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Hyperfibrinolysis and Hypofibrinogenemia Diagnosed With Rotational Thromboelastometry in Dogs Naturally Infected With *Angiostrongylus vasorum*

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Background: The pathomechanism of *Angiostrongylus vasorum* infection-associated bleeding diathesis in dogs is not fully understood.

Objective: To describe rotational thromboelastometry (ROTEM) parameters in dogs naturally infected with *A. vasorum* and to compare ROTEM parameters between infected dogs with and without clinical signs of bleeding.

Animals: A total of 21 dogs presented between 2013 and 2016.

Methods: Dogs with *A. vasorum* infection and ROTEM evaluation were retrospectively identified. Thrombocyte counts, ROTEM parameters, clinical signs of bleeding, therapy, and survival to discharge were retrospectively retrieved from patient records and compared between dogs with and without clinical signs of bleeding.

Results: Evaluation by ROTEM showed hyperfibrinolysis in 8 of 12 (67%; 95% CI, 40–86%) dogs with and 1 of 9 (11%; 95% CI, 2–44%) dogs without clinical signs of bleeding ($P = .016$). Hyperfibrinolysis was associated with severe hypofibrinogenemia in 6 of 10 (60%; 95% CI, 31–83%) of the cases. Hyperfibrinolysis decreased or resolved after treatment with 10–80 mg/kg tranexamic acid. Fresh frozen plasma (range, 14–60 mL/kg) normalized follow-up fibrinogen function ROTEM (FIBTEM) maximal clot firmness in 6 of 8 dogs (75%; 95% CI, 41–93%). Survival to discharge was 67% (14/21 dogs; 95% CI, 46–83%) and was not different between dogs with and without clinical signs of bleeding ($P = .379$).

Conclusion and Clinical Importance: Hyperfibrinolysis and hypofibrinogenemia were identified as an important pathomechanism in angiostrongylosis-associated bleeding in dogs. Hyperfibrinolysis and hypofibrinogenemia were normalized by treatment with tranexamic acid and plasma transfusions, respectively.

Key words: Angiostrongylosis; Bleeding diathesis; Canine; Fibrinogen.

Angiostrongylus vasorum infection in dogs is an emerging disease that has high morbidity and mortality.[1–3] The adult worm (13–21 mm) is a metastrongylid nematode that resides in the pulmonary arteries and right heart in dogs and other wild canids.[4–6]

Infected dogs may exhibit clinical signs of verminous pneumonia, coagulopathies, neurological deficiencies, and pulmonary hypertension.[7–13] Naturally infected dogs may show clinical or internal signs of bleeding with associated complications.[1,3,9,11,13,14]

Several studies have evaluated biochemical, hematological, and coagulation profiles in dogs both naturally and experimentally infected with *A. vasorum*.[7,8,10,11,13,15,16] Thrombocytopenia, decreased factor V activity, and increased factor VIII activity were identified during parasitic infection, and coagulation abnormalities have been attributed mainly to disseminated intravascular coagulation (DIC).[8,11,15,16] Deposits of fibrinogen within the pulmonary vessels further suggest severe intravascular coagulation.[7] However, the exact pathomechanism of *A. vasorum*-associated bleeding has not been elucidated. Plasmatic coagulation profiles and thrombocyte counts may be normal or hypercoagulable despite clinical or radiographic signs of bleeding in naturally infected dogs.[11,17,18] Recently, low fibrinogen concentrations and diagnosis of hypocoagulability based on thromboelastography (TEG) analysis were identified.[11] Hyperfibrinolysis has been suggested as a possible pathomechanism in response to this publication,[19] but was not described in the original study.[11] Hyperfibrinolysis identified on TEG analysis is described in a case report from a dog with *A. vasorum* infection, but lysis was attributed to severe DIC rather than to an independent pathomechanism.[20]

Abbreviations:

CFT	clot formation time
CI	confidence interval
CT	clotting time
DIC	disseminated intravascular coagulation
FDP	fibrinogen degradation product
FFP	fresh frozen plasma
MCF	maximum clot firmness
ML	maximum lysis
ROTEM	rotational thromboelastometry
TEG	thromboelastography

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This study was conducted at the Vetsuisse Faculty, University of Zurich, Switzerland.

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Incidence, magnitude, and clinical relevance of hyperfibrinolysis in dogs infected with *A. vasorum* have neither been systematically described in clinically or experimentally infected dogs nor have they been compared to clinical signs of bleeding in these patients.

