12.2**
Pekingese	0/19	0/19	1/61	1/99	6.7	0.9-48.6
Pomeranian	**1/46**	**0/44**	**2/65**	**3/155**	**13.2**	**4.2–42.2**
Shetland Sheepdog	**2/52**	**1/15**	**2/66**	**5/133**	**26.8**	**10.7–66.9**
Shih Tzu	1/251	0/302	1/565	2/1118	1.2	0.3-4.8
Springer Spaniel	0/615	1/491	0/1286	1/2392	0.3	0.0-1.9
Toy Poodle	0/36	0/15	1/80	1/131	5.1	0.7-36.5
Weimeraner	0/118	0/63	1/211	1/392	1.7	0.2-12.0
Yorkshire Terrier	1/285	0/136	0/410	1/831	0.8	0.1-5.6
X-breed	0/965	4/1275	1/3564	5/5804	0.5	0.2

Odds ratio are presented for each breed with 95% confidence limits. Breeds displayed in bold typeface have 95% confidence intervals that do not cross 1.0 and are considered predisposed breeds.

2.2 | Statistical analysis

An online statistical analysis program was used for calculations[2]. Histograms and the Shapiro-Wilk test were used to evaluate data distributions for normality. Categorical data and clinicopathologic variables which had non-Gaussian distributions were analyzed by use of non-parametric statistical analyses. Descriptive statistics for these variables were expressed as median values and total or interquartile ranges. Categorical data were expressed as frequencies for each group. The Mann-Whitney test was used to detect differences in median age, bodyweight, and clinicopathologic variables between the BTs and non-BTs and BTs and cBTs.

Fisher's exact test was performed to evaluate the differences between groups (BTs versus non-BTs and BTs versus cBTs,) with regards to sex and neuter status and individual endocrinopathy incidence. Fisher's exact test was also used to compare frequency of presentation of specific clinical signs (vomiting, lethargy, inappetence, abdominal pain, icterus, and diarrhea), gall bladder rupture status, frequency of histopathologic abnormalities (neutrophilic cholangitis, cholangiohepatitis, and cholecystitis), and outcome at different time intervals in BTs versus non BTs.

After censoring of euthanized animals, dogs diagnosed with GBM were classified as medically managed or surgically managed (underwent cholecystectomy). Clinical signs and outcome data for medically managed and surgically managed dogs were compared using a Fisher's exact test. A Mann-Whitney test was used to compare the number of clinical signs between medically and surgically managed dogs.

A significance level of $P \leq .05$ was modified with a Bonferroni correction when comparing age, weight, and clinicopathologic variables such that $P \leq .0016$ was considered significant. Statistical significance was set at a p value of ≤.0029 for the Fisher's exact test when comparing frequency of clinical signs, histopathological abnormalities, gall bladder rupture status, and outcome (after application of the Bonferroni correction).

3 | RESULTS

A total of 64,640 dogs greater than 2 years of age were presented to the three centers over the respective study periods. Of these, 848 (1.3%) were BTs. Ninety-nine dogs (51 BTs and 48 non-BTs including 4 Affenpinschers, 3 Bichon Frises, 3 Cavalier King Charles Spaniels, 3 Jack Russell Terriers, 3 Labrador Retrievers, 6 Miniature Schnauzers, 3 Pomeranians, 5 Shetland Sheepdogs, 2 Shih-Tzus, 5 cross-breed dogs, and 11 other individual breeds) were diagnosed

[2]GNU Project (2015). GNU PSPP for GNU/Linux (Version 0.8.5). Boston, MA: Free Software Foundation. Available from: https://www.gnu.org/software/pspp/documentation.html

TABLE 2 Frequency of clinical signs in dogs with gall bladder mucoceles separated to allow comparison according to breed [BTs vs other breeds (non-BTs)] and management approach (medical vs surgical)

Clinical signs	BT (n = 51)	Non-BT (n = 48)	*P* value	Medical management (n = 12)	Surgical management (n = 77)	*P* value
Vomiting	44 (86%)	35 (73%)	.13	5 (42%)	67 (87%)	.001
Diarrhea	8 (16%)	16 (33%)	.06	3 (25%)	19 (25%)	1.0
Abdominal pain	20 (39%)	14 (29%)	.40	1 (8%)	28 (36%)	.094
Inappetance	38 (75%)	30 (63%)	.28	5 (42%)	55 (71%)	.052
Lethargy	38 (75%)	31 (65%)	.38	6 (50%)	57 (74%)	.10
Hypersalivation	2 (4%)	1 (2%)	1.0	0 (0%)	2 (3%)	1.0
Weight loss	5 (10%)	4 (8%)	1.0	0 (0%)	8 (10%)	.59
Icterus	20 (39%)	15 (31%)	.53	0 (0%)	31 (40%)	.0067

Values in bold typeface were significantly different (Bonferroni corrected *P* value ≤ .0029).
Ten dogs that were not treated specifically for their GBM were censored from the comparison of medically and surgically-managed dogs.

with a GBM based on ultrasound alone (34) or ultrasound with histopathologic confirmation (65).

