

Standard Article

J Vet Intern Med 2017;31:69–80**Hemorrhagic, Hemostatic, and Thromboelastometric Disorders in 35 Dogs with a Clinical Diagnosis of Leptospirosis: A Prospective Study**

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Background: Leptospirosis in dogs is occasionally associated with a hemorrhagic syndrome, the pathophysiology of which is not fully understood.

Hypothesis/Objectives: To characterize hematologic, hemostatic, and thromboelastometric abnormalities in dogs with leptospirosis and to study their association with hemorrhagic diatheses and outcomes.

Animals: Thirty-five client-owned dogs.

Methods: A prospective observational single cohort study was conducted. Results from the CBC, coagulation tests (prothrombin, activated partial thromboplastin and thrombin times, fibrinogen, fibrin(ogen) degradation products, and D-dimer concentrations), rotational thromboelastometry (TEM), signalment, hemorrhagic diatheses, occurrence of disseminated intravascular coagulation (DIC) at admission, and survival to discharge were recorded.

Results: The most common hematologic and hemostatic abnormalities were anemia (30/35), thrombocytopenia (21/35), and hyperfibrinogenemia (15/35). Eight dogs were diagnosed with DIC. A normal TEM profile was found in 14 dogs, a hypercoagulable profile in 14 dogs, and a hypocoagulable profile in 7 dogs. The 8 dogs with hemorrhagic diatheses at admission had significantly decreased platelet counts ($P = .037$) and increased D-dimer concentrations ($P = .015$) compared with other dogs. Dogs with a hypocoagulable profile exhibited more hemorrhagic diatheses compared with the dogs that had normal and hypercoagulable profiles ($P = .049$). The mortality rate was lower in dogs with a hypercoagulable profile than in those with a hypocoagulable profile (21% vs 57%; $P = .043$). Disseminated intravascular coagulation was not a significant prognostic factor.

Conclusions and Clinical Importance: Thromboelastometric parameters were altered in dogs with both hypercoagulable and hypocoagulable profiles. A hypocoagulable profile was significantly correlated with hemorrhagic diathesis and higher mortality rate.

Key words: Bleeding; Canine; Coagulation; DIC; Leptospirosis; Thrombocytopenia.

Leptospirosis is a reemerging widespread zoonosis caused by pathogenic members of the genus *Leptospira*.¹ The clinical manifestations of leptospirosis range from a mild illness to an acute, life-threatening multisystemic disorder.¹ Leptospirosis is commonly associated with acute kidney injury, liver dysfunction, pulmonary involvement, and, less commonly, a hemorrhagic syndrome.^{1,2} In previous retrospective studies of dogs, this hemorrhagic syndrome was characterized by the occurrence of hematuria (with an incidence of

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Abbreviations:

A10	amplitude 10 minutes after CT
aPTT	activated partial thromboplastin time
CFT	clot formation time
CT	clotting time
DIC	disseminated intravascular coagulation
exTEM	extrinsic thromboelastometry
FDPs	fibrinogen and fibrin degradation products
fibTEM	fibrinogen function thromboelastometry
G	shear elastic modulus strength
Li45	lysis index 45 minutes after CT
MAT	microagglutination test
MCF	maximum clot firmness
ML	maximum lysis
NSAIDs	nonsteroidal anti-inflammatory drugs
PCR	polymerase chain reaction
PT	prothrombin time
TEM	thromboelastometry
TPI	thrombodynamic potential index
TT	thrombin time

6–31%),^{3–7} melena (3–9%),^{4,6,8} petechial hemorrhages (11.5–12.5%),^{6,9} hemorrhagic nasal discharge (19%),⁶ hematochezia (3%),⁴ and spontaneous hemorrhage (2.5%).¹⁰

Although the hemorrhagic potential of leptospirosis was observed as early as 1886,¹¹ the causes and mechanisms of hemorrhage have not been clearly elucidated. First, leptospirosis is assumed to cause systemic vasculitis, which could be an important mechanism of tissue damage and hemorrhagic tendencies.¹² Inflammation of the vascular endothelium may be a consequence of

direct invasion by infectious agents^{12–14} or of immune mechanisms.^{15–17} Second, thrombocytopenia is a well-documented feature of leptospirosis (14–58% in naturally occurring leptospirosis in dogs).^{4–6,8,18,19} The underlying mechanisms of thrombocytopenia are not fully understood.¹⁶ Thrombocytopenia may be the result of decreased thrombopoiesis^{20,21} or increased platelet consumption related to immune or nonimmune causes,^{16,18,22,23} Kupffer cell phagocytosis,²⁴ or a combination of these causes.¹⁶ Third, leptospirosis can be associated with disseminated intravascular coagulation (DIC).^{1,2,6,16,25,26} In 2 previous retrospective studies partially focused on coagulation disorders in naturally occurring leptospirosis in dogs, DIC was diagnosed in 7 of 16⁶ and 38 of 209 dogs.² Disseminated intravascular coagulation was not associated with a poor outcome in the first study⁶ but was associated with a negative outcome (death or euthanasia) in the second study.² Multivariate analysis conducted in 2 prospective studies of humans^{25,26} did not identify DIC as an independent factor for clinical hemorrhage, and there was no significant association between DIC scores and hemorrhagic diathesis. In these 2 studies, DIC was not associated with outcome.^{25,26}

Rotational thromboelastometry (TEM) can be used to assess the viscoelastic properties of clot formation in whole blood under low shear conditions, which provides information on global hemostatic function from the beginning of clot formation through fibrinolysis.²⁷ The shape of the TEM profile defines a patient's hemostatic condition as normal, hypercoagulable, or hypocoagulable.^{27,28} To our knowledge, no study has been performed to assess the thromboelastometric findings in leptospirosis in humans or in dogs.

The aim of our study was to describe the clinical hemorrhagic signs and the hematologic, hemostatic, and thromboelastometric abnormalities observed in dogs with naturally occurring leptospirosis at a referral center in France by evaluation of traditional and global coagulation tests including TEM.

Materials and Methods

Dogs presented to the intensive care unit (SIAMU) of VetAgro Sup campus vétérinaire de Lyon, France, between January 2013 and June 2015 with a clinical diagnosis of leptospirosis were eligible for inclusion in this prospective, observational, single cohort study. Owner consent was obtained before enrollment in the study. Institutional ethical approval was obtained before the start of the study.

Inclusion and Exclusion Criteria

Dogs were suspected to have leptospirosis if they had clinical signs consistent with the disease (acute kidney injury, glycosuria without hyperglycemia, acute hepatitis, hemorrhagic syndrome, and hemorrhagic gastroenteritis) and if other causes were ruled out or appeared unlikely. Dogs were diagnosed with leptospirosis if they fulfilled at least 1 of the following 3 criteria: single microagglutination test (MAT) titer $\geq 1 : 800$ for nonvaccine serovars or $\geq 1 : 1600$ for vaccine serovars (from the serogroups icterohaemorrhagiae and canicola);^{29,30} a 4-fold increase in convalescent titers;

or a positive urine or blood polymerase chain reaction (PCR) test. These criteria were applied regardless of vaccination status. The MAT assays were performed at the Laboratoire des Leptospores.⁴ Twenty-four serovars representing the following 13 serogroups were tested icterohaemorrhagiae, australis, autumnalis, ballum, bataviae, canicola, grippityphosa, hebdomadis, panama, pomona, pyrogenes, sejroe, and tarassovi.