Viscoelastic tests such as rotational thromboelastometry (ROTEM) measure the viscoelastic properties of whole blood under low shear conditions and display the changes in viscoelasticity in a graph.[21] Several parameters are automatically determined. The clotting time (CT) describes the time until fibrin formation starts. The clot formation time (CFT) displays the kinetics of clot formation and depends mainly on fibrinogen concentration and thrombocyte numbers. The maximum clot firmness (MCF) describes the maximal strength of the fibrin/thrombocyte clot in mm. Fibrinolysis can be assessed by maximum lysis (ML) in % within 60 minutes of measurement.

Several coagulation activators are available from the manufacturer. The EXTEM test investigates clot formation by activation of the coagulation cascade through proprietary tissue factor (extrinsic pathway), whereas the INTEM test is activated by a contact activator (intrinsic pathway). In the APTEM test, fibrinolysis is inhibited by added aprotinin, an antifibrinolytic, to the EXTEM reagent. Comparison of the APTEM to the EXTEM graph confirms hyperfibrinolysis and allows for diagnosis of hyperfibrinolysis in bleeding human patients.[23] The FIBTEM tracing enables determination of fibrinogen concentrations by adding cytochalasin D, a platelet inhibitor, to the EXTEM reagent.[21] The resultant MCF of the FIBTEM tracing represents the functional fibrinogen and correlates to fibrinogen concentrations determined by the Clauss method.[22]

The first aim of our retrospective study was to describe EXTEM and FIBTEM parameters of dogs naturally infected with *A. vasorum*. Secondly, we aimed to compare findings, specifically presence of hyperfibrinolysis and hypofibrinogenemia, between dogs with and without clinical signs of bleeding. Additionally, treatment of dogs with clinical signs of bleeding with fresh frozen plasma (FFP) and tranexamic acid as an antifibrinolytic drug is described and evaluated.

Our hypothesis was that EXTEM and FIBTEM parameters would be different between dogs with and without clinical signs of bleeding and that hyperfibrinolysis would normalize after treatment with tranexamic acid.

Materials and Methods

The hospital database was searched for *A. vasorum*-positive dogs that had at least 1 ROTEM analysis performed. Dogs were included if they either had a positive fecal analysis result (*A. vasorum* first-stage larvae detected by the Baermann-Wetzel technique), a positive serum antigen result by ELISA,[24] a positive rapid in-clinic assay[a,25] confirmation of *A. vasorum* infection at necropsy or some combinations of these, and if they had a ROTEM analysis performed at presentation. Dogs pretreated with fenbendazole or moxidectin were excluded.

Patient records were searched for additional information including signalment, presenting complaints, treatment before presentation, clinical signs at presentation including signs of respiratory distress, respiratory rate, and presence or history of cough, or pulmonary changes on thoracic radiographs. Thrombocyte numbers, coagulation test results within 12 hours of presentation, and ROTEM parameters during hospitalization were retrieved, as well as diagnosis, therapy, duration of hospital stay, and survival to discharge. The ROTEM[b] analysis was performed within 30 minutes of blood collection according to the manufacturer's instructions with 3.2% citrated whole blood in a 1 : 9 ratio. Briefly, 300 μL of 37.0° warm, citrated whole blood was incubated for 5 seconds with a single-portion EXTEM-S[c] or FIBTEM-S[d] reagent, followed by transferring the incubated blood sample into the ROTEM cuvette[e] and attaching the cuvette to the pin. An automated pipette was used and samples were analyzed for 60 minutes. After measurement, all ROTEM tracings were visually evaluated for artifacts. Of the parameters measured and calculated by the ROTEM device, EXTEM CT, CFT, MCF, ML, and FIBTEM MCF were further evaluated in our patients. The FIBTEM MCF was used as parameter representing functional whole-blood fibrinogen concentration.[21] In some dogs, serum fibrinogen concentration was measured by the Clauss method.

For statistical analysis, an undetectable FIBTEM MCF (green line) was defined as a FIBTEM MCF of 0 mm. An infinite EXTEM CFT due to the MCF not reaching 20 mm was defined as 3,600 seconds (60 minutes). Hyperfibrinolysis was defined as the ML at 60 minutes exceeding the in-house reference interval of 0–10%.

Based on ROTEM results, dogs were classified as hypocoagulable if ≥2 of the following parameters were abnormal compared to the in-house reference intervals: prolonged CT or CFT, low EXTEM or low FIBTEM MCF.

Statistical Analysis

Data were entered into a spreadsheet, and analyses were performed by the statistical software program SPSS[f]. Distribution of data was assessed by Shapiro-Wilk. Non-normally distributed continuous variables were analyzed with Mann-Whitney tests, and normally distributed continuous variables were analyzed with *t*-tests. The frequency distributions of the categorical or ordinal (score) variables were derived. Confidence intervals (CI) were calculated based on the Wilson procedure without correction for continuity by an online calculator (http://www.vassarstats.net/prop1.html). For comparison of coagulation parameters in dogs with and without bleeding diathesis, the patient population was grouped into dogs with and without clinical signs of bleeding at admission. Associations between categorical variables were evaluated by the chi-square test. When both variables were binary and expected cell frequencies were <5, a 2-sided Fisher's exact test was used. A value of $P < .05$ was considered significant.

