

Evaluation of Hemostatic Abnormalities in Canine Spirocerosis and Its Association with Systemic Inflammation

P. Pazzi, A. Goddard, A.T. Kristensen, and E. Dvir

Background: Canine spirocerosis is caused by the nematode *Spirocerca lupi* and is characterized by esophageal fibro-inflammatory nodules that may undergo neoplastic transformation. No sensitive and specific laboratory assays other than histopathology have been reported to differentiate non-neoplastic from neoplastic disease.

Hypothesis/Objectives: Dogs with spirocerosis will have evidence of hypercoagulability based on thromboelastography (TEG)-derived maximal amplitude (MA); increased MA will be correlated with increased acute phase protein (APP) concentrations (C-reactive protein [CRP] and fibrinogen); increased MA and APPs will be exacerbated with neoplastic spirocerosis.

Animals: Thirty-nine client-owned dogs with naturally occurring spirocerosis and 15 sex-matched healthy controls.

Methods: A prospective comparative study evaluating TEG, activated partial thromboplastin time, prothrombin time, antithrombin (AT) activity, platelet count and D-dimer concentration, and APPs of dogs with non-neoplastic (n = 24) and neoplastic (n = 15) spirocerosis compared to control dogs.

Results: Median MA was significantly increased in the non-neoplastic group ($P < .01$) and neoplastic group ($P < .01$) compared to the controls. Both APPs were significantly increased in the neoplastic group compared to the non-neoplastic and control groups. MA was strongly correlated with fibrinogen ($r = 0.85$, $P < .001$) and CRP ($r = 0.73$, $P < .001$). An MA >76 mm provided 96% specificity and 73% sensitivity for differentiation of disease state.

Conclusions and Clinical Importance: Canine spirocerosis is associated with increased TEG variables, MA and α , and decreased AT activity, which may indicate a hypercoagulable state seemingly more severe with neoplastic transformation. MA was correlated with APP in dogs with spirocerosis and can be used as an adjunctive test to support the suspicion of neoplastic transformation.

Key words: Hemostasis; Hypercoagulable; Inflammation; Spirocerca.

S*pirocerca lupi* (*S. lupi*) is a canine nematode infesting the esophageal wall resulting in the formation of fibro-inflammatory nodules.¹ The nodules may undergo neoplastic transformation into osteosarcoma (60–85% of neoplastic cases), fibrosarcoma, or anaplastic sarcoma in up to 26% of dogs.^{1–3} Early diagnosis of neoplastic transformation, preferentially by noninvasive means, is crucial to facilitate early and improved intervention which includes surgical excision and chemotherapy. Excluding histopathology of the nodule, no sensitive and specific laboratory assays have been identified to confirm a diagnosis of neoplastic transformation.

Neoplastic transformation has been reported to be closely linked with inflammation.^{4–6} Evidence of systemic inflammation in spirocerosis includes pyrexia,

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Submitted October 20, 2012; Revised August 19, 2013; Accepted September 10, 2013.

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10.1111/jvim.12220

Abbreviations:

APP	acute phase proteins
aPTT	activated partial thromboplastin time
AT	antithrombin
CBC	complete blood count
CRP	C-reactive protein
G	global clot strength
Ht	hematocrit
IQR	interquartile range
K	clotting time
LY30	clot lysis at 30 minutes
LY60	clot lysis at 60 minutes
MA	maximal amplitude
PT	prothrombin time
r	correlation coefficient
ROC	receiver operator curve
R	reaction time
<i>S. lupi</i>	<i>Spirocerca lupi</i>
TEG	thromboelastography
α	angle

leukocytosis as well as increased C-reactive protein (CRP) and IL-8 concentrations in the blood.^{7–9} Systemic inflammation has been reported to result in activation of hemostasis,¹⁰ and subsequent alterations in the inflammatory and hemostatic variables in spirocerosis are expected. Definitions of hypercoagulability based on TEG variables have not yet been established for animals and numerous variables are used in different studies to define hypercoagulability, including maximal amplitude (MA) or global clot strength (G) alone or in combination with shortened reaction time (R), increased angle (α), or both.^{11,12} Increased MA has been reported

to be indicative of hypercoagulability in studies on animals,^{11,13} and a single variable may be easier to interpret and may have greater clinical application. Evidence to support hemostatic alterations in spirocercosis includes three case reports of aorto-iliac thromboemboli that were attributed to the migration of *S. lupi* larvae and associated vascular injury.¹⁴⁻¹⁶ Studies have reported decreased AT activity in dogs with spirocercosis, suggesting the presence of systemic hemostatic changes in this disease.^{17,18}

We hypothesized that (1) dogs with spirocercosis will have evidence of hypercoagulability based on TEG-derived variable, MA; (2) increased MA will be correlated with increased APP concentrations (CRP and fibrinogen); and (3) increased MA and APP concentrations will be exacerbated in dogs with neoplastic spirocercosis and may be used to differentiate dogs with neoplastic transformation.

Materials and Methods

A prospective clinical study was performed on 41 client-owned dogs with naturally occurring spirocercosis that were presented to the Onderstepoort Veterinary Academic Hospital. This study was approved by the Faculty Research Committee and the Animal Ethics committee of the University of Pretoria, South Africa.

All dogs included in the study were older than 6 months, weighed more than 6 kg and signed consent was obtained from owners for enrollment in the study. Upon admission, a complete history, clinical examination, urinalysis, fecal flotation, complete blood count (CBC), serum biochemistry profile, and thoracic radiographs were performed. Diagnosis of spirocercosis was based on positive fecal flotation,¹⁹ thoracic radiographs (at least 2 of the following radiographic signs: a caudodorsal mediastinal mass, spondylitis of the caudal thoracic vertebrae or undulation of the lateral border of the descending aorta), or esophageal endoscopy.

Infected dogs were divided into 2 groups, non-neoplastic or neoplastic. The diagnosis of non-neoplastic spirocercosis was based on the endoscopic visualization of esophageal nodules with smooth surfaces and regression of the nodules at follow-up endoscopy, 6 weeks after treatment. Neoplastic transformation was based on histopathology with samples obtained by endoscopically guided biopsy, surgical excision or at necropsy. Fifteen healthy sex-matched dogs were included as controls.

