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Research Article

Evaluation of Clinical and Haematological Findings of Mono- and Co-Infection with Hepatozoon canis in Dogs

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ABSTRACT

Hepatozoon canis (*H.canis*) which is transmitted by *Rhipicephalus sanguineus*, is the most common cause of canine hepatozoonosis in Europe, Asia, Africa and Latin America. The objective of this study was to evaluate risk factors and clinical signs in 32 *H. canis* infected dogs (16 dogs with *H. canis* mono-infection, 15 dogs with *H. canis and Ehrlichia canis* co-infection and one dog with *H. canis and Babesia canis co-infection)* and compare the haematological findings between the groups. For this purpose, signalment, anti-parasitic drug application, living environment, the presence of tick and general condition of the dogs were noticed, clinical and haematological variables were determined. Nine out of 16 (56.25%) mono infected dogs and ten out of 16 (62.5%) co-infected dogs were living in the house. Nine out of 16 (56.25%) mono-infected and 12 out of 16 (75%) co-infected dogs were unfrequently treated with anti-parasitic drugs. Consequently, 4 out of 16 (25%) mono- and 9 out of 16 (56.25%) co-infected dogs had tick infestation on clinical examination. In both groups, the most common clinical signs included Inappatence (93,75%), lymphadenopathy (59,38%), tachypnoea (53,13%) and fever (50%). Thrombocytopenia (84,38%) and anaemia (56,25%) were the most frequent haematological abnormalities. There were no significant differences in the haematological variables between the groups. In conclusion, H. canis mono and co-infection should be considered in the diagnosis of the dogs presenting inappetence, lymphadenopathy, tachypnea, fever, thrombocytopenia and anaemia with previous tick exposure.

Keywords: H. canis, mono- and co-infection, clinical and haematological findings

Hepatozoon canis ile Mono ve Ko-Enfeksiyonlu Köpeklerde Klinik ve Hematolojik Bulgularının Değerlendirilmesi

ÖZET

Rhipicephalus sanguineus tarafından bulaştırılan *Hepatozoon canis (H. canis),* Avrupa, Asya, Afrika ve Latin Amerika'daki köpeklerde hepatozoonosis'in en yaygın nedenidir. Bu çalışmada, *H. canis* ile enfekte 32 köpekte (*H. Canis* ile mono enfekte 16 köpek, *H. canis* ve *Ehrlichia canis* ile ko-enfekte 15 köpek ve *H. Canis* ve *Babesia canis* ile ko-enfekte bir köpek) risk faktörleri ve klinik bulguların değerlendirilmesi ve gruplar arasında hematolojik bulguların karşılaştırılması amaçlanmaktadır. Bu amaçla, köpeklerin eşkâlleri, anti-paraziter ilaç uygulamaları, yaşadıkları ortam, kene varlığı ve genel durumları not edilmiş, klinik ve hematolojik değişkenler belirlenmiştir. Mono-enfekte 16 köpekten 9'u (%56,25) ve ko-enfekte 16 köpekten 10'u (% 62,5) ev ortamında yaşıyordu. Mono-enfekte 16 köpekten dokuzu (%56.25) ve ko-enfekte 16 köpekten 12'sinin (%75) anti-paraziter ilaç uygulamaları düzensizdi ve buna bağlı olarak, 16 mono enfekte köpeğin 4'ünde (% 25) ve 16 ko-enfekte köpeğin 9'unda (% 56.25) klinik muayene sırasunda kene görüldü. Her iki grupta da en sık görülen klinik bulgular iştahsızlık (% 93,75), lenfadenopati (% 59,38), taşipne (% 53,13) ve ateş (% 50) olarak tespit edildi. Trombositopeni (% 84,38) ve anemi (% 56,25) en yaygın görülen hematolojik anormalliklerdi ve gruplar arasında hematolojik değişkenler açısından anlamlı bir fark bulunmadı. Sonuç olarak, daha önce keneye maruz kalmış, iştahsızlık, lenfadenopati, taşipne, ateş, trombositopeni ve anemi görülen köpeklerin tanısında *H. canis* mono ve koenfeksiyonlarının da dikkate alınması gerektiği kanısına varıldı..

