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STANDARD ARTICLE

Ultrasonographic patterns, clinical findings, and prognostic variables in dogs from Asia with gallbladder mucocele

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Abstract

Background: Gallbladder mucocele (GBM) is a common biliary disorder in dogs that can be categorized into 6 types, but the value of this classification scheme remains unknown. Cholecystectomy is associated with high death rates and warrants additional interrogation.

Objectives: Investigate the clinical value of ultrasonographic diagnosis of type of GBM and identify prognostic factors in dogs with GBM undergoing cholecystectomy.

Animals: Two hundred sixteen dogs.

Methods: Retrospective cohort study. Dogs with GBM diagnosed from 2014 to 2019 at 6 veterinary referral hospitals in Asia. Ultrasonogram images were reviewed and a GBM type (ie, types I-VI) assigned.

Results: Dogs with GBM type V as compared to I (OR, 8.6; 95% CI, 2.6-27.8; $P < .001$) and III (OR, 10.0; 95% CI, 2.5-40.8; $P = .001$), and dogs with type VI compared to I (OR, 10.5; 95% CI, 1.8-61.2; $P = .009$) and III (OR, 12.3; 95% CI, 1.8-83.9; $P = .01$) were more likely to exhibit signs of biliary tract disease. Independent predictors of death after cholecystectomy included age (OR, 2.81; 95% CI, 1.41-5.59; $P = .003$) and intraoperative systolic blood pressure (SBP) nadir. There was an interaction between SBP nadir and gallbladder rupture; SBP nadir in dogs with (OR, 0.92; 95% CI, 0.89-0.94; $P < .001$) and without (OR, 0.88; 95% CI, 0.82-0.93; $P < .001$) gallbladder rupture.

Conclusion and Clinical Importance: Increasing developmental stage of GBM could be associated with an increased likelihood of biliary tract related clinical signs. Nadir SBP deserves further investigation as a prognostic or potentially modifiable variable, particularly in the presence of gallbladder rupture.

Abbreviations: AFAST, abdominal focused assessment with sonography for trauma; GBM, gallbladder mucocele; IQR, interquartile range; SBP, systolic blood pressure.

KEYWORDS
biliary, blood pressure, canine, cholecystectomy, hypotension, survival

1 | INTRODUCTION

Gallbladder mucocele (GBM) is a common extrahepatic biliary disease in dogs. It is characterized by gallbladder epithelial mucus hypersecretion and hypokinesia that can result in emergent clinical disease associated with extrahepatic bile duct obstruction, cholecystitis (sterile or infectious), biliary rupture, and systemic inflammatory response syndrome.[1-4] Surgical intervention to remove the gallbladder has an approximate in-hospital death rate of 20% (range, 7%-31%).[2,5-8] The specific disease etiology of GBM remains unknown; however, affected dogs have a high incidence of concurrent endocrinopathies and hyperlipidemia.[9,10] Further, the disease is over-represented in purebred dogs such as Shetland sheepdog, border terrier, miniature schnauzer, and beagles.[6,11] These associations and breed predispositions are suggestive of a multifactorial pathogenesis with both genetic and metabolic influences.

The ultrasonographic classification of GBM in dogs has evolved since it was first described more than 20 years ago,[12] with 6 types of GBM based on unique ultrasonographic patterns.[13] This classification scheme, combined with the widespread use of ultrasonography, allows for earlier recognition and intervention in dogs with GBM. In addition, the identification of these GBM types could have clinical value. There are associations between GBM type and the presence of gallbladder rupture and risk of death, respectively.[13,14] The aforementioned studies are the only reported literature that investigated the potential clinical value of GBM type in dogs.

Gallbladder rupture is a relatively common complication of GBM occurring in approximately 25% (range, 19%-38%) of dogs with signs of biliary tract disease.[2,5,7,8,15] There are conflicting reports regarding the clinical importance of gallbladder rupture in dogs with GBM. A recent large, retrospective study found that the presence of gallbladder rupture in dogs with GBM that had a cholecystectomy was associated with in-hospital death.[2] In contrast, 2 small retrospective studies did not find a difference in survival to hospital discharge when intraoperative rupture was identified.[15,16] Understanding this relationship is important because of the common occurrence of gallbladder rupture in dogs with clinical signs of GBM.

Intraoperative hemodynamics in humans is an important factor in the development of postoperative complications, including death, in noncardiac surgeries.[17] One study in dogs with GBM showed that the risk of death increased by 20 times with the presence of postoperative hypotension.[15] Another study found no association between the presence of intraoperative hypotension and survival to discharge in dogs with GBM.[18] However, the aforementioned study evaluated intraoperative hypotension as a categorical (ie, present/absent) variable. Currently, there are no studies that have evaluated intraoperative blood pressure as a continuous variable, which could provide greater actionable and prognostic value.

The present study had 2 objectives. The first was to determine if ultrasonographic GBM type was associated with several relevant clinical variables. Our second objective was to investigate the prognostic value of gallbladder rupture and intraoperative systolic blood pressure (SBP) nadir as well as several other clinically relevant variables in dogs with GBM undergoing cholecystectomy.

2 | MATERIALS AND METHODS

2.1 | Selection of cases

A retrospective, multi-center, cohort study was performed. Multiple institutions throughout Asia were contacted directly for participation in this study. Medical records of dogs that had a diagnosis of GBM made via ultrasonography, gross or histopathology, or both between January 2014 and January 2019 were eligible for inclusion. Medical records were searched at each institution for terms including “gallbladder mucocele/mucocoele,” “mucocele/mucocoele,” and “cholecystectomy.” Dogs without gross pathology or histopathologic confirmation of GBM were excluded if retrospective evaluation of available ultrasonogram images did not confirm the original diagnosis. Specific approval by an Ethical Review Committee was not required by any institution. Dog owners signed consent on institution admission documents that provided permission for use of data found in medical records. Cholecystectomies were performed by board-certified small animal surgeons or trained experts mandated by the region. Anesthesia was performed by trained individuals dictated by the standards of the region. Histopathologic interpretations were made by board-certified veterinary pathologists or similarly trained experts. Ultrasound examinations were performed by veterinary clinicians trained in ultrasonography.

