

## Article

# Coagulation Status in Dogs Naturally Infected with *Angiostrongylus vasorum*

Nadja E. Sigrist <sup>1,\*</sup>, Lucienne Tritten <sup>2</sup>, Claudia Kümmerle-Fraune <sup>3</sup>, Natalie Hofer-Inteeworn <sup>3</sup>,  
Rahel Jud Schefer <sup>4</sup>, Manuela Schnyder <sup>2</sup> and Annette P. N. Kutter <sup>5</sup>

<sup>1</sup> Department for Small Animals, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland

<sup>2</sup> Institute of Parasitology, University of Zurich, 8057 Zurich, Switzerland; lucienne.tritten@uzh.ch (L.T.); manuela.schnyder@uzh.ch (M.S.)

<sup>3</sup> Department for Small Animals, Clinic for Small Animal Medicine, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland; ckummerle@vetclinics.uzh.ch (C.K.-F.); nhofer@vetclinics.uzh.ch (N.H.-I.)

<sup>4</sup> Department for Small Animals, Division of Emergency and Critical Care, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland; rjud@vetclinics.uzh.ch

<sup>5</sup> Department for Clinical Diagnostics and Services, Section Anaesthesiology, Vetsuisse Faculty, University of Zurich, 8057 Zurich, Switzerland; akutter@vetclinics.uzh.ch

\* Correspondence: nsigrist@vetclinics.uzh.ch

**Abstract:** *Angiostrongylus vasorum* infection has been associated with coagulopathies including hyperfibrinolysis. We compared coagulation status including thromboelastometry (ROTEM) parameters in dogs naturally infected with *A. vasorum* versus healthy dogs to determine clinicopathological parameters associated with bleeding, hypocoagulopathy, and hyperfibrinolysis. Clinical signs, white blood cell count, platelet count, hematocrit, plasmatic coagulation tests (PT, aPTT, fibrinogen concentration), D-dimer, and ROTEM S parameters (Ex-tem, In-tem, Fib-tem, Ap-tem) were analysed and compared between bleeding, nonbleeding, and control dogs and between hypo- and normocoagulable animals. Clinical signs of bleeding were present in 6/9 (67%) hypocoagulable and 1/9 (11%) normocoagulable dogs. PT, fibrinogen concentration, and several ROTEM parameters were significantly different between hypocoagulable and normocoagulable *A. vasorum* infected dogs. Hyperfibrinolysis was identified in 44% of infected dogs and was significantly more common in bleeding and hypocoagulable dogs. Hyperfibrinolysis was significantly associated with low MCFFib-tem but not with low fibrinogen concentration or increased D-dimers. CFTEEx-tem > 248 swas 100% sensitive and 89% specific to predict hyperfibrinolysis. Hyperfibrinolysis, hypocoagulability and bleeding are common in *A. vasorum* infected dogs. Only Ex-tem and Fib-tem parameters and potentially PT were associated with bleeding or hypocoagulability. Ex-tem analysis enables detection of bleeding, hypocoagulability and hyperfibrinolysis within minutes.

**Keywords:** angiostrongylosis; bleeding diathesis; fibrinogen



**Citation:** Sigrist, N.E.; Tritten, L.; Kümmerle-Fraune, C.; Hofer-Inteeworn, N.; Jud Schefer, R.; Schnyder, M.; Kutter, A.P.N. Coagulation Status in Dogs Naturally Infected with *Angiostrongylus vasorum*. *Pathogens* **2021**, *10*, 1077. <https://doi.org/10.3390/pathogens10091077>

Academic Editor: Magda Dunowska

Received: 30 July 2021

Accepted: 20 August 2021

Published: 25 August 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

The canine heart- and lungworm *Angiostrongylus vasorum* (Baillet 1866) causes serious infections in dogs and is associated with a high mortality rate if not diagnosed and treated in due time [1]. The disease often causes a broad spectrum of clinical signs, including unspecific clinical signs. Bleeding diathesis, present in about 60% of clinical cases, can lead to rapid life-threatening complications [2] and may occur within the central nervous system, the lung, body cavities, mucous membranes, or in the integument [3,4]. The mechanisms and pathogenesis of these coagulopathies are poorly understood and several unresolved hypotheses such as chronic disseminated intravascular coagulation (DIC), FV and FVIII deficiency, immune-mediated thrombocytopenia, anticoagulants secreted by the parasite or activation of the fibrinolytic system of the host have been formulated [2,4–9]. A recent study evaluating serum proteome of experimentally infected dogs did not support an underlying

DIC but rather consumption of some key factors of the coagulation and complement cascade [10]. Interestingly, a previous retrospective study identified hyperfibrinolysis (HFL) and associated hypofibrinogenemia in 67% of dogs with an *A. vasorum* infection and simultaneous clinical signs of bleeding [6]. Hyperfibrinolysis, which can be diagnosed with rotational thromboelastometry (ROTEM) [11], but not with plasmatic coagulation tests, may explain the bleeding diathesis seen in some dogs with *A. vasorum* infection [6].

Eventually, the identification of dogs at risk for bleeding diathesis remains a challenge. In a previous study, 73% of dogs with *A. vasorum* infection were hypocoagulable based on thromboelastography (TEG, which is comparable to ROTEM), with TEG parameters being significantly different between bleeding and nonbleeding dogs [2]. These authors found no association between other coagulation parameters (fibrinogen concentration, antithrombin activity, D-dimers, von Willebrand factor) and hypocoagulable parameters on TEG.

To our knowledge, clinical signs of bleeding, plasmatic coagulation times, true prevalence of HFL and its association with bleeding diathesis had not been prospectively evaluated in dogs with *A. vasorum* infection.

The present study aimed to describe the coagulation status in dogs naturally infected with *A. vasorum*, to establish the incidence of HFL and to determine clinicopathological parameters indicating the presence of HFL by comparing coagulation parameters of infected dogs to a healthy control group. An additional objective was to evaluate clinicopathological differences between hypocoagulable and normocoagulable *A. vasorum* infected dogs.

We hypothesized that dogs naturally infected with *A. vasorum* and with clinical signs of bleeding would show changes in coagulation parameters, and that ROTEM parameters would be superior predictors of HFL than plasmatic coagulation tests or clinical signs at presentation.

## 2. Results

*Angiostrongylus vasorum* positive dogs included five Beagles, three mixed breed dogs and one each of the following breeds: Belgian Shepherd, Bernese Mountain Dog, Chihuahua, Dalmatian, Doberman, German Shepherd, French Bulldog, Golden Retriever, Hovawart, and Schnauzer. The control group included three mixed breed dogs and one Beagle, one Belgian Shepherd, one German Pointer, one Golden Retriever, and one Nova Scotia Duck Tolling Retriever. Breed ( $p = 0.483$ ), sex ( $p = 0.230$ ), age ( $p = 0.154$ ), and weight ( $p = 0.441$ ) were not significantly different between dogs with *A. vasorum* infection and control dogs.

