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Description of dogs naturally infected with *Hepatozoon canis* in the Aegean region of Turkey

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Abstract: Clinical and laboratory findings recorded in 10 dogs naturally infected with *Hepatozoon canis* in the Aegean region of Turkey were reported. The diagnosis was made by finding *H. canis* gamonts within leucocytes in Giemsa-stained blood smears. *H. canis* parasitaemia level was calculated manually by counting 500 neutrophils in blood smears. Parasitaemia varied from 1% to 23% of the circulating neutrophils. Anorexia, fever, depression, weight loss, and lymphadenopathy are the main clinical signs in infected dogs. All dogs had microcytic normochromic anaemia; however, 7 of the dogs had neutrophilia, 1 had lymphopenia, 2 had monocytosis, 1 had eosinophilia, and 9 had thrombocytopenia. Abnormal serum biochemical values were hyperproteinaemia (7 of 10 dogs), hypoalbuminaemia (9 of 10 dogs), hyperglobulinaemia (7 of 10 dogs), increased serum alkaline phosphatase activity (2 of 10 dogs), and increased serum creatinine kinase activity (8 of 10 dogs). Concurrent infections such as *Ehrlichia canis, Anaplasma phagocytophilum*, and *Anaplasma platys* were detected with a polymerase chain reaction test and 8 dogs had 2 or 3 of these concurrent infections.

Key words: *Hepatozoon canis,* clinico-pathological findings, *Rhipicephalus sanguineus*

Türkiye'de Ege bölgesinde *Hepatozoon canis* ile doğal enfekte köpeklerin tanımlanması

Özet: Bu çalışmada, Türkiye'de Ege bölgesinde *Hepatozoon canis* ile doğal enfekte olan 10 köpekte kaydedilen klinik ve laboratuvar bulguların tanımlanması amaçlanmıştır. *H. canis* enfeksiyonunun tanısı Giemsa ile boyanmış kan frotilerinin mikroskobik bakısında lökositler içerisinde gamontların görülmesi ile konuldu. Hazırlanan kan frotilerde bir alanda 500 nötrofil sayılarak *H. canis*'in parazitemi düzeyi belirlendi. *H. canis* ile enfekte köpeklerin parazitemi düzeyi % 1 ile % 23 arasında değişkenlik gösterdi. *H. canis* ile enfekte köpeklerde en önemli klinik bulgu olarak anoreksi, ateş, depresyon, kilo kaybı ve lenfadenopati belirlendi. Hematolojik muayenede bütün köpeklerde mikrositik hipokromik anemi belirlenirken, 10 köpeğin 7'sinde nötrofili, 1'inde lenfopeni, 2'sinde monositozis, 1'inde eozinofili, 9'unda ise trombositopeni tespit edildi. Biyokimyasal muayenede ise 10 köpeğin 7'sinde hiperproteinemi, 9'unda hipoalbuminemi, 7'sinde hiperglobulinemi, 2'sinde serum alkalen fosfataz aktivitesinde artış, 8'inde ise serum kreatin kinaz aktivitesinde artış belirlendi. *H. canis* ile enfekte 8 köpekte *Ehrlichia canis, Anaplasma phagocytophilum* ve *Anaplasma platys* gibi konkurent enfeksiyonların tanısı polimerize zincir reaksiyon testiyle konuldu. *H. canis* ile enfekte 8 köpekte konkurent enfeksiyonlardan 2 veya 3' ü belirlendi.

Anahtar sözcükler: *Hepatozoon canis,* klinikopatolojik bulgular, *Rhipicephalus sanguineus*

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Introduction

Canine hepatozoonosis is a protozoal disease caused by *Hepatozoon canis* that affects mainly domestic dogs (1). *H. canis* was first described in a dog by Bentley (2) in India, and since then it has been reported in dogs throughout the world (3-7). Studies performed on the prevalence of *H. canis* parasitemia in different regions of world revealed a range from 1% to 39.2% (1,5,8-11). *H. canis* has also been reported in Turkey (11-13). Serologic surveys conducted in Turkey indicated that 36.8% of 349 dogs were seropositive for *H. canis* (11).

