THROMBOELASTOGRAPHIC PLATELET MAPPING IN DOGS WITH COMPLICATED *BABESIA ROSSI* INFECTION

PLATELET MAPPING IN CANINE BABESIOSIS

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ABSTRACT

Background: Dogs with *Babesia rossi* infection display a normocoagulable thromboelastogram, despite being markedly thrombocytopenic. This is purportedly due to large-scale platelet activation. Thromboelastographic platelet mapping (TEG-PM) evaluates individual contributions of thrombin, fibrinogen and platelets to clot formation, and may elucidate some of the pathomechanisms of thrombocytopenia-associated hemostatic alterations.

Objective: This study investigated potential differences in TEG-PM variables in dogs with complicated *B. rossi* infection compared to healthy controls, and whether these variables correlated with indices of platelet activation.

Methods: The maximum amplitude (MA) following thrombin generation ($MA_{Thrombin}$) was determined using kaolin-activated TEG. TEG-PM variables included MA following addition of platelet agonists arachidonic acid (MA_{AA}) and adenosine diphosphate (MA_{ADP}), and MA due to fibrin alone (MA_{Fibrin}). In addition, platelet indices and fibrinogen concentration were determined.

Results: Thirteen dogs with complicated *B. rossi* infection and five healthy controls were included. The median MA_{Fibrin} and fibrinogen concentration were significantly higher ($P < .01$ for both) and platelet count was significantly lower ($P < .01$) in the babesiosis group versus controls. No significant differences were found for $MA_{Thrombin}$ and $MA_{AA/ADP}$. MA_{Fibrin} was positively correlated with fibrinogen concentration ($r = 0.735$), mean platelet volume ($r = 0.517$) and mean platelet mass ($r = 0.498$), and negatively correlated with hematocrit ($r = –0.685$), platelet count ($r = –0.476$) and plateletcrit ($r = –0.479$) ($P < .05$ for all).

Conclusions: This study suggests that the presence of hyperfibrinogenemia offsets the severe thrombocytopenia associated with *B. rossi* to result in normal thromboelastograms and lack of overt clinical bleeding.

Keywords

Dogs; Hyperfibrinogenemia; MA_{Fibrin}; Thrombocytopenia

INTRODUCTION

Canine babesiosis is an intra-erythrocytic tick-borne protozoal disease, of which *Babesia rossi* is the most virulent form.[1] The pathogenesis involves a marked pro-inflammatory host response which is likely responsible for the development of complicated disease.[2,3] Severe thrombocytopenia is common in canine babesiosis, yet such cases seldom display overt signs of hemorrhage[4,5], and have been reported to be normocoagulable.[5] While evidence exists that platelet activation secondary to the inflammatory process may offset the thrombocytopenia[5–7], the receptor-mediated mechanisms by which this occurs are unknown.

Platelet activation occurs when an agonist binds to a receptor, leading to activation of downstream signaling pathways to result in degranulation, translocation of negatively charged membrane phospholipids to the outer leaflet, and activation of the glycoprotein $(GP)_{IIbIIIa}$ receptor responsible for binding fibrinogen. Agonists include thrombin, collagen, adenosine diphosphate (ADP), and thromboxane A_2 (TxA_2) generated from arachidonic acid (AA).[8] Activated platelets provide a procoagulant surface for large-scale thrombin generation.[8,9]

Thromboelastography (TEG) is a global assessment of hemostasis[10], and relies on thrombin generation to cleave fibrinogen and activate platelets.[11] The maximum amplitude (MA) is a function of the dynamic interaction between platelets and fibrin(ogen).[10] This modality provides information regarding the patient's maximum hemostatic potential, but is non-specific for platelet activity.[10] Thromboelastographic platelet mapping (TEG-PM) evaluates the individual contributions of thrombin, fibrin and platelet activity to clot formation.[12] Heparinized blood, in combination with an activator, bypasses thrombin activity, allowing for assessment of the contributions of fibrin and milder platelet agonists such as ADP and AA.[11,12]

In people, TEG-PM is frequently employed to monitor the efficacy of antiplatelet therapy.[13] TEG-PM has been reported to be useful for identifying alterations in platelet receptor function during cardiopulmonary bypass[14], traumatic brain injury[15] and multisystem trauma.[16] There is a paucity of literature regarding TEG-PM in veterinary medicine. To date, pilot studies have been conducted in dogs[17] and cats[11], and a small number of studies have used TEG-PM to assess the effects of various medications on platelet function.[18–20] Only one study has focused on TEG-PM findings in dogs with a clinical disease associated with hypercoagulability, namely hyperadrenocorticism (HAC).[21]

The objectives of this study were to investigate possible differences in TEG-PM variables in dogs with complicated *B. rossi* infection compared to healthy controls, and determine whether TEG-PM variables correlated with any hematological variables.

MATERIALS AND METHODS

This prospective study was conducted on client-owned dogs presenting to the Onderstepoort Veterinary Academic Hospital (OVAH) of the University of Pretoria, South Africa. The babesiosis group consisted of dogs naturally infected with *B. rossi* with clinical or laboratory evidence of complicated disease. The initial diagnosis of babesiosis was made by demonstrating the presence of intra-erythrocytic trophozoites on a stained thin blood film. Infection with *B. rossi* was confirmed by means of PCR and reverse line blot (RLB).[22,23] Healthy dogs presenting for routine sterilization or blood donation were included as controls. This study was approved by the University of Pretoria's Animal Ethics Committee (Protocol number V098-15; 30 October 2015). Signed owner consent was required to enroll an animal in the study.