Data retrieved from medical records included: breed; sex (including altered status); age; weight; reason for ultrasonogram; time (days) from the onset of clinical signs to surgery; description of clinical signs; concurrent surgical procedures; duration of surgery; presence of gallbladder rupture; culture positivity; intraoperative SBP nadir; presence of hyperadrenocorticism, hypothyroidism, and diabetes mellitus; blood biochemical data (total bilirubin, triglyceride, and cholesterol concentrations); survival to hospital discharge; and cause of death.

Gallbladder mucocele was diagnosed via ultrasonography, gross appearancehistopathology, or both. Gallbladder mucocele was diagnosed with ultrasonography if a gallbladder contained gravity-independent and immobile material that did not change with position of the dog. Available ultrasonogram static images, video clips, or both were retrospectively reviewed by a single board-certified veterinary radiologist (KS) and a GBM type (ie, types I-VI) designation was made based on previously established criteria (Figure 1).[7,13] The radiologist was blinded to corresponding clinical information at the time the images and video clips were reviewed. A GBM diagnosis could also be made based on the appearance of a distended gallbladder with accumulation of thick, inspissated, amorphous mucus in combination with cystic mucosal hyperplasia/hypertrophy histologically.[19] Gallbladder rupture was diagnosed intraoperatively if a perforation was identified or bile leakage was observed, perforation/rupture was identified histopathologically, or both. Intraoperative SBP measurements were obtained via Doppler or oscillometric techniques in accordance with previously established guidelines.[20] Hypotension was defined as an SBP of <90 mm Hg.[21] Dogs were considered to have hyperadrenocorticism, hypothyroidism, and diabetes mellitus based on published criteria.[6] Dogs were considered to be clinical or subclinical based on the presence or absence, respectively, of any signs of biliary tract disease within the 7 days preceding hospital admission: vomiting, diarrhea, lethargy, anorexia, hyporexia, abdominal pain or distension, jaundice, weakness, hypodipsia, adipsia, and fever. Outcome was defined as in-hospital death.

2.2 | Statistical analysis

Statistical analysis was performed using commercially available software (STATA 17, StataCorp LLC, College Station, TX). Data with a non-normal distribution were presented as median and interquartile range (IQR). Data with a normal distribution were presented as mean and SD. Categorical data were reported as proportions. Associations of GBM type to clinical status, fold change in total bilirubin, hyperadrenocorticism, hypothyroidism, gallbladder rupture, age, and weight were evaluated using Fisher's exact tests for categorical variables, ANOVA for normal continuous variables, and Kruskal-Wallis for non-normal continuous variables. Only dogs with clinical signs associated with GBM were included in statistical tests related to clinical status. All analyses of blood total bilirubin concentration were made based on the fold change with respect to the upper limit of the corresponding reference interval (ie, blood total bilirubin concentration/upper limit of the reference interval). A significant test of variance was followed by a Dunn's test with Bonferroni correction for multiple comparisons. Logistic regression clustered on institution was performed to determine predictors of death in-hospital after cholecystectomy. Univariable exact logistic regression was performed for variables selected based on clinical plausibility (Table 1), and multivariable models were created using variables with a *P*-value of ≤0.2 in univariable exact logistic regression using a combination of backwards selection and theory based on prior literature. Competing models were evaluated using likelihood ratio, Akaike Information Criterion, Bayesian Information Criterion tests, and the best model validated using link test and Pearson goodness-of-fit tests. A *T*-test was used to compare the SBP nadir between dogs that died and those that survived for dogs with and without gallbladder rupture. The level of significance was set at $P < .05$.

3 | RESULTS

3.1 | Cohort group

Two hundred and sixteen dogs from 3 academic veterinary hospitals and 3 private practice specialty hospitals from Japan, South Korea, Taiwan, and Hong Kong were included in this study (Figure 2). No dogs were excluded.