Dogs were excluded from the study if they had received any macrocyclic lactone in the previous 6 months, showed signs of

trauma or wounds or had been previously diagnosed with cardiac, hepatic, or renal disease. Any dogs showing concurrent systemic or inflammatory disease conditions not known to be associated with spirocercosis on clinical examination, hematology, serum biochemistry, urinalysis, and thoracic radiographs were excluded. In addition, if signs of concurrent disease were present after endoscopic regression of the nodule in the non-neoplastic group or concurrent disease was found at necropsy in the neoplastic group, the dogs were excluded from the study. Any previously diagnosed inherited coagulopathy or treatment with medication known to interfere with normal hemostasis (including corticosteroids of any kind, nonsteroidal anti-inflammatory drugs, aspirin, clopidogrel, or heparin products) either during hospitalization or 4 weeks before presentation also led to exclusion from the study.

Sampling

After diagnosis, before any treatment, serum, 3.2% sodium citrate, and EDTA samples were collected from the jugular vein of each patient with a 21-gauge needle by careful venipuncture with minimum stasis into vacutainers. CBC was repeated if the diagnosis of spirocercosis was made on a day different from admission. Blood samples were collected in the order described above to avoid tissue factor contamination of the citrate sample. The sodium citrate tube was filled to ensure the correct anticoagulant-to-blood ratio (1 : 9). Citrated-blood was left to stabilize for 30 minutes after collection before TEG measurements, a fixed time point after sampling was chosen to minimize inter-assay variation.²⁰ TEG was performed using a computerized thromboelastograph^a according to a previously described method using human recombinant tissue factor^b as the activator.²⁰ Thromboelastograms were obtained for 90 minutes at 37°C. Recorded TEG variables included time to initiation of clot formation (R), time for the tracing to achieve a predetermined clot strength (K), speed of clot propagation (α), greatest clot strength (MA), calculated G value and degree of fibrinolysis at 30 and 60 minutes after MA was reached (Ly30 and Ly60, respectively; Fig 1).

The remaining citrated and serum samples were centrifuged at 2100 g for 8 minutes within 2 hours of collection and the serum or plasma removed, aliquoted and stored at -80°C for the determination of CRP concentration and other coagulation assays as a batch at a later date.^{21,22} Reference intervals for all assays included were available from the Clinical Pathology laboratory, but a healthy control group also was included for the hemostatic assays to minimize analytical variation.

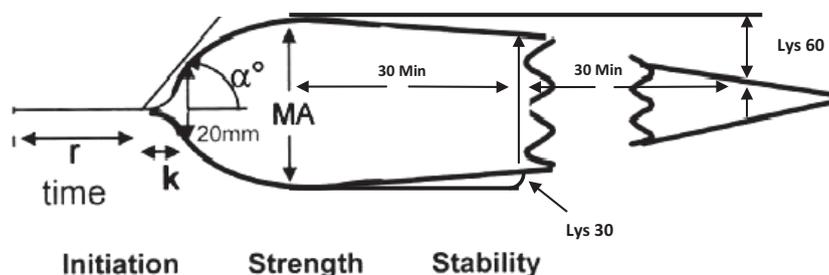


Fig 1. Variables of the thromboelastograph. Reaction time (R) represents the latency period from the start of the assay until a preset fibrin formation is reached. K (clotting time) is a measurement of the rapidity of clot development.²⁰ The angle (α) represents the rapidity of fibrin buildup and cross linking (clot formation). The MA is the maximum distance in millimeters between the 2 diverging branches and represents the final clot strength. The degree of clot lysis is determined at 30 and 60 minutes and is represented as LY30 and LY60, respectively. (Modified from Mallett SV, Cox DJA. Thromboelastography. Br J Anaesth 1992;69:307-313, Figure 2. Reproduced by permission of Oxford University Press/British Journal of Anesthesia).

Coagulation Assays

PT and aPTT assays were performed on the ST art 4 analyzer^c using the Neoplastine CI Plus^c reagent kit for PT, and the C.K. Prest^d reagent kit for aPTT, according to the manufacturers' instructions. D-dimer assays were performed, using an immunometric flow-through principle.^e Fibrinogen assays were performed on the ST art 4 analyzer^c using the Sta-Fib 2^c reagent kit. AT activity in plasma was measured on the Cobas Integra^c utilizing a thrombin-dependent chromogenic substrate assay.^f Serum C-reactive protein was determined using an automated CRP immunoturbidometric immunoassay for humans on the Cobas Integra.^{c,[23]} All assays were performed only once and calibrated according to the manufacturers' recommendations for human samples. Commercially available human control reagents for the respective assays were analyzed as internal controls together with pooled plasma from 5 healthy dogs.

Endoscopy and Histopathology

In addition to the previously mentioned standard diagnostics, esophageal and gastric endoscopy was performed under general anesthesia in all dogs except those where the owner declined endoscopy because of the radiographic findings of pulmonary metastasis before endoscopy. Endoscopy was performed before blood sample collection. The procedure was performed using a video endoscopy unit.^g Endoscopically guided biopsies were performed on any luminal esophageal nodule that was not smooth and showed any signs of ulceration or cauliflower-like appearance commonly seen in neoplastic transformation of esophageal nodules. A standard sampling procedure and anesthetic protocol was maintained for all spirocercosis dogs in an attempt to prevent artifactually affecting hemostatic variables. A follow-up endoscopy was performed on all dogs not euthanized, approximately 6 weeks after initial treatment. A standard necropsy was performed on all euthanized dogs as well as histopathologic examination of all esophageal nodules because of *S. lupi*.