Animals

Dogs of any breed and either sex with demonstrable parasitemia were considered for inclusion into the babesiosis group, provided they were older than six months and weighed more than 3.5 kg. The infected dogs had to demonstrate one or more of the following manifestations of complicated babesiosis: secondary immune-mediated hemolytic anemia (IMHA; marked spherocytosis, positive warm in-saline agglutination or Coombs test result), acute kidney injury (AKI; oliguria/anuria and persistent azotemia unresponsive to appropriate fluid therapy), hypoglycemia (blood glucose < 3.3 mmol/L), cerebral babesiosis (neurological signs not attributable to any other cause), hepatopathy with cholestasis (icterus, marked bilirubinuria, hyperbilirubinemia, raised liver enzyme activities), acute respiratory distress syndrome (ARDS; dyspnea, adventitious lung sounds, frothy blood-tinged nasal discharge, blood-gas evidence of ventilation-perfusion mismatch, radiological evidence of edema and lung consolidation), hemoconcentration (congested mucous membranes, high-normal or raised HCT with evidence of concurrent intravascular hemolysis), and pancreatitis (vomiting, cranial abdominal pain, melena and icterus; raised serum lipase activity; or ultrasonographic evidence of acute pancreatitis).[1,24,25] The *Babesia*-infected dogs were treated according to the standard protocol in use at the OVAH. This included treatment with diminazene aceturate (Berenil RTU 0.07 g/mL, Intervet South Africa, Spartan, Kempton Park, South Africa), transfusion with blood products, or intravenous fluid therapy as deemed necessary. Complications were treated as needed. Dogs in the control group were deemed clinically healthy based on a physical examination, CBC and blood smear evaluation, serum biochemical profile, and urine analysis, and if they were free of any parasitemia as determined by PCR and RLB. The same requirements regarding age and weight, as mentioned for the babesiosis group, applied.

For both groups, dogs were excluded if they had clinical evidence of any concurrent inflammatory or neoplastic condition, or if any evidence of trauma or wounds was present. Dogs treated with any medications known to interfere with platelet function within four weeks prior to presentation were excluded. Dogs were also excluded if they were found to be co-infected with other tick-borne agents, such as *Ehrlichia canis, B. vogeli, Theileria* and *Anaplasma* species, as determined by PCR and RLB.

Sample collection

At presentation and prior to any treatment (including blood products or intravenous fluids), blood was collected from the jugular vein from each dog using a 21-gauge needle by careful venipuncture with minimum stasis. Blood was collected into serum, sodium citrate, lithium heparin and EDTA vacutainer tubes (BD Biosciences, Franklin Lakes, NJ, USA), in this order. All tubes were filled, using vacuum assistance, to ensure the correct ratio of anticoagulant to blood. The EDTA sample was used to perform the CBC and the PCR/RLB analysis. Standard kaolin-activated TEG was performed on the sodium citrate sample, after which the remainder was centrifuged within one hour at 2100 *g* for 8 minutes. The plasma was stored at –80 °C for determination of fibrinogen concentration and antithrombin activity at a later stage. The lithium heparin sample was used to perform the TEG-PM analysis.

Thromboelastographic platelet mapping analysis

Two thromboelastograph hemostasis analyzers (TEG 5000 Thrombelastograph Hemostasis System, Haemonetics Corporation, Braintree, MA, USA) were used. The sodium citrate and lithium heparin samples were left to equilibrate at room temperature for 30 minutes post-

collection while four TEG cups were pre-warmed to 37 °C. Following equilibration, 1 mL of citrated blood was added to a kaolin-coated vial, which was gently inverted five times. Following addition of 20 µL $CaCl_2$ to the cup in channel 1, 340 µL of citrated kaolin blood was transferred to this cup. Standard TEG analysis was performed to determine MA due to the action of thrombin ($MA_{Thrombin}$). To determine the contribution of cross-linked fibrin to clot formation, 10 µL of the activator substance (Activator F, Haemonetics Corporation, Braintree, MA, USA), a reagent containing reptilase (an enzyme with thrombin-like activity capable of cleaving fibrinogen to fibrin) and Factor XIII (required for fibrin cross-linking) were transferred to the cup in channel 2, followed by 360 µL of heparinized blood. The mixture in the cup was gently mixed by partially aspirating the contents three times, after which MA_{Fibrin} was measured. To evaluate the contribution of platelet agonist receptors to clot formation, 10 µL of Activator F and 360 µL of heparinized blood were added to channels 3 and 4. Then, 10 µL of ADP were added to channel 3 to yield a final ADP concentration of 2 µmol/L. Similarly, 10 µL of AA were added to channel 4 to result in a final AA concentration of 1 mmol/L. Both samples were gently mixed, after which the MA_{ADP} and MA_{AA} were determined.

Hematology variables

A CBC was performed on an optical-based automated hematology system (ADVIA 2120, Siemens, Munich, Germany) and variables of interest included HCT, platelet count (PLT), plateletcrit (PCT), MPV, mean platelet mass (MPM), and mean platelet component concentration (MPC). Analysis was performed within 30 minutes of sample collection.

