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ORIGINAL STUDY

Comparison of healthy blood donor Greyhounds and non-Greyhounds using a novel point-of-care viscoelastic coagulometer

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Abstract

Objective: To measure and compare viscoelastic coagulation in 2 canine blood donor populations using a novel, point-of-care device (VCM Vet Analyzer, VCM).

Design: Cohort study.

Setting: Academic and commercial veterinary blood banks.

Animals: Non-Greyhounds from community-based blood donor program and Greyhounds from a blood bank colony.

Intervention: Blood was collected from all dogs via direct venipuncture for a complete hemogram, biochemistry, and point-of-care viscoelastic coagulation.

Measurement and main results: All biochemical measurements for all dogs in Group NG (n = 38, non-Greyhounds) and Group G (n = 53, Greyhounds) were within local reference intervals. Hematology data showed significant statistical differences between groups in hemoglobin, RBC, platelet, and WBC concentrations. Group G demonstrated lower maximum clot firmness (MCF) with 17 VCM units (26 VCM units in Group NG), increased lysis with 30 VCM units at 30 minutes (LI30) and 27 VCM units at 45 minutes (LI45) (86 VCM units LI30 and 85 VCM units LI45 in Group NG), and decreased amplitude of 13 VCM units 10 minutes (A10) after clot time (CT) and 6 VCM units 20 minutes after CT (A20) (18 VCM units [A10] and 22 VCM units [A20] in Group NG).

Conclusion: This study found differences between healthy Greyhound and non-Greyhound blood donors in measures of clot strength and fibrinolysis as measured by the VCM. Whereas Greyhound have unique hematologic and hemostatic profiles, these measured viscoelastic differences are important to note prior to and following surgical intervention to aid in clinical decision-making if bleeding complications develop.

KEYWORDS
blood donor program, canine, clot formation, coagulopathy, hemostasis

ABBREVIATIONS: A10, amplitude at 10 minutes after CT; A20, amplitude at 20 minutes after CT; AA, alpha angle; CFT, clotting formation time; CT, clotting time; Hgb, hemoglobin; LI30, lysis 30 minutes after MCF; LI45, lysis 45 minutes after MCF; MCF, maximum clot firmness; ROTEM, rotational thromboelastometry; TEG, thromboelastography; VCM, Vet viscoelastic coagulometer.

1 | INTRODUCTION

Viscoelastic tests provide data about clot formation and its physical strength, in addition to any dissolution by following the change in elasticity over time using a mechanical detection system. They also provide another window into hemostasis not achievable via traditional coagulation tests (eg, prothrombin time, partial thromboplastin time, and fibrinogen concentration),[1] through the detection of abnormalities such as hypercoagulability and hyperfibrinolysis.[2] The most common viscoelastic test systems currently used in veterinary medicine are thromboelastography (TEG) and rotation thromboelastometry (ROTEM), both of which have limited use due to the expense and required technical skill.[3,4]

The differences between TEG and ROTEM have been published in the veterinary and human medical literature.[3–6] These technologies measure the viscoelastic properties of clotting blood in a sample cup. The ROTEM rotates the pin within the blood in the stationary sample cup, whereas the TEG pin is stationary and the cup rotates. As clot formation ensues, fibrin fibrils physically link the pin to the cup. ROTEM traces are produced from a deflection in the angle of light directed at the pin/wire transduction system while the rotational movement of the pin in TEG is sensed via the mechanical-electrical transducer and converted into an electrical signal for display.[4]

A novel, point-of-care viscoelastic device[a] uses frosted glass discs held in parallel on flexible plastic arms within a cartridge. The narrow space between these discs holds the blood sample introduced via capillary action from a detachable sample cup. This surface triggers coagulation through contact activation. The plastic arms interact with optical sensors within the analyzer to assess the differences in proportional movement between the stationary and oscillatory arms, and differences in these movements over time are calculated via device software and graphically displayed. Similar to the other viscoelastic technologies, the VCM displays fibrinolysis as a percentage of maximum clot firmness (MCF) measured at 30 and 45 minutes after clot time (CT).

