ORIGINAL ARTICLE

Hypercoagulability identified in dogs with chronic enteropathy using a point-of-care viscoelastic assay

S. I. Barth*, S. M. DeMonaco[†,‡], B. J. Conner[‡] and A. R. Wilkinson [‡,1]

*Department of Small Animal Clinical Sciences, Fetch Specialty and Emergency Veterinary Centers in Brandon, Florida, USA
[†]Department of Small Animal Clinical Sciences, Long Island University College of Veterinary Medicine, Brookville, New York, USA
[‡]Department of Small Animal Clinical Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, Virginia, USA

[1]Corresponding author email: ashleyrw@vt.edu

Objectives: **Thromboelastography (TEG) using the TEG 6s, a point-of-care viscoelastic assay, was prospectively evaluated in dogs with chronic enteropathy (CE). Additionally, the study determined whether disease activity, assessed using the Canine Chronic Enteropathy Clinical Activity Index (CCECAI), correlated with TEG 6s parameters.**

Materials and Methods: **A CCECAI score and TEG using the TEG 6s (Haemonetics®) was performed on 19 dogs with CE. In a separate study, TEG using the TEG 6s was performed on 40 healthy adult dogs, which served as the control group. For statistical analysis, normally distributed data were analysed using the two-sample *t*-test. Non-Gaussian data were analysed using the Wilcoxon rank sum test. Correlations between TEG 6s parameters and the CCECAI scores were assessed using the Pearson test for data with Gaussian distribution and the Spearman test for data with non-Gaussian distribution.**

Results: **Dogs with CE had significantly shortened mean clot kinetics, prolonged mean reaction time (R) and increased alpha angle (angle), maximum amplitude (MA) and RapidTEG™ MA compared to healthy dogs. Dogs with CE had a significant median increase in Functional Fibrinogen MA compared to healthy dogs. The CCECAI moderately positively correlated with angle.**

Clinical Significance: **With the exception of prolonged R, dogs with CE have several TEG 6s alterations suggestive of hypercoagulability.**

Journal of Small Animal Practice (2025); **66**, 365–371
DOI: 10.1111/jsap.13841

Accepted: 20 January 2025; Published online: 13 February 2025

INTRODUCTION

Chronic enteropathies (CE) predispose dogs to a hypercoagulable state and thromboembolic disease (Dixon et al., 2021; Goodwin et al., 2011; Jacinto et al., 2017; Wennogle et al., 2021). A CE is gastrointestinal disease that has been present for 3 weeks or longer. Additionally, extra-intestinal disease leading to gastrointestinal signs and gastrointestinal neoplasia and parasitism have been excluded in patients with CE. There are different forms of CE, including food-responsive enteropathy, antibiotic-responsive enteropathy, immunosuppressant-responsive enteropathy and non-responsive enteropathy. A subset of dogs with chronic gastrointestinal signs may have intestinal lymphangiectasia as well (Dandrieux & Mansfield, 2019). Chronic enteropathy severity is often assessed using serum albumin concentration or a clinical scoring system, such as the Canine Chronic Enteropathy Clinical Activity Index (CCECAI) (Allenspach et al., 2007). The haemostatic derangements experienced by dogs with CE are a significant cause of morbidity and mortality in this patient population (Jacinto et al., 2017). Similar findings are observed in humans with inflammatory bowel disease (Arvanitakis et al., 2021; Yonghua et al., 2020). Unfortunately, identifying canine patients with CE that are hypercoagulable and at risk for thromboembolism remains challenging in daily practice.

Thromboelastography (TEG) is a whole blood assay that identifies patients with features of both hypo- and hypercoagulability.

Previous studies evaluated TEG in dogs with CE and largely identified TEG alterations suggestive of hypercoagulability. The previous studies used a traditional model of TEG, the TEG 5000 Thromboelastograph (Haemonetics®), a benchtop viscoelastic analyzer (Dixon et al., 2021; Goodwin et al., 2011; Wennogle et al., 2021). The TEG 5000 requires manual precision pipetting of the samples and reagents, which is tedious, time consuming and increases the risk of human error during sample analysis. It must be maintained on a level surface, which prevents it from being easily moved and transported, and requires quality control every day of use that is only valid for 8 hours (Volod & Runge, 2023). In a busy practice setting, the logistics associated with running and maintaining the TEG 5000 are often too cumbersome, making TEG unavailable in most practices.

A newer commercially available TEG unit, the TEG 6s Hemostasis Analyzer (Haemonetics®) would likely be more practical in a busy practice setting. Advantages include its portability, cartridge-based system, which negates the need for manual precision pipetting and ability to provide rapid results at the site of care. The global haemostasis cartridge associated with the TEG 6s provides a Kaolin TEG assay, which includes reaction time (R), clot kinetics (K), alpha angle (angle) and maximum amplitude (MA). Additional assays within the global haemostasis cartridge include Kaolin TEG with Heparinase (R), RapidTEG™ (MA) and TEG Functional Fibrinogen (MA and level) (Haemonetics Corporation, 2024). Additionally, agreement between the TEG 6s and TEG 5000 was previously assessed in dogs (Wheeler et al., 2022). The following databases (Medline (PubMed), Ovid, Google Scholar and Science Direct) have been searched with the following keywords: TEG 6s dog, TEG 6s canine, TEG 6s chronic enteropathy dog and TEG 6s inflammatory bowel disease dog on 01/07/2025. No other reports evaluating the TEG 6s in a population of ill dogs or those with CE were found doing these searches.