Plasma fibrinogen concentration and antithrombin activity

Plasma fibrinogen concentration was determined by the Clauss method using an automated coagulometric analyzer (ACL Elite, Instrumentation Laboratory, Bedford, MA, USA). Plasma antithrombin (AT) activity was assessed using a thrombin-dependent chromogenic substrate assay (Precimat Chromogen, Roche, Basel, Switzerland) on an automated analyzer (Cobas Integra 400 Plus, Roche, Basel, Switzerland). A normal pooled control sample, with a presumed activity of 100%, was run with each batch of tests, and the patient AT activity was normalized against the pooled control sample.

Statistical analysis

Statistical analyses were performed using a commercial software package (SPSS Statistics 24.0 Software; SPSS Inc., Armonk, NY, USA). Data were inspected for normality using the Shapiro-Wilk test and found not to display a normal distribution. Differences between groups were assessed using the Mann-Whitney *U* test. Correlations were determined using Spearman's rank correlation coefficient. Gender proportions between groups were compared using the Fisher's exact test. Data are presented as median and interquartile range (IQR). In addition, interindividual CV for patient and control groups were calculated for the TEG-PM variables by dividing the group standard deviation by the group mean and multiplying this by 100. A *P* value of $< .05$ was considered statistically significant.

RESULTS

Animals

Thirteen dogs were included in the babesiosis group, and five dogs in the control group. There were no significant differences in age between the groups, with the median patient age 24

months (12–52) and the median control age 48 months (19–82). The ratio of male: female for the *Babesia*-infected dogs was 7: 6 and the controls 3: 2, with no significant difference between the groups. The following complications were observed either singly or in combination: IMHA (*n* = 4), ARDS (*n* = 2), hepatopathy with cholestasis (*n* = 3), AKI (*n* = 2), neurological signs (*n* = 4), hypoglycemia (*n* = 1) and hemoconcentration (*n* = 3).

Thromboelastographic platelet mapping analysis

The TEG-PM variables are shown in Table 1. Median $MA_{Thrombin}$ did not differ significantly between the groups (Figure 1A); however, MA_{Fibrin} was significantly higher in the babesiosis group compared to the control group (Figure 1B). No significant differences were observed between groups for median MA_{ADP} (Figure 1C) and MA_{AA} (Figure 1D). Inter-individual CV for the babesiosis and control groups were 21.1% and 5.9%, respectively for $MA_{Thrombin}$; 38.9% and 41.9%, respectively for MA_{Fibrin}; 48.3% and 92.9%, respectively for MA_{ADP}; and 38.7% and 41.6%, respectively for MA_{AA}. Representative TEG-PM tracings from a dog with babesiosis and a control dog are also shown to illustrate these findings (Figure 2).

Hematological variables

Results are shown in Table 1. The median HCT, PLT and PCT were significantly decreased, with the MPV and MPM significantly increased in the babesiosis group compared to the control group. No significant difference was noted for MPC.

Table 1. Descriptive statistics of thromboelastographic platelet mapping and hematology variables for the *Babesia*-infected and healthy control dogs.

Variable (Unit)	Control dogs (n = 5) Median (IQR) Range	*Babesia*-infected dogs (n = 13) Median (IQR) Range	*P*-value
$MA_{Thrombin}$ (mm)	56.3 (55.9–61.0) 55.8–63.9	51.0 (43.1–62.2) 31.8–67.3	0.218
MA_{Fibrin} (mm)	5.6 (3.4–7.8) 2.5–8.4	28.3 (23.5–36.6) 13.9–58.1	0.001*
MA_{ADP} (mm)	15.9 (7.8–60.5) 7.6–69.4	29.5 (24.2–48.1) 11.0–67.5	0.402
MA_{AA} (mm)	44.8 (28.6–61.4) 16.6–65.7	44.9 (29.4–58.9) 15.3–67.7	0.805
Hematocrit (L/L)	0.49 (0.47–0.56) 0.47–0.58	0.18 (0.14–0.34) 0.10–0.63	0.007*
Platelet count ($\times 10^9$/L)	225 (155–347) 151–467	29 (22–50) 5–166	0.003*
Plateletcrit (%)	0.22 (0.18–0.43) 0.18–0.55	0.06 (0.04–0.11) 0.01–0.22	0.003*
Mean platelet volume (fL)	11.8 (10.6–12.8) 9.6–13.6	21.1 (16.6–23.2) 9.8–28.1	0.007*
Mean platelet mass (pg)	2.24 (1.74–2.42) 1.60–2.53	3.09 (2.68–3.48) 1.67–3.62	0.012*
Mean platelet component concentration (g/dL)	21.5 (18.6–23.0) 16.9–24.0	19.7 (19.0–22.0) 12.8–23.9	0.402
Fibrinogen (g/L)	2.6 (1.9–3.0) 1.7–3.0	6.9 (6.3–7.0) 1.2–7.0	0.006*
Antithrombin activity (%)	106 (99–109) 94–110	78 (71–96) 54–108	0.016*

*Denotes statistical significance between groups.

Data are represented as medians (IQR), followed by the minimum-maximum range.
MA indicates maximum amplitude; IQR, interquartile range.