The proportion of dogs presenting with a GBM for each breed to each center are shown in Table 1.

Of the 51 BTs with GBM, there were 4 (8%) intact males, 18 (35%) neutered males, 6 (12%) intact females, and 23 (45%) neutered females. The BTs had a median age of 9 years 11 months (range 4 years 11 months to 13 years 11 months), and a median bodyweight of 10.0 kg (range 4.9–13.6). The 48 non-BTs with GBM included 9 (19%) intact males, 22 (46%) neutered males, 3 (6%) intact females, and 14 (29%) neutered females. They had a median age of 9 years 1 month (range 2 years 8 months to 15 years 0 month) and median bodyweight of 9.7 kg (range 2.7–37.9). There were no significant differences in age (*P* = .24), bodyweight (*P* = .77), sex (*P* = .044), or neuter status (*P* = .63) between BTs and non-BTs.

Eighty-seven cBTs that met the inclusion criteria were identified. The median age of the cBTs was 8 years 8 months (range 1 years 7 months to 15 years 11 months) which was younger than the BT group (*P* = .037). cBTs were diagnosed with a wide range of different conditions that were broadly grouped into the following categories: gastrointestinal (n = 29), neurological (16), hepatic/pancreatic (11), oncological (7), renal (7), endocrine (6), cardiorespiratory (4), hematologic (2), orthopedic (2), ophthalmic (1), reproductive (1), or dermatologic (1) disease.

The most frequent clinical signs at initial presentation in all 99 dogs with GBM were vomiting (80%), lethargy (70%), inappetence (69%), icterus (35%), abdominal pain (34%), and diarrhea (24%). Table 2 shows a comparison of the frequency of the clinical signs in BTs and non-BTs. No significant differences in clinical signs were found between these groups.

A total of 24 dogs (4 BTs, 14 non-BTS, and 6 cBTs) were presented with a single previously confirmed endocrinopathy and 5 dogs (1 BT, 3 non-BTS, and 1 cBT) had been previously diagnosed with two endocrine conditions (Table 3). Incidence rates for individual endocrinopathies were not significantly different between either the BT and non-BT groups or between BTs and cBTs (Table 3).

Leucocytosis was present in 40% (35/88) of dogs with GBM at presentation as well as neutrophilia (48%; 41/86), lymphopenia (41%; 35/86), monocytosis (51%; 44/86), eosinopenia (35%; 30/86), and thrombocytosis (29%; 25/86)/thrombocytopenia (3%; 3/86). The most common serum biochemical abnormalities found in dogs with GBM were increased ALP activity(97%; 90/93), hypercholesterolemia (93%; 81/87), increased ALT activity (91%; 82/90), GGT activity (85%; 61/72), AST activity (82%; 36/44), hyperbilirubinemia (72%; 65/90), hypertriglyceridemia (66%; 25/38), and hypokalemia (20%; 14/70). About 56% of dogs (10/18) with GBM tested, had low serum total thyroxine (T_4).

Median total bilirubin was significantly greater in BTs than non-BTs (*P* = .001) and the proportion of hyperbilirubinemic BTs (42/48, 88%) was greater than the non-BTs (23/42, 55%). Median cholesterol was not significantly different between BTs and non-BTs (*P* = .66). Median values (and interquartile ranges) for the hematologic and biochemical variables of BTs, non-BTs and cBTs are shown in Tables 4 and 5. P values for Mann Whitney comparisons between BTs and non-BTs and BTs and cBTs are also shown. The incidence of values outside the reference range are also included.

About 65/77 dogs with GBM that underwent cholecystectomy had gall bladder histopathology and 54/65 had liver histopathology. Neutrophilic cholangitis was present in 23/28 BTs and 14/26 non-BTs (*P* = .040), cholangiohepatitis in 13/28 BTs and 17/26 non-BTs (*P* = .18) and cholecystitis in 11/37 BTs and 9/28 non-BTs (*P* = 1.0).