Results

Thirty dogs with suspicion of *A. vasorum* infection and ROTEM analysis were identified; 4 dogs were excluded due to lack of a definitive diagnosis of *A. vasorum* infection and 5 were excluded because ROTEM analysis was performed after initiation of anthelmintic treatment, missing ROTEM analysis at presentation or both. One dog that presented with clinical signs of bleeding and that had ROTEM analysis performed 6 h after PO fenbendazole administration was included. Twenty-one dogs with a confirmed diagnosis of

A. vasorum infection and ROTEM analysis at presentation subsequently were evaluated; 6 dogs that were previously described[13] were included in our study because ROTEM parameters of these dogs had not been described previously.

The cohort consisted of various breeds (Table S1); 3 dogs were Cavalier King Charles Spaniels (3/21, 14.3%), 2 were Chihuahuas (2/21, 9.5%), and the remaining 16 dogs represented different breeds. Median age was 3.2 years (range, 0.6–10.3 years) and mean weight was 18.6 ± 13 kg (range, 2.8–42 kg); 9 of 21 dogs (43%) were male (6 intact, 3 castrated), and 12 dogs (57%) were female (7 intact, 5 spayed).

Dogs were presented with various complaints (Table S1). Clinically visible bleeding was identified in 12 of 21 (57%) dogs at presentation; 9 of 21 dogs (43%) presented with dyspnea, and 3 additional dogs (14%) were panting at presentation. Coughing was identified in 12 of 21 (57%) of the dogs, with 9 of 21 (43%) presenting with acute and 3 of 21 (14%) with chronic cough. Radiographic signs compatible with parasitic bronchopneumonia[26] could be identified on 19 of 20 (95%) thoracic radiographs; 5 of 21 (24%) dogs presented with neurological signs (Table S1).

Nine of 21 dogs (43%) showed hyperfibrinolysis on the EXTEM tracing (Fig 1). Three additional dogs showed a very slow rising and weak (<30 mm) MCF (Table S2, Fig 2). Although 13 of 21 (62%) dogs presented with thrombocytopenia, only 2 (10%) had thrombocyte counts that potentially may be associated with spontaneous bleeding (2 dogs with thrombocyte counts of 27,000/μL each).

Eight of 18 dogs (44%) that had FIBTEM performed at presentation had a FIBTEM MCF of 0 mm. Overall, 10 of 20 (50%) dogs were hypofibrinogenemic at presentation.

Comparison of ROTEM Parameters in Dogs With and Without Clinical Signs of Bleeding

Twelve of 21 (57%; 95% CI, 37–76%) dogs presented with various clinical signs of bleeding. The 2 groups were comparable in terms of median age (4.9 years; range, 0.9–10.3 years and 1.9 years; range, 0.6–9.8 years; $P = .148$) and median weight (5.1 kg; range, 2.8–35.0 kg and 21.4 kg; range, 0.6–42 kg; $P = .058$). The nonbleeding group included 5 of 9 (56%) male and 4 of 9 (44%) female dogs whereas the group with clinical signs of bleeding consisted of 4 of 12 (33%) male and 8 of 12 (67%) female dogs ($P = .185$). Hospitalization time was 2.5 days (range, 0.5–11 days) in nonbleeding dogs vs 4.5 days (range, 0.5–8 days) in dogs with clinical signs of bleeding ($P = .310$). Presence of dyspnea at presentation ($P = .595$) and history of coughing ($P = .757$) were similar in both groups (Table 1).

The ROTEM abnormalities in dogs with and without clinical signs of bleeding diathesis are summarized in Tables 1 and 2. The ROTEM evaluation showed hypocoagulability in 11 of 12 (92%; 95% CI, 65–99%) dogs with clinical signs of bleeding and was significantly