The VCM has the potential to reach broader veterinary audiences as it does not require sample manipulation. As soon as untreated whole blood sample is loaded into the cartridge, the sample is monitored in real-time in a self-contained automated system. It is less expensive, requires less technical skill, and uses smaller amounts (0.25-0.5 mL) of fresh whole blood compared to TEG and ROTEM. VCM reference intervals have been established for both dogs and cats.[b,c]

Greyhound dogs possess unique hematologic and coagulation characteristics that are uncommonly seen in other breeds. Greyhounds have higher mean values for hemoglobin, hematocrit, and mean corpuscular volume and mean lower values for neutrophils, lymphocytes, monocytes, eosinophils, and platelets compared to other breeds.[7,8] Greyhounds, compared to other breeds, have slower clotting kinetics and a weaker clot strength as measured with TEG.[9] One study also documented 26% of Greyhounds to have postoperative bleeding likely due to enhanced fibrinolysis.[10] It is currently unknown if the VCM can detect differences in coagulation or fibrinolysis between Greyhound and non-Greyhound breeds.

Our study aims to measure and compare viscoelastic coagulation in 2 canine blood donor populations (Greyhounds vs non-Greyhounds) using VCM. We hypothesized that Greyhounds have weaker clot strength and hyperfibrinolysis compared to non-Greyhound blood donors.

2 | METHODS

Healthy, non-Greyhounds from the community-based UC Davis Veterinary Blood Bank (Group NG) and group-housed Australian Greyhounds from Formosa Vet Canine and Feline Blood Center of Taiwan (Group G) were enrolled. Group NG has Institutional Animal Care and Use Committees approval for inclusion in this study; Group G received local clinical trials review board approval. Dogs with missing clinical or clinicopathological data, less than 100,000 platelets/μL, hemoglobin less than 10 g/dL, WBC concentration $< 4000/\mu$L, or undetected VCM values were excluded. For all dogs, 5 mL of blood was collected into a syringe from direct venipuncture of the cephalic or jugular vein using a 22- or 23-Ga needle. According to VCM operator instructions, 300 μL of this whole blood sample was placed into the sample addition cup of the VCM cartridge within 4 minutes of venipuncture. When blood has completed its flow into the cartridge, as noted by visualization through the cartridge window, the sample addition cup is removed before immediate insertion of the cartridge into the device. The residual blood was equally divided into EDTA tubes (for CBC) and serum tubes (for biochemistry panel) and analyzed within 24 hours using commercially available hematology and chemistry analyzers[d,e] for Group G and Group NG.[f,g]

Descriptive statistics were completed. All data were assessed for normality using a Shapiro–Wilk normality test. If possible, non-normally distributed data were transformed after viewing results from a ladder of power test to determine the best transformation of the data to achieve a normally distributed data set. Normally distributed data are presented as a mean and standard deviation while non-normally distributed data are presented as a median and range. To look for differences in CBC and VCM parameters, a *t*-test or a Wilcoxon rank-sum test was done for normally and non-normally distributed data, respectively, and $P < 0.05$ was considered statistically significant. Statistics were completed using commercial software.[h]

3 | RESULTS

The sex distribution favored intact dogs in Group G (n = 53) over Group NG (n = 38). Group NG comprised 1 intact male, 18 neutered males, 4 intact females, and 15 neutered females. Group G had 12 intact males, 23 neutered males, and 18 intact females. Multiple VCM devices were used for measurements. Each one passed daily quality assurance checks. Group NG dogs were measured on 2 devices, and Group G dogs were measured on 3 different devices. VCM cartridges for each site were from the same unique batch and lot number with the same expiry date.