Dogs were excluded from the study if blood parameters could not be assessed at the time of admission or if anticoagulants (unfractionated or low-molecular-weight heparin or antiplatelet medications [aspirin or clopidogrel]) were given by the primary veterinarian during the month preceding admission.

Clinical Information

Clinical information collected for each case included signalment, primary or referred consultation, vaccination status for leptospirosis, previous treatments (anticoagulants, antibiotics, corticosteroids, and nonsteroidal anti-inflammatory drugs [NSAIDs]), hemorrhagic diatheses, duration of clinical signs, duration of hospitalization, and outcomes (survival, natural death, or euthanasia). Clinical signs used to define hemorrhagic diatheses were hematochezia, melena, hematemesis, macroscopic hematuria, hemorrhagic nasal discharge, hemoptysis, skin or mucosal hematomas or hemorrhages, and petechial hemorrhages. The same clinician (AB) performed all physical examinations of each suspected dog at admission.

Blood and Urine Sample Collection

At admission, in each case of suspected leptospirosis, the dog underwent venipuncture of the lateral saphenous vein with a 21-gauge needle connected to a syringe to collect 10 mL of venous whole blood. Blood samples were divided into the following tubes: citrate tube for coagulation profiling and TEM, ethylenediaminetetraacetic acid tube for CBC and blood PCR assays, serum tube for MAT, and lithium heparin tube for biochemical profiles. Citrated whole blood samples were collected in 3.2% buffered sodium citrate in a 1 : 9 ratio of citrate to blood (final concentration 10.8 mM citrate). Urine was collected by ultrasound-guided cystocentesis and placed in 2 glass tubes: 1 for the urinalysis and 1 for the urine PCR assay.

Analyses

Blood analyses included CBC,^b biochemical profile,^c coagulation profile,^{d,e} and TEM.^f The CBC and coagulation profiles were performed according to the manufacturers' recommendations. If anemia was present, a microscopic examination of the blood smear was performed by a trained technician to manually determine the reticulocyte count to discriminate between regenerative (reticulocytes $> 100 \times 10^9/L$), hyporegenerative (reticulocytes between 40 and $100 \times 10^9/L$), and nonregenerative (reticulocytes $< 40 \times 10^9/L$) anemia. The coagulation profile included prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen and fibrin(ogen) degradation products (FDPs), and D-dimer concentrations.

Rotational TEM was performed exactly 30 minutes after blood sample collection. Two technicians performed all of the analyses according to the manufacturer's instructions. Prewarmed (37°C) citrated whole blood samples (300 μL each) were added to clotting reagents^g with the supplied electronic pipette. Two profiles were conducted as follows: extrinsic TEM (exTEM)^h to evaluate the extrinsic pathway (with tissue factor activation) and fibrinogen function TEM (fibTEM)ⁱ to assess fibrinogen function (with tissue factor activation and platelet inactivation with cytochalasin D).

These 2 profiles were run a total of 60 minutes. The data obtained from the manufacturer's modeling software included clotting time (CT), clot formation time (CFT), amplitude 10 minutes after CT (A10), maximum clot firmness (MCF), α -angle (α), lysis index 45 minutes after CT (Li45), and maximum lysis (ML). The reference ranges for these TEM parameters were previously established at our institution in 53 healthy dogs by the same protocol as that described above.¹ Furthermore, 2 global coagulation indices based on the exTEM assay were calculated: the shear elastic modulus strength (G), which is a measure of clot firmness expressed in metric units calculated from MCF as follows: $G = (5,000 \times \text{MCF}) / (100 - \text{MCF})$, and the thrombodynamic potential index (TPI), which was calculated from maximum clot elasticity (MCE; $\text{MCE} = (100 \times \text{MCF}) / (100 - \text{MCF})$) using the following equation: $\text{TPI} = \text{MCE} / \text{CFT}$. Reference ranges for G and TPI were defined at our institution as 4,025–11,129 dynes/cm², and 0.38–2.81, respectively.¹

Definitions of Coagulation Profiles

Dogs were categorized as DIC-positive if at least 3 of the following criteria were met at the time of admission: decreased platelet count ($<200 \times 10^9/\text{L}$), prolonged PT (>10 s), prolonged aPTT (>20 s), prolonged TT (>25 s), reduction of fibrinogen concentration (<1.5 g/L), increased FDP concentration (>20 $\mu\text{g}/\text{mL}$), or increased D-dimer concentration (>0.8 $\mu\text{g}/\text{mL}$).

A hypercoagulable profile based on the exTEM assay was defined as $G > 11,129$ dynes/cm² and $\text{TPI} > 2.81$, and a hypocoagulable profile was defined as $G < 4,025$ dynes/cm² and $\text{TPI} < 0.38$.

Finally, an APPLEfast scoring system was calculated in each dog to stratify illness severity by mortality risk as previously described.³¹

Statistical Analysis

All data are presented as the median (range). Normality was assessed by the d'Agostino and Pearson omnibus test. Comparisons between survivors and nonsurvivors, between dogs with and without DIC, and between dogs with and without hemorrhagic diatheses, were performed by Student's t -test for normally distributed data and the Mann-Whitney U -test for non-normally distributed data. Comparisons among dogs with hypocoagulable, normal, and hypercoagulable profiles were performed by one-way analysis of variance (ANOVA). Qualitative data were compared by Fisher's exact test and by calculating the odds ratios (ORs) associated with the 95% confidence interval (95% CI). No correction for multiple comparisons was made in this pilot exploratory study. Survival was assessed by Kaplan-Meier product limit estimates, and differences among groups were assessed by the log-rank test. Statistical analyses were performed by a commercially available statistical program.^k A P -value $\leq .05$ was considered statistically significant.

Results

Leptospirosis Diagnosis and Vaccination

Between January 2013 and June 2015, a clinical diagnosis of leptospirosis was established in 45 dogs. Ten dogs were excluded because TEM could not be performed at the time of admission. Thirty-five dogs were included in the study. Leptospirosis was diagnosed by MAT in 30 dogs (as single MAT titers in 25 dogs [median of the highest titer, 1 : 800; range, 1 : 800–1 : 3200] and a 4-fold rise in convalescent titers in 5 dogs [median of the highest titer, 1 : 3200; range,

1 : 3200–1 : 6400]), by PCR in 3 dogs (positive urine and blood PCR in 1 dog and positive blood PCR in 2 dogs), and by a combination of MAT and positive urine PCR in 2 dogs.

Thirty-four dogs (97%) had been vaccinated against leptospirosis within the last year. Only bivalent vaccines were used against the serogroups canicola and icterohaemorrhagiae.^{1,m,n} Based on single MAT titers at admission, the most commonly represented serogroups were australis, autumnalis, and panama (Table 1).

Characteristics of the Study Population

The median age of the dogs was 5.0 years (range, 4 months to 12 years), and their median weight was 29.4 kg (range, 5.8–58.7 kg). Twenty-six dogs (74%) were males (23 intact [66%] and 3 castrated [9%]) and 9 were females (26%) (4 intact [12%] and 5 neutered [14%]). Thirty-two dogs (91%) were purebred and belonged to 22 breeds. The most commonly represented breeds were German Shepherds (4/35, 11%) and Jack Russell Terriers, Golden Retrievers, and Labrador Retrievers (3/35 each, 9%). Thirty-two dogs (91%) were referred by their primary veterinarians. Twenty-three dogs (66%) had received antibiotics before admission. Three dogs (9%) had received glucocorticoids before admission (dexamethasone in 2 dogs and methylprednisolone in 1 dog). Two dogs (6%) had received meloxicam before admission. None of the dogs had received anticoagulants by the primary veterinarian.