Anahtar Kelimeler: H. canis, mono- ve ko-enfeksiyon, klinik ve hematolojik bulgular

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Introduction

Hepatozoon species are apicomplexan parasites from the family of Hepatozoidae and are phylogenetically closely related to the piroplasms and haemosporinids (Ivanov & Tsachev, 2008; Baneth, 2011; Aydin et al., 2015) Two *Hepatozoon* species have been identified in dogs: *Hepatozoon Canis* (*H. canis*) and *Hepatozoon americanum* (*H. americanum*) (Gavazza et al., 2003; Little et al., 2009; Baneth, 2011; Aydin et al., 2015; Senthil et al., 2015). While *H. canis* is mainly transmitted by *Rhipicephalus sanguineus*, *H. americanum* is transmitted by *Amblyoma maculatum* (Baneth, 2011; Paşa et al., 2011; Aktas et al., 2015). *H. canis* is the most common species associated with canine hepatozoonosis in Europe, Asia, Africa and Latin America. (Baneth & Vincent-Johnson, 2005; Karagenc et al., 2006; Littleet al., 2009; Baneth, 2011; Aktas 2014; Kaewkong et al., 2014; Aydin et al., 2015). Tüzdil (1933) was firstly described canine hepatozoonosis in Turkey, and then the small number of epidemiological surveys in the different region of Turkey was published (Voyvoda et al., 2004; Karagenc et al., 2006; Pasa et al., 2009; Aktas et al., 2013; Aktas et al., 2015; Aydin et al., 2015). In these studies, the prevalence of canine hepatozoonosis caused by *H. canis* ranged from 3.61 % to 36.8 % by the different methods.

Hepatozoonozis in dogs may be asymptomatic or display a severe, life-threatening illness, with fever, lethargy, weight loss, lymphadenomegaly, and anaemia in varying combinations, depending on the level of parasitaemia (Baneth et al., 1995, Baneth and Weigler 1997, Harrus et al., 1997; Moreira et al., 2003). The severe clinical signs are characteristic for high parasitaemia reaching 100% and often, is associated with marked leukocytosis (up to150,000 /µl) whereas the low parasitaemia with gamonts in less than 5% of neutrophils is generally related to asymptomatic or mild illness (Baneth et al., 1995, 1997). Furthermore, co-infections with other vector-borne pathogens (VBPs) or intrinsic factors specific to the host (age, breed, physical condition, immune status, or stress) may contribute to a more severe expression of the disease by impairing the host immune responses (Baneth et al., 1998, 2001). In this regard, co-infections of *H. canis* with *E. canis* (Baneth and Weigler, 1997), *B. canis* (Cardoso et al., 2010) and *L. İnfantum* (Rioux et al., 1964) are described. In endemic areas, canine vector-borne disease-causing pathogens may infect the same dog with three (*H. canis, Babesia spp., E. canis*) [Karagenc et al., 2006; Sasanelli et al., 2009] or even four agents (*H. canis, Babesia spp., E. canis, L. infantum*) [Otranto et al., 2010]. In this content, *H.canis* and *E. canis* are two of the most common and widely distributed canine haemopatogens. Anaemia and leucocytosis with neutrophilia are the most common blood abnormalities in dogs that have been naturally or experimentally infected with *H. canis* (M'ghirbi et al., 2009).

Veterinarians easily misdiagnose *hepatozoon canis* infection as general symptoms are similar to those seen in other tick-borne diseases such as ehrlichiosis and babesiosis (McCully et al., 1975; Murata et al., 1991, Spolidorio et al., 2011). Additionally, scarce information is available regarding the implication of co-infections with other vector-borne pathogens. The purposes of the study were therefore to evaluate risk factors and clinico-haematological findings in IFAT-confirmed naturally H. *canis*-infected dogs around the Aegean Region, Turkey, and to assess the potential impact of co-infections with other vector-borne pathogens.

Table 1. Breeds distribution of *H.canis* mono- and co-infected dogs

Total		Mono-infected Dogs		Co-infected Dogs	
Breed	**n**	**Breed**	**n**	**Breed**	**n**
Crossbreed	8	Crossbreed	4	Crossbreed	4
Terrier	5	German Shepherd	2	Terrier	4
Golden Retriever	3	Boxer	2	Golden Retriever	2
German Shepherd	3	Terrier	1	German Shepherd	1
Boxer	2	Golden Retriever	1	Cocker Spaniel	1
Cocker Spaniel	2	Cocker Spaniel	1	Doberman	1
Pekinese	1	Bull Terrier	1	Kangal	1
Bull Terrier	1	Basset Hound	1	Pekinese	1
Basset Hound	1	Rottweiler	1	Siberian husky	1
Rottweiler	1	Dogo Argentino	1		
Dogo Argentino	1	King Charles	1		
King Charles	1				
Kangal	1				
Siberian husky	1				
Doberman	1				