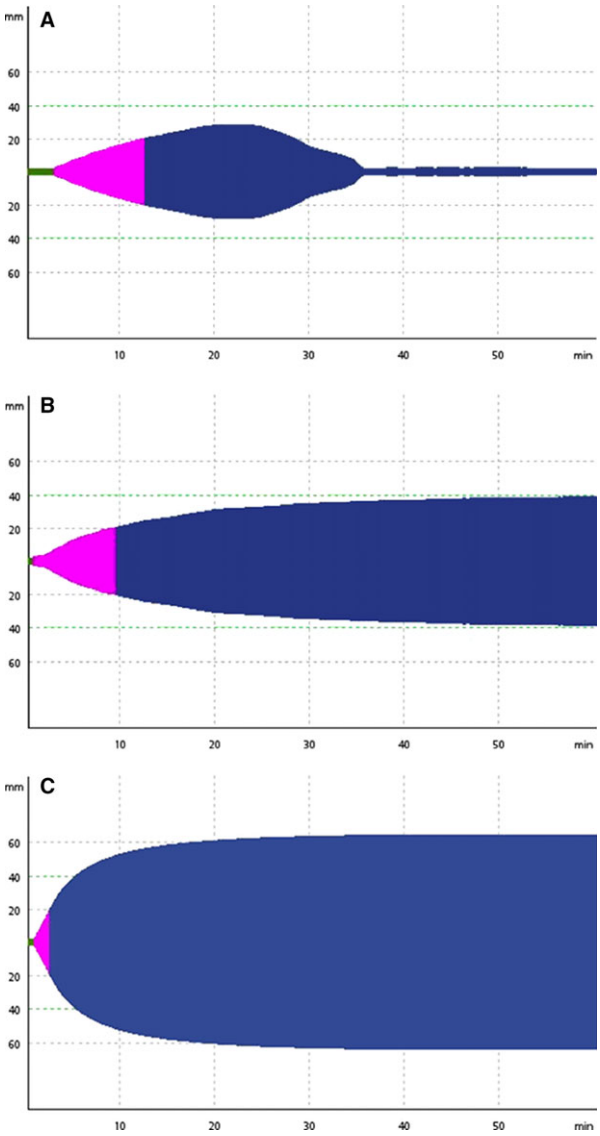

Fig 1. EXTEM tracing of a dog with clinical signs of bleeding and hyperfibrinolysis before (A) and after (B) treatment with tranexamic acid. C shows a normal rotational thromboelastometry (ROTEM) tracing of a dog with *Angiostrongylus vasorum* infection without clinical signs of bleeding.

different from nonbleeding dogs ($P = .002$); (Table 1). Presence of thrombocytopenia ($P = .397$) and median thrombocyte numbers ($P = .370$) were not significantly different between dogs with and without clinical signs of bleeding (Tables 1 and 2).

Eight of the 12 dogs with clinical signs of bleeding showed hyperfibrinolysis on the EXTEM tracing, corresponding to a 67% (95% CI, 40–86%) incidence of hyperfibrinolysis (ML range, 62–100%) in bleeding dogs. In contrast, only 1 of 9 (11%; 95% CI, 2–44%) of the nonbleeding dogs showed increased lysis (ML 12%); (Table 1, $P = .016$).

The FIBTEM MCF at presentation was significantly lower in dogs with clinical signs of bleeding compared to dogs without bleeding ($P = <.001$); (Table 2). A FIBTEM MCF of 0 mm or serum fibrinogen concentration

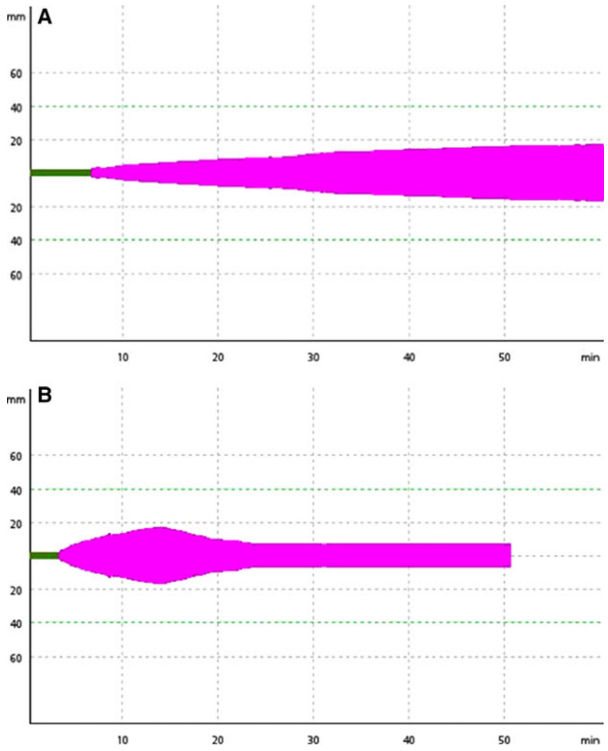

Fig 2. EXTEM tracings of 2 dogs with *Angiostrongylus vasorum* infection and clinical signs of bleeding. A shows a severely prolonged clot formation time and a low maximum clot firmness that does not reach maximal amplitude at 60 minutes, B additionally shows hyperfibrinolysis.

