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Monocytic Ehrlichiosis in Aegean Region Dogs: Clinical and Haematological Findings

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Abstract: The objective of the present study was to evaluate clinical and haematological findings among 63 dogs solely diagnosed canine monocytic ehrlichiosis (CME) within IFAT application. For this purpose; age, breed, sex, anti-parasitic drug application, living environment, the presence of tick and general condition of the dogs were noticed, clinical and haematological variables were determined. Fourteen out of 63 (22.22%) dogs were regularly treated with anti-parasitic drugs while other 49 dogs (77.77%) were irregularly treated with anti-parasitic drugs. Forty-two (66.66%) of the dogs were living on garden conditions while 21 (33.33%) of the dogs were living in the house and 36 (57.14%) of the dogs had tick found on clinical referral. The most commonly observed clinical signs were anorexia (87.30%), lymphadenopathy (80.95%), pale mucous membranes (47.61%) and fever (42.85 %). Besides tachypnoea (36.50%), tachyarrhythmia (34.92%), haemorrhagia (22.22%), eye lesions (22.22%), skin lesions (7.93%), neurological signs (3.17%) and arthritis (19.04%) were other observed clinical signs. Thrombocytopenia (63.49%) and anaemia (46.03%) were most commonly detected haematological findings. In conclusion, it was suggested that CME was prevalent in our region and CME must be taken into consideration among dogs presenting aforementioned clinical and haematological findings.

Keywords: *Ehrlichia canis*, Dog, Haematological and clinical findings.

Ege Bölgesindeki Köpeklerde Monositik Ehrlichiosis: Klinik ve Hematolojik Bulgular

Öz: Bu çalışmanın amacı IFAT uygulaması ile sadece köpek monositik ehrlichiosis (KME) tanısı konulmuş 63 köpekteki klinik ve hematolojik bulguların değerlendirilmesidir. Bu amaçla; yaş, ırk, cinsiyet, anti-paraziter ilaç uygulamaları, yaşadığı ortam, kene varlığı ve genel durumla ilgili bilgiler kaydedilip, klinik ve hematolojik değişkenler belirlendi. Altmış üç köpekten 14'ünde (%22.2) anti-paraziter ilaç uygulamaları düzenliyken, 49 (%77.7) köpekte ise anti-paraziter ilaç uygulamaları düzensizdi. Köpeklerden 42'sinin (%66.6) bahçe ortamında, 21'inin (%33.3) ev ortamında yaşadığı ve 36 köpeğin (%57.1) kliniğe getirildiğinde üzerinde kene olduğu belirlendi. En sık gözlenen klinik bulgular; anoreksi (%87.30), lenfadenopati (%80.95), soluk mukoz membranlar (%47.61) ve ateş olarak tespit edilmiştir (%42.85). Ayrıca, Taşipne (%36.50), taşiaritmi (%34.92), hemoraji (%22.22), göz lezyonu (%22.22), deri lezyonu (%7.93), nörolojik bulgular (%3.17) ve arthritis (%19.04) gözlemlenen diğer klinik bulgular olarak belirlendi. Trombositopeni (%63.49) ve anemi (%46.03) en yaygın belirlenen hematolojik bulgular olarak dikkat çekmiştir. Sonuç olarak bölgemizde KME'in yaygın olarak görüldüğü ve yukarda belirtilen klinik ve hematolojik bulguların birlikte görüldüğü köpeklerde KME'inde dikkate alınması gerektiği kanısına varıldı.

Anahtar kelimeler: *Ehrlichia canis,* Hematolojik ve klinik bulgular, Köpek.

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INTRODUCTION

Tick-borne bacteria and parasites are significant pathogens of domestic dogs and potentially are of public health significance. *Ehrlichia canis* (*E.canis*), a Gram-negative obligate intracellular bacterium is the primary causative agent of canine monocytic ehrlichiosis (CME) (1-3). The agent is predominantly transmitted by the brown dog tick Rhipicephalus sanguineus (2,3).

Canine monocytic ehrlichiosis is an important canine disease with a worldwide distribution. CME has been reported throughout the world, with a higher frequency in tropical and subtropical regions (1-3). CME results in a variety of acute, chronic or subclinical syndromes with different phases of the disease course and multiple clinical manifestations. Clinical manifestations of the disease often include fever, pale mucous membranes, depression, anorexia, haemorrhagia, weight loss and ocular signs (2,4,5). Laboratory findings most frequently determined are thrombocytopenia, leukopenia, anaemia, and hypergammaglobulinemia (4,6,7). All breeds may be infected with E. canis. However, the German Shepherd Dogs seems to be more prone to develop clinical CME (4).