Treatment

Treatment was initiated after all samples and diagnostic tests were completed and it was based on a published treatment protocol of 400 µg/kg doramectin^h given by SC injection 2 weeks apart for 3 treatments.²⁴

Data Analysis

Statistical analysis was performed using SPSS 20ⁱ and Graphpad Instat 3.^j The data were checked for normality using the Kolmogorov–Smirnov test. The Kruskal–Wallis test was used to determine significance across the 3 groups for each parameter. If significance was present, Dunn's multiple comparison was used as posthoc analysis. Correlation between inflammatory and hemostatic variables was determined using the Spearman's rank correlation test. Data are presented as median and range of values. $P < .05$ was considered significant. P values are reported as $<.05$, $<.01$ or $<.001$ as per the limits of the software available.

Results

Study Population

Two of the 41 dogs admitted to the study were excluded: the first because of concurrent pyothorax, and the second because of severe thrombocytopenia secondary to septic pneumonia. The remaining 39 dogs

were deemed free of any concurrent inflammatory conditions based on results of urinalysis, CBC, serum biochemistry, thoracic radiographs, and complete regression of clinical signs at the 6-week follow-up endoscopy or lack of concurrent disease based on postmortem examination. Twenty-four of the 39 dogs were included in the non-neoplastic group and 15 in the neoplastic group. No dogs received fluid therapy before blood collection. Three dogs in the neoplastic group did not have endoscopy performed. Seventeen breeds were represented with a significant difference in age between the control group and the neoplastic group (Table 1). The ratios of male : female for each group were as follows: control (6 : 9), non-neoplastic (13 : 11), and neoplastic (5 : 10) with no significant difference found among groups. Neoplastic transformation of the *S. lupi* nodule was confirmed with histopathology resulting in the diagnosis of 14 osteosarcomas and 1 anaplastic sarcoma. Six dogs with neoplastic spirocercosis had metastatic lesions at necropsy and 20% (3/15) of the neoplastic group had hypertrophic osteopathy.

Hemostatic and Inflammatory Laboratory Variables

Compared to the control group, α and MA were significantly increased and K significantly decreased in both the non-neoplastic and neoplastic groups, consistent with a hypercoagulable state (Table 1, Fig 2). When the neoplastic group was compared to the non-neoplastic group, α and MA were significantly increased, consistent with a greater degree of hypercoagulability in the neoplastic group. Based on our laboratory reference intervals for MA, 33% (8/24) of the non-neoplastic group and 80% (12/15) of the neoplastic group had increased MA. In contrast, when the infected group medians were compared to the control median, 92% (22/24) of the non-neoplastic and 100% (15/15) neoplastic groups had increased MA. None of the dogs were hypocoagulable and none of the dogs in the control group had an MA outside of the laboratory reference interval. There was no significant difference between groups for R, LY30 and LY60. In an attempt to differentiate neoplastic from non-neoplastic dogs, a cutoff value of >76 mm was used for the MA in receiver operating curve (ROC) analysis resulting in a sensitivity of 73% and specificity of 96% for the differentiation of disease state (Fig 3).

The median CRP and fibrinogen concentrations were significantly increased in the neoplastic group compared to the non-neoplastic group ($P < .01$) as well as between the neoplastic and control group ($P < .01$; Table 1). When ROC analysis was applied to fibrinogen concentration to differentiate neoplastic from non-neoplastic dogs at a cutoff value of >4.2 g/L, a sensitivity of 80% and specificity of 75% were found (Fig 3).

Total leukocyte count ($P < .01$), mature neutrophil count ($P < .01$) as well as platelet count ($P < .01$) were significantly increased in the neoplastic group compared to the non-neoplastic group (Table 1), and hematocrit (Ht) was significantly decreased ($P < .05$). There was a

Table 1. Age, hemostatic, and inflammatory variables for dogs with non-neoplastic and neoplastic spirocercosis and the healthy controls.

Parameter	Reference interval	Control Median			Non-neoplastic Median			Neoplastic Median		
		(range)	% >RI	% <RI	(range)	% >RI	% <RI	(range)	% >RI	% <RI
Age (months)	N/A	49 (6–96)	57 (6–133)	N/A	50	72 (48–132)	N/A	N/A	87	
Hematocrit (L/L) ^a	0.37–0.55	0.51 (0.38–0.58) ^c	0.46 (0.36–0.47) ^d	0	20.8	0.35 (0.23–0.55)	0	60	6.7	
Total leukocyte count ($\times 10^9/L$) ^a	6–15	10 (6–20) ^c	10 (5–23) ^d	8.3	50	22 (8–51)	66.7	0	73.3	
Mature neutrophil count ($\times 10^9/L$) ^a	2.5–15.4	6.6 (2.3–10.66) ^c	6.6 (3.3–17.3) ^d	4.2	50	15.9 (5.2–43.8)	53.3	0	80	
Band neutrophil count ($\times 10^9/L$) ^a	0–0.5	0 (0–0.4)	0.1 (0–3.5)	12.5	70.8	0.2 (0–3.8)	33.3	0	100	
Platelet count ($\times 10^9/L$) ^a	200–500	318 (212–457)	298 (174–494) ^d	0	37.5	415 (227–862)	13.3	0	86.7	
PT (seconds) ^a	4.4–9.0	6.7 (5–10.7) ^c	7.1 (4.7–8.1)	0	70.8	7.1 (6.1–7.8)	0	0	80	
aPTT (seconds)	9.9–12.6	11.3 (10.3–12.5)	11.5 (10.3–12.7)	4.2	62.5	12.2 (10.5–14.9)	46.7	0	66.7	
AT (%) ^a	95.6–154.6	117.0 (107.1–143.9) ^{b,c}	90.3 (58–124.1)	0	8.3	84.3 (58.5–111.6)	0	73.3	0	
D-Dimer (mg/L)	<0.5	0.2 (0.1–0.4)	0.1 (0–1.5)	4.2	16.7	0.1 (0.1–2.0)	6.7	93.3	13.3	
Fibrinogen (g/L) ^a	1.3–3.9	2.6 (1.7–4.4) ^c	3.3 (1.3–8.9) ^d	29.2	66.7	5.1 (2.9–14.8)	80	0	100	
CRP (mg/L) ^a	<35	20.1 (4.2–71.5) ^c	17.5 (11.4–249.9) ^d	41.6	58.3	85.7 (6.1–151.6)	73.3	26.7	80	
R (minutes)	1–11	5.8 (3–13.8)	4.9 (3.8–10.2)	0	25	5.2 (3–7.7)	0	0	20	
K (minutes) ^a	1–5	2.5 (1.4–5.4) ^{b,c}	1.6 (1.1–3.7)	0	16.7	1.3 (0.8–2.7)	0	20	6.7	
α (degrees) ^a	34–77	45.5 (26.3–70.2) ^{b,c}	68.9 (36.1–75.2) ^d	0	95.8	72.5 (57.1–78.3)	13.3	0	100	
MA (mm) ^a	42–71	58.6 (42.0–66.2) ^{b,c}	66.3 (49.7–79.7) ^d	33.3	91.7	78.9 (61.5–89.9)	80	0	100	
G (d/s) ^a	2.4–11.3	7.1 (3.7–10.0) ^{b,c}	9.9 (4.9–19.7) ^d	33.3	91.7	18.7 (8–44.3)	80	0	100	
Ly 30 (%)	0–6	0 (0–6.1)	0 (0–16.1)	4.2	37.5	0.1 (0–3)	0	0	46.7	
Ly 60 (%)	0–11	2.4 (0–13.1)	1.2 (0–20.6)	4.2	33.3	1.8 (0–7)	0	0	40	

