Effect of clinical signs, endocrinopathies, timing of surgery, hyperlipidemia, and hyperbilirubinemia on outcome in dogs with gallbladder mucocele

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Abstract

Gallbladder mucocele (GBM) is a common extra-hepatic biliary syndrome in dogs with death rates ranging from 7 to 45%. Therefore, the aim of this study was to identify the association of survival with variables that could be utilized to improve clinical decisions. A total of 1194 dogs with a gross and histopathological diagnosis of GBM were included from 41 veterinary referral hospitals in this retrospective study.

Dogs with GBM that demonstrated abnormal clinical signs had significantly greater odds of death than subclinical dogs in a univariable analysis (OR, 4.2; 95% CI, 2.14–8.23; $P < 0.001$). The multivariable model indicated that categorical variables including owner recognition of jaundice (OR, 2.12; 95% CI, 1.19–3.77; $P = 0.011$), concurrent hyperadrenocorticism (OR 1.94; 95% CI, 1.08–3.47; $P = 0.026$), and Pomeranian breed (OR, 2.46; 95% CI 1.10–5.50;

P = 0.029) were associated with increased odds of death, and vomiting was associated with decreased odds of death (OR, 0.48; 95% CI, 0.30–0.72; *P* = 0.001). Continuous variables in the multivariable model, total serum/plasma bilirubin concentration (OR, 1.03; 95% CI, 1.01–1.04; *P* < 0.001) and age (OR, 1.17; 95% CI, 1.08–1.26; *P* < 0.001), were associated with increased odds of death. The clinical utility of total serum/plasma bilirubin concentration as a biomarker to predict death was poor with a sensitivity of 0.61 (95% CI, 0.54–0.69) and a specificity of 0.63 (95% CI, 0.59–0.66). This study identified several prognostic variables in dogs with GBM including total serum/plasma bilirubin concentration, age, clinical signs, concurrent hyperadrenocorticism, and the Pomeranian breed. The presence of hypothyroidism or diabetes mellitus did not impact outcome in this study.

Keywords
Canine Cushing's; Cholecystectomy; Gallbladder mucocoele; Hypothyroidism; Survival

Introduction

Gallbladder mucocele (GBM) in dogs is defined as a distended gallbladder with an abnormal accumulation of thick amorphous mucin in conjunction with histologic evidence of mucosal hypertrophy, formation of mucus-filled cysts, and long, thin branching fronds of well differentiated gallbladder epithelial cells (Gookin et al., 2015, Kesimer et al., 2015, Pike et al., 2004; Stalker et al., 2007). The syndrome has emerged as one of the most commonly identified causes of biliary disease in dogs over the last 10 years (Gookin et al., 2015). The clinical importance of GBM relates to the morbidity and mortality associated with extrahepatic biliary duct obstruction, gallbladder rupture, biliary infection, and systemic inflammatory response syndrome that can occur secondary to gallbladder wall necrosis, infarction, or perforation (Jaffey et al., 2018).

The optimal time for surgical intervention in dogs with GBM that demonstrate clinical signs associated with biliary tract disease has not been defined. Over the last 20 years there has been a shift towards early surgical intervention in people with acute cholecystitis (Lo et al., 1998). Performing cholecystectomy in people with acute cholecystitis within 72 h of time of onset of symptoms significantly decreases the overall incidence of complications, including perioperative blood loss, length of hospital stay, wound infection, overall morbidity, and mortality (Cao et al., 2016, Gonzalez-Rodriguez et al., 2009, Lau et al., 2006). While not directly comparable to canine GBM, some of these concerns regarding cholecystitis in people are likely to be of relevance, particularly as cholecystitis and regional inflammation has been observed in some dogs with GBM (Malek et al., 2013, Pike et al., 2004, Tamborini et al., 2016, Wennogle et al., 2019, Rogers et al., 2019).

The average time from the onset of clinical signs to surgery in dogs with GBM according to one small study is approximately 8 days (Worley et al., 2004). However, the relationship between timing of cholecystectomy and outcome has not been investigated in dogs with GBM. Furthermore, there is limited information related to the impact of abnormal clinical signs associated with biliary tract disease in dogs with GBM on survival. In a recent retrospective study, dogs that demonstrated one or more clinical sign attributable to biliary tract disease had a significantly greater mortality rate than dogs that were subclinical at the

time of cholecystectomy (Youn et al., 2018). However, this study did not investigate the effect of specific clinical signs and survival in a multivariable model.

Hyperbilirubinemia is reported to occur in approximately 52%–100% of dogs with GBM (Crews et al., 2009, Malek et al., 2013, Youn et al., 2018). Hyperbilirubinemia is a preoperative risk factor for death in dogs undergoing biliary surgery and total serum/plasma bilirubin concentrations predict a shorter survival time in Shetland sheepdogs with gallbladder disease and in dogs with acute and chronic hepatopathies (Aguirre et al., 2007, Amsellem et al., 2006, Dereszynski et al., 2008, Ferguson et al., 2011, Gomez Selgas et al., 2014, Kilpatrick et al., 2016, Lester et al., 2016, Poldervaart et al., 2009). There is limited information regarding the utility of total bilirubin concentration as a biomarker for survival in dogs with GBM despite hyperbilirubinemia being a common clinicipathologic abnormality in dogs with GBM and its association with decreased survival in dogs with biliary surgery.

