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Original Article

Prevalence of *Ehrlichia Canis* Infection in Uşak and Investigation of Clinical, Hematological and Biochemical Signs in Infected Dogs

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Canine ehrlichiosis which becomes widespread worldwide is generated by a pathogen bacteria called *Ehrlichia Canis*. The prevalence of ehrlichiosis changes according to geographic regions in veterinary practice. The aim of this study was to determine the prevalence of the canine ehrlichiosis in Uşak, Turkey, together with clinical, hematological and biochemical signs. 100 dogs found in 6 different regions of Uşak city were checked for the presence of *Ehrlichia Canis*. Clinical signs most frequently described in 7 dogs (7%) having ehrlichiosis were anorexia, weight loss, thinness, fever and lymphadenopathy. Hematological signs most commonly detected in the dogs having ehrlichiosis were anemia, thrombocytopenia and leukopenia. Increase on hypoalbuminemia, alkaline phosphatase and alanine aminotransferase enzyme activities were recorded as biochemical changes most frequently seen in the dogs having ehrlichiosis. This is the first study in which the prevalence of the canine ehrlichiosis is determined in Uşak, Turkey, together with clinical, hematological and biochemical signs.

Keywords: Canine ehrlichiosis, clinical signs, hematological indices, biochemical profile, Uşak

Ehrlichia canis is a bacterium of the genus Ehrlichia in the family Rikettsia (Anaplas-matacea), which is obligatory, intracellular, gram-negative, pleomo-rphic and has an obligate intracellular conchoidal structure (1-3). *E. canis* causes infection in humans and animal species such as dog, cat, sheep, goat, horse (4-5). There are two different types of leukotropic diseases that are caused by ehrlichia bacteria in dogs. These are "Canine Monocytic Ehrlichiosis" caused by *E. canis* that is frequently encountered, and "Canine Granulocytic Ehrlich-iosis" caused by *E. ewingi*. The incubation period of the illness is 7-21 days in general and it has three stages as acute, sub-clinical, and chronic (6). During acute stage in dogs, bleeding diathesis (mucous membrane petechiae resulting

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from thrombocy-topenia), dyspnea, vestibular dysfunction, rarely moderate nose bleeding, hypersensitivity, central nervous system diseases occur together with fever, depression, extreme weight loss, anorexia, lymphadenopathy (7). Besides, 40% of the infected dogs have tick infestation (8). In the acute stage, they settle in mononuclear phagocytic tissues within active liver, spleen and lymph nodes and into the circulating mononuclear cells. Then, infected cells reach other organs such as lungs, kidney and brain through blood and cause vasculitis adhering to the vascular endothelium (9-10). They cause thrombocytopenia by leading to immunological destruction of platelets (11-13). They also cause central nervous system abnormalities in dogs. Furthermore, leukocyte count decreases leading to anemia as a result of erythrocyte production decrease (14-15).

The aim of the present study was to examine the disease in terms of clinical, hematological and blood biochemical parameters and to find out possible changes in terms of internal diseases occurring during the course of the disease, investigate the level of organs affection, and determine the prevalence of the disease in Uşak, Turkey.

Materials and methods

The study material consisted of 100 dogs of different sex whose ages was ranging between 1 and 10 years and which were owned and found in the city center and Banaz, Eşme, Ulubey, Sivaslı and Karahallı towns of Uşak. Clinical examination was performed on all animals. Hematological and serum biochemical indices were examined in all blood samples in the laboratory.

This study was conducted within the scope of ethical rules of Afyon Kocatepe University Animal Testing Ethics Committee with the reference number: AKUHADYEK 395-14.

Statistical analysis

Statistical analysis of the obtained data was performed with Chi square (X^2) test by using SPSS for Windows program (2011). Data are presented as mean ± SD, and $P< 0.05$ was considered as statistically significant.

