Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
29 (4): 381-390, 2023
DOI: 10.9775/kvfd.2023.29395

Research Article

Thromboelastographic Evaluation of Coagulation Profile in Dogs with Subclinical and Clinical Ehrlichiosis

Meriç KOCATÜRK [1 (*)] Zeki YILMAZ [1] Ryou TANAKA [2] Jose Joaquin CERÓN [3]

[1] Department of Internal Medicine, Faculty of Veterinary Medicine, Bursa Uludag University, TR-16059 Bursa - TÜRKİYE
[2] Tokyo University of Agriculture & Technology, 3-5-8 Saiwai-Cho, Fuchu, 183-8590, Tokyo, JAPAN
[3] Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, 30100, Espinardo, Murcia, SPAIN

(*) **Corresponding author:** Meriç KOCATÜRK
Phone: +90 224 294 0817
Fax: +90 224 294 0873
E-mail: merick@uludag.edu.tr

How to cite this article?

Kocatürk M, Yılmaz Z, Tanaka R, Cerón JJ: Thromboelastographic evaluation of coagulation profile in dogs with subclinical and clinical ehrlichiosis. *Kafkas Univ Vet Fak Derg,* 29 (4): 381-390, 2023.
DOI: 10.9775/kvfd.2023.29395

Article ID: KVFD-2023-29395
Received: 20.03.2023
Accepted: 10.07.2023
Published Online: 17.07.2023

ABSTRACT

Canine monocytic ehrlichiosis (CME), a vector-borne disease of worldwide distribution, causes coagulopathy in dogs. Thromboelastography-TEG measures the efficiency of coagulation. However, there is lack of knowledge about TEG evaluation in different stages of CME. Thus, this study aimed to evaluate the coagulation status and viscoelastic properties of blood using TEG parameters in dogs with naturally-occurring CME, and their potential to discriminate between subclinical and clinical forms of the disease. The relationship between TEG parameters and C-reactive protein (CRP) was also investigated. For these purposes, 29 *E. canis*-seropositive dogs were used (12 subclinical and 17 clinical forms), and 10 healthy dogs as controls. Kaolin-activated TEG was performed in all dogs. Platelets were lower ($P<0.01$), but CRP was higher ($P<0.001$) in clinical form than in subclinical form. TEG-reaction (R) and clot-formation times (K) decreased ($P<0.01$), whereas α-angle and coagulation index (CI) increased ($P<0.01$) in both forms of CME compared to controls. The magnitude of decreases in R- and K-times and increases in α-angle and CI were higher in dogs with subclinical form compared to clinical form. CRP was correlated negatively ($P<0.05$) with TEG-Ly30. In conclusion, among TEG parameters, R-time, K-time, α-angle, and CI values may be used to differentiate subclinical form of CME from clinical form. Hypercoagulability is especially frequent in dogs with subclinical CME and may be associated with systemic inflammation.

Keywords: Coagulation, C-reactive protein, Dogs, Ehrlichiosis, Thromboelastography

Introduction

Canine monocytic ehrlichiosis (CME) is a rickettsial disease caused by *Ehrlichia canis* transmitted by the brown dog tick, *Rhipicephalus sanguineus*. The clinical symptoms of CME vary according to the host's immune response, infectious dose of the pathogen, and co-infections [1]. Ehrlichiosis can be divided into three stages based on the presence of the clinical signs: acute (early disease), subclinical (asymptomatic), and clinical (chronic) infection [2]. The most reliable parameter for the diagnosis of subclinical CME was mild thrombocytopenia without severe anemia [3]. In the chronic stage, dogs are suffering from pyrexia, jaundice, lymphadenopathy, and bleeding disorders along with thrombocytopenia [4,5].

Platelet (PLT) functions could also be altered during CME because of bone marrow suppression, increased PLT destruction and consumption, and the presence of anti-PLT antibodies [6-9]. Thrombocytopenia and PLT dysfunction in dogs with CME may lead to bleeding disorders due to primary hemostasis abnormality [6]. Thus, it seems to be essential to evaluate the coagulation status in dogs with CME regardless of the clinical stage.

Coagulation status has been evaluated by conventional screening tests such as PLT counts, prothrombin time (PT), activated partial thromboplastin time (aPTT), and d-dimer levels [10,11]. In practice, the changes in PLT indices (mean PLT volume [MPV], plateletcrit [PCT], and PLT distribution width [PDW] are also investigated to evaluate coagulation in dogs with CME [12,13]. However, these tests provide little information on the vital interaction between PLTs and the coagulation cascade [10,11,13-16].

Thromboelastography (TEG) measures the efficiency of blood coagulation from initial clot formation to fibrinolysis, thereby yielding superiority over traditional coagulation screening methods [14]. TEG evaluates clot kinetic by

reaction (R-time) and coagulation times (K-time), clot strengthening by α-angle, PLT function by maximum amplitude (MA), and clot stability by the percentage of lysis at 30 min (Ly30) [17,18]. In a previous study [11], TEG results showed hypercoagulation and hypofibrinolysis in dogs with experimentally-induced ehrlichiosis. Currently, there is lack of information about TEG evaluation in different stages of CME in dogs. Thus, this study aimed to evaluate the coagulation status and global viscoelastic properties of blood clot formation using TEG in dogs with naturally occurring CME, and their discriminating potential between clinical forms of the disease. C-reactive protein (CRP), a non-specific inflammatory marker, was reported to play a possible pro-coagulant role in humans [19], and dogs [20]. CRP can enhance thrombogenesis [19] and inhibit fibrinolysis with the inflammatory response in humans [21]. A positive correlation between TEG-MA value and serum CRP in dogs with spirocercosis [22] and patients with poor prognosis [23] were previously reported. Thus, we also investigated the possible relationship between TEG parameters and serum CRP levels in the dogs studied.

Material and Methods

Ethical Statement

The present study was approved by the Ethics and Welfare Committee of Bursa Uludag University (Bursa, Turkey). A signed information consent form was obtained from the dog owners enrolled in the study between 2010-2016 (Decision number: 2010-06/10; Date: 24.08.2010).

Dogs and Groups

Dogs from different breeds, ages, body weights, and gender were included in the study *(Table 1)*. The dogs with or without clinical signs at admission to the clinic were screened using a combined commercial enzyme-linked immunosorbent assay (ELISA) kit for the antibody against *E. canis*, *Anaplasma marginale* and *Borrelia burgdorferi*, and antigen against *Dirofilaria immitis* (Anigen Rapid®, CaniV-4, Bionote, Korea) in Veterinary Teaching Hospital (Bursa/Türkiye). Dogs were also screened to exclude *Leishmania infantum* (Leishmania Ab Test Kits or CaniV-4 [Leish], Anigen Rapid®, Bionote, Korea). Dogs with comorbidities were not included in the study, as reported in our [8,24] and other previous studies [11].