Materials And Methods

The investigation was carried out on 32 *H. canis* infected dogs of both sexes, different breeds and ages. All of the animals were from the Aegean region of Turkey (Aydın, İzmir, Denizli,

Table 2. Risk factors of *H.canis* mono- and co-infected dogs

Risk factors	Total	Mono-infected	Co-infected
Age			
< 1	1/32 (3.13 %)	0/16 (0%)	1/16 (6.25%)
≥ 1	31/32 (96.88 %)	16/16 (100%)	15/16 (93.75%)
Sex			
Male	17/32 (53.13 %)	9/16 (56.25%)	8/16 (50%)
Female	15/32 (46.88 %)	7/16 (43.75%)	8/16 (50%)
Residing conditions			
House	19/32 (59.38 %)	9/16 (56.25%)	10/16 (62.50%)
Garden	13/32 (40.63 %)	7/16 (43.75%)	6/16 (37.5%)
Anti-parasitic Application			
Frequently	11/32 (34.38%)	7/16 (43.75%)	4/16 (25%)
Infrequently	21/32 (65.63%)	9/16 (56.25%)	12/16 (75%)
Presence of tick			
Existent	13/32 (43.75%)	4/16 (25%)	9/16 (62.5%)
Nonexistent	18/32 (56.25%)	12/16 (75%)	7/16 (43.75%)

Muğla, Manisa) and all dogs were owned. The dogs were admitted to the Department of İnternal Medicine, Faculty of Veterinary Medicine, the University of Adnan Menderes for vaccination, clinical examination and therapy applications. As being informed by the owner's age, breed, sex, anti-parasitic drug application, residing conditions, presence of tick and general condition of the dogs were recorded. All animals were examined for general clinical investigation and all data were recorded.

Blood samples for haematological, serological and molecular analysis were obtained by cephalic venipuncture into tubes with and without anticoagulant. Anti-coagulated bloods were analysed shortly after collection for haematological analysis. Haematological analysis (erythrocyte count, haemoglobin concentration, haematocrit, leucocyte and platelet count) were performed using the Abacus Junior Vet haematology cell counter (Diatron MI Ltd, Hungary).

Serology of *H. canis* was performed by use of the indirect fluorescent antibody test (IFAT) (Shkap et al., 1994; Baneth et al., 1998). Briefly sera were diluted at 1:32 as the cut off titre for IgG seropositivity determined previously (Shkap et al., 1994, Baneth et al., 1998). Rabbit-antidog IgG fluorescein conjugate (Sigma–Aldrich Biotechnology LP) was used at 1.100 dilution.

IFAT was used to detect *E. canis* IgG antibodies. The latter technique was applied according to the manufacturer's recommendations (VMRD®, Inc.). 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 E0133) PBS according to the manufacturer's recommendations (Karagenç et al., 2005)

The other laboratory procedures included the diagnosis of *Babesia canis* with PCR (Kırlı, 2006), serological screening for *Leishmania infantum* (*L. infantum*) by immunofluorescent antibody test (Abranches et al., 1991).

Statistical analysis was performed using SPSS version 19.0 for Windows (SPSS, Armonk, NY: IBM Corp). Independent-samples T-test was used to compare haematology parameters between mono- and co-infected dogs. Chi-squared tests (x^2) were conducted to examine whether the dogs' breed, sex, age, residing conditions, ant parasitic application and presence of tick were associated with mono- and co-infected dogs. $P < 0.05$ was considered significant. Results were given as mean ± standard deviation.

Results

Out of 32 dogs, 16 dogs were mono-infected and 16 dogs were co-infected (co-infection of 15 dogs with *H. canis* and *E. canis* and one dog with *H. canis* and *B. canis*).

The most commonly represented breed was crossbreed (n=8), followed by Terrier (n=5) and Golden Retriever (n =3) (Table 1). Dogs were evaluated against risk factors such as age, sex, residing conditions, tick presence, and anti-parasitic applications (Table 2).