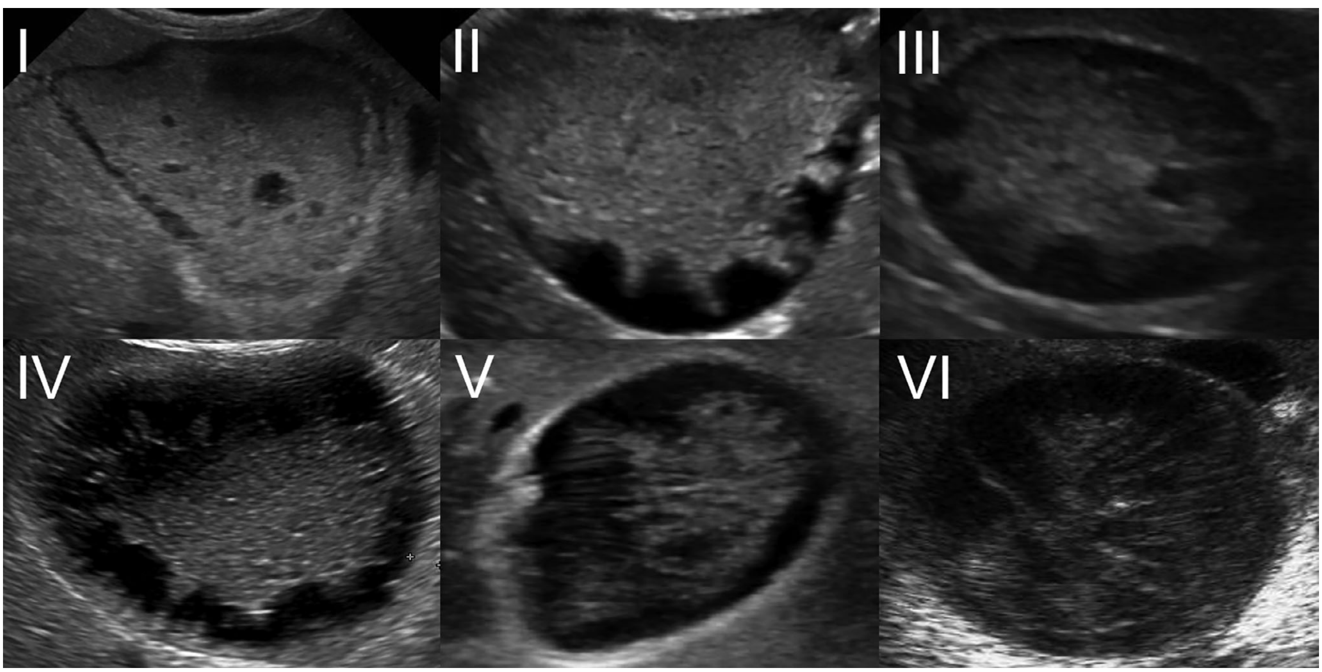

FIGURE 1 Representative ultrasonogram images of 6 gallbladder mucocele types: (I) organized echogenic debris occupying >30% of lumen, (II) combination of organized echogenic debris with partial stellate strands adhered to the gallbladder wall, (III) stellate pattern, (IV) combination of stellate and kiwi pattern, (V) kiwi pattern with echogenic debris, and (VI) kiwi pattern

TABLE 1 Results of univariable analyses (exact logistic regression) and final multivariable model for association with death (9/85) in dogs with gallbladder mucocele undergoing cholecystectomy

Variable category	Yes	No	Died	Univariable OR (95% CI)	Univariable *P*-value	Multivariable OR (95% CI)	Multivariable *P*-value[a]
Signalment							
Sex female	48/85 (56%)	37/85 (44%)	5/48 (10%)	1.11 (0.21-6.42)	1.00		
Neutered	74/85 (87%)	11/85 (13%)	7/74 (9%)	0.41 (0.04-5.09)	.60		
Weight (kg)	Continuous (range, 1.5-25)			1.00 (0.79-1.23)	.90		
Age (years)	Continuous (range, 5-17)			1.41 (1.02-2.06)	.03[b]	2.81 (1.41-5.59)	.003
Breed (6 most frequent)							
Pomeranian	14/85 (16%)	71/85 (84%)	3/14 (21%)	1.92 (0.23-13.84)	.71		
Toy poodle	10/85 (12%)	75/85 (88%)	2/10 (20%)	1.78 (0.14-13.52)	.84		
Miniature schnauzer	7/85 (8%)	78/85 (92%)	0/7	0.90 (0.00-7.97)	.94		
Chihuahua	6/85 (7%)	79/85 (93%)	0/6	2.11 (0.00-21.74)	1.00		
Maltese	6/85 (7%)	79/85 (93%)	1/6 (17%)	1.15 (0.02-22.72)	1.00		
Shetland sheepdog	5/85 (6%)	80/85 (94%)	1/5 (20%)	3.29 (0.05-71.99)	1.00		
Clinical signs							
Time clinical (days)	Continuous (range, 1-42)			0.96 (0.80-1.11)	.76		
Any sign	71/85 (84%)	14/85 (16%)	8/71 (11%)	1.85 (0.18-96.45)	1.00		
Jaundice	5/85 (6%)	80/85 (94%)	0/5	5.00 (0-195)	1.00		
Hyporexia	6/85 (7%)	79/85 (93%)	0/6	1.00 (0-39)	1.00		
Painful abdomen	6/85 (7%)	79/85 (93%)	2/6 (33%)	9.98 (0.58–190.66)	.12[b]		
Anorexia	43/85 (51%)	42/85 (49%)	6/43 (14%)	3.03 (0.48-23.41)	.31		
Diarrhea	4/85 (5%)	81/85 (95%)	1/4 (25%)	3.27 (0.04-82.45)	.81		
Vomiting	55/85 (65%)	30/85 (35%)	7/55 (13%)	2.63 (0.42-29.85)	.44		
Lethargy	27/85 (32%)	58/85 (68%)	2/27 (7%)	0.56 (0.05-3.57)	.79		
Clinicopathological findings/comorbidity							
Fold change in total serum/plasma bilirubin	Continuous (range, 0-25.4)			1.06 (0.94-1.19)	.26		
Hyperbilirubinemia	42/84 (50%)	42/84 (50%)	6/42 (14%)	2.14 (0.33-18.11)	.60		
Hypertriglyceridemia	27/46 (59%)	19/46 (41%)	3/27 (11%)	1.94 (0.13-112.37)	1.00		
Hypercholesterolemia	37/71 (52%)	34/71 (48%)	4/37 (11%)	1.22 (0.16-10.61)	1.00		
Diabetes mellitus	2/85 (2%)	83/85 (98%)	1/2 (50%)	8.37 (0.08-841.74)	.49		
Hypothyroidism	2/85 (2%)	83/85 (98%)	0/2	1.84 (0-23.79)	1.00		
Hyperadrenocorticism	2/85 (2%)	83/85 (98%)	1/2 (50%)	8.87 (0.09-861.56)	.45		

TABLE 1 (Continued)

Variable category	Yes	No	Died	Univariable OR (95% CI)	Univariable P-value	Multivariable OR (95% CI)	Multivariable P-value[a]
Surgical							
Systolic blood pressure nadir (mm Hg)	Continuous (range, 41-103)			0.89 (0.81–0.97)	.002[b]	Rupture absent: 0.88 (0.82-0.93); rupture present: 0.92 (0.89-0.94)	<.001
Duration of surgery (minutes)	Continuous (range, 43-240)			1.00 (0.99-1.02)	.68		
Hypotension	67/78 (86%)	11/78 (14%)	7/67 (10%)	2.84 (0.14-230.52)	.88		
Gallbladder rupture	22/85 (26%)	63/85 (74%)	5/22 (23%)	4.02 (0.65–30.24)	.16[b]		
Concurrent surgical procedure	70/85 (82%)	15/85 (18%)	4/70 (6%)	0.15 (0.02-0.99)	.05[b]		
Positive culture	8/58 (14%)	50/58 (86%)	0/8	13.5 (0.0-526.5)	1.00		

Note: Variables associated with death ($P \leq .20$) in univariable analyses were included in initial multivariable regression analysis. Variables significantly associated with death ($P < .05$) were retained in the final multivariable regression analysis. For all binary variables, the referent is the absence of the condition (ie, no). Mean unbiased estimates provided for variables where no deaths occurred.