The epidemiology of canine ehrlichiosis has been widely reported. However, concerning the clinical and haematological analysis of canine monocytic ehrlichiosis, little is documented in the veterinary literature in Turkey. The objective of this study was to evaluate clinical and haematology findings in Aegean Region for E. canis.

MATERIALS AND METHODS

Animal Design

This study was approved and performed under the guidelines of Ethics Committee for Animal Use of University of Adnan Menderes (B.30.2.ADÜ.0.06.00.00/124-HEK/2006/0058).

Two hundred twenty-four dogs, of various breed and both sexes, admitted to the Department of Internal Medicine, Faculty of Veterinary Medicine, the University of Adnan Menderes for vaccination, clinical examination and therapy applications were enrolled in this study. All of the animals were from the Aegean region of Turkey (Aydın, İzmir, Denizli, Muğla, Manisa) (Figure 1) and all dogs were owned. The age, breed, sex, anti-parasitic drug application, living area, the presence of tick and general condition of the dogs were noticed.

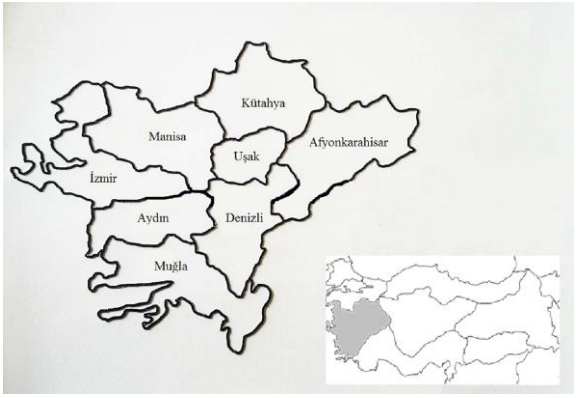

Figure 1. Geographical locations where dogs were sampled.

Şekil 1. Köpeklerin örneklendiği coğrafi lokasyonlar.

A total of 81 dogs were detected in exposed to *E. canis* as determined by indirect fluorescent antibody test (IFAT) application. Among those 81 dogs, only 63 of them were solely infected with *E. canis*. Other 18 dogs co-infected with Leishmania infantum, Babesia canis and Hepatozoon canis were excluded from the study.

Haematology

After the puncture of the vena cephalica antebrachii, blood samples were collected into serum separator, and EDTA-treated glass tubes from above mentioned 224 dogs. Blood cell counts were immediately determined partially from EDTA sampled whole blood. Another part of EDTA-treated

samples was then used for necessary PCR analysis. Serum samples were obtained after clotting (at room temperature for 1 hour) and centrifugation (3000g for 10 minutes at room temperature) then stored at -200 C until analysis.

Haematological examination (Erythrocyte count, haemoglobin concentration, haematocrit, leukocyte and platelet count) were performed using the Abacus Junior Vet haematology cell counter (Diatron MI Ltd, Hungary).

Serology and PCR amplification

IFAT was used to detect *E. canis* IgG antibodies. The latter technique was applied according to the manufacturer's recommendations (VMRD® Pullman, USA). Sera were diluted at 1:100 in saline solution and the used conjugate was a rabbit IgG anti-dog IgG, diluted in 0.01% concentrated Evans Blue (Sigma-Aldrich E0133, Saint Louis, USA). PBS according to the manufacturer's recommendations (8).

Leishmania infantum, *Babesia canis* and *Hepatozoon canis* were detected by IFAT, Polymerase Chain Reaction (PCR), IFAT, respectively, applied as previously described (9,10,11).

Statistical Analysis

Descriptive statistics were used to determine the frequency of the variable occurrences. For this purpose, data were analysed using the SPSS version 19.0 for Windows (Statistical Package for the Social Sciences, IBM SPSS Statistics, Chicago, IL, ABD).

RESULTS

In the present study 63 *E. canis* seropositive dogs, of various breed and age, were analysed throughout clinical and haematological signs. Dogs were evaluated according to the characteristic such as breed, age, sex, living environment, anti-parasitic applications and tick presence (Table 1). Among *E. canis* seropositive dogs most commonly represented breeds were crossbred, followed by Rottweiler (n=7) and Terrier (n=7) (Table 1). Fourteen out of 63 (22.22%) dogs were frequently treated with antiparasitic drugs while other 49 dogs (77.77%) were infrequently treated with antiparasitic drugs (Table 1). Forty-two (66.66%) of the dogs were living on garden conditions while 21 (33.33%) of the dogs were living in the house and only roaming outside for urination and exercise (Table 1). Thirty-six (57.14%) of the dogs had tick found on clinical referral whereas 27 (42.85%) dogs showed no presence of tick (Table 1). Among those dogs 40 (63.49%) were male, and 51 (80.95%) of them were below one year of age (Table 1).

