

# THE SPREAD OF CANINE MONOCYTTIC EHRLICHIOSIS IN TURKEY TO CENTRAL ANATOLIA

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## ABSTRACT

*Ehrlichia canis*, a rickettsial agent, causes canine monocytotropic ehrlichiosis (CME) in dogs characterized by fever, anorexia, weakness, and lymphadenopathy. In most cases, after the acute stage, the disease regresses to an asymptomatic form which may progress into a more severe chronic and often fatal form. Once transmitted by *Rhipicephalus sanguineus* ticks, it reaches the spleen, liver, lymph nodes via blood and lymph vessels. In the present study, dogs of different breeds and ages submitted to our clinics for various health problems, many of them with tick infestations, were examined serologically for ehrlichiosis. Blood samples were collected from a total of 122 dogs (66 females and 56 males) and analyzed using a commercial immunofluorescence antibody test kit. Total blood count was conducted using an automated cell counter. Seropositivity was detected in 18 dogs (14.75%), among which 17 had no clinical findings while one had a non-specific cough. The seropositive dogs had no specific clinical or hematological signs. It was concluded that these dogs were probably in the subclinical phase of the disease. Seropositivity in male and female dogs was 12.12% and 17.85%, respectively. The result of this study is demonstrates the expansion of canine ehrlichiosis into the dry regions of Anatolia. Therefore, we recommend that dogs especially those with tick infestations should be examined serologically for ehrlichiosis as subclinically infected dogs usually exhibit no clinical signs or hematological changes and may for unknown reasons revert to the chronic severe and usually fatal form of CME.

**Key Words:** Dog, *Ehrlichia canis*

## INTRODUCTION

Canine monocytotropic ehrlichiosis (CME) is a serious infectious disease in dogs caused by *Ehrlichia canis*, a rickettsial organism. Since its first report in Algeria in 1935 by Donatien and Lestoquard, the disease has been described in many tropical and subtropical regions around the world (1-2). The infection results from infestation by the "brown dog tick", *Rhipicephalus sanguineus*. *Dermacentor variabilis*, the American dog tick, has also been shown to induce experimental CME (3). The vector tick contaminates the feeding sites with salivary secretions during blood sucking. The incubation period of *E. canis* varies from 8 to 20 days, during which time the organisms multiply in macrophages of the monocytic phagocytic system throughout the body, especially in the liver, spleen and lymph nodes (3-4). The disease causes significant changes in hematological parameters along with bone marrow suppression and/or destruction and depending of the stage of the disease is associated with a deficiency in production of one or more blood elements (3). The infected dogs exhibit several

clinical signs depending on the phase of the disease. The most frequent clinical signs during the acute phase include high fever, anorexia, emaciation, hepatomegaly, splenomegaly, and lymphadenopathy. The diseased animals may also exhibit cardiac and respiratory impairment as well as nervous and ocular disorders (2).

When a dog is infected by *E. canis*, the disease may progress through three subsequent phases: acute, subclinical and chronic. Each phase is characterized by various degrees of clinical and hematologic abnormalities (5). The severity of clinical signs in the acute phase vary from mild to severe, and the symptoms include non-specific clinical signs including fever, anorexia, weight losses, depression, dyspnea, ocular disturbances, petechiae, ecchymoses and epistaxis as well as neurological disorders (4). Thrombocytopenia, mild anemia, and mild leucopenia are among the hematological abnormalities seen in the acute phase. The diseased animal in acute phase can recover completely if the animal is treated appropriately; however, the disease may progress to the subclinical phase,

lasting for years in the absence of an appropriate treatment protocol (6). The diseased animals in the subclinical phase appear clinically healthy although mild thrombocytopenia may still exist (6). The infected dog in the subclinical phase may either remain as a persistent carrier or for unknown reasons enter the chronic phase of the disease (3). In the chronic phase, dogs exhibit clinical signs including weakness, depression, emaciation, and fever (6). The chronic phase in its severe form is associated with pancytopenia that results from bone marrow hypoplasia and deficiency in bone marrow derived blood elements. Dogs with pancytopenia suffer from severe nonregenerative anemia, leucopenia, and thrombocytopenia. The diseased dogs in this stage do not respond to antibiotic treatment and subsequently die of secondary infections and bleeding (3).

Many diagnostic procedures have been described and used for CME including the Indirect Fluorescence Antibody Technique (IFA), the Enzyme-linked immunosorbent assay (ELISA), Western Blot and Polymerase Chain Reaction (PCR) test (7-8).