Culture results were available for bile (46 dogs), gall bladder wall (18 dogs), and liver (13 dogs). Among the BTs 1/25 had a positive bile culture (*Enterococcus faecalis*) and the same dog had a positive culture of gall bladder wall (1/12); all 7 liver cultures were negative. Concerning the non-BTs, 3/21 had a positive bile culture (2 *Escherichia coli* and

TABLE 3 Number of previously diagnosed endocrinopathies in BTs, other breeds (non-BTs), and cBTs

Cases of endocrine disease	Dogs with GBM		Dogs without GBM
	BTs (n = 51)	Non BTs (n = 48)	cBTs (n = 87)
Diabetes mellitus	2 (3.9%)	4 (8.3%)	3 (3.5%)
Hyperadrenocorticism	3 (5.9%)	8 (16.7%)	3 (3.5%)
Hypothyroidism	1 (2.0%)	5 (10.4%)	1 (1.2%)

About 5 dogs (1 BT, 3 non-BTS and 1 cBT) were diagnosed with two endocrine conditions. 2 dogs (1 BT and 1 non-BT) had hypothyroidism and diabetes mellitus, 2 non-BT had hypothyroidism and hyperadrenocorticism and 1 cBT was diagnosed with hyperadrenocorticism and diabetes mellitus.

1 *Enterobacteriacae* sp.) and 1/6 non-BTs had a positive culture of gall bladder wall (*E. coli*); 1/6 liver cultures was positive (*Clostridium* sp.).

Of the 99 dogs with GBM, 8 dogs [3 BTs (6%) and 5 non-BTs (10%)] were euthanized within 3 days of obtaining an ultrasonographic confirmation of GBM and without further treatment. Two non-BTs, a Shih-Tzu with concurrent gastric neoplasia and a Weimeraner with a diagnosis of mediastinal squamous cell carcinoma were discharged without treatment for their GBM and euthanized after 27 and 54 days, respectively, for reasons unrelated to the GBM. Seventy-seven dogs [43 BTs (84%) and 34 non-BTs (71%)] underwent cholecystectomy and 12 dogs [5 BTs (10%) and 7 non-BTs (15%)] were managed medically. Gall bladder rupture was identified in 18/51 (35%) of BTs and 14/48 (29%) of non-BTs and was not significantly different (P = .53) between BTs and non-BTs. Of the 77 surgically managed dogs, 9 dogs (7 BTs and 2 non-BTs) died in the first week after cholecystectomy (7-day surgical case fatality rate of 11.7%). Three (2 BTs, 1 non-BT) of the 9 dogs that died within 7 days of cholecystectomy had gall bladder rupture identified at surgery. Overall 3/30 dogs with gall bladder rupture died within 7 days which was not significantly different from the 6/59 dogs without gall bladder rupture that died in the same period (P = 1.0) (Table 6).

Of the 12 dogs with an ultrasonographic diagnosis of GBM managed medically, concurrent endocrinopathies were seen in 4/12 dogs (3 dogs with hyperadrenocorticism and 1 with diabetes mellitus). Ursodeoxycholic acid and s-Adenosylmethionine (1), trilostane (3), insulin (1), or omeprazole (1) were used in 9/12 dogs.

Dogs that underwent cholecystectomy presented a median of 4/6 (range 0/6 to 6/6) clinical signs which was significantly (P < .001) more than the medically managed dogs (median 1/6, range 0/6 to 4/6). Table 2 shows a comparison of the frequency of the clinical signs in medically and surgically managed GBMs and the p values for the Fisher's Exact comparison. Dogs managed by cholecystectomy were significantly more likely to have presented for vomiting at or prior to presentation (P < .001).

About 9/10 (90%) medically managed and 39/49 (80%) surgically managed GBM cases survived greater than 1 year (Table 7); two additional dogs were alive at the end of the study period but the one-year survival criteria had not yet been reached. There were no significant differences in the proportions of dogs surviving to 7 days, 6 months, or 1 year according to breed status or management approach (Table 7). Median survival times could not be calculated for any of the groups (<50% case fatality rate).

4 | DISCUSSION

In this study 99 cases of GBM were identified across three centers. More than half of these cases were BTs with similar proportions at each site indicating that this is not an issue pertaining to a single location, although might be unique to the United Kingdom. The strongly suspected BT breed predisposition was validated by an OR of 85 (95% confidence interval 56.9–126.8). Odds ratios, by nature of their calculation, tend to overestimate relative risk but, given the low incidence of GBM in all breeds in this study population (compatible with the rare disease assumption), any difference should be minimal.[23]

BT have not been previously identified as being predisposed to GBM. The authors are aware of only three BTs with GBM included in previous studies.[9,24] This could, however, be an underestimate given that a complete breakdown of breeds was not always reported. The recent recognition of GBM within this breed might partly reflect the increasing size of the UK's BT population. The Kennel Club 2015 report highlighted that the most popular 10% of BT sires were responsible for 1 in every 3 BT puppies born since 2001 raising concerns as to potential loss of genetic diversity within the breed. Pedigree analysis was not performed in our study but, on this basis, merits further investigation.

