Received: 18 November 2020 | Accepted: 15 June 2021

DOI: 10.1111/jvim.16203

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

Evaluation of coagulation parameters in dogs with gallbladder mucoceles

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Funding information
Companion Animal Health Fund at Cummings School of Veterinary Medicine at Tufts University

Abstract

Background: Gallbladder mucocele (GBM) is a common biliary disorder in dogs. Limited information is available on the coagulation status of dogs with GBM.

Hypothesis/Objectives: To determine patterns of coagulation alterations in dogs with GBM and correlate them with clinicopathologic abnormalities and ultrasonographic findings of disease severity.

Animals: Twenty-three dogs with GBM identified on ultrasound examination were prospectively enrolled.

Methods: At the time of GBM identification, blood and urine were collected for CBC, serum biochemical panel, urinalysis, prothrombin time, activated partial thromboplastin time (aPTT), factor VIII, protein C (PC), von Willebrand's factor (vWF), antithrombin activity, fibrinogen, D-dimers, and thromboelastrography (TEG). Gallbladder mucoceles were classified into ultrasound types 1 to 5. Medical records were reviewed for clinical presentation, underlying conditions and to determine if systemic inflammatory response syndrome (SIRS) was present.

Results: Based on TEG parameters, maximal amplitude, and *G*, 19/23 (83%) of dogs with GBM had evaluations consistent with hypercoagulability. On plasma-based coagulation testing, dogs with GBM had increased total PC activity (20/23, 87%), fibrinogen (9/23, 39%), platelet count (9/23, 39%), and D-dimers (6/15, 40%) as well as prolongations in aPTT (9/22, 41%) and low vWF activity (5/21, 24%). No correlation was found between TEG *G* value and any coagulation or clinical pathology variables, ultrasound stage of GBM or disease severity as assessed by the presence of SIRS.

Conclusions and Clinical Importance: Dogs with ultrasonographically identified GBM have changes in whole blood kaolin-activated TEG supporting a hypercoagulable state although traditional plasma-based coagulation testing suggests that a complex state of hemostasis exists.

Abbreviations: aPTT, activated partial thromboplastin time; AT, antithrombin; GBM, gallbladder mucocele; MA, maximum amplitude; PC, protein C; PT, prothrombin time; PTE, pulmonary thromboembolism; SIRS, systemic inflammatory response syndrome; TEG, thromboelastography; vWF, von Willebrand's factor.

KEYWORDS
biliary, hepatic, hypercoagulable, liver, thromboelastography, thrombosis

1 | INTRODUCTION

Gallbladder mucoceles (GBMs) are an important cause of biliary disease in dogs.[1-16] Cholecystectomy is recommended for dogs with GBM that are clinically or biochemically affected or in dogs that have static GBM for 4 to 6 months despite medical management.[3,4,12,14,15] Surgery can be associated with short-term perioperative morbidity and mortality that ranges from 0% to 40%.[1-4,8,10,11-15,16] Overt evidence of macrothrombosis, such as pulmonary thromboembolism (PTE), splenic vein thrombosis, or brain or gallbladder wall infarction, has been reported in dogs with GBM.[3,14-16] In addition, disorders associated with microthrombosis, such as pancreatitis, acute renal failure, peritonitis, and disseminated intravascular coagulation, may occur.[7,9,10,14,16]

Over the last decade, the complex alterations in coagulation that occur in human patients with hepatobiliary disease have been recognized.[17-19] These alterations involve changes in pro- and anticoagulants, as well as changes in factors involved in fibrinolysis. The net result is that human patients with liver disease have laboratory and clinical features of both hypo- and hypercoagulability. Prolongations in prothrombin time (PT) and activated partial thromboplastin time (aPTT) and bleeding from provocative procedures suggest hypocoagulable tendencies. Increases in clot strength, D-dimers and von Willebrand's factor (vWF) and factor VIII activity, decreases in antithrombin (AT) and protein C (PC) activity, and an increased incidence of thrombosis in the splanchnic circulation suggest hypercoagulable tendencies. The presence of commensurate changes in both pro- and antihemostatic pathways has led to the concept of rebalanced hemostasis in human patients with liver disease. In patients with rebalanced hemostasis, coagulation is more easily disrupted than in normal individuals. Whether such patients have clinical bleeding or thrombosis depends on factors that shift the coagulation state one way or the other. Hemorrhage can be precipitated by infection, uremia, hypervolemia, or acidosis, whereas thrombosis occurs with aggravated portal hypertension, corticosteroid use, proinflammatory states or with other concurrent pro-thrombotic conditions. Emerging evidence suggests that such a rebalanced state of coagulation also occurs in dogs with liver disease.[20-22] This conclusion is supported by reports of bleeding and thrombotic tendencies in dogs with liver disease and evidence that their coagulation profiles involve changes in pro- and anticoagulants and factors controlling fibrinolysis.[1,17-19]

Despite many reported cases of GBM in dogs, little is known about their coagulation status.[1-16] Studies either have not reported on coagulation parameters or provided only limited information, including PCV, platelet count, PT, aPTT, or some combination of these.[4,7,11,16] One study in dogs with extrahepatic bile duct obstruction that included 2 dogs with GBM[1] determined that all dogs in the study were hypercoagulable based on thromboelastographic (TEG) parameters and many had increases in D-dimers (80%) and fibrinogen (70%). Another study that evaluated dogs undergoing biliary surgery and included dogs with GBM found that nonsurvivors had significantly prolonged aPTT.[7] In Shetland sheep dogs with different types of gallbladder pathology, increases in fibrinogen (50%) and D-dimers (40%) were reported.[16] These studies suggest that dogs with GBM have hypercoagulable tendencies, but studies assessing a more comprehensive panel of coagulation parameters in dogs with GBM are needed to test this hypothesis.

Our objective was to evaluate PT, aPTT, factor VIII activity, fibrinogen, D-dimers, TEG parameters, and PC, AT and vWF activities in dogs with ultrasonographically identified GBM. We hypothesized that dogs with GBM would have coagulation parameters compatible with a hypercoagulable state. A secondary aim was to determine if plasma-based coagulation variables, clinical pathologic findings, the presence of systemic inflammatory response syndrome (SIRS), or GBM ultrasonographic patterns correlated with coagulation state.