Signs of Bleeding at Admission

The median time from the onset of clinical signs to admission to our facility was 4 days (1–30 days). Eight dogs (23%) were presented with hemorrhagic diatheses, 5 of 8 dogs exhibited a combination of 2 hemorrhagic clinical signs, and 1 of 8 dogs was presented with a combination of 3 hemorrhagic clinical signs. The hemorrhagic clinical signs included hematochezia (3/35, 9%), macroscopic hematuria (3/35, 9%), melena (2/35, 6%), hematemesis (2/35, 6%), spontaneous mucous membrane hemorrhages (2/35, 6%, 1 oral bleeding and 1 scleral hemorrhage), hemoptysis (1/35, 3%), petechial hemorrhages (1/35, 3%), and skin hematomas (1/35, 3%).

Hospitalization and Outcomes

The median hospitalization time was 7 days (range, 0–21 days). Twenty-two dogs (63%) were discharged. Thirteen dogs (37%) died during hospitalization. Six dogs died naturally (4 secondary to respiratory distress not responding to supportive care and 2 during hemodialysis), and 7 were euthanized. The reasons for euthanasia were the following: development of respiratory distress associated with severe hemoptysis and hemorrhagic nasal discharge (2 dogs), presence of intestinal intussusception (2 dogs), severe deterioration of general condition (2 dogs), and failure to introduce a hemodialysis central venous catheter into the jugular

Table 1. Description of the serogroups and serovars identified by MAT in 35 dogs with leptospirosis based on admission single MAT titers ≥ 1 : 1600 for vaccine serovars (from the serogroups icterohaemorrhagiae and canicola) or ≥ 1 : 800 for nonvaccine serovars.

Serogroup	Serovar	All dogs (n = 35)	With hemorrhagic diatheses (n = 8)	Without hemorrhagic diatheses (n = 27)	With a hypercoagulable profile (n = 14)	With a normal profile (n = 14)	With a hypocoagulable profile (n = 7)
Icterohaemorrhagiae	Icterohaemorrhagiae	3	0	3	0	1	2
	Copenhagen	6	1	5	3	2	1
Australis	Australis	13	1	12	1	6	6
	Bratislava	13	2	11	1	6	6
	Munchen	18	2	16	9	8	1
Autumnalis	Autumnalis	4	0	4	1	3	0
	Bim	10	1	9	1	6	3
Ballum	Castellonis	0	0	0	0	0	0
Bataviae	Bataviae	0	0	0	0	0	0
Canicola	Canicola	0	0	0	0	0	0
Grippotyphosa	Grippotyphosa	3	2	1	0	1	2
	Vanderhoedoni	2	0	2	0	1	1
Hebdomadis	Hebdomadis	0	0	0	0	0	0
	Kremastos	0	0	0	0	0	0
Panama	Mangus	11	2	9	1	6	4
	Panama	2	0	2	0	2	0
Pomona	Mozdok	1	0	1	0	0	1
	Pomona	1	0	1	0	1	0
Pyrogenes	Pyrogenes	2	1	1	1	0	1
Sejroe	Sejroe	0	0	0	0	0	0
	Saxkoebing	0	0	0	0	0	0
	Hardjo	0	0	0	0	0	0
Tarassovi	Tarassovi	0	0	0	0	0	0

MAT, microagglutination test.

veins because of the presence of thrombi in each jugular vein (1 dog).

Hematologic, Hemostatic, and Thromboelastometric Findings at Admission

Hematologic abnormalities were found in 32 dogs (91%). Abnormal CBC findings at admission included anemia (30/35, 86%; all with a reticulocyte count $< 40 \times 10^9/L$), thrombocytopenia (21/35, 60%), leukocytosis (6/35, 17%), leukopenia (2/35, 6%), and mildly increased in hematocrit (1/35, 3%; Table 2).

Hemostatic abnormalities were found in 29 dogs (83%). Abnormal findings in the coagulation profile at admission included hyperfibrinogenemia (15/35, 43%), prolonged aPTT (8/35, 23%), hypofibrinogenemia (7/35, 20%), prolonged PT (5/35, 14%), prolonged TT (5/35, 14%), and increased FDP concentration (4/35, 11%). D-dimer concentration was only measured in 23 dogs and was increased in 9 dogs (39%; Table 2). Based on these alterations of the coagulation profile and thrombocytopenia, 8 dogs (23%) were diagnosed with DIC.

Abnormal findings in the exTEM profile at admission included an increase in α -angle (19/35, 54%), A10 (14/35, 40%), MCF (14/35, 40%), CT (8/35, 23%), and CFT (7/35, 20%) and a decrease in CFT (16/35, 46%), A10, α -angle, and MCF (7/35 for each, 20%). The TPI and G were increased in 14 dogs (40%) and decreased

in 7 dogs (20%; Table 3). Based on the values of G and TPI, 14 dogs (40%), 14 dogs (40%), and 7 dogs (20%) were defined as having a hypercoagulable profile, normal TEM profile, and hypocoagulable profile, respectively.

Abnormal findings in the fibTEM profile at admission included an increase in A10 (20/35, 57%), MCF (19/35, 54%), CT (5/35, 14%), and ML (1/35, 3%) and a decrease in A10 and MCF (3/35 for each, 9%) and CT (1/35, 3%; Table 4).

Comparison Between Dogs With and Without Bleeding Diatheses

No association was found between serogroup and the presence of hemorrhagic diathesis (Table 1).

Compared with the 28 dogs without hemorrhagic diathesis, the 8 dogs (23%) with hemorrhagic diatheses had significantly lower platelet counts ($P = .037$) and significantly higher D-dimer concentrations ($P = .015$; Table 2). The exTEM assay indicated that the CT and CFT were significantly prolonged in dogs with hemorrhagic diatheses compared with dogs without hemorrhagic diatheses ($P = .047$ and $P = .014$, respectively), whereas A10 ($P = .007$), α -angle ($P = .025$), MCF ($P = .006$), TPI ($P = .009$), and G ($P < .001$) were significantly decreased (Table 3). The fibTEM assay indicated that no parameter was different between dogs with and without hemorrhagic diatheses (Table 4).

Table 2. Results of the CBC and coagulation profile (median (range)) in 35 dogs with leptospirosis.

