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RESEARCH ARTICLE

Serological Investigation of Some Vector-Borne Parasitic and Rickettsial Agents in Dogs in the Western Part of Turkey

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ABSTRACT

Many infectious pathogens are transmitted to dogs by vector arthropods. Some of the most well-known agents causing canine vector-borne diseases (CVBDs) are *Anaplasma* spp., *Ehrlichia canis*, *Leishmania infantum* and *Dirofilaria immitis*. These rickettsial and parasitic agents pose a public health risk due to their zoonotic significance. This study was planned to determine the prevalence of *Anaplasma* spp., *E. canis*, *L. infantum* and *D. immitis* in dogs in the western part of Turkey. For this purpose, blood samples were collected from 248 dogs brought to private veterinary clinics with non-specific clinical symptoms such as anaemia, anorexia, fatigue, weight loss and loss of appetite. By using an immunochromatographic immunoassay, the presences of *D. immitis* antigens, and the anti-*E. canis*, anti-*L. infantum* and anti- *A. platys*/*A. phagocytophilum* antibodies were investigated, and the total prevalence of these vector-borne pathogens was determined as 37.1% (n:92). The prevalences of *D. immitis*, *E. canis*, *L. infantum* and *Anaplasma* spp. were determined as 1.2, 19.8, 14.9 and 8.5%, respectively. Dual co-infection rate by *E. canis*+*L. infantum* and *E. canis*+*Anaplasma spp.* were detected as 6.5%. A triple co-infection case caused by *D. immitis*, *E. canis*, and *Anaplasma* spp. was also detected in a dog from Aydın province. This study demonstrates the common presence of parasitic and rickettsial pathogens causing CVBDs in dogs in the western part of Turkey. Therefore, preventive measures against infection-transmitting arthropod vectors are recommended for animal welfare and the public health perspective.

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INTRODUCTION

Vector-borne diseases (VBDs) are widely distributed in tropical and subtropical zone countries, including Turkey (Inci *et al*., 2016). The epidemiology of VBDs is influenced by various factors such as climatic changes and extreme mobility of animal populations. Constant spread of VBDs increases the significance of these diseases for veterinary and human medicine (Beugnet and Marie, 2009; Ali *et al*., 2020a). Almost one million human deaths have annually occurred due to VBDs, and the close relationship between pet animals and humans increase the importance of VBDs in the veterinary field (Otrando, 2018; Peter, 2020).

Canine vector-borne diseases represent a large group of VBDs in animals, and they are caused by bacterial, parasitic, rickettsial and viral pathogens transmitted mainly by haematophagous arthropods such as fleas, lice, black flies, ticks, mosquitoes, phlebotomine sand flies, kissing bugs. Ticks and mosquitoes are the primary vectors of these diseases (Parola *et al*., 2005; Otrando, 2018). Anaplasmosis, bartonellosis, babesiosis, borreliosis, dirofilariasis, ehrlichiosis, rickettsiosis, leishmaniosis and thelaziosis constitute a VBDs complex in dogs. Most of these diseases cause life-threatening clinical symptoms in dogs and affect human beings (Baneth *et al*., 2012; Mahmood *et al*., 2020).

The incidence of CVBDs is increasing depending on the factors influencing the epidemiology of these diseases in Turkey. Anaplasmosis, dirofilariasis, ehrlichiosis and leishmaniosis are some of the most common diseases of dogs in Turkey (Inci *et al*., 2018; Duzlu *et al*., 2020).

Dirofilariasis is a nematode infection, and the etiological agents of the infection are *Dirofilaria immitis* (*D. immitis*), *D. repens* and *D. tenuis*. Mosquitoes play a significant role in the transmission of dirofilariasis (Inci *et al*., 2018). The primary tick-transmitted pathogens, *Anaplasma* and *Ehrlichia* species belong to the Anaplasmataceae family, also cause infections in dogs. *Anaplasma platys*, *A. phagocytophilum* and *Ehrlichia canis* cause infections, namely canine cyclic thrombocytopenia, canine granulocytic anaplasmosis and canine monocytic ehrlichiosis in dogs, respectively (Saito and Walker, 2016; Ceylan *et al*., 2021). *Leishmania infantum* is the primary etiological agent of canine leishmaniosis, and phlebotomine sand flies are the vectors of this protozoan infection (Ribeiro *et al*., 2018). There has been an increase in the number of investigations aimed to determine the prevalence of all these diseases mentioned above in recent years in Turkey (Duzlu *et al*., 2020; Ceylan *et al*., 2021).

Serological studies are of significance in terms of obtaining data on the epidemiology of diseases and taking necessary precautions against diseases. This study was conducted to determine the serological prevalences of *A. platys*/*A. phagocytophilum*, *D. immitis*, *E. canis* and *L. infantum* in dog blood samples collected especially from various provinces located in the Aegean and Mediterranean coastline in the western part of Turkey.