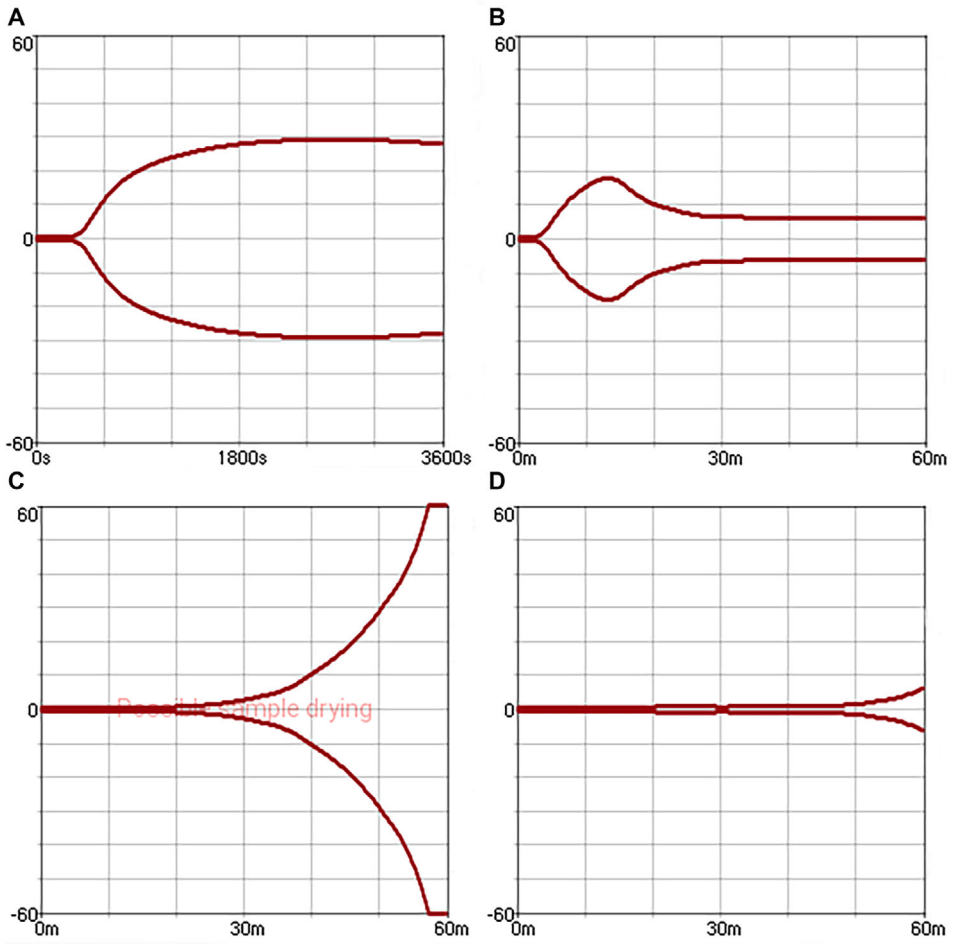

FIGURE 1 Representative VCM traces from healthy blood donor dogs where the X axis is VCM units and the Y axis is time in seconds (s) or minutes (m). (A) Non-Greyhound, (B) Greyhound, (C) Greyhound with "possible sample drying" artifact, and (D) Greyhound with poor separation. Multiple VCM measurements are unobtainable in (C) and (D) and are examples of excluded data. range. Dots represent outlying values. VCM, Vet viscoelastic coagulometer

Group NG initially comprised 42 dogs, and Group G comprised 69 dogs. Four dogs in Group NG were excluded due to missing clinical or clinicopathological data. The total number of exclusions in Group G was 16 dogs: missing clinical or clinicopathological data (n = 2), less than 100,000 platelets/μL (n = 2), leukocytes < 4000/μL (n = 4), or undetected VCM values (n = 8). Unusual shapes of these VCM traces with undetected values (n = 6) showed no separation (eg, no alpha angle measurement) of the lines suggesting inappropriate initial fibrin formation. The other VCM traces with missing values displayed a "possible sample drying" warning indicator (n = 2). These traces had a missing alpha angle, MCF, or lysis parameter data. Figure 1 provides examples of representative traces for each group in addition to examples of traces with undetected values.