2 | MATERIALS AND METHODS

Ours was a prospective study to measure and interpret coagulation parameters in dogs with GBMs identified on ultrasound examination. The study was conducted over 18 months from 2017 to 2018 at the Foster Hospital for Small Animals at the Cummings Veterinary Medical Center at Tufts University. Dogs were included if they had an ultrasound diagnosis of GBM.[6,8,13] The GBM were diagnosed by the presence of characteristic immobile, echogenic bile patterns, as previously described.[6,8,11] Additional inclusion criteria included a CBC, serum biochemistry panel and urinalysis within 24 hours before or after the ultrasound diagnosis, and body weight > 5 kg. Exclusion criteria included administration of vitamin K, blood products, or any medications known to affect coagulation such as nonsteroidal anti-inflammatory drugs, corticosteroids, heparin, clopidogrel, free fatty acids, or hydroxyethyl starch within 2 weeks of the ultrasound diagnosis. Additionally, Greyhounds were excluded because of known alterations in clot formation.[23] Abdominal ultrasound examination was performed by a radiology resident or board-certified radiologist. At the time of ultrasound examination, detailed information regarding the ultrasonographic appearance of the gallbladder and its contents, including stage of GBM development and the presence or absence of pericholecystic effusion or hyperechoic fat, were recorded. The latter findings were considered consistent with possible loss of gallbladder wall integrity.[3,10] The GBM pattern was classified into 5 types.[6] Type 1 pattern had echogenic immobile bile occupying the gallbladder. Type 2 pattern was an incomplete stellate pattern characterized by a few hypoechoic bile casts along the gallbladder wall with central

echogenic sludge. Type 3 pattern was a typical stellate pattern characterized by many hypoechoic bile casts along the gallbladder wall with central echogenic bile. Type 4 pattern was a kiwi fruit-like pattern and stellate combination characterized by hypoechoic bile casts accompanied by fine striations with a central echogenic region. Type 5 pattern was a kiwi fruit-like pattern with residual central echogenic bile.[6] All ultrasound images were reviewed by a single board-certified radiologist (D.G. Penninck).

Whole blood collected from the patients was placed into 3 tubes containing 3.2% sodium citrate to obtain a dilution of blood-to-sodium citrate of 9 : 1. After a 30-minute hold period at room temperature, a trained operator performed a kaolin-activated TEG (TEG 500 Thromboelastograph, Haemonetics Corp, Braintree, Massachusetts) as previously described.[17-19] The following TEG variables were recorded: *R* (a measure of initial fibrin formation), *K* (indicative of clot formation time), angle (indicative of rapidity of fibrin cross-linking), maximum amplitude (MA, indicative of overall clot strength), LY30 (percentage of clot lysis 30 minutes after MA was reached), and LY60 (percentage of clot lysis 60 minutes after MA was reached). The *G* value, a mathematical manipulation of MA, was calculated. Dogs were classified as hypercoagulable (defined as $G > 8.44$ dynes/s, MA > 64.2 mm, $R < 2$ minutes, or some combination of these), normocoagulable, or hypocoagulable (defined as $G < 3.88$ days/s, MA < 45.3 mm, $R > 8$ minutes, or some combination of these).[18-20]

The remaining citrated plasma was stored at −80°C for analysis of PT, aPTT, fibrinogen, D-dimers, and AT, PC, factor VIII and vWF activity. All coagulation testing, except for factor VIII, was conducted in the Coagulation Laboratory at the Cummings School of Veterinary Medicine at Tufts University. Coagulation parameters were evaluated using the ELITE ACL analyzer and Hemosil reagents according to the manufacturer's recommendations (Instrumentation Laboratories, Bedford, Massachusetts). The PT was determined using RecomiPlusTin 2G reagents and aPTT using the SYthASil Kit. Fibrinogen was determined using a quantitative Clauss assay with bovine thrombin. Both D-dimer and vWF antigen concentrations were measured using enhanced automated latex immunoassays with mouse monoclonal antibodies against D-dimer or vWF epitopes, respectively. Antithrombin activity was determined by a 2-step assay involving incubation with a factor Xa reagent followed by use of a synthetic chromogenic substrate (N-alpha-Z-D-Arg-GLy-Arg-p nitroaniline). Total PC activity likewise was determined in a 2-step manner. Plasma was incubated with a PC activator (venom from Agkistrodon C contortrix) followed by quantitative assay using a synthetic chromogenic substrate (pyro Glu-Arg-p nitroaniline). Reference values for dogs were established on the ELITE using a group of 75 normal healthy dogs. Manufacturer's quality control samples were utilized in combination with frozen (−80°C) pooled normal dog plasma that was thawed before use.

Factor VIII clotting activity was measured at the Cornell University Animal Health Diagnostic Laboratory using a 1-stage aPTT with human factor VIII-deficient substrate plasma (George King Biomedical, Overland Park, Kansas). Clotting time values were log-transformed to calculate factor activity compared with dilutions of a pooled canine plasma standard. The standard had an assigned value of 100% FVIII : C and was prepared from 20 healthy dogs and stored in single use aliquots at −70°C.

Medical records were reviewed to evaluate clinical presentation and determine the presence of concurrent conditions. The presence of SIRS, which was used as a crude measure of disease severity, was determined for each dog at the time of enrollment based on the finding of ≥2 of the following abnormalities: temperature >39.2°C or <37.8°C, heart rate >120 beats/min, respiratory rate >20 breaths/min, and leukocyte counts above or below upper or lower limits of normal (<4900/μL or >16 900/μL) or the presence of band cells (>3% of the total white blood cell count).[17,24] Survival to discharge was recorded for each dog.

Statistical analysis was performed to determine if the data were normally or non-normally distributed by constructing histograms and using tests for kurtosis and skewness. Means ± SD or medians with ranges were computed depending on data distribution. The TEG variables in dogs with GBM were compared to normal control reference range values established in the Clinical Coagulation Laboratory using paired Student's *t* tests or Wilcoxon Rank tests. The control reference ranges were defined in 75 normal dogs.[19] Correlations of TEG *G* with other coagulation parameters (fibrinogen, D-dimers, PCV, platelet count, and AC, PC, vWF and factor VIII activity) were done using Pearson's correlation coefficient with log transformation, if necessary. The association of SIRS and ultrasound findings with coagulation parameters was assessed using Fisher's exact test. A *P* value of <.05 was considered significant.

3 | RESULTS

3.1 | Clinical findings

Twenty-three dogs were enrolled in the study. There were 14 castrated males, 6 spayed females, and 3 intact males. The average age was 10 ± 3.0 years. The average weight was 15.3 ± 9.4 kg. There were 9 mixed breed dogs and 12 different purebred dogs. These included Collies (2), Maltese (2), and 1 each of Jack Russell terrier, Pug, Fox terrier, Pomeranian, American Staffordshire terrier, English bulldog, West Highland white terrier, Shetland sheep dog, Beagle and Black, and Tan coonhound.