MATERIALS AND METHODS

Study area and collection of blood samples: The study material consisted of 248 owned dog blood samples collected from various western provinces of Turkey between March and June 2021. Blood samples were taken from dogs brought to private veterinary clinics in Adana, Ankara, Antalya, Aydın, Balıkesir, Bursa, Denizli, İstanbul, İzmir, Kocaeli, Muğla and Sakarya provinces of Turkey. Anorexia, anaemia, fatigue, weight loss and loss of appetite were the main clinical signs of sampled dogs. However, fever, jaundice and incoordination were rarely detected as well. In the study, 128 female and 120 male dogs aged between 2 months and 14 years (56 of dogs <2 years-old, 192 of dogs >2 years-old) were sampled from many breeds including American Bully (n:1), Beagle (n:1), Belgian Shepherd (n:1), Border Collie (n:1), Chihuahua (n:1), Doberman (n:2), Dogo Argentino (n:1), English Pointer (n:1), French Bulldog (n:1), German Hunting Terrier (n:1), German Shepherd (n:3), Golden Retriever (n:12), Husky (n:1), Jack Russell Terrier (n:1), Kangal (n:11), King Charles Cavalier (n:1), Labrador (n:3), Pitbull (n:2), Poodle (n:1), Pointer (n:4), Pug (n:1), Rottweiler (n:5), Russian Tsvetnaya Bolonka (n:1), Maltese Terrier (n:1), Kurzhaar (n:1), Samoyed (n:2), Setter (n:5), Shar Pei (n:1), Staph (n:2), Shepherd dog (n:2), Terrier (n:4) and Crosbred dogs (n:173). The dogs were individually bled through the *Vena cephalica antebrachii*, and 1-4 mL blood sample from each dog was collected into a tube containing ethylenediaminetetra-acetic acid (EDTA). Detailed information about the sampled provinces and the number of the samples are shown in Fig. 1.

Serological detection: An immunochromatographic immunoassay (Antigen Rapid Caniv-4 (Leish) Test Kit, Bionote) was used for the simultaneous detection of *D. immitis* antigens, anti-*A. platys*/*phagocytophilum*, anti-*E. canis* and anti-*L. infantum* antibodies. All reagents and blood samples were kept at room temperature (15-30°C) before running the assay. The immunochromatographic test kit was removed from its foil pouch and placed on a flat and dry surface. 20 µL of whole blood sample was then added into each sample hole. Three drops of assay diluent were added into each sample hole, and the results were interpreted after 15 minutes. A second band formed in front of the control band was considered as positive.

Statistical analysis: SPSS version 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical program was used to analyze all data. P and chi-square values (χ^2) were calculated to determine statistical significance, if any, among different gender and age groups of dogs and among samples from different provinces.

Ethical statement: Informed consent was obtained from the owners of the dogs for sampling, and all experimental procedures were performed following the ethical guidelines of the Experimental Animals Production and Research Center Ethics Committee of Veterinary Faculty of Selcuk University (Decision number: SUVDAMEK-2021/39).

Fig. 1: Sampled provinces and the number of samples according to the provinces; 1-Adana (n: 31), 2-Ankara (n: 7), 3-Antalya (n: 13), 4-Aydın (n: 26), 5-Balıkesir (n: 11), 6- Bursa (n: 7), 7-Denizli (n: 10), 8-İstanbul (n: 25), 9- İzmir (n: 12), 10-Kocaeli (n: 36), 11-Muğla (n: 29), 12-Sakarya (n: 41).

RESULTS

Mono infections (30.2%), dual co-infections (6.5%) and a triple co-infection (0.4%) were detected in 92 (37.1%) of 248 dog blood samples examined in the study. The presence of *D. immitis* antigen was only detected in three blood samples (1.2%). The seroprevalence of *A. platys*/*phagocytophilum*, *E. canis* and *L. infantum* was determined as 8.5, 19.8 and 14.9, respectively. Dual co-infections caused by *E. canis*+*A. platys*/ *phagocytophilum* and *E. canis*+*L. infantum* were detected in 16 dog blood samples, and a case of triple co-infection by *D. immitis*, *E. canis*, and *Anaplasma* spp. was also detected in a dog. Graphical presentation of infections is given in Fig. 2. Detailed information about the number of seropositive dogs according to provinces is shown in Table 1. Immunochromatographic test kits showing positive reactions for all agents detected in the study are indicated in Fig. 3.

No statistically significant difference was determined between the gender and the prevalence of each pathogen in the present study (Fig. 4). However, the situation was found to be different for the age factor. Although no statistically significant difference between the age factor and the prevalence of each pathogen was also determined for *D. immitis* and *Anaplasma* spp., a statistically significant relationship was detected for *E. canis* and *L. infantum*. Seroprevalences were detected at higher rates in dogs older than 2 years of age. Detailed information regarding the number of infections according to the age of dogs is indicated in Fig. 5. The statistical similarities or differences of the infection rates according to provinces are indicated in Table 2.