All biochemical measurements for all dogs in Groups NG and G were within local reference intervals (data not shown). Hematology data (see Table 1) show statistical differences between groups in the following parameters: hemoglobin (P = 0.03), WBC (P < 0.0001), platelets (P = 0.003), and RBCs (P < 0.0001). Non-normally distributed data (platelet, clot formation time [CFT], clot firmness at 20 minutes after clot time [CT, A20], amplitude of the clot at 30 [LI30] and 45 [LI45] minutes after CT as a percentage of MCF in Group NG, and platelet, cCFT, alpha angle [AA], A20, LI30, LI45 in Group G) was log or inverse transformed for analysis (see Tables 1 [CBC] and 2 [VCM] and Figure 2).

The comparison of data for each VCM parameter is shown in Table 2. Statistically significant differences exist between groups NG and G in the values related to clot strength and stability.

4 | DISCUSSION

Greyhounds showed weaker clot strength and increased lysis as measured by a novel point-of-care viscoelastic testing device. Speculated reasons for a weaker clot strength in Greyhounds are due to their lower platelet concentration and function, lower enzymatic function, and slow clot kinetics.[9]

TABLE 1 Summarized CBC results of Group NG (non-Greyhound dogs, n = 38) and Group G (Greyhounds, n = 53)

	Group NG		Group G		
Parameter (Unit)	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	*P*-value
Hgb (g/dL)*	17.1 (1.7)	17.3 (13.6-20)	18.1 (2.5)	17.9 (13.9-23.9)	0.03
RBC (M/μL)*	6.8 (0.8)	6.7 (5.0-8.6)	9.5 (1.4)	9.5 (6.9-12.3)	<0.0001
WBC (/μL)*	7,409 (1921)	7,105 (4,360-12,960)	10,098 (2,230)	10,200 (5,830-15,550)	<0.0001
PLT (K/μL)‖	207 (48)	193 (117-300)	265 (98)	247 (102-595)	0.003

Normally distributed parameters are indicated by the (*). Parameters that were analyzed after statistical transformation are indicated by (§) if they became normal or (‖) if they remained non-normally distributed.

TABLE 2 Summarized VCM results of Group NG (non-Greyhound dogs, n = 38) and Group G (Greyhound dogs, n = 53)

	Group NG		Group G		
Parameter (Unit)	Mean (SD)	Median (Range)	Mean (SD)	Median (Range)	*P*-value
CT (seconds)*	288.7 (84.6)	293.5 (138-458)	275.4 (52.2)	276 (144-390)	0.36
CFT (seconds)‖	255.9 (102.5)	249.5 (161-810)	242.7 (114.6)	204 (114-798)	0.08
AA (degrees)§	51.2 (8.4)	53 (30-63)	50.5 (8.0)	53 (26-65)	0.66
A10 (VCM units)*	17.6 (3.0)	17.5 (8-24)	13.3 (5.9)	13 (4-27)	0.0001
A20 (VCM units)‖	21.8 (6.3)	23 (3-31)	6.5 (3.9)	5 (3-20)	<0.0001
MCF (VCM units)*	26.0 (5.7)	26.5 (13-38)	17.1 (4.4)	17 (10-29)	<0.0001
LI30 (%)‖	86.4 (21.0)	93 (17-100)	29.5 (13.9)	25 (12-100)	<0.0001
LI45 (%)‖	84.9 (24.7)	98 (15-100)	27.3 (12.9)	25 (9-95)	<0.0001

Normally distributed parameters are indicated by the (*). Parameters that were analyzed after statistical transformation are indicated by (§) if they became normal or (‖) if they remained non-normally distributed.