Chronic hypercortisolemia (Worley et al., 2004), hypothyroidism (Ekmektzoglou and Zografos, 2006, Ladenson et al., 1984, Safer, 2013, Safer et al., 2004), and diabetes mellitus (DM; Ekmektzoglou and Zografos, 2006, Hickman et al., 1988) have been shown to negatively impact wound healing and substantially increase the risk of surgery-related morbidity and death in a variety of species. Dogs with hyperadrenocorticism (i.e. hypercortisolemia; Gookin et al., 2015, Mesich et al., 2009), hypothyroidism (Mesich et al., 2009), and hyperlipidemia (Kutsunai et al., 2014) have significantly higher odds of having a GBM. However, the possibility of an association between hyperadrenocorticism, hypothyroidism, and hyperlipidemia and outcome in dogs with GBM has not been evaluated. Understanding this relationship is clinically important because of how common GBM occur in dogs with hyperadrenocorticism, hypothyroidism, DM, and hyperlipidemia.

The present study had four objectives. The first was to determine if timing of cholecystectomy after recognition of clinical signs impacted survival. Our second objective was to investigate if there was a difference in outcome between dogs with or without clinical signs attributable to GBM. The third objective was to determine if a diagnosis of concurrent hyperadrenocorticism, hypothyroidism, DM, or hyperlipidemia was associated with increased death. The final objective of our study was to evaluate total serum/plasma bilirubin concentration as a prognostic biomarker. We hypothesized that greater time (days) from initial recognition of clinical signs to cholecystectomy in dogs with GBM would be significantly associated with death. Further, we hypothesized that dogs with clinical signs attributable to GBM at the time of cholecystectomy would be more likely to die than dogs without clinical signs. Next, we hypothesized that dogs with comorbidities (hyperadrenocorticism, hypothyroidism, DM, and hyperlipidemia) would be more likely to die than dogs without these comorbidities. Lastly, we hypothesized that total serum/plasma bilirubin concentration could be used to predict death.

Materials and methods

Criteria for selection of cases

A retrospective, multi-center, case series was performed. Multiple institutions throughout the United States of America, Canada, Australia, Asia, South America and Europe were contacted for participation in this study. This was achieved by direct communication via

email with board-certified small animal internists with contact information available in the American College of Veterinary Internal Medicine and European College of Veterinary Internal Medicine – Companion Animal directory. Only institutions with at least one boarded small animal specialist (i.e. internal medicine, surgeon, clinical pathologist, or criticalist) were eligible for participation. Medical records of dogs that had gross and histopathologic diagnosis of GBM after cholecystectomy examined between January 2007 and January 2017 were requested. Cases were identified at each institution by searching medical records, histopathology and surgery logs for 'gallbladder mucocele/mucocoele', 'mucocele/mucocoele', and 'cholecystectomy'. Some data from this cohort was previously reported in studies with different objectives (Allerton et al., 2018, Jaffey et al., 2018, Rogers et al., 2019). This study was approved by the Clinical Research Ethical Review Board of the University of Edinburgh (# 132.17; date of approval 11th November 2017) and University of Nottingham (# 2149.171108; date of approval 7th November 2017). The remaining institutions obtained dog owner signed consent on standard hospital admission documents that outline permission for use of data from medical records for research. All histologic interpretations were made by board-certified veterinary pathologists or the equivalently trained expert as dictated by the scientific standards of the geographic region. Likewise, all cholecystectomies were performed by, or under the direct supervision of, a boarded-veterinary surgeon or the equivalently trained expert as dictated by the scientific standards of the geographic region.

The data retrieved from medical records included: beginning and ending dates of medical records undergoing review; age; breed; sex; histologic features consistent with GBM; time (days) from the onset of clinical signs to surgery; description of clinical signs; the presence of hyperadrenocorticism, hypothyroidism, DM, or combination thereof; treatment for hyperadrenocorticism, hypothyroidism, and DM; blood biochemical data (total bilirubin, triglyceride, and cholesterol concentrations); survival to discharge; and cause of death. All biochemical data were obtained during the hospitalization associated with cholecystectomy or death.

A diagnosis of GBM was made based on gross appearance of a distended gallbladder with an abnormal accumulation of thick amorphous mucin in conjunction with histologic evidence of mucosal hypertrophy, formation of mucus-filled cysts, and long, thin branching fronds of well differentiated gallbladder epithelial cells (Gookin et al., 2015, Kesimer et al., 2015, Pike et al., 2004). A dog was considered to have hypothyroidism or hyperadrenocorticism if a historical diagnosis was present at the time of cholecystectomy. A dog was considered to have hypothyroidism if serum (total or free) T4 concentration was less than the lower limit of the reference interval and concurrent serum TSH concentration was greater than the upper limit of the reference interval of the respective laboratory (Mesich et al., 2009). A dog was considered to have hyperadrenocorticism if a low-dose dexamethasone suppression test or ACTH stimulation test results were positive according to the laboratory-derived cutoffs used for hyperadrenocorticism and consistent clinical signs were present (e.g. polyuria, polydipsia, polyphagia, panting, abdominal distension; Mesich et al., 2009). A dog was considered to have DM if a historical diagnosis was present or if hyperglycemia, glucosuria, and clinical signs consistent with DM were identified at the time of evaluation (Mesich et al., 2009). Hyperlipidemia was defined as an increase in the blood concentration of cholesterol, triglycerides, or both (De Marco et al., 2017, Lee et al., 2017). Both

cholesterol and triglyceride concentrations were required to be less than the upper limit of each reference interval to rule out hyperlipidemia in order to minimize potential false negatives related to missing data for either parameter. Dogs were considered to have clinical signs attributable to biliary tract disease if the owner reported vomiting, lethargy, anorexia, hyporexia, pain, jaundice, diarrhea, hypodipsia, adipsia, weakness, fever, and abdominal distension in the 7 days prior to presentation. Death-in-hospital was the primary outcome of interest, and was defined collectively, as in-hospital death or euthanasia in dogs with GBM that had undergone a cholecystectomy. Survival after hospital discharge was not assessed in this study.