Fifteen of 23 (65%) dogs had clinical signs related to gallbladder disease, including vomiting (13/15, 87%), anorexia (8/15, 53%), lethargy (6/15, 40%), or abdominal pain (2/15, 13%). Three of 23 (13%) of the dogs were presented for elective cholecystectomy based on a history of a previously diagnosed GBM. Five of these 23 (22%) dogs were presented for problems unrelated to their GBM including 1 each for coughing, aspiration pneumonia, tenesmus related to prostatomegaly, poorly controlled diabetes mellitus, and collapsing episodes.

Nine of 23 (39%) dogs had known concurrent medical conditions. Four of these 23 (17%) dogs had a previous diagnosis of hyperadrenocorticism. Two were not being treated at the time GBM was identified. Three of 23 (13%) dogs had diabetes mellitus (2 of which

were also being treated for hyperadrenocorticism). Three of 23 (13%) had collapsing trachea. One dog had well-controlled hypothyroidism, heart disease, and International Renal Interest Society stage 2 chronic kidney disease, and 1 dog had idiopathic epilepsy with seizures that were well controlled by phenobarbital.

3.2 | Clinical pathology

Selected clinico pathological variables are presented in Table 1. Common abnormalities included increases in the serum activities of alanine aminotransferase (20/23, 87%), alkaline phosphatase (20/23, 87%), gamma-glutamyl transferase (15/23, 65%), and aspartate aminotransferase (14/23, 60%). Hypercholesterolemia, mature leukocytosis, and hyperbilirubinemia were present in 16/23 (70%), 12/23 (52%), and 11/23 (47%) of dogs, respectively. Urinalysis was performed on 21/23 (91%) dogs. Eight of 21 (38%) dogs had isosthenuria and 2/21 (9.5%) were hyposthenuric. Twelve of 23 (52%) had some proteinuria either 3+ (4/21), 2+ (3/21), 1+ (4/21), or trace (1/21). No dog had a urine protein : creatinine ratio performed. Urine cultures in 3 dogs were negative.

Plasma-based coagulation parameters are summarized in Table 2. Results for PC activity and factor VIII activity were available for all 23 dogs; PT, aPTT, fibrinogen, and AT activity were available for 22 dogs; vWF activity was available for 21 dogs; and D-dimers for 15 dogs. Many dogs had increases in total PC activity (20/23, 87%), AT activity (9/22, 39%), platelet count (9/23, 39%), and fibrinogen (9/22, 41%). The PT was prolonged in 3/22 (14%) and the aPTT was

TABLE 1 Select clinical pathological values in 23 dogs with gallbladder mucoceles

Variable	Median (range) or mean ± SD	#/(%) Increased[a]	#/(%) Decreased[a]	Reference range
Hematocrit (%)	45 ± 7.53	0	1 (4)	38-55
White blood cells (K/uL)	14.7 (7.33-42.4)	9 (39)	0	4.4-15.1
Neutrophils (K/uL)	12.5 (4.42-37.4)	12 (52)	0	2.8-11.5
BUN (mg/dL)	17 (4-82)	2 (9)	3 (13)	8-30
Creatinine (mg/dL)	0.7 (0.5-5)	1 (4)	4 (18)	0.6-2
Total protein (g/dL)	6.2 (4.4-9.2)	1 (4)	3 (13)	5.5-7.8
Albumin (g/dL)	3.4 ± 0.56	0	3 (13)	2.8-4
Total bilirubin (mg/dL)	0.2 (0-4.8)	11 (48)	0	0-0.3
ALP (U/L)	1898 (65-6980)	20 (87)	0	12-127
GGT (U/L)	22 (1-272)	15 (65)	0	0-10
ALT (U/L)	391 (37-6342)	20 (87)	0	14-86
AST (U/L)	70 (20-680)	14 (61)	0	9-54
Cholesterol (mg/dL)	490 (270-2334)	16 (70)	0	82-355
Triglycerides (mg/dL)	242 (44-1378)	7 (39)	0	30-338

Abbreviations: ALP, serum alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate amino transferase; BUN, blood urea nitrogen; GGT, gamma-glutamyl transferase .
[a]Number of dogs in which the clinical parameter was increased above the upper limit of the reference range or decreased below the lower limit of the reference range.

TABLE 2 Coagulation parameters in 23 dogs with gallbladder mucoceles

Variable	Median (range) or mean ± SD	#/(%) Increased[a]	# /(%) Decreased[a]	Reference range
Platelet count ($\times 10^9$/L) (n = 23)	427 ± 200	9/(39)	0	173-486
PT (s) (n = 22)	7.9 (6.4-14.2)	3 (14)	0	5.98-9.36
aPTT (s) (n = 22)	18.6 (15-35)	9 (41)	0	9.90-20.4
Fibrinogen (mg/dL) (n = 22)	358.6 ± 186.0	9 (41)	0	73.4-410
Antithrombin (%) (n = 22)	108.5 (58.6-66.7)	9 (41)	4 (18)	75-112
Protein C (%) (n = 23)	195.6 ± 76.0	20 (87)	1 (4)	64.9-130
D-dimers (ng/mL) (n = 15)	306 (163-967)	6 (40)	0	55-533
Factor VIII (%) (n = 23)	108 (51-463)	3 (9)	0	50-200
von Willebrand factor (%) (n = 21)	74.5 ± 43.3	0	5 (24)	43-141

Abbreviations: aPTT, activated partial thromboplastin time; PT, prothrombin time.
[a]Number of dogs in which the clinical parameter was increased above the upper limit of the reference range or decreased below the lower limit of the reference range.

prolonged in 9/22 (41%) dogs. No dog had a shortened PT or aPTT. D-dimers were increased in 6/15 (40%) and factor VIII activity in 3/23 (12%) dogs. Five of 21 (24%) dogs had low vWF activity. No dog had increased vWF activity.

All 23 dogs had TEG analysis performed (Figure 1). Overall, when compared to the hospital-generated control reference range population, dogs with GBM had significantly decreased *K* values and increased angle, MA, and *G* ($P < .001$). Based on MA and *G* value, 19/23 dogs (83%) were classified as hypercoagulable and 4/23 (17%) were classified as normocoagulable. Four of 23 (17%) of dogs were classified as hyperfibrinolytic based on LY60 values >8.5%. The TEG *G* values did not correlate with any of the plasma-based coagulation parameters (Table 3).

The increase in total PC activity seen in many dogs was not correlated with TEG *G* value. Because increases in PC activity have been reported with hyperlipidemia in human patients,[25-27] we examined the relationship between PC activity and serum lipids in dogs with GBM. Serum PC activity was moderately positively correlated with serum cholesterol ($r = 0.431$, $P = .05$), but not serum triglyceride ($r = 0.178$, $P = .44$) concentrations.