Our study also offers further supportive evidence for important breed predilection among Shetland Sheepdogs, Pomeranians, and Miniature Schnauzers as previously described.[2,4] Cocker spaniels (English and American) were underrepresented (OR < 1) in contrast to American Cocker Spaniels in Japan that had an OR of 8.94.[4] Cocker Spaniel populations from different continents might represent different genotypes with divergent predisposition to GBM. Indeed, American Cocker Spaniels in Japan present a phenotypically different form of chronic hepatitis to that reported in Europe and The United States.[25]

The OR for GBM diagnosis in Affenpinschers [179.8 (55.7–517.3)] was even higher than that for BTs although the total cohort size was small (20) meaning that the findings should be interpreted with caution due to risk of a type I error. Identification of a disease-susceptible population can help guide the search for contributing genes via genome-wide association studies (GWAS) especially if multiple affected breeds can be used.[26] This study highlights several candidate breeds for such investigations.

In our study, pre-existing hyperadrenocorticism, hypothyroidism, and diabetes mellitus were reported in 5.9%, 2%, and 3.9% of BTs and 17%, 10.4%, and 8.3% of non-BTs, respectively. Previous studies have reported incidence rates of hyperadrenocorticism in 13% and 21% dogs with GBM, hypothyroidism in 13% and 14%, and diabetes mellitus in 11.7% and 2%.[2,8,9,24,27] Both hypothyroidism, and hyperadrenocorticism have been shown to be significant risk factors for GBM formation[8,9] and also for gall bladder sludge accumulation.[28] A previous study found that the incidence of diabetes mellitus in GBM cases was not different from the matched control group[8] refuting a role of diabetes mellitus in GBM formation. No differences in the frequencies of individual endocrinopathies was found between BTs and cBTs in our study suggesting that these conditions might not be associated with GBM formation in this breed.

Interestingly, in another study, 56% of cases of hyperadrenocorticism and 45% of hypothyroidism cases were suspected at initial presentation but only confirmed in the 6 months after GBM diagnosis.[8] Due to a lack of detailed follow-up, endocrinopathies diagnosed after GBM diagnosis were not included in our study and any role of endocrine disease in our study might be underestimated.

There were statistically significant differences in the median values of the white blood cells for BTs compared with the cBTs. The median leucocyte, neutrophil, and monocyte counts were greater in BTs while median eosinophil and lymphocyte numbers were lower compared with the cBTs. This combination of differences could represent an exaggeration of the non-specific stress leucogram expected in

TABLE 4 Median values and interquartile ranges for hematologic and biochemical variables for BTs, other breeds (non-BTs) and cBTs and number of dogs in each group that are above (H) or below (L) the reference range (RR) for each variable

		Dogs with GBM (n = 99)				Dogs without GBM (n = 87)			
	Reference range (RR)	BTs median (and interquartile range)	BTs outside RR	Non-BTs median (and interquartile range)	Non-BTs outside RR	cBTs median (and interquartile range)	cBTs outside RR	MW *P* value BTs v non-BTs	MW *P* value BTs v cBTs
RBCs ($\times 10^{12}$/L)	5.39-8.70	7.0 (6.3–7.4)	1/44 **H** 2/44 **L**	6.5 (5.7-7.2)	2/43 **H** 7/43 **L**	6.7 (6.2–7.2)	2/84 **H** 5/84 **L**	.040	.13
Hemoglobin (g/dL)	13.4–20.7	15.9 (14.7–17.1)	0/45 **H** 6/45 **L**	14.9 (13.5–16.6)	0/45 **H** 11/45 **L**	15.2 (13.8–17.3)	1/85 **H** 19/85 **L**	.057	.27
Hematocrit (l/L)	38.3–56.5	47.3 (42.2–50.0)	9/45 **H** 4/45 **L**	43.8 (39.0–48.0)	6/46 **H** 6/46 **L**	45.0 (40.7–51.0)	16/85 **H** 6/85 **L**	.078	.34
MCV (fL)	59.0–76.0	67.7 (65.0–71.2)	0/44 **H** 3/44 **L**	69.1 (64.2–74.0)	1/42 **H** 3/42 **L**	69.1 (66.3–72.1)	1/84 **H** 2/84 **L**	.53	.087
MCH (pg)	21.9–26.1	23.4 (22.4–23.9)	7/41 **L**	23.4 (22.7–24.1)	6/41 **L**	23.7 (22.8–24.2)	6/77 **L**	.90	.24
MCHC (g/dL)	32.6–39.2	34.8 (33.3–35.8)	11/45 **L**	33.9 (32.6–35.1)	5/42 **L**	34.0 (33.0–35.2)	14/80 **L**	.099	.11
WBCs ($\times 10^{9}$/L)	4.9–17.6	18.8 (11.9–25.4)	13/45 **H** 0/45 **L**	12.0 (9.2–18.9)	22/43 **H** 0/43 **L**	12.3 (9.2–16.2)	18/84 **H** 0/84 **L**	.011	<.001
Neutrophils ($\times 10^{9}$/L)	2.94-12.67	**14.0 (9.7–21.0)**	17/44 **H** 0/44 **L**	9.1 (6.7–15.1)	24/42 **H** 0/42 **L**	**9.1 (6.4–12.8)**	22/83 **H** 0/83 **L**	.0065	**<.001**
Lymphocytes ($\times 10^{9}$/L)	1.06-4.95	**1.1 (0.8-1.4)**	0/44 **H** 15/44 **L**	1.4 (1.0–2.2)	2/42 **H** 20/42 **L**	**1.6 (1.3-2.3)**	1/83 **H** 11/83 **L**	.011	**<.001**
Monocytes ($\times 10^{9}$/L)	0.13-1.15	1.4 (0.9-2.3)	17/44 **H** 1/44 **L**	1.0 (0.3-1.7)	27/42 **H** 2/42 **L**	0.8 (0.5-1.3)	22/81 **H** 3/81 **L**	.027	<.001
Eosinophils ($\times 10^{9}$/L)	0.07-1.49	**0.1 (0.0-0.3)**	1/44 **H** 12/44 **L**	0.2 (0.0-0.4)	0/42 **H** 18/42 **L**	**0.3 (0.2-0.8)**	5/82 **H** 11/82 **L**	.65	**<.001**
Platelets ($\times 10^{9}$/L)	150–450	404.0 (324–458)	10/44 **H** 0/44 **L**	329.0 (238–436)	15/42 **H** 3/42 **L**	333.0 (255–541)	20/83 **H** 4/83 **L**	.0051	.022

