

Silent Threat: Subclinical Canine Monocytic Ehrlichiosis in Stray Dogs in Turkey

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Abstract: Canine monocytic ehrlichiosis has been recognized as an important world-wide canine infectious disease. Thrombocytopenia, leucopenia and normocytic, normochromic anemia are among the major laboratory findings. In this study, 160 stray dogs randomly selected to represent the stray dog population in Ayvalık, Burhaniye, Dikili, Edremit, Bergama region, were tested using IFA to detect antibodies against *Ehrlichia canis*. The development of methods of in vitro cultivation of *E. canis* led to the use of the IFA serologic test for detection and titration of anti-*E. canis* antibodies in dogs. One hundred and eleven of 160 dogs were found seropositive (69.4%) including 69.9% (65/93) of the males and 68.7% (46/67) of the females. There were no significant sex differences. All the dogs were clinically healthy. Thrombocytopenia ($<200000 \mu\text{l}^{-1}$) was the most common hematological abnormality ($p < 0,001$) (80 %). Total leukocyte counts of the seropositive dogs were lower than in seronegative dogs ($p < 0.001$), although none of the dogs were leukopenic except in two cases.

The results of the present study highlight the importance of canine monocytic ehrlichiosis in Aegean region of Turkey and suggest the need for further impact studies.

Key Words: *Ehrlichia canis*, Dog, IFA, Thrombocytopenia, Turkey.

Sessiz Tehdit: Türkiye'deki Sokak Köpeklerinde Subklinik Canine Monositik Ehrlichiosis

Özet: Canine monositik ehrlichiosis köpeklerin dünya çapında karşılaşılan önemli bir bulaşıcı hastalığı olarak kabul edilmektedir. Trombositopeni, lökopeni ve normositik, normokromik anemi en önemli laboratuvar bulgularıdır. Bu çalışmada Ayvalık, Burhaniye, Dikili, Edremit, Bergama bölgelerindeki sokak köpeği popülasyonundan rastgele seçilen 160 sokak köpeğinde *Ehrlichia canis*'in varlığını belirlemek için IFA testi uygulandı. IFA köpeklerde anti - *E. canis* antikorlarının tespiti ve titrasyonunda kullanılan bir serolojik test metodudur. Tüm köpekler klinik olarak sağlıklıydı. Köpeklerin 111'inin (% 69,4) seropozitif oldukları; seropozitiflik oranının erkek köpeklerde % 69.9 (65/93) ve dişilerde % 68.7 (46/67) olduğu belirlenmiştir. Trombositopeni ($<200.000 \mu\text{l}^{-1}$) ($p < 0,001$) (% 80) en yaygın hematolojik anormallik olarak saptanmıştır. Seropozitif köpeklerde seronegatiflere oranla total lökosit sayılarının daha düşük olduğu ($p < 0.001$) saptanmasına rağmen iki köpek haricinde hiçbir köpekte lökopeni tespit edilmemiştir.

Bu çalışmanın sonuçları, Türkiye'nin Ege bölgesinde canine monositik ehrlichiosisün önemini vurgulamakta ve daha fazla etkili çalışmaların yapılmasına yönelik ihtiyacın olduğunu göstermektedir.

Anahtar Kelimeler: *Ehrlichia canis*, Köpek, IFA, Trombositopeni, Türkiye.

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Introduction

Canine monocytic ehrlichiosis (CME) has been recognized as an important world-wide canine infectious disease²¹. *Ehrlichia* are tick-transmitted, obligately intracellular, gram-negative bacteria associated with serious and sometimes fatal disease in humans, some domestic and wild animals^{1,12,25}. Thrombocytopenia^{5,8,13}, leucopenia¹¹ and normocytic, normochromic anemia²⁷ are among the major laboratory findings. Thrombocytopenia is an almost consistent finding in dogs infected experimentally and naturally with *Ehrlichia canis*. It has been suggested that platelet destruction is related to an immunological response¹⁰.

The development of methods of in vitro cultivation of *E. canis* led to the use of the IFA serologic test for detection and titration of anti-*E. canis* antibodies in dogs. The IFA test described by Ristic et al.²⁰ in 1972 has since become the serological "gold standard" test, indicating exposure to *E. canis*¹⁷.

Previous seroprevalence studies have suggested the presence of *E. canis* or related *Ehrlichia* spp. infecting dogs throughout East Mediterranean and Aegean regions^{3,4,15,16}. The first case of CME in Turkey was diagnosed in 1997⁹. Seroprevalence of *E. canis* among dogs in Turkey^{2,26} was previously reported. But until now, there is a gap in information regarding seroprevalence for *E. canis* among stray dogs. In addition, tick vectors may potentially serve to spread the infection from stray dogs to owned ones and, exceptionally to humans¹⁸. Consequently; we attempted to determine the anti-*E. canis* IgG antibody titers in the stray dogs living in these regions, in order to contribute to a better understanding of the importance of subclinical CME in this areas.