Seventeen dogs (53.13%) were male and 15 dogs (46.87%) were female. Only one dog was smaller than 1-year-old and it was also co-infected with *B. canis*. Nine (56.25%) mono infected dogs and 10 (62.5%) co-infected dogs were living in the house. Nine out of 16 (56.25%) mono-infected and 12 out of

Table 3. Clinical signs *of H.canis* mono- and co-infected dogs

Clinical Signs	Total	Mono-infected	Co-infected
Inappetence	30/32 (93.75%)	15/16 (93.75%)	15/16 (93.75%)
Lymphadenopathy	19/32 (59.38%)	9/16 (56.25%)	10/16 (62.5%)
Tachypnoea	17/32 (53.13%)	10/16 (62.50%)	7/16 (43.75%)
Fever	16/32 (50%)	9/16 (56.25%)	7/16 (43.75%)
Tachyarythmia	10/32 (31.25%)	10/16 (62.50%)	7/16 (43.75%)
Pale mucose membranes	9/32 (28.13%)	7/16 (43.7%)	2/16 12.50(%)
Arthritis	6/32 (18.75%)	2/16 (12.5%)	4/16 (25%)
Eye lesions	5/32 (15.63%)	3/16 (18.75%)	2/16 (12.50%)
Skin lesions	4/32 (12.5%)	3/16 (18.75%)	1/16 (6.25%)
Haemorrhage	3/32 (9.38%)	2/16 (12.5%)	1/16 (6.25%)
Neurological signs	1/32 (3.13%)	1/16 (6.25%)	0/16 (0%)
Haematuria	1/32 (3.13%)	0/16 (0%)	1/16 (6.25%)

16 (75%) co-infected dogs were unfrequently treated with anti-parasitic drugs. Consequently, 4 (25%) mono- and 9 (62.50%) co-infected dogs had tick infestation on clinical examination (Table 2).

The data about clinical signs are summarised in Table 3. In both groups, the most common clinical signs included inappetence (93.75%), lymphadenopathy (59.38%), tachypnea (53.13%), fever (50%), tachyarrhythmia (31.25%), pale mucose membranes (28.13%). Other findings were observed arthritis, eye lesions, skin lesions, haemorrhage, neurological signs and haematuria.

Haematological variables and Haematological abnormalities of *H. canis* mono- and co-infected dogs are shown in Table 4 and Table 5, respectively. Thrombocytopenia (84.38%) and anaemia (56.25%) were the most frequent haematological abnormalities in both of the groups and there were no significant differences in the haematological variables between the groups ($p>0.05$). Other haematological findings were leucocytosis (25%), leukopenia (25%) and pancytopenia (21.88%).

Discussion

Hepatozoon infection in the dog, caused by *H. canis* is widely spread in Europe, Asia, Africa and Latin America (Ivanov & Tsachev, 2008; Baneth 2011) and presence of this disease has been reported in various regions of Turkey (Voyvoda et al. 2004; Karagenç et al. 2006; Paşa et al. 2009; Aktaş et al., 2013; Aktaş et al., 2015; Aydin et al., 2015).

Habitat, environmental conditions and epidemiological factors, such as the presence of the vector are essential factors in the development of *H. canis* infection (Craig, 1990; Gavazza et al. 2003; Baneth & Vincent- Johnson, 2005; Paşa et al., 2011). *Rhipicephalus sanguineus* is widely distributed in the world, but it is mainly in tropical and subtropical regions and also well adapted to the indoor environment where owned dogs are kept (Uspensku & Ioffe-Uspensky, 2002; Dantas-Torres, 2010; M Ansari-Mood et al., 2015). In this study 21 dogs (% 65.625) were infrequently treated with ectoparasitic drugs, and as a result of this situation, 43.75 % of dogs infected ticks. Co-infected dogs (56.50%) were exposed to ticks more than mono-infected dogs (25%). Co-infection with other hematozoa can be attributed to the presence of the common tick vector, *Rhipicephalus sanguineus,* which is also a transmitter of *E. canis* and *B. canis* (Gondim et al., 1998; O'Dwyer et al., 2001; Mundim et al., 2008). Several studies indicate that a small percentage of ticks are responsible for harbouring multiple pathogens and successfully transmitting all pathogens to host (Kaur et al., 2011, Chhabra et al., 2013).

Although male dogs were slightly more affected than females by canine hepatozoonosis, gender differences were not significant in the present study. This situation is consistent with previous reports of no correlation of gender with the presence of infection (Gomes et al., 2010, Aktaş et al., 2015). Furthermore, no significant difference was found between mono- and co-infected dogs concerning gender in our study.