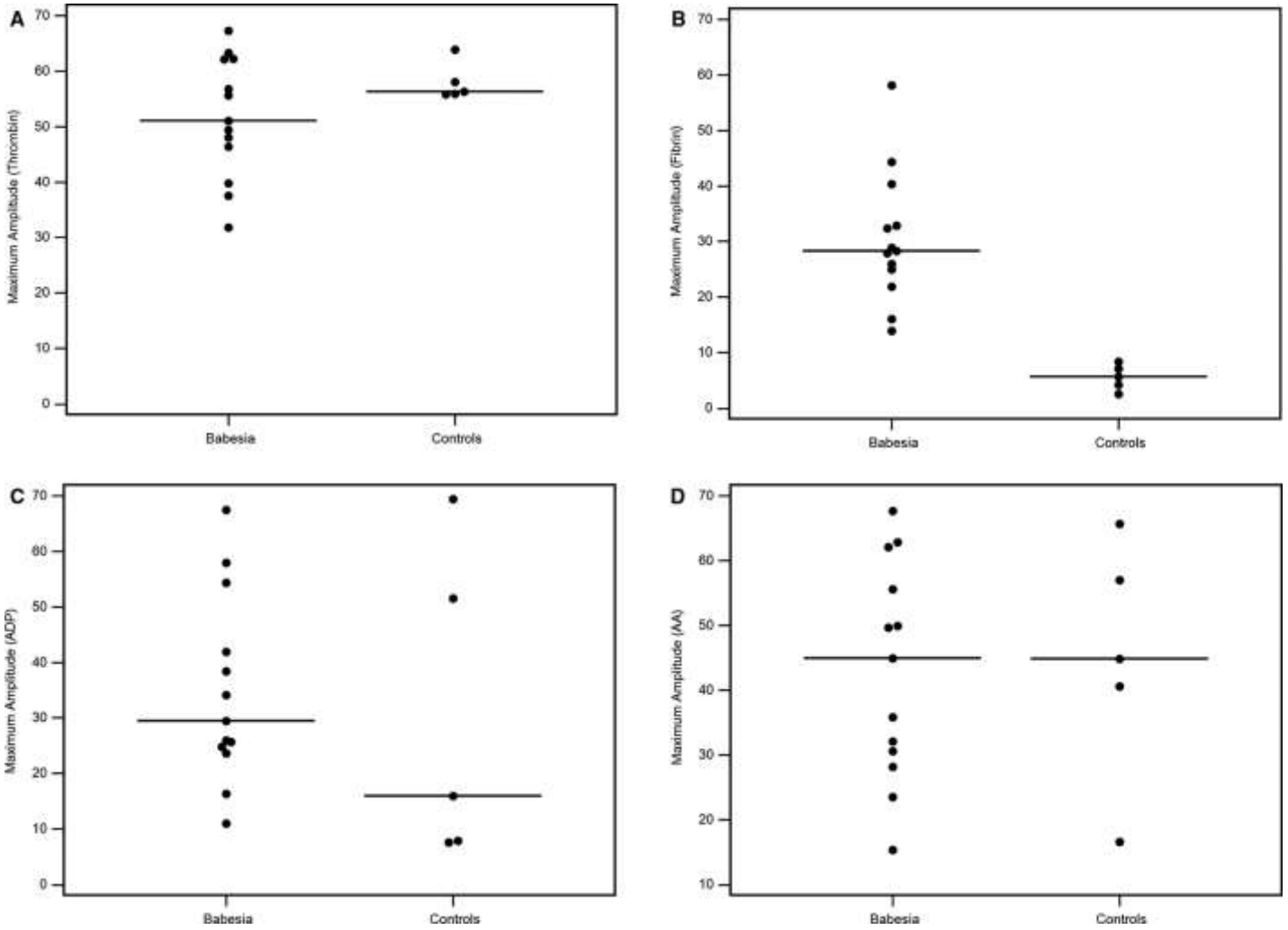

Figure 1. Dot plots of the thromboelastographic platelet mapping variables (A) $MA_{Thrombin}$, (B) MA_{Fibrin}, (C) MA_{ADP}, (D) MA_{AA} of the *Babesia*-infected group compared with the control group. Each dot represents an individual result, and the group medians are indicated by the horizontal lines. Maximum amplitude = MA; ADP = adenosine diphosphate; AA = arachidonic acid