3.3 | Ultrasound findings

All dogs had ultrasonographic evidence of GBM. Thirteen of 23 dogs (57%) had pericholecystic hyperechoic fat or effusion, a finding

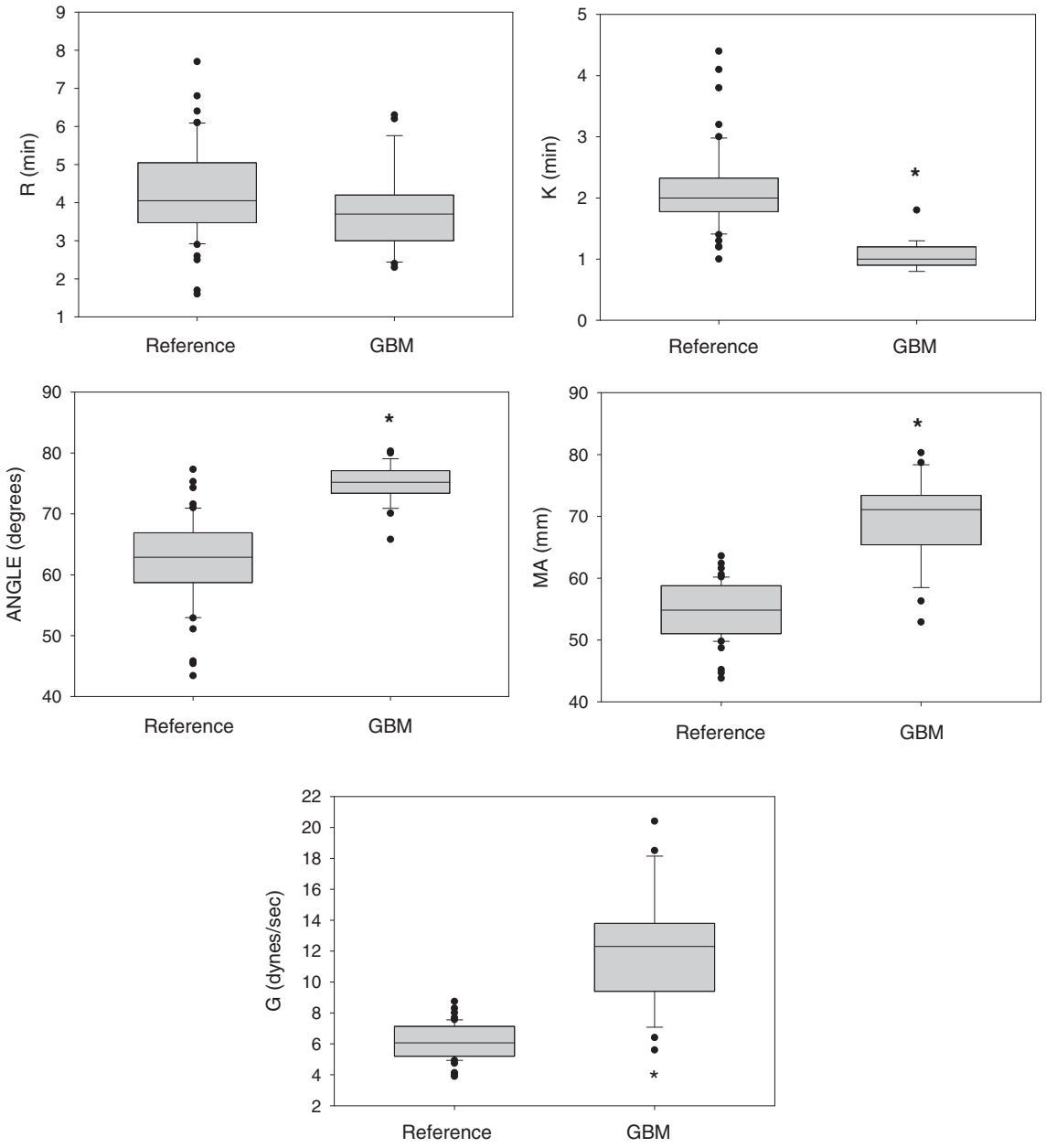

FIGURE 1 F Box and whisker plot of thromboelastographic parameters in 23 dogs with gallbladder mucocele compared to control reference range (n = 50) including *R* (A), *K* (B), angle (C), maximum amplitude (MA, D), and *G* (E). Kaolin-activated thromboelastography was performed on 23 dogs with an ultrasound diagnosis of gallbladder mucocele (GBM). Bar inside each box indicates median, with lower and upper edges marking 25th and 75th percentiles, respectively. The asterisk indicates that the value is significantly difference from the value in the reference control population, $P < .001$

common with compromised GBM integrity.[3,10] No difference was found in TEG *G* between dogs with and without these ultrasound changes. Ultrasound GBM type was classified for 22/23 dogs. Two of 22 (8.7%) dogs had type 1 GBM, 4/22 (17%) dogs had type 2, 3/22 (13%) dogs had type 3, 7/22 (30%) dogs had type 4, 6/22 (26%) dogs had type 5 and 1 dog was not classified. Dogs were divided into early mucoceles (types 1, 2, and 3; n = 9) and late mucoceles (types 4 and 5; n = 13) for comparison with coagulation parameters. No difference was found in TEG *G*, fibrinogen, PC, AT, vWF or factor VIII activity, PT, aPTT or platelet count between the 2 groups (Table 4).

TABLE 3 Correlation between TEG *G* value and plasma-based coagulation parameters in dogs with gallbladder mucoceles

Coagulation parameter	*R* (Pearson's correlation coefficient)	*P* value[a]
PT (s)	0.075	.65
aPTT (s)	0.014	.17
Fibrinogen (mg/dL)	0.289	.09
Protein C (%)	0.157	.57
Antithrombin (%)	−0.067	.49
vWF (%)	0.112	.66
Platelet count (× 10^9/L)	0.075	.71
Factor VIII (%)	−0.156	.48
D-dimers	0.359	.22

Abbreviations: aPTT, activated partial thromboplastin time; PT, prothrombin time; TEG, thromboelastrography; vWF = von Willebrand's factor.
[a]*P* value for Pearson's correlation.

3.4 | Association with SIRS

Eleven of 23 (47.8%) dogs met the criteria for SIRS. Dogs with SIRS had significantly higher total PC activity and fibrinogen concentration than dogs without SIRS. There was no difference in ultrasound imaging, TEG *G*, platelet count, D-dimer, factor VIII, vWF, or AT activity results between dogs with and without SIRS (Table 5).

3.5 | Outcome

Fifteen of 23 (65%) dogs survived to discharge, and 8/23 (35%) dogs were euthanized. Five dogs, all with clinical signs, were euthanized at the time of GBM identification without further treatment. One dog with a GBM (treated medically) was euthanized because of respiratory failure.