Blood parameter	Reference intervals	All dogs (n = 35)	Survivors (n = 22)	Nonsurvivors (n = 13)	P value ^a	With hemorrhagic diatheses (n = 8)	Without hemorrhagic diatheses (n = 27)	P value ^b
PT (s)	6–10	7.5 (6.5–69.2)	7.2 (6.5–12.9)	7.9 (6.7–69.2)	.083	8.2 (6.7–12.9)	7.4 (6.5–69.2)	.102
aPTT (s)	10–20	15.5 (9.6–180)	15.5 (9.8–180)	15 (9.6–180)	.561	18.3 (12.9–180)	14.5 (9.6–180)	.116
TT (s)	10–25	15.7 (6.2–60)	15.3 (6.2–60)	17.1 (12.5–60)	.833	17.4 (12.8–60)	15.0 (6.2–60)	.336
Fibrinogen (g/L)	1.5–4	3.1 (0.9–7.3)	4.1 (0.9–7.3)	2.8 (1–5.6)	.134	1.6 (0.9–7.3)	3.4 (0.9–6.0)	.512
FDPs (µg/mL)	<20	<5 (<5–>20)	<5 (<5–>20)	5–20 (<5–>20)	NA	5–20 (<5–>20)	<5 (<5–>20)	NA
D-dimer (µg/mL)	<0.8	0.6 (0.3–2.8)	0.6 (0.3–1.6)	0.9 (0.4–2.8)	.166	1.6 (0.4–2.8)	0.6 (0.3–1.5)	.015
		(n = 13)	(n = 13)	(n = 10)		(n = 7)	(n = 16)	
Hematocrit (L/L)	0.37–0.54	0.29 (0.09–0.6)	0.29 (0.16–0.45)	0.29 (0.09–0.6)	.609	0.25 (0.16–0.60)	0.30 (0.09–0.45)	.121
Leukocytes (×10 ⁹ /L)	6–17	12.7 (2.1–29.8)	12.7 (2.8–29.8)	12.7 (2.1–23.9)	.608	12.3 (6.0–23.6)	12.7 (2.1–29.8)	.798
Platelets (×10 ⁹ /L)	200–500	177 (38–460)	192 (76–422)	175 (38–460)	.535	107 (38–273)	189 (77–460)	.037

aPTT, activated partial thromboplastin time; FDPs, fibrin(ogen) degradation products; NA, not applicable; PT, prothrombin time; TT, thrombin time.

^aComparison between survivors and nonsurvivors.

^bComparison between dogs with and without bleeding diatheses.

The bold values are corresponding to P -value $\leq .05$

Among the 8 dogs with hemorrhagic diatheses at admission, 5 were presented with a hypocoagulable TEM profile (5/7, 71%), 2 with a normal profile (2/14, 14%), and 1 with a hypercoagulable profile (1/14, 7%).

Comparison Between Dogs With and Without DIC

Disseminated intravascular coagulation was diagnosed in 8 dogs (23%) at admission. Four dogs with DIC (50%, 4/8) and 4 dogs without DIC (15%, 4/27) had hemorrhagic diatheses at admission. The DIC was not associated with the presence of hemorrhagic diatheses ($P = .161$). Hematemesis and melena at admission were the only hemorrhagic signs that were significantly associated with the presence of DIC (OR = 21.2; 95% CI = 0.9–496.2; $P = .047$ for each).

The exTEM assay indicated that CT and CFT were significantly prolonged in dogs with DIC compared with dogs without DIC ($P < .0001$ and $P = .005$; respectively), whereas A10, α -angle, and MCF were significantly decreased ($P < .0001$ for each; Fig 1). The fibTEM assay showed that CT and ML were significantly increased in dogs with DIC compared with dogs without DIC ($P < .0001$ and $P = .041$, respectively), whereas A10 ($P = .0002$) and MCF ($P = .0002$) were significantly decreased (Fig 2).

Values for G and TPI were significantly decreased in dogs with DIC compared with dogs without DIC ($P < .0001$ for each; Fig 1). Among the 8 dogs with DIC, 75% (6/8) had a hypocoagulable profile and 25% (2/8) had a normal profile based on the exTEM assay. Among the 27 dogs without DIC, 52% (14/27) had a hypercoagulable profile, 44% (12/27) had a normal profile, and 4% (1/27) had a hypocoagulable profile based on the exTEM assay. The only dog exhibiting a hypocoagulable profile without DIC was presented with thrombocytopenia ($38 \times 10^9/L$) and increased D-dimer concentration ($2.78 \mu g/mL$).

Comparison Among Dogs With Hypocoagulable, Normal, and Hypercoagulable Profiles

No association was found between serogroup and a hypocoagulable, normal, or hypercoagulable TEM profile (Table 1).

The dogs with a hypocoagulable profile had significantly more hemorrhagic signs than dogs with normal and hypercoagulable profiles (OR = 9.17; 95% CI = 1.15–73.3; $P = .049$). Macroscopic hematuria was documented significantly more frequently in dogs with a hypocoagulable profile than in dogs with normal and hypercoagulable profiles (OR = 22.56; 95% CI = 1.97–524.8; $P = .026$).

Platelet counts and fibrinogen concentration were significantly decreased in dogs with a hypocoagulable profile compared with dogs with a normal profile ($P = .010$ and $P = .028$, respectively), in dogs with a hypocoagulable profile compared with dogs with a hypercoagulable profile ($P < .001$ and $P = .005$, respectively), and in dogs with a normal profile compared

Table 3. Results of exTEM (extrinsic thromboelastometry) parameters (median (range)) in 35 dogs with leptospirosis.

exTEM parameter	Reference intervals	All dogs (n = 35)	Survivors (n = 22)	Nonsurvivors (n = 13)	<i>P</i> value ^a	With hemorrhagic diatheses (n = 8)	Without hemorrhagic diatheses (n = 27)	<i>P</i> value ^b
CT (s)	33–70	47 (33–3600)	39 (33–3600)	53 (36–2025)	.119	79 (34–3600)	40 (33–2025)	.047
CFT (s)	78–231	79 (18–3600)	67 (20–3600)	88 (18–3600)	.130	291 (20–3600)	67 (18–3600)	.014
A10 (mm)	33–59	55 (0–75)	62 (0–75)	52 (0–75)	.099	22 (0–72)	57 (0–75)	.007
α (°)	51–75	78 (0–87)	81 (0–86)	73 (0–87)	.115	33 (0–86)	79 (0–87)	.025
MCF (mm)	45–69	62 (5–80)	68 (5–80)	57 (5–79)	.035	30 (5–77)	67 (5–80)	.006
Li45 (%)	88–100	100 (97–100)	100 (99–100)	100 (97–100)	.369	100 (99–100)	100 (97–100)	.746
ML (%)	0–41	5 (0–17)	5 (0–16)	1.5 (0–17)	.288	1 (0–16)	5 (0–17)	.133
TPI	0.38–2.81	1.81 (0–20.90)	2.94 (0–16.74)	1.77 (0–20.90)	.066	0.09 (0–16.74)	2.61 (0–20.90)	.009
G (dynes/cm ²)	4,025–11,129	8,158 (0–20,000)	10,625 (0–20,000)	6,628 (0–18,810)	.037	2,239 (0–16,739)	10,151 (0–20,000)	< .001

A10, amplitude 10 minutes after CT; CFT, clot formation time; CT, clotting time; G, shear elastic modulus strength; Li45, lysis index 45 minutes after CT; MCF, maximum clot firmness; ML, maximum lysis; TPI, thrombodynamic potential index.

^aComparison between survivors and nonsurvivors.

^bComparison between dogs with and without bleeding diatheses.