As the tick population in the Central Anatolia has increased for the recent years, the number of individual reports on tick-borne diseases such as Crimean Congo haemorrhagic fever and canine monocytic ehrlichiosis has increased (9-10). As CME may occur subclinically with no clinical and hematological signs in dogs, we considered that it was critical to determine the prevalence of *E. canis* among the dog population with no clinical and hematological signs in Kirikkale Province, a non-tropical region in the Central Anatolia.

## MATERIALS AND METHODS

Blood samples were collected from 122 dogs (66 female; 56 male) that had been housed in various locations of the Kirikkale Province including Balı̇eyh, Keskin, Bahşılı, Yahşihan and Kalecik. Blood samples were withdrawn by venipuncture of the vena cephalica antebrachium into tubes with EDTA and without anti-coagulants, respectively. Sera were obtained from blood samples without anticoagulant by centrifugation and placed in a -20°C freezer. Complete blood count was performed in blood samples in EDTA tubes using an automatic blood analyzer calibrated specifically for canine blood (MS9-3V Melet Schloesing Laboratoires, Switzerland).

Using a commercial *E. canis* IgG IFA test kit (*Product Number: ECG-120 Fuller Laboratories Fullerton, CA USA*), serum samples were analyzed for the presence of IgG antibodies against *E. canis*. Serum samples as well as positive and negative controls were diluted at a ratio of 1:50 in PBS. The fluorescence reaction at 1:800 was used as the cut-off level as a positive reaction in this study. The tested dilution of sera is given in Table 1. The test procedure was followed according to the protocol recommended by the manufacture. The test slides were visualized using a fluorescence microscope (Olympus BX50, Japan) at a magnification of X400.

The results were evaluated based on the system defined in the commercial test kit. As indicated by the test kit, homogenous red or green staining was considered negative; on the other hand, presence of uniform light green granules in cytoplasm of the cells was considered positive (Figure 1). Using the t-test, serologic results were statistically compared between male and female dogs.

## RESULTS

Of the 122 samples, 18 (14.75%) were determined seropositive for *E. canis* (Figure 1A). The titers are given in Table 1.

Of the 18 seropositive dogs, 10 were males (56%) and 8 were females (44%). Seropositivity rates among male and female dogs were 17.85% and 12.12%, respectively (Table 3) ( $p>0.05$ ). Of the seropositive dogs, 17 (94.4%) appeared clinically healthy while one dog had non-specific cough. None of the seropositive dogs had altered hematological parameters (Table 2).

The age of the seropositive dogs varied from 1 to 7 years. The oldest dog included in the study was 7 years. No seropositivity was detected in dogs younger than a year old. Of the 18 seropositive dogs, 12 (21.81%) were cross-breed dogs and 4 (10%) were Turkish Anatolian Kangal sheep dogs. One German shepherd 1 and one Setter 1 were also seropositive.

## DISCUSSION

The first case of CME in Turkey was reported in 1997 by Dodurka and Aydın (11). Later, Batmaz and his colleagues (12) investigated the seroprevalence of *E. canis* based on the presence of *E. canis* antibodies in dog populations in Turkey. In Batmaz et al. (12) study, the blood samples were collected from various locations of the Western and Southern Turkey where the weather is warmer and the climate is similar to that of the tropical regions. In our study, blood samples were collected in various locations of the Kirikkale Province located in the Central Anatolia (Kirikkale region) where the climate is mainly terrestrial. In recent years, the tick population has significantly increased especially throughout the Central Anatolia (9-10), which may be the most important epidemiological background for the expansion of canine ehrlichiosis towards the Central Anatolia as evidenced by the present study. Thus, the present study points out that the Central Anatolia should also be included in the map of the risk regions for canine ehrlichiosis as the seropositivity rate in our study (14.75%) is quite comparable to those reported from the Western and Southern Turkey (20.78%) (12), Mexico (19%) (13), Spain (19.2%) (14).