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] $P < .05$ in final multivariable regression analysis.

[b] $P \leq .20$ in univariable analysis and included in initial multivariable regression analysis.

There were 197 purebred dogs and 19 mixed breed dogs included (Table S1). The most common purebred breeds included (ie, > 10 dogs) were Chihuahua (23/216, 10.6%), Maltese (20, 9.3%), toy poodle (20, 9.3%), Pomeranian (19, 8.8%), miniature schnauzer (12, 5.6%), and Shetland sheepdog (12, 5.6%). The median age and weight were 11.0 years (IQR, 9.0-13.0) and 5.1 kg (IQR, 3.4-8.5), respectively. The age of 1 dog was not recorded. There were 78 male-castrated, 34 male-entire, 88 female-spayed, and 16 female-entire dogs.

Most dogs with GBM were diagnosed via ultrasonogram alone 63.4% (137/216) or in conjunction with gross pathology/histopathology 35.6% (77/216; Figure 2). Two dogs had abdominal focused assessment with sonography for trauma (AFAST) examinations performed without complete abdominal ultrasound examinations before cholecystectomy. All dogs with gross pathology/histopathology after cholecystectomy confirmed the pre-surgical GBM diagnosis made via ultrasonogram. Eighty-six percent (185/214) of dogs that had an ultrasonogram performed had sufficient static images or video clips available to designate a GBM type (ie, I-VI). Of the 185 dogs with sufficient images available for review, 43.2% (80/185) had video clips and 56.8% (105/185) had static images alone. Ultrasonogram images were not available from the 2 dogs that had AFASTs performed. The remaining 29 dogs that had official abdominal ultrasound examinations performed did not have images or video clips available for review or were insufficient to definitively designate a GBM type. Twenty-nine percent (9/31) of those dogs had a cholecystectomy performed and subsequent gross pathology/histopathologic confirmation of GBM, including the 2 dogs that had AFAST examinations. Static ultrasonogram images or video clips were available from the remaining 22 dogs for retrospective review confirming a GBM diagnosis; however, a clear GBM type designation could not be made.

The most common GBM types identified in this cohort were type II (55/185, 30%), type IV (44, 23.7%), and type I (32, 17.3%; Figure 2). Fifty-five percent (118/216) of dogs had clinical signs. Eighty-four percent (99/118) of those dogs had ≥1 biliary tract clinical sign directly related to GBM, and those most commonly reported included vomiting 72% (71/99), anorexia 60% (59/99), and lethargy 33% (33/99). Information related to the duration of clinical signs before hospital-admission was available in 97% (96/99) of dogs. Clinical signs were present for a median of 5.0 days (IQR; 3.0-10.0) before evaluation. There were 16.1% (19/118) of dogs with clinical signs that were not directly related to GBM including unclear etiology (3), immune mediated hemolytic anemia (2), severe acute pancreatitis (2), and 1 each of splenic mass/hemoabdomen, chronic pancreatitis, diabetes mellitus, acute gastroenteritis, focal hepatic mass, inflammatory bowel disease, intrathoracic mass, multicentric lymphoma, cutaneous lymphoma, multifocal liver masses, primary neurologic disease, and pneumonia.

A total of 65.7% (142/216) and 43.9% (95/216) of dogs had cholesterol or triglycerides measured, respectively. All dogs with triglyceride measurements also had concurrently measured cholesterol. Hypercholesterolemia was identified in 42.2% (60/142) and hypertriglyceridemia was found in 43% (41/95) of dogs. Fourteen percent (13/95) of dogs had both hypercholesterolemia and hypertriglyceridemia. Total blood bilirubin concentration was measured in 78.7% (170/216) of dogs, of which the median was 0.5 times the upper limit of the reference interval (IQR,