The primary aim of this study was to assess TEG using the TEG 6s, a point-of-care viscoelastic assay, in dogs with CE. A secondary aim was to determine whether disease activity in dogs with CE, assessed using the CCECAI, correlates with TEG parameters. We hypothesise that dogs with CE have one or more TEG 6s alterations suggestive of hypercoagulability, including shortened R, shortened K, increased angle, increased MA, increased RapidTEG™ MA and increased Functional Fibrinogen MA, compared to healthy dogs. Additionally, we hypothesise that R and K will correlate negatively, and angle, MA, RapidTEG™ MA and Functional Fibrinogen MA will correlate positively with CCECAI scores in dogs with CE.

METHODS AND MATERIALS

Study design and inclusion criteria

Dogs 1 year of age or older of any sex presenting for evaluation of chronic gastrointestinal disease to the *Virginia Maryland College of Veterinary Medicine Veterinary Teaching Hospital* in *Blacksburg, Virginia* were recruited for this prospective observational study. To qualify for enrolment, dogs had to have one or more of the following clinical signs for 3 weeks or longer: Hyporexia, small or mixed bowel diarrhoea, vomiting or weight loss. Small bowel diarrhoea was defined as normal to large stool volume, normal to mildly increased defecation frequency and/or the presence of melena. Mixed bowel diarrhoea was defined as any of the previously listed clinical signs combined with increased frequency of defecation, tenesmus, urgency to defecate, dyschezia, mucoid stool and/or haematochezia. Dogs were not eligible for enrolment if they were a sighthound breed or Cavalier King Charles Spaniel (Pedersen et al., 2002; Vilar et al., 2008). Additionally, dogs previously diagnosed with a condition known to alter haemostasis, including von Willebrand disease, haemophilia, diabetes mellitus, hyperadrenocorticism, renal disease, malignant neoplasia, hepatic disease, pancreatitis or other major concurrent illness, could not be enrolled (Ceriello, 1993; Fry et al., 2017; Jergens et al., 1987; Ke et al., 2023; Nielsen et al., 2019; Othman et al., 2009; Park et al., 2013). Dogs could not receive medications known to alter haemostasis within a week of enrolment, including clopidogrel, aspirin, warfarin, factor Xa inhibitors, heparin, non-steroidal anti-inflammatory drugs, calcium channel blockers, beta-blockers, fish oil and corticosteroids (Bae et al., 2019; Fresno et al., 2005; McLaughlin et al., 2017; Mehta, 1985; Neff-Davis et al., 1981; Probst et al., 1988; Rose et al., 2011; Saati et al., 2018; Westgarth et al., 2018). Written informed owner consent was obtained prior to study enrolment and the study was approved by *Virginia Tech* Institutional Animal Care and Use Committee (IACUC) (protocol 21-126).

Eligible dogs underwent a comprehensive diagnostic evaluation to exclude systemic disease and definitively diagnose them with CE. Comprehensive blood work included a complete blood cell count, serum or plasma biochemistry, and urinalysis. If there was evidence of hypoalbuminemia, fasting and post-prandial serum bile acids were recommended to rule out hepatic dysfunction. Patients with elevated pre- and/or post-prandial serum bile acids were excluded. If there was evidence of proteinuria, a urine protein: creatinine ratio (UPC) was performed. Mild proteinuria (1+) confirmed with sulfosalicylic acid testing in well concentrated urine (urine specific gravity of 1.035 or greater) was considered acceptable and UPC was not performed. Dogs with a UPC of greater than 0.5 were excluded. Since thrombocytopenia and anaemia affect TEG results, dogs with a platelet count less than 100,000 cells/uL or haematocrit less than 25% were excluded (Brooks et al., 2014). To screen for gastrointestinal parasitism, a centrifugal faecal flotation using zinc sulphate was performed on each patient and those diagnosed with parasitism were excluded. Testing for hypoadrenocorticism was at the discretion of the attending clinician. If the patient's basal serum cortisol was ≤2 µg/dL, an adrenocorticotrophin (ACTH) stimulation test was performed. If dogs had an abnormal ACTH stimulation test, they were excluded. Dogs with a basal serum cortisol ≥2 µg/dL did not receive an ACTH stimulation test and hypoadrenocorticism was ruled out. Abdominal ultrasonography was performed and/or reviewed in all dogs by a board-certified radiologist

or chief radiology resident. Dogs with focal gastrointestinal masses on abdominal ultrasound were excluded. All medically stable dogs underwent a strict hydrolysed or novel protein diet trial for a minimum of 2 weeks. Dogs were not enrolled if their clinical signs resolved on a hydrolysed or novel protein diet trial. Dogs with mixed bowel diarrhoea underwent esophagogastroduodenoscopy and colonoscopy with endoscopic biopsies collected from the stomach, duodenum, colon and when possible, ileum, while dogs with small bowel diarrhoea underwent esophagogastroduodenoscopy with endoscopic biopsies collected from the stomach and duodenum. Gastrointestinal endoscopy was performed by a board-certified small animal internal medicine (SAIM) specialist, chief SAIM resident or a SAIM resident under the direct supervision of a board-certified SAIM specialist. All gastrointestinal histopathology was reviewed by a board-certified pathologist through the *Virginia Tech Animal Laboratory Services* laboratory. Dogs with inflammatory infiltrate (lymphoplasmacytic, lymphocytic, eosinophilic and/or neutrophilic) within the stomach, small intestines and/or colon on histopathology were included. Dogs with intestinal lymphangiectasia with or without intestinal inflammatory infiltrates were included. Dogs with normal gastrointestinal histology or evidence of neoplasia or infectious disease on gastrointestinal histopathology were excluded. All diagnostics associated with the study were performed within 1 month of gastrointestinal endoscopy.