Results

Clinical Findings

Out of 100 dogs studied in this study, 49 had tick infestation, 27 had respiratory disorders, 31 had dehydration, 52 had lose weight, 41 had anorexia, 3 presented mucous membrane petechial bleedings, and 43 had high fever.

Among 100 investigated dogs, *Ehrlichia Canis* infection was present in 7 dogs (2 in Banaz, 2 in the city center, 2 in Ulubey and 1 in Eşme). Statistical comparisons of the body temperatures, heart and respiration frequencies of the dogs with *Ehrlichia Canis* infection are represented in Table 1.

Body temperatures and heart frequencies of *E. canis* positive (+) group was significantly higher ($P< 0.05$) than the *E. canis* negative (-) group. No significant difference between the respiration

Table 1. Comparison of clinical features of the dogs with and without *E. canis* infection

Groups/Parameter	Erlichia (+) (n=7)	Erlichia (-) (n=93)	Reference	P
T (°C)	39.51± 1.20[a]	38.6 ± 1.04[b]	37.5-38.6	< 0.05
P (frequency/min)	126.52± 8.64[a]	118.02± 4.23[b]	70-120	< 0.05
R (frequency/min)	28.06± 1.45	27.85± 1.08	15-30	> 0.05

T: temperature; P: heart beat; R: respiration. Data are expressed as mean± SD. [a,b] Difference between the mean of control groups that have different letters in the same column is statistically significant (P<0.05).

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frequency of the *E. canis* (+) and the *E. canis* (-) group was observed (P> 0.05).

Hematological examination

Hematological findings are shown in Table 2. Accordingly, white blood cells, monocytes and granulocytes count of the *E. canis* (+) group were significantly lower than the *E. canis* (-) group (P< 0.05), although they were within the reference limits in both groups. Also, red blood cells count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet levels of the *E. canis* (+) group were below the reference limits and significantly lower than the average of the *E. canis* (-) group (P< 0.05). The amount of red blood cell distribution width (RDW) of the *E. canis* (+) group was above the reference limits and significantly higher than the average of the *E. canis* (-) group (P <0.05). Although within the reference limits, the mean number of lymphocytes in *E. canis* (+) group was insignificantly higher than the *E. canis* (-) group (P> 0.05). Also, the average number of monocytes was within the reference limits, and the average monocytes number of the *E. canis* (+) group was lower than the *E. canis* (-) group without being significantly different (P >0.05).

Metabolic profile evaluation

Elements determining the metabolic profile, were evaluated in all studied animals. Accordingly, alanine transaminase (ALT) levels of 15 dogs (1 in Banaz, 6 in Karahallı, 2 in the city center, 4 in Sivas, 2 in Ulubey) were above the reference limits but others' (n=85) levels were within the reference limits. Alkaline phosphate (ALP) levels of 36 dogs (8 in Banaz, 6 in Eşme, 5 in Karahallı, 6 in the city center, 5 in Sivaslı, 6 in Ulubey) were above reference values but others' (n=64) levels were within the reference limits. Blood urea nitrogen (BUN) values of 5 dogs (1 in Banaz, 3 in Eşme, 1 in Sivaslı) were below the reference limits, values of 12 of them (1 in Banaz, 3 in Eşme, 2 in Karahallı, 2 in the city center, 1 in Sivaslı, 3 in Ulubey) were above the reference limits and others' (n=83) values were within the reference limits. Regarding