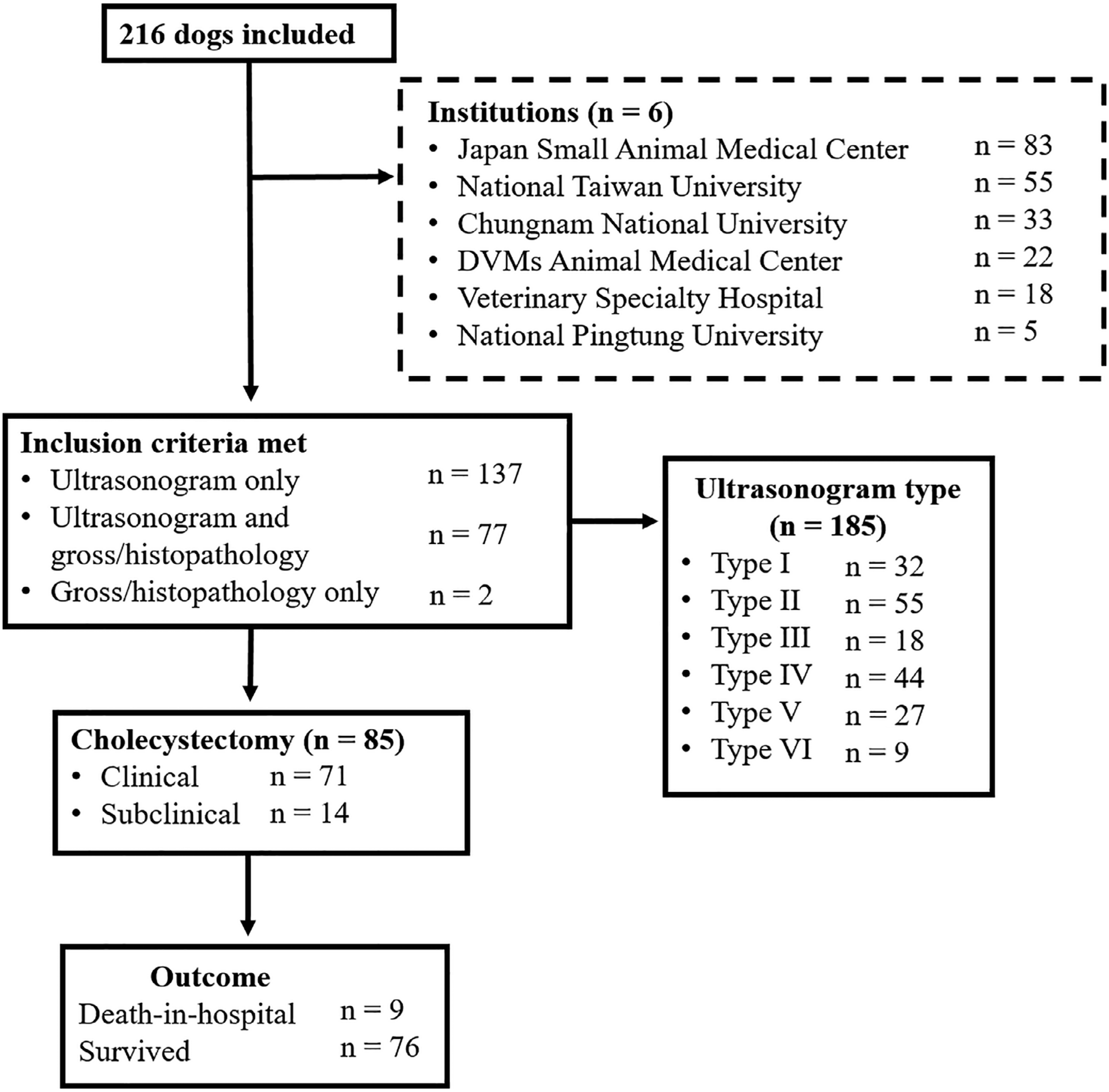

FIGURE 2 Flow diagram. A total of 216 dogs from 6 veterinary referral hospitals in Asia with gallbladder mucoceles were included in the study

0.20-2.02). Ninety percent (89/99) of dogs clinical for GBM had total blood bilirubin measured and the median in those dogs was 1.2 times the upper limit of the reference interval (IQR, 0.37-5.2).

Endocrinopathies including hyperadrenocorticism, hypothyroidism, and diabetes mellitus were relatively uncommon (13%; 28/216) to have been present at the time of hospital admission. Fourteen percent (4/28) of these dogs had 2 concurrent endocrinopathies including 2 each of hyperadrenocorticism with hypothyroidism and hyperadrenocorticism with diabetes mellitus. Hyperadrenocorticism was present in 6.4% (14/216) of dogs, of which 79% (11/14) were being treated with trilostane at the time of hospital-admission. Hypothyroidism was present in 5.1% (11/216) of dogs; of which 64% (7/11) were being treated at the time of admission. Diabetes mellitus was present in only 3.2% (7/216) of dogs.

Cholecystectomy was performed in 39.4% (85/216) of dogs with 84% (71/85) and 16% (14/85) of them being clinical or subclinical, respectively. Eighty-nine percent (76/85) of dogs that had a cholecystectomy performed had a pre-operative GBM type designation. The distribution of GBM types among those dogs were type II (30%; 23/76), type IV (27%; 21/76), type V (20%; 15/76), type III (9%; 7/76), type I (7%; 5/76), and type VI (7%; 5/76). Ninety-three percent (79/85) of dogs that underwent a cholecystectomy had gallbladders submitted for gross pathology/histopathologic examination. Preoperative GBM types of the 6 dogs without gross pathology/histopathologic submission included type II (3), and 1 each of type III, type IV, and type VI. All dogs with gross pathology/histopathologic examination after cholecystectomy confirmed the pre-surgical GBM diagnosis made via ultrasonogram.

Cholecystectomy was not performed in any of the 19 dogs that had clinical signs unrelated to GBM. Twenty-eight percent (28/99) of dogs that had signs directly attributable to GBM did not have a cholecystectomy. Thirty-two percent (9/28) of these dogs did not survive to hospital discharge [died (7), euthanized (2)]. The remaining 68% (19/28) of these clinical dogs survived to hospital discharge. The owners of 9 dogs declined surgery and these dogs were lost to follow-up. Two dogs were discharged and had a cholecystectomy performed at a different veterinary hospital, and 1 dog was discharged but euthanized the next day. Cholecystectomy was aborted in 1 dog after cardiac arrest and successful intraoperative resuscitation. Follow-up information was available from 6 dogs that were treated medically because owners declined surgery. The median follow-up time was 212.5 days (IQR, 17.5-365; range, 7-365 days). One dog died from acute pancreatitis 365 days after hospital discharge and the remaining 5 dogs were alive at last known follow-up.

The indication for surgery in the 14 subclinical dogs included prophylactic surgery after incidental ultrasonographic GBM identification in 71% (10/14), failure of improvement in liver enzyme activities in conjunction with a lack of ultrasonographic resolution of GBM after medical management in 14% (2/14), and 1 each of either worsened ultrasonographic appearance of GBM or failure to resolve after medical management. Eighty-two percent (70/85) of dogs had ≥1 surgical procedure in addition to cholecystectomy performed. Liver biopsies were the most common additional surgical procedure alone in 61% (43/70) or in combination with ≥1 procedure 23% (16/70) of dogs.