Dogs that were found healthy based on clinical and hematobiochemical examinations along with seronegative test results were used as controls (n=10). Seropositive dogs only for *E. canis* were enrolled in the study as a subclinical form, if they did not show any clinical signs but had thrombocytopenia (n=12); and as a clinical form if they had at least one or more symptoms (loss of appetite, lethargy, depression, and/or exercise intolerance, etc.) with the presence of both thrombocytopenia and anemia (n=17) [9,11,25,26]. None of the dogs with subclinical CME were either leukopenic or neutropenic, and they had normal erythrogram values (RBC, Hct, Hgb, MCV, MCH, MCHC, and RDW) at the sampling time. Serum levels of hepato-renal injury markers were within the range of reference. Eight of the subclinical dogs had increased serum globulin concentrations (>3.7 g/dL) [11]. Additionally, all dogs included in the study did not receive any medication or vaccination at least for 10 days before being admitted to the clinic, as reported in our previous studies [8,24].

Sampling and Measurements

All dogs included in this study had initial anamnesis, physical examination, and laboratory analysis as part of the routine diagnostic procedures.

For hemogram (5-parts CBC differential with 22 parameters, VetScan® HM5, Abaxis, USA) and serum biochemistry analysis (Comprehensive profile, VetScan® VS2, Abaxis, USA), blood samples were collected from cephalic veins into the vacutainer tubes with or without anticoagulant (EDTA), respectively. Also, a total of 2 mL

Table 1. *Signalment and some clinical parameters in the dogs with subclinical and clinical ehrlichiosis and healthy dogs in the control group*

Parameter	Healthy n=10 (mean ± sd)	Subclinical Ehrlichiosis n=12 (mean ± sd)	Clinical Ehrlichiosis n=17 (mean ± sd)
Body weight (kg)	10.5±3.8^{a}	25.9±5.8^{b**}	22.4±8.8^{b*}
Gender (M/F)	5F + 5M	5F + 7M	7F + 10M
Age (months)	51.3±22.9^{a}	68.0±37.4^{a}	54.3±13.7^{a}
Temperature (°C)	37.9±0.7^{a}	38.6±0.4^{a}	38.7±0.8^{a}
Pulsation bpm	121±8^{a}	123±6^{a}	113±7^{a}
Respiration rpm	20±14^{a}	22±17^{a,b}	99±14^{b***}

M: *male,* ***F:*** *female,* ***bpm:*** *beat per minute,* ***rpm:*** *respiration per minute*
^{a,b} *Different letter in the same line represent a statistically significant change between variables*
**P<0.05 **P<0.01 *** P<0.001 compared to healthy controls*

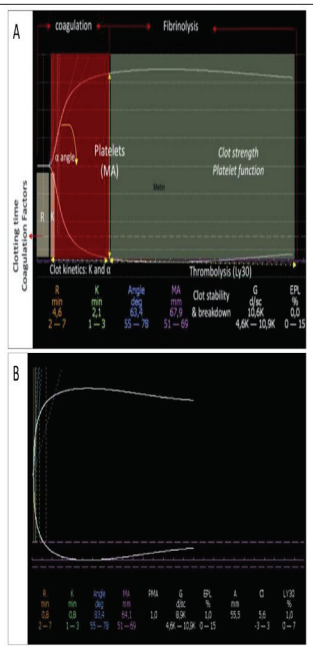

Fig 1. Two examples of thromboelastography (TEG) trace. **A-** From a healthy dog (Golden retriever, 5 years, male) with a schematic illustration of TEG trace including coagulation activation (R time and K time), clot kinetics (K time and a-angle), maximum clot stability (MA) and thrombolysis or fibrinolysis (Ly30), and **B-** From *E. canis* seropositive dog in the subclinical phase (Golden retriever, 4 years, male) showing hypercoagulable states due to shortened R- and K-time as well as increased a-angle, G value and coagulation index (CI), thereby increasing risk for intravascular coagulation (pro-thrombotic state)

blood was sampled for coagulation analysis (TEG®5000, Haemoskope, MA, USA) into vacutainer tubes with 3.2% sodium citrate (BD Vacutainer System, BD Diagnostics, NJ, USA), and were gently inverted at least 5 times to allow proper mixing, as previously described in our studies [17,18,27].

Comprehensive serum biochemistry panels included albumin, alkaline phosphatase (ALP), alanine amino-transferase (ALT), amylase, total bilirubin, blood urea nitrogen (BUN), calcium (Ca), phosphate (Phos), creatinine (Cr), glucose (Glu), sodium (Na), potassium (K), total protein (TP) and globulin. In addition, CRP in all dogs was measured to evaluate the acute phase response by the technique described in our previous study [28].

For TEG analysis, within 30 min following the blood sampling, dogs were examined for coagulation status by kaolin-activated TEG parameters *(Fig. 1, Table 2)*, namely R time and K time (clot kinetics), α-angle (clot strength), as well as parameters for PLT function (MA, projected maximum amplitude [PMA], and maximal clot strength (G value, dyn/cm^2]), clot stability (A and LY30: Percentage of lysis 30 minutes after MA, estimated potential lysis [EPL], coagulation index [CI]), as reported in our previous studies [17,18]. TEG was halted after maximal fibrin clot strength was recorded, and the G value of the clot stability was calculated from the MA value via TEG software, using the following formula: G = (5000 × MA)/(100 - MA). Coagulation status (hypercoagulable and hypocoagulable states) was characterized by TEG, as suggested [29]. The hypercoagulable and hypocoagulable states were defined by shortened reaction time (R- and/or K-time), increased α-angle, and/or MA values; whereas by prolonged R- and K- time, and/or decreased α-angle and MA values, respectively [29,30].

In this study, doxycycline (10 mg/kg, PO, q24h for 28 days) and supportive care were the treatment of choices for all *E. canis* seropositive dogs, as suggested [1,11], and they were not monitored thromboelastographically during and after the treatment.

Statistical Analysis

Statistical analysis was performed by routine descriptive statistical procedures and software (SigmaPlot® Statistical and Graphing Software, California, USA). Changes in the analytes between healthy dogs and dogs with either subclinical or clinical ehrlichiosis were assessed by One-way Analysis of Variance (ANOVA). When the normality test failed, the Mann-Whitney Rank Sum test was used. The Holm-Sidak test was used for both pairwise comparisons versus a control group. The Pearson correlation coefficient *(r)* was used for measuring a linear correlation between TEG parameters and PLT indices, and serum CRP levels. All data were expressed as mean±sd. Values of $P<0.05$ were considered significant.

Results

Signalement and Clinical Examination

A total of 39 dogs comprised of naturally infected clinical, and subclinical form of ehrlichiosis, and healthy control dogs of different breeds, ages, gender, and body weights were used in the study *(Table 1, Table 3, and Table 4)*. There was no statistical difference in age, temperature, and pulsation between the groups, but the respiratory rate increased ($P<0.01$) in dogs with clinical ehrlichiosis, compared to controls. Control dogs did not have any clinical and/or pathological findings based on the physical examination and hemato-biochemistry evaluations *(Table 1-6)*.