The bold values are corresponding to *P*-value $\leq .05$

Table 4. Results of fibTEM (fibrinogen function thromboelastometry) parameters (median (range)) in 35 dogs with leptospirosis.

fibTEM parameter	Reference intervals	All dogs (n = 35)	Survivors (n = 22)	Nonsurvivors (n = 13)	<i>P</i> value ^a	With hemorrhagic diatheses (n = 8)	Without hemorrhagic diatheses (n = 27)	<i>P</i> value ^b
CT (s)	32–98	44 (27–3600)	40.5 (27–3600)	52 (37–3600)	.045	71 (35–3600)	42 (27–3600)	.057
CFT (s)	NA	NA	NA	NA	NA	NA	NA	NA
A10 (mm)	3–13	15 (0–36)	16 (0–36)	14 (3–32)	.186	5 (0–35)	17 (0–36)	.078
α (°)	NA	NA	NA	NA	NA	NA	NA	NA
MCF (mm)	3–14	15 (0–36)	17 (0–36)	13 (0–32)	.154	5 (0–35)	18 (0–36)	.069
Li45 (%)	64–100	100 (90–100)	99 (90–100)	100 (93–100)	.405	100 (93–100)	100 (90–100)	1
ML (%)	0–48	2 (0–84)	2 (0–84)	2 (0–13)	.963	12 (0–84)	1 (0–18)	.076

A10, amplitude 10 minutes after CT; CFT, clot formation time; CT, clotting time; Li45, lysis index 45 minutes after CT; MCF, maximum clot firmness; ML, maximum lysis; NA, not applicable.

^aComparison between survivors and nonsurvivors.

^bComparison between dogs with and without bleeding diatheses.

The bold values are corresponding to *P*-value $\leq .05$

with dogs with a hypercoagulable profile ($P = .008$ and $P = .002$, respectively). The PT, aPTT, and D-dimer concentration were significantly increased in dogs with a hypocoagulable profile compared with dogs with a normal profile ($P = .006$, $P = .004$, and $P = .042$, respectively) and in dogs with a hypocoagulable profile compared with dogs with a hypercoagulable profile ($P = .002$, $P < .001$, and $P = .018$, respectively). The TT was significantly prolonged in dogs with a hypocoagulable profile compared with dogs with a hypercoagulable profile ($P < .001$) and in dogs with a normal profile compared with dogs with a hypercoagulable profile ($P = .020$; Fig 3).

The APPLEfast score was not significantly different among these 3 groups (Table 5).

Comparison Between Survivors and Nonsurvivors

None of the CBC or classic coagulation profile parameters were significantly different between survivors and nonsurvivors (Table 2).

The exTEM assay showed that MCF and G were significantly increased in the survivors compared with the nonsurvivors ($P = .035$ and $P = .037$, respectively; Table 3; Fig 4), whereas CT according to the fibTEM assay was significantly decreased ($P = .045$; Table 4; Fig 4). The areas under the curve (AUC) were calculated for these 3 parameters and were 0.715, 0.713, and 0.592, respectively, for MCF and G according to the exTEM assay and for CT according to the fibTEM assay.

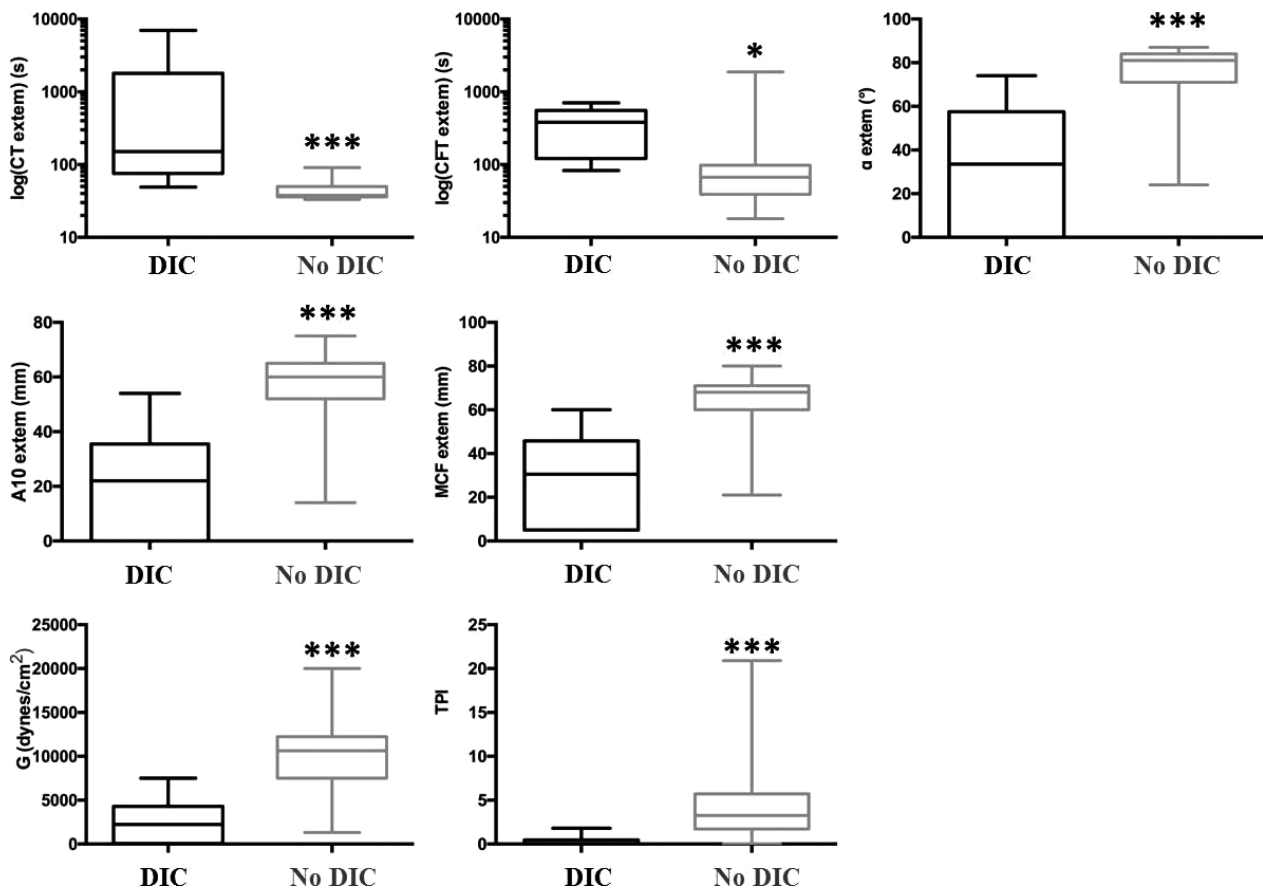


Fig 1. Comparison of the distribution of exTEM (extrinsic thromboelastometry) parameters among 35 dogs with leptospirosis between dogs with DIC ($n = 8$) and dogs without DIC ($n = 27$) at admission. CT and CFT are presented on a log scale for clarity. A10, amplitude 10 minutes after CT; CFT, clot formation time; CT, clotting time; DIC, disseminated intravascular coagulation; G, shear elastic modulus strength; MCF, maximum clot firmness; TPI, thrombodynamic potential index. * $P < .05$, ** $P < .001$, *** $P < .0001$ between the dogs with DIC and the dogs without DIC.

The difference between mortality rate in dogs with hemorrhagic diatheses compared with those without hemorrhagic diatheses was not significant (63% [8/8] and 30% [8/27], respectively; $P = .051$). Mortality rates were significantly lower in dogs with a hypercoagulable profile than in dogs with a hypocoagulable profile (21% [3/14] and 57% [4/7], respectively; $P = .043$; Fig 5). Mortality rates were not significantly different between dogs with a normal TEM profile and those with a hypercoagulable profile or between dogs with a normal TEM profile and those with a hypocoagulable profile ($P = .847$ and $P = .195$, respectively). The mortality rates were not different between dogs with and without DIC ($P = .433$).