Anesthesia medical records were available to determine intraoperative SBP nadir and surgery time in 92% (78/85) and 89% (76/85) of dogs, respectively. Eighty-six percent (67/78) of dogs had intraoperative hypotension recorded and the median SPB nadir was 76.0 mm Hg (IQR, 68-84.5). The median duration of surgery time was 76.5 minutes (IQR, 55.3-125.8). Gallbladder wall, luminal contents, or both were submitted for bacterial culture in 68% (58/85) dogs and only 14% (8/58) were positive for growth. One organism was cultured in each of the 8 dogs including *Escherichia coli* (2), *Enterococcus faecium*, *Lactobacillus fermentum*, *Staphylococcus pseudointermedius*, *Stenotrophomonas maltophilia*, *Salmonella* sp, and *Streptococcus* sp. Gallbladder rupture was diagnosed in 26% (22/85) of dogs.

Death-in-hospital occurred in 11% (9/85) of dogs that underwent cholecystectomy for GBM, with a 95% CI of 5-19. Eighty-nine percent (8/9) of those dogs died, while only 1 dog was euthanized. The aforementioned dog was euthanized because of the development of severe postoperative seizures. Death-in-hospital occurred in 11% (8/71; 95% CI, 5-21) of dogs that had signs related to biliary tract disease at the time of cholecystectomy, compared to 7% (1/14; 95% CI, 0-34) of subclinical dogs.

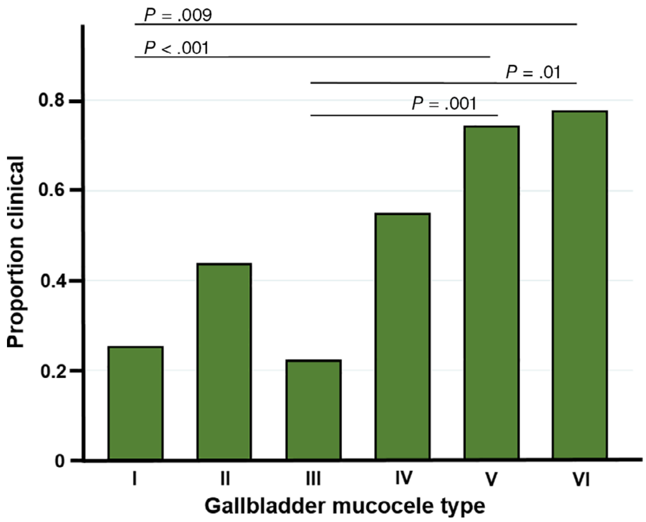

FIGURE 3 Box plot illustrating associations between ultrasonographic gallbladder mucocele type and clinical status

3.2 | Parameter association with ultrasonographic gallbladder mucocele type

Fisher's exact test was used to evaluate the association of clinical status ($P < .001$), hyperadrenocorticism ($P = .32$), hypothyroidism ($P = .90$), and gallbladder rupture ($P = .21$) with GBM type. Dogs with GBM type V as compared to I (OR 8.6; 95% CI 2.6-27.8; $P < .001$) and III (OR 10.0; 95% CI 2.5-40.8; $P = .001$), and dogs with type VI compared to I (OR 10.5; 95% CI 1.8-61.2; $P = .009$) and III (OR 12.3; 95% CI 1.8-83.9; $P = .01$) were more likely to exhibit signs of biliary tract disease (Figure 3). There was no significant difference in age ($P = .52$), weight ($P = .62$), or fold change in total bilirubin ($P = .14$) between GBM types (Figure S1).

3.3 | Risk factors for death in dogs undergoing cholecystectomy

Variables that were significant in univariable screening ($P \leq .20$; Table 1) for increased odds of in-hospital death for the 9 dogs that died of the 85 dogs that underwent cholecystectomy included age (OR, 1.41; 95% CI, 1.02-2.06; $P = .03$), painful abdomen (OR, 9.98; 95% CI, 0.58-190.66; $P = .12$), intraoperative SBP nadir (OR, 0.89; 95% CI, 0.81-0.97; $P = .002$), gallbladder rupture (OR, 4.02; 95% CI, 0.65-30.24; $P = .16$), and concurrent surgical procedure (OR, 0.15; 95% CI, 0.02-0.99; $P = .05$).

Variables selected from screening that remained statistically significant ($P < .05$; Table 1) in the final multivariable logistic regression model clustered on institution included age (OR, 2.81; $P = .003$) and intraoperative SBP nadir. The odds of death associated with intraoperative SBP nadir were different for dogs with (OR, 0.92; $P < .001$) and without (OR, 0.88; $P < .001$) gallbladder rupture. Intraoperative SBP nadir was lower for dogs with gallbladder rupture that died (n = 5; mean, 58.0 mm Hg; SD, 12.9) compared to dogs with

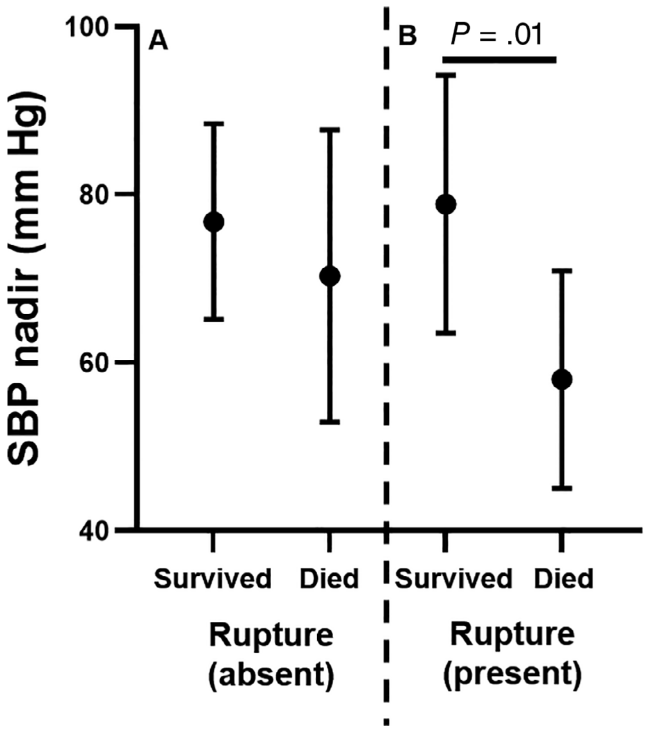

FIGURE 4 Comparison of intraoperative systolic blood pressure (SBP) nadir in dogs with gallbladder mucocele that underwent cholecystectomy without gallbladder rupture that survived (n = 53) and died (n = 3; A) and in dogs with gallbladder rupture that survived (n = 17) and died (n = 5; B). Enclosed circles represent the mean. Error bars represent SD above and below the mean