Hemato-Biochemistry Panel

CBC values and serum biochemistry panel of all dogs (n=39) were given in detail *(Table 4, Table 5)*. As for the aim of the study, WBC, RBC, Hct, PLT count, and serum CRP levels were presented as follows: There was no statistically significant change in total WBC count between the groups. RBC was lower ($P<0.01$) in dogs with clinical CME compared to control, and Hct values in dogs with clinical CME were lower ($P<0.001$) than in

Table 2. *Coagulation panel measured by kaolin activated thromboelastography (TEG) in the dogs with subclinical and clinical ehrlichiosis and dogs in the control group.*

Parameter	Healthy n=10 (mean ± sd)	Subclinical Ehrlichiosis n=12 (mean ± sd)	Clinical Ehrlichiosis n=17 (mean ± sd)
R time (min)	5.1±1.3^{a} (2.9-7.9)	1.1±0.6$^{b\#***}$ (0.2-2.3)	2.8±1.3^{c***} (0.5-5.8)
K time (min)	2.9±1.0^{a} (1.4-5.8)	1.4±1.5^{b**} (0.8-5.4)	1.8±1.1^{b*} (0.8-4.7)
a angle (degree)	55.9±6.4^{a} (45.2-64.9)	76.7±12.0$^{b***,\#**}$ (40.1-85.2)	65.2±13.5^{c**} (29.6-83.4)
MA (mm)	56.2±6.6^{a} (44.1-66.3)	63.9±12.7^{a} (36.7-79.1)	55.4±15.3^{a} (19.8-77.6)
PMA mm	0.05±0.2^{a} (0.0-1.0)	0.6±0.4$^{b***,\#*}$ (0.0-1.0)	0.2±0.4^{a} (0.0-1.0)
G (dyn/cm^2)	6.6±1.7^{a} (4.0-9.9)	10.4±5.0^{b*} (2.9-18.9)	7.4±4.0^{ab} (1.2-17.4)
EPL %	0.2±0.7^{a} (0.0-2.8)	0.1±0.3^{a} (0.0-0.7)	0.3±0.6^{a} (0.0-2.1)
CI value	-0.7±2.1^{a} (-4.1-3.6)	4.5±2.9$^{b***,\#**}$ (-2.5-7.6)	1.7±3.4^{c*} (-7.0-6.4)
A mm	51.7±9.2^{a} (30.5-64.3)	62.1±11.5^{a} (40.3-78.4)	53.6±14.6^{a} (21.5-88.0)
Ly30 (min)	0.7±2.3^{a} 0.0-10.0	0.1±0.3^{a} (0.0-0.7)	0.3±0.6^{a} (0.0-1.7)

^{a,b,c} *between healthy dogs and subclinical and clinical ehrlichiosis (there is a significant difference between groups containing different letters in the same line);* $^{\#}$ *between subclinical and clinical ehrlichiosis*
**P<0.05.* ** *P<0.01. and* *** *P<0.001 compared to healthy controls*
R: *Reaction time,* ***K:*** *Coagulation time,* ***α-angle:*** *Alpha angle,* ***MA:*** *Maximum amplitude,* ***PMA:*** *Projected maximum amplitude,* ***G:*** *G value calculated,* ***EPL:*** *Estimated potential lysis,* ***A and LY30:*** *Percentage of lysis 30 minutes after MA,* ***CI:*** *Coagulation index*

subclinical form and in healthy controls. The mean PLT count was lower in dogs with clinical CME than that of subclinical CME (P<0.01), and control dogs (P<0.001) *(Table 3)*. Serum CRP levels in dogs with clinical CME was higher (P<0.001) than that of dogs with subclinical CME, and healthy controls *(Table 3)*.

Thromboelastography Analysis

TEG parameters including their minimum and maximum levels were presented in *Table 2*. There were statistically significant differences (P<0.05) in R time, α-angle, PMA, and CI between subclinical and clinical forms. R- and K-time decreased (P<0.05) but α-angle, PMA, G, and CI values increased (P<0.05) in dogs with subclinical CME, compared to those with clinical CME, and healthy controls. The magnitude of decrease in R- and K-time and increase in α-angle, PMA, and CI values were higher in dogs with subclinical form of CME, compared to those with clinical form.

In individual evaluation of TEG, 6 subclinical and 4 clinical dogs had higher G values from the maximum levels detected in healthy controls (9.9 dyn/cm^2). Similarly, higher CI values in 9 subclinical, and 6 clinical dogs were found compared to maximum levels measured in healthy controls.

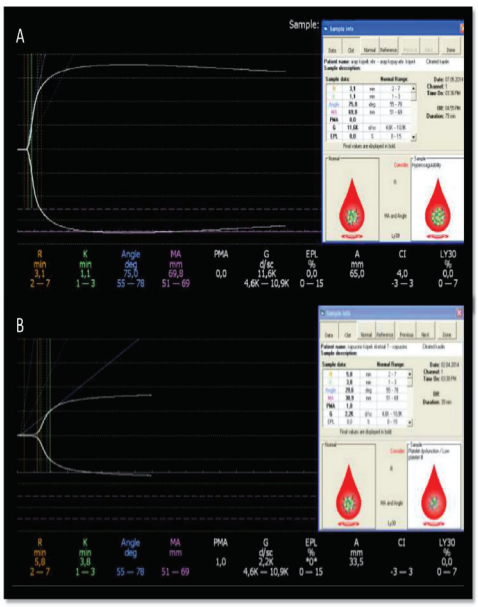

Fig 2. Thromboelastography (TEG) profiles from two dogs with clinical (chronic) ehrlichiosis. **A** shows hypercoagulable states due to shortened K time and increased maximum amplitude (MA), G value, and coagulation index (CI), **B** shows a low platelet/platelet dysfunction and/or hypofibrinogenemia characterized by low a-angle, MA, G value, and CI

Table 3. *Some hemogram parameters and serum C-reactive protein levels in the dogs with subclinical and clinical ehrlichiosis and healthy dogs in the control group*

Parameter	Healthy n=10 (mean ± sd)	Subclinical Ehrlichiosis n=12 (mean ± sd)	Clinical Ehrlichiosis n=17 (mean ± sd)
WBC (x10^3/uL)	12.2±0.6^a	12.8±7.1^a	7.8±6.6^a
RBC (x10^6/uL)	6.3±0.8^a	6.0±1.4^{ab}	4.0±0.9^{b**}
Hct (%)	41.4±7.2^a	38.4±9.0^a	25.1±6.7$^{b\#***}$
Platelet count (x10^3/uL)	257±61^a	149±22^{b**}	58.7±43$^{c\#***}$
Serum CRP (ug/mL)	15.4±12.3^a	31.9±19.8^{b**}	70.7±21.2$^{b\#***}$