<1 g/L was identified at presentation in 10 of 11 dogs (91%; 95% CI, 62–98%) with clinical bleeding diatheses but in none of the dogs without clinical signs of bleeding (P = <.001, Table 1). All 6 hyperfibrinolytic dogs in the bleeding group that had FIBTEM performed at presentation showed concurrent hypofibrinogenemia. Hypofibrinogenemia was significantly associated with hyperfibrinolysis (P = .010).

A total of 12 of 21 (57%; 95% CI, 37–76%) dogs had repeated ROTEM analysis performed after initiation of therapy (Table S2). As only 3 dogs in the group without clinical signs of bleeding had repeat ROTEM results, follow-up ROTEM parameters were not evaluated statistically.

Treatment

All dogs were treated with fenbendazole or moxidectin and prednisolone and additional symptomatic medications as deemed necessary by the clinician in charge; 1 dog treated with fenbendazole (50 mg/kg) 6 hours before ROTEM analysis was included in the study because fenbendazole is not expected to have a clinically relevant anthelmintic effect within 6 h.[27]

One dog (11%; 95% CI, 2–44%) without and 9 of 12 (75%; 95% CI, 47–91%) with clinical signs of bleeding received FFP (Table 3). A median dose of 29 mL/kg (range, 14–60 mL/kg) was administered; 8 dogs with clinical signs of bleeding and concurrent hypofibrinogenemia received FFP, which normalized follow-up FIBTEM MCF in 6 of 8 dogs (75%; 95% CI, 41–93%). However, 4 of the 6 dogs required >1 FFP transfusion for normalization of FIBTEM MCF (Table S2).

Twelve of 21 dogs (57%; 95% CI, 37–76%) were treated with tranexamic acid[g], including 2 of 9 (22%; 95% CI, 6–55%) dogs without and 10 of 12 (83%; 95% CI, 55–95%) dogs with clinical signs of bleeding diathesis (P = .002). Tranexamic acid was administered as a 10–20 mg/kg bolus over 15–20 minutes and was repeated after 6–8 hours or sooner if indicated by follow-up EXTEM evaluation. Maximum lysis decreased in all 7 hyperfibrinolytic dogs treated with tranexamic acid (Table 3, Fig 1); 2 dogs with hyperfibrinolysis (100 and 99%, respectively) required >20 mg/kg tranexamic acid because this dose decreased EXTEM ML only to 72 and 77%, respectively. Hyperfibrinolysis resolved on day 1 in 1 of these dogs, whereas the other died (progression to status epilepticus) before an additional EXTEM could be performed. Two other dogs with hyperfibrinolysis did not receive tranexamic acid because hyperfibrinolysis was not correctly identified at the time of ROTEM analysis; 1 was euthanized and the other died within 8 hours after admission, both as a consequence of severe, progressive neurological signs.

Fourteen of 21 dogs survived to discharge (67%; 95% CI, 46–83%) which was not significantly different

Table 1. Frequencies of clinical signs, thrombocyte number, and rotational thromboelastometry (ROTEM) parameters at presentation in dogs with *Angiostrongylus vasorum* infection with (bleeding dogs) and without (nonbleeding dogs) clinical signs of bleeding diathesis.

	Nonbleeding dogs (n = 9)			Bleeding dogs (n = 12)			
Parameter at presentation	n/N	%	95% CI	n/N	%	95% CI	*P*-value
Dyspnoea	5/9	55.6	27–81	4/12	33.3	14–61	.595
History of coughing	6/9	67	35–88	6/12	50.0	25–75	.379
Thrombocytopenia	5/9	55.6	27–81	9/12	75.0	47–91	.397
EXTEM lysis (maximum lysis >10%)	1/9	11	2–44	8/12	67	39–86	.016
Low fibrinogen[a]	0/9	0	0–3	10/11	91	62–98	<.001
Lysis in combination with low FIBTEM	0/9	0	0–3	7/11	64	35–85	.004
ROTEM hypocoagulable	2/9	22	6–55	11/12	92	65–99	.002

[a]Low fibrinogen, defined as FIBTEM MCF<3 mm or fibrinogen measured by Clauss <1.0 g/L.

Table 2. Hematocrit (Hct), thrombocyte count, and ROTEM parameters at presentation in dogs with *Angiostrongylus vasorum* infection with (bleeding dogs) and without (nonbleeding dogs) clinical signs of bleeding diathesis.