gallbladder rupture that survived (n = 17; mean, 78.9 mm Hg; SD, 15.3; *P* = .01; Figure 4), but was not different for dogs without gallbladder rupture that died (n = 3; mean, 70.3 mm Hg; SD, 17.4) compared to those that survived (n = 53; mean, 76.8 mm Hg; SD, 11.6; *P* = .36; Figure 4). The change in OR for age in multivariable regression as compared with univariable was associated with both gallbladder rupture and intraoperative SBP nadir. An OR of 2.3 was found for age in the full model (all variables with a *P*-value ≤.20 in univariable regression), with the OR increasing slightly to 2.8 as non-significant variables were removed during the process of backwards stepwise regression. Further investigation of the effect of variables from the full model paired with age on the OR for age showed that intraoperative SBP nadir, gallbladder rupture, and duration of surgery increased the OR for age, with the OR of 1.91 ($P < .001$), 1.44 ($P = .12$), and 1.91 ($P < .001$), respectively. The remaining variables had no effect on the OR for age.

4 | DISCUSSION

This multi-center study is the largest and most comprehensive investigation of dogs from Asia with GBM. One of the objectives of this study was to determine if unique ultrasonographic gallbladder patterns in dogs with GBM were associated with several relevant clinical variables. Collectively, dogs with more advanced developmental stage of GBM (ie, higher ultrasonographic GBM type) were more likely to have clinical signs of GBM. This study also investigated the prognostic value of several variables in dogs with GBM that received a cholecystectomy in a multivariable survival model. The results from this model indicate that the continuous variables age and intraoperative SBP nadir (especially dogs with gallbladder rupture) were independently associated with in-hospital death.

Ultrasonographic biliary patterns were associated with clinical status in dogs with GBM in this study. Dogs with either GBM type V or type VI were more likely to be clinical than dogs with types I or III. Collectively, these results suggest that dogs with a more advanced developmental stage of GBM could be at a higher risk to develop signs of biliary tract disease. This finding is important because dogs with GBM that have clinical signs typically require emergent surgical intervention and 2 retrospective studies have shown an association between clinical status and in-hospital death.[6,18] Two previous studies found no association between GBM type and clinical status.[7,13] One potential explanation for the lack of association in the previously mentioned studies is that statistical comparisons were not sufficiently powered (ie, type II error). Each of the aforementioned studies only included 43 and 62 dogs, respectively, which were then allocated into even smaller groups based on GBM type and clinical status.[7,13] Another possible reason is the distribution of GBM types in those studies. There were no dogs in either study classified as GBM type VI. Moreover, the majority of each cohort consisted predominately of dogs with less advanced GBM (ie, types I-III; 65%-69%).[7,13] A specific explanation for the link between ultrasonographic biliary pattern and clinical status was not established in this study. The amount and organization/consolidation of intraluminal inspissated material generally increases with developmental stage of GBM. Therefore, it is possible higher GBM types are more likely to lead to gallbladder distension, which impairs mural circulation and predisposes to cholecystitis as well as wall necrosis and perforation. Importantly, these results cannot be extrapolated for use in clinical situations until future large studies investigating the clinical value of ultrasonographic biliary pattern in dogs with GBM are performed.

Intraoperative SBP nadir was an independent predictor of in-hospital death in dogs with GBM undergoing cholecystectomy. These results are in contrast to 2 recent retrospective studies that found no association between intraoperative hypotension and death in dogs undergoing cholecystectomy.[18,21] The dissimilar statistical interrogation of intraoperative blood pressure could explain the conflicting results. The aforementioned studies evaluated intraoperative blood pressure categorically (eg, hypotension [present/absent] and hypotension lasting >10 minutes [present/absent]). Evaluating predictors as categorical is less powerful than evaluating them as continuous variables. This supposition is supported by the lack of significant association between the presence of hypotension as a categorical variable and outcome in the current study. Our results are similar to studies in humans that demonstrate hypotension during noncardiac surgeries is a strong determinant of death.[17,22]

The association between intraoperative SBP nadir and in-hospital death identified in this study is important for 2 reasons. Firstly, clinically relevant hypotension is common in dogs undergoing cholecystectomy. Eighty-six percent (67/78) of dogs in this study developed intraoperative hypotension, which is comparable to previous studies (55%-74%) that evaluated intraoperative blood pressure in dogs undergoing cholecystectomy.[18,21,23] This is much higher than the 7% to 10% incidence reported in general small animal anesthesia.[24,25] Reasons a dog might develop hypotension during anesthesia are often complex and multifactorial, but the high incidence during cholecystectomy indicates that biliary disease itself is contributory. Experimental models of obstructive jaundice in dogs suggest that bilirubinemia and cholemia (increased blood bile acids) decrease total peripheral resistance by reducing responsiveness and functional activity of vascular α1-receptors and by decreasing ventricular contractility.[26-28] Moreover, the volume of blood loss needed to induce substantial hypotension in dogs with obstructive jaundice is half that of controls.[29] This suggests that hemodynamic derangements could be exaggerated intraoperatively in dogs with biliary disease given blood loss as well as exposure to inhaled and intravenous anesthetics (eg, sevoflurane, isoflurane, propofol). The second reason the link between blood pressure and outcome found in the current study is important is that, unlike most baseline characteristics that are fixed, intraoperative blood pressure can usually be controlled; making it an important therapeutic target that could improve survival.