Discussion

Evidence of hemorrhage in naturally occurring leptospirosis in dogs has been inconsistently reported and poorly characterized. Our prospective study provides an in-depth investigation of this clinical feature and the associated hemostatic disorders. To our knowledge, ours is the first study to describe the

thromboelastometric profiles of dogs suspected to have acute leptospirosis in combination with classic hemostatic parameters and the first to compare them between dogs with (23%) and without hemorrhagic diatheses. Among the population as a whole, the most common hematologic abnormalities were anemia (86%) and thrombocytopenia (60%). Abnormalities in the coagulation profile were observed in 83% of the cases. Disseminated intravascular coagulation occurred in 23% of the dogs and was not a negative prognostic factor. According to the results of TEM, 40% of the dogs had a hypercoagulable profile, 40% had a normal profile, and 20% had a hypocoagulable profile. The mortality rate was significantly lower in dogs with a hypercoagulable profile than in those with a hypocoagulable profile.

Thrombocytopenia was a common hematologic finding in the present study, with a slightly higher prevalence (60%) than previously reported (14–58%).^{4–6,8,18,19} Thrombocytopenia is a well-documented feature of leptospirosis in humans and dogs. The underlying mechanisms of thrombocytopenia are not fully understood. Thrombocytopenia may be the result of platelet consumption due to activation,

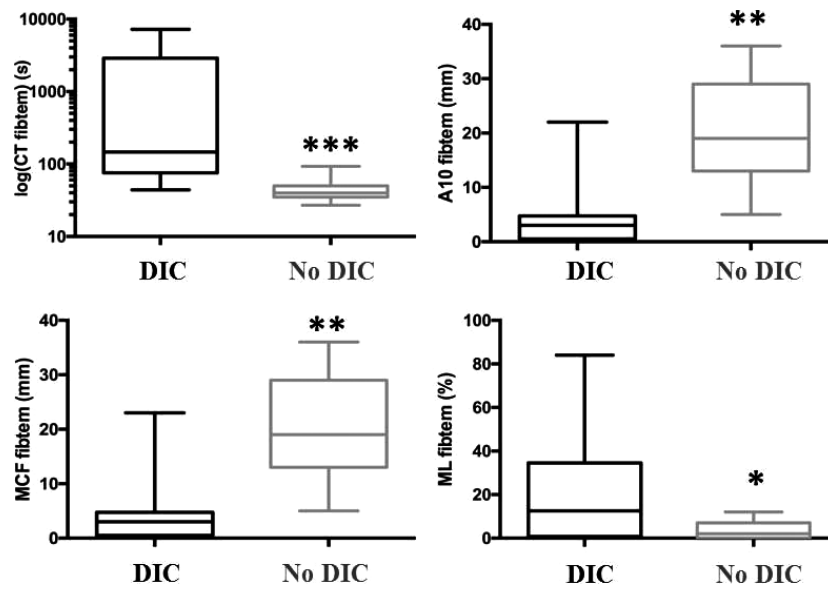


Fig 2. Comparison of the distribution of fibTEM (fibrinogen function thromboelastometry) parameters among 35 dogs with leptospirosis between dogs with DIC (n = 8) and dogs without DIC (n = 27) at admission. CT is presented on a log scale for clarity. A10, amplitude 10 minutes after CT; CT, clotting time; DIC, disseminated intravascular coagulation; MCF, maximum clot firmness; ML, maximum lysis. * $P < .05$, ** $P < .001$, *** $P < .0001$ between the dogs with DIC and the dogs without DIC.

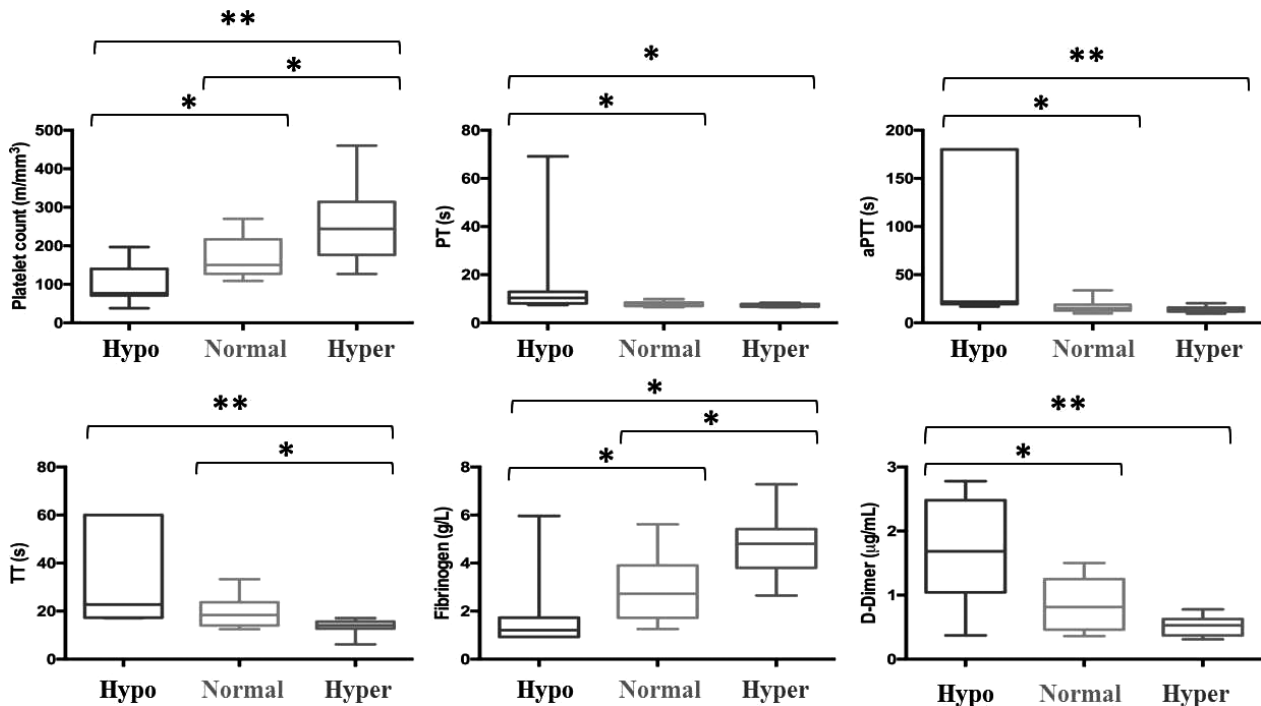


Fig 3. Comparison of the distribution of the platelet count, PT, aPTT, TT, fibrinogen, and D-dimer concentration among 35 dogs with leptospirosis between dogs with a hypocoagulable profile (n = 7), a normal profile (n = 14), and a hypercoagulable profile (n = 14) at admission. The accolades represent a comparison between 2 profile types. aPTT, activated partial thromboplastin time; hyper, hypercoagulable profile; hypo, hypocoagulable profile; PT, prothrombin time; TT, thrombin time. * $P < .05$, ** $P < .001$ between the different groups.

adhesion, and aggregation due to a stimulated vascular endothelium,³² increased platelet consumption due to immune causes,^{16,18,22,23} hemophagocytic syndrome,^{24,33} or some combination of these factors. Weak evidence of

bone marrow suppression secondary to a direct toxic effect of *Leptospira* has also been documented.²¹ According to the results of our study, thrombocytopenia may partially explain the hemorrhagic signs

Table 5. Results of APPLEfast scoring system in 35 dogs with leptospirosis.