Table 2. Hematological examination of *E. canis* positive and negative dogs

Group	WBC (10^3/ µL)	RBC (10^6/ µL)	HGB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (10^3/ µL)	RDW %	LYMPH (10^3/ µL)	MONO (10^3/ µL)	GRAN (10^3/ µL)	EOS (10^3/ µL)
Erlichia (+)	9.68± 2.10^a	3.50± 0.80^a	8.14± 1.16^b	21.55± 4.40^b	58.57± 5.60^b	19.11± 3.43^b	31.91± 3.68^b	90.71± 8.68^b	19.89± 3.18^a	2.82± 0.32	0.67± 0.02	7.31± 1.22^b	4.34± 0.60
Erlichia (-)	12.79± 2.46^b	6.02± 0.24^b	13.36± 2.12^a	39.69± 5.10^a	66.33± 7.48^a	22.16± 5.68^a	33.31± 6.48^a	274.33±32.2^a	16.91± 4.16^b	2.34± 0.50	0.85± 0.04	9.37± 2.16^a	4.67± 0.86
Reference*	6-17	5.5-8.5	12-18	37-55	60-77	19.5-24.5	32-36	200-500	11.6-14.8	1-4.8	0.15-1.35	3-11.5	0.10–1.25
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	> 0.05	> 0.05	< 0.05	0.05

WBC: white blood cells; RBC: red blood cells; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelet; RDW: red blood cell distribution width; LYMPH: lymphocytes; MONO: monocytes; GRAN: granulocytes; EOS: eosinophils. Data are expressed as mean± SD. ^{a,b} Difference between control groups that have different letters in the same column is statistically significant (P< 0.05).

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creatinine (CREA) values, one dog (in Eşme) was below the reference limits, 14 dogs (2 in Banaz, 1 in Eşme, 3 in Karahallı, 3 in the city center, 2 in Sivaslı, 3 in Ulubey) were above the reference limits and values for the remaining (n=85) were within the reference limits. In terms of total protein (TP) values, 2 dogs (1 in Karahallı and 1 in Ulubey) were below the reference values, 25 (5 in Banaz, 3 in Eşme, 6 in Karahallı, 4 in the city center, 3 in Sivaslı, 4 in Ulubey) showed levels above the reference values and others (n=73) were within the reference levels. Lastly, albumin (ALB) values of 52 dogs (11 in Banaz, 9 in Eşme, 9 in Karahallı, 7 in the city center, 9 in Sivaslı, 7 in Ulubey) were below the reference values and others' (n=48) values were within the reference values.

Statistical analyzes of elements of the metabolic profile are shown in Table 3. ALT, ALP, BUN, and CREA levels of the *E. canis* (+) dogs was above the reference values and significantly higher than the *E. canis* (-) dogs (P< 0.05). Total protein amounts did not differ significantly between the 2 groups (P> 0.05) and were within the reference values. However, the average of ALB levels in *E. canis* (+) dogs was significantly lower than the average of ALB in *E. canis* (-) dogs (P< 0.05).

Discussion

In the present study, 7 out of 100 examined dogs (7%) were *E. canis* (+) and all *E. canis* (+) dogs were infested with ticks. Compared to other researches conducted in Aegean region, this rate is surprisingly low. Hence, it is pointed out that brown dog tick called *Rhipicephalus sanguineus* causing ehrlichiosis in dogs can live in all climate conditions in Turkey and may be found in sheep, goat, horse, donkey, dog, cat, cattle, buffalo, camel, warthog, and humans (16). The low rate (7%) of ehrlichiosis found in the present study, may be due to the effective fight against ticks in the region, recently.

We observed that dogs infected with *E. canis* have generally pale mucous membranes and 2 of 7 dogs had mucous membranes bleedings. In the study conducted by Rodriguez et al. in Mexico, paleness and bleedings in mucous membranes were reported among common clinical symptoms of the dogs infected with *E. canis*(17). In the present study, all *E. canis* infected dogs had anorexia.

Dodurka and Bakırel reported high fever in the first case they encountered (14). We detected high fever which is one of the most significant clinical signs of Ehrlichiosis in 6 of 7 dogs infected with *E. canis*. In a study conducted in USA, Suksawat et al. reported that 6 out of 44 dogs infected with *E. canis* had high fever (18).