WBC: *White blood cell count,* ***RBC:*** *Red blood cell count,* ***Hct:*** *Hematocrit,* ***CRP:*** *C-reactive protein*
$^{a,b\,c}$ *Different letters in the same line represent a statistically significant change between variables*
** P<0.05. **P<0.01. and *** P<0.001 compared to healthy controls. # compared to Subclinical Ehrlichiosis*

Table 4. *Complete blood cell counts in the dogs with subclinical and clinical ehrlichiosis and healthy dogs in the control group*

Parameter	Healthy n=10 (mean ± sd)	Subclinical Ehrlichiosis n=12 (mean ± sd)	Clinical Ehrlichiosis n=17 (mean ± sd)
WBC (K/uL)	12.2±0.6^a	12.8±7.1^a	7.8±6.6^a
Lymphocyte (K/uL)	3.2±0.3^a	1.7 ±1.6^{a,b}	0.7±0.5^{b**}
Monocyte (K/uL)	0.5±0.0^a	0.7 ±0.5^a	0.6±1.0^a
Neutrophile (K/uL)	8.0±0.5^a	8.1±4.0^a	4.9±3.4^a
Eosinophile (K/uL)	0.30±0.00^a	0.81±0.23^a	0.06±0.04^a
Basophile (K/uL)	0.01±0.02^a	0.04±0.03^a	0.01±0.00^a
RBC (x10^{12}/L)	6.3±0.8^a	6.0±1.4^a	4.0±0.9^{b**}
Hgb (g/dL)	13.2±2.1^a	12.3±3.3^a	7.7±2.2^{b*}
HCT (%)	41.4±7.2^a	38.4±9.0^a	25.1±6.7^{b***}
MCV (fL)	65.4±3.6^a	64.7±6.1^a	62.1±6.5^a
MCH (pg)	20.8±1.1^a	20.4±2.2^{a,b}	18.8±2.1^{b,*}
MCHC (g/dL)	31.9±0.8^a	31.4±1.7^{a,b}	29.5±3.0^{b**}
RDW (%)	15.9±0.8^a	16.4±2.9^a	15.9±6.9^a
PLT (x10^9/L)	257±61^a	149±22^{b**}	58.7±43c***
MPV (fL)	7.9±0.2^a	9.5±1.6^a	8.1±2.0^a
PCT (%)	0.22±0.05^a	0.18±0.01^{a,b**}	0.05±0.04^{b***}
PDW (fL)	29.5±0.6^a	37.2±8.7^a	32.8±9.2^a

^{a,b,c} *Different letters in the same line represent a statistically significant change between variables*
**P<0.05 ** P<0.01 *** P<0.001 compared to healthy controls*
WBC: *White blood cell count,* ***RBC:*** *Red blood cell count,* ***Hgb:*** *Hemoglobin,* ***HCT:*** *Hematocrit,* ***MCV:*** *Mean corpuscular volume,* ***MCH:*** *Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration,* ***RDW:*** *RBC distribution width,* ***PLT:*** *Platelet count,* ***MPV:*** *Mean platelet volume, PCT: Plateletcrit,* ***PDW:*** *Platelet distribution width*

In addition, the TEG analyzer showed hypercoagulable states (n=19; 11 in subclinical and 8 in clinical CME), normocoagulable states (n=18, 1 from subclinical CME, and 7 from clinical CME, and 10 from healthy controls) in dogs studied (*Fig. 1*, *Fig. 2*). Although none of the dogs did not fit into thromboelastographic definition of the hypocoagulable state, the TEG - PLT mapping assay displayed the presence of PLT dysfunction and/or hypofibrinogenemia with a low MA, α-angle, and/or CI value in two dogs with clinical CME. In addition, these two dogs had lower G and CI levels than healthy controls.

Correlations

Statistically significant correlations between TEG parameters and PLT indices and serum CRP values were as follows: Negative correlations between PLT count and α-angle (P<0.01); PDW and MA (P<0.05), G (P<0.05) and CI (P<0.01); PCT and R time, and positive correlations

Table 5. *Serum biochemistry of the dogs with subclinical and clinical ehrlichiosis and dogs in the control group*

Parameter	Healthy n=10 (mean ± sd)	Subclinical Ehrlichiosis n=12 (mean ± sd)	Clinical Ehrlichiosis n=17 (mean ± sd)
Albumin (g/dL)	2.9±0.4^{a,b}	3.1±0.7^{a}	2.1±0.5^{b*}
ALP (U/L)	57.1±36.7^{a}	60.7±32.0^{a}	161.1±96.9^{b**}
ALT (U/L)	25.8±5.5^{a}	45.6±22.5^{a}	133.7±162.3^{a}
Amylase (U/L)	468±131^{a}	696.8±407.9^{a}	805.8±278.0^{a}
Total Bilirubin (mg/dL)	0.16±0.05a	0.35±0.07^{a}	0.94±1.71^{b,**}
BUN (mg/dL)	12.0±5.6^{a}	15.6±9.8^{a}	17.7±15.8^{a}
Ca (mg/dL)	9.2±1.9^{a}	10.2±0.7^{a}	9.5±0.8^{a}
P (mg/dL)	5.5±2.4^{a}	6.1±4.5^{a}	5.3 ±1.2^{a}
Cr (mg/dL)	0.7±0.2^{a}	0.8±0.2^{a}	0.7±0.2^{a}
Glucose (mg/dL)	110±21^{a}	93±32^{a}	88±33^{a}
Na (mmol/L)	137±0^{a}	138±3^{a}	139±2^{a}
K (mmol/L)	4.2±0.1^{a}	4.7±0.2^{a}	4.5±0.1^{a}
TP (g/dL)	5.9±0.9^{a}	7.2±1.1^{a}	7.0±1.8^{a}
Globulin (g/dL)	2.2±0.7^{a}	4.0±1.1^{b**}	4.8±1.7^{b**}
CRP (ug/mL)	15.4±12.3^{a}	31.9±19.8^{b**}	70.7±21.2^{b***}

ALP: *Alkaline phosphatase,* ***ALT:*** *Alanine amino transferase,* ***BUN:*** *Blood urea nitrogen,* ***Ca:*** *Calcium,* ***P:*** *Phosphate,* ***Cr:*** *Creatinine,* ***Na:*** *Sodium,* ***K:*** *Potassium,* ***TP:*** *Total protein,* ***CRP:*** *C-reactive protein*
^{a,b} *Different letters in the same line represent a statistically significant change between variables*
**P<0.05, **P<0.01. and ***P<0.001 compared to healthy controls*

Table 6. *Pearson correlation coefficient (r) results between serum C-reactive protein (CRP) and platelet (PLT) count and TEG parameters in all dogs studied (n=39)*