Statistical analysis

Statistical analysis was performed using commercially available software (STATA SE, v.15.1, StataCorp, College Station, TX). The Shapiro-Wilk test was used to assess normality of continuous numerical variables. The data with non-normal distribution were presented as median, Q1, Q3, and range. Analyses of total serum/plasma bilirubin concentration were made based on the fold change with respect to the upper limit of the corresponding reference interval (i.e. total serum/plasma bilirubin concentration / upper limit of the reference interval). A receiver-operating characteristic (ROC) curve was used to determine the area under the curve (AUC) and select the optimum cut-off value of the fold change in total serum/plasma bilirubin concentration for prediction of death-in-hospital that maximized the Youden's J statistic for sensitivity and specificity reporting.

Patient data were considered clustered within hospital; and all regression models were fitted as random-effects logit models with death as the binary outcome of interest. Coefficients in the models were exponentiated to produce odds ratios. Numerical variables, age and duration of clinical signs before surgery, were assessed in regression as continuous variables and also categorized into quartiles and quintiles, respectively, based on natural breaks in the data. Model comparison was based on Akaike information criteria (AIC) and Bayesian information criteria (BIC), and for both variables analysis as continuous variables was chosen as equivalent or superior to division into quartiles/quintiles.

Univariate random-effects logistic regression analyses were performed to screen for the association between death and the independent variables of interest. Comorbidities as hyperadrenocorticism, hypothyroidism, DM, hyperlipidemia, hypercholesterolemia, hypertriglyceridemia were included in regression analyses as dichotomous variables with absence of the comorbidity as the referent. Covariates screened individually using univariable logistic regression and having a liberal *P*-value cutoff, i.e. $P < 0.20$, were included in the final multivariable analysis. The level of significance was set at $P < 0.05$ for the multivariable analysis, as well as all other statistical tests, and only variables with *P*-values <0.05 were retained in the final reported model. Confounding was assessed by determining if individual variable odds ratios changed by >20% with inclusion/removal of additional variables.

Results

Animal population

A total of 1194 dogs from 38 academic veterinary hospitals and 3 private practice specialty hospitals in 20 countries were included in the study. Collaborators were recruited through direct contact with referral centers. Medical record system changes altered the study period from four institutions (Supplemental Table S1). No dogs were excluded.

There was a total of 1006 purebred dogs and 188 mixed breed dogs (Supplemental Table S2). The most commonly represented breeds (i.e. those comprising >30 dogs) were Mixed breed dogs, Shetland sheepdog, Cocker spaniel, Miniature schnauzer, Bichon frise, Beagle, Pomeranian, Border terrier, Chihuahua, and Shih tzu (Supplemental Table S1). The median age was 10 years (Q1, Q3, range; 8.0, 12.0, 2.0–17.0 years). There were 546 spayed females, 496 castrated males, 86 intact males and 66 intact females.

All 1194 dogs had a cholecystectomy performed. Medical records detailing the presence or absence of clinical signs attributable to biliary tract disease (i.e. clinical or subclinical) at the time of cholecystectomy were available for 99.9% (1193/1194) dogs (Fig.1). A total of 17.6% (211/1194) of dogs did not exhibit clinical signs at the time of cholecystectomy. The reason for cholecystectomy was available for 84.8% (179/211) of dogs that were subclinical. The indication for surgery in these 179 dogs included prophylactic surgery after an incidental ultrasonographic identification of GBM in 62.6% (112/179), failure of improvement in liver enzyme activities or lack of ultrasonographic resolution of GBM after medical management in 12.8% (23) of dogs, and concomitant increase in liver enzyme activities and ultrasonographic identification of GBM in 6.7% (12). A total of 17.9% (32/179) of dogs had a planned cholecystectomy in conjunction with another surgery or it was performed at the discretion of a surgeon during an unrelated procedure (Supplemental Table S3).

Eighty-two percent (982/1194) of dogs with GBM demonstrated at least one clinical sign attributable to biliary tract disease, and those most commonly reported (i.e. in more than >10% of dogs) included vomiting 80.0% (786/982), lethargy 46.7% (458/982), anorexia 46.2% (454/982), diarrhea 18.3% (180/982), painful abdomen 16.7% (164/982), hyporexia 11.2% (110/982), and jaundice 9.8% (96/982). The remaining distribution of clinical signs are provided in Supplemental Table S4. Information on the duration of clinical signs prior to cholecystectomy was available in 98.4% (966/982) of dogs; the median time was 4 days (Q1, Q3, range; 2.0, 7.0, 1.0–730 days).

A total of 84.4% (1008/1194), 19.4% (232/1194), and 19.0% (227/1194) dogs had cholesterol and triglyceride concentrations, or both measured, respectively. Hyperlipidemia was identified in 90.8% (585/644) dogs. Hypercholesterolemia was found in 55.4% (559/1008) and hypertriglyceridemia was identified in 48.3% (112/232) of dogs. Twenty-six percent (59/227) of dogs had normocholesterolemia and normotriglyceridemia. Total serum/plasma bilirubin concentration was measured in 92.4% (1103/1194) of dogs, of which the median was 2.3 times the upper limit of the reference interval (Q1, Q3, range; 0.67, 11.2, 0.0–135).