Of the 18 dogs with *A. vasorum* infection, 7 presented with clinical or magnetic resonance imaging (MRI) signs of bleeding (39%). Bleeding occurred in the brain ( $n = 3$ ), episcleral ( $n = 2$ ), lip ( $n = 2$ ), lung ( $n = 1$ ), and nose ( $n = 1$ ). Three of seven dogs showed more than one bleeding location. All three dogs with signs of central nervous system (CNS) bleeding on MRI presented with episcleral bleeding.

Nine of 18 (50%) infected dogs were hypocoagulable. Six of 7 (86%) bleeding dogs and 3/11 (27%) of nonbleeding dogs were hypocoagulable ( $p = 0.050$ ) while clinical signs of bleeding were present in 6/9 (67%) hypocoagulable and 1/9 (11%) normocoagulable dogs ( $p = 0.050$ ). Hypocoagulable dogs exhibited a median of four different hypocoagulable parameters (2–7).

Differences between control, infected bleeding and infected nonbleeding dogs are summarized in Tables 1 and 2. None of the evaluated clinical parameters at presentation was significantly different between groups (Table 1). The group of dogs with bleeding diathesis was thrombocytopenic, had a prolonged prothrombin time (PT) and hypocoagulable Ex-tem and Fib-tem parameters compared to both nonbleeding and control dogs.

**Table 1.** Haematological, plasmatic coagulation, and ROTEM parameters in 8 control dogs and 18 dogs with *A. vasorum* infection with and without signs of bleeding.

Parameter	Reference Interval	Groups									Comparison between Groups			
		Control (n = 8)			<i>A. vasorum</i> Infected Nonbleeding (n = 11)			<i>A. vasorum</i> Infected Bleeding (n = 7)			All Groups	Control vs. Nonbleeding	Control vs. Bleeding	Bleeding vs. Nonbleeding
		n/N	Median	Range	n/N	Median	Range	n/N	Median	Range	p *	P <sup>+</sup>	P <sup>+</sup>	P <sup>+</sup>
Haematological and plasmatic coagulation results														
Haematocrit (%)	42–55	8/8	48	39–51	11/11	42	36–52	6/7	41	32–46	0.064			
White blood cells (10 × 9/L)	4.7–11.3	8/8	7.7	5.1–10.7	11/11	11.7	9.2–21.1	6/7	13.5	10.9–18	0.001	0.003	0.002	1.000
Platelet count (10 × 9/L)	150–399	7/8	275	213–403	10/11	280	81–421	6/7	130	87–174	0.014	1.000	0.030	0.023
PT (s)	6.5–8.7	8/8	7.2	6.3–7.8	9/11	7.7	7.0–9.9	2/7	17.3	10.5–24.1	0.010	0.126	0.015	0.351
aPTT (s)	10.2–13.8	8/8	11.8	9.6–14.2	9/11	12.1	10.0–15.5	2/7	20.4	14.1–26.7	0.091	NA	NA	NA
Fibrinogen Clauss (g/L)	1.2–2.8	8/8	1.9	1–2.3	9/11	1.9	0.7–2.8	3/7	0.9	0.1–1.2	0.052	NA	NA	NA
D-dimer (mg/dL)	<0.3	8/8	0.1	0.1–1.7	8/11	0.7	0.1–3.8	2/7	1.4	0.9–1.8	0.123	NA	NA	NA
ROTEM parameters														
CT <sub>Ex-tem</sub> (s)	23–87	8/8	34	25–94	11/11	29	24–113	7/7	138	80–251	0.001	1.000	0.012	0.001
CFT <sub>Ex-tem</sub> (s)	85–357	8/8	188	82–264	11/11	180	53–541	7/7	411	210–3600	0.008	1.000	0.017	0.019
Alpha <sub>Ex-tem</sub> (°)	42–77	8/8	58	52–74	11/11	58	35–83	7/7	41	18–53	0.011	1.000	0.019	0.029
MCF <sub>Ex-tem</sub> (mm)	32–65	8/8	50	41–66	11/11	50	28–72	7/7	29	14–47	0.008	1.000	0.020	0.014
MCE <sub>Ex-tem</sub> (%)	45–142	8/8	100	70–197	11/11	99	38–254	7/7	41	17–89	0.007	1.000	0.019	0.013
ML <sub>Ex-tem</sub> (%)	0–12	8/8	5	1–9	11/11	3	0–11	7/7	32	0–100	0.079	NA	NA	NA
CT <sub>In-tem</sub> (s)	133–210	8/8	160	151–206	10/11	184	149–258	1/7	(289)	NA	0.116	NA	NA	NA
CFT <sub>In-tem</sub> (s)	59.201	8/8	102	59–121	10/11	84	51–289	1/7	(294)	NA	0.207	NA	NA	NA
Alpha <sub>In-tem</sub> (°)	58–78	8/8	71	67–78	10/11	73	47–79	1/7	(48)	NA	0.279	NA	NA	NA
MCF <sub>In-tem</sub> (mm)	52–71	8/8	60	57–68	10/11	63	40–72	1/7	(41)	NA	0.235	NA	NA	NA
MCE <sub>In-tem</sub> (%)	108–242	8/8	153	130–211	10/11	169	67–260	1/7	(69)	NA	0.197	NA	NA	NA
ML <sub>In-tem</sub> (%)	0–3	8/8	1	0–5	10/11	0	0–80	1/7	(0)	NA	0.568	NA	NA	NA
CT <sub>Fib-tem</sub> (s)	21–112	8/8	33	22–464	10/11	36	23–3600	7/7	3600	278–3600	0.002	1.000	0.006	0.005
MCF <sub>Fib-tem</sub> (mm)	2–9	8/8	4	2–8	11/11	6	0–14	7/7	0	0–2	0.001	1.000	0.028	0.001
MCE <sub>Fib-tem</sub> (%)	2–9	8/8	4	3–9	11/11	7	0–16	7/7	0	0–2	0.001	1.000	0.024	0.001
CT <sub>Ap-tem</sub> (s)	21–75	8/8	29	23–51	9/11	34	24–95	3/7	238	112–265	0.019	1.000	0.016	0.070
CFT <sub>Ap-tem</sub> (s)	99–485	8/8	198	127–371	9/11	162	49–428	3/7	1044	476–3600	0.023	1.000	0.063	0.020
Alpha <sub>Ap-tem</sub> (°)	42–79	8/8	66	41–78	9/11	59	43–78	3/7	23	17–36	0.023	1.000	0.021	0.056
MCF <sub>Ap-tem</sub> (mm)	32–65	8/8	49	37–57	9/11	52	30–70	3/7	26	16–37	0.034	1.000	0.093	0.031
MCE <sub>Ap-tem</sub> (%)	47–175	8/8	98	59–132	7/11	96	43–231	3/7	34	19–59	0.034	0.980	0.085	0.033

Table 1. Cont.