4.1 | Lysis

Our study found significant differences in lysis parameters on the VCM (LI30 and LI45) as well as MCF, A10, and A20 between the 2 blood donor groups. Clinical experience and research documentation finds a significant proportion of Greyhounds with postoperative bleeding after routine gonadectomy[10] and other surgical interventions. Increased postoperative bleeding in Greyhounds has been attributed to increased fibrinolysis and not a primary or secondary hemostatic disorder.[10] In this subpopulation, lower concentrations of preoperative antiplasmin were documented, indicating enhanced fibrinolysis, but viscoelastic testing was not reported, and lysis-specific biomarkers (eg,

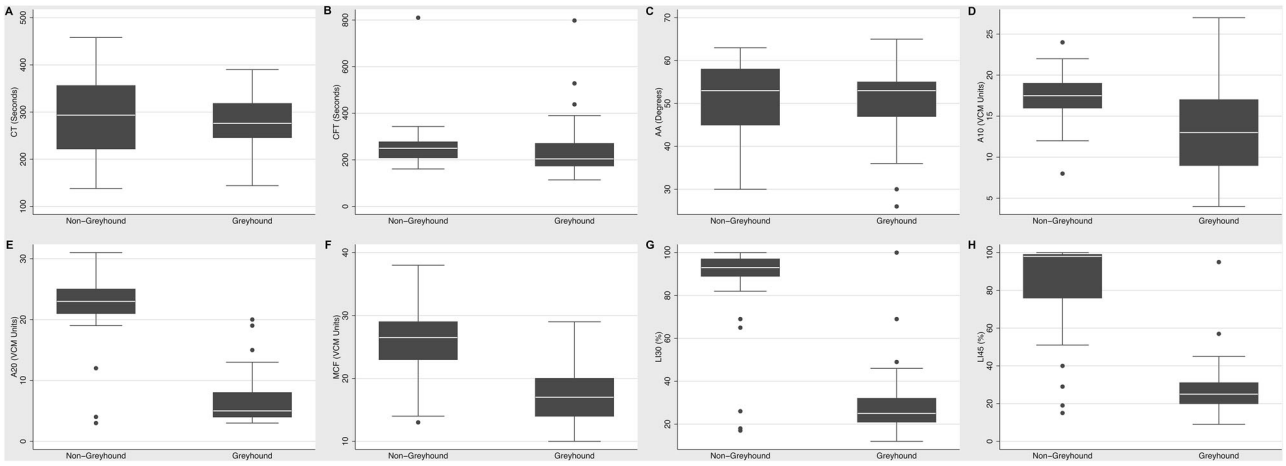

FIGURE 2 Box and whisker plots of the 8 VCM measurements for both groups: NG (non-Greyhounds) and G (Greyhounds). Each panel displays a box that demonstrates the 25th and 75th percentile with a horizontal line as median. The length of the whiskers represents the highest or lowest value that is less than or equal to 1.5 times the interquartile. VCM, Vet viscoelastic coagulometer

antiplasmin, D-dimers, or plasminogen) were not measured.[10] Vilar et al found all TEG parameters different between bleeders and nonbleeders in their study of retired racing Greyhounds except for the R time and Ly60 using $CaCl_2$ as an activator.[9] Viscoelastic testing provides a unique method to document fibrinolysis in veterinary patients.[3,4] However, Fletcher et al were also unable to demonstrate significant fibrinolysis using TEG in citrated native whole blood in dogs with spontaneous hemoperitoneum or controls.[11] It was not until the addition of 50 U/mL tissue plasminogen activator that differences were found in lysis parameters.[11] It is unknown if the VCM technology is more sensitive to detect fibrinolysis compared to the TEG or ROTEM. Although the measurements are not identical or directly interchangeable due to different technologies, both determined weaker clot strength in Greyhounds.

No inferences can be made because thromboelastography provides complementary diagnostic information that is not directly translatable from traditional coagulation testing.[1] Future studies to detail these and other biomarkers may be indicated to uncover the biological reason for such profound lysis in this group of clinically normal Greyhounds.

The VCM can be easily used in Greyhounds as well as in other breeds. A normal canine reference interval was recently established for the VCM with its measurements correlated to TEG measurements (with and without tissue factor activation).[i] These data are very similar to our Group NG data, although the dog breeds were not identified in this abstract. Our Group G measurements of A10, A20, and MCF are less than their reference interval, also suggesting weaker clot strength for our Greyhounds when compared to their 48 healthy dogs assessed. Lysis data were not reported in this study.