Results of APPLEfast	All dogs (n = 35)	Dogs with a hypercoagulable profile (n = 14)	Dogs with a normal profile (n = 14)	Dogs with a hypocoagulable profile (n = 7)
Median	21	19.5	21.5	21
Range	15–30	15–30	17–26	19–22
<i>P</i> value ^a			.64	

^aComparison between dogs with a hypercoagulable, a normal, and a hypocoagulable profile.

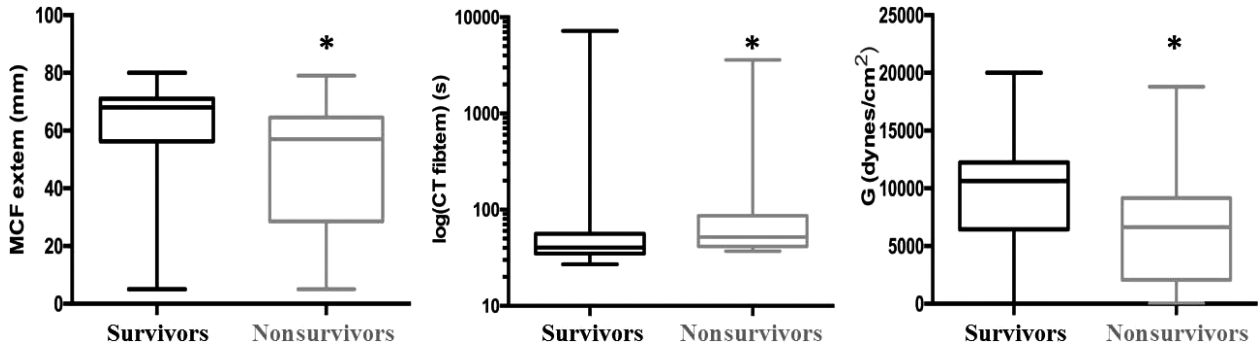


Fig 4. Comparison of the distribution of MCF and G based on the exTEM (extrinsic thromboelastometry) assay and CT from the fibTEM (fibrinogen function thromboelastometry) assay between the surviving dogs (n = 22) and the nonsurviving dogs (n = 13) with leptospirosis at admission. CT from the fibTEM assay is presented on a log scale for clarity. CT, clotting time; G, shear elastic modulus strength; MCF, maximum clot firmness. **P* < .05 between the survivors and nonsurvivors.

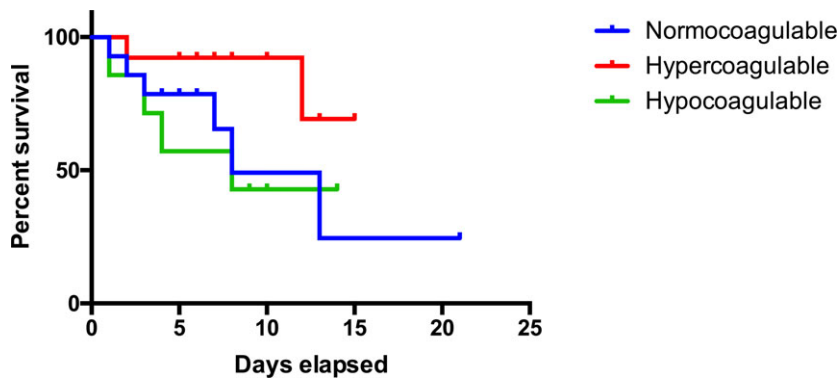


Fig 5. Comparison of the Kaplan-Meier curves for the 35 dogs with leptospirosis between dogs with a hypercoagulable profile (n = 14), a normal thromboelastometric profile (n = 14), and a hypocoagulable profile (n = 7).

observed in leptospirosis in dogs. In our study, the platelet count was significantly lower in dogs with hemorrhagic diatheses than in dogs without hemorrhagic diatheses, and dogs with a hypocoagulable profile exhibited significantly more hemorrhagic diatheses than dogs with normal and hypercoagulable profiles. Furthermore, a hypocoagulable profile was associated with a low platelet count and a low fibrinogen concentration. In humans, thrombocytopenia is an indicator of severe disease and a risk factor for hemorrhage in leptospirosis, and it is the only independent hemostasis factor that has been associated with clinical hemorrhage.^{25,26}

Disseminated intravascular coagulation occurred in 8 of 35 dogs (23%), 4 of 8 of which had hemorrhagic diathesis. In a previous retrospective study, DIC (defined

by the same criteria as those listed in the Materials and Methods section) was observed in 7 of 16 dogs (44%).⁶ In a second study, DIC was diagnosed in 38 of 209 dogs based on the presence of at least 2 abnormal hemostatic parameters among the 4 routinely assessed parameters (platelet count, PT, aPTT, and plasma fibrinogen concentration).² Disseminated intravascular coagulation is commonly considered as a cause of bleeding diatheses in leptospirosis.^{1,2,6,16,25,26} However, the link between DIC and bleeding signs in spontaneous and experimental cases of leptospirosis remains unclear because DIC has been inconsistently documented in hemorrhagic animals. In an experimental model of leptospirosis in guinea pigs, neither platelet thrombi nor fibrin thrombi were found in the liver, lungs, or kidneys by morphological

observation, although D-dimer and FDP concentrations were significantly increased.²⁴ In 2 prospective studies of humans, DIC was diagnosed in 10 of 46 patients (22%)²⁵ and in 36 of 49 (73%) patients²⁶ based on the overt DIC score developed by the DIC scientific subcommittee of the International Society for Thrombosis and Hemostasis.³⁴ However, DIC did not appear as an independent factor of hemorrhagic diatheses based on a multivariate analysis,²⁶ and there was no significant association between DIC scores and hemorrhagic diatheses.²⁵ As reported in our study, DIC was not found to be a negative prognostic factor in these dogs with leptospirosis.^{6,25,26} However, DIC was associated with a negative outcome (death or euthanasia) in another study of dogs (OR = 7.9; 95% CI = 3.4–18.4).²

Our results highlight the complexity of the hemostatic imbalance in leptospirosis by documenting elements favoring the occurrence of both hypercoagulable and hypocoagulable profiles. Some dogs exhibited an increase in FDPs (11%) and D-dimer (39%) concentrations at the time of admission. However, almost no fibrinolysis TEM parameter was altered (except ML in the fibTEM assay), which could represent a lack of sensitivity of TEM needed to identify alterations in fibrinolysis. The results of a previous prospective study performed in dogs with spontaneous hemoperitoneum showed that enhancing *in vitro* fibrinolysis with 50 U/mL of tissue plasminogen activator increased the ability of the thromboelastographic assay to identify differences in fibrinolysis.³⁵ Furthermore, among the dogs with a normal TEM profile (40%), the majority exhibited alterations in primary (thrombocytopenia) and secondary hemostasis. Additional studies are needed to characterize this group of dogs to establish whether they are normocoagulable or are in a transitional phase between hypercoagulable and hypocoagulable profiles.