TEG Parameters	PLT Count		MPV		PDW		PCT		Serum CRP	
	r	P	*r*	P	*r*	P	*r*	P	*r*	P
R time	0.189	0.33	0.025	0.89	0.321	0.09	-0.399	0.03	0.100	0.61
K time	0.146	0.45	0.171	0.386	0.519	0.004	-0.207	0.29	0.030	0.87
a-angle	-0.485	0.01	-0.213	0.27	0.049	0.80	0.475	0.01	-0.425	0.02
MA	0.159	0.42	-0.066	0.73	-0.363	0.05	-0.106	0.59	0.262	0.17
PMA	-0.084	0.66	-0.096	0.62	-0.087	0.65	0.271	0.16	-0.009	0.96
G	0.01	0.95	-0.106	0.59	-0.409	0.03	-0.014	0.94	0.133	0.49
EPL	-0.298	0.12	-0.073	0.70	0.066	0.73	0.296	0.12	-0.302	0.11
A	0.003	0.98	-0.000	0.97	-0.356	0.06	-0.026	0.89	0.113	0.56
CI	-0.112	0.57	-0.158	0.42	-0.523	0.004	0.286	0.14	0.006	0.97
LY30	-0.344	0.07	-0.086	0.66	0.046	0.81	0.470	0.01	-0.350	0.05

Underlined results show statistically significant correlation at least P<0.05 between the related parameters
TEG: *Thromboelastography,* ***PLT:*** *Platelet,* ***MPV:*** *Mean platelet volume,* ***PDW:*** *Platelet distribution width,* ***PCT:*** *Plateletcrit,* ***CRP:*** *C-reactive protein,* ***R:*** *Reaction time,* ***K:*** *Coagulation time,* ***α-angle:*** *Alpha angle,* ***MA:*** *Maximum amplitude,* ***PMA:*** *Projected maximum amplitude,* ***G:*** *G value calculated,* ***EPL:*** *Estimated potential lysis,* ***A and LY30:*** *Percentage of lysis 30 min after MA,* ***CI:*** *Coagulation index*

between PDW and K time (P<0.01), and PCT and α-angle (P<0.01), and Ly30 (P<0.01). Additionally, a negative correlation (P<0.05) between serum CRP and α-angle, and Ly30 *(Table 6);* and serum CRP level with PLT count (*r* = - 0.631, P<0.001) were determined.

Discussion

In the present study, TEG changes in *E. canis*-infected dogs, which is a disease known to cause a decrease in the number of PLTs but its effect in other aspects of coagulation is not well known. There were statistically significant differences in TEG parameters between healthy dogs, and dogs with subclinical and clinical CME. The hypercoagulable state was common in both stages, especially in the subclinical form of CME; however, PLT dysfunction and/or hypofibrinogenemia could be observed in some dogs with clinical CME.

TEG was used in this report to evaluate the coagulation, since it provides detailed information compared to traditional assays such as PT and aPTT on associations between PLTs and their effects on aggregation, clot strength, fibrin cross-linking, and the fibrinolysis process of the hemostasis [14]. Hitherto, there is no published data or study on thromboelastographic evaluation of coagulation status in dogs with naturally occurring CME. Also, the potential of TEG as a diagnostic tool to distinguish subclinical from clinical CME is almost unknown.

In this study, there were statistically significant differences ($P<0.05$) in TEG parameters (R time, α-angle, PMA, and CI) between subclinical and clinical forms. The magnitude of decreases in R- and K-time, and increases in α-angle, PMA, and CI values were higher in dogs with subclinical form compared to those of clinical form of CME. While considering TEG parameters referring to each specific step of coagulation, observed decreases in R- and K-time may be related to shortened time for the enzymatic portion of clot formation (R time) and fibrin-cross linking or clot kinetics (K time) in dogs with CME. In addition, increasing MA, PMA, α-angle, and CI were associated with increasing PLT function/aggregation (MA and PMA), and clot strength and propagation (α-angle) in dogs, especially with the subclinical form of CME. These observations showed that several distinguished TEG parameters (R time, K time, α-angle, PMA, and CI) may be used to discriminate clinical form of CME from its subclinical form in dogs.

TEG analysis has the potential to show the presence of hypercoagulation earlier than other laboratory tests. When MA increases, it means that the patient's blood is in a hypercoagulable state, and is prone to thrombosis, and *vice versa*, when the MA decreases, the patient's blood is diluted and prone to bleeding [23]. In the present study, shortened R- and K-times and high α-angle and/or MA values indicated a hypercoagulable state in 19 out of 29 dogs with CME, as reported earlier [11]. In a previous study [11], a hypercoagulable state was reported in dogs with experimentally induced ehrlichiosis, where the dogs were not divided into subclinical and clinical subgroups. Our results from the dogs with naturally occurring CME confirmed the findings of the hypercoagulation detected after experimental *E. canis* infection in dogs [11], and also, extended the current knowledge by indicating that hypercoagulable state might be more common in the subclinical form than the clinical form of the disease. Instead of more TEG parameters, only based on the TEG G value, a measurement of global clot strength, the hypercoagulable state was characterized in humans (>11 dyn/cm^2) [31], and dogs (>7.2 dyn/cm^2) [32]. Thus, the increase in TEG G value in dogs of our study supporting the hypercoagulation tendency in dogs is also in parallel to these findings [32]. Our cut-off of G value (4.0-9.9 dyn/cm^2) used in the present study seems to be higher than that of Wiinberg et al.[32], due to the possible differences between TEG activation methods used (kaolin- *vs* tissue factor-activation).

CI is used to the assessment of overall coagulation derived from other indices including R time, K time, MA, and α-angle. In the present study, the observed increase in CI value was associated with hypercoagulation, as reported in humans with trauma [33], and dogs with critical illnesses [34]. Mean TEG CI value (4.5±2.9) in dogs with subclinical CME was similar to the results (4.2±1.9) of our previous study in dogs with dilated cardiomyopathy [18] from the aspect of the presence of a hypercoagulable state.

Although the changes in TEG parameters in dogs studied did not fit the definition of a hypocoagulable state, the TEG PLT mapping showed the presence of PLT dysfunction and/or hypofibrinogenemia in two dogs with clinical CME. Observed decreases in TEG G and CI values might be suggestive of bleeding tendency (hypocoagulation) in these dogs. Also, although there was not a statistically significant difference in Ly30 value between the clinic stages of the disease, observed decreases in Ly30 may be related to a tendency for hypofibrinolysis in dogs with CME, as reported in a previous study [11]. In this study, why the dogs with clinical CME did not show bleeding signs despite severe thrombocytopenia could be explained by a common observation of hemostatic phenotype rather than a bleeding phenotype in *E. canis*-infected dogs [11].