4.2 | Platelets

Greyhounds possess higher PCV, hemoglobin concentration, RBC concentration and, therefore, blood viscosity compared to other breeds.[7,12–14] WBC, platelet, and neutrophil concentrations are lower than other breeds. Our data concur with these reports except that the Greyhounds in the current study had a statistically significant but clinically insignificant higher platelet concentration when compared to the non-Greyhounds. The difference in platelet concentration found between groups in this study is clinically insignificant; however, it may influence variability of clot strength and overall hemostasis. Platelets contribute to fibrinolysis via binding of fibrinolytic proteins to the cell surface. For example, the plasminogen binding capacity of platelets is very robust, leading to localized and enhanced fibrinolytic activity.[15] A recent abstract found dog platelets have increased adhesion to fibrinogen and the highest contractile forces compared to human, porcine, and mouse platelets,[j] which may also lead to enhanced clot contraction and subsequent lysis.[16] It is difficult to identify the specific contribution of platelets within the overall hemostatic profile during viscoelastic testing unless using preanalytical modifications and specific reagents. As this technology has not been adapted to use various activators and relies on native blood directly from the patient as a true point-of-care device, preanalytical sample manipulation would be difficult should one attempt to create any modifications for analysis conditions.

Platelets have a larger effect in the measurement of clot strength (MA as measured on the TEG, MCF on the VCM) compared to the reaction time (R) where initial fibrin formation is the key contributor.[4] Weaker overall clot strength has been documented where no difference in lysis times was found between Greyhounds and other breeds.[9] Our study found weaker clot strength in spite of a higher concentration of platelets in Greyhounds, which may have contributed to the increase in fibrinolysis over the non-Greyhound group.

Visualization of the blood smears to confirm or refute the machine platelet count was not completed. However, platelet clumps were not detected or noted by either cytometer.

4.3 | PCV

Higher PCV may lead to less plasma per unit volume of blood, which is known to decrease clot strength and kinetics in vitro even in platelet-free samples.[17] The influence of higher PCVs, which are typical in Greyhounds and other sighthounds, on viscoelastic measures is a concern as the blood viscosity increases and the relative amount of plasma is decreased.[9] It is not known if this effect on viscoelastic measurements is a direct result of the high PCV or an intrinsic limitation of the technology.[4] Our Greyhounds exhibited higher PCVs than the non-Greyhounds as anticipated. An in vitro study that explored the effect of hematocrit and viscosity (adjusted using alginate or saline and not autologous blood) found TEG variables were influenced by whole blood viscosity independently of hematocrit.[18] The traces in this study appeared hypercoagulable with decreasing hematocrit. The opposite may be true with our Greyhounds, comparatively hypocoagulable (decreased A10, A20, and MCF) with higher PCVs. The influence of viscosity or hematocrit has not yet been studied using the VCM.

It is unknown what may contribute to the unique hemostatic profile of this group of healthy blood donor Greyhounds. They are retired racing Greyhounds from Australia that are phenotypically nonbleeders; however, they have not undergone major surgical challenge to their hemostatic balance except for castration (n = 23 of 53). They may also have a genetic predisposition or unique diet that predisposes them to weaker clot strength or increased fibrinolysis. The non-Greyhounds in this study are from a community-based blood donor program and are truly diverse in their genetics and diets. Most have undergone at least 1 surgery (n = 33 of 38) and are phenotypically also nonbleeders. Racing Greyhounds fed a raw meat diet have been reported to have a cutaneous and renal vasculopathy[19] that clinically appears as postoperative bleeding. The diet of the Greyhounds in this study includes approximately 60% cooked chicken or beef mixed with 40% dry kibble. As these Greyhound blood donors are prospectively monitored and may eventually undergo surgery, data on their hemostasis will be documented to investigate similar bleeding tendencies to the previously reported retired racing Greyhounds.[10]

Another theory for the considerable difference in lysis parameters may be clot separation from the plates within the VCM cartridge. This apparent fibrinolytic profile may to some extent be related to the loss of contact with the frosted plates within the VCM cartridge, as evidenced by a rapid decrease in clot strength from MCF to LI30 and LI45. Inspection of the cartridges and the clot produced between the plates may be valuable in future studies.