Mann–Whitney *P* values for the BT vs non-BT and BT vs cBT comparisons of median values are also shown (MW *P* value). values in bold typeface were significantly different (Bonferroni corrected *P* value ≤.0016).

Number of dogs with MCH and MCHC above the reference range not included.

TABLE 5 Median values and interquartile ranges for hematologic and biochemical variables for BTs, other breeds (non-BTs) and cBTs and number of dogs in each group that are above (H) or below (L) the reference range (RR) for each variable

		Dogs with GBM				Dogs without GBM			
	Reference range (RR)	BTs median (and interquartile range)	BTs outside RR	Non-BTs median (and interquartile range)	Non-BTs outside RR	cBTs median (and interquartile range)	cBTs outside RR	MW *P* value BTs v non-BTs	MW *P* value BTs v cBTs
Total Protein (g/dL)	5.49-7.53	6.6 (5.9-6.9)	3/46 **H** 10/46 **L**	6.4 (5.9-6.9)	3/47 **H** 3/47 **L**	6.2 (5.8-6.6)	1/87 **H** 15/87 **L**	.92	.083
Albumin (g/dL)	2.63-3.82	3.0 (2.5-3.3)	0/47 **H** 16/47 **L**	2.9 (2.7-3.3)	0/47 **H** 11/47 **L**	3.0 (2.6-3.3)	1/85 **H** 19/85 **L**	.55	.59
Globulin (g/dL)	2.63-3.82	4.2 (3.1–3.8)	5/46 **H** 5/46 **L**	3.4 (3.1–3.8)	1/47 **H** 2/47 **L**	3.1 (2.8-3.4)	8/85 **H** 1/85 **L**	.87	.0068
Urea (mg/dL)	8.68-30.2	9.8 (7.3–17.1)	4/45 **H** 14/45 **L**	12.9 (10.1–18.1)	4/45 **H** 4/45 **L**	12.9 (9.9–23.0)	4/87 **H** 21/87 **L**	.052	.077
Creatinine (mg/dL)	0.22-1.59	0.7 (0.57-0.86)	2/46 **H** 0/46 **L**	0.7 (0.59-0.82)	0/44 **H** 0/44 **L**	0.9 (0.67-0.93)	2/86 **H** 0/86 **L**	.76	.025
ALT (U/L)	19.8–124	**577.0 (230.0–1157.8)**	44/44 **H**	410.0 (140.0–982.0)	38/46	**53.0 (33.9–89.8)**	12/86	.18	**<.001**
ALP (U/L)	<130	**3577.0 (1830–6259)**	46/46 **H**	1694.0 (442.0–4485.0)	44/47	**131.0 (59.0–325.1)**	43/85	.0076	**<.001**
GGT (U/L)	2.5–10.6	**69.4 (35.3–131.0)**	35/39 **H**	33.0 (16.0–59.0)	26/33	**5.2 (1.0–8.8)**	11/53	.0034	**<.001**
AST (U/L)	10.0–50.0	**188.0 (102.0–349.0)**	19/21 **H**	79.0 (50.5–165.0)	17/23	**36.0 (27.0–51.0)**	10/37	.053	**<.001**
Total bilirubin (mg/dL)	0-0.3	**4.1 (1.6-6.3)**	42/48 **H**	**0.4 (0.19-5.0)**	23/42 **H**	**0.2 (0.11-0.39)**	19/75 **H**	**.0013**	**<.001**
Cholesterol (mg/dL)	123.8–240.0	**448.9 (354.5–518.6)**	46/46 **H** 0/46 **L**	441.2 (295.7–539.1)	35/41 **H** 0/41 **L**	**220.6 (177.6–249.4)**	25/76 **H** 3/76 **L**	.66	**<.001**
Triglycerides (mg/dL)	26.5–106	132.5 (76.4–238.4)	12/18 **H** 0/18 **L**	158.9 (82.6–485.4)	13/20 **H** 0/20 **L**	79.5 (51.0–153.0)	15/46 **H** 0/46 **L**	.31	.006