When TEG parameters are interpreted with serum CRP improves the quality of clinical decision making, ultimately, the estimation of patient outcomes [23]. As a result of the inflammatory responses that occur after rickettsial infections, an increase in acute-phase proteins is observed as a physiological response [35,36]. In our study, a significant increase of serum CRP was observed in dogs with CME, especially in the clinical stage, indicating that CRP may be a useful indicator to detect inflammation in response to *E. canis* infection and to discriminate clinical from the subclinical stage. The higher concentrations of serum CRP in the clinical phase of ehrlichiosis in dogs could be a result of the severity of tissue damage and inflammation [35].

In the present study, the correlations showed statistically significant interactions between TEG parameters, PLT indices, and serum CRP. Circulating PLT count was correlated negatively with α-angle in dogs studied. Similarly, Zhou et al.[37] reported that despite decreased PLT count, TEG parameters of α-angle and MA increased significantly. While PLT counts decrease, PLT volume (MPV) and size (PDW) may increase to compensate for thrombocytopenia in dogs [38], thereby giving rise to a

normal coagulation profile in these patients. In parallel to this observation, in the present study, normocoagulation was defined in eight dogs with CME despite the presence of different severity of thrombocytopenia. These results showed that PLT's role in the hemostasis processes starting from clot initiation to fibrinolysis may have been more than expected, as reported in our previous study [39].

A significant negative correlation between serum CRP level and PLT count showed that thrombocytopenia was accompanied by elevated serum CRP levels, indicating that the severity of inflammation could have a role in the decrease in PLTs in this disease. There were negative correlations between serum CRP level and TEG parameter α-angle and Ly30 in the present study. This may be explained by the pro-coagulant effects of CRP because CRP was reported to be associated with the hypercoagulability of plasma and increased PLT reactivity [40]. CRP may play a key role to alter the coagulation process during CME, as reported in different inflammatory diseases in humans [20] and dogs [21].

One of the limitations of the study is that traditional tests such as PT and aPTT were not applied together with TEG analysis. However, while considering the abilities of TEG, it evaluates primary, secondary, and tertiary hemostasis. Despite the lack of additional tests evaluating coagulation status, the results of the study are thought to be inclusive and significant [11,18,27]. Also, these tests give no information on the vital interaction between PLTs and the coagulation cascade. Some patients having normal PT/aPTT values may have active bleeding because of abnormal hemostasis, therefore, PT and aPTT tests may be inadequate for coagulation monitoring [14]. This study included 29 dogs with CME, whereas, in a previous experimental study, 5 healthy beagle dogs were used to show the alteration in TEG analysis after *E. canis* inoculation [11]. Thus, the number of samples in this study seemed to be adequate for the study's aims when compared to others with smaller sample size [11]. We did not monitor the coagulation of the dogs during/after the treatment, since this was out of the scope of this study.

Currently, the indirect fluorescent antibody test (IFAT) is considered the "gold standard" method for diagnosis of CME [3]; however, cross-reactive antibodies, lack of standardization of test and trained personnel, and expensive equipment requirements were reported to limit the reliability and applicability of IFAT for this purpose [41]. Polymerase chain reaction (PCR), another test for the diagnosis of CME, is limited usage because of generally allowing detection of dogs in the acute phase of the disease [42]. In practice, combined commercial ELISA kits from different companies (Idexx Laboratories, Antech Diagnostics, and Abaxis, etc.) are commonly used to detect antibodies to *E. canis* [8] with high specificity (99-100%) and sensitivity (96.2-97.6%) in dogs [43-45]. Thus, in this study, using an in-clinic ELISA kit was thought adequate for the accurate diagnosis of CME, as performed in our [8,24] and other previous studies [11,46-50].

Our results showed that there are changes in coagulation conditions in different stages of dogs with CME. Based on the TEG values, clot stability, clot kinetics, clot strength, and fibrinolysis may vary in dogs with CME, regardless of clinical form; being some TEG parameters of potential use to distinguish subclinical from clinical ehrlichiosis in dogs. Hypercoagulability (a state of increased risk for thrombosis) is more common in dogs with CME, especially in the subclinical form, whereas PLT dysfunction/hypocoagulability (a state of increased risk for bleeding) may be seen in some cases of clinical CME. Inflammation (represented by elevated CRP) may be associated with hypercoagulation in dogs with CME. Additionally, further studies with larger sample sizes are needed to investigate the PLT dynamics in thrombocytopenic dogs with ehrlichiosis.

Availability of Data and Materials

The data that support the findings of this study are available on request from the corresponding author (M. Kocatürk).

Acknowledgements

We thank to Pınar Levent (DVM, PhD) and Ahmet Sarıl (DVM, PhD) because of their performing the analysis of the TEG analysis in Coagulation Lab at the Veterinary Teaching Hospital (Bursa Uludag University Veterinary Faculty, Bursa, Turkey).

Funding Support

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing Interests

None of the other authors have any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Authors' Contributions

ZY and MK conceived and supervised this study, completed the main laboratory analysis, collected and analyzed data. ZY, MK, RT and JJC wrote the first draft of the manuscript. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

References

1. Neer TM, Breitschwerdt EB, Greene RT, Lappin MR: Consensus statement on ehrlichial disease of small animals from the infectious disease study group of the ACVIM. *J Vet Intern Med*, 16, 309-315, 2002. DOI: 10.1111/j.1939-1676.2002.tb02374.x

2. Codner EC, Farris-Smith LL: Characterization of the subclinical phase of ehrlichiosis in dogs. *J Am Vet Med Assoc,* 189, 47-50, 1986.

3. Waner T, Harrus S, Bark H, Bogin E, Avidar Y, Keysary A: Characterization of the subclinical phase of canine ehrlichiosis in experimentally infected beagle dogs. *Vet Parasitol,* 69, 307-317, 1997. DOI: 10.1016/s0304-4017(96)01130-2

4. Batmaz H, Nevo E, Waner T, Sentürk S, Yilmaz Z, Harrus S: Seroprevalence of *Ehrlichia canis* antibodies among dogs in Turkey. *Vet Rec,*

148, 665-666, 2001. DOI: 10.1136/vr.148.21.665

5. Parashar R, Sudan V, Jaiswal AK, Srivastava A, Shanker D: Evaluation of clinical, biochemical and haematological markers in natural infection of canine monocytic ehrlichiosis. *J Parasit Dis*, 40, 1351-1354, 2016. DOI: 10.1007/s12639-015-0688-7

6. Harrus S, Waner T, Weiss DJ, Keysary A, Bark H: Kinetics of serum antiplatelet antibodies in experimental acute canine ehrlichiosis. *Vet Immunol Immunopathol*, 51, 13-20, 1996. DOI: 10.1016/0165-2427(95)05516-9

7. Harrus S, Waner T, Eldor A, Zwang E, Bark H: Platelet dysfunction associated with experimental acute ehrlichiosis. *Vet Rec*, 139, 290-293, 1996. DOI: 10.1136/vr.139.12.290

8. Escribano D, Cihan H, Martínez-Subiela S, Levent P, Kocaturk M, Aytug N, Cerón JJ, Tvarijonaviciute A, Yilmaz Z: Changes in serum proteins in dogs with *Ehrlichia canis* infection. *Microb Pathog*, 113, 34-39, 2017. DOI: 10.1016/j.micpath.2017.10.024

9. Harrus S, Waner T, Aizenberg I, Bark H. Therapeutic effect of doxycycline in experimental subclinical canine monocytic ehrlichiosis: Evaluation of a 6-week course. *J Clin Microbiol*, 36, 2140-2142, 1998. DOI: 10.1128/JCM.36.7.2140-2142.1998

10. Macotpet A, Pattarapanwichien E, Suksawat F: Hemostatic disorders in canine monocytic ehrlichiosis. *KKU Vet J*, 18, 11-18, 2018.