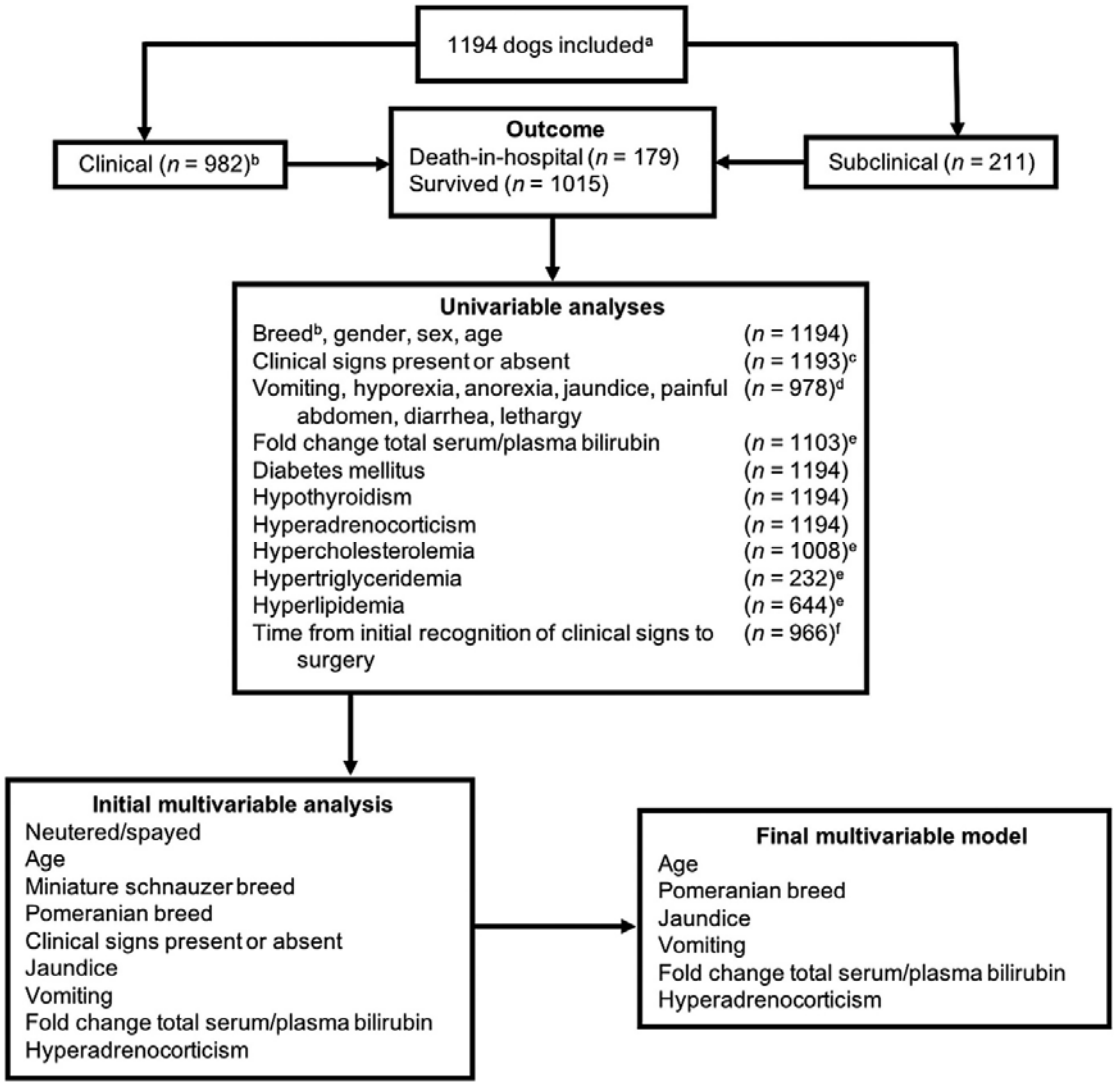

Fig. 1. Flow diagram. A total of 1194 dogs with gallbladder mucoceles were included in the study. [a]There were no dogs excluded. Several potentially clinically significant variables were included in univariable logistic regression analyses. [b]The 10 most common breeds were included (i.e. Mixed breed, Shetland sheepdog, Cocker spaniel, Miniature schnauzer, Bichon frise, Pomeranian, Border terrier, and Chihuahua). [c]One dog did not have a clear distinction if clinical signs were present or absent in the 7-days preceding cholecystectomy. [d]The seven most commonly reported clinical signs (i.e. vomiting, hyporexia, anorexia, jaundice, painful abdomen, diarrhea, and lethargy). [e]Biochemical parameters (total serum/plasma bilirubin, cholesterol, and triglyceride concentrations) were performed at the discretion of the attending clinician, which resulted in missing data for these variables. [f]Time (days) from initial recognition of clinical signs to cholecystectomy was missing in 16/982 clinical dogs. Significant variables in the univariable analyses ($P < 0.20$) were included in the initial multivariable model. The final multivariable model retained significant variables ($P < 0.05$) with 915 total observations.

Of the 10.1% (120/1194) of dogs diagnosed with hyperadrenocorticism, 61.7% (74/120) were undergoing medical management at the time of cholecystectomy. Of the 6.1% (73/1194) of dogs diagnosed with hypothyroidism, all (73/73) were undergoing medical management at the time of cholecystectomy. Of the 5.8% (69/1194) of dogs diagnosed with DM, all (69/69) were undergoing medical management at the time of cholecystectomy.

Death-in-hospital occurred in 15.0% (179/1194; 95% CI 13.0–17.1%) of dogs that underwent cholecystectomy for GBM. Ninety-six of these dogs were euthanased and 83 died. Death-in-hospital occurred in 17.1% (168/982) of dogs that demonstrated clinical signs attributable to biliary tract disease at the time of cholecystectomy, compared to 4.7% (10/211) of dogs that were subclinical. The one dog without a clinical status prior to surgery available, died following cholecystectomy. Of the dogs that were subclinical and had cholecystectomy performed for prophylactic purposes in absence of a concurrent procedure 5.4% (6/112) had death-in-hospital. The reason for euthanasia and cause for death was reported for all (96/96) and 45.8% (45/83) of dogs, respectively (Supplementary Table S5).