Materials and Methods

Population of the Study

Dogs (n = 160) included in this study were selected randomly among stray dogs and consisted of totally 93 male (58.1%) and 67 female (41.9%). Dogs were picked up from streets from north-eastern Aegean towns (Ayvalik, Burhaniye, Edremit, Dikili and Bergama) and brought to Ayvalik dog shelter. Samples were collected from all dogs in their first week in the shelter. The sex distribution of dogs used in the study among regions is shown in Table I. The regions of Ayvalik (33 Male, 27 Female),

Burhaniye (20 Male, 10 Female), Edremit/ Balikesir (12 Male, 13 Female) and Dikili/ Izmir (9 Male, 11 Female) and Bergama/Izmir (19 male, 6 female) are under the influence of a typical Mediterranean climate with mild and rainy winters and hot, dry summers.

Hematology

The blood samples collected into 2 ml anticoagulated (EDTA) tubes, were immediately submitted to automatic analysis (CELL-DYN 3500R, Abbott Diagnostics, Santa Clara, CA) to obtain complete blood cell (CBC) and platelet counts. All the blood samples were collected between June to August.

Serology

Blood for serological tests was collected from dogs in plastic tubes without anticoagulant, allowed to clot for 2–3 h and then centrifuged (800 g for 3 min) to separate the serum. Serum was stored at –20 °C until used for IFA.

Formol-inactivated suspension of cells (2 cells/ml) infected with the bacteria *E. canis*, was used for anti-*E. canis* specific antibodies titration by IFA test. *Ehrlichia canis* antigen supplied by Synbiotics Europe (Lyon) was placed on 12 wells slides, and then dipped into cold acetone at -20°C during four hours. Slides ready-to-use were stored at -20°C. Sera were 20-fold diluted and treated with the reference method. Examination with an ultraviolet microscope shows fluorescent intracytoplasmic morulae in case of positive sample. Samples were screened for a test with a positive threshold set at 1/80 as advised by *American College of Veterinary Medicine*¹⁷.

Statistical Analysis

Statistical difference between the average thrombocyte count, white blood cell (WBC), red blood cell (RBC), haemoglobin (HGB), mean corpuscular volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) of seropositive and seronegative dogs to *E. canis* was calculated using *t-test*.

Results

All dogs were clinically healthy. Overall, 69.4% (111/160) of the dogs were seropositive, including 69.9% (65/93) of the males and 68.7% (46/67) of the females.

All subclinically infected dogs had IFA antibody titers to *E. canis* at a dilution varying from 1:80 to 1:5120. The antibody titer distribu-

tion was 1:80 in fourteen dogs, 1:160 in five dogs, 1:320 in two dogs, 1:640 in ten dogs, 1:1280 in eight dogs, 1:2560 in thirteen dogs and 1:5120 in 59 dogs (Figure 1).

Thrombocytopenia ($<200000 \mu\text{l}^{-1}$) was the most common hematological finding ($p < 0.001$) (80 %) in seropositive dogs in the present study.

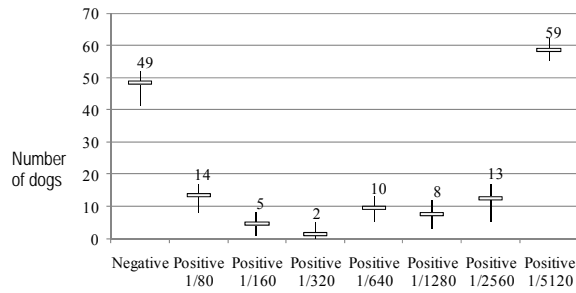


Figure 1.

IFA titers for Ehrlichia canis.

Şekil 1.

Ehrlichia canis için IFA titreleri

Total leukocyte counts of the seropositive dogs were lower than in seronegative dogs ($p < 0.001$), although none of the dogs were leukopenic except in two cases ($<6400 \text{ WBC } \mu\text{l}^{-1}$)¹⁴. Packed cell volume ($p = 0.006$), total erythrocyte ($p = 0.002$) and neutrophil counts ($p = 0.058$) were lower in seropositive dogs compared to seronegative dogs. There were no statistical differences between seropositive and seronegative dogs for eosinophil and monocyte values. Hemoglobin values were in reference ranges in seronegative dogs, however, hemoglobin concentration was lower than reference ranges ($\text{Hgb} < 11.9 \text{ g/dl}$) in seropositives ($p < 0.001$).

Serologic results for *E. canis* among regions in this study are shown in Table 1.

Table 1. Distribution of serologic results of *E. canis* according to the regions.