11. Shropshire S, Olver C, Lappin M: Characteristics of hemostasis during experimental *Ehrlichia canis* infection. *J Vet Intern Med*, 32, 1334-1342, 2018. DOI: 10.1111/jvim.15130

12. Özata F, Ural K: Thrombocyte indices in dogs infected with *Ehrlichia canis* and *Anaplasma phagocytophilum*. *Revista MVZ Córdoba*, 19, 4277-4288, 2014.

13. Pasa S, Ural K, Gultekin M: Interpretation of coagulation tendency contributing to thrombosis in Vector-Borne Diseases (Ehrlichiosis, Anaplasmosis, Leishmaniosis, and Dirofilariasis) among dogs. *Acta Scientiae Vet,* 45:7, 2017. DOI: 10.22456/1679-9216.80033

14. Ahmad A, Kohli M, Malik A, Kohli M, Bogra J, Abbas H, Gupta R, Kushwaha BB: Role of thromboelastography versus coagulation screen as a safety predictor in pre-eclampsia/eclampsia patients undergoing lower-segment caesarean section in regional anaesthesia. *J Obstet Gynaecol India,* 66, 340-346, 2016. DOI: 10.1007/s13224-016-0906-y

15. Erdoğan H, Paşa S, Ural K, Gültekin M, Parlatır Y, Toplu S, Balıkçı C: Ehrlichiosis'li köpeklerde D-dimer/fibrinojen oranı. *Atatürk Univ Vet Bil Derg*, 13, 28-33, 2018. DOI: 10.17094/ataunivbd.298252

16. Atikyılmaz G, Cingi CC: Determination of vitamin D and D-dimer levels in dogs with *Ehrlichia canis* infection. *Indian J Anim Res*, 55, 226-229, 2021. DOI: 10.18805/IJAR.B-1223

17. Eralp O, Yilmaz Z, Failing K, Moritz A, Bauer N: Effect of experimental endotoxemia on thrombelastography parameters, secondary and tertiary hemostasis in dogs. *J Vet Int Med,* 25, 524-531, 2011. DOI: 10.1111/j.1939-1676.2011.0698.x

18. Yilmaz Z, Kocaturk M, Inan OE, Levent P: Thromboelastographic evaluation of hemostatic function in dogs with dilated cardiomyopathy. *Turk J Vet Anim Sci*, 41, 372-379, 2017. DOI: 10.3906/vet-1608-4

19. Bisoendial RJ, Kastelein JJ, Levels JH, Zwaginga JJ, van den Bogaard B, Reitsma PH, Meijers JC, Hartman D, Levi M, Stroes ES: Activation of inflammation and coagulation after infusion of C-reactive protein in humans. *Circ Res*, 96, 714-716, 2005. DOI: 10.1161/01.RES.0000163015.67711.AB

20. Cheng T, Mathews KA, Abrams-Ogg AC, Wood RD: Relationship between assays of inflammation and coagulation: A novel interpretation of the canine activated clotting time. *Can J Vet Res,* 73, 97-102, 2009.

21. Zouaoui Boudjeltia K, Piagnerelli M, Brohée D, Guillaume M, Cauchie P, Vincent JL, Remacle C, Bouckaert Y, Vanhaeverbeek M: Relationship between CRP and hypofibrinolysis: Is this a possible mechanism to explain the association between CRP and outcome in critically ill patients? *Thromb J,* 2:7, 2004. DOI: 10.1186/1477-9560-2-7

22. Pazzi P, Goddard A, Kristensen AT, Dvir E: Evaluation of hemostatic abnormalities in canine spirocercosis and its association with systemic inflammation. *J Vet Intern Med*, 28, 21-29, 2014. DOI: 10.1111/jvim.12220

23. Xuan J, Wang J, Wei B: Diagnostic value of thromboelastography (TEG) for the diagnosis of death in infected patients. *Clin Appl Thromb Hemost,* 27:10760296211047231, 2021. DOI: 10.1177/10760296211047231

24. Rubio CP, Yilmaz Z, Martínez-Subiela S, Kocaturk M, Hernández-Ruiz J, Yalcin E, Tvarijonaviciute A, Escribano D, Ceron JJ: Serum antioxidant capacity and oxidative damage in clinical and subclinical canine ehrlichiosis. *Res Vet Sci*, 115, 301-306, 2017. DOI: 10.1016/j.rvsc.2017.06.004

25. Cihan H, Temizel EM, Davoust B, Marie JL, Casali F, Parzy D, Aytuğ N: Silent threat subclinical canine monocytic ehrlichiosis in stray dogs in Turkey. *Uludag Univ J Vet Med Fac*, 29, 15-19, 2010.

26. Rodríguez-Alarcón CA, Beristain-Ruiz DM, Olivares-Muñoz A, Quezada-Casasola A, Pérez-Casio F, Álvarez-Martínez AA, Tapia-Alanís J, Lira-Amaya JJ, Rivera-Barreno R, Cera-Hurtado OS, Ibancovichi-Camarillo JA, Soon-Gómez L, Adame-Gallegos JR, Figueroa-Millán JV: Demonstrating the presence of *Ehrlichia canis* DNA from different tissues of dogs with suspected subclinical ehrlichiosis. *Parasites Vectors,* 13:518, 2020. DOI: 10.1186/s13071-020-04363-0

27. Bauer N, Eralp O, Moritz A: Establishment of reference intervals for kaolin-activated thromboelastography in dogs including an assessment of the effects of sex and anticoagulant use. *J Vet Diagn Invest,* 21, 641-648, 2009. DOI: 10.1177/104063870902100508

28. Kocatürk M, Martinez S, Eralp A, Tvarijonaviciute A, Ceron JJ, Yilmaz Z: Prognostic value of serum acute-phase proteins in dogs with parvoviral enteritis. *J Small Anim Pract*, 51, 478-483, 2010. DOI: 10.1111/j.1748-5827.2010.00965.x

29. Müller MC, Meijers JC, Vroom MB, Juffermans NP: Utility of thromboelastography and/or thromboelastometry in adults with sepsis: A systematic review. *Crit Care,* 18 (1):R30, 2014. DOI: 10.1186/cc13721

30. Hartmann J, Sikorski RA: Thromboelastography (TEG 5000 and TEG 6s Hemostasis Analysers with TEG Manager Software). **In,** Moore HB, Neal MD, Moore EE (Eds): Trauma Induced Coagulopathy. Springer, Cham. 2021.