Disease severity was assessed using the CCECAI scoring system, which was calculated as previously described at the time of enrolment (Allenspach et al., 2007). All study dogs were managed by the admitting clinician at presentation, and not necessarily the study investigators.

Control population

In a separate study, 40 healthy dogs (≥1 year old) weighing 6 kg or greater of any sex were recruited from faculty, staff and students at the *Virginia Maryland College of Veterinary Medicine* in *Blacksburg, Virginia.* Any breed of dog was included except sighthounds and Cavalier King Charles spaniels (Pedersen et al., 2002; Vilar et al., 2008). Dogs were deemed healthy based on unremarkable physical examination, history, plasma biochemistry (Beckman AU480, Beckman Coulter, Inc., Brea, CA, USA), hemogram (Sysmex XN-1000v, Sysmex Corporation, Kobe, Japan), urinalysis and negative SNAP 4Dx testing (IDEXX®, Westbrook, Maine). These diagnostics were performed through *Virginia Tech Animal Laboratory Services*. Additionally, control dogs could not receive any medication, except heartworm, flea and tick prevention, at the time of enrolment. Written informed owner consent was obtained prior to study enrolment and the study was approved by *Virginia Tech* IACUC (protocol 21-071).

Thromboelastography

The procedures for blood sample collection and TEG analyses were informed by the Partnership on Rotational ViscoElastic Test Standardization recommendations for veterinary viscoelastic testing (Flatland et al., 2014). All blood samples for TEG analyses were collected by a licensed veterinary technician or one of the study investigators using atraumatic technique and a 21-gauge needle from a jugular vein. If blood collection from a jugular vein was not possible, blood was collected from the cephalic or lateral saphenous veins alternatively. Within the dogs with CE group, all blood samples were collected within 24 hours of gastrointestinal endoscopy and prior to general anaesthesia. After collection, the blood sample was immediately transferred into 3.2% sodium citrate siliconised vacutainer tubes and gently inverted three to four times. The whole blood to anticoagulant ratio was 9:1 (vol/vol). The sodium citrate samples were allowed to rest at room temperature for 30 minutes prior to analysis with the TEG 6s.

Thromboelastography was performed using the TEG 6s Hemostasis Analyzer (Haemonetics Corp., Boston, MA, USA) and associated global haemostasis cartridges according to manufacturer guidelines. The global haemostasis cartridges provided a Kaolin TEG assay, RapidTEG™ assay, Heparinase assay and TEG Functional Fibrinogen assay. For the Kaolin TEG assays, R (minutes), K (minutes), angle (degrees) and MA (mm) were measured. The TEG 6s Heparinase assay measured R (minutes), the RapidTEG™ assay measured MA (mm) and the TEG Functional Fibrinogen assay measured MA (mm) and a functional fibrinogen level (mg/dL). All TEG assays were performed by two of the investigators. Since none of the patients enrolled in this study received heparin, the Heparinase assay results were not reported for the purpose of this study.

Statistical analysis

For group comparisons, distribution properties of the data were assessed using normal probability plots. Normally distributed data were analysed using the two-sample *t*-test. Non-Gaussian data were analysed using the Wilcoxon rank sum test. When a result was at the upper or lower bound of the machine-specific reportable value, the value of the limit was reported. Assessment for correlations between TEG 6s parameters and the CCECAI scores in CE dogs was performed using the Pearson test for data with Gaussian distribution. The Spearman test was performed to assess for correlations when the data had a non-Gaussian distribution. For correlation testing, a statistically significant correlation of 0.3 to 0.5 was considered a weak correlation, 0.5 to 0.7 was considered a moderate correlation and 0.7 to 1.0 was considered a strong correlation. Statistical analysis was performed using SAS version 9.4 (Cary, NC). Significance was set at $P < 0.05$.

In a separate study, a reference interval for the TEG 6s was generated using the 40 healthy control dogs. A minimum of 40 dogs were enrolled to comply with the American Society for Veterinary Clinical Pathology guidelines (Friedrichs et al., 2012). Reference intervals for TEG 6s parameters were computed using open-source software and a non-parametric method with the minimum and maximum as the reference limits (with 90% bootstrap confidence intervals) (Geffre et al., 2011; Le Boedec, 2019). For the reference interval analysis, distribution properties of the data were assessed using the Shapiro-Wilk test with a threshold of $P > 0.2$. Outliers were identified using Tukey's method.

RESULTS

Patient population

From July 2021 to April 2023, a total of 102 dogs presenting with clinical signs suggestive of CE presented to the *Virginia Maryland College of Veterinary Medicine Veterinary Teaching Hospital.* Nineteen dogs were enrolled in the study and 83 dogs were deemed ineligible during pre-enrolment screening. Reasons for ineligibility included resolution of clinical signs after receiving a hydrolysed or novel protein diet trial ($n = 37$), significant concurrent disease ($n = 17$), previous diagnosis of a CE and receiving corticosteroids ($n = 10$), clinical signs suggestive of only large bowel disease ($n = 7$), further diagnostics were declined ($n = 5$), gastrointestinal parasitism ($n = 2$), resolution of gastrointestinal signs with empirical or no treatment ($n = 2$), under 1 year of age ($n = 2$) and gastrointestinal neoplasia ($n = 1$).