Total serum/plasma bilirubin concentration as a biomarker to predict to death-in-hospital

The area under the ROC curve for total serum/plasma bilirubin concentrations in relation to survival-to-discharge in dogs with GBM was 0.64 (95% CI, 0.60–0.69). The optimal cutoff was 4.3 times the upper limit of the reference interval, with a greater increase being consistent with death-in-hospital. Using this cutoff, a sensitivity of 0.61 (95% CI, 0.54–0.69) and a specificity of 0.63 (95% CI, 0.59–0.66) for the prediction of death-in-hospital was achieved (Table 1).

Table 1. Sensitivity and specificity of fold change, with respect to the upper reference interval, of total serum/plasma bilirubin concentration at time of presentation for prediction of death-in-hospital for dogs following cholecystectomy for gallbladder mucocoele management using different cutoff values.

Cutoff (fold increase)	Sensitivity	95% CI	Specificity	95% CI
0.01	1.00	0.98–1.00	0.01	0.01–0.02
1.32	0.77	0.70–0.83	0.42	0.39–0.46
2.00	0.70	0.62–0.77	0.51	0.48–0.54
3.00	0.66	0.58–0.73	0.58	0.55–0.61
4.00	0.63	0.55–0.71	0.61	0.58–0.65
4.30	0.61	0.54–0.69	0.63	0.59–0.66
8.60	0.47	0.39–0.55	0.74	00.71–0.76
10.10	0.42	0.34–0.49	0.76	0.73–0.79
15.00	0.28	0.21–0.35	0.83	0.81–0.86
20.20	0.19	0.14–0.26	0.88	0.86–0.90
71.67	0.01	0.00–0.04	1.00	0.98–1.00

CI, confidence interval.

Univariable analysis

Variables pertaining to patient signalment that were significant in univariate screening ($P < 0.20$; Table 2; Fig.1) for increased odds of death-in-hospital included being neutered/spayed (OR, 1.69; 95% CI, 0.95–3.01; $P = 0.072$) and increasing age (OR, 1.14; 95% CI, 1.07–1.22; $P < 0.001$). Breeds with significant associations included reduced odds of death for Miniature schnauzers (OR, 0.27; 95% CI, 0.08–0.88; $P = 0.030$) and increased odds of death for Pomeranians (OR, 1.67; 95% CI, 0.82–3.42; $P = 0.159$; Table 3).

Table 2. Results of univariable analyses related to signalment and final multivariable model for association with death-in hospital. Variables associated with death-in hospital ($P < 0.20$) in univariable analyses were included in initial multivariable regression analysis. Variables significantly associated with death-in hospital ($P < 0.05$) were retained in the final multivariable regression analysis.

Signalment	Variable		Univariable OR (95% CI)	Univariable *P-value*	Multivariable OR (95% CI)	Multivariable *P*[b]
Sex	Female: 93/612 (15.2%)	Male: 86/582 (14.8%)	1.02 (0.73–1.41)	0.925		
Neutered status	Neutered: 162/1042 (15.6%)	Entire: 17/152 (11.2%)	1.69 (0.95–3.01)	0.072[a]		
Age (years)	Continuous (range 2–17)		1.14 (1.07–1.22)	<0.001[a]	1.17 (1.08–1.26)	<0.001

OR, odds ratio; CI, confidence interval.

[a] $P < 0.20$ in univariable analysis and included in initial multivariable regression analysis.

[b] $P < 0.05$ in final multivariable regression analysis.

Table 3. Results of univariable analyses related to the 10 most frequent breeds and final multivariable model for association with death-in hospital. Variables associated with death-in hospital ($P < 0.20$) in univariable analyses were included in initial multivariable regression analysis. Variables significantly associated with death-in hospital ($P < 0.05$) were retained in the final multivariable regression analysis.

Breed (10 most frequent)	Yes	No	Univariable	Univariable	Multivariable	Multivariable
			OR (95% CI)	*P*-value	OR (95% CI)	*P-value*
Mixed breed	25/188 (13.2%)	154/1006 (15.3%)	0.93 (0.58–1.50)	0.768		
Shetland sheepdog	19/126 (15.1%)	160/1068 (15.0%)	1.10 (0.65–1.88)	0.730		
Cocker spaniel	11/74 (14.9%)	168/1120 (15.0%)	0.92 (0.47–1.83)	0.821		
Miniature schnauzer	3/63 (4.8%)	176/1131 (15.6%)	0.27 (0.08–0.88)	0.030[a]		
Bichon frise	6/58 (10.3%)	173/1136 (15.2%)	0.59 (0.24-1.42)	0.240		
Beagle	7/54 (13.0%)	172/1140 (15.1%)	0.84 (0.37–1.93)	0.687		
Pomeranian	11/48 (22.9%)	168/1146 (14.7%)	1.67 (0.82–3.42)	0.159[a]	2.46 (1.10–5.50)[b]	0.029
Border terrier	8/41 (19.5%)	171/1153 (14.8%)	1.58 (0.65–3.87)	0.315		
Chihuahua	3/36 (8.3%)	176/1158 (15.2%)	0.51 (0.15–1.69)	0.269		
Shih tzu	4/34 (11.8%)	175/1160 (15.1%)	0.72 (0.24–2.11)	0.547		

OR, odds ratio; CI, confidence interval.

[a]$P < 0.20$ in univariable analysis and included in initial multivariable regression analysis.

[b]Confounding present: Pomeranian breed confounded by variables of jaundice and vomiting.