RI, Reference interval, CM, Control median, N/A, not applicable.

^aKruskal–Wallis showed significance between the 3 groups compared, $P < .05$.

^bSignificance between control and non-neoplastic groups.

^cSignificance between control and neoplastic groups.

^dSignificance between non-neoplastic and neoplastic groups.

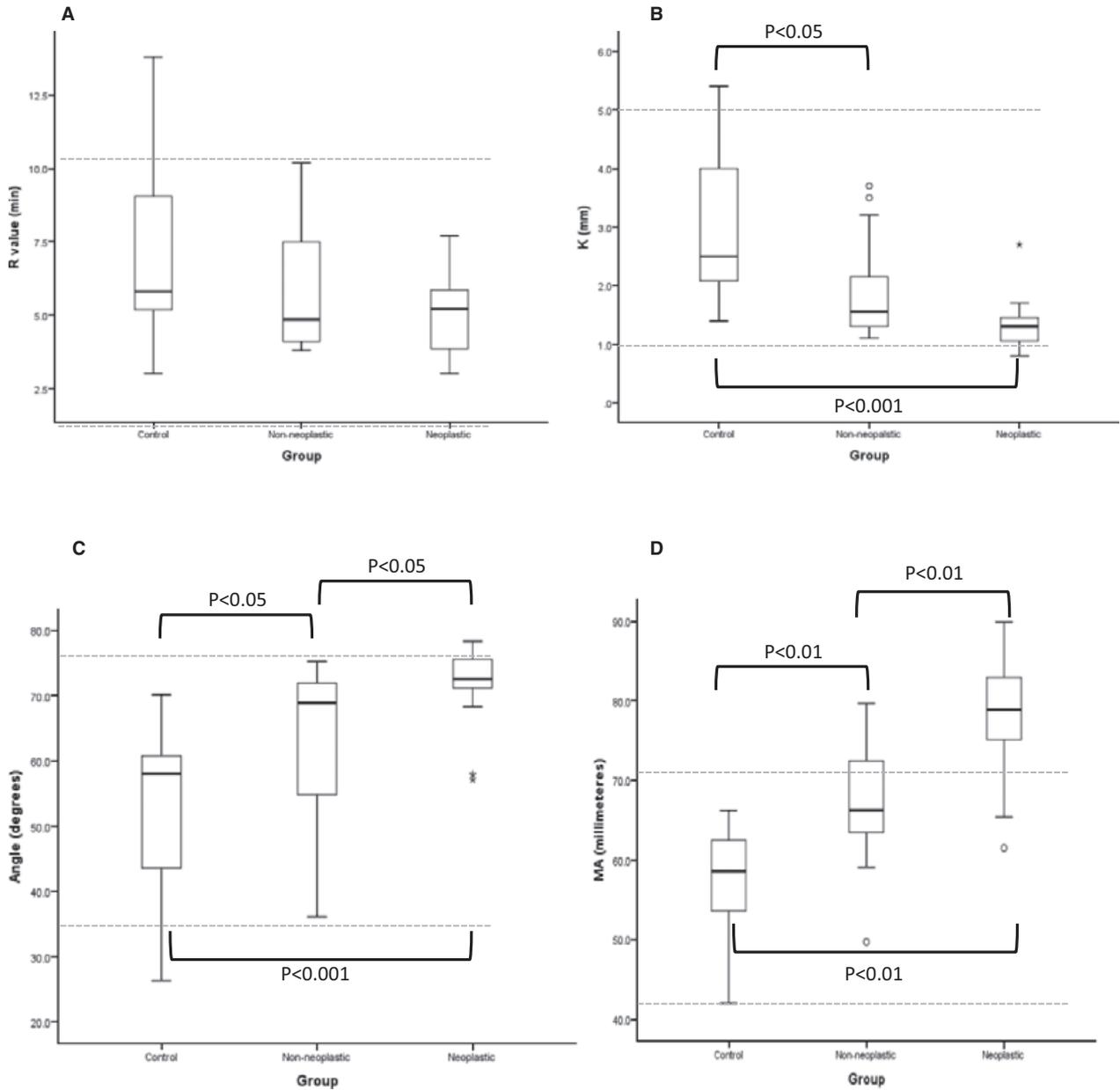


Fig 2. Box plot (representing the interquartile range [IQR]) of the TEG variables in dogs with neoplastic and non-neoplastic spirocercosis, as well as healthy control dogs. The box incorporates the middle 50% of the observations with the line inside the box as the median. The whiskers denote the range extending to 1.5 times the IQR from the upper and lower quartiles. Outliers, values that are 1.5 times removed from the interquartile range, are plotted separately as circles. Outlier values greater than 3 times the IQR are denoted as an asterisk. Reference interval for each parameter is represented by a dashed line. (A) R value, (B) K value, (C) α value, and (D) MA. Statistical significance between groups is indicated on each graph (*P* values).

significant increase in total leukocyte count ($P < .01$) and mature neutrophil count ($P < .05$) between the neoplastic and control groups, and a significant decrease between the 2 groups for Ht ($P < .001$).