As mentioned by the Partnership on Rotational ViscoElastic Test Standardization (PROVETS), there is insufficient evidence to recommend how a hypercoagulable or a hypocoagulable profile should be defined in dogs based on TEM parameters.^{36,37} This definition is most often based on the values of 4 measured parameters (CT, CFT, α -angle, and MCF) or using *G* values.³⁷ In our study, we categorized the TEM profiles based on *G* and TPI values according to the results of a previous preliminary retrospective study.⁹ In this previous study, the 4 measured parameters and 5 calculated indices (maximal clot elasticity, *G*, TPI, global index, and coagulation index) were evaluated to establish their sensitivity and specificity for identifying an exTEM hypocoagulable or hypercoagulable profile. An exTEM profile was arbitrarily considered abnormal if ≥ 2 of the measured parameters changed in the same direction (e.g., a shortened CT or CFT or an increased α -angle or MCF to identify a hypercoagulable profile; or a prolonged CT or CFT or decreased α -angle or MCF to identify a hypocoagulable profile).³⁷ According to the results of this previous study,⁹ *G* and TPI appeared to be the best indices for identifying hypercoagulable (AUC = 0.998 and AUC = 0.991, respectively) and hypocoagulable profiles (AUC = 0.987 and AUC = 0.999, respectively).

Some differences were obtained when we compared the TEM parameters that were measured by the exTEM assay and the fibTEM assay between surviving and nonsurviving dogs and between dogs with and without hemorrhagic diatheses. The fibTEM assay was useful for assessing fibrinogen functions (with tissue factor activation) by irreversibly inhibiting the platelets with cytochalasin D, a potent inhibitor of actin polymerization. Except for the fibrinolytic parameters, all of the exTEM parameters were significantly different between dogs with and without hemorrhagic diatheses, whereas no statistical differences were observed for the fibTEM parameters between these 2 groups. These results suggest that leptospirosis-induced hypocoagulability is at least in part a reflection of the availability of fibrinogen and platelets (fibrinogen concentrations and platelet counts were lower in dogs with a hypocoagulable profile than in dogs with a hypercoagulable profile; Fig 3), but platelet availability appeared to play a greater role than fibrinogen concentration in the occurrence of hypocoagulability. The results of our study suggest the relevance of systematically performing an exTEM assay in association with a fibTEM assay to identify the TEM disorders in dogs with leptospirosis.

The number of hypercoagulable TEM profiles observed in this study may have been influenced by the high frequency of anemia (86%). Some studies suggest that a hypercoagulable profile induced by decreased hematocrit may have a nonspecific influence in viscoelastic tests, which may result in an artifactual hypercoagulable profile.^{38–40} In addition, 43% of dogs in our study had hyperfibrinogenemia, which could favor hypercoagulable profiles.³⁹ Three dogs received some glucocorticoids before admission to our facility. The results of a previous study showed that chronically administering prednisone in healthy mixed-breed dogs led to increased clot strength and decreased clot lysis when a thromboelastographic approach was used.⁴¹ In our study, the dog that received methylprednisolone had a normal profile, and of the 2 dogs that received dexamethasone, 1 had a hypercoagulable profile, and 1 had a hypocoagulable profile. Thus, the administration of glucocorticoids did not appear to have influenced the TEM profiles. Finally, 2 dogs received meloxicam before admission. These dogs were not excluded from the study, in accordance with the results of a previous study in which the administration of meloxicam did not seem to alter hemostasis according to the methods evaluated (TEM and whole blood platelet aggregation) in dogs.⁴²

In our study, the mortality rate was 37%. This result is similar to that reported in previous studies with mortality rates between 17 and 52.5%.^{2–8,10,18} The mortality rate was lower in dogs with a hypercoagulable profile than in those with a hypocoagulable profile, and *G* values were significantly higher in survivors than in nonsurvivors. Other studies demonstrated that dogs with a hypocoagulable profile had a higher mortality rate than those with a hypercoagulable profile in DIC,⁴³ in crocotalid snake envenomation,⁴⁴ and in immune-mediated hemolytic anemia.⁴⁵ This relationship brings into question the role of coagulation in inflammatory processes. In the case of sepsis associated with leptospirosis,

activation of coagulation could foster compartmentalization of bacteria in microvessels and decrease bacterial invasion into tissue.⁴⁶ Conversely, hypocoagulability could facilitate enhanced spread of infection and subsequently mortality.⁴⁶

Our study had several limitations. First, the diagnosis of leptospirosis based on a single MAT titer, especially in vaccinated dogs, is very controversial. Vaccination can result in titers $\geq 1 : 6400$ to nonvaccine serovars several months after vaccination.⁴⁷ However, a previous study has demonstrated that the administration of leptospirosis-inactivated bivalent vaccine induced a relatively low- and short-lived antibody response.⁴⁸ Therefore, it remains possible that some dogs in our study had other causes of their illness. Second, the dogs belonged to a referral population and are likely to represent a more severely affected population than those seen in general practice, and it is unclear whether our results can be extrapolated to all dogs with leptospirosis. The third limitation is the small sample size, which decreased the power of the statistical tests. The frequency of DIC in our study should be interpreted cautiously because the diagnosis of DIC has not been standardized in dogs. A model-based scoring system was developed in veterinary medicine,⁴⁹ but the application of this scoring system was not possible in this study, because it used reference intervals for tests performed at 1 specific laboratory. Finally, no discard tube was used to collect the blood samples, unlike the recommended standard.^{36,37} It is possible that this method of blood collection led to preanalytical bias.

In conclusion, hemostatic derangements are an important but still poorly characterized aspect of leptospirosis. The results of our study suggest that the hemorrhagic tendency observed in leptospirosis is the result of an imbalance in the hemostatic equilibrium, with alterations in both primary and secondary hemostasis. This imbalance may lead to DIC, which occurred in more than 20% of dogs in this case series. However, DIC was not a negative prognostic factor, and mortality rates were lower among dogs with a hypercoagulable TEM profile compared to those with a hypocoagulable profile. Our results suggest that further study is warranted to determine what triggers this disordered hemostasis, which specific components of inflammation and hemostasis are involved, and whether identifying a hypocoagulable TEM profile in an individual dog with leptospirosis has therapeutic implications.

Footnotes

^a Laboratoire des Leptospire, VetAgro Sup, Campus vétérinaire de Lyon, 1 avenue Bourgelat, Marcy l'Etoile, France

^b Xt-2000iV, Sysmex, Kobe, Japan

^c VetTest, IDEXX, Westbrook, ME, US

^d STart, Stago, Asnières sur Seine, France

^e Konelab 30, ThermoFisher, Cergy Pontoise, France

^f ROTEM, Pentapharm GmbH, Munich, Germany

^g starTEM, Pentapharm GmbH, Munich, Germany

^h exTEM, Pentapharm GmbH, Munich, Germany

ⁱ fibTEM, Pentapharm GmbH, Munich, Germany

^j Barthélemy A, Rannou B, Verwaerde P, et al. Establishment of reference intervals for extrinsic rotational thromboelastometry (exTEM) parameters in normal dogs: assessment of sex and blood component influences. *J Vet Emerg Crit Care* 2014; 24:S32

^k Prism 6, GraphPad Software, La Jolla, USA, CA

^l Nobivac[®] lepto, MSD Santé Animale, Beaucouzé, France

^m Canigen[®], Virbac France, Carros, France

ⁿ Eurican[®], Merial, Lyon, France

^o Barthélemy A, Verwaerde P, Rannou B, et al. Specificity and sensitivity of rotational thromboelastometry (ROTEM[®]) parameters and indices to identify hypercoagulable or hypocoagulable states in dogs. *J Vet Emerg Crit Care* 2014; 24:S30-S31

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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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