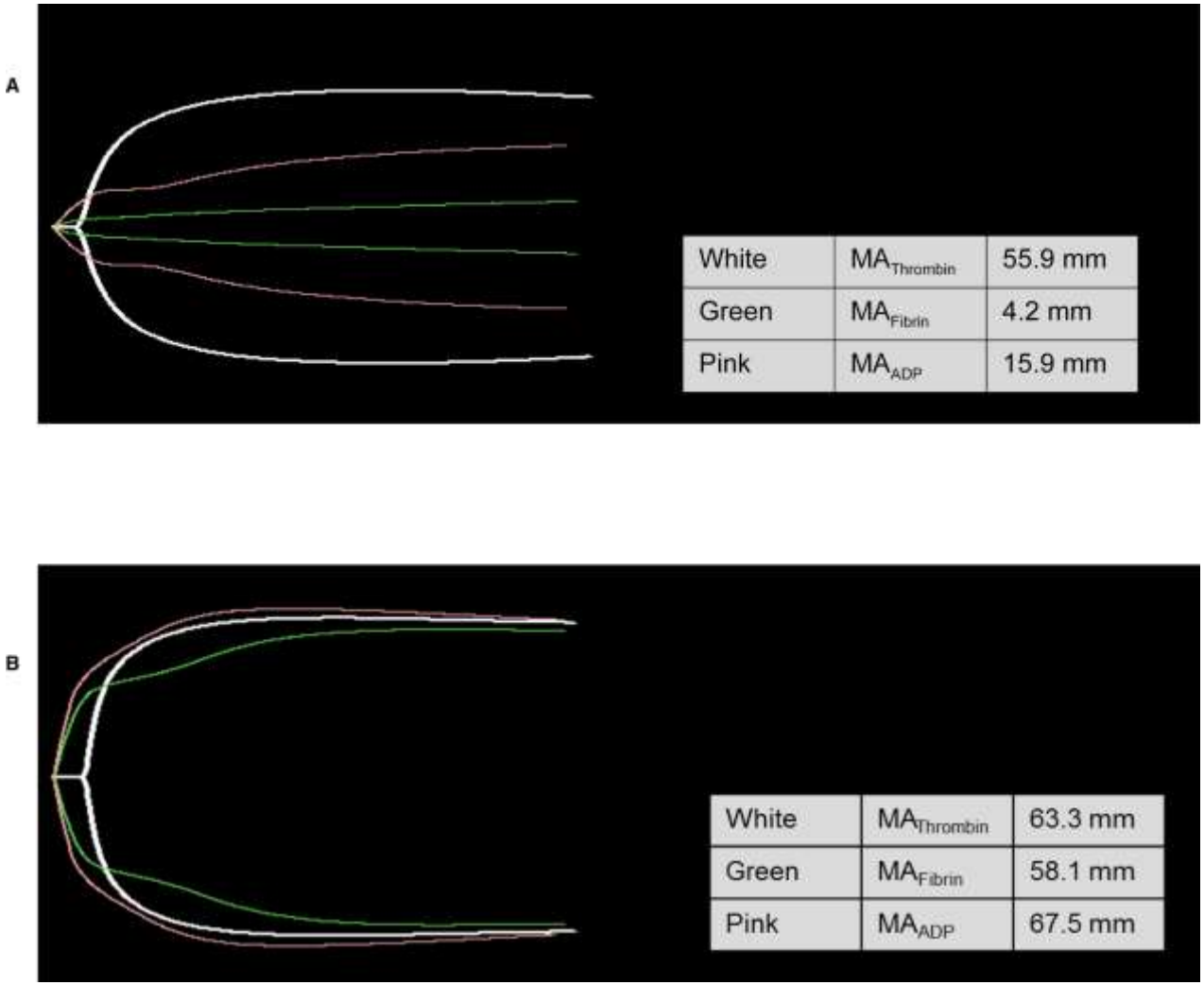

Figure 2. Thromboelastographic platelet mapping tracings from (A) a healthy control dog and (B) a dog with complicated *Babesia rossi* infection. The white line indicates the maximum amplitude due to thrombin generation ($MA_{Thrombin}$); the green and pink lines denote the maximum amplitude due to fibrin (MA_{Fibrin}) and the maximum amplitude due to adenosine diphosphate (MA_{ADP}), respectively

Plasma fibrinogen concentration and antithrombin activity

Results are shown in Table 1. The median fibrinogen concentration was significantly increased and the AT activity was significantly decreased in the babesiosis group compared to the control group.

Correlation analysis

The $MA_{Thrombin}$ had significant strong positive correlations with PLT ($r = 0.709$; $P = 0.001$) and PCT ($r = 0.707$; $P = 0.001$). The MA_{Fibrin} had significant moderate to strong positive correlations with MPV ($r = 0.523$; $P = 0.026$), MPM ($r = 0.509$; $P = 0.031$) and fibrinogen concentration ($r = 0.742$; $P < 0.001$), and significant moderate to strong negative correlations with HCT ($r = –0.690$; $P = 0.002$), PLT ($r = –0.478$; $P = 0.045$), and PCT ($r = –0.481$; $P = 0.044$).

DISCUSSION

This is the first report to show the effect of hyperfibrinogenemia, using TEG-PM, on the hemostatic function of dogs with complicated babesiosis. The findings suggest that the presence of marked hyperfibrinogenemia and the resultant contribution of fibrin to clot strength offsets the severe thrombocytopenia associated with babesiosis, to result in a lack of clinical bleeding.

The study showed no significant difference for $MA_{Thrombin}$ between the *Babesia*-infected and healthy control dogs. $MA_{Thrombin}$ is the maximum amplitude determined using standard kaolin-activated TEG. Our results are similar to a previous report on uncomplicated babesiosis, using tissue factor-activated TEG.[5] The authors in that study suggested two possible reasons for their findings. Since TEG is influenced by HCT, PLT, platelet function, fibrinogen concentration and AT activity, the normocoagulable thromboelastograms could have been the result of the opposing forces of these variables on the overall tracing. The second explanation was that