Parameter	Reference Interval	Groups									Comparison between Groups			
		Control (n = 8)			<i>A. vasorum</i> Infected Nonbleeding (n = 11)			<i>A. vasorum</i> Infected Bleeding (n = 7)			All Groups	Control vs. Nonbleeding	Control vs. Bleeding	Bleeding vs. Nonbleeding
		n/N	Median	Range	n/N	Median	Range	n/N	Median	Range	<i>p</i> *	<i>P</i> <sup>+</sup>	<i>P</i> <sup>+</sup>	<i>P</i> <sup>+</sup>
ML <sub>p-tem</sub> (%)	0–10	8/8	5	0–8	9/11	1	0–13	3/7	0	0–100	0.247	NA	NA	NA
Delta MCF <sub>Ap-tem</sub>	NA	8/8	−14	−22–33	9/11	8	−9–71	3/7	4	−5–14	0.312	NA	NA	NA
−MCF <sub>Ex-tem</sub> (%)														

*p*\*, Kruskal–Wallis test; *P*<sup>+</sup>, post hoc analysis with Dunn–Bonferroni. aPTT, activated partial thromboplastin time; CT, clotting time; CFT, clot formation time; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness; ML, maximum lysis; NA, not assessed; PT, prothrombin time; MCE, maximum clot elasticity; MCF, maximum clot firmness; ML, maximum lysis; PT, prothrombin time.

**Table 2.** Respiratory clinical and radiographic signs, haematological and ROTEM abnormalities and treatment in 8 control, 11 infected nonbleeding, and 7 infected bleeding dogs with *A. vasorum* infection.

Parameter	Groups			All Groups	Comparison between Groups *		
	Control (n = 8)	<i>A. vasorum</i> Infected Nonbleeding (n = 11)	<i>A. vasorum</i> Infected Bleeding (n = 7)		Control vs. Nonbleeding	Control vs. Bleeding	Bleeding vs. Nonbleeding
	n/N (%)	n/N (%)	n/N (%)	<i>p</i>	<i>p</i>	<i>p</i>	<i>p</i>
Respiratory clinical and radiographic signs indicative for canine angiostrongylosis							
Cough	0/8	5/11 (46%)	2/7 (28%)	0.063	NA	NA	NA
Respiratory distress	0/8	2/11 (18%)	1/7 (14%)	1.000	NA	NA	NA
Interstitial pattern on chest radiographs	NA	4/6 (67%)	4/4 (100%)	0.467	NA	NA	NA
Abnormal haematological and plasmatic coagulation parameters							
Thrombocytopenia (<150 × 10 <sup>9</sup> /L)	1/8 (13%)	1/10 (10%)	5/6 (83%)	0.004	1.000	0.026	0.008
PT > 8.7 s	0/8	2/9 (22%)	2/2 (100%)	0.017	0.471	0.022	0.109
aPTT > 13.8 s	1/8 (13%)	1/9 (11%)	2/2 (100%)	0.053	1.000	0.067	0.055
Fibrinogen Clauss <1.2 g/L	0/8	1/9 (11%)	2/3 (67%)	0.046	1.000	0.055	0.127
D-dimers > 0.3 mg/dL	2/8 (25%)	4/8 (50%)	2/2 (100%)	0.171	0.608	0.133	0.467

Table 2. Cont.

Parameter	Groups			All Groups	Comparison between Groups *		
	Control (n = 8)	<i>A. vasorum</i> Infected Nonbleeding (n = 11)	<i>A. vasorum</i> Infected Bleeding (n = 7)		Control vs. Nonbleeding	Control vs. Bleeding	Bleeding vs. Nonbleeding
	n/N (%)	n/N (%)	n/N (%)		p	p	p
Hypocoagulable ROTEM parameters							
CT <sub>Ex-tem</sub> > 87 s	1/8 (13%)	1/11 (9%)	6/7 (86%)	0.001	1.000	0.010	0.002
CFT <sub>Ex-tem</sub> > 328 s	0/8	3/11 (27%)	6/7 (86%)	0.002	0.228	0.001	0.050
Alpha <sub>Ex-tem</sub> < 42°	0/8	1/11 (9%)	4/7 (57%)	0.011	1.000	0.026	0.047
MCF <sub>Ex-tem</sub> < 32 mm	0/8	1/11 (9%)	5/7 (71%)	0.002	1.000	0.007	0.013
MCE <sub>Ex-tem</sub> < 45	0/8	1/11 (9%)	5/7 (71%)	0.002	1.000	0.007	0.013
ML <sub>Ex-tem</sub> > 12%	0/8	0/11	4/7 (57%)	0.002	NA	0.026	0.011
CT <sub>In-tem</sub> > 211 s	0/8	3/10 (30%)	1/1 (100%)	0.063	0.216	0.111	0.364
CFT <sub>In-tem</sub> > 130 s	0/8	2/10 (20%)	1/1 (100%)	0.075	0.477	0.111	0.273
Alpha <sub>In-tem</sub> > 58°	0/8	1/10 (10%)	1/1 (100%)	0.105	1.000	0.111	0.182
MCF <sub>In-tem</sub> < 57 mm	0/8	2/10 (20%)	1/1 (100%)	0.075	0.477	0.111	0.273
MCE <sub>In-tem</sub> < 108	0/8	2/10 (20%)	1/1 (100%)	0.075	0.477	0.111	0.273
ML <sub>In-tem</sub> > 5%	0/8	2/10 (20%)	0/1	0.532	0.477	NA	1.000
CT <sub>Fib-tem</sub> > 112 s	3/8 (38%)	1/10 (10%)	7/7 (100%)	0.001	0.275	0.026	<0.001
MCF <sub>Fib-tem</sub> 0 mm	0/8	1/11 (9%)	6/7 (86%)	<0.001	1.000	0.001	0.002
MCF <sub>Ap-tem</sub> > MCF <sub>Ex-tem</sub>	2/8 (25%)	2/9 (18%)	0/3	1.000	1.000	1.000	1.000
Treatment							
Plasma transfusion	NA	0/11	4/7 (57%)	NA	NA	NA	0.011
Tranexamic acid	NA	0/11	5/7 (71%)	NA	NA	NA	0.002

\* chi<sup>2</sup> test; aPTT, activated partial thromboplastin time; CT, clotting time; CFT, clot formation time; AUC, area under the curve; CI, confidence interval; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness; ML, maximum lysis; PT, prothrombin time; MCE, maximum clot elasticity; MCF, maximum clot firmness; ML, maximum lysis; NA, not assessed; PT, prothrombin time.

Differences between hypocoagulable and normocoagulable *A. vasorum* infected dogs are summarized in Tables 3 and 4. None of the clinical signs occurred in different frequency between hypo- and normocoagulable dogs. Platelet count (but not the presence of thrombocytopenia), PT, and several ROTEM parameters were significantly different between hypocoagulable and normocoagulable *A. vasorum* infected dogs. Clinical signs and the localization of clinical manifestations were not significantly different between hypo- and normocoagulable dogs.

**Table 3.** Differences in haematological, plasmatic coagulation and ROTEM parameters between hypo- vs. normocoagulable *A. vasorum* infected dogs.