Within the study population, nine dogs were spayed females, eight dogs were neutered males, one dog was an entire female and one dog was an entire male. The median age of the CE dogs was 5 years (range 1 to 11 years). Breeds enrolled include mixed breed ($n = 8$) and one of each of the following: Basset Hound, Chihuahua, dachshund, English Bulldog, French Bulldog, German shepherd dog, Labrador retriever, Miniature poodle, Papillon, Yorkshire terrier and shih-tzu. Median body weight was 12.9 kg (range 3.8 to 39.6 kg).

Dogs in the healthy control group were enrolled from July 2021 to October 2022 in a separate study. Within the healthy control group ($n = 40$), 21 dogs were spayed females, 14 were neutered males, 3 were entire females and 2 were entire males. The median age of the healthy control group was 3.5 years (range 1 to 9 years), and median body weight was 21.2 kg (8 to 47 kg). Breeds in the healthy control group included mixed breed ($n = 19$), beagle ($n = 5$), golden retriever ($n = 3$), Dobermann Pinscher ($n = 2$), Labrador retriever ($n = 2$) and one of each of the following: Coonhound, English Pointer, English Setter, Miniature Australian Shepherd, Miniature dachshund, Newfoundland, Pembroke Welsh Corgi, Pug and Standard poodle.

The mean haematocrit of the dogs with CE population was 48.2% (range 34.1% to 57.8%). Within the healthy control group, the mean haematocrit was 49.7% (range 42.1% to 58.3%). The median platelet count of the dogs with CE population was 309,000 cells/μL (range 106,000 to 1,086,000 cells/μL). Within the healthy control group, the median platelet count was 230,500 cells/μL (range 117,000 to 525,000 cells/μL).

Six dogs with CE had a serum or plasma albumin concentration below the reference interval at <2.8 g/dL and 13 dogs had a normal serum or plasma albumin concentration ≥2.8 g/dL. Median serum albumin for all dogs with CE was 3.2 g/dL (range 1 to 3.6 g/dL), and median serum albumin for hypoalbuminemic dogs was 1.4 g/dL (range 1 to 2.2 g/dL). Median CCECAI was 9 (range 4 to 19). Pre- and post-prandial bile acids were normal in the six dogs with CE and hypoalbuminemia. Six dogs with CE in total required UPC measurement due to proteinuria, and all had a UPC of 0.3 or less. Sixteen of the 19 dogs received testing to rule out hypoadrenocorticism.

On abdominal ultrasound, three dogs with CE had hyperechoic mucosal striations, six had evidence of small intestinal wall thickening, five had abdominal lymphadenopathy and three had peritoneal effusion. Four dogs had normal abdominal ultrasounds.

Nine dogs underwent esophagogastroduodenoscopy and 10 dogs underwent esophagogastroduodenoscopy with colonoscopy, all under general anaesthesia. No dogs had significant complications during, or immediately post-anaesthesia and all dogs survived to discharge. Fifteen dogs had evidence of lymphoplasmacytic, eosinophilic, neutrophilic and/or lymphocytic inflammatory infiltrate on endoscopic biopsies of the stomach, duodenum, ileum and/or colon. Three dogs had evidence of intestinal lymphangiectasia. Two of the three dogs with intestinal lymphangiectasia had concurrent inflammatory infiltrate on gastrointestinal histopathology.

Thromboelastography findings

All 19 dogs with CE had TEG analysis performed. The R, K and angle values were available for all 19 dogs and MA, RapidTEG™ MA and Functional Fibrinogen MA values were available for 17 dogs. Functional Fibrinogen level results were not available for 15 dogs, so further analysis of Functional Fibrinogen level was not performed. Data was normally distributed for all TEG parameters except Functional Fibrinogen MA. Overall, dogs with CE had a shorter K and increased R, angle, MA, RapidTEG™ MA and Functional Fibrinogen MA compared to healthy dogs (Table 1).

Overall, K was shorter and angle and MA were higher in CE dogs with hypoalbuminemia compared to those without hypoalbuminemia. The remaining TEG parameters, including R, Rapid TEG MA and Functional Fibrinogen MA were not significantly different between CE dogs with and without hypoalbuminemia (Table 2).

The portion of dogs with CE that have TEG values above and below the previously established TEG 6s reference interval are reported in Table 3. The portion of dogs with CE and hypoalbuminemia that have TEG values above and below the previously established reference interval are reported in Table 4. Hypercoagulability, defined as one or more TEG 6s alterations that include shortened R, shortened K, increased angle, increased MA, increased RapidTEG™ MA and increased

Table 1. TEG 6s results in dogs with CE and healthy adult dogs

TEG 6s variable	Difference between means (95% CI)	P value
R (min)	0.7 (0.2 to 1.2)	0.006*
K (min)	−0.4 (−0.8 to −0.1)	0.006*
Angle (degrees)	2.8 (0.4 to 5.2)	0.022*
MA (mm)	7.6 (4.3 to 10.9)	<0.001*
RapidTEG™ MA (mm)	8.8 (5.2 to 12.5)	<0.001*
FF MA (mm)	4.8 (3.6 to 5.5)†	<0.001*

Data was analysed using a two-sample *t*-test
ANGLE Alpha angle, CE Chronic enteropathy, CI Confidence interval, FF Functional fibrinogen, K Clot kinetics, MA Maximum amplitude, min Minutes, mm Millimetres, R Reaction time
*P values <0.05 were considered statistically significant
†Median reported rather than mean since data not normally distributed