Babesia-infected dogs remained normocoagulable, despite severe thrombocytopenia, due to marked platelet activation associated with inflammation. Subsequently, studies confirmed the presence of activated platelets in dogs with babesiosis based on the presence of known and surrogate markers of platelet activation, namely increased circulating platelet-monocyte aggregates and increased MPV and MPM.[6,7] Although $MA_{Thrombin}$ may be affected by PLT, platelet function and plasma fibrinogen concentration[10], our study showed a significant correlation with PLT only, but not with any of the markers for platelet activity. HCT is also an important variable to consider, because it has been shown that decreased HCT results in more hypercoagulable viscoelastic tracings.[26,27] However, similar to a previous report, no correlation was seen with HCT in our study cohort.[5]

MA_{Fibrin} was significantly increased in the *Babesia*-infected dogs compared to the controls. Rotational thromboelastometry (ROTEM) studies have demonstrated that adequate fibrinogen concentrations are critical for clot formation.[28–30] Even in the face of severe thrombocytopenia, the maximum clot elasticity (MCE) determined by ROTEM increased exponentially when increasing concentrations of fibrinogen were added.[29] Moreover, the use of ROTEM, modified by the addition of cytochalasin-D (FIBTEM) to isolate the contribution of fibrinogen to clot strength, showed that MCE_{Fibtem} displayed a similar exponential increase as fibrinogen concentrations increased.[29] Canine babesiosis is characterized by an acute phase response with increases in acute phase proteins, including fibrinogen[5,31–33], and all but one of the *Babesia*-infected dogs in our study were hyperfibrinogenemic. The strong positive correlation between MA_{Fibrin} and plasma fibrinogen concentration in our study supports previous reports on the effect of hyperfibrinogenemia in coagulation, especially in the presence of thrombocytopenia. Similar findings have been reported in dogs with pituitary-dependent HAC,

in which affected dogs had hyperfibrinogenemia and higher median MA_{Fibrin} values than healthy controls at all assessed time points.[21] The HAC study also reported a weak positive correlation between MA_{Fibrin} and plasma fibrinogen concentration.

Interestingly, the contribution of platelets to clot formation increases in a similar fibrinogen concentration-dependent pattern. Each platelet bears numerous $GP_{IIbIIIa}$ receptors on its surface, and when activated by thrombin, platelets are able to bind high numbers of fibrinogen molecules.[34] Thus, each individual platelet may become increasingly engaged with fibrinogen via its $GP_{IIbIIIa}$ receptors as the overall platelet count decreases and platelet volume increases.[29] Given the inflammatory nature of the disease which could potentially result in both hyperfibrinogenemia and platelet activation, it is plausible that this mechanism is also at play in dogs with babesiosis. This may explain the significant and positive correlation of MA_{Fibrin} with MPV and MPM. Moreover, enhanced thrombopoiesis and platelet regeneration in response to the inflammatory process and thrombocytopenia may have also resulted in increased MPV and MPM. Younger platelets tend to be larger and more hemostatically active, and both platelet size and immaturity are independent determinants of platelet function.[35,36] The negative correlation between MA_{Fibrin} and PLT is also noteworthy and supports the theory of platelets becoming increasingly engaged with fibrinogen as their numbers decrease, as previously stated.[29] A possible explanation for the negative correlation with HCT is that a defined volume of anti-coagulated blood is used for the TEG assay and erythrocytes act as a functional diluent for plasma, limiting the volume of plasma and plasma coagulation proteins, included in the assay. A whole blood sample with a low HCT will consequently allow for more plasma coagulation proteins, specifically fibrinogen, to be included in the assay.[26,27]

Besides hyperfibrinogenemia, other reasons for high MA_{Fibrin} include prior platelet sensitization and AT deficiency.[11] Although prior platelet sensitization during blood collection cannot be excluded[11,17], a standardized sampling method was employed for our study, which makes this an unlikely reason for the significant difference between the groups. Since heparin requires AT to inhibit thrombin, it is conceivable that the lower median AT activities observed in dogs with babesiosis could have contributed to the increased MA_{Fibrin} values. However, no significant correlation was observed between MA_{Fibrin} and AT activity in our study. In addition, although the study on dogs with HAC reported persistently elevated MA_{Fibrin} compared to controls, the median AT activity was within the reference interval, which further mitigates against low AT activity as a possible cause.

Previous studies in dogs[17], cats[11] and horses[18] showed that the MA_{Fibrin} is prone to a high degree of intra-and inter-individual variability, with extremely high CVs being obtained in the dog study (> 100%). Similarly, the HAC study found significant overlap between MA_{Fibrin} in affected dogs and controls.[21] In our study, the inter-individual CVs for both the babesiosis and control groups were significantly lower (38.9% and 41.6%, respectively) than previously reported values; and although the sample sizes were small, no overlap was seen between the groups. This, together with a plausible underlying pathomechanism to explain the increased MA_{Fibrin}, suggests that the difference between the groups is due to disease and not biological or analytical variation. Nevertheless, assay analytical precision, as well as biological variation, should be investigated further.