Parameter	Normocoagulable (n = 9)			Hypocoagulable (n = 9)			Reference Interval	p *
	n/N	Median	Range	n/N	Median	Range		
Haematological and plasmatic coagulation parameters								
Haematocrit (%)	9/9	44	32–52	9/9	41	36–46	42–55	0.423
White blood cells (10 <sup>9</sup> /L)	9/9	11.7	9.2–15.2	8/9	13.7	10.9–21.1	4.7–11.3	0.139
Platelet count (10 <sup>9</sup> /L)	8/9	292	137–421	8/9	136	81–286	150–399	0.007
PT (s)	6/9	7.6	7.0–8.5	5/9	9.9	7.7–24.1	6.5–8.7	0.017
aPTT (s)	6/9	12.1	10.0–12.6	5/9	14.1	11.6–26.7	10.2–13.8	0.082
Fibrinogen Clauss (g/L)	7/9	1.9	1.2–2.8	5/9	0.9	0.1–2.3	1.2–2.8	0.073
D-dimer (mg/dL)	6/9	0.7	0.1–3.8	4/9	0.9	0.5–1.8	<0.3	1.000
ROTEM parameters								
CT <sub>Ex-tem</sub> (s)	9/9	29	24–80	9/9	134	27–251	23–87	0.001
CFT <sub>Ex-tem</sub> (s)	9/9	175	53–359	9/9	411	180–3600	85–357	0.001
Alpha <sub>Ex-tem</sub> (°)	9/9	58	47–83	9/9	41	18–58	42–77	0.003
MCF <sub>Ex-tem</sub> (mm)	9/9	50	35–72	9/9	29	14–54	32–65	0.002
MCE <sub>Ex-tem</sub>	9/9	99	53–254	9/9	41	17–117	45–142	0.002
ML <sub>Ex-tem</sub> (%)	9/9	2	0–4	9/9	11	0–100	0–12	0.063
Ct <sub>In-tem</sub> (s)	7/9	173	149–233	4/9	226	168–289	133–210	0.230
CFT <sub>In-tem</sub> (s)	7/9	83	51–105	4/9	255	79–294	59–201	0.073
Alpha <sub>In-tem</sub> (°)	7/9	73	69–79	4/9	56	47–74	58–78	0.073
MCF <sub>In-tem</sub> (mm)	7/9	63	62–72	4/9	44	40–64	52–71	0.073
MCE <sub>In-tem</sub>	7/9	172	164–260	4/9	79	67–177	108–242	0.073
ML <sub>In-tem</sub> (%)	7/9	0	0–22	4/9	1	0–80	0–3	0.412
CT <sub>Fib-tem</sub> (s)	8/9	36	25–278	9/9	3600	23–3600	21–112	0.008
MCF <sub>Fib-tem</sub> (mm)	9/9	6	2–14	9/9	0	0–5	2–9	<0.001
MCE <sub>Fib-tem</sub>	9/9	7	2–16	9/9	0	0–5	2–9	<0.001
CT <sub>Ap-tem</sub> (s)	6/9	32	24–45	6/9	104	26–265	21–75	0.041
CFT <sub>Ap-tem</sub> (s)	6/9	125	59–193	6/9	452	238–3600	99–485	0.002
Alpha <sub>Ap-tem</sub> (°)	6/9	67	55–78	6/9	40	17–52	42–79	0.002
MCF <sub>Ap-tem</sub> (mm)	6/9	57	49–70	6/9	34	16–49	32–65	0.002
MCE <sub>Ap-tem</sub>	4/9	152	94–231	6/9	51	19–96	47–175	0.019
ML <sub>Ap-tem</sub> (%)	6/9	1	0–2	6/9	0	0–100	0–10	0.818
Delta								
MCF <sub>Ap-tem</sub> -MCF <sub>Ex-tem</sub> (%)	6/9	9.5	1.7–71.4	6/9	5.6	−9.3–14.3		0.240

\* p determined by Mann–Whitney U-test; aPTT, activated partial thromboplastin time; CT, clotting time; CFT, clot formation time; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness; ML, maximum lysis; PT, prothrombin time; MCE, maximum clot elasticity; MCF, maximum clot firmness; ML, maximum lysis; PT, prothrombin time.

**Table 4.** Comparison of the occurrence of clinicopathological variables between normo- and hypocoagulable *A. vasorum* infected dogs.

Parameter	Normocoagulable (n = 9)	Hypocoagulable (n = 9)	p
	n/N (%)	n/N (%)	
Localization of clinical manifestation			
Mucocutaneous bleeding	1/9 (11%)	2/9 (22%)	0.086
Lung	4/9 (44%)	2/9 (22%)	
CNS	0	4/9 (44%)	
Incidental diagnosis	4/9 (44%)	1/9 (11%)	
Respiratory clinical and radiographic signs and bleeding indicative for canine angiostrongylosis			
Cough	4/9 (44%)	2/9 (22%)	0.620
Respiratory distress	1/9 (11%)	2/9 (22%)	0.500
Alveolar-interstitial pattern on chest radiographs	4/5 (80%)	4/5 (80%)	1.000
Clinical signs of bleeding	1/9 (11%)	6/9 (67%)	0.050
Hematological parameters correlated with coagulation			
Thrombocytopenia	1/8 (13%)	5/8 (63%)	0.119
PT > 8.7 s	0/6	4/5 (80%)	0.015
aPTT > 13.8 s	0/6	3/5 (60%)	0.061
Fibrinogen Clauss < 1.2 g/L	0/7	3/5 (60%)	0.045
D-dimer > 0.3 mg/dL	3/6 (50%)	3/4 (75%)	0.571
ROTEM parameters			
CT <sub>Ex-tem</sub> > 87 s	0/9	7/9 (78%)	0.002
CFT <sub>Ex-tem</sub> > 328 s	1/9 (11%)	8/9 (89%)	0.003
MCF <sub>Ex-tem</sub> < 32 mm	0/9	6/9 (67%)	0.009
alpha <sub>Ex-tem</sub> < 42°	0/9	5/9 (56%)	0.029
MCE <sub>Ex-tem</sub> < 45	0/9	6/9 (67%)	0.009
ML <sub>Ex-tem</sub> > 12%	0/9	4/9 (44%)	0.082
CT <sub>In-tem</sub> > 211 s	2/7 (29%)	2/4 (50%)	0.576
CFT <sub>In-tem</sub> > 130 s	0/7	3/4 (75%)	0.024
MCF <sub>In-tem</sub> < 57 mm	0/7	3/4 (75%)	0.024
alpha <sub>In-tem</sub> > 58°	0/7	2/4 (50%)	0.109
ML <sub>In-tem</sub> > 5%	1/7 (14%)	1/4 (25%)	1.000
MCE <sub>In-tem</sub> < 108	0/7	3/4 (75%)	0.024
CT <sub>Fib-tem</sub> > 112 s	1/8 (13%)	7/9 (78%)	0.015
MCF <sub>Fib-tem</sub> 0 mm	0/9	7/9 (78%)	0.002
Treatment			
Plasma transfusion	0/9	4/9 (44%)	0.082
Tranexamic acid	0/9	5/9 (56%)	0.029

aPTT, activated partial thromboplastin time; CT, clotting time; CFT, clot formation time; AUC, area under the curve; CI, confidence interval; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness; ML, maximum lysis; PT, prothrombin time; MCE, maximum clot elasticity; MCF, maximum clot firmness; ML, maximum lysis; PT, prothrombin time.