TABLE 4 Comparison of coagulation parameters in dogs with gallbladder mucocele that have early (type 1/2/3) or late (type 4/5) ultrasonographic appearance

Coagulation parameter	Type 1/2/3 (n = 9)	Type 4/5 (n = 13)	*P* value[a]	Reference interval
TEG *G* (dynes/s) (n = 23)	12.5 ± 3.0	12.3 ± 3.7	.76	4.6-10.9
Platelet (× 10^9/L) (n = 23)	413 ± 201	436 ± 231	.8	173-486
PT (s) (n = 22)	7.7 (6.9-14)	8.1 (6.4-9.8)	.65	5.98-9.36
aPTT (s) (n = 22)	18.6 (16.6-27)	21.4 (12.6-35.6)	.5	9.9-20.4
Protein C (%) (n = 23)	222 ± 67	186 ± 76	.26	64.9-130
Antithrombin (%) (n = 22)	101 (86-138)	123 (56-138)	.18	75-112
Factor VIII (%) (n = 23)	139 (51-301)	86 (78-230)	.08	50-200
D-dimers (μg/mL) (n = 15)	262 (163-694)	411 (288-941)	.19	55-533
Fibrinogen (mg/dL) (n = 22)	375 ± 171	401 ± 199	.76	73.4-410
vWF activity (%) (n = 21)	82 ± 36	56 ± 45	.22	43-141

Abbreviations: aPTT, activated partial thromboplastin time; PT, prothrombin time; TEG, thromboelastography; vWF, von Willebrand's factor.
[a]*P* value for comparison of coagulation parameters in dogs with type 1,2,3 and type 4-5 gallbladder ultrasound appearance.

TABLE 5 Coagulation parameters in dog with gallbladder mucoceles with and without systemic inflammatory response syndrome

Parameter	SIRS+ (n = 11)	SIRS− (n = 12)	*P* value[a]	Reference interval
Platelet (× 10^9/L)	390 ± 165	464 ± 215	.49	173-486
Protein C (%)	277 ± 81	166 ± 61	.05	64.9-130
Fibrinogen (mg/dL)	492 ± 175	301 ± 140	.01	73.4-410
Antithrombin (%)	111 ± 16	94 ± 36	.21	75-112
D-dimers (μg/mL)	559 ± 320	431 ± 229	.41	55-533
Factor VIII (%)	117 ± 69	140 ± 107	.46	50-200
vWF (%)	60 ± 45	74 ± 39	.47	43-141
TEG *G* (dynes/s)	13.2 ± 4.3	11.5 ± 3.2	.32	4.6-10.9

Abbreviations: SIRS, systemic inflammatory response syndrome; TEG, thromboelastrography; vWF, von Willibrand's factor.
[a]*P* value for comparison of parameters between dog with SIRS+ and without SIRS.

TABLE 6 Summary of coagulation parameters in 23 dogs with gallbladder mucoceles

Coagulation parameters associated with thrombotic tendencies		Coagulation parameters associated with bleeding tendencies	
Increased TEG G/MA	(83%, 19/23)	Prolonged aPTT	(41%, 9/22)
Increased fibrinogen	(41%, 9/22)	Decreased vWF activity	(24%, 5/21)
Increased D-dimers	(40%, 6/15)	Prolonged PT	(14%, 3/22)
Thrombocytosis	(39%, 9/23)	Increased LY60	(17%, 4/23)
Decreased AT activity	(17%, 4/23)	Prolonged TEG *K*	(4.3%, 2/23)
Increased factor VIII activity	(13%, 2/23)	Increased PC activity	(4.3%, 1/23)
Decreased PC activity	(4.3%, 1/23)	Decreased fibrinogen	(4.5%, 1/22)

Abbreviations: aPTT, activated partial thromboplastin time; AT, antithrombin; MA, maximum amplitude; PC, protein C; PT, prothrombin time; TEG, thromboelastography; vWF, von Willebrand's factor.

Thirteen dogs had cholecystectomy performed, and 11 survived. Two dogs were euthanized postoperatively. One experienced cardiopulmonary arrest during surgery and was resuscitated, but was not responsive to postoperative assisted ventilation. Pulmonary thromboembolism was suspected because of a dilated right atrium on echocardiogram. No necropsy was performed. A second dog with preoperative renal azotemia was euthanized postoperatively because of the development of oliguric renal failure. One additional dog developed postoperative aspiration pneumonia and pancreatitis, but survived to discharge.

4 | DISCUSSION

Our objective was to complete a detailed assessment of coagulation parameters in dogs with ultrasonographically diagnosed GBM. We found that many dogs had coagulation parameters compatible with a hypercoagulable state (Table 6), including increased MA and *G* on TEG (83%), hyperfibrinogenemia (41%), and thrombocytosis (39%). A smaller number of dogs, however, had results consistent with hypocoagulability including prolongations in aPTT (41%) or PT (14%), decreases in vWF activity (24%), or increased LY60 on TEG (13%). Thus, our results mirror those seen in humans with hepatobiliary disease and indicate a complex state of coagulation in dogs with GBM. The discovery of strong hypercoagulable tendencies, however, suggests that the rebalanced state of coagulation in the dogs with GBM might be shifted more toward thrombosis than hemorrhage.

The current veterinary literature supports the concept that dogs with GBM are predisposed to thrombotic complications. A recent large multicenter retrospective study of dogs undergoing cholecystectomy found that 14/179 (7.8%) of dogs died from documented thrombotic events.[14] The occurrence of PTE was noted in our study and has been sporadically reported as a cause of postoperative death in other studies examining outcome in dogs undergoing cholecystectomy for GBM.[3,4,7,16] In our study, 1 dog died with clinical suspicion of PTE. Additional evidence for a role for thrombosis in dogs with GBM comes from the observation that microthrombi and associated necrosis of the gallbladder wall occur as histopathologic findings in many dogs with GBM.[3,10,28]

If macro- or microthrombi do contribute to morbidity and mortality during the management of dogs with GBM, then therapeutic strategies to mitigate thrombosis would be indicated. If microthrombi contribute to the progression of gallbladder damage, then antiplatelet or anticoagulant treatment may be indicated. If dogs with GBM are hypercoagulable before surgery as our study would suggest, this hypercoagulable state may be potentiated by surgical cholecystectomy.[29] Thus, perioperative strategies to control thrombotic complications could improve morbidity and mortality in dogs with GBM undergoing cholecystectomy. These strategies might include the perioperative use of anticoagulants or antiplatelet drugs. In addition, switching from open surgical procedures for cholecystectomy to laparoscopic approaches, which are associated with a diminished acute phase response, might minimize thrombotic tendencies.[29-32] The technique for successful laparoscopic removal of the gallbladder has been described in the veterinary literature.[33-35]