The lack of significant difference between groups for MA_{ADP} and MA_{AA} was unexpected, given that platelet activation has been reported to occur in dogs with canine babesiosis. However, the inter-individual variation for both variables was significant, which is similar to previous

studies in dogs[17,37] and cats.[11] There are several potential explanations for the lack of significance, such as the use of suboptimal agonist concentrations.[17] The TEG-PM kit contains 2 μmol/L ADP and 1 mmol/L AA, compared to 5–20 μmol/L of ADP and 1 mmol/L AA typically required in aggregometry.[20] An additional reason could be that the functions of the receptors for ADP and TXA_2 may not be altered appreciably in all dogs during *B. rossi* infection, especially considering the clinical and clinicopathologic heterogeneity seen in infected dogs.[24] However, the study cohort included dogs with complicated disease, which is associated with an excessive pro-inflammatory response.[3] Since ADP is released by platelet dense granules during platelet activation to augment the activation response initiated by other agonists[8], and AA is an important inflammatory mediator, it would seem unlikely that the function of these receptors was unaltered in this population of dogs. It is also possible that the variation in the results is due to biologic variability, as a great deal of heterogeneity exists regarding the response of platelets to various agonists in dogs.[37–39] The lower HCT could also have affected both the MA_{AA} and MA_{ADP} of the *Babesia*-infected group; however, no significant correlation with HCT was observed.

Our study had some limitations. Sample sizes were small due to financial constraints, which may have resulted in failure to detect significant changes in the MA_{ADP} and MA_{AA}. Due to the nature of sample collection from clinical cases, sampling at a standardized time point in the disease course was not possible. This may also have introduced heterogeneity into the study population. More reliable assays for determination of platelet activation, such as flow cytometry or aggregometry, were not performed, thus it was not possible to show conclusively, using surrogate markers of platelet activation such as raised MPM and MPV, that platelet activation occurred in the dogs of this study. In addition, the TEG-PM assay has not been validated for use

in dogs, but several published studies have used this methodology.[11,17–21] Lastly, the analytical variation of the variables is presently unknown.

In conclusion, the findings of this study suggest that hyperfibrinogenemia in the presence of large platelets, secondary to either concurrent activation or regeneration, overrides the severe thrombocytopenia associated with canine babesiosis to result in normal thromboelastograms and absence of clinical bleeding.

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REFERENCES

1. Jacobson LS. The South African form of severe and complicated canine babesiosis: Clinical advances 1994–2004. *Vet Parasitol.* 2006;138:126–139.

2. Welzl C, Leisewitz AL, Jacobson LS, Vaughan-Scott T, Myburgh E. Systemic inflammatory response syndrome and multiple-organ damage/dysfunction in complicated canine babesiosis. *J*

S Afr Vet Assoc. 2001;72:158–162.

3. Goddard A, Leisewitz AL, Kjelgaard-Hansen M, Kristensen AT, Schoeman JP. Excessive pro-inflammatory serum cytokine concentrations in virulent canine babesiosis. *Plos One.* 2016;11:e0150113.

4. Kettner F, Reyers F, Miller D. Thrombocytopaenia in canine babesiosis and its clinical usefulness. *J S Afr Vet Assoc.* 2003;74:63–68.

5. Liebenberg C, Goddard A, Wiinberg B, et al. Hemostatic abnormalities in uncomplicated babesiosis (*Babesia rossi*) in dogs. *J Vet Intern Med.* 2013;27:150–156.

6. Goddard A, Leisewitz AL, Kristensen AT, Schoeman JP. Platelet indices in dogs with *Babesia rossi* infection. *Vet Clin Pathol.* 2015;44:493–497.

7. Goddard A, Leisewitz AL, Kristensen AT, Schoeman JP. Platelet activation and platelet-leukocyte interaction in dogs naturally infected with *Babesia rossi*. *Vet J.* 2015;205:387–392.

8. Wiinberg B, Jessen LR, Tarnow I, Kristensen AT. Diagnosis and treatment of platelet hyperactivity in relation to thrombosis in dogs and cats. *J Vet Emerg Crit Care.* 2012;22:42–58.

9. Hoffman M, Monroe DM. A cell-based model of hemostasis. *Thromb Haemost.* 2001;85:958–965.

10. Mallett S, Cox D. Thrombelastography. *Br J Anaesth.* 1992;69:307–313.

11. Blois SL, Banerjee A, Wood RD. Evaluation of thrombelastographic platelet-mapping in healthy cats. *Vet Clin Pathol.* 2012;41:223–227.

12. Craft RM, Chavez JJ, Bresse SJ, Wortham DC, Cohen E, Carroll RC. A novel modification of the thrombelastograph assay, isolating platelet function, correlates with optical platelet aggregation. *J Lab Clin Med.* 2004;143:301–309.

13. Collyer TC, Gray DJ, Sandhu R, Berridge J, Lyons G. Assessment of platelet inhibition

secondary to clopidogrel and aspirin therapy in preoperative acute surgical patients measured by Thrombelastography® Platelet Mapping™. *Br J Anaesth.* 2009;102:492–498.

14. Weitzel NS, Weitzel LB, Epperson LE, Karimpour-Ford A, Tran ZV, Seres T. Platelet mapping as part of modified thromboelastography (TEG ®) in patients undergoing cardiac surgery and cardiopulmonary bypass. *Anaesthesia.* 2012;67:1158–1165.

15. Davis PK, Musunuru H, Walsh M, et al. Platelet dysfunction is an early marker for traumatic brain injury-induced coagulopathy. *Neurocrit Care.* 2013;18:201–208.

16. Wohlauer MV, Moore EE, Thomas S, et al. Early platelet dysfunction: An unrecognized role in the acute coagulopathy of trauma. *J Am Coll Surg.* 2012;214:739–746.