For the infected groups, MA had significant positive correlations with CRP concentration ($r = 0.73$, $P < .001$), fibrinogen concentration ($r = 0.85$, $P < .001$; Fig 4), total leukocyte count ($r = 0.42$, $P < .001$), mature neutrophil count ($r = 0.40$, $P < .01$), and platelet count ($r = 0.39$, $P = .014$). Moreover, MA of the

infected group had significant negative correlations with Ht ($r = -0.60$, $P < .001$) and AT activity ($r = -0.34$, $P = .037$).

Of the traditional hemostatic variables, only PT and AT activity showed significant differences between groups (Table 1). Compared to the control group, the median AT activity was significantly decreased in both the non-neoplastic ($P < .001$) and neoplastic ($P < .001$) groups. For PT, a significant difference was found only between the neoplastic group and the control group

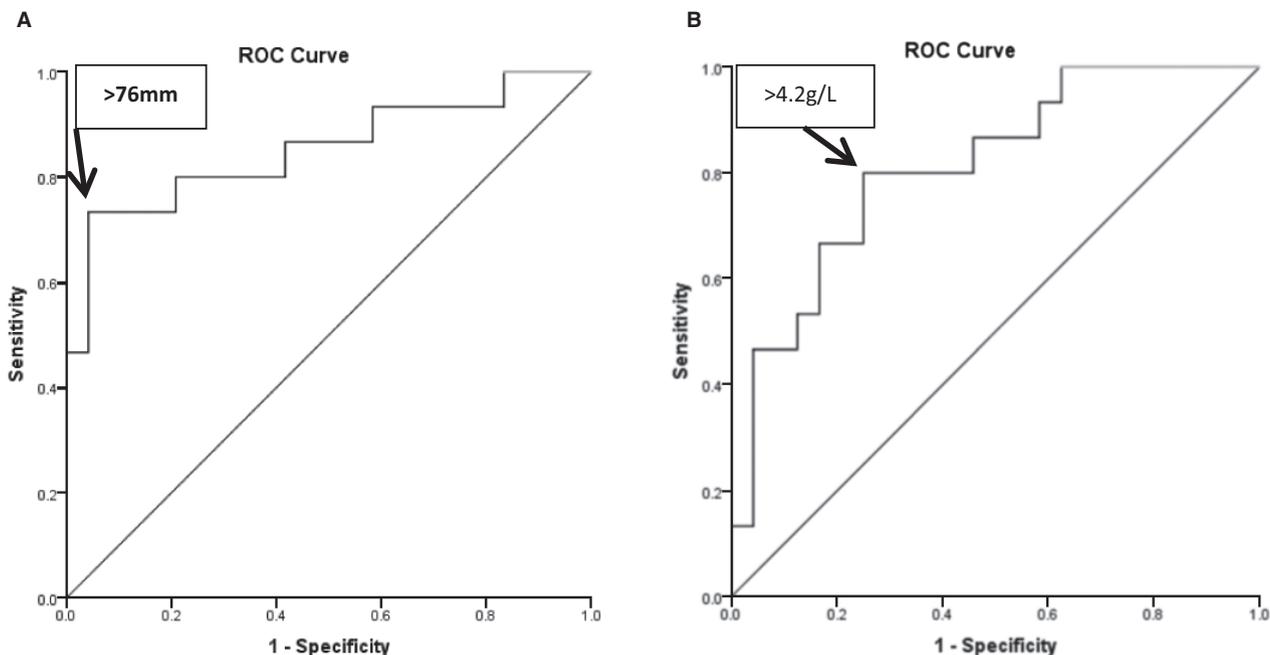


Fig 3. Receiver operator curve (ROC) for differentiation of neoplastic transformation with (A) MA value of >76 mm; a sensitivity of 73% and specificity of 96% is obtained, area under the curve is 0.853 and (B) fibrinogen concentration >4.2 g/L; a sensitivity of 80% and specificity of 75% is obtained. Area under the curve is 0.811.