Table 2. TEG 6s results in CE dogs with and without hypoalbuminemia

TEG 6s variable	Difference between means (95% CI)	P value
R (min)	−0.4 (−1.9 to 1.1)	0.60
K (min)	−0.5 (−1 to −0.1)	0.013*
Angle (degrees)	5 (1 to 9)	0.018*
MA (mm)	6.5 (0.4 to 12.6)	0.037*
RapidTEG MA (mm)	6.6 (1.2 to 14.4)	0.09
FF MA	0 (0 to 0.03)†	0.20

ANGLE alpha angle, CE chronic enteropathy, CI confidence interval, FF functional fibrinogen, K clot kinetics, MA maximum amplitude, min minutes, mm millimetres, R reaction time
*P values <0.05 were considered statistically significant
†Median reported rather than mean since data not normally distributed

Table 3. Dogs with CE with TEG 6s parameters outside reference interval

TEG 6s variable	RI	Number below RI	Number above RI
R (min)	1.31 to 3.6	1/19 (5%)	8/19 (42%)
K (min)	0.81 to 4.17	3/19 (16%)	0 (0%)
Angle (degrees)	53.85 to 77.19	0 (0%)	3/19 (16%)
MA (mm)	40.04 to 65.12	0 (0%)	5/17 (29%)
RapidTEG MA (mm)	40 to 62.47	0 (0%)	4/17 (24%)
FF MA (mm)	34.01 to 52	0 (0%)	14/17 (82%)†
G (dyn/cm^2)	2576.9 to 5416.7	0 (0%)	17/17 (100%)

ANGLE alpha angle, FF functional fibrinogen, K clot kinetics, MA maximum amplitude, min minutes, mm millimetres, R reaction time, RI reference interval
†Portion of dogs at upper bound of RI

Table 4. Dogs with CE and hypoalbuminemia with TEG 6s results outside reference interval

TEG 6s variable	RI	Number below RI	Number above RI
R (min)	1.31 to 3.6	0/6 (0%)	3/6 (50%)
K (min)	0.81 to 4.17	2/6 (33%)	0/6 (0%)
Angle (degrees)	53.85 to 77.19	0/6 (0%)	2/6 (33%)
MA (mm)	40.04 to 65.12	0/5 (0%)	2/5 (40%)
RapidTEG™ MA (mm)	40 to 62.47	0/5 (0%)	2/5 (40%)
FF MA (mm)	34.01 to 52	0/5 (0%)	5/5 (100%)†

ANGLE alpha angle, FF functional fibrinogen, K clot kinetics, MA maximum amplitude, MIN minutes, MM millimetres, R reaction time, RI reference interval
†Portion of dogs at upper bound of RI

Functional Fibrinogen MA, was identified in 14 of the 19 dogs with CE. When Functional Fibrinogen MA was not taken into account, 7 of the 19 dogs with CE were defined as hypercoagulable.

The CCECAI was moderately positively correlated with angle ($r = 0.509$, $P = 0.025$). The CCECAI was not correlated with K ($P = 0.05$), R ($P = 0.75$), MA ($P = 0.11$), RapidTEG™ MA ($P = 0.17$) or Functional Fibrinogen MA ($P = 0.38$).

DISCUSSION

In our study, dogs with CE had several TEG 6s alterations compared to healthy dogs. Overall, dogs with CE had significantly prolonged R, shortened K, increased angle, increased MA and increased Functional Fibrinogen MA compared to healthy dogs. These TEG alterations are suggestive of hypercoagulability except for prolonged R, which is typically observed with hypocoagulability due to a quantitative or qualitative coagulation factor deficiency (Burton & Jandry, 2020). Humans with inflammatory bowel disease also experience several TEG alterations, including shortened R, shortened K, increased angle and increased MA compared to healthy controls (Yonghua et al., 2020). Previous studies evaluating TEG parameters in dogs with CE using the TEG 5000 found that CE dogs had shortened K, increased angle and increased MA as well. In contrast to the present study, the previous studies found R to be shortened in their populations of dogs with CE (Goodwin et al., 2011; Wennogle et al., 2021).

It is unclear why there is a difference in the R values between studies. Dogs with CE may have decreased production or function of coagulation factors that the TEG 6s was able to detect. There is a high prevalence of vitamin K and D deficiency in humans with inflammatory bowel disease, which is likely due to malabsorption of fat-soluble vitamins (Kuwabara et al., 2009). Vitamin K deficiency can lead to decreased production of vitamin K-dependent coagulation factors, including clotting factors II, VII, IX and X and antithrombotic protein C and protein S (Girolami et al., 2018). Dogs with CE also have lower levels of vitamin D compared to healthy dogs (Titmarsh et al., 2015). To the authors' knowledge, vitamin K status has not been evaluated in dogs with CE. Future studies may be aimed at evaluating prothrombin time, partial thromboplastin time, protein induced by vitamin K absence-II concentration, individual coagulation factor concentrations and/or protein C in dogs with CE. Additionally, protein C or protein S deficiency due to vitamin K deficiency could serve as a mechanism for hypercoagulability and thrombosis in dogs with CE (Kelly et al., 2020).

Another explanation for the discrepancy in R values among studies is a difference in technology. A previous study revealed that the TEG 5000 and TEG 6s measurements are not directly interchangeable in dogs. This may be explained by how the TEG 5000 uses a cup-and-pin method to assess clot strength and stability, whereas the TEG 6s uses a resonance-frequency method (Wheeler et al., 2022). In a busy practice setting, the TEG 6s cartridge-based system offers a practical option for rapidly assessing the coagulation status of patients in comparison to traditional TEG units, such as the TEG 5000. To the author's knowledge, the current study was the first to use the TEG 6s in a population of dogs with naturally occurring illness.