		Nonbleeding dogs (n = 9)			Bleeding dogs (n = 12)			
Parameter	Reverence interval	n	Median Mean ± SD	Range (min–max)	n	Median Mean ± SD	Range (min–max)	*P*-value
Hct (%)	40–55	9	44 ± 8.5	31–60	11	34.4 ± 14.1	18–67	.077
Thrombocytes (10^6/μL)	130–394	9	109	27–299	11	87	27–279	.370
EXTEM CT (s)	26–57	9	37 ± 11	27–56	12	189 ± 89	41–388	<.001
EXTEM CFT (s)	53–153	9	182 ± 141	63–520	12	1,465 ± 1,349	165–3,600	<.001
EXTEM α-angle (°)	62–82	9	70	57–82	12	27.5	9–59	<.001
EXTEM MCF (mm)	46–62	9	55	30–71	12	28	14–57	<.001
EXTEM ML (%)	0–10	9	2	0–12	12	61	0–100	.015
FIBTEM MCF (mm)	3–9	9	12	3–16	9	0	0–8	<.001

CFT, clot formation time; CT, clotting time; ML, maximum lysis; ROTEM, rotational thromboelastometry.

Table 3. Treatment and outcome of 21 dogs with *Angiostrongylus vasorum* infection with (bleeding dogs) and without (nonbleeding dogs) clinical signs of bleeding diathesis.

	Nonbleeding dogs (n = 9)			Bleeding dogs (n = 12)			
Parameter	n/N	%	95% CI	n/N	%	95% CI	*P*-value
FFP administration	1/9	11	2–44	9/12	75	47–91	.008
FFP for low fibrinogen	0/9	0	0–30	8/11	73	43–90	.001
Normalization of fibrinogen after FFP	0/1	0	0–80	6/8	75	41–93	.533
TXA administration	2/9	22	6–55	10/12	83	55–95	.008
TXA for hyperfibrinolysis	1/9	11	2–44	6/12	50	25–75	.078
Decreased hyperfibrinolysis after TXA	1/1	100	21–100	6/6	100	61–100	NA
Survival to discharge	5/9	55.6	27–81	9/12	75.0	47–91	.379

FFP, fresh frozen plasma; TXA, tranexamic acid; CI, confidence interval; NA, not assessed.

between dogs with and without clinical signs of bleeding at presentation (Table 1, $P = .379$); 6 of 7 dogs with a diagnosis of hyperfibrinolysis that were treated with tranexamic acid survived to discharge.

Discussion

Ours is the first study to systematically describe hyperfibrinolysis and its treatment with tranexamic acid in dogs naturally infected with *A. vasorum*. Distinct hyperfibrinolysis was identified in 67% of the dogs with clinical signs of bleeding whereas only 1 dog without clinical signs of bleeding showed hyperfibrinolysis. We therefore suspect hyperfibrinolysis to be a major underlying pathomechanism in dogs with bleeding diathesis as a consequence of *A. vasorum* infection.

Hyperfibrinolysis has been reported in humans with various diseases, including trauma,[28–30] and is a reason for severe uncontrollable clinical bleeding.[29,31] It has been documented previously in a dog with *A. vasorum* infection,[20] a dog with acute traumatic coagulopathy[32] and in dogs with spontaneous hemoperitoneum.[33] Hyperfibrinolysis may develop independently of activation of the coagulation cascade and occurs when plasmin generation exceeds the neutralizing capacity of antiplasmins.[34] Hyperfibrinolysis leads to rapid degradation of cross-linked fibrin and possibly to overt bleeding diathesis. The degradation of fibrin and cross-linked fibrin leads to increased concentrations of fibrinogen degradation products (FDPs) and D-dimers.[34] Fibrinogen degradation products were not measured in our study, and D-Dimer concentrations were determined in only 3 dogs (data not shown).

The factors leading to induction of hyperfibrinolysis in *A. vasorum* infection remain to be determined. Hyperfibrinolysis may result from depletion of plasminogen activator inhibitor 1 by increased concentrations of activated protein C, the latter being excessively produced by thrombomodulin upregulation.[30] Thrombomodulin upregulation has been associated with hypoperfusion in human trauma patients,[30] and hyperlactatemia has been associated with hyperfibrinolysis in dogs with spontaneous hemoperitoneum[33] and in humans with trauma.[28,30] Hyperfibrinolysis may further be induced by endothelial plasminogen activator released after endothelial trauma.[35] Impairment of host coagulation also has been reported in *Dirofilaria immitis* infections. *Dirofilaria immitis*-derived antigens have been shown to activate fibrinolysis by binding to plasminogen and leading to generation of plasmin, but also by enhancing plasminogen activators.[36,37] Adult *A. vasorum* worms and first-stage larvae or their metabolic products also may lead to mechanical or chemical injury of endothelial cells in the right ventricle and pulmonary arteries with subsequent release of plasminogen activator. They also may directly release a plasminogen

activator, as described for *D. immitis*.[36–38] A high percentage of *A. vasorum*-infected dogs show classical radiographic signs of verminous bronchopneumonia and suspicion of pulmonary bleeding.[26,39] Several of our dogs that initially presented with hemoptysis but without dyspnea showed worsening of pulmonary function and severe postmortem evidence of pulmonary bleeding. These findings support the hypothesis that the pathogen itself directly contributes to coagulopathy primarily in the pulmonary vessels with or without concurrent systemic activation of the coagulation cascade.