Sodium (mEq/L)	135–155	148.0 (145.0–150.2)	0/36 **H** 2/36 **L**	144.9 (143.0–147.2)	0/32 **H** 1/32 **L**	147.0 (145.6–149.8)	3/77 **H** 1/77 **L**	.012	.67
Potassium (mEq/L)	3.6-5.6	**3.6 (3.4-4.3)**	0/37 **H** 13/37 **L**	**4.6 (4.0–5.0)**	0/33 **H** 1/33 **L**	**4.4 (4.1–4.8)**	0/76 **H** 3/76 **L**	**<.001**	**<.001**
Chloride (mEq/L)	100–116	108.5 (105.3–112.0)	0/34 **H** 2/34 **L**	109.0 (104.5–111.8)	0/30 **H** 3/34 **L**	112.0 (110.0–113.8)	3/66 **H** 3/66 **L**	.72	.0053
Inorganic phosphate (mg/dL)	2.48-4.96	4.7 (3.9-5.3)	11/36 **H** 0/36 **L**	4.3 (3.7-5.1)	10/37 **H** 0/37 **L**	4.3 (3.5-4.9)	13/70 **H** 0/70 **L**	.50	.15
Calcium (mg/dL)	9.44-11.36	10.0 (9.4–10.8)	3/38 **H** 4/38 **L**	10.4 (9.9–10.7)	1/35 **H** 1/35 **L**	10.0 (9.4–10.4)	7/76 **H** 12/76 **L**	.32	.38
Glucose (mg/dL)	64.8–126	100.8 (94.5–118.0)	2/32 **H** 0/32 **L**	102.6 (95.4–106.2)	3/29 **H** 1/29 **L**	100.8 (88.0–110.1)	5/62 **H** 1/62 **L**	.56	.18
Thyroxine (μg/dL)	1.01–3.98	0.9 (0.82-1.00)	0/9 **H** 8/9 **L**	1.2 (0.79-1.54)	0/9 **H** 2/9 **L**	1.4 (1.20-1.55)	0/19 **H** 2/19 **L**	.12	**.0013**

Mann–Whitney *P* values for the BT vs non-BT and BT vs cBT comparisons of median values are also shown (MW *P* value). Values in bold typeface were significantly different (Bonferroni corrected *P* value ≤.0016). Number of dogs with ALT, ALP, GGT, and AST activities and total bilirubin below the reference range not included.

ill animals due to acute endogenous corticosteroid production.[2] An increased neutrophil count may also reflect the inflammatory response to concurrent cholangitis, cholecystitis, or bile peritonitis.[2,29] Unfortunately, cytological review of a blood smear was inconsistently performed and, thus morphological features of the neutrophils (toxic change, presence of a left shift) were not included in this study.

A higher leucocyte count in dogs with GBM was found in non-survivors compared with survivors in two previous studies.[2,30] However, these findings have not been replicated in other studies[23] and the prognostic value of leucocytosis remains uncertain. While the median neutrophil count of the BTs was significantly higher than the non-BTs in this study there was a greater proportion of non-BTs with neutrophilia. This contradiction limits interpretation of the significance of the higher median neutrophil count in the BTs. Neutrophilic leucocytosis has been associated with bile peritonitis[2] although the incidence of gall bladder rupture was also not different between the groups in our study.