Complete blood count has a huge importance in ehrlichiosis diagnosis. In the acute stage of the disease, a severe thrombocytopenia stands out in the blood chart. Leukopenia and anemia accompany thrombocytopenia in this stage. In the present study,

Table 3. Metabolic profile of *E. canis* positive and negative dogs

Group	ALT (U/L)	ALP (U/L)	BUN	CREA (mg/dl)	TP (g/l)	ALB (g/L)
Erlichia (+)	124.14±22.4^{a}	124.85±24.1^{a}	54.99±12.82^{a}	2.24±0.44^{a}	6.95±1.00^{b}	2.11±0.44^{b}
Erlichia (-)	89.23±26.55^{b}	92.87±24.67^{b}	33.18±14.16^{b}	1.87±0.83^{b}	7.07±2.43^{a}	3.3±1.02^{a}
Reference*	10-94	10-90	7-32	0.5-1.4	5.3-7.6	3.2-4.2
P	<0.05	<0.05	<0.05	<0.05	>0.05	<0.05

ALT: alanine transaminase; ALP: alkaline phosphate; BUN: blood urea nitrogen; CREA: creatinine; TP: total protein; ALB: albumin. Data are expressed as mean± SD. ^{a,b} Difference between control groups that have different letters in the same column is statistically significant (P< 0.05).

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hematological examination signs demonstrated that thrombocytopenia and leukopenia were present in dogs having *Ehrlichia canis*. Similar signs were reported by Dodurka and Bakırel (14). In another study, Frank and Breitschwerdt reported that 77% of the dogs infected with *E. canis* have thrombocytopenia (19). Similarly, Suksawat et al. detected thrombocytopenia in 13 out of 44 dogs infected with *E. Canis* (18). Cihan et al. emphasized that thrombocytopenia was the most obvious distinguishing feature in the blood chart of the dogs infected with *E. Canis* and it occurred in 80% of 111 *E. Canis* (+) dogs (20).

Thrombocytopenia which is at the top of the most important diagnostic parameters, was also observed clearly by Harrus et al. (10). Similarly, we found that the average platelet count of *E. canis* (+) dogs was below 100000 /µl (90710/µl).

Thrombocytopenia, anemia, eosinopenia. left deviation in neutrophils, are the hematological signs frequently encountered in canine ehrlichiosis (15, 21-22). According to the hematological indices found in the present study (Table 2), erythrocyte count, and hemoglobin and hematocrit levels of the dogs having ehrlichiosis were quite low ($P< 0.05$) compared to the dogs without ehrlichiosis. Also, normocytic and normochromic anemia were present in dogs with ehrlichiosis. Similar results were reported previously (9, 23).

BUN and CREA levels obtained in the present study indicate the presence of kidney disease. It was previously reported that ehrlichiosis accompanied by hyperglobulinemia and thrombocytopenia leads to renal damage and causes renal amyloidosis in dogs, and high levels of BUN and CREA are significant markers of this damage (10, 24). In the present study, serum ALT and ALP activities were significantly increased in dogs with ehrlichiosis. These high enzyme levels are considered as important signs of liver and possibly renal damage (25-27).

We also found that there was no significant difference ($P> 0.05$) in TP levels between dogs with *E. canis* and those without infection, which is in agreement with the results obtained by Paşa and Azizoğlu (28), but it differs from the study of Reardon and Pierce (29) reporting that TP levels of the dogs with *E. canis* was decreased. Our data on ALB levels correspond to other reports in which hypoalbuminemia developed in the dogs with *E. canis* (28-29).

The present report is an important and reference study determining the prevalence of E. canis in Uşak for the first time. In the region, compared to other literatures, low incidence of the disease (7%) is an important finding in terms of regional livestock and effectiveness of the struggle. Moreover, clinical, hematological and blood biochemical parameters' measurements together with bacteria isolation may provide significant advantages in terms of determining the severity, the course, and the prognosis of the disease and consequently the treatment that will be applied.

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Conflict of interest

The authors declared no conflict of interest.

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