Overall incidence of HFL defined by maximum lysis above the reference interval in any of the ROTEM tracings was 44% in dogs with *A. vasorum* infection (8/18 dogs) and was significantly higher in bleeding dogs ( $p = 0.011$ ; Table 2) and hypocoagulable dogs ( $p = 0.015$ , Table 4). Four of 7 (57%) bleeding dogs showed increased Ex-tem lysis compared to none of the nonbleeding dogs ( $p = 0.011$ ). Hyperfibrinolysis was significantly associated with low MCF<sub>Fib-tem</sub> ( $p = 0.001$ ) but not with low fibrinogen concentration ( $p = 0.088$ ). AUROC results for prediction of HFL are shown in Table 5. CFT<sub>Ex-tem</sub> > 248 s was 100% sensitive and 89% specific and CT<sub>Ex-tem</sub> > 103 s was 75% sensitive and 99% specific. Hyperfibrinolysis was not associated with high D-dimers ( $p = 0.559$ ).

**Table 5.** AUROC analysis for specific factors to predict hyperfibrinolysis in 18 dogs with *A. vasorum* infection.

Parameter	AUC	95% CI	<i>p</i>
CT <sub>Ex-tem</sub>	0.781	0.552–1.000	0.024
CFT <sub>Ex-tem</sub>	0.951	0.874–1.000	<0.001
PT	0.808	0.593–1.000	0.064
Fibrinogen Clauss	0.336	0.000–0.702	0.321
MCF <sub>Ex-tem</sub>	0.042	0.000–0.115	<0.001
ML <sub>Ex-tem</sub>	0.125	0.000–0.273	0.003
Platelet count	0.250	0.050–0.450	0.072

AUC, area under the curve; CI, confidence interval; CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness; ML, maximum lysis; PT, prothrombin time.

All dogs were discharged from the hospital. Four dogs had been treated with fresh frozen plasma (20–30 mL/kg BW) and additionally received tranexamic acid (20–60 mg/kg). These four dogs were bleeding, hypocoagulable, and lacked clot formation in the Fib-tem tracing. An additional bleeding dog was treated with tranexamic acid. Plasma transfusion was significantly associated with low fibrinogen Clauss ( $p = 0.045$ ), low MCF<sub>Fib-tem</sub> ( $p = 0.011$ ), CT<sub>Ex-tem</sub> prolongation ( $p = 0.011$ ), and bleeding ( $p = 0.011$ ) but not with hypocoagulability ( $p = 0.082$ ).

Ap-tem tracings showed both weaker and stronger MCFs than Ex-tem profiles. MCF<sub>Ap-tem</sub> was not stronger (indicating HFL) in any of the 5 dogs with HFL diagnosed in Ex-tem or In-tem but was > 15% stronger in 4/15 dogs with no HFL and ML's within the reference interval.

### 3. Discussion

The present prospective study identified bleeding diathesis in 39% of dogs naturally infected with *A. vasorum*. Among those, 86% were diagnosed hypocoagulable by thromboelastometry and plasmatic coagulation tests. Hyperfibrinolysis was present in 44% of all infected dogs and in 56% of those dogs with bleeding diathesis.

The true incidence of *A. vasorum* associated bleeding diathesis remains unknown, as this study was based mostly on referred cases. However, our incidence of 39% is in the range of previously reported incidences between 35% and 57% [6,12].

The main clinical signs of dogs naturally infected with *A. vasorum* are coughing and tachypnea/dyspnea together with unspecific signs such as lethargy, anorexia, and vomiting/diarrhea [2,4,12–15]. Haemorrhage and neurological signs are less frequently observed [4,12]. None of the clinically evaluated parameters in the current study were significantly associated with the presence of haemorrhage, as previously observed [2].

ROTEM analysis identified several parameters that were significantly different between dogs with bleeding diathesis compared to infected dogs without bleeding and healthy control dogs. Prolonged CT<sub>Ex-TEM</sub>, CFT<sub>Ex-TEM</sub>, decreased MCF<sub>Ex-tem</sub> and MCF<sub>Fib-tem</sub>, and Ex-TEM HFL were significantly associated with the presence of bleeding. These findings indicate that both clot formation and clot strength are affected in dogs with *A. vasorum* induced coagulopathy.

In contrast to a previous report [2], our study population of dogs with bleeding diathesis showed a significantly lower median platelet count compared to both nonbleeding dogs and healthy control dogs. The platelet count of bleeding dogs was below the reference interval but not in a range that would explain spontaneous bleeding. Several studies and case reports demonstrated thrombocytopenia and prolonged clotting times [8,16–18], but experimental studies indicate that such findings are inconsistent over time [7,9,13]. Thrombocytopenia was significantly associated with the presence of bleeding in our study population and 83% of dogs with bleeding were thrombocytopenic ( $n = 5/6$ ), while this was observed in only 10% ( $n = 1/10$ ) of nonbleeding infected dogs. However, no reasonable platelet count cut-off value to identify dogs with bleeding diathesis could be identified and whether the decreased platelet count is the cause or the result of bleeding is unknown. Together with the identified mild thrombocytopenia, we interpret this finding as evidence



that thrombocytopenia is a consequence rather than the cause of bleeding, indicating that mechanisms other than thrombocytopenia are causing bleeding diathesis.

The PT, aPTT, and fibrinogen concentration were not significantly associated with bleeding, indicating that viscoelastic testing is superior to classical plasmatic coagulation times for the identification of bleeding in *A. vasorum* infected dogs.

While overt clinical signs of bleeding will guide the clinician to investigate the coagulation status, internal bleeding in the brain or lung may be fatal if hypocoagulability is not detected and treated [17,19]. The recognition of dogs at risk for bleeding diathesis remains a challenge [6]. Therefore, the identification of valid prediction parameters to detect hypocoagulability are of relevant clinical interest.

We therefore analysed clinical and laboratory parameters to detect hypocoagulability in *A. vasorum* infected dogs. Of interest, only two-thirds of hypocoagulable dogs showed clinical signs of bleeding and more than one-quarter of nonbleeding dogs were hypocoagulable and hence at risk to develop bleeding during hospitalization. Cough, pulmonary changes consistent with radiographic signs of *A. vasorum* infection [20] on radiographs and presence of respiratory signs were not associated with hypocoagulability, suggesting that respiratory signs and the typical interstitial-alveolar pattern seen on thoracic radiographs are not caused by haemorrhage; consequently, thoracic radiographs will not aid in the identification of hypocoagulable dogs. This finding is supported by a study evaluating radiographic, CT and necropsy findings in experimentally infected dogs and linking them to granulomatous inflammation rather than haemorrhage [20].

Of note, all dogs with neurological signs were hypocoagulable. Such *A. vasorum* infected dogs commonly do not present with classical pulmonary signs and internal haemorrhage may not be apparent [17]. However, the three dogs with neurological signs all presented with episcleral bleeding, a finding that should be investigated in a larger study population. Until then, it is important to not only include *A. vasorum* infection as a differential diagnosis to central nervous clinical signs but to further investigate the coagulation status for hypocoagulability if the dogs are infected.