Although the TEG *G* value characterized most dogs with GBM as hypercoagulable, whether a hypercoagulable *G* value can be used as a biomarker for the presence of clinical thrombosis is controversial. Although some studies have suggested a correlation,[36,37] others have failed to show TEG abnormalities in dogs with confirmed thrombosis.[38,39] In human patients with liver disease, stroke, neoplasia, or in those undergoing surgery, TEG parameters can predict the occurrence of thrombosis.[40-45] In dogs, indirect evidence for an association of high TEG *G* values with thrombosis comes from the observation that conditions associated with thrombosis, such as immune-mediated hemolytic anemia, protein-losing enteropathy, protein-losing nephropathy, hyperadrenocorticism, and pancreatitis, have TEG parameters compatible with hypercoagulabilty.[36,46] Studies that link potential coagulation biomarkers of hypercoagulability in dogs with GBM with actual indices of clinical thrombosis are needed, but will be hampered by the difficulty of making an antemortem diagnosis of thrombosis.

Alterations in several plasma-based coagulation parameters associated with hypercoagulability occurred in dogs with GBM, although none clearly identified dogs with high TEG *G* values. Despite the occurrence of increases in fibrinogen, platelet number, and D-dimers in approximately one-third of the dogs, none of these variables correlated with TEG *G* value. Decreases in AT and PC activity and increases in factor VIII activity, all of which can lead to a pro-thrombotic state, were documented in only a few dogs and again none of these variables correlated with TEG *G*.[46,47]

Several coagulation parameters, not investigated in our study, could have contributed to a hypercoagulable state in dogs with GBM. In human patients with cholestatic liver disease, platelet hyper-reactivity contributes to a hypercoagulability.[48,49] We did not investigate platelet function. Decreases in a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS-13) concentrations accompany liver disease in people and predict high TEG *G* values and the presence of portal thrombosis.[50-53] The decrease in ADAMTS-13, a circulating protease that cleaves vWF, results in the formation of ultra large vWF multimers that are capable of spontaneous thrombi formation.[53] Increased concentrations of plasminogen activator inhibitor-1 occur in liver disease in humans and are associated with hypofibrinolysis and thrombosis.[54,55] Thus, there are several unexplored aspects of coagulation that might contribute to hypercoagulability in dogs with GBM that should be investigated in future studies.

Concurrent pro-thrombotic conditions likely influenced the coagulation status of some dogs in our study. Inflammation is known to promote a pro-thrombotic state.[36,46] Forty percent of the dogs in our study had an increase in the acute phase protein and fibrinogen, and 52% had leukocytosis. Both clinicopathologic findings suggest the presence of systemic inflammation. Seven of 23 dogs (30%) had pro-thrombotic conditions including hyperadrenocorticism (n = 4), congestive heart failure (n = 1), neoplasia (n = 1), and a history of splenectomy (n = 1), which could have contributed to the hypercoagulable TEG findings. However, it is unlikely that these predisposing conditions alone accounted for the hypercoagulability because all 8 asymptomatic dogs without underlying conditions also had high TEG *G* values.

Increases in total PC activity in 87% of the dogs with GBM was an unexpected finding because decreases, rather than increases, in total PC activity, have been reported in dogs with hepatobiliary disorders.[17-19,46] The clinical relevance of these increases is unknown. Increased activated PC activity is implicated in the pathogenesis of trauma-induced coagulopathy and associated hemorrhage.[56,57] The bleeding tendencies are associated with activated PC-induced inhibition of factors V, VIII, and plasminogen activator inhibitor. No dogs in our study had bleeding tendencies, and reports of hemorrhage in patients with GBM are rare. It is noteworthy that we measured total PC activity not total activated PC activity. Although studies in humans suggest that increased total PC activity correlates with a concomitant increase in activated PC activity, it is unknown if the same is true in dogs.[58,59] Future studies to examine if total activated PC activity is increased in dogs with GBM are necessary.

Besides its role as an anticoagulant, PC also has anti-inflammatory and cytoprotective properties.[25] Increases in total PC activity occur as a reaction to systemic inflammation. This observation might fit with our finding that PC activity was higher in dogs that met the criteria for SIRS. In humans, increases in total PC also accompany hyperlipidemia.[26,27] Many dogs in our study were hypercholesterolemic and a significant positive correlation was foud between PC activity and serum cholesterol concentration. Because hyperlipidemia is known risk factor for the development of GBM in dogs,[16,60] the role of increased PC activity and its association with hypercholesterolemia in the pathogenesis of GBD in dogs should be further explored.

Our study had several limitations. We evaluated a relatively small heterogenous population of dogs with GBM that varied in whether or not they had clinical signs, inflammation or necrosis of the gallbladder wall, hyperlipidemia, or comorbid conditions. The small population limited our data analysis so that complex multivariate modeling such as principal component analysis was not feasible. However, such analysis will likely be necessary to clarify the nature of the complex coagulopathy that accompanies GBM. One-third of the dogs had missing data points for D-dimers because of hyperlipidemia and interference with the assay endpoint. Our study only evaluated kaolin-activated TEG and whole blood or tissue factor-activated TEG may have yielded different results. We did not examine the relationship between coagulation abnormalities and survival and thus there was no stratification of illness severity beyond classification based on SIRS and ultrasound criteria. Coagulation parameters could be different in dogs with more severe inflammation, sepsis, or multiple organ damage. Although fibrinogen is an acute phase reactant in the dog, more specific markers of inflammation, such as C-reactive protein, were not investigated in our study. Last, we studied a diverse population of older dogs that could have had undiagnosed diseases compatible with hypercoagulability. For example, many dogs had proteinuria on presentation and whether it was transiently related to the acute inflammation associated with the GBM or from chronic underlying protein-losing nephropathy was not clarified. A recent study also reported on the presence of proteinuria in dogs with GBM, suggesting that the occurrence of renal lesions may represent part of the metabolic disease process in affected dogs.[61]

In conclusion, dogs with GBM have a complex pattern of changes in coagulation parameters. The pattern is most compatible with a hypercoagulable state. This observation and emerging evidence that both micro- and macrothrombi may promote disease progression, complicate surgical management or both, suggest further investigation into coagulation status in these dogs is warranted so that decisions on the most appropriate perioperative antithrombotic treatment can be made.

ACKNOWLEDGMENT

Funding for this study was provided by the Companion Animal Health Fund at Cummings School of Veterinary Medicine at Tufts University.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

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

Approved by the Clinical Science Review Committee at the Cummings School of Veterinary Medicine at Tufts University.