Hyperfibrinolysis as a pathomechanism for bleeding diathesis in our dogs with *A. vasorum* infection is supported by the concurrent presence of severe hypofibrinogenemia.[19] Most dogs with hyperfibrinolysis had a nondetectable FIBTEM MCF (green line) whereas none of the dogs without clinical signs of bleeding diathesis showed evidence of hypofibrinogenemia. We suspect hypofibrinogenemia to be a sign of increased loss of fibrinogen as a result of severe hyperfibrinolysis, resulting from increased consumption due to continuous clot lysis.[40] Blood loss as a cause for hypofibrinogenemia seems less likely because the hematocrit and thrombocyte numbers would be expected to be substantially decreased, which was not the case in our dogs. Fibrinogen, an acute-phase protein produced during the inflammatory stages of the disease, was decreased in many of our dogs. Hyperfibrinogenemia as a sequel of the inflammatory reaction to adult worms, larvae, or their metabolic products would be expected in dogs with *A. vasorum* infection.[7] Disseminated intravascular coagulation has been suspected as the main pathomechanism leading to bleeding diathesis in previous studies and may be associated with both hyperfibrinolysis and hypofibrinogenemia.[1,8,11,15,16,20] We cannot completely rule out DIC as the reason for distinct hyperfibrinolysis and hypofibrinogenemia because increased fibrinolytic activity has been stated to be a feature of DIC.[41] However, studies showing hyperfibrinolysis in dogs with suspected DIC are lacking and a recent study of humans did not identify hyperfibrinolysis and hypofibrinogenemia (indicated by low FIBTEM MCF) in patients with DIC.[42] Furthermore, dogs with DIC rarely show very low fibrinogen concentrations.[17,43] Additionally, previous studies in naturally infected dogs reported plasmatic coagulation profiles and thrombocyte counts to be normal despite clinical or radiographic signs of bleeding.[11,17,18] A different, previously undetected pathomechanism leading to bleeding diathesis in *A. vasorum* infection therefore is expected. Based on our findings, hyperfibrinolysis and associated hypofibrinogenemia seem to be important pathomechanisms of bleeding diathesis and are induced by the parasite itself rather than being a consequence of DIC.

Other pathomechanisms for bleeding diathesis have been discussed. Thrombocytopenia alone does not explain the bleeding diathesis because several dogs with clinical bleeding had normal thrombocyte numbers.[11] This finding is in agreement with those of our study in which median thrombocyte counts were not different between dogs with and without clinical signs of bleeding. Thrombocytopathia has been suggested as a cause for bleeding because both thrombocyte numbers and secondary coagulation parameters can be normal.[11] To our knowledge, thrombocytopathia has not specifically been investigated so far and should be included in future studies.

The identification of hyperfibrinolysis as a main pathomechanism for *A. vasorum*-associated bleeding diathesis offers a new treatment modality. Treatment of hyperfibrinolysis with tranexamic acid as an antifibrinolytic drug has become common practice in human trauma patients and surgery-related bleeding and has led to a substantial decrease in the use of blood products.[44,45] Tranexamic acid is a readily available antifibrinolytic agent that competitively binds to lysine-binding sites on plasminogen and plasmin, therefore interfering with the formation of plasmin from its precursor plasminogen.[46,47] Although hyperfibrinolysis in *A. vasorum* infection has been described in a dog,[20] our study is the first to describe treatment of *A. vasorum*-induced bleeding with tranexamic acid. All but 2 dogs with hyperfibrinolysis were treated with tranexamic acid immediately after identification of hyperfibrinolysis. Our results indicate that hyperfibrinolysis decreased or normalized after treatment with tranexamic acid. The dosage of tranexamic acid required to normalize hyperfibrinolysis in our study was derived empirically from data in humans and own clinical experience with repeated monitoring of hyperfibrinolytic patients. The dosage remains speculative because no pharmacological studies evaluating the minimal required dosage to inhibit hyperfibrinolysis have been performed. In a study using an in vitro model of hyperfibrinolysis induced by tissue plasminogen activator, dosages up to 10 times those used in humans were suggested to be necessary to inhibit induced hyperfibrinolysis in dogs.[48] Our dogs received dosages of 10–20 mg/kg q6–8h, which decreased hyperfibrinolysis in all dogs. However, several dogs needed repeated doses of tranexamic acid (up to 80 mg/kg) until fibrinolysis normalized. Although most dogs treated with tranexamic acid for hyperfibrinolysis also received FFP as treatment for severe concurrent hypofibrinogenemia, FFP itself is not expected to inhibit hyperfibrinolysis. In the previously mentioned case report describing a dog with *A. vasorum* infection and hyperfibrinolysis, treatment with antiparasitic drugs and FFP did not resolve hyperfibrinolysis completely.[20] We therefore conclude that tranexamic acid was successful in treating dogs with hyperfibrinolysis and bleeding diathesis. All dogs that reached normal ML after treatment of hyperfibrinolysis survived to discharge.