Dogs with GBM that demonstrated clinical signs attributable to biliary tract disease had significantly greater odds of death than subclinical dogs in a univariable analysis (OR, 4.20; 95% CI, 2.14–8.23; $P < 0.001$; Table 4). Owner recognition of jaundice was associated with increased odds of death-in-hospital (OR, 2.33; 95% CI, 1.40–3.89; $P = 0.01$), whereas vomiting was associated with decreased odds of death (OR, 0.52; 95% CI, 0.34–0.78; $P = 0.001$; Table 4). Total serum/plasma bilirubin concentration (OR, 1.03; 95% CI, 1.02–1.04; $P < 0.001$) and hyperadrenocorticism (OR, 1.68; 95% CI, 1.04–2.70; $P = 0.035$; Table 5)

were associated with increased odds of death-in-hospital, but hyperlipidemia, hypercholesterolemia, duration of clinical signs, and presence of other endocrinopathies were not associated with odds of death (Table 5; Fig.1)

Table 4. Results of univariable analyses related to clinical signs and final multivariable model for association with death-in hospital. Variables associated with death-in hospital ($P < 0.20$) in univariable analyses were included in initial multivariable regression analysis. Variables significantly associated with death-in hospital ($P < 0.05$) were retained in the final multivariable regression analysis.

Clinical sign	Present	Absent	Univariable OR (95% CI)	Univariable *P-value*	Multivariable OR (95% CI)	Multivariable P^b-value
Time clinical (days)	Continuous (range 0–135)		0.99 (0.99–1.00)	0.450		
Any sign	168/982 (17.1%)	10/211 (4.7%)	4.20 (2.14–8.23)	<0.001[a]	Collinear with jaundice/vomiting	
Jaundice	29/96 (30.2%)	139/882 (15.8%)	2.33 (1.40–3.89)	0.001[a]	2.12 (1.19–3.77)	0.011
Hyporexia	23/110 (20.9%)	145/868 (16.7%)	1.23 (0.72–2.17)	0.451		
Painful abdomen	25/164 (15.2%)	143/814 (17.6%)	0.85 (0.52–1.40)	0.531		
Anorexia	78/454 (17.2%)	90/524 (17.2%)	1.05 (0.73–1.50)	0.785		
Diarrhea	36/180 (20.0%)	132/798 (16.5%)	1.19 (0.78–1.82)	0.419		
Vomiting	121/786 (15.4%)	47/192 (24.5%)	0.52 (0.34–0.78)	0.001[a]	0.48 (0.31–0.75)	0.001
Lethargy	71/458 (15.5%)	97/520 (18.7%)	0.80 (0.56–1.14)	0.214		

OR, odds ratio; CI, confidence interval.

[a] $P < 0.20$ in univariable analysis and included in initial multivariable regression analysis.

[b] $P < 0.05$ in final multivariable regression analysis.

Table 5. Results of univariable analyses related to clinicopathological findings/comorbidity and final multivariable model for association with death-in hospital. Variables associated with death-in hospital ($P < 0.20$) in univariable analyses were included in initial multivariable regression analysis. Variables significantly associated with death-in hospital ($P < 0.05$) were retained in the final multivariable regression analysis.

Clinicopathological findings/comorbidity	Present	Absent	Univariable	Univariable	Multivariable	Multivariable
			OR (95% CI)	*P-value*	OR (95% CI)	P^b-value
Fold change in total serum/plasma bilirubin	Continuous (range 0–135)		1.03 (1.02-1.04)	<0.001[a]	1.03 (1.01–1.04)	<0.001
Hyperlipidemia	77/585 (13.2%)	12/59 (20.3%)	0.62 (0.29–1.31)	0.210		
Hypertriglyceridemia	19/112 (17.0%)	15/120 (12.5%)	1.61 (0.69–3.79)	0.273		
Hypercholesterolemia	74/559 (13.2%)	67/449 (14.9%)	0.93 (0.64–1.35)	0.695		
Diabetes mellitus	13/69 (18.8%)	166/1125 (14.8%)	1.39 (0.73–2.66)	0.315		
Hypothyroidism	12/73 (16.4%)	167/1121 (14.9%)	1.20 (0.62–2.30)	0.595		
Hyperadrenocorticism	27/120 (22.5%)	152/1074 (14.2%)	1.68 (1.04–2.70)	0.035[a]	1.94 (1.08–3.47)	0.026

OR, odds ratio; CI, confidence interval.

[a] $P < 0.20$ in univariable analysis and included in initial multivariable regression analysis.

[b] $P < 0.05$ in final multivariable regression analysis.

Multivariable analysis

Variables selected from screening that remained statistically significant ($P < 0.05$) in the final multivariable regression model included age (OR 1.17; $P < 0.001$), Pomeranian breed (OR 2.46; $P = 0.029$), presence of owner-identified jaundice (OR 2.12; $P = 0.011$), quantitative increase in total serum/plasma bilirubin concentration (OR 1.03; $P < 0.001$), and concurrent hyperadrenocorticism (OR 1.94; $P = 0.026$) all increasing the odds of death, and presence of vomiting decreasing the odds of death (OR 0.48; $P = 0.001$; Table 2, Table 3, Table 4, Table 5; Fig.1).

Discussion

This international, multi-center study included over 1000 dogs, making it the largest GBM study to date. This study investigated important variables with clinical plausibility to impact survival-to-discharge including time from initial recognition of clinical signs to cholecystectomy, presence or absence of clinical signs attributable to biliary tract disease, presence of hyperlipidemia, total serum/plasma bilirubin concentration, age, sex, breed, and presence of endocrinopathy (i.e. DM, hyperadrenocorticism, hypothyroidism). The results from this study indicate that the continuous variables total serum/plasma bilirubin concentration and age, as well as the categorical variables concurrent hyperadrenocorticism, Pomeranian breed, and owner recognition of jaundice at the time of initial evaluation, were associated with increased odds of death. Unexpectedly, owner recognition of vomiting at the time of evaluation conferred a protective effect in our population. Lastly, while increasing total serum/plasma bilirubin concentration was associated with increased odds of death in this study, it proved to be a poor biomarker to predict death.