17. Blois SL, Banerjee A, Wood RD, Park FM. Thromboelastography platelet mapping in healthy dogs using 1 analyzer versus 2 analyzers. *Can J Vet Res.* 2013;77:231–236.

18. Brooks MB, Divers TJ, Watts AE, et al. Effects of clopidogrel on the platelet activation response in horses. *Am J Vet Res.* 2013;74:1212–1222.

19. Conner BJ, Hanel RM, Hansen BD, Motsinger-Reif AA, Asakawa M, Swanson CR. Effects of acepromazine maleate on platelet function assessed by use of adenosine diphosphate activated- and arachidonic acid-activated modified thromboelastography in healthy dogs. *Am J Vet Res.* 2012;73:595–601.

20. Brainard BM, Kleine SA, Papich MG, Budsberg SC. Pharmacodynamic and pharmacokinetic evaluation of clopidogrel and the carboxylic acid metabolite SR 26334 in healthy dogs. *Am J Vet Res.* 2010;71:822–830.

21. Park FM, Blois SL, Abrams-Ogg ACG, et al. Hypercoagulability and ACTH-dependent hyperadrenocorticism in dogs. *J Vet Intern Med.* 2013;27:1136–1142.

22. Matjila PT, Penzhorn BL, Bekker CPJ, Nijhof AM, Jongejan F. Confirmation of occurrence

of *Babesia canis vogeli* in domestic dogs in South Africa. *Vet Parasitol.* 2004;122:119–125.

23. Matjila PT, Leisewitz AL, Jongejan F, Penzhorn BL. Molecular detection of tick-borne protozoal and ehrlichial infections in domestic dogs in South Africa. *Vet Parasitol.* 2008;155:152–157.

24. Jacobson L, Clark I. The pathophysiology of canine babesiosis - New approaches to an old puzzle. *J S Afr Vet Assoc.* 1994;65:134–145.

25. Koster LS, Steiner JM, Suchodolski JS, Schoeman JP. Serum canine pancreatic-specific lipase concentrations in dogs with naturally occurring *Babesia rossi* infection. *J S Afr Vet Assoc.* 2015;86:Art.#1297. http:// dx.doi.org/10.4102/jsava. v86i1.1297.

26. McMichael MA, Smith SA, Galligan A, Swanson KS. *In vitro* hypercoagulability on whole blood thromboelastometry associated with *in vivo* reduction of circulating red cell mass in dogs. *Vet Clin Pathol.* 2014;43:154–163.

27. Smith SA, McMichael MA, Gilor S, Galligan AJ, Hob CM. Correlation of hematocrit, platelet concentration, and plasma coagulation factors with results of thromboelastometry in canine whole blood samples. *Am J Vet Res.* 2012;73:789–798.

28. Fries D, Innerhofer P, Reif C, et al. The effect of fibrinogen substitution on reversal of dilutional coagulopathy: An in vitro model. *Anesth Analg.* 2006;102:347–351.

29. Lang T, Johanning K, Metzler H, et al. The effects of fibrinogen levels on thromboelastometric variables in the presence of thrombocytopenia. *Anesth Analg.* 2009;108:751–758.

30. Zentai C, Solomon C, van der Meijden PEJ, et al. Effects of fibrinogen concentrate on thrombin generation, thromboelastometry parameters, and laboratory coagulation testing in a 24-hour porcine trauma model. *Clin Appl Thromb Hemost.* 2016;22:749–759.

31. Goddard A, Wiinberg B, Schoeman JP, Kristensen AT, Kjelgaard-Hansen M. Mortality in virulent canine babesiosis is associated with a consumptive coagulopathy. *Vet J.* 2013;196:213–217.

32. Schetters TPM, Kleuskens JAGM, De Crommert JV, De Leeuw PWJ, Finizio A, Gorenflot A. Systemic inflammatory responses in dogs experimentally infected with *Babesia canis*; a haematological study. *Vet Parasitol.* 2009;162:7–15.

33. Koster LS, van Schoor M, Goddard A, Thompson PN, Matjila PT, Kjelgaard-Hansen M. C-reactive protein in canine babesiosis caused by *Babesia rossi* and its association with outcome. *J S Afr Vet Assoc.* 2009;80:87–91.

34. Wagner C, Mascelli M, Neblock D, Weisman H, Coller B, Jordan R. Analysis of GPIIb/IIIa receptor number by quantification of 7E3 binding to human platelets. *Blood.* 1996;88:907–914.

35. Thompson C, Jakubowski J, Quinn P, Deykin D, Valeri C. Platelet size as a determinant of platelet-function. *J Lab Clin Med.* 1983;101:205–213.

36. Thompson C, Jakubowski J, Quinn P, Deykin D, Valeri C. Platelet size and age determine platelet-function independently. *Blood.* 1984;63:1372–1375.

37. Blois SL, Lang ST, Wood RD, Monteith G. Biologic variability and correlation of platelet function testing in healthy dogs. *Vet Clin Pathol.* 2015;44:503–510.

38. Clemmons R, Meyers K. Acquisition and aggregation of canine blood-platelets - Basic mechanisms of function and differences because of breed origin. *Am J Vet Res.* 1984;45:137–144.

39. Nielsen LA, Zois NE, Pedersen HD, Olsen LH, Tarnow I. Platelet function in dogs: Breed differences and effect of acetylsalicylic acid administration. *Vet Clin Pathol.* 2007;36:267–273.