Disease severity, assessed with the CCECAI and the presence of hypoalbuminemia, had a mild impact on TEG 6s parameters. The CCECAI was moderately correlated with angle and had no correlation with R, K, MA, RapidTEG™ MA or Functional Fibrinogen MA in CE dogs. A previous study found no association between hypercoagulability and Canine Inflammatory Bowel Disease Activity Index scores in dogs with CE (Dixon et al., 2021). In the present study, K was shortened and angle and MA were increased in CE dogs with hypoalbuminemia compared CE dogs with normal serum or plasma albumin concentration. In contrast to the present study, Dixon

found no difference in K, MA and angle between dogs with CE and hypoalbuminemia and those with normal serum or plasma albumin while using the TEG 5000 (Dixon et al., 2021). Overall, disease severity is not a consistent predictor of TEG alterations in CE dogs.

Of our 19 dogs with CE, 7 (37%) were assessed as hypercoagulable based on established criteria. When including CE dogs with Functional Fibrinogen MA at the upper bound of the reference interval, 14 (82%) dogs with CE were identified as hypercoagulable. Using a previously published definition for hypercoagulability where the G value is ≥25% of the upper end of the reference interval (Dixon et al., 2021), 10 (53%) of our dogs with CE were hypercoagulable. However, since the G value is a mathematical alteration of the MA only, several other potential markers for hypercoagulability are not included. Although a universally accepted definition for hypercoagulability assessed by TEG does not yet exist, for clinical purposes, significant alterations in any of the four main parameters are often used to suggest presence of hypercoagulability. Robust studies assessing the predictive value of hypercoagulability on TEG for thrombotic complications do not yet exist in veterinary medicine, making the consideration and reporting of all variables important in both research and clinical settings (Hanel et al., 2014). While the data reported here is insufficient to make recommendations regarding thromboprophylaxis, it may be considered for dogs with CE. Until more studies are completed, clinicians might consider viscoelastic testing results in combination with all other findings to make more informed therapeutic decisions.

There are several limitations in the present study. Some dogs had TEG 6s parameters that were at the upper or lower bound of the machine-specific reportable value. When this inherent limitation of the TEG 6s occurred, the value of the limit was reported. Older models of TEG, including the TEG 5000, also have limits of detection, which can be problematic while using them as well. Additionally, due to analytical error, Functional Fibrinogen level was not available for 15 CE dogs, so this TEG 6s parameter was not reported.

The TEG Functional Fibrinogen assay eliminates the platelet contribution to clot strength with the use of a platelet inhibitor, abciximab. When used in conjunction with the Kaolin TEG assay in humans, the TEG Functional Fibrinogen assay allows for estimation of the fibrinogen contribution to clot strength. In contrast to humans, abciximab has no effect on TEG tracings in dogs (Brainard et al., 2011). The TEG Functional Fibrinogen assay should potentially be avoided in dogs due to this limitation. The TEG Functional Fibrinogen level could not be assessed in this study due to analytic error likely associated with dogs having values that significantly exceeded the upper limit of the analytic range. Similar to a previous study evaluating the TEG 6s in healthy dogs, the majority of CE dogs had TEG Functional Fibrinogen MA values that exceeded the upper limit of the analytic range (Wheeler et al., 2022). The lack of platelet inhibition likely leads to stronger clot strength in dogs compared to humans and ultimately higher Functional Fibrinogen MA values in dogs. Additionally, there are no data evaluating the interpretation of TEG Functional Fibrinogen MA levels in ill dogs with hypercoagulability. Due to the limitations of the TEG Functional Fibrinogen assay, the significance of finding elevated Functional Fibrinogen MA in 82% of dogs with CE is overall unclear and may not truly represent hypercoagulability.

The present study had relatively small case numbers for determining correlations between TEG 6s parameters and the CCECAI scores, which may have resulted in a type II statistical error. The control group, which included 40 healthy adult dogs, was not developed specifically for the purpose of this present study. Although not a direct comparison with the study population, the control group was developed using the exact same TEG 6s unit, which was operated by one of the investigators in the present study and patients were enrolled from the same referral hospital population. Additionally, data was collected for the TEG 6s control group during an overlapping time period with the present study.

In conclusion, dogs with CE have several TEG 6s alterations suggestive of hypercoagulability, except for prolonged R time. Additionally, the TEG 6s is a point-of-care viscoelastic assay that can be easily implemented into clinical practice to identify hemostatic derangements in ill dogs, such as those with CE.

Acknowledgements

The authors thank past and current veterinary technicians, including Lauren Scaletta, Lindsay Bartowski and Kris Wagher at the Virginia-Maryland College of Veterinary Medicine that helped make the study possible. The authors would also like to thank Dr. Katie Boes for her support throughout the study.

Author contributions

S. I. Barth: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); resources (lead); writing – original draft (lead); writing – review and editing (lead). **S. M. DeMonaco:** Conceptualization (supporting); data curation (supporting); formal analysis (supporting); investigation (equal); methodology (equal); resources (equal); supervision (equal); writing – review and editing (equal). **B. J. Conner:** Data curation (supporting); formal analysis (equal); investigation (supporting); methodology (equal); resources (equal); writing – review and editing (equal). **A. R. Wilkinson:** Conceptualization (lead); data curation (lead); formal analysis (lead); funding acquisition (lead); investigation (lead); methodology (lead); project administration (lead); resources (lead); supervision (lead); writing – original draft (lead); writing – review and editing (lead).