In contrast, none of the dogs with an incidental diagnosis of *A. vasorum* infection was hypocoagulable. We suspect that these dogs were either not infected long enough to develop coagulopathies or with too few worms to show hypocoagulability or clinical signs of bleeding. Experimentally inoculated dogs with higher worm burdens showed earlier and more severe respiratory signs and more pronounced coagulopathies [13]. The magnitude of parasite burden was unknown in our study population; therefore, a direct comparison cannot be made. In addition, individual differences between dogs cannot be excluded: the study of dog serum proteome in experimentally infected dogs revealed that the lectin pathway and the coagulation cascades were particularly affected; these may be additionally associated with individual differences that altogether may explain the onset or absence of hypocoagulability [10].

In the present work, not all hypocoagulable dogs exhibited identifiable signs of bleeding, highlighting the need for laboratory analysis of coagulation status to identify dogs at risk of bleeding. Hypocoagulable dogs had significantly longer PT and abnormal Ex-tem, In-tem, and Fib-tem parameters but no significant thrombocytopenia or prolonged aPTT. Previously, no associations could be identified between coagulation parameters (fibrinogen concentration, antithrombin activity, D-dimers, von Willebrand factor) and hypocoagulable thromboelastography parameters [2], indicating that viscoelastic tests may be more appropriate to identify hypocoagulable dogs.

We recently retrospectively identified HFL and associated hypofibrinogenemia in *A. vasorum* infected dogs [6], two findings that are supported by the data presented here. Hyperfibrinolysis was identified in 44% of dogs naturally infected with *A. vasorum* and 5/9 (56%) of bleeding dogs showed increased Ex-tem or In-tem lysis compared to 1/9 (11%) nonbleeding dogs. Both bleeding and hypocoagulable dogs had low to no fibrin clot formation compared to nonbleeding dogs. Hypofibrinogenemia was significantly more common in hypocoagulable dogs. Bleeding dogs displaying decreased fibrinogen

concentration have also been described previously [2]. As suggested earlier [5,6], the cause of decreased fibrin clot formation in the Fib-tem tracing may be explained by HFL and not by low fibrinogen concentrations. Hyperfibrinolysis was significantly associated with  $MCF_{Fib-TEM}$  but not with fibrinogen concentration measured by Clauss. Identification and treatment of HFL therefore seems to be of clinical relevance. Neither platelet count nor plasmatic coagulation times are able to predict or diagnose HFL. ROTEM analysis; however, was able to predict HFL by means of prolonged CT and CFT within minutes.

The initial study protocol envisaged Ap-tem profiles to confirm HFL identified in Ex-tem profiles. Our study, showing inconsistent Ap-tem results, supports earlier evidence that Ap-tem assays are not helpful in the diagnosis of HFL in dogs [6,11].

Elevated D-dimer concentrations in dogs with *A. vasorum* infection have also been described [2,17,21]. Although expected to increase with fibrinolysis, D-dimer levels were not different between hypo- and normocoagulable dogs in our study population and were not associated with HFL. A reason for this finding may be that the presence of D-dimers requires lysis of crosslinked fibrin while *A. vasorum* associated lysis of fibrinogen or fibrin strands may occur prior to crosslinking to a stable fibrin clot [6]. Fibrinogen, being an acute phase protein, is expected to be present at high levels in dogs with *A. vasorum* infections. Another possible explanation for the lack of stable fibrinogen clot is FXIII deficiency or malfunction. FXIII is needed for cross-linking of fibrin and congenital or acquired deficiency leads to unexpected bleeding in people [22,23]. In line with this, a recent time-course proteomics study performed on sera of *A. vasorum* infected dogs identified FXIII-B, a subunit of FXIII, as downregulated among other candidates of the coagulation cascade and complement pathway compared to before inoculation with the parasite [10].

While significantly more hypocoagulable dogs were treated with tranexamic acid, hypocoagulability was not significantly associated with administration of a plasma transfusion. Significantly more plasma transfusions were given to bleeding dogs, however, a plasma transfusion was not deemed necessary in 3/7 dogs with bleeding, based on coagulation parameters. Together with the wide variety of identified ROTEM abnormalities in hypocoagulable dogs, this indicates that coagulation testing the complete set of coagulation parameters is crucial to identify not only dogs at risk for bleeding but also to treat the coagulation disorder with specific therapeutic measures.

In former studies up to 30% of *A. vasorum* infected dogs died [2,12], while all dogs in this study were discharged from the hospital. It is possible that early identification of coagulation problems and early therapeutic intervention has led to the excellent survival rate in our study population. As all patients were treated based on ROTEM results as well as at the discretion of the clinician in charge, the effect of treatment on survival cannot be investigated further.

This study included only a small group of *A. vasorum* infected dogs and only part of them showed bleeding diathesis. Unfortunately, bleeding dogs were often presented during emergency hours when plasmatic coagulation testing was not available. We however showed that results of plasmatic coagulation testing should be interpreted with caution. Additionally, when ROTEM tests were chosen by the clinician in charge during emergency service hours, in several patients In-tem analysis was not performed. In-tem parameters between bleeding and nonbleeding *A. vasorum* infected dogs could therefore not be compared. Bleeding was furthermore based on visible clinical signs of bleeding and MRI, with the possibility of having falsely assigned an internally bleeding dog to the nonbleeding group.

#### 4. Materials and Methods

Dogs presenting to the Small Animal Clinic, Vetsuisse Faculty, University of Zurich between March 2016 and July 2019 and being diagnosed with *A. vasorum* infection by either serological ELISA antigen or rapid-assay (AngioDetect™) [24,25] and/or copromicroscopic identification of first stage larvae by the Baermann funnel method were eligible to enter this and a concurrent study. Healthy dogs were used as a control group. Control

dogs had a normal complete blood cell and chemistry panel, plasmatic coagulation times including fibrinogen measured by Clauss and were negative for *A. vasorum* infection. The project was approved by the ethics committee on animal research of the canton of Zurich (ZH001/16,27384) and informed owner consent was obtained for both healthy and infected dogs, in which additional blood tests were performed for the study.

Dogs were excluded if they were pre-treated with antifibrinolytics, blood products or NSAIDS within five days of presentation and if ROTEM analysis was not performed within 30 min after blood sampling.

Signalment, sex, weight, presenting complaint, clinical signs including the presence of bleeding were determined at presentation. The infected dogs were assigned to the bleeding or the nonbleeding group based on the presence of clinical signs of bleeding or MRI signs compatible with bleeding in dogs undergoing MRI exam. In addition, clinical signs were categorized as mucocutaneous bleeding, lung or CNS localization or incidental diagnosis without clinical signs (localization of clinical manifestation). Diagnostic imaging findings and survival to discharge were extracted later from patient records.

Approximately 8–12 mL of venous blood were drawn at presentation or immediately after diagnosis of *A. vasorum* infection using a 20 G hypodermic needle. Blood samples were transferred to tubes in the following order: two 3.2% citrate and one EDTA tube (all Sarstedt AG, Sevelen, Switzerland). One citrate and the EDTA tube were sent to the in-house laboratory for plasmatic coagulation tests (PT, aPTT, TT, fibrinogen<sub>Clauss</sub>, and D-dimers) (Start 4 STAGO CH SA, Glattbrugg, Switzerland) and complete blood cell status (Sysmex-XT 2000iV, Sysmex Cooperation, Kobe, Japan). Another citrate tube was placed within the warming plate of the ROTEM device for 10–20 min.