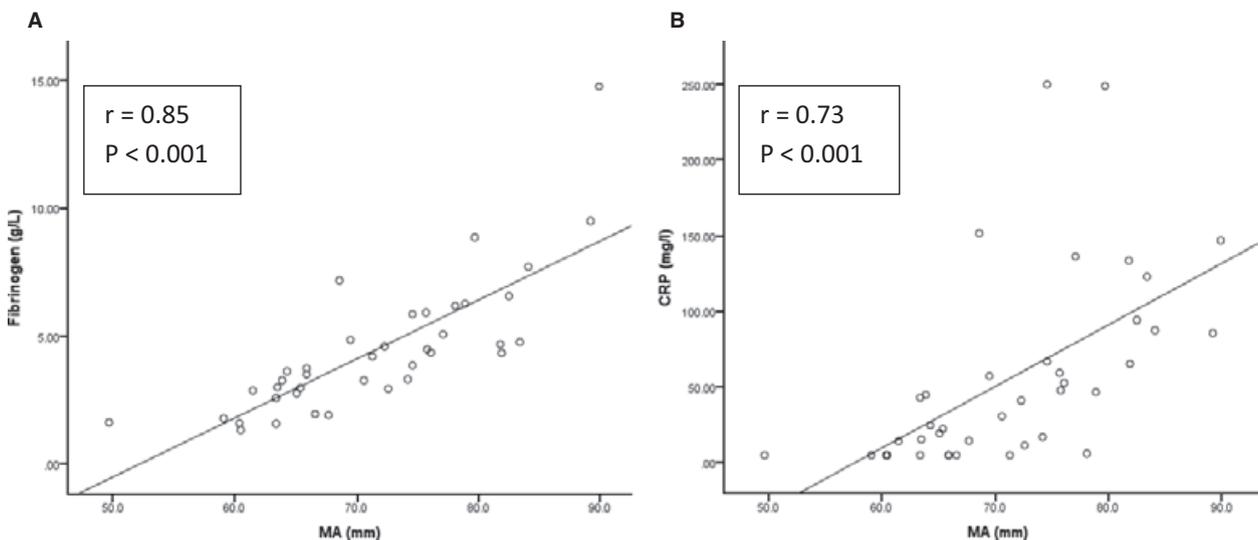


Fig 4. Scatter plot and positive linear correlations (solid line depicts the linear correlation whereas the open circles represent each case) between (A) MA and fibrinogen for all infected animals; and (B) MA and CRP for all infected animals. The effect of outliers on the Spearman's correlation coefficient is less than that would be seen if the data were parametric.

($P < .05$). No significance was found between groups for D-dimer concentration.

Of the euthanized dogs (14 neoplastic), only 2 dogs had any evidence of thromboemboli at necropsy; both dogs had chronic infarcts in the kidney (1 unilaterally, the other bilaterally).

Discussion

This study demonstrated that dogs with spirocercosis had increased α and MA and decreased K on the

thromboelastogram, increased fibrinogen concentration, and decreased AT activity compared to healthy dogs. These hemostatic changes are supportive of a hypercoagulable state, and were more pronounced in dogs where the esophageal nodule had undergone neoplastic transformation. Indicators of inflammation, the APPs, total leukocyte and mature neutrophils were correlated with the thromboelastogram-derived MA. These findings are in agreement with previous studies of other inflammatory and neoplastic conditions in which hypercoagulability was reported based on the

presence of thromboemboli or changes in TEG variables.^{11,12,25}

The previously reported tendency of neoplastic spirocercosis to have an increased female ratio¹⁰ was also shown in this study, but sex has been reported not to influence coagulation based on kaolin-activated TEG,²⁶ and the same can only be suspected with tissue-activated TEG. The effect of age on TEG variables has not been described and may be considered a confounder in this study as the neoplastic group had a median age that was significantly higher than the control group, and it is unclear whether the advanced age may have contributed to the increased MA of the neoplastic group. Although no dogs received fluid therapy before blood collection, the majority of dogs in the spirocercosis groups did undergo anesthesia or endoscopy. The effect of these procedures on coagulation is unclear and is likely to be minimal. MA has been reported to be indicative of hypercoagulability in previous studies in dogs,^{11,13} although the predictive value of MA for thrombotic events is questionable. The number of infected dogs with MA significantly higher than the control median was more than when the MA of infected dogs were compared to the reference interval. This finding is supportive of increased MA in spirocercosis in a controlled setting, but is of questionable clinical utility with established reference ranges, especially in the non-neoplastic group. The higher median MA in the neoplastic group compared to the non-neoplastic group allows for MA to be considered as an additional tool to differentiate between neoplastic and non-neoplastic disease. Although overlap did exist between the infected groups, an MA value of >76 mm achieved a specificity of 96% for neoplastic transformation, resulting in a very low false positive rate for neoplastic transformation above the cutoff value of 76 mm. One must be aware of the limitations when employing such a method for differentiation of disease state. Spirocercosis should be diagnosed before performing TEG because many other diseases also result in hypercoagulability. Moreover, if spirocercosis is confirmed, the presence of other inflammatory conditions must be excluded to interpret the MA value with confidence. The MA should never be viewed in isolation, but rather used as an adjunctive test in dogs where ambiguity exists. The sensitivity of the test is poor (73%) and may result in many false negative results for neoplastic transformation. Therefore, an MA >76 mm will help support the suspicion of neoplastic transformation, but will not exclude neoplastic transformation if the value is <76 mm. Histopathology remains the gold standard for diagnosis of neoplastic transformation of *S. lupi* esophageal nodules.

The concentrations for CRP and fibrinogen were significantly increased in the neoplastic group, and both showed strong positive correlation with MA. Significant correlation among MA, fibrinogen concentration, and platelet count has been reported in both normal and hypercoagulable people and dogs.^{27–29} Fibrinogen has been reported to increase MA in both humans and dogs because of the fact that MA

is primarily dependent on the cross binding of platelets and fibrinogen.^{30,31} Fibrinogen may be considered one of the “driving forces” behind inflammation because it directly influences the production of proinflammatory cytokines and chemokines,^{10,32,33} and this is further supported in this study by the positive correlations among MA, total leukocyte count and mature neutrophil count—cells responsible for the production of cytokines and chemokines. The increased fibrinogen concentration in dogs with spirocercosis is believed to promote the inflammatory response, therefore fibrinogen is suspected of playing an important role in the increase seen in MA in these dogs. The significant difference in CRP concentration between neoplastic and non-neoplastic groups agrees with previous reports on spirocercosis.⁹ When the CRP concentration was compared between the non-neoplastic and control groups, no significant difference was found, which is surprising considering the inflammation that is observed histologically in the non-neoplastic nodules.^{1,34} The reason for this finding is unclear and is contrary to previous reports on CRP concentration in spirocercosis.⁹ The close correlation of both APPs with MA supports the interrelationship between inflammation and hemostasis and, although a consistent trend was found, the individual variation from the trend line was large, making prediction of exact values difficult. The ROC analysis of a fibrinogen cutoff concentration of >4.2 g/L to differentiate between neoplastic and non-neoplastic dogs had a very similar sensitivity compared to MA at a cutoff of 76 mm, but fibrinogen concentration had a lower specificity (75%). This would imply that the possibility of a false positive diagnosis of neoplastic transformation is greater utilizing fibrinogen concentration compared to MA. The cutoffs and subsequent sensitivity and specificities used in this study will vary among laboratories, and the establishment of laboratory-specific cutoffs to differentiate between disease states are likely to be more appropriate for the local population and laboratory.