The main vector in *H. canis* infection is the brown dog tick *Rhipicephalus sanguineus,* although the infection may also be transmitted by other dog tick species such as *Haemaphysalis longicornus*, *Haemaphysalis flavas* (1,14), and possibly *Amblyomma ovale* (15). The dog becomes infected with the ingestion of a tick containing sporulated oocysts. The ingested sporozoites are then released in the dog's intestinal tract, penetrate the gut wall, and are carried by blood or lymph to various tissues, where merogony occurs. Some merozoites enter neutrophils or monocytes and develop into gametocytes (8,16).

The clinical signs of *H. canis* infection vary depending on age of the host, degree of infection, and the presence of concurrent infections (1). *H. canis* is often found in apparently healthy dogs but occasionally can cause serious problems and death. Fever and emaciation are among the most frequently observed clinical signs (14,17). Other signs such as anorexia, depression, weight loss, pale mucous membranes, and lymphadenopathy have also been indicated in some reports (7,14). Haematological and biochemical abnormalities detected in dogs infected with *H.* canis include anaemia, leucocytosis, thrombocytopenia, hypoalbuminemia, hyper-globulinemia, and increased serum alkaline phosphatase activity (8,14).

Routine diagnosis of *H. canis* is carried out by the detection of gamonts within neutrophils and monocytes in stained peripheral blood smears (17). An indirect fluorescent antibody test (11) and polymerase chain reaction (PCR) (11,18) can also be used for diagnosis.

Although *H. canis* has been demonstrated to exist in Turkey (11-13), the information regarding the pathogenesis and epidemiology of canine hepatozoonosis is rather limited. With this in mind, the aim of the present study was to describe the clinical and laboratory findings in 10 dogs naturally infected with *H.* canis in the Aegean region in Turkey.

Materials and methods

Ten dogs of mongrel breed and of both sexes (3 males and 7 females), aged between 2 and 8 years, naturally infected by *H.* canis, were studied (Table 1). All of the animals were from the Aegean region in Turkey. Each dog was submitted to a rigorous clinical examination to correctly evaluate its health. All the dogs were examined for the presence of ticks. None of the dogs had received any medication before the diagnosis.

Blood samples were taken from the cephalic vein into tubes with and without anticoagulant for identification of haemoparasites, haematological and biochemical analysis, and parasitaemia level of the animals. *H. canis* gamonts were determined by microscopy in blood smears stained with Giemsa (Figure). *H. canis* parasitemia level was calculated manually by counting 500 neutrophils in blood smears. Haematological parameters were determined by an automated blood cell counter (Beckman-Coulter-Gens) and included haematocrit (PCV), haemoglobin (Hb), white blood cell counts (WBC),

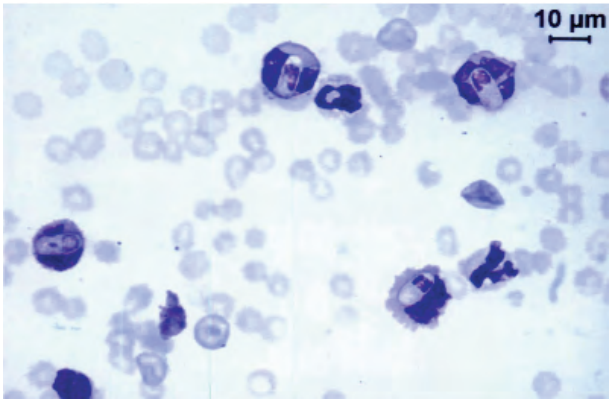

Figure. Gamont of *Hepatozoon canis* in neutrophils from peripheral blood smear.

mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), and platelets (PLT). Differential leucocyte counts (lymphocytes, monocytes, eosinophils, segmented neutrophils, band neutrophils) were carried out by standard methods (19). The serum biochemical analysis including total protein (TP), albumin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine kinase (CK), blood urea nitrogen (BUN), and creatinine was performed with an ILA B_{900} autoanalyser. Plasma globulin concentration was obtained by subtracting the value of albumin from total protein concentration.