HUMAN ETHICS APPROVAL DECLARATION

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

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REFERENCES

1. Mayhew PD, Savigny MR, Otto CM, et al. Evaluation of coagulation in dogs with partial or complete extrahepatic biliary tract obstruction by means of thromboelastography. *J Am Vet Med Assoc*. 2013;242:778-785.
2. Worley DR, Hottinger HA, Lawrence HJ. Surgical management of gallbladder mucoceles in dogs: 22 cases (1999-2003). *J Am Vet Med Assoc*. 2004;225:1418-1422.
3. Pike FS, Berg J, King NW, et al. Gallbladder mucocele in dogs: 30 cases (2000-2002). *J Am Vet Med Assoc*. 2004;224:1615-1622.
4. Malek S, Sinclair E, Hosgood G, et al. Clinical findings and prognostic factors for dogs undergoing cholecystectomy for gallbladder mucocele. *Vet Surg*. 2013;42:418-426.
5. Uno T, Okamoto K, Onaka T, et al. Correlation between ultrasonographic imaging of the gallbladder and gallbladder content in eleven cholecystectomised dogs and their prognoses. *J Vet Med Sci*. 2009;71:1295-1300.
6. Choi J, Kim A, Keh S, et al. Comparison between ultrasonographic and clinical findings in 43 dogs with gallbladder mucoceles. *Vet Radiol Ultrasound*. 2014;55:202-207.
7. Mehler SJ, Mayhew PD, Drobatz KJ, et al. Variables associated with outcome in dogs undergoing extrahepatic biliary surgery: 60 cases (1988-2002). *Vet Surg*. 2004;33:644-649.
8. Besso JG, Wrigley RH, Gliatto JM, et al. Ultrasonographic appearance and clinical findings in 14 dogs with gallbladder mucocele. *Vet Radiol Ultrasound*. 2000;41:261-271.
9. Amsellem PM, Seim HB, MacPhail CM, et al. Long-term survival and risk factors associated with biliary surgery in dogs: 34 cases (1994-2004). *J Am Vet Med Assoc*. 2006;229:451-1457.
10. Crews LJ, Feeney DA, Jessen CR, et al. Clinical ultrasonographic, and laboratory findings associated with gallbladder disease and rupture in dogs: 45 cases (1997-2007). *J Am Vet Med Assoc*. 2009;234:359-366.
11. Parkanzky M, Grimes J, Schmiedt C, Secrest S, Bugbee A. Long-term survival of dogs treated for gallbladder mucocele by cholecystectomy, medical management, or both. *J Vet Intern Med*. 2019;33:2057-2066.
12. Allerton F, Swinbourne F, Barker L, et al. Gall bladder mucoceles in Border terriers. *J Vet Intern Med*. 2018;32:1618-1628.
13. Jaffey JA, Graham A, VanEerde E, et al. Gallbladder mucocele: variables associated with outcome and the utility of ultrasonography to identify gallbladder rupture in 219 dogs (2007-2016). *J Vet Intern Med*. 2018;32:195-200.
14. Jaffey JA, Pavlick M, Webster CR, et al. Effect of clinical signs, endocrinopathies, timing of surgery, hyperlipidemia, and hyperbilirubinemia on outcome in dogs with gallbladder mucocele. *Vet J*. 2019;251:105350.
15. Youn G, Waschak MJ, Kunkel KAR, et al. Outcome of elective cholecystectomy for the treatment of gallbladder disease in dogs. *J Am Vet Med Assoc*. 2018;252:970-975.
16. Aguirre AL, Center SA, Randolph JF, et al. Gallbladder disease in Shetland sheepdogs: 38 cases (1995-2005). *J Am Vet Med Assoc*. 2007;231:79-88.
17. Caldwell S, Carlini LE. Coagulation homeostasis in liver disease. *Clin Liv Dis*. 2020;16:137-141.
18. Lisman T, Hernandez-Gea V, Magnusson M, et al. The concept of rebalanced hemostasis in patients with liver disease: communication from the ISTH SSC working group on hemostatic management of patients with liver disease. *J Thromb Haemost*. 2021;19:1116-1122.
19. Stravitz RT. Algorithms for managing coagulation disorders in liver disease. *Hepatol Int*. 2018;12:390-401.
20. Kelley D, Lester C, Shaw S, et al. Thromboelastographic evaluation of dogs with acute liver disease. *J Vet Intern Med*. 2015;29:1053-1062.
21. Kelley D, Lester C, DeLaforcade A, et al. Thromboelastographic evaluation of dogs with congenital portosystemic shunts. *J Vet Intern Med*. 2013;7:1262-1267.
22. Fry W, Lester C, Etedali NM, et al. Thromboelastography in dogs with chronic hepatopathies. *J Vet Intern Med*. 2017;31:419-426.
23. Vilar P, Couto CG, Westendorf N, et al. Thromboelastographic tracings in retired racing greyhounds and in non-greyhound dogs. *J Vet Intern Med*. 2008;22:374-379.
24. Hauptman JG, Walshaw R, Olivier NB. Evaluation of the sensitivity and specificity of diagnostic criteria for sepsis in dogs. *Vet Surg*. 1997;26:393-397.
25. Kohli S, Al-Dabet MM, Isermann B. Cell biology of activated protein C. *Curr Opin Hematol*. 2019;26:41-50.
26. Bruckert E, Ankri A, Jung M, et al. Mild liver abnormalities associated with elevated plasma factor VII and protein C in hypertriglyceridaemic patients. *Eur J Med*. 1993;2:461-465.
27. Mannucci PM, Valsecchi C, Bottasso B, et al. High plasma levels of protein C activity and antigen in the nephrotic syndrome. *Thromb Haemost*. 1986;55:31-33.
28. Rogers E, Jaffey JA, Graham A, et al. Prevalence and impact of cholecystitis on outcome in dogs with gallbladder mucocele. *J Vet Emerg Crit Care*. 2020;30:97-101.
29. Tsiminikakis N, Chouillard E, Tsigris C, et al. Fibrinolytic and coagulation pathways after laparoscopic and open surgery: a prospective randomized trial. *Surg Endosc*. 2009;23:2762-2769.
30. Sista F, Schietroma M, Carlei F, et al. The neutrophils response after laparoscopic and open cholecystectomy. *Ann Ital Chir*. 2013;84:153-158.
31. Schietroma M, Carlei F, Mownah A, et al. Changes in the blood coagulation, fibrinolysis, and cytokine profile during laparoscopic and open cholecystectomy. *Surg Endosc*. 2004;18:1090-1096.
32. Zezos P, Christoforidou A, Kouklakis G, et al. Coagulation and fibrinolysis activation after single-incision versus standard laparoscopic cholecystectomy: a single-center prospective case-controlled pilot study. *Surg Innov*. 2014;21:22-31.
33. Simon A, Monnet E. Laparoscopic cholecystectomy with single port access system in 15 dogs. *Vet Surg*. 2020;49(suppl 1):O156-O162.
34. Kanai H, Hagiwara K, Nukaya A, et al. Short-term outcome of laparoscopic cholecystectomy for benign gall bladder diseases in 76 dogs. *J Vet Med Sci*. 2018;23(80):1747-1753.
35. Scott J, Singh A, Mayhew PD, et al. Perioperative complications and outcome of laparoscopic cholecystectomy in 20 dogs. *Vet Surg*. 2016;45:O49-O59.
36. de Laforcade A, Bacek L, Blais MC, et al. Consensus on the rational use of antithrombotics in veterinary critical care (CURATIVE): domain 1: defining populations at risk. *J Vet Emerg Crit Care*. 2019;29:37-48.
37. Koch BC, Motta L, Wiinberg B, et al. D-dimer concentrations and thromboelastography in five dogs with ischemic stroke. *Front Vet Sci*. 2019;6:255. https://doi.org/10.3389/fvets.2019.00255.
38. Thawley VJ, Sánchez MD, Drobatz KJ, et al. Retrospective comparison of thromboelastography results to postmortem evidence of thrombosis in critically ill dogs: 39 cases (2005-2010). *J Vet Emerg Crit Care*. 2016;26:428-436.
39. Marschner CB, Kristensen AT, Rozanski EA, et al. Diagnosis of canine pulmonary thromboembolism by computed tomography and mathematical modelling using haemostatic and inflammatory variables. *Vet J*. 2017;229:6-12.
40. Krzanicki D, Sugavanam A, Mallett S. Intraoperative hypercoagulability during liver transplantation as demonstrated by thromboelastography. *Liver Transpl*. 2013;19:852-861.