The moderate negative correlation between MA and Ht found in this study supports the interrelationship between Ht and MA previously reported,^{35–37} and it is likely that the interpretation of MA in the neoplastic group may have been affected by the significant decrease in Ht in that group compared to the non-neoplastic and control groups. Consequently, a decrease in Ht should be considered an important contributor to the change in MA. It is most likely, however not the only contributor because the correlations between MA and other variables, such as fibrinogen and CRP, were stronger than that between MA and Ht. Moreover, the role of other factors besides Ht affecting MA is supported by the significantly higher MA of the non-neoplastic group compared to the control group, despite the Ht between the 2 groups not being significantly different.

With the exception of AT activity, the traditional variables of hemostasis have been reported to be poor indicators of hypercoagulability,^{11,12} and also proved

to be poor indicators of hypercoagulability in this study. AT is considered a negative APP and is associated with hypercoagulable states in humans, predisposing patients to thrombotic events.^{17,18,38} The negative correlation of AT activity with MA in this study also supports the presence of a hypercoagulable state in dogs with spirocercosis.

Local and systemic inflammation associated with the esophageal nodules, aortic intimal damage caused by larval migration (as evidenced by 3 case reports describing aorto-iliac thromboembolism¹⁴⁻¹⁶), subsequent aortic aneurysms, and turbulent blood flow are all likely to contribute to hypercoagulability and the possible development of thrombosis in spirocercosis. No clinical suspicion existed for the presence of thromboemboli in the infected dogs of this study; however, 2 of the 14 dogs euthanized had renal infarcts as evidence of the presence of thromboemboli, but the infarcts appeared chronic and may not have been directly related to concurrent spirocercosis. Based on the low number of reported dogs with spirocercosis-related thromboemboli, as well as the low number of dogs with signs of thrombosis at necropsy in this study, clinical thrombosis associated with spirocercosis would appear to be an uncommon clinical complication despite the presence of increased MA. An alternative hypothesis is the possibility of an artifactually increased MA, secondary to anemia and hyperfibrinogenemia (particularly in the neoplastic group), but these 2 factors alone are unlikely to result in the changes seen in MA because TEG provides a holistic view of hemostasis, and hemostatic variables other than TEG also were indicative of a hypercoagulable state, further supporting the multifactorial increase in MA in spirocercosis. Regardless, spirocercosis, particularly neoplastic spirocercosis, should be a differential diagnosis of hypercoagulable states in endemic areas. The prevalence of subclinical thrombi and the effect of anticoagulant therapeutic intervention in spirocercosis are unknown and may be areas of future investigation.

In conclusion, this study identified laboratory variables indicative of hypercoagulability in canine spirocercosis with a higher degree of hypercoagulability with neoplastic transformation. A MA of >76 mm provided a specificity of 96% and sensitivity 73% for the differentiation of disease states. Fibrinogen concentration (at a cutoff of >4.2 g/L) was not as useful to differentiate with a similar sensitivity of 80%, but specificity of 75%. Factors shown to correlate with the final MA in this study included fibrinogen and CRP concentrations, Ht, total leukocyte count, mature neutrophil count, platelet count, and AT. The indicators of inflammation were most strongly positively correlated with MA, supporting the interrelationship between inflammation and hemostasis in spirocercosis. TEG can be used as an adjunctive test to support the suspicion of neoplastic transformation of the esophageal nodule as well as to determine the overall hemostatic status of the patient and assist in the therapeutic management of spirocercosis.

Footnotes

- ^a TEG[®] 5000 Thrombelastograph[®] Hemostasis System; Hemoscope, Niles, IL
^b Innovin; Siemens, Marburg, Germany
^c Cobus Integra 400 Plus Analyser; Roche, Basel, Switzerland
^d Diagnostica Stago, Roche, Asnières-sur-Seine, France
^e D-dimer Single Test; NYCOCARD Reader, ILEX, Johannesburg, Gauteng, South Africa
^f Cobas Integra 400 Plus Analyser; Roche Antithrombin (A), Mannheim, Germany
^g Storz endoscopy Unit; Karl Storz Endoscopy, Tuttlingen, Germany
^h Dectomax[®]; Pfizer, Johannesburg, South Africa
ⁱ SPSS Statistics 17.0[®] Software; SPSS Inc, Armonk, NY
^j Graphpad Instat, [®]San Diego, CA
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Acknowledgment

The study was funded by the Faculty of Veterinary Science, University of Pretoria, South Africa.

Conflict of Interest Declaration: The authors disclose no conflict of interest.

References

- Dvir E, Clift SJ, Williams MC. Proposed histological progression of the *Spirocerca lupi*-induced oesophageal lesion in dogs. *Vet Parasitol* 2010;168:71–77.
- Seibold HR, Bailey WS, Hoerlein BF, et al. Observations on the possible relation of malignant esophageal tumors and *Spirocerca lupi* lesions in the dog. *Am J Vet Res* 1955;18:5–14.
- Dvir E, Kirberger RM, Malleczek D. Radiographic and computed tomographic changes and clinical presentation of spirocercosis in the dog. *Vet Radiol Ultrasoun* 2001;42:119–129.
- Weller RE, Wolf AM, Oyejide A. Transitional cell carcinoma of the bladder associated with cyclophosphamide therapy in a dog. *J Am Anim Hosp Assoc* 1979;15:733–736.
- Macy DW, Withrow SJ, Hoopes J. Transitional cell carcinoma of the bladder associated with cyclophosphamide administration. *J Am Anim Hosp Assoc* 1983;19:965–969.
- Vascellari M, Melchiotti E, Bozza MA, Mutinelli F. Fibrosarcomas at presumed sites of injection in dogs: Characteristics and comparison with non-vaccination site fibrosarcomas and feline post-vaccinal fibrosarcomas. *J Vet Med* 2003;50:286–291.
- Dvir E, Kirberger RM, Mukorera V, et al. Clinical differentiation between dogs with benign and malignant spirocercosis. *Vet Parasitol* 2008;155:80–88.
- Dvir E, Mellanby RJ, Kjelgaard-Hansen M, Schoeman JP. Plasma IL-8 concentrations are increased in dogs with spirocercosis. *Vet Parasitol* 2012;190:185–190.
- Mukorera V, Dvir E, van der Merwe LL, Goddard A. Serum C-reactive protein concentration in benign and malignant canine spirocercosis. *J Vet Intern Med* 2011;25:963–966.
- Levi M, Poll T. Inflammation and coagulation. *Crit Care Med* 2010;38:S26–S34.
- Otto CM, Rieser TM, Brooks MB, Russell MW. Evidence of hypercoagulability in dogs with parvoviral enteritis. *J Am Vet Med Assoc* 2000;217:1500–1504.
- Kristensen AT, Wiinberg B, Jessen LR, et al. Evaluation of human recombinant tissue factor-activated thromboelastography in 49 dogs with neoplasia. *J Vet Intern Med* 2008;22:140–147.