The other laboratory procedures included diagnosis of *Ehrlichia canis, Anaplasma phagocytophilum*, and *Anaplasma platys* with PCR, serological screening for *Leishmania infantum* by immunofluorescent antibody test, and diagnosis of *Dirofilaria immitis* infection using a commercial kit (Canine Heartworm Antigen, Idexx). In order to detect the presence of each *Ehrlichia* spp., a nested PCR was performed using DNA extracted from 200 µL of EDTA-anticoagulated whole blood by using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA). A primer set, S8FE (5′- GGA ATT CAG AGT TGG ATC MTG GYT CAG) and B-GA1B- 5′- biotin- CGG GAT CCC GAG TTT GCC GGG ACT TCT), which amplify the 16S rRNA gene (20), was used in the first round of PCR. This was followed by the second round PCR for each *Ehrlichia* species. *E. canis*-specific primer (5′ - CAA TTA TTT ATA GCC TCT GGC TAT AGG A) and an *Ehrlichia* genus-specific primer, HE3, (5′ – TAT AGG TAC CGT CAT TAT CTT CCC TAT) were used for *E. canis* amplifications (21), *A. platys*- specific primer (5′ -AAG TCG AAC GGA TTT TTG TCG TAG CTT) (22) with some modification and HE3 were used for *A. platys* amplifications, and *A. phagocytophilum*-specific primer (5′ -TAG CTT GCT ATA AAG AAT AAT TAG TGG (23) and HE3 were used for *A. phagocytophilum* amplifications. PCR conditions were as described previously (21-23).

Results

The clinical findings recorded in all dogs naturally infected with *H. canis* are summarised in Table 1. Anorexia, fever, weight loss, depression, and lymphadenopathy were recorded in all dogs. Pale mucous membranes were detected in 5 of the 10 dogs, bilateral seromucous ocular discharge were detected in 2, and skin problems such as allopecia, scaling, crust and erosion in 8. Brown dog ticks on the surface of the skin were found in 7 of the 10 dogs. The diagnosis of *H. canis* infection was made by direct observation of the gamonts in circulating leucocytes on blood smears stained with Giemsa. Parasitemia levels ranged between 1% and 23% (Table 1).

Table 1. Description of dogs naturally infected with *H. canis*.

Dog	Breed	Age (years)	Sex	Clinical Signs	Parasitaemia %
1	Mongrel	4	Male	WL, ANOR, DEPR, LA, SP, FEV, PMM,	3.5
2	Mongrel	3	Female	WL, ANOR, DEPR, LA, SP, FEV, PMM,	1
3	Mongrel	8	Female	WL, ANOR, DEPR, LA, SP, FEV,	5
4	Mongrel	4	Female	WL, ANOR, DEPR, LA, SP, FEV	2
5	Mongrel	2	Male	WL, ANOR, DEPR, LA, SP, FEV, PMM, OD	3
6	Mongrel	5	Male	WL, ANOR, DEPR, LA, SP, FEV, PMM, OD	4
7	Mongrel	3	Female	WL, ANOR, DEPR, LA, SP, FEV,	6
8	Mongrel	6	Female	WL, ANOR, DEPR, LA, SP, FEV, PMM, OD	23
9	Mongrel	2	Female	WL, ANOR, DEPR, LA, FEV, PMM	2.5
10	Mongrel	7	Female	WL, ANOR, DEPR, LA, FEV	13

WL, weight loss; ANOR, anorexia; DEPR, depression; LA, lymphadenopathy; SP, skin problems; FEV, fever; PMM, pale mucous membranes; OD, ocular discharge

The haematological findings are shown in Table 2. All dogs had microcytic normochromic anaemia. The differential leucocyte count revealed neutrophilia in 7 of the 10 dogs. Monocytosis was observed in 2 dogs, eosinophilia in 1, lymphopenia in 1, and thrombocytopenia in 9.

The results of the biochemical examination are presented in Table 3. A high concentration of TP was observed in 7 of the 10 dogs. Hypoalbuminemia was observed in 9 dogs and hyperglobulinemia in 7. Increased serum ALP activity was detected in 2 dogs. Serum CK activity was elevated in 8 dogs. Serum AST, BUN, and creatinine concentrations were within the reference ranges in all infected dogs.

Concurrent infections were present in 8 of the dogs infected with *H. canis* (Tables 2 and 3). Other haemoparasites such as *E. canis* (8 of 10 dogs), *A. platys* (6 of 10 dogs), and *A. phagocytophilum* (4 of 10 dogs) were also detected (Tables 2 and 3).