Hypofibrinogenemia in our dogs was treated successfully with FFP transfusions. As described in the human medical literature, large amounts of FFP (mean, 29 mL/kg; range, 14–60 mL/kg) were required to increase FIBTEM MCF's into the lower end of the reference interval.[49–51]

An interesting finding was the very low EXTEM MCF that was combined with slow clot formation in 3 dogs without evident hyperfibrinolysis (Fig 2). The low EXTEM MCF may be a sign of

thrombocytopathia.[11,19] However, because all 3 dogs had substantial improvement in CFT and EXTEM MCF after therapy with tranexamic acid and FFP, we suspect that concurrent clot lysis during clot formation without evident hyperfibrinolysis caused the weak clot.

The ROTEM evaluation identified 91% of the clinically bleeding dogs as being hypocoagulable. By TEG, 94% of bleeding dogs were identified as being hypocoagulable.[11] We defined the group of bleeding dogs based on clinically visible signs of bleeding, and patients with suspicion of internal bleeding (pulmonary or intracranial) were not included in this group. Therefore, we believe that the results are comparable and that viscoelastic tests are suitable for the identification of hypocoagulability in *A. vasorum*-infected dogs.

Being retrospective in nature, our study has several limitations. The ROTEMs were not repeated at standardized timepoints, with some dogs having only 1 measurement available. In addition, treatment was not standardized, and therefore, conclusions regarding treatment effects are limited. Furthermore, hyperfibrinolysis identified on ROTEM tracings was not compared to APTEM tracings. Because all EXTEM tracings with hyperfibrinolysis improved or normalized after treatment of dogs with tranexamic acid, this in vivo antifibrinolytic treatment indicates the initial presence of hyperfibrinolysis. Furthermore, distinct hyperfibrinolysis as seen in our dogs with *A. vasorum* infection is a rare finding in EXTEM tracings in our hospitalized dogs with other diseases (data not shown) and is not commonly identified on standard ROTEM analysis.[51]

Survival after treatment was 67% and not different between dogs with and without clinical signs of bleeding at presentation; 2 of the dogs with severe hyperfibrinolysis were not treated with tranexamic acid, because hyperfibrinolysis was not recognized at the time, and both dogs died. In 5 of 12 dogs, treatment with tranexamic acid was initiated without signs of bleeding or diagnosis of hypocoagulable state or hyperfibrinolysis. Doing so might be detrimental in cases that are in a hypercoagulable state even though tranexamic acid has not been associated with severe adverse effects.[46,52] On the other hand, 2 dogs with normal ROTEM parameters and no clinical signs of bleeding at presentation developed severe pulmonary bleeding during hospitalization, leading to death. Prophylactic treatment of hyperfibrinolysis in these dogs may have prevented death.

Although hyperfibrinolysis seems to be an important pathomechanism in *A. vasorum*-associated bleeding diathesis, several questions still remain. A prospective, randomized study with a larger population of dogs in various stages of infection is required to fully address the complete pathomechanism of *A. vasorum*-associated bleeding and to evaluate whether treatment of hyperfibrinolysis with tranexamic acid alone may be sufficient to decrease bleeding diathesis.

Conclusion

We identified hyperfibrinolysis and severe hypofibrinogenemia associated with clinical signs of hemorrhage in dogs naturally infected with *A. vasorum*. Hyperfibrinolysis and hypofibrinogenemia were treated successfully with tranexamic acid and FFP transfusions, respectively. A prospective clinical trial with consistent diagnostic evaluation and treatment protocols is warranted.

Footnotes

[a] Angio Detect, IDEXX Laboratories, Westbrook, ME
[b] ROTEM-Delta, TEM Innovations GmbH, Munich, Germany
[c] TEM Innovations GmbH
[d] TEM Innovations GmbH
[e] Cup and Pin Pro, TEM Innovations GmbH
[f] SPSS, version 23, SPSS Inc, Chicago, IL
[g] Tranexamic acid, Cyklokapron, Pfizer Corporation Austria Ges.m.b.H, Vienna, Austria

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Clinical signs upon presentation, diagnoses and survival of 21 *A. vasorum*-infected dogs.

Table S2. ROTEM parameters, thrombocyte numbers and fibrinogen concentration and effect of therapy on follow-up ROTEM parameters in 21 dogs with *A. vasorum*-infection.