31. Kupcinskiene K, Trepenaitis D, Petereit R, Kupcinskas J, Gudaityte R, Maleckas A, Macas A: Monitoring of hypercoagulability by thrombo-elastography in bariatric surgery. *Med Sci Monit,* 15, 1819-1826, 2017. DOI: 10.12659/msm.900769

32. Wiinberg B, Jensen AL, Johansson PI, Rozanski E, Tranholm M, Kristensen AT: Thromboelastographic evaluation of hemostatic function in dogs with disseminated intravascular coagulation. *J Vet Intern Med*, 22, 357-365, 2008. DOI: 10.1111/j.1939-1676.2008.0058.x

33. Liu C, Guan Z, Xu Q, Zhao L, Song Y, Wang H: Relation of thromboelastography parameters to conventional coagulation tests used to evaluate the hypercoagulable state of aged fracture patients. *Medicine (Baltimore)*, 95:e3934, 2016. DOI: 10.1097/MD.0000000000003934

34. Han HJ, Kim JH: Correlation between D-dimer concentrations and thromboelastography in dogs with critical illness: A retrospective, cross-sectional study. *Front Vet Sci*, 14:844022, 2022. DOI: 10.3389/fvets.2022.844022

35. Mylonakis ME, Ceron JJ, Leontides L, Siarkou VI, Martinez S, Tvarijonaviciute A, Koutinas AF, Harrus S: Serum acute phase proteins as clinical phase indicators and outcome predictors in naturally occurring canine monocytic ehrlichiosis. *J Vet Intern Med*, 25, 811-817, 2011. DOI: 10.1111/j.1939-1676.2011.0728.x

36. Erdogan S, Basbug O, Aydogdu U, Pasa S, Erdogan H, Ural K: Diagnostic value of C-reactive protein and soluble urokinase plasminogen activator receptor in canine monocytic ehrlichiosis. *Thai J Vet Med*, 51, 567-575, 2021.

37. Zhou W, Zhou W, Bai J, Ma S, Liu Q, Ma X: TEG in the monitoring of coagulation changes in patients with sepsis and the clinical significance. *Exp Ther Med,* 17, 3373-3382, 2019. DOI: 10.3892/etm.2019.7342

38. Yilmaz Z, Eralp O, Ilcol YO: Evaluation of platelet count and its association with plateletcrit, mean platelet volume, and platelet size distribution width in a canine model of endotoxemia. *Vet Clin Pathol,* 37, 159-163, 2008. DOI: 10.1111/j.1939-165X.2008.00023.x

39. Levent P, Kocaturk M, Akgun E, Saril A, Cevik O, Baykal AT, Tanaka R, Ceron JJ, Yilmaz Z: Platelet proteome changes in dogs with congestive heart failure. *BMC Vet Res*, 16:466, 2020. DOI: 10.1186/s12917-020-02692-x

40. Lu D, Owens J, Kreutz RP: Plasma and whole blood clot strength

measured by thrombelastography in patients treated with clopidogrel during acute coronary syndromes. *Thromb Res*, 132 (2):e94-8, 2013. DOI: 10.1016/j.thromres.2013.07.012

41. Singla LD, Singh H, Kaur P, Singh ND, Singh NK, Juyal PD: Serodetection of *Ehrlichia canis* infection in dogs from Ludhiana district of Punjab. *India J Parasit Dis*, 35, 195-198, 2011. DOI: 10.1007/s12639-011-0055-2

42. René-Martellet M, Lebert I, Chêne J, Massot R, Leon M, Leal A, Badavelli S, Chalvet-Monfray K, Ducrot C, Abrial D, Chabanne L, Halos L: Diagnosis and incidence risk of clinical canine monocytic ehrlichiosis under field conditions in Southern Europe. *Parasit Vectors,* 6:3, 2015. DOI: 10.1186/s13071-014-0613-4

43. Cardoso L, Mendão C, Madeira de Carvalho L: Prevalence of *Dirofilaria immitis, Ehrlichia canis, Borrelia burgdorferi* sensu lato, *Anaplasma* spp. and *Leishmania infantum* in apparently healthy and CVBD-suspect dogs in Portugal - A national serological study. *Parasites Vectors*, 5:62, 2012. DOI: 10.1186/1756-3305-5-62

44. Parmar C, Pednekar R, Jayraw A, Gatne M: Comparative diagnostic methods for canine ehrlichiosis. *Turk J Vet Anim Sci*, 37, 282-290, 2013. DOI: 10.3906/vet-1201-12

45. Aziz MU, Hussain S, Song B, Ghauri HN, Zeb J, Sparagano OA: Ehrlichiosis in dogs: A comprehensive review about the pathogen and its vectors with emphasis on south and east Asian countries. *Vet Sci*, 10:21, 2023. DOI: 10.3390/vetsci10010021

46. Gospodinova K, Zhelev G, Petrov V: Comparıson of a rapid enzyme-linked ımmunosorbant assay test with an indirect immunofluorescent antibody test in diagnosing *Ehrlichia* and *Anaplasma* ınfections in dogs. *Trakia J Sci*, 4, 346-352, 2019.

47. Petruccelli A, Ferrara G, Iovane G, Schettini R, Ciarcia R, Caputo V, Pompameo M, Pagnini U, Montagnaro S: Seroprevalence of *Ehrlichia* spp., *Anaplasma* spp., *Borrelia burgdorferi sensu lato*, and *Dirofilaria immitis* in stray dogs, from 2016 to 2019, in Southern Italy. *Animals*, 11 (1):9, 2021. DOI: 10.3390/ani11010009

48. Laboso J, Kihurani D, Kimeli P, Shah D: Serological diagnosis of canine ehrlichiosis in Kenya and Tanzania, *Research Square*, 1-10, 2023. DOI: 10.21203/rs.3.rs-2453148/v1

49. Roopali B, Kasaralikar VR, Patil NA, Ravindra BG, Sandeep H: Spot diagnosis of canine monocytic ehrlichiosis: Need of the hour. *J Entomol Zool Stud*, 8 (2): 366-368, 2020.

50. Alberigi B, Labarthe N, Cardoso F, Cunha C, Almeida C, Souza C, Mendes-de-Almeida F: Serological evidence of canine arthropod-borne infections in an ecotone area of a natural reserve at the Pantanal, Brazil. *Braz J Vet Med*, 41 (1):e103719, 2019. DOI: 10.29374/2527-2179.bjvm103719