Discussion

Canine hepatozoonosis caused by *H. canis* is a major health problem for canine populations in Africa, Southern Europe, and Asia (26,27). The habitat, environmental conditions, and the presence of ticks are among the essential factors in the development of canine hepatozoonosis (14). In this study, all dogs were living in a dog shelter and 7 dogs had a history of tick infestation. Hepatozoonosis is a chronic disease, and dogs of all ages can be infected (1,8). Gavazza et al. (14) reported that *H. canis* infection was found in dogs from 9 months to 7 years old. Baneth and Weigler (7) found that *H. canis* infection was most prevalent in dogs less than 6 months and in dogs 5 to 10 years old. Our results showed that dogs infected with *H. canis* were aged between 2 and 8 years. It has also been reported that *H. canis* infection can be seen in both sexes (14). However, the results of the present study suggest that females are more prone to *H. canis* infection than males.

Intermittent fever and emaciation are the most frequent clinical signs observed in dogs infected with *H. canis* (8,17). Other clinical signs such as anaemia, anorexia, weight loss, and oculonasal discharge have also been reported in some studies (14,17). The most frequent clinical signs detected in cases of canine hepatozoonozis reported in the present study were anorexia, depression, weight loss, fever, and lymphadenopathy. Alterations of clinical signs in dogs infected with *H. canis* vary depending mainly on age of the host, degree of infection, and association with concurrent infection (1). The type and number of

Table 2. Haematological findings in dogs naturally infected with *H. canis*.

Parameters	Dog number										Reference values
	1 □◊	2	3 □O	4 □◊O	5	6 □O	7 □◊O	8 □◊	9 □O	10 □O	
PCV (%)	26.5	34.2	19.3	34.0	28.9	33.1	36.0	27.1	32.6	36.0	37-55
Hb (g/dL)	9.2	11.6	6.7	11.6	9.8	10.4	12.2	9.1	10.7	12.1	12-18
WBC (/μL)	12.800	12.300	11.600	8.590	6.470	10.900	15.100	13.200	14.200	13.400	6.000-17.000
MCV(fL)	59	51	57	56	58	62	60	60	55	62	66-77
MCHC (g/dL)	34.5	32.8	34.6	34.2	32.3	35.1	33.8	33.6	32.4	32.6	32-36
Platelets (10^3/μL)	21	114	33	45	30	82	275	40	65	141	175-400
Lymphocytes (/μL)	390	2.706	1.508	1.546	2.459	3.488	3.322	1.320	1.420	4.556	1.000-4.800
Monocytes (/μL)	1.400	1.476	464	258	194	327	755	528	852	402	150-1.350
Eosinophils (/μL)	250	492	580	687	453	436	604	396	994	670	100-750
Segmented neutrophils (/μL)	10.250	7.134	7.888	5.584	3.235	6.540	9.815	9.768	9.372	7.504	3.000-11.500
Band neutrophils (/μL)	510	492	1.160	515	129	109	604	1.188	1.562	268	0-300

Reference values according to the reports by Duncan and Prasse (24), Morgan (25)
□: mixed infection with *Ehrlichia canis*, ◊: mixed infection with *Anaplasma phagocytophilium*, O: mixed infection with *Anaplasma platys*

Table 3. Biochemical findings in dogs naturally infected with *H. canis*.

Parameters	Dog number										Reference values
	1 □◊	2	3 □O	4 □◊O	5	6 □O	7 □◊O	8 □◊	9 □O	10 □O	
TP (g/dL)	5.7	6.7	3.6	8.0	8.9	8.7	8.9	7.4	7.6	7.2	5.0-7.1
Albumin (g/dL)	1.4	1.7	1.0	2.5	2.1	2.4	2.4	2.4	2.3	2.8	2.8-4.0
Globulin (g/dL)	4.3	5.0	2.6	5.5	6.8	6.3	6.5	5.0	5.3	4.4	3.0-4.7
AST (U/I)	33	25	33	35	16	39	32	28	32	38	10-50
ALP (U/I)	184	25	80	173	40	40	35	42	74	55	20-150
CK (U/I)	209	216	514	379	91	296	255	83	267	304	30-120
BUN (mg/dL)	16	11	14	14	18	13	10	12	17	11	10-25
Creatinine (mg/dL)	0.6	0.7	0.6	0.6	0.9	0.7	0.8	0.7	0.8	0.8	0.6-2

Reference values according to the reports by Duncan and Prasse (24), Morgan (25)
□: mixed infection with *Ehrlichia canis*, ◊: mixed infection with *Anaplasma phagocytophilium*, O: mixed infection with *Anaplasma platys*

clinical signs in dogs with *H. canis* were also different in this study. Some investigators (3) have considered *H. canis* to be non-pathogenic, and attributed clinical signs of infected dogs to other causes such as ehrlichiosis, leishmaniasis, anaplasmosis, babesiosis, or generalised demodicosis (14,17,28). In a previous study, 4 dogs naturally infected with *H. canis* had concurrent infection, which is most likely to affect the severity of the pathology observed (14). In the present study, 8 of the 10 dogs infected with *H. canis* had 2 or three of the concurrent infections (8 dogs had *E. canis*, 6 dogs had *A. platys*, and 4 dogs had *A. phagocytophilum*). Therefore, clinical signs were mainly associated with primary *H. canis* infection in 2 dogs. Clinical signs reported in these 2 cases are in agreement with those reported in the literature (13,14,17).