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Allenspach, K., Wieland, B., Gröne, A. & Gaschen, F. (2007) Chronic enteropathies in dogs: evaluation of risk factors for negative outcome. *Journal of Veterinary Internal Medicine*, **21**, 700–708.

Arvanitakis, K.D., Arvanitaki, A.D., Karkos, C.D., Zintzaras, E.A. & Germanidis, G.S. (2021) The risk of venous thromboembolic events in patients with inflammatory bowel disease: a systematic review and meta-analysis. *Annals of Gastroenterology*, **34**, 680–690.

Bae, J., Kim, H., Kim, W., Kim, S., Park, J., Jung, D. et al. (2019) Therapeutic monitoring of rivaroxaban in dogs using thromboelastography and prothrombin time. *Journal of Veterinary Internal Medicine*, **33**, 1322–1330.

Brainard, B., Abed, J. & Koenig, A. (2011) The effects of cytochalasin D and abciximab on hemostasis in canine whole blood assesse by thromboelastography and the PFA-100 platelet function analyzer system. *Journal of Veterinary Diagnostic Investigation*, **23**, 698–703.

Brooks, A.C., Guillaumin, J., Cooper, E.S. & Couto, G.C. (2014) Effects of hematocrit and red blood cell-independent viscosity on canine thromboelastographic tracings. *Transfusion*, **54**, 727–734.

Burton, A. & Jandry, K. (2020) Use of thromboelastography in clinical practice. *Veterinary Clinics of North America. Small Animal Practice*, **50**, 1397–1409.

Ceriello, A. (1993) Coagulation activation in diabetes mellitus: the role of hyperglycaemia and therapeutic prospects. *Diabetologia*, **36**, 1119–1125.

Dandrieux, J. & Mansfield, C. (2019) Chronic enteropathy in canines: prevalence, impact and management strategies. *Veterinary Medicine (Auckland, N.Z.)*, **10**, 203–214.

Dixon, A., Hall, E.J., Adamantos, S., Kathrani, A., McGarth, C. & Black, V. (2021) Hypercoagulability in dogs with chronic enteropathy and association with serum albumin concentration. *Journal of Veterinary Internal Medicine*, **35**, 860–866.

Flatland, B., Koenigshof, A., Rozanski, E., Goggs, R. & Wiinberg, B. (2014) Systematic evaluation of evidence of veterinary viscoelastic testing part 2: sample acquisition and handling. *Journal of Veterinary Emergency and Critical Care*, **24**, 30–36.

Fresno, L., Moll, J., Peñalba, B., Espada, Y., Andaluz, A., Prandi, D. et al. (2005) Effects of preoperative administration of meloxicam on whole blood platelet aggregation, buccal mucosal bleeding time, and haematological indices in dogs undergoing elective ovariohysterectomy. *Veterinary Journal*, **170**, 138–140.

Friedrichs, K.R., Harr, K.E., Freeman, K.P., Szladovits, B., Walton, R.M., Barnhart, K.F. et al. (2012) ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. *Veterinary Clinical Pathology*, **41**, 441–452.

Fry, W., Lester, C., Etedali, N.M., Shaw, S., DeLaforcade, A. & Webster, C.R.L. (2017) Thromboelastography in dogs with chronic hepatopathies. *Journal of Veterinary Internal Medicine*, **31**, 419–426.

Geffre, A., Concordet, D., Braun, J.P. & Trumel, C. (2011) Reference value advisor: a new freeware set of macroinstructions to calculate reference intervals with Microsoft Excel. *Veterinary Clinical Pathology*, **40**, 107–112.

Girolami, A., Ferrari, S., Cosi, E., Santarossa, C. & Randi, M.L. (2018) Vitamin K-dependent coagulation factors that may be responsible for both bleeding and thrombosis (FII, FVII, FIX). *Clinical Applications of Thrombosis and Hemostasis: CATHE*, **24**, 42S–47S.

Goodwin, L.V., Goggs, R., Chan, D.L. & Allenspach, K. (2011) Hypercoagulability in dogs with protein-losing enteropathy. *Journal of Veterinary Internal Medicine*, **25**, 273–277.

Haemonetics Corporation. (2024) TEG®6s hemostasis system user manual. Boston, MA: Haemonetics Corporation.

Hanel, R.M., Chan, D.L., Conner, B., Gauthier, V., Holowaychuk, M., Istvan, S. et al. (2014) Systemic evaluation of evidence on veterinary viscoelastic testing part 4: definitions and data reporting. *Journal of Veterinary Emergency and Critical Care*, **24**, 47–56.

Jacinto, A.M.L., Ridyard, A.E., Aroch, I., Watson, P.J., Morrison, L.R., Chandler, M.L. et al. (2017) Thromboembolism in dogs with protein-losing enteropathy with non-neoplastic chronic small intestinal disease. *Journal of the American Animal Hospital Association*, **53**, 185–192.

Jergens, A.E., Turrentine, M.A., Kraus, K.H. & Johnson, G.S. (1987) Buccal mucosa bleeding times of healthy dogs and of dogs in various pathologic states, including thrombocytopenia, uremia, and von Willebrand's disease. *American Journal of Veterinary Research*, **48**, 1337–1342.