Clinical findings of dogs with *H. canis* vary from mild to severe signs, depending on the parasitemia and the dog immune status. Dogs with a low parasitemia may be normal or show only mild clinical signs, whereas more severe clinical signs including fever, lethargy and emaciation are noted with high parasitemia. Most frequently observed clinical signs in both groups were inappetence, lymphadenopathy, tachypnea, fever, tachyarrhythmia, pale mucose membranes (Table 3). These findings showed similarity to other researchers (Paşa et al., 2009; Chhabra et al., 2013). Some investigators reported that *H. canis* to be non-pathogenic and attributed clinical signs of infected dogs to other causes such as ehrlichiosis, leishmaniasis or babesiosis, (McCully et al., 1975; Banrth et al., 2003; Gavazza et al., 2003; Mylonakis et al., 2004; Voyvoda et al., 2004; Paşa et al., 2011). In contrast these report, clinical findings in 16 mono infected dogs in this study were associated with primary H. canis infection. None of the signs was attributable to concurrent

Table 4. Haematological variables of *H. canis* mono- and co-infected dogs([a] Raskin & Wardrop, 2010)

Parameters	Mono	Dual	Referances Value
WBC (×10³ cells/µL)	4.73-24.40 13,00±6.49	4.48-28.67 12,04±7.07	6-17[a]
RBC (×10⁶ cells/µL)	1.40-10.90 5,69±2,37	4.25-7.89 5,56±1,02	5.5-8.5[a]
Hct (%)	10.60-59.30 36,72±14,24	27.70-45.76 35,82±6,31	37-55[a]
PLT (x10³/µL)	0-322 121.63±98.67	0-366 108.88±107.94	200-500[a]

Table 5. Haematological abnormalities of *H. canis* mono- and co-infected dogs

Haematological results	Total	Mono-infected	Co-infected
Thrombocytopenia	27/32 (84.38%)	14/16 (87.5%)	13/16 (81.25%)
Anaemia	18/32 (56.25%)	8/16 (50%)	10/16 (62.5%)
Leucocytosis	8/32 (25%)	5/16 (31.25%)	3/16 (18.75%)
Leukopenia	8/32 (25%)	4/16 (25%)	4/16 (25%)
Pancytopenia	7/32 (21.88%)	3/16 (18.8%)	3/16 (18.75%)

disease states, and we were observed similar clinical signs in both groups.

The most common haematological abnormalities was thrombocytopenia in both groups (Table 5). In co-infected dogs, thrombocytopenia can be caused by *E. canis* infection which thrombocytopenia is most common hematologic findings in each stage of the disease (Tuna & Ulutaş, 2009; Carlos, et al., 2011; Maazi et al., 2014). Thrombocytopenia that is observed in *E. canis* infection 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 (Woody & Hoskins, 1991; Gaunt et al., 2010; Maazi et al., 2014) Mechanisms of thrombocytopenia in *H. canis* mono-infected dogs are not well understood, but it may be the result of general causes of thrombocytopenia. There was no significant difference found between mono- and co-infected dogs concerning thrombocyte count in current study. Some authors report that common haematological abnormality in *H. canis* infected dogs with or without concurrent infection; have been anaemia (Baneth &Weigler, 1997; Gondim et al., 1998; Kontos and Koutinas, 1991; O'Dwyer et al., 2006, Mundim et al., 2008; Marchetti et al., 2009, Baneth, 2011, O'Dwyer, 2011). Similar to these reports, anaemia also was common hematologic findings in our study. The leucocyte count is usually normal or increased in *H. canis*-infected dogs (Gaunt et al., 1983; Baneth, 2006, Miyama et al., 2011). The alterations in leukocyte count may be due to the parasite's invasion and multiplication of the parasite in animal's organ, leading to an inflammatory response exacerbated by secondary bacterial infections. Gaunt et al. (1983) reported that anaemia and neutrophilia are probably secondary to necrosis and inflammation of the spleen, lymph nodes, liver and lungs.

These results may provide important information about risk factors clinical and haematological abnormalities in *H.canis* mono- and co-infected Dogs. *H. canis* infection should be considered in tick existence and/or a history of tick infestation. In dogs with thrombocytopenia and anaemia, *H. canis* infection should also be taken into consideration.

Conflict of interest

The authors declare that they have no competing interests.

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