Table 1. Demographic characteristics and risk factors in *E. canis* seropositive 63 dogs.
Tablo 1. *E. canis* seropozitif 63 köpekteki demografik özellikler ve risk faktörleri.

Demographic characteristics and risk factors				
	n	%	Breed	n
Age			Crossbreed	15
< 1	12/63	19.04	Rottweiler	7
≥ 1	51/63	80.95	Terrier	7
			Golden Retriever	6
Sex				
Male	40/63	63.49	Kangal	6
Female	23/63	36.50	German Shepherd	5
			English Pointer	3
Living environment				
House	21/63	33.33	Boxer	3
Garden	42/63	66.66	Cooker	3
			Russian Lapdog	2
Antiparasitic application				
Frequently	14/63	22.22	Chow Chow	1
Infrequently	49/63	77.77	English Setter	1
			Labrador Retriever	1
Presence of tick				
Existent	36/63	57.14	Poodle	1
Nonexistent	27/63	42.85	Siberian Husky	1
			Tibetan Spaniel	1

Apparent clinical signs among 63 naturally *E. canis* infected dogs were presented in Table 2. Fifty-four out of 63 dogs referred to the clinic with at least one of the clinical signs as follows; anorexia, depression, lethargy, weight loss, rhinorrhagia, gum

haemorrhage, lameness, rash, scaling, pruritus and vomiting. Nine other dogs were referred for routine health controls and vaccinations. The most commonly observed clinical signs were Anorexia (55/63, 87.30%), lymphadenopathy (51/63, 80.95%), pale mucous membranes (30/63, 47.61%) and fever (27/63, 42.85%). Besides tachypnoea (23/63, 36.50%), tachyarrhythmia (10/63, 15.87%), haemorrhagia (14/63, 22.22%), eye lesions (14/63, 22.22%), skin lesions (5/63, %7.93), neurological signs (2/63, 3.17%) and arthritis (12/63, 19.04%) other observed clinical signs (Table 2).

Table 2. Clinical and haematological findings in 63 *E. canis* seropositive dogs.
Tablo 2. *E. canis* seropozitif 63 köpekteki klinik ve hematolojik bulgular.

Clinical Signs	n	%
Anorexia	55	87.30
Lymphadenopathy	51	80.95
Pale mucose membranes	30	47.61
Fever	27	42.85
Tachypnoea	23	36.50
Eye lesions	14	22.22
Haemorrhagia	14	22.22
Arthritis	12	19.04
Tachyarrhythmia	10	15.87
Skin lesions	5	7.93
Neurological signs	2	3.17
Haematological findings		
Thrombocytopenia	40	%63.49
Anaemia	29	%46.03
Pancytopenia	14	%22.22
Leucocytosis	9	%14.28
Leukopenia	5	%7.63

Haematological results were presented in Table 2. Thrombocytopenia (40/63, 63.49%) and anaemia (29/63, 46.03%) were most commonly detected haematological analysis results. Besides pancytopenia was observed 14 out of 63 dogs (22.22%) while leucocytosis and leukopenia were determined in 9 (14.28%) and 5 (7.63%) dogs, respectively.

DISCUSSION and CONCLUSION

Ehrlichia canis, the most important species of *Ehrlichia* in canine species, is the etiological agent responsible for canine monocytic ehrlichiosis worldwide. The presence of the disease has been reported in Asia, Africa, Europe and America continents (12-15). Several studies regarding ehrlichiosis have been reported in several parts of Turkey (16-19), in which most of them were seroprevalence studies. Concerning clinical and haematological analysis of canine monocytic ehrlichiosis, little is documented in the veterinary literature especially in Turkey.

To compare clinical features and assess risk factors for canine monocytic ehrlichiosis we enrolled owned cases in which most of them were infected with ticks. Parasitism by *R. sanguineous* is a well-known risk factor for ehrlichiosis (7), and dogs infected with ticks showed a higher risk for seropositivity against canine ehrlichiosis (1,5,7,20). In our study among 63 *E. canis* seropositive dogs, 49 were infrequently medicated with ectoparasitic drugs. In our opinion, infrequent antiparasitic applications result in higher risk for tick exposure. Therefore a total of 36 dogs (57.14%) showed tick presence on referral. Clinical and demographic characteristics were assessed for cases with laboratory-confirmed cases of CME, and risk factors were evaluated. A previous study reported that dogs living outside showed a greater incidence and seropositivity in contrast to dogs living at home (21). In our study among seropositive dogs, 42 were living on garden conditions whereas 21 of them were living at home. We suggest that living outside may cause a greater exposure the ticks, therefore, a higher risk for *E. canis* infection.