Hematologic findings reported in this study indicated that all of the dogs had microcytic normochromic anaemia. This may be a consequence of iron deficiency caused by chronic blood loss (29). It should be noted, however, that serum iron concentrations were not measured in the present study. It has been reported that leucocytosis with neutrophilia is observed in dogs infected with *H. canis* (7,30). In this study, none of the dogs had neutrophilic leucocytosis. This finding is in agreement with the observations reported by Elias and Homans (17), who found that dogs did not have neutrophilic leucocytosis. Concurrent infections may have inhibited the haematological response. Lymphocyte counts were low in one dog infected with *H. canis*. This is in agreement with previous observations (30). Moreover, in contrast to another report (14), lymphocytosis was not found in any of the dogs. In this study, monocyte counts were elevated in 2 dogs. This was related to *H. canis* infection. This finding is in agreement with the observations reported by Gavazza et al. (14). Eosinophilia was observed in 1 dog infected with *H. canis*. This is in agreement with previous observations (14). Thrombocytopenia was also detected in 9 dogs infected with *H. canis*. The mechanism of thrombocytopenia associated with *H. canis* is not well understood, but it may well be the result of general causes of thrombocytopenia (19) or may be related to the presence of concurrent infections (14).

Serum biochemical abnormalities include hyperproteinemia, hypoalbuminemia, hyperglobulinemia, increased ALP activity, and increased serum CK activity. These findings are in agreement with previous observations (7,19). Elevated serum TP concentration has been reported in dogs with *H. canis* (7). In this study, serum TP concentration was elevated in 7 dogs. This may be due to increased production of globuins and/or a decrease in albumin concentration (7). The results of the present study also revealed that

most of the dogs (9/10) with *H. canis* had hypoalbuminemia. This might be due to a chronic inflammatory disease, anorexia, or decreased protein intake (14). Hyperglobulinemia reported in the present study (7 out of the 10 dogs) can probably be attributed to a chronic state of the disease (15). Elevated serum ALP activity has been reported in dogs with *H. canis* because of increased osteoblastic activity (7). In this study, serum ALP activity was elevated in 2 dogs. An increase in serum CK activity was determined in 8 dogs, which could be related to recumbency owing to lethargy (7).

Our results showed that the presence of *H. canis* parasitaemia ranged from 1% to 23%. The level of parasitaemia determined in all dogs in the present study is in close agreement with some *H. canis* cases reported in other countries (5,8,31). However, Gondim et al. (30) indicated that the parasitaemia of the infected dogs was lower than 0.5%. According to Baneth and Weigler (7), animals with high parasitemia have more severe clinical manifestations of infection. Further studies are required to determine the pathogenesis and relationship between levels of *H. canis* parasitaemia and severity of the disease.

Concurrent infections involving *H. canis* and other canine pathogens such as *E. canis, Babesia canis, Leishmania infantum*, and *A. phagocytophilum* have been reported in different countries (10,14,28,31). Concurrent infections with *H. canis* and other pathogens may be explained by several mechanisms. As *Rhipicephalus sanguineus* is the common vector of *H. canis* and *E. canis*, *H. canis* may increase the susceptibility to or promote the clinical manifestations of other diseases. Immunosuppression caused by a concurrent infection may predispose to *H. canis* infection or allow manifestation of a subclinical infection (14). In this study, 8 of the 10 dogs were confirmed to have concurrent infections (8 had *E. canis*, 6 had *A. platys*, and 4 had *A. phagocytophilum*).

In conclusion, the results of the present study indicate that *H. canis* infection should be considered in the differential diagnosis when the existence of ticks or a history of tick infestation is recorded. Clinical and laboratory findings in this study could be useful for monitoring the clinical response of the disease in treated animals and for improving the epidemiological approach in endemic areas.

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