Biochemical findings in both the BTs and non-BTs were similar to those described for dogs with GBM.[1,2,24,27,31] Increased liver serum enzyme activities, particularly ALP and GGT, were seen in greater than 85% cases and this combination of findings is considered highly specific (94%) for hepatobiliary disease.[32] Hyperbilirubinemia was recorded in 65/90 GBM cases but was only manifested as visible icterus in 35/99 cases reflecting the higher optical threshold for clinical icterus.[33] BTs had higher median total bilirubin and were more likely to be hyperbilirubinaemic than non-BTs, potentially reflecting an increased incidence of biliary obstruction/cholestasis, pancreatitis, sepsis, or bacterial cholangitis in this cohort.[29] Cannulation of the common bile duct is commonly performed during cholecystectomy.[24] It was not possible to ascertain the degree of difficulty of bile duct catheterization from the surgical records in our study or whether this procedure was more frequently performed in the BTs. Future studies could look to evaluate breed particularities associated with biliary obstruction and the therapeutic advantage of biliary decompression.

Hyperlipidemia was identified in 84/87 GBM cases (3 non-BTs had hypertriglyceridemia without hypercholesterolemia). Median cholesterol and triglyceride values were higher in BTs than cBTs although cBTs may have been more likely to be appropriately fasted prior to blood sampling. Post-prandial hyperlipidemia is considered unlikely to have played a significant role, as the majority of GBM dogs were inappetent at presentation. Our statistical analysis compared median values for the different groups of dogs; this approach might overlook significant differences in the proportions of dogs with hypercholesterolemia.

Marked hypercholesterolemia in the context of GBM is suggestive of bile duct obstruction[1] although other conditions, including endocrinopathies (hyperadrenocorticism, hypothyroidism, and diabetes mellitus), protein losing nephropathy, pancreatitis, or obesity may also have contributed to this biochemical abnormality in the dogs included in this study. Given that excretion in the bile is the principal pathway for cholesterol elimination from the body,[34] any impairment of gall bladder motility, as thought to occur with GBM[12] could be expected to cause hypercholesterolemia. Hypercholesterolemia could also have a contributory role in GBM formation. Supersaturation of bile with cholesterol has been associated with impaired gallbladder motility in people[35] and sludge formation in dogs[4] and thus, may be a precursor to GBM formation. However, gall bladder sludge, while prevalent in older dogs, has not been shown to progress toward significant biliary disease over the course of a year[36] and might not represent a risk factor in its own right. A recent study into the mechanisms of GBM formation identified a disproportionate increase in the gel-forming mucin Muc5ac relative to Muc5b, defective mucin un-packaging and altered levels of mucin-interacting proteins as potential contributory factors.[3] The role of cholesterol in these processes remains uncertain although the expression of certain mucin genes has been linked to cholesterol-associated gallbladder disease in people.[37]

TABLE 6 Seven-day survival data for dogs with gall bladder mucoceles according to gall bladder rupture status separated to allow comparison according to breed [BTs vs other breeds (non-BTs)]

Dogs with GBM	Proportion of animals that died within 7 days		
	With gall bladder rupture	Without rupture	*P* value
BTs	2/17	5/31	$P = 1.0$
Non BTs	1/13	1/28	$P = .54$
All dogs	3/30	6/59	$P = 1.0$

The higher prevalence of primary hypercholesterolemia in the Shetland Sheepdog[38] and hypertriglyceridemia with or without hypercholesterolemia in the Miniature Schnauzer[34] may, at least partially account for these breeds' predisposition to GBM. However, not all Shetland Sheepdogs with GBM manifest hyperlipidemia[2] and cholesterol concentrations were not significantly different in GBM cases compared with control dogs.[4] Our study did not establish an association of hypercholesterolemia with GBM in BTs. However, the precise role of cholesterol as contributor to, or consequence of GBM formation merits further interrogation. Further longitudinal studies are necessary to confirm the importance of hypercholesterolemia as a risk factor for GBM.

Potassium concentrations were significantly lower in the BTs compared with non-BTs and cBTs. This electrolyte has previously been suggested to have prognostic importance in GBM cases as non-survivors had lower potassium compared with survivors.[2] The lower total thyroxine (T_4) levels in BTs compared with cBTs could reflect a greater incidence of undiagnosed hypothyroidism as cTSH measurements (or wider thyroid panels) were not routinely performed. An alternative explanation would be non-thyroidal illness secondary to the complications of hepatobiliary disease. Undiagnosed hypothyroidism could also have contributed to the high prevalence of hypercholesterolemia among BTs, although, this may also be associated with cholestasis in the context of the GBM. Future prospective studies serially interrogating the thyroid axis should clarify this important point more fully. Follow-up data establishing thyroid status was unfortunately not available to permit clarification in the current cohort.