Slightly male dogs were more frequently affected than female dogs in the current study. We may suggest that this difference may be due to gender popularity rather than a true predisposition for CME. According to some researchers (12,21), sex predilection has no significant influence on CME. In the present study among *E. canis* seropositive dogs, 63.49% were male, and we showed that male dogs were more prone to *E. canis* infection in contrast to female dogs. All dog breeds may be infected with

CME. Indeed German Shepherd dogs were more prone to this disease (22). In our study, *E. canis* seropositive dogs showed a great breed distribution. The most commonly represented dogs were crossbred which were follow the by Rottweiler and Terrier breed.

The most commonly represented clinical signs in this study showed similarity to those previously reported in CME (3-5,7). Anorexia, lymphadenopathy, pale mucous membranes and fever occurred more frequently in our cases. CME may cause variable and non-specific clinical signs, which makes the diagnosis more challenging. Several factors might cause this condition such as *E. canis* strain pathogenicity, immune status of the host, dose of the infectious organism and stages of the disease (2,13).

Ocular signs may be presented in all clinical phases of CME as accompanying clinical manifestations of this disease, indeed may also be the only presenting complaint (23,24). Eye lesions such as keratitis, uveitis and retinal haemorrhagia were observed in a total of 14 (22.22%) dogs in the present study. Regarding pathogenesis of ocular lesions in association with CME has not been investigated. However, CME is frequently associated with thrombocytopenia and widespread immune complex formation/deposition (23,24). Intraocular inflammation caused by immune-mediated mechanisms and bleeding tendencies due to thrombocytopenia may lead to ocular lesions in CME.

Thrombocytopenia and anaemia most common clinical signs in this study and showed similarity to those previously reported by Rodriguez‐Vivas et al. (13), Borin et al. (25), M'Ghirbi et al. (6), Carlos et al., (7). Thrombocytopenia is observed in the all stage of the disease but in the chronic phase; thrombocytopenia is more severe as a result of bone marrow hypoplasia (2,26). Thrombocytopenia may be due to destruction and consumption of platelets, increased hepatic or splenic platelet sequestration, decreased platelet production following bone marrow hypoplasia and production of antiplatelet antibodies (1,17). Thrombocytopenia was detected in 77% of dogs with ehrlichiosis in a retrospective serological study in the United States of America (27). In Israel, ehrlichiosis is more commonly observed in contrast to the United States of America whereas in a serological study only 27% of dogs had thrombocytopenia (28). In our study thrombocytopenia was observed among 63.49% of *E. canis* seropositive dogs. The varieties among seroprevalence may be due to differences of etiological agent pathogenicity or sampling diversity as reported by Dagnone et al., (20).

The prevalence of anemia among dogs with ehrlichiosis showed varieties such as 41 to 90% (20,26-28). Several mechanisms such as the effects of the mononuclear phagocytic system, suppression of erythropoiesis at the bone marrow and cell lysis mediated by the complement system may lead to anaemia (7). In the present study, anaemia was determined in 46.03% of dogs enrolled. In our opinion, the diversity of the prevalence of anaemia may be related to differences in strain pathogenicity or the phase of the disease. Cell lysis mediated by the complement system and suppression of erythropoiesis at the bone marrow may lead to anaemia (7).

Pancytopenia detected in *Ehrlichia canis* infections may occur in chronic phase due to bone marrow aplasia (29). Mylonakis et al. (30), determined pancytopenia in 17 out of 19 dogs (90%) with chronic ehrlichiosis. In our study pancytopenia was evident in 14 dogs (22.22%), which might be related to the chronic phase of the disease.

In conclusion, taking into consideration of the endemic status of *E. canis* in Aegean region, canine monocytic ehrlichiosis must be on the list of differential diagnosis in dogs referred to the clinic with the presence of tick, anorexia, lymphadenopathy, pale mucous membranes and fever and with laboratory analysis such as thrombocytopenia, anaemia or pancytopenia. Therefore clinically based, detailed laboratory

analysed studies with large dog populations are necessary to understand the pathogenesis of canine monocytic ehrlichiosis better and to provide novel treatment application.

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