41. Walsh M, Moore EE, Moore H, et al. Use of viscoelastography in malignancy-associated coagulopathy and thrombosis: a review. *Semin Thromb Hemost*. 2019;45:354-372.
42. Wang C, Liu Q, Sun L, et al. Application of thromboelastography in primary total knee and total hip replacement: a prospective 87 patients' study. *Blood Coagul Fibrinolysis*. 2019;30:281-290.
43. Liang Y, Wu J, Liu J, et al. The clinical implications of thromboelastography in the diagnosis of acute cerebral infarction. *Clin Lab*. 2018;64:147-152.
44. Sumislawski JJ, Moore HB, Moore EE, et al. Not all in your head (and neck): stroke after blunt cerebrovascular injury is associated with systemic hypercoagulability. *J Trauma Acute Care Surg*. 2019;87:1082-1108.
45. Kashuk JL, Moore EE, Sabel A, et al. Rapid thromboelastography (*r*-TEG) identifies hypercoagulability and predicts thromboembolic events in surgical patients. *Surgery*. 2009;146:764-772.
46. Webster CR. Hemostatic disorders associated with hepatobiliary disease. *Vet Clin North Am Small Anim Pract*. 2017;47:601-615.
47. Bauer N, Moritz A. Characterization of changes in the haemostasis system in dogs with thrombosis. *J Small Anim Pract*. 2013;54:129-136.
48. Pihusch R, Rank A, Göhring P, et al. Platelet function rather than plasmatic coagulation explains hypercoagulable state in cholestatic liver disease. *J Hepatol*. 2002;37:548-555.
49. Ben-Ari Z, Panagou M, Patch D, et al. Hypercoagulability in patients with primary biliary cirrhosis and primary sclerosing cholangitis evaluated by thromboelastographic. *J Hepatol*. 1997;26:554-559.
50. Lee KCL, Baker L, Mallett S, et al. Hypercoagulability progresses to hypocoagulability during evolution of acetaminophen-induced acute liver injury in pigs. *Sci Rep*. 2017;7:9347.
51. Mikuła T, Kozłowska J, Stańczak W, et al. Serum ADAMTS-13 levels as an indicator of portal vein thrombosis. *Gastroenterol Res Pract*. 2018;2018:3287491. https://doi.org/10.1155/2018/3287491.
52. Lancellotti S, Basso M, Veca V, et al. Presence of portal vein thrombosis in liver cirrhosis is strongly associated with low levels of ADAMTS-13: a pilot study. *Intern Emerg Med*. 2016;11:959-967.
53. Driever EG, Stravitz RT, Zhang J, et al. VWF/ADAMTS13 imbalance, but not global coagulation or fibrinolysis, is associated with outcome and bleeding in acute liver failure. *Hepatology*. 2021;73(5):1882-1891.
54. Blasi A, Patel VC, Adelmeijer J, et al. Mixed fibrinolytic phenotypes in decompensated cirrhosis and acute-on-chronic liver failure with hypofibrinolysis in those with complications and poor survival. *Hepatology*. 2020;71:1381-1390.
55. Ciavarella A, Gnocchi D, Custodero C, et al. Translational insight into prothrombotic state and hypercoagulation in nonalcoholic fatty liver disease. *Thromb Res*. 2020;198:139-150.
56. Haas T, Cushing MM. Hemostatic balance in severe trauma. *Front Pediatr*. 2020;8:600501. https://doi.org/10.3389/fped.2020.600501.
57. Duque P, Mora L, Levy JH, et al. Pathophysiological response to trauma-induced coagulopathy: a comprehensive review. *Anesth Analg*. 2020;130:654-664.
58. Espana F, Zuazu I, Vicente V, et al. Quantification of circulating activated protein C in human plasma by immunoassays—enzyme levels are proportional to total protein C levels. *Thromb Haemost*. 1996;75:56-66.
59. Arishima T, Ito T, Yasuda T, et al. Circulating activated protein C levels are not increased in septic patients treated with recombinant human soluble thrombomodulin. *Thromb J*. 2018;16:24. https://doi.org/10.1186/s12959-018-0178-0.
60. Kutsunai M, Kanemoto H, Fukushima K, et al. The association between gall bladder mucoceles and hyperlipidaemia in dogs: a retrospective case control study. *Vet J*. 2014;199:76-79.
61. Lindaberry C, Vaden S, Aicher KM, et al. Proteinuria in dogs with gallbladder mucocele formation: a retrospective case control study. *J Vet Intern Med*. 2021;35:878-886.

How to cite this article: Pavlick M, DeLaforcade A, Penninck DG, Webster CRL. Evaluation of coagulation parameters in dogs with gallbladder mucoceles. *J Vet Intern Med*. 2021;35(4):1763–1772. https://doi.org/10.1111/jvim.16203