Transport and handling of the cartridges under different conditions may also add another level of uncertainty to the veracity of our data as each group was housed in a different site.

To fully appreciate these results, the limitations of this study must be acknowledged. As previously mentioned, this study was conducted using different blood donor populations and different VCM devices in 2 countries. Due to the different sites and devices used, a variability between the devices used on each group may exist. However, daily quality assurance tests were completed and passed for each device. Individual cartridges may also have created undue variation based on any potential manufacturing flaw; however, lot and expiry dates were the same within each group. Eight Greyhounds were excluded due to undetected VCM values whereas there were none in the non-Greyhound group. These exclusions were due to abnormal VCM traces with missing values for parameters such as alpha angle, MCF, or lysis parameters.

Operator error may have been another reason for the undetected values as each group was sampled and measured by different people. Two of these samples had "possible sample drying" warnings displayed, which may also have been operator-induced. If the sample dries inappropriately within the cartridge during measurement, the data could be affected as this would alter the viscoelastic properties of clot formation and dissolution over time in that sample. The groups also have a different sex distribution, and the influence of hormonal status cannot be fully excluded. As expected, the baseline hematology data are different between groups, with Greyhounds possessing higher PCV and RBC concentration, as examples. We also excluded different amounts of data from Group NG (n = 4) and Group G (n = 16), decreasing our numbers significantly in Group G and making a Type II error more likely. A power analysis was not calculated in advance to detect the sample size needed as we did not have a predicted effect size. However, the overall statistical results suggest that the data are statistically significantly different for measures of clot strength (MCF) and lysis (LI30, LI45). Finally, we did not pair the VCM data with other viscoelastic testing (such as TEG or ROTEM), analysis of platelet function, or biomarkers of hemostasis/fibrinolysis.

Because the VCM data demonstrated considerable hyperfibrinolysis in Greyhounds, future studies to assess the effect of various antifibrinolytic medications may be helpful to optimize treatment. More detailed biological information on the other potential causes of a weaker clot strength and fibrinolysis should also be characterized in future investigations.

This study found differences between healthy Greyhound and non-Greyhound blood donors in measures of clot strength and fibrinolysis as measured by the VCM. Whereas Greyhounds have unique hematologic and hemostatic profiles, these measured viscoelastic differences are important to note prior to and following surgical intervention to aid in clinical decision-making if bleeding complications develop.

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ENDNOTES

[a] VCM, VCM Vet, Entegrion, Inc, Research Triangle Park, NC.
[b] Stata 14.4, Stata Corporation, College Station, TX.
[c] Copeland R, Oshinowo OT, Sakurai Y, et al. A comparative medicine study of platelet biophysics among hemostasis models of different species. American Society of Hematology Annual Meeting, San Diego, CA, December 2018. *Blood*. 2018;132(Suppl 1):869.
[d] IDEXX proCyte Dx, Westbrook, ME.
[e] IDEXX Catalyst Dx, Westbrook, ME.
[f] Roche Cobas c501 Clinical Chemistry Analyzer, Indianapolis, IN.
[g] Siemens ADVIA 120 Hematology System, Munich, Germany.
[h] Stata 14.4, Stata Corporation, College Station, TX.
[i] Stata 14.4, Stata Corporation, College Station, TX.
[j] Copeland R, Oshinowo OT, Sakurai Y, et al. A comparative medicine study of platelet biophysics among hemostasis models of different species. American Society of Hematology Annual Meeting, San Diego, CA, December 2018. *Blood*. 2018;132(Suppl 1):869.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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