Hyperbilirubinemia is a preoperative risk factor for death in dogs undergoing biliary surgery and total serum/plasma bilirubin concentrations predict a shorter survival time in Shetland sheepdogs with gallbladder disease and in dogs with acute and chronic hepatopathies (Aguirre et al., 2007, Amsellem et al., 2006, Dereszynski et al., 2008, Ferguson et al., 2011, Gomez Selgas et al., 2014, Kilpatrick et al., 2016, Lester et al., 2016, Poldervaart et al., 2009). Likewise, total serum/plasma bilirubin concentration in the present study conferred a significant increase in odds of death-in-hospital. Hyperbilirubinemia in dogs with GBM likely reflects more severe or progressive disease with the presence of complications such as gallbladder rupture, pancreatitis, partial or complete extrahepatic bile duct obstruction, significant functional hepatobiliary disease, or endotoxin-induced (cholestasis of sepsis), which have the potential to compromise survival after cholecystectomy (Buote et al., 2006, Jaffey et al., 2018, Mayhew et al., 2002, Wang and Yu, 2014). The aforementioned variables were not evaluated in this study but warrant future investigations to better understand the pathogenesis and impact hyperbilirubinemia has on survival in dogs with GBM. While increasing total serum/plasma bilirubin concentrations proved to be significantly associated with death in this study, its utility as a clinically reliable biomarker to predict death was unacceptably poor. In other words, increasing total serum/plasma bilirubin concentrations in dogs with GBM can be interpreted as a negative prognostic indicator but cannot be relied upon to predict outcome.

Dogs with GBM that demonstrated clinical signs attributable to biliary tract disease at the time of cholecystectomy had 4.2 times the odds of death than dogs that were subclinical in a univariable logistic regression analysis. When evaluated in a multivariable model, the presence of clinical signs (present/absent) lost significance when adjusted for specific clinical signs. The final multivariable model indicated that owner recognition of jaundice was associated with 2.1 times the odds of death. The sclera and skin have detectable icterus when serum bilirubin is >51.3–68.4 µmol/L [>3–4 mg/dl] in dogs (Willard and Twedt, 2012). Therefore, the magnitude of hyperbilirubinemia was likely severe if untrained dog owners were capable of recognizing it and as identified in our study, increasing total serum/plasma bilirubin concentration was associated with increased odds of death. Unexpectedly, dog

owner recognition of vomiting was associated with 0.46 times the odds of death than when vomiting was not reported. It is possible that dogs evaluated for GBM without vomiting had more advanced disease. This interesting finding warrants additional investigation. The clinical progression of dogs with incidentally discovered GBM is poorly understood as is the impact of elective cholecystectomy on survival in these dogs (Clemente et al., 2017, Kesimer et al., 2015). Moreover, the long-term benefit of conservative medical therapy in dogs with GBM is scarce. One retrospective study found that none of the 15 dogs with hyperadrenocorticism treated with trilostane for 6 months had resolution of GBM (Kim et al., 2017). However, there is a report that describes GBM resolution in two dogs following medical management (Walter et al., 2008). Therefore, the finding that once clinical signs develop survival decreases suggests prospective studies evaluating the impact of early surgical intervention for incidentally discovered GBM on survival are warranted.

Dog breeds that have been reported to be over-represented for GBM include Shetland sheepdog, Cocker spaniel, Miniature schnauzers, and Border terriers (Aguirre et al., 2007, Allerton et al., 2018, Jaffey et al., 2018). Further, one study reported that Shetland sheepdogs with GBM formation were 9.3 times more likely to have been treated with imidacloprid than Shetland sheepdogs without GBM (Gookin et al., 2015). However, to the authors' knowledge, previous studies have not identified breed associations with survival in dogs with GBM. Pomeranian breed dogs had 2.3 times the odds of death than other breeds with GBM in the present study. A specific pattern for cause of death in the Pomeranians in our study could not be established because the majority experienced cardiopulmonary arrest of unknown etiology. However, of the Pomeranian dogs that were euthanased or died, the most common reasons were hypoxemia and refractory hypotension. Pomeranians are one of the most common breeds affected by tracheomalacia, which could have contributed to post-operative hypoxemia and refractory hypotension (Buback et al., 1996, Macready et al., 2007). Future studies are needed to investigate the cause for increased odds of death in Pomeranian dogs with GBM.

Advancing age was associated with increased odds of death-in-hospital in our study, consistent with many different studies investigating risk factors for diseases in dogs (Hanson et al., 2007, Mattin et al., 2018). Survival studies in veterinary literature are subject to the confounding variables that influence euthanasia. It is possible dogs with advanced age in this study were more likely to develop a severe complication related to accrued comorbidities that were not evaluated in our study. However, it is also possible that clinicians and dog owners perceived advanced age negatively and opted towards euthanasia if complications arose. Therefore, the authors caution against utilization of advanced age as a stand-alone negative prognostic indicator in dogs with GBM.

A concurrent diagnosis of hyperadrenocorticism at the time of cholecystectomy in dogs with GBM yielded an approximately 2-fold increase in odds of death-in-hospital than dogs without a diagnosis of hyperadrenocorticism. There are several possible explanations for this finding. We did not determine the severity of illness, duration of illness, remission status or evaluate for evidence of iatrogenic hypoadrenocorticism in dogs with hyperadrenocorticism in this study. Additionally, dogs were categorized as having hyperadrenocorticism only if identified before the time of cholecystectomy. It is possible that the dogs with a pre-operative diagnosis of hyperadrenocorticism had more severe

hypercortisolemia and more advanced or severe disease for a longer period of time, which prompted their earlier diagnosis. Only 10% of dogs in this study had a pre-operative diagnosis of hyperadrenocorticism and the majority of those dogs were being medically managed at the time of cholecystectomy. This small subset of dogs made statistical evaluation of the impact of medical management of hyperadrenocorticism on outcome in dogs with GBM unreliable and was not pursued. Hypercortisolemia in dogs with hyperadrenocorticism without medical management or poor control of disease can result in several potential deleterious consequences including hypercoagulability (Park et al., 2013, Rose et al., 2013), decreased wound healing (Wang et al., 2013), and immune system dysregulation (Mori et al., 2009). Conversely, medically managed dogs could be susceptible to either relative or absolute iatrogenic hypoadrenocorticism at the time of cholecystectomy. Eucortisolemia or true hypocortisolemia could have produced a physiologic syndrome similar to critical illness related corticosteroid insufficiency (i.e. a state of relative adrenal insufficiency), which has been associated with decreased survival in critically ill dogs (Burkitt et al., 2007). Future prospective studies are required to investigate the role of medical management of hyperadrenocorticism in dogs with GBM prior to cholecystectomy.