The seropositive dogs in the present study appeared clinically healthy, only one had nonspecific cough. In addition, hematological parameters were found unaltered in the seropositive dogs. Studies indicated that dogs in the sub-clinical stage of canine ehrlichiosis are frequently

thrombocytopenic and they may also be anemic and leukopenic (15-16). However, dogs in subclinical stage of ehrlichiosis often clinically appear healthy with normal hematological parameters (17). For unknown reasons the disease may progress and enters the chronic stage during which the animal may develop severe pancytopenia, as well as secondary pulmonary hemorrhage, thromboembolism, hepatomegaly, splenomegaly, renal and reproductive disease, polyarthritis, anterior uveitis, retinal disorders, meningoencephalitis, and death as a result of hypotensive shock (17). Thus, the prognosis in the chronic stage is worse than acute stage as the animal does not respond to antibiotic treatment. It is not difficult to imagine that the seropositive dogs found in the present study would possibly enter chronic stage later in their lives. Thus, serological investigation of the dogs for ehrlichiosis is critically important, especially in regions with a high tick population. We also strongly recommend that dogs with tick infestation should also be serologically examined even if the clinical and hematological parameters seem normal. Shipov et al (6) reported that dogs in subclinical stage of the disease might express mild hematological changes such as thrombocytopenia and anemia. However, none of the seropositive dogs in our study had altered hematological parameters.

There are contradictory reports on the gender differences for the frequency of the seropositivity for canine ehrlichiosis. Batmaz et al. (12) and Leib and Monroe (18) reported that the frequency was greater in males than females. In contrast, Baneth et al. (19) and Botros et al. (20) found no sex differences among seropositive dogs. In our study, the frequency was higher in males (17.85%) than females (12.12%); however, the difference tends to be negligible ( $p > 0.05$ ).

## CONCLUSION

The seroprevalence of *Ehrlichia canis* in the Province of Kirikkale, a non-tropical region of Turkey, is as high as 14.75%. These subclinically infected dogs expressed neither a specific clinical signs nor hematological changes. The result of this study is also important to show expansion of canine ehrlichiosis toward the regions of terrestrial climate, coinciding with the increased tick population. In addition to preventative measures to control tick populations and tick contamination, we consider it is also critical to conduct seroprevalence studies in areas with a high tick population, suggestively at the beginning of the summer and winter. Importantly, dogs with a history of tick infestation should also be examined serologically for ehrlichiosis despite absence of any clinical signs and altered hematological signs.

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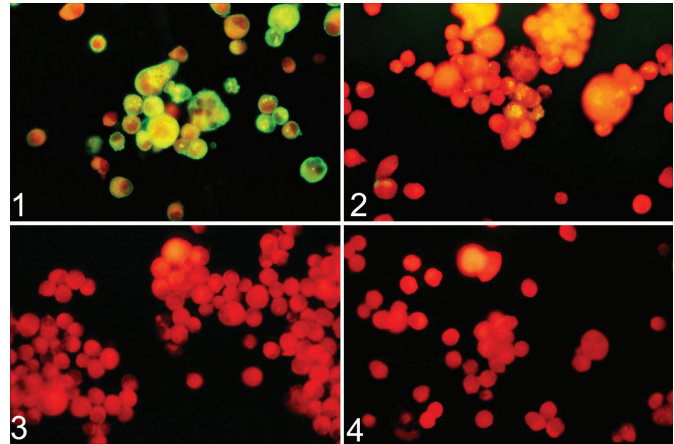
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**Fig. 1:** IFA test for *E. canis* antibodies: Homogenous red or green staining was considered negative; presence of uniform light green granules in cytoplasm of the cells was considered positive.

**TABLES**

**Table 1.**

Total blood count parameters in *Ehrlichia canis* positive dogs (n=18) based on IFA test

Parameters (reference range)	Mean (SD)	Min.	Max.
Total leucocytes (6-17 10 <sup>3</sup> /mm <sup>3</sup> )	14.09 (3.01)	9.9	18.2
Erythrocytes (5.5-8.5 10 <sup>6</sup> /mm <sup>3</sup> )	7.42 (1.76)	6.14	11.2
Haematocrit (35-55%)	43.03 (4.52)	35.8	48.4
Haemoglobin (10-18 g/dL)	14.39 (1.52)	11.8	16.18
Mean corpuscular volume (58-73 fL)	59.5 (8.45)	43.21	68.81
Mean corpuscular haemoglobin concentration (28-40 g/dL)	33.35 (0.52)	32.5	34.2
Thrombocytes (120-600 10 <sup>3</sup> /mm <sup>3</sup> )	331.71 (66.05)	260	426

SD = standard deviation

**Table 2.**

*Ehrlichia canis* IFA test positive titres

Dilution rate	Number of seropositive animals	(%)
1/50	9	50
1/200	1	5.56
1/400	6	33.33
1/800	-	-
1/1600	2	11.11

**Table 3.**

IFA test for male and female *Ehrlichia canis* positive dogs.

Gender	Dogs examined	Seropositive dogs	%
Female	66	8	12.12
Male	56	10	17.85
Total	122	18	14.75