Increasing age was associated with increased odds of in-hospital death in this study with each year increase in age found to increase the odds of death by 43% when age was considered alone. These results are consistent with previous studies investigating risk factors for GBM and other diseases in dogs.[6,30,31] Most survival studies in the veterinary literature are susceptible to the confounding variables that influence euthanasia; however, only 1 dog in this study was euthanized and the remaining dogs died in-hospital from various complications. It is possible dogs with advanced age are more likely to accumulate comorbidities that increase the risk for major complications. The odds of death associated with age increased when the variables of gallbladder rupture, intraoperative SBP nadir, and duration of surgery were considered, particularly with the interaction between gallbladder rupture and SBP nadir that was included in the final multivariable model. However, this study did not include potentially confounding age-related comorbidities into the survival model such as myxomatous mitral valve disease, systemic hypertension, chronic kidney disease, proteinuria, airway or pulmonary parenchymal disease.[32-36] 36 Therefore, until additional studies are performed, clinicians must avoid the utilization of advanced age alone as a negative prognostic indicator in dogs with GBM.

Gallbladder rupture was identified in 26% (22/85) of dogs undergoing cholecystectomy in this study, similar to several recent studies (19%-38%).[2,5,7,8] Identification of gallbladder rupture was significantly associated with in-hospital death on univariable screening but lost significance when adjusted for in the multivariable model in this study. However, the presence of gallbladder rupture did have a significant effect on survival through its interaction with intraoperative SBP nadir. While intraoperative SBP nadir was significant for all dogs, it was especially important in dogs with gallbladder rupture. The specific reason for the interaction between intraoperative SBP nadir and gallbladder rupture on outcome is unclear and given the relatively small number of deaths must be interpreted with caution, but the various sequela of bile peritonitis is surmised to be contributory. Bile components are toxic and incite necrosis and increased vascular permeability resulting in transudation of fluid and translocation of bacteria into the peritoneum.[37] In addition, the innate immune response is compromised in dogs with bile peritonitis.[38] Taken together, these sequelae likely increase the risk for various complications that can affect blood pressure including systemic inflammatory response syndrome, septic shock, and multiple organ dysfunction. While this model cannot distinguish between causal versus predictive variables, our results in combination with the findings of prior literature suggest that vigilant intraoperative blood pressure monitoring is needed in dogs with GBM and gallbladder rupture undergoing cholecystectomy. Future studies are needed to determine if specific intraoperative blood pressure thresholds could be targeted to improve survival.

Studies have yielded conflicting results concerning the prognostic value of gallbladder rupture as an independent variable in dogs with GBM receiving cholecystectomy, with some reports suggesting no association with survival and a large study reporting a nearly 3-fold increase in the likelihood of in-hospital death.[2,15,16] A recent study even reported a survival benefit for preoperative suspicion for gallbladder rupture on ultrasound examination.[14] Many dogs in that study did not end up having rupture identified intraoperatively, but the authors surmised the preoperative suspicion for rupture was the nexus for aggressive case management yielding a protective effect. The sequela of bile peritonitis likely increases the risk for death in dogs, as it does in humans.[39] Therefore, importantly, the lack of association between gallbladder rupture as an independent variable and outcome in our study should not dissuade clinicians from promptly intervening with surgery in dogs with GBM and suspected or confirmed rupture.

This study had several limitations that were largely associated with its retrospective design. Outcome for dogs undergoing cholecystectomy in the current study was defined as in-hospital death, which could have underestimated the overall rate of death. Several dogs were discharged from the hospital alive without undergoing cholecystectomy as recommended by the attending clinician and later died or were lost to follow-up. Case-selection bias could have also led to an underestimation of the death rate because our study focused specifically on dogs with GBM that underwent cholecystectomy; therefore, potentially severely affected dogs that died or were euthanized without surgery were not included. The use of static ultrasonographic images could have led to the misidentification of GBM type in some dogs. Moreover, the quantity and location within the gallbladder that static images were procured was not controlled and could have influenced ultrasonographic typing. In addition, retrospective review of static images did not allow for confirmation that intraluminal echogenic material was immobile. Therefore, it is possible that some of the dogs, primarily those with a less advanced GBM type (ie, type I), did not have a GBM. However, all official abdominal ultrasound examinations were performed by clinicians trained in ultrasonography as dictated by the geographic region. It is standard ultrasound technique to confirm immobility of echogenic material with varied positions at each of the institutions included in this study. Ultrasonogram video recordings allow for a more global and accurate assessment of the gallbladder but were not available for all dogs. There were several factors related to intraoperative blood pressure assessment that could have been influential that warrant elucidation. Confounding variables not accounted for included relevant preexisting comorbid diseases, blood volume status, quantification of blood loss, duration of hypotension, and types of interventions to correct hypotension. A future study accounting for these potentially influential variables are needed. Anesthesia was performed by trained experts, which is standard for the region because a board-certified system for veterinary anesthesia has not yet been established in Asia. The method of blood pressure assessment (eg, Doppler or oscillometric) was not standardized in this study, as it was retrospective, which could have introduced variation. The robust size of this study including various institutions in Asia provided a comprehensive regional assessment of dogs with GBM, but it is unknown if these results apply to dogs in other geographic regions. The findings from the survival model should be interpreted cautiously, given the relatively small number of deaths and the number of independent variables in the model. When the number of events per variable is low, type II errors are more likely than type I, although there is decreased concern for plausible and highly significant associations.[40] Exact logistic regression was used in univariable analysis to help compensate for the low number of events, but it was not possible to do this for the multivariable model, as it was not possible to evaluate the interaction term suggested by model validation using exact logistic regression. This may be a reason that some variables previously associated with death in dogs with GBM were not significant here.

5 | CONCLUSION

The findings of the current study indicate that increasing developmental stage of GBM determined by ultrasonography could be associated with an increased likelihood of signs of biliary tract disease. Results from our multivariable survival model indicate that increasing age and decreasing intraoperative SBP nadir (especially in dogs with gallbladder rupture) as continuous variables were independent predictors of in-hospital death in dogs with GBM undergoing cholecystectomy.

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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

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

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

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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