Performing cholecystectomy in people with acute cholecystitis within 72 h of the onset of symptoms significantly decreases overall morbidity and mortality (Cao et al., 2016, Gonzalez-Rodriguez et al., 2009, Gutt et al., 2013, Johansson et al., 2003, Johansson et al., 2005, Kolla et al., 2004, Lai et al., 1998, Lau et al., 2006, Saber and Hokkam, 2014). In contrast to people with acute cholecystitis, time (days) from the initial recognition of abnormal clinical signs to cholecystectomy was not associated with survival in dogs with GBM in this study when evaluated as a continuous or categorical variable. The problems associated with delayed surgical intervention (i.e. >72 h) in people with acute cholecystitis are speculated to arise from prolonged regional inflammation and life-threatening complications including gallbladder perforation, bile peritonitis, pericholecystic abscess, and the development of a biliary fistula (Kimura et al., 2007). While acute cholecystitis in people is not directly comparable to dogs with GBM, it is reasonable to suspect the sequela of both diseases could be similar. The reliance on dog owners' perception of when clinical signs started was subject to various uncontrolled variables related to the dog owner (e.g. medical background, perceptiveness, multiple owners with conflicting opinions, and accurate memory). However, in the absence of a canine GBM experimental model dependence on dog owners' historical perception of their dog's clinical signs while a limitation, is essential, and clinically applicable. As it is not possible to reliably determine whether cholecystitis is present alongside GBM then owners should be advised of a likely increased risk of adverse outcome were cholecystitis present and cholecystectomy not performed with urgency. The frequency of concurrent cholecystitis in dogs with GBM is reported to range from 9% to 68% (median, 25%; Pike et al., 2004, Malek et al., 2013, Wennogle et al., 2019, Rogers et al., 2019).

The main limitations of this study are associated with its retrospective nature. Outcome in the present study was defined as death-in-hospital, which might have underestimated the overall death rate attributed to dogs with GBM undergoing cholecystectomy. The inclusion criteria of gross and histopathologically confirmed GBM were intentionally strict to optimally investigate the primary aims. This strict approach likely omitted dogs that did not

have histopathology performed, or died or were euthanased intra- or post-operatively then had histopathologic evaluation withdrawn. Not every dog in this cohort was screened for an endocrinopathy or had the necessary biochemical parameters evaluated to diagnose hyperlipidemia. On that note, the decision to perform various types of diagnostics varied with clinician preference. The decision to evaluate cholesterol and/or triglycerides could have been biased towards grossly lipemic samples. Biochemical parameters were evaluated using different analyzers with various reference intervals. This was mitigated by evaluating cholesterol and triglyceride in relation to the upper limit of the laboratory reference interval and categorizing them as either hypercholesterolemia or hypertriglyceridemia. Moreover, total serum/plasma bilirubin concentration analyses were made based on the fold change with respect to the upper limit of each laboratory reference interval. Survival analyses in our study did not include previously identified prognostic variables in dogs with GBM including lymphocyte count (Crews et al., 2009), gallbladder rupture (Jaffey et al., 2018), postoperative serum lactate concentrations, and postoperative hypotension, which was a limitation (Malek et al., 2013). The inclusion of euthanized dogs in our survival analyses was a limitation. It is possible some of these dogs were euthanized for reasons unrelated to severity of disease (e.g. owner finances, owner objection to treatment, or clinician recommendation). Inclusion of dogs that were euthanized in the death-in-hospital group precludes our ability to truly determine if dogs with certain risk variables are more likely to die naturally. For example, there might be an underlying perception by attending clinicians that clinical signs coupled with hyperbilirubinemia in this population is associated with a poorer outcome. Relaying this perception to owners might have increased the likelihood of euthanasia, when a portion of these dogs could have survived. A prospective study that allows clinicians to provide a detailed description of the circumstances leading to euthanasia would be able to provide a better understanding of the link between some of these variables and survival. However, despite the inherent biases and data issues associated with a multi-center retrospective study, the large volume of cases, and diverse geographic source of care included make the conclusions robust.

Conclusions

The findings of the present study indicate that increased total serum/plasma bilirubin concentration is a negative prognostic indicator but is an unacceptably poor biomarker for predicting death-in-hospital in dogs with GBM at the time of cholecystectomy. The presence of clinical signs attributable to biliary tract disease at the time cholecystectomy yielded significantly greater odds of death-in-hospital than subclinical disease status in a univariable analysis. In the final multivariable model, owner recognition of jaundice was associated with increased odds of death-in-hospital. Pomeranian breed dogs were more likely to die in hospital following cholecystectomy than other breeds with GBM in our study. Concurrent hyperadrenocorticism was associated with nearly a 2-fold increase in odds for death in this study. The presence of comorbidities including hyperlipidemia, DM, hypothyroidism, and time (days) from initial recognition of clinical signs to cholecystectomy, were not associated with survival.

Conflict of interest statement

The authors have no financial or personal relationships that could inappropriately influence of bias the content of the paper.

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