Ke, C.H., Liu, C.C., Wang, S.L. & Lin, C.S. (2023) Paired analysis of d-dimer and its correlated hemostatic parameters in 30 dogs with neoplasmas after tumorectomy. *Animals (Basel)*, **13**, 969.

Kelly, D., Juvet, F. & Moore, G. (2020) Congenital protein C deficiency and thrombosis in a dog. *Journal of Veterinary Internal Medicine*, **34**, 1300–1303.

Kuwabara, A., Tanaka, K., Tsugawa, N., Nakase, H., Tsuji, H., Shide, K. et al. (2009) High prevalence of vitamin K and D deficiency and decreased BMD in inflammatory bowel disease. *Osteoporosis International*, **20**, 935–942.

Le Boedec, K. (2019) Reference interval estimation of small sample sizes: a methodologic comparison using a computer-simulation study. *Veterinary Clinical Pathology*, **48**, 335–346.

McLaughlin, C.M., Marks, S.L., Dorman, D.C., Motsinger-Reif, A. & Hanel, R.M. (2017) Thromboelastographic monitoring of the effect of unfractionated heparin in healthy dogs. *Journal of Veterinary Emergency and Critical Care*, **27**, 71–81.

Mehta, J.L. (1985) Influence of calcium-channel blockers on platelet function and arachidonic acid metabolism. *American Journal of Cardiology*, **55**, 158b–164b.

Neff-Davis, C.A., Davis, L.E. & Gillette, E.L. (1981) Warfarin in the dog: pharmacokinetics as related to clinical response. *Journal of Veterinary Pharmacology and Therapeutics*, **4**, 135–140.

Nielsen, L., Holm, J., Rozanski, E., Meola, D., Price, L.L. & de Laforcade, A. (2019) Multicenter investigation of hemostatic dysfunction in 15 dogs with acute pancreatitis. *Journal of Veterinary Emergency and Critical Care*, **29**, 264–268.

Othman, M., Powell, S., Chirinian, Y., Hegadorn, C., Hopman, W. & Lillicrap, D. (2009) Thromboelastography reflects global hemostatic variation among severe haemophilia A dogs at rest and following acute exercise. *Haemophilia*, **15**, 1126–1134.

Park, F.M., Blois, S.L., Abrams-Ogg, A.C.G., Wood, R.D., Allen, D.G., Nykamp, S.G. et al. (2013) Hypercoagulability and ACTH-dependent hyperadrenocorticism in dogs. *Journal of Veterinary Internal Medicine*, **27**, 1136–1142.

Pedersen, H.D., Häggström, J., Olsen, L.H., Christensen, K., Selin, A., Burmeister, M.L. et al. (2002) Idiopathic asymptomatic thrombocytopenia in Cavalier King Charles spaniels is an autosomal recessive trait. *Journal of Veterinary Internal Medicine*, **16**, 169–173.

Probst, A., Lill, H. & Strein, K. (1988) Beta-adrenergic receptor mediated release of tissue plasminogen activator in anaesthetized dogs. *Thrombosis Research*, **50**, 9–17.

Rose, L.J., Dunn, M.E., Allegret, V. & Bedard, C. (2011) Effect of prednisone administration on coagulation variables in healthy beagle dogs. *Veterinary Clinical Pathology*, **40**, 426–434.

Saati, S., Abrams-Ogg, A.C.G., Blois, S.L. & Wood, R.D. (2018) Comparison of multiplate, platelet function Analyzer-200, and plateletworks in healthy dogs treated with aspirin and clopidogrel. *Journal of Veterinary Internal Medicine*, **32**, 111–118.

Titmarsh, H., Gow, A.G., Kilpatrick, S., Sinclair, J., Hill, T., Milne, E. et al. (2015) Association of vitamin D status and clinical outcome in dogs with a chronic enteropathy. *Journal of Veterinary Internal Medicine*, **29**, 1473–1478.

Vilar, P., Couto, C.G., Westendorf, N., Iazbik, C., Charske, J. & Marin, L. (2008) Thromboelastographic tracings in retired racing greyhounds and in non-greyhound dogs. *Journal of Veterinary Internal Medicine*, **22**, 374–379.

Volod, O. & Runge, A. (2023) Mesaphy. In: Favaloro, E.J. & Gosselin, R.C. (Eds.) Hemostasis and thrombosis methods and protocols, 2nd edition. New York: Springer Science+Business Media, LLC, pp. 709–724.

Wennogle, S.A., Olver, C.S. & Shropshire, S.B. (2021) Coagulation status, fibrinolysis, and platelet dynamics in dogs with chronic inflammatory enteropathy. *Journal of Veterinary Internal Medicine*, **35**, 892–901.

Westgarth, S., Blois, S.L., Wood, R.D., Verbrugghe, A. & Ma, D.W. (2018) Effects of omega-3 polyunsaturated fatty acids and aspirin, alone and combined, on canine platelet function. *Journal of Small Animal Practice*, **59**, 272–280.

Wheeler, L.R., Edwards, T.H., Heinz, J.A., Scott, L.L.F., Grantham, L.E., 2nd, Keesee, J.D. et al. (2022) Comparison of assessment of coagulabion in healthy dogs by the TEG 6s and TEG 5000 viscoelastic analyzers. *Journal of Veterinary Diagnostic Investigation*, **34**, 780–788.

Yonghua, S., Shi, L., Zhang, J., Zhu, H., Yao, Y., Liu, Z. et al. (2020) Thromboelastography in patients with inflammatory bowel disease. *Gastroenterology Research and Practice*, **2020**, 1–5.