Ex-tem S, In-tem S, Fib-tem S, and Ap-tem S analysis (TEM International GmbH, Munich) were performed by the primary investigators according to an institutional protocol based on the manufacturer's instruction and international guidelines [26,27]. Briefly, 300 µL citrated whole blood were incubated with the lyophilized reagent before placement in the cup and start of measurement. An automated pipetting system was used. Tracings were run for 60 min. All tracings were checked for artefacts by two of the authors (N.E.S, A.P.N.K). Clotting time (CT), clot formation time (CFT), alpha angle (α), maximum clot firmness (MCF), maximum clot elasticity (MCE) and maximum lysis (ML) were extracted from the ROTEM database. A green line in the fib-tem tracing was defined as an MCF<sub>Fib-tem</sub> of 0 mm. If MCF did not reach 20 mm, CFT was defined as 3600 s. Hypofibrinogenemia was defined as fibrinogen measured by Clauss < 1.2 g/L (in-house reference interval) or MCF<sub>Fib-tem</sub> < 2 mm.

Based on ROTEM profiles, dogs were categorized as hypocoagulable if  $\geq 2$  of the following parameters were hypocoagulable compared to reference intervals [28]: CT<sub>Ex-tem</sub> or PT, CT<sub>In-tem</sub> or aPTT, CFT<sub>Ex-tem</sub> / In-tem or  $\alpha$  Ex-tem / In-tem, MCF<sub>Ex-tem</sub> / In-tem / Fib-tem or MCE<sub>Ex-tem</sub> / In-tem / Fib-tem. Hyperfibrinolysis was defined as ML > reference interval in any of the tracings. Dogs were treated at the discretion of the clinician in charge, generally based on a standardized protocol including 50 mg/kg BW fenbendazole daily for three weeks and 1 mg/kg BW prednisolone daily for seven days was administered; strict exercise constraint was advised. Fresh frozen plasma (20 mL/kg BW) was transfused for hypofibrinogenemia and tranexamic acid was prescribed to treat HFL (20 mg/kg BW, repeated until HFL resolved) as needed.

### Statistical Analysis

Data were entered into a spreadsheet and analyses were performed using the statistical software program IBM SPSS (Version 23.0. IBM Corp, Armonk, NY, USA). Continuous data is presented as median (min-max). Clinical signs and coagulation parameters were compared between dogs with and without bleeding diathesis and control dogs using Kruskal–Wallis tests followed by post hoc analysis with Dunn-Bonferroni for continuous variables and chi<sup>2</sup> test for categorical variables. In a second step dogs with *A. vasorum* infection were grouped as hypo- or normocoagulable and clinical signs and coagulation

parameters were compared across groups using Mann–Whitney U-test or Fisher’s exact test. The accuracy of standard coagulation tests and ROTEM parameters to determine dogs with HFL was evaluated using receiver operating characteristics (ROC) and the area under the ROC curve (AUROC). Youden’s J statistic was used to select the optimum cut-off point of the ROC curves. A value of  $p < 0.05$  was considered significant.

## 5. Conclusions

In conclusion, HFL, hypocoagulability and bleeding are commonly occurring in *A. vasorum* infected dogs. None of the evaluated clinical parameters are significantly associated with bleeding or hypocoagulability, and plasmatic coagulation tests did not allow clear discrimination between bleeding and nonbleeding *A. vasorum* infected dogs. In contrast, most ROTEM parameters were significantly different between groups, allowing the differentiation between bleeding and nonbleeding *A. vasorum* infected dogs. Ex-tem analysis was able to detect bleeding, hypocoagulability, and HFL within a few minutes after blood draw and may therefore be recommended.

**Author Contributions:** Conceptualization, N.E.S., A.P.N.K., L.T. and M.S.; Methodology, N.E.S., A.P.N.K., L.T. and M.S.; Formal analysis, N.E.S.; Investigation, N.E.S., R.J.S., N.H.-I. and C.K.-F.; Resources, N.E.S. and M.S.; Data curation, N.E.S.; Writing—original draft preparation, N.E.S.; Writing—review and editing, L.T., A.P.N.K., C.K.-F., R.J.S., M.S. and N.H.-I.; Visualization, N.E.S., M.S., A.P.N.K., L.T., N.H.-I. and R.J.S.; Supervision, M.S.; Project administration, N.E.S.; Funding acquisition, N.E.S., R.J.S. and M.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was partly funded by the Small Animal Foundation of the Vetsuisse Faculty (ROTEM material) and the ‘Privatdozierende’ Foundation of the University of Zurich (lab costs).

**Institutional Review Board Statement:** The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the ethics committee on animal research of the canton of Zurich (ZH001/16, 27384).

**Informed Consent Statement:** Informed consent was obtained from all dogs involved in the study in which additional blood tests were performed for the study.

**Data Availability Statement:** The data presented in this study is available on request from the corresponding author.

**Acknowledgments:** We would like to thank Axonlab AG, Switzerland for a generous discount on ROTEM material.

**Conflicts of Interest:** The authors declare no conflict of interest. M. Schnyder is guest editor of this journal but was not involved in the reviewing process or decision taking regarding publication of this study. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

## References

1. Staebler, S.; Ochs, H.; Steffen, F.; Naegeli, F.; Borel, N.; Sieber-Ruckstuhl, N.; Deplazes, P. Autochthonous infections with *Angiostrongylus vasorum* in dogs in Switzerland and Germany. *Schweiz. Arch. Tierheilkd.* **2005**, *147*, 121–127. [[CrossRef](#)]
2. Adamantos, S.; Waters, S.; Boag, A. Coagulation status in dogs with naturally occurring *Angiostrongylus vasorum* infection. *J. Small Anim. Pract.* **2015**, *56*, 485–490. [[CrossRef](#)] [[PubMed](#)]
3. Glaus, T.; Sigrist, N.; Hofer-Inteeworn, N.; Kuemmerle-Fraune, C.; Mueller, C.; Geissweid, K.; Beckmann, K.; Wenger, M.; Matos, J.N. Unexplained bleeding as primary clinical complaint in dogs infected with *Angiostrongylus vasorum*. *Schweiz. Arch. Tierheilkd.* **2016**, *158*, 701–709. [[CrossRef](#)]
4. Koch, J.; Willesen, J. Canine pulmonary angiostrongylosis: An update. *Vet. J.* **2009**, *179*, 348–359. [[CrossRef](#)]
5. Zoia, A.; Caldin, M. Coagulation status in dogs with naturally occurring *Angiostrongylus vasorum* infection and primary hyperfibrinolysis. *J. Small Anim. Pract.* **2015**, *56*, 728. [[CrossRef](#)] [[PubMed](#)]
6. Sigrist, N.; Hofer-Inteeworn, N.; Schefer, R.J.; Kuemmerle-Fraune, C.; Schnyder, M.; Kutter, A. Hyperfibrinolysis and hypofibrinogenemia diagnosed with rotational thromboelastometry in dogs naturally infected with *Angiostrongylus vasorum*. *J. Vet. Intern. Med.* **2017**, *31*, 1091–1099. [[CrossRef](#)]