13. Fenty RK, Laforcade AM, Shaw SP, O'Toole TE. Identification of hypercoagulability in dogs with primary immune-mediated hemolytic anemia by means of thromboelastography. *J Am Vet Med Assoc* 2011;238:463–467.
14. Kirberger RM, Zambelli A. Imaging diagnosis – aortic thromboembolism associated with spirocercosis in a dog. *Vet Rad Ultrasoun* 2007;48:418–420.
15. Gal A, Kleinbart S, Aizenberg Z, Baneth G. Aortic thromboembolism associated with *Spirocerca lupi* infection. *Vet Parasitol* 2005;130:331–335.
16. Alvarenga J, Saliba AM. Iliac embolism in a dog. *Mod Vet Pract* 1971;52:37–38.
17. Kuzi S, Segev G, Haruvi E, Aroch I. Plasma antithrombin activity as a diagnostic and prognostic indicator in dogs: A retrospective study of 149 dogs. *J Vet Intern Med* 2010;24:587–596.
18. Green RA. Clinical implications of antithrombin III deficiency in animal diseases. *Compendium* 1984;6:537–546.
19. Christie J, Schwan EV, Bodenstern LL, et al. The sensitivity of direct faecal examination, direct faecal flotation, modified centrifugal faecal flotation and centrifugal sedimentation/flotation in the diagnosis of canine spirocercosis. *J S Afr Vet Assoc* 2011;82:71–75.
20. Wiinberg B, Jensen AL, Rojkaer R, et al. Validation of human recombinant tissue factor-activated thromboelastography on citrated whole blood from clinically healthy dogs. *Vet Clin Path* 2005;34:389–393.
21. Aziz N, Fahey JL, Detels R, Butch AW. Analytical performance of a highly sensitive C-reactive protein-based immunoassay and the effects of laboratory variables on levels of protein in blood. *Clin Diagn Lab Immunol* 2003;10:652–657.
22. Bateman SW, Mathews KA, Abrams-Ogg AC, et al. Evaluation of the effect of storage at -70 degrees C for six months on hemostatic function testing in dogs. *Can J Vet Res* 1999;63:216–220.
23. Kjelgaard-Hansen M, Jensen AL, Kristensen AT. Evaluation of a commercially available human C-reactive protein (CRP) turbidometric immunoassay for determination of canine serum CRP concentration. *Vet Clin Path* 2003;32:81–87.
24. Lavy E, Aroch I, Bark H, et al. Evaluation of doramectin for the treatment of experimental canine spirocercosis. *Vet Parasitol* 2002;109:65–73.
25. Sinnott VB, Otto CM. Use of thromboelastography in dogs with immune-mediated hemolytic anemia: 39 cases (2000–2008). *J Vet Emerg Crit Care* 2009;19:484–488.
26. Bauer N, Eralp O, Moritz A. Establishment of reference intervals for kaolin-activated thromboelastography in dogs including an assessment of the effects of sex and anticoagulant use. *J Vet Diag Invest* 2009;21:641–648.
27. Wagg CR, Boysen SR, Bedard C. Thromboelastography in dogs admitted to an intensive care unit. *Vet Clin Path* 2009;38:453–461.
28. Kang YG, Martin DJ, Marquez J, et al. Intraoperative changes in blood coagulation and thromboelastographic monitoring in liver transplantation. *Anesth Analg* 1985;64:888–896.
29. Caprini JA, Zuckerman L, Cohen E, et al. The identification of accelerated coagulability. *Thromb Res* 1976;9:167–180.
30. Tanaka KA, Taketomi T, Szlam FM, et al. Improved clot formation by combined administration of activated factor VII (NovoSeven^(R)) and fibrinogen (Haemocomplettan^(R) P). *Anesth Analg* 2008;106:732–738.
31. 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.
32. Porta C, Larghi P, Rimoldi M, et al. Cellular and molecular pathways linking inflammation and cancer. *Immunobiology* 2009;214:761–777.
33. Moss SF, Blaser MJ. Mechanisms of disease: Inflammation and the origins of cancer. *Nat Clin Pract Oncol* 2005;2:90–97.
34. Dvir E, Schoeman JP, Clift SJ, et al. Immunohistochemical characterization of lymphocyte and myeloid cell infiltrates in spirocercosis-induced oesophageal nodules. *Parasite Immunol* 2011;33:545–553.
35. Spiezia L, Radu C, Marchioro P, et al. Peculiar whole blood rotation thromboelastometry (Rotem) profile in 40 sideropenic anaemia patients. *Thromb Haemost* 2008;100:1106–1110.
36. Ogawa S, Szlam F, Bolliger D, et al. The impact of hematocrit on fibrin clot formation assessed by rotational thromboelastometry. *Anesth Analg* 2012;115:16–21.
37. Vilar P, Couto CG, Westendorf N, et al. Thromboelastographic tracings in retired racing Greyhounds and in non-greyhound dogs. *J Vet Intern Med* 2008;22:374–379.
38. Dhainaut JF, Marin N, Mignon A, Vinsonneau C. Hepatic response to sepsis: Interaction between coagulation and inflammatory processes. *Crit Care Med* 2001;29:S42–S47.