Cholecystitis occurs in 5%-33% of dogs with GBM[24,27,31] and in 80% of Shetland Sheepdogs.[2] The identification of cholecystitis in 11/37 (30%) BTs in this study does not support a primary role for gall bladder inflammation in GBM formation in this breed. Cholangitis was

TABLE 7 Outcome data for dogs with gall bladder mucoceles separated to allow comparison according to breed [BTs vs other breeds (non-BTs)] and management approach (medical versus surgical)

	BTs	Non-BTs	*P* value	Medically managed dogs	Surgically managed dogs	*P* value
7 day survival	41/48 (85%)	39/41 (95%)	.17	12/12 (100%)	68/77 (88%)	.60
6 month survival	33/41 (80%)	31/35 (89%)	.37	10/11 (91%)	54/65 (83%)	.68
1 year survival	26/34 (76%)	22/25 (88%)	.33	9/10 (90%)	39/49 (80%)	.67

About 3 BTs and 7 non-BTs were euthanized without treatment and are excluded from survival analysis.
About 13 dogs were still alive at time of writing but had survival times less than 6m.
About 29 dogs were still alive at time of writing but had survival times greater than 6 m but less than 1 year (includes the 13 dogs above).

found in 23/28 (82%) BTs in this study compared with 30%-45% of dogs with GBM in previous studies.[2,24] Gallbladder disease has been implicated as a primary cause of intrahepatic cholangitis in dogs and can occur with GBM without concurrent cholecystitis.[39] The 10% (6/63) incidence of positive culture of gallbladder contents (bile or gall bladder wall) is within the 3%-70% range of previous reports[2,24,27,30,31,40] and might have been underestimated by pre-operative antibiotic therapy[24,27,40] (not investigated in this study). Pathogens identified in the gallbladder contents and liver in this study have been reported.[1,29,39,40]

Dogs lacking clinical signs referable to hepatobiliary disease and in which the GBM was an incidental finding have previously been reported.[2,27,41] In our study 12 dogs were managed medically. These dogs presented fewer clinical signs and a lower incidence of vomiting than dogs that underwent cholecystectomy. Our study demonstrates that appropriately selected cases can be managed without surgery as 9/10 cases have survived for more than 1 year. However, outcome comparison is subject to an inherent bias through targeted selection of less clinically affected animals for medical therapy. Two previously reported cases had complete resolution of GBM within 4 months without surgical intervention.[42] Follow-up ultrasound reports were rarely available for the medically managed cases in our study prohibiting an objective evaluation of treatment efficacy beyond survival status.

The 11.7% case fatality rate at 7 days is similar to other reports; case fatality rates for GBM dogs undergoing cholecystectomy have been between 7% and 32%.[2,24,27,31,43] Gall bladder rupture was not shown to have a negative effect on survival although this could reflect a type II error due to the low numbers of dogs with rupture in this study. A recent larger study found that dogs with rupture of the gall bladder were 2.7 times more likely to die than dogs without rupture.[40] In this study, dogs that underwent cholecystectomy and survived the initial perioperative period had an extended survival time (MST still not reached), in agreement with previous studies.[27]

Our study did have limitations. The multicenter approach meant that blood work was performed at more than one laboratory with slightly different reference ranges. Data has been presented as absolute values, rather than percentages above/below the reference range to facilitate interpretation. Endocrine disease testing prior to, or, at presentation was often incomplete leading to potential underestimation of the incidence of these disorders. Furthermore, there was an absence of consistent long-term follow up meaning that endocrinopathies subsequently diagnosed could not be included in the analysis. Ultrasonographic changes affecting the gall bladder consistent with early or developing GBMs were also excluded potentially leading to an underestimation of GBM cases. Peculiarities in the non-BT group could have skewed any comparison with the BT group given the possibility of an increased incidence of particular metabolic or endocrinopathic conditions therein (eg, hyperlipidemia in Miniature Schnauzers). This could have led to false association (or lack thereof) between underlying diseases or biochemical abnormalities. A larger non-BT group would be required to help interrogate this further. A clear limitation of the cBT group is that they were not a healthy cohort. Therefore, despite the lack of ultrasonographic evidence for GBM, any comparison between groups in relation to clinical signs or blood-work abnormalities must be interpreted with caution. The BT and cBT groups were not age-matched; the cBT group were younger. This therefore raises the question as to whether some of these cBT would have developed GBM should they have been presented at an older age. As with the previous point, this limits the ability to identify risk factors.

In conclusion, our study provides robust evidence for a predisposition to GBM in the BT breed. The Affenpinscher is also potentially predisposed and merits evaluation of a greater number of cases. Similar to findings from previous studies in non-BT breeds, the etiology of GBM formation in BTs, is likely to be multi-factorial, reflecting the presence of one or more risk factors in a predisposed individual. Further studies of BTs are warranted to identify factors influencing this predisposition. The BT represents a suitable candidate breed for future genetic association studies.

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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

Ethical approval was obtained from the institutional scientific and ethical review committees for each of the participating centers.

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