7. Schelling, C.; Greene, C.; Prestwood, A.; Tsang, V. Coagulation abnormalities associated with acute *Angiostrongylus vasorum* in dogs. *Am. J. Vet. Res.* **1987**, *47*, 2669–2673.
8. Ramsey, I.; Littlewood, J.D.; Dunn, J.K.; Herrtage, M.E. Role of chronic disseminated intravascular coagulation in a case of canine angiostrongylosis. *Vet. Rec.* **1996**, *138*, 360–363. [[CrossRef](#)]
9. Cury, M.; Lima, W.; Guimarães, M.; Carvalho, M. Hematological and coagulation profiles in dogs experimentally infected with *Angiostrongylus vasorum* (Baillet, 1866). *Vet. Parasitol.* **2001**, *104*, 139–149. [[CrossRef](#)]
10. Tritten, L.; Gillis-Germitsch, N.; Kockmann, T.; Schnyder, M. Quantitative proteomics analysis of *Angiostrongylus vasorum*-induced alterations in dog serum sheds light on the pathogenesis of canine angiostrongylosis. *Sci. Rep.* **2021**, *11*, 1–11. [[CrossRef](#)]
11. Sigrist, N.; Schefer, R.J.; Kutter, A. Characteristics of hyperfibrinolysis in dogs and cats demonstrated by rotational thromboelastometry (ROTEM). *Vet. J.* **2018**, *242*, 67–73. [[CrossRef](#)] [[PubMed](#)]
12. Chapman, P.; Boag, A.K.; Guitian, J.; Boswood, A. *Angiostrongylus vasorum* infection in 23 dogs (1999–2002). *J. Small Anim. Pract.* **2004**, *45*, 435–440. [[CrossRef](#)]
13. Schnyder, M.; Fahrion, A.; Riond, B.; Ossent, P.; Webster, P.; Kranjc, A.; Glaus, T.; Deplazes, P. Clinical, laboratory and pathological findings in dogs experimentally infected with *Angiostrongylus vasorum*. *Parasitol Res.* **2010**, *107*, 1471–1480. [[CrossRef](#)]
14. Morgan, E.; Jefferies, R.; van Otterdijk, L.; McEniry, R.; Allen, F.; Bakewell, M.; Shaw, S. *Angiostrongylus vasorum* infection in dogs: Presentation and risk factors. *Vet. Parasitol.* **2010**, *173*, 255–261. [[CrossRef](#)]
15. Holmes, S.; Paine, P.; Wright, I.; Morgan, E.R.; Elsheikha, H.M. Risk factors and predictors of angiostrongylosis in naturally infected dogs in the southeast of England. *Companion Anim.* **2020**, *25*, 233–240. [[CrossRef](#)]
16. Helm, J.R.; Morgan, E.; Jackson, M.W.; Wotton, P.; Bell, R. Canine angiostrongylosis: An emerging disease in Europe. *J. Vet. Emerg. Crit. Care* **2010**, *20*, 98–109. [[CrossRef](#)]
17. Gredal, H.; Willesen, J.L.; Jensen, H.; Nielsen, O.L.; Kristensen, A.T.; Koch, J.; Kirk, R.K.; Pors, S.; Skerritt, G.C.; Berendt, M. Acute neurological signs as the predominant clinical manifestation in four dogs with *Angiostrongylus vasorum* infections in Denmark. *Acta Vet. Scand.* **2011**, *53*, 43–48. [[CrossRef](#)] [[PubMed](#)]
18. Willesen, J.; Bjornvad, C.; Koch, J. Acute haemoabdomen associated with *Angiostrongylus vasorum* infection in a dog: A case report. *Ir. Vet. J.* **2008**, *61*, 591–593. [[CrossRef](#)]
19. Yamakawa, Y.; McGarry, J.W.; Denk, D.; Dukes-McEwan, J.; Macdonald, N.; Mas, A.; McConnell, F.; Tatton, B.; Valentine, E.G.; Wayne, J.; et al. Emerging canine angiostrongylosis in northern England: Five fatal cases. *Vet. Rec.* **2009**, *164*, 149–152. [[CrossRef](#)]
20. Dennler, M.; Makara, M.; Kranjc, A.; Schnyder, M.; Ossent, P.; Deplazes, P.; Ohlerth, S.; Glaus, T.M. Thoracic computed tomography findings in dogs experimentally infected with *Angiostrongylus vasorum*. *Vet. Radiol. Ultrasound* **2011**, *52*, 289–294. [[CrossRef](#)]
21. Kruse, B.D.; Hartmann, K.; Groth, A.; Schulz, B.; Wehner, A. Disseminated intravascular coagulopathy in a dog with *Angiostrongylus vasorum* infection. *Tierarztl. Prax. Ausg. K Kleintiere Heimtiere* **2013**, *41*, 401–407. [[CrossRef](#)] [[PubMed](#)]
22. Wettstein, P.; Haerberli, A.; Stutz, M.; Rohner, M.; Corbetta, C.; Gabi, K.; Schnider, T.; Korte, W. Decreased factor XIII availability for thrombin and early loss of clot firmness in patients with unexplained intraoperative bleeding. *Anesth. Analg.* **2004**, *99*, 1564–1569. [[CrossRef](#)]
23. Theusinger, O.M. The Inhibiting effect of factor XIII on hyperfibrinolysis. *Anesth. Analg.* **2012**, *114*, 1149–1150. [[CrossRef](#)] [[PubMed](#)]
24. Schnyder, M.; Jefferies, R.; Schucan, A.; Morgan, E.R.; Deplazes, P. Comparison of coprological, immunological and molecular methods for the detection of dogs infected with *Angiostrongylus vasorum* before and after anthelmintic treatment. *Parasitology* **2015**, *142*, 1270–1277. [[CrossRef](#)]
25. Schnyder, M.; Stebler, K.; Naucke, T.J.; Lorentz, S.; Deplazes, P. Evaluation of a rapid device for serological in-clinic diagnosis of canine angiostrongylosis. *Parasites Vectors* **2014**, *7*, 72. [[CrossRef](#)]
26. De Laforcade, A.; Goggs, R.; Wiinberg, B. Systematic evaluation of evidence on veterinary viscoelastic testing Part 3: Assay activation and test protocol. *J. Vet. Emerg. Crit. Care* **2014**, *24*, 37–46. [[CrossRef](#)] [[PubMed](#)]
27. Flatland, B.; Koenigshof, A.M.; Rozanski, E.A.; Goggs, R.; Wiinberg, B. Systematic evaluation of evidence on veterinary viscoelastic testing Part 2: Sample acquisition and handling. *J. Vet. Emerg. Crit. Care* **2014**, *24*, 30–36. [[CrossRef](#)] [[PubMed](#)]
28. Schefer, R.J.; Heimgartner, L.; Stirn, M.; Sigrist, N.E. Determination of reference intervals for single vial rotational thromboelastometry (ROTEM) parameters and correlation with plasmatic coagulation times in 49 clinically healthy dogs. *Res. Vet. Sci.* **2020**, *129*, 129–136. [[CrossRef](#)]