Tablo1. Bölgelere göre *E. canis*'in serolojik sonuçlarının dağılımı

Serologic results	Ayvalik	Burhaniye	Edremit	Dikili	Bergama
1/80	-	1M/1F	1M/3F	1M/2F	4M/1F
1/160	-	1M	2F	-	2M
1/320	1F	-	-	1M	-
1/640	2M	2M/1F	2M	1M/2F	-
1/1280	2M/2F	1M	1M	1M/1F	-
1/2560	5M/4F	2M	-	2M	-
1/5120	18M/13F	7M/4F	6M/5F	2M/4F	-

M= Male (Erkek) F= Female (Dişi)

Discussion

CME is widely distributed around the world, more particularly in tropical and subtropical areas^{3,4,6,16,19,29}. However, the prevalence of CME varies as a function of climatic conditions, due to certain epidemiological factors, such as distribution of the vector, animal behaviour, age range of the studied population²², and management practices and habitat where the animals live²³. Dogs in this study were selected from Ayvalik, Burhaniye, Edremit, Dikili and Bergama regions in Turkey. These regions are under the influence of a typical Mediterranean climate with mild and rainy winters and hot, dry summers. Seroprevalence studies have suggested the presence of *E. canis* or related *Ehrlichia* spp. infecting dogs throughout East Mediterranean and Aegean regions^{3,4,15,16}. In a previous study, seroprevalence of *E. canis* antibodies among dogs in west, south, and south eastern parts of Turkey was 20.8%². In the study², it was reported that five of 38 dogs (13,16%) were seropositive for *E. canis* in Balıkesir, and thirteen of 32 dogs (40,63%) in Izmir city, which are located in Aegean region in Turkey. Acute and chronic phases of CME are well diagnosed and documented, probably due to the fact that dogs suffering from these clinical forms are often ill and presented to veterinary clinics for treatment. Conversely, dogs in the subclinical phase appear healthy, and generally do not show overt clinical pathological changes²¹. In the present study, we found a high seroprevalence of *E. canis* (69.4 %) and our clinical examination results showed that all of these seropositive dogs were healthy. Waner et al.²⁸ noted that the most reliable parameter for judging possible subclinical ehrlichial infection in beagle dogs was a persistent high antibody titer to *E. canis*. In their study, all subclinically infected dogs had IFA antibody titers to *E. canis* at a dilution varying from 1:2560 to 1:20480. In our study, high IFA titers of dogs with subclinical ehrlichiosis was higher than 1:2560 in 36.9% of the dogs tested and in 53.2% of the seropositive ones. In some sera, the antibody titer was found higher than 1/100000. In the present study, the number of dogs with titer equal or higher 1:5120, was consistent with the findings of Codner and Farris-Smith⁷ and other reports^{6,28}, describing a prolonged duration of infection and chronic antigenic stimulation for subclinical infections.

In our study, infection rate in males (69.9%) and females (68.7%) was similar with

the reports by Botros et al.⁴, Baneth et al.³, Watanabe et al.²⁹ and Rodriguez-Vivas et al.²², where no significant sex differences were detected.

Mild thrombocytopenia is an important hematological finding in subclinical phase of CME²⁸. Codner and Farris-Smith⁷ studying naturally infected dogs of various breeds, found that 50% of subclinically infected dogs were thrombocytopenic. Recent studies have demonstrated that its mechanism may have an immune basis related to the overproduction of natural antiplatelet antibodies¹⁰. In our study, the most prominent hematological finding was thrombocytopenia ($p < 0.001$) (80 %).

Leucopenia has been reported in 31% and 25% of dogs with natural acute CME³⁰. Waner et al.²⁸ found leukocyte counts were reduced in 78% of the dogs with subclinical ehrlichiosis compared with their preinfection values. Codner and Farris-Smith⁷ found that 30% of dogs with subclinical CME had an absolute neutropenia. However, in that study, it was shown that none of the dogs were found to be either leukopenic or neutropenic. In our study, it was found that total leukocyte counts of the seropositive dogs were significantly ($p < 0.001$) lower than seronegative dogs' values, although leukocyte counts were in normal limits except in two dogs.

Anemia was observed which is frequently related to CME^{8,24}. However, infected dogs presented mild to moderate hematological changes in acute experimental infection for just a few weeks. The tendency for the hematological parameters to return to normal was evident at the end of the experiment. This result may be a consequence of transient suppression of bone marrow activity due to *E. canis* infection^{5,28} found that none of the experimentally infected subclinical dogs were overtly anemic, although declines in packed cell volume, hemoglobin concentration and total erythrocyte count were detected in an inconsistent manner among the dogs. Similarly, in the present study, none of the dogs was anemic although packed cell volume, hemoglobin concentration and total erythrocyte count were significantly lower in seropositive dogs when compared to seronegative ones. This can be interpreted in a way similar to Waner et al.²⁸ who suggested that erythrocyte parameters are not good indicators to suspect subclinical CME.

Hematological and serological tests prove that subclinical Ehrlichiosis is an incontrovertible

reemergent threat both for other animals and also for people. This is especially important for practicing veterinarians because early diagnosis before the animal enters the chronic phase of the disease, leads to a relatively good prognosis. Subclinical forms should be treated in order to reduce the transition to chronic or acute forms of the disease and to decrease the animal reservoirs.

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