DISCUSSION

Vector-borne diseases of dogs are becoming more important as they also threaten human health (Otrando, 2018). Many seroepidemiological and molecular epidemiological studies regarding CVBDs have been carried out both abroad and in Turkey (Alanazi *et al*., 2020; Aslan Celik *et al*., 2020; Diaz-Reganon *et al*., 2020). It is a fact that this situation parallels the prevalence of diseases. Changing climatic conditions and increased animal activity are the most critical factors affecting the prevalence of CVBDs (Beugnet and Marie, 2009; Hager *et al*., 2020; Ali *et al*., 2020b).

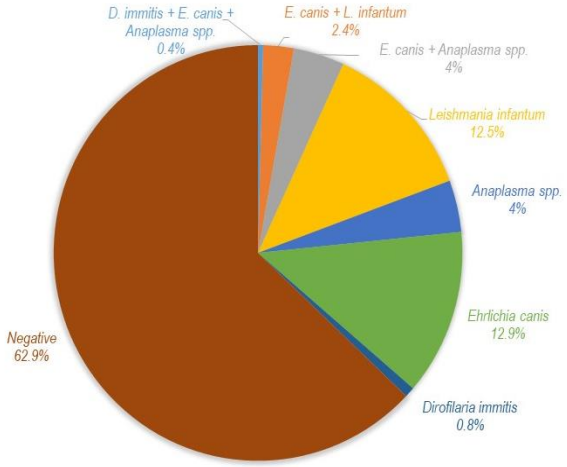

Fig. 2: Immunochromatographic test results indicating the global distribution of mono and co-infections.

Table 1: Distribution of single and mixed infections in dog blood samples

City	n	Mono infections				Dual co-infections		Triple co-infections	Total
		D. immitis	*E. canis*	*Anaplasma* spp.	*L. infantum*	*E. canis* *Anaplasma* spp.	*E. canis* *L. infantum*	*D. immitis* *E. canis* *Anaplasma spp.*	
		Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Adana	31	-	6	2	-	3	-	-	11
Ankara	7	-	2	-	3	-	-	-	5
Antalya	13	-	-	-	6	-	-	-	6
Aydın	26	-	2	-	2	2	3	1	10
Balıkesir	11	1	2	-	2	-	1	-	6
Bursa	7	-	3	-	1	-	-	-	4
Denizli	10	-	-	-	5	-	-	-	5
İstanbul	25	-	8	1	-	1	-	-	10
İzmir	12	-	1	1	3	-	1	-	6
Kocaeli	36	1	2	4	2	1	-	-	10
Muğla	29	-	5	-	7	1	1	-	14
Sakarya	41	-	1	2	-	2	-	-	5
Total (%)	248	2 (0.8%)	32(12.9%)	10(4%)	31(12.5%)	10(4%)	6(2.4%)	1(0.4%)	92(37.1%)

Table 2: Statistical comparison of the seroprevalences of *D. immitis*, *E. canis*, *Anaplasma* spp., and *L. infantum* according to the sampled provinces

		Adana	Ankara	Antalya	Aydın	Balıkesir	Bursa	Denizli	İstanbul	İzmir	Kocaeli	Muğla	Sakarya	P	χ^2
D. immitis	-	31^{1}	7^{1}	13^{1}	25_{a}	10_{a}	7^{1}	10^{1}	25^{1}	12^{1}	36^{1}	29^{1}	40_{a}		
	+	0^{1}	0^{1}	0^{1}	1_{a}	1_{a}	0^{1}	0^{1}	0^{1}	0^{1}	0^{1}	0^{1}	1_{a}	0.512	9.830
E. canis	-	22_{a}	$5_{a,b,c}$	13^{1}	18_{a}	$8_{a,b,c}$	4_{a}	10^{1}	16_{a}	$10_{a,b,c}$	$33_{b,c}$	$22_{a,b}$	38_{c}		
	+	9_{a}	$2_{a,b,c}$	0^{1}	8_{a}	$3_{a,b,c}$	3_{a}	0^{1}	9_{a}	$2_{a,b,c}$	$3_{b,c}$	$7_{a,b}$	3_{c}	0.013	23.973
Anaplasma	-	26_{a}	7^{1}	13^{1}	23_{a}	11^{1}	7^{1}	10^{1}	23_{a}	11_{a}	31_{a}	28_{a}	37_{a}		
spp.	+	5_{a}	0^{1}	0^{1}	3_{a}	0^{1}	0^{1}	0^{1}	2_{a}	1_{a}	5_{a}	1_{a}	4_{a}	0.572	9.507
L. infantum	-	31^{1}	4_{a}	7_{a}	$21_{a,b}$	8_{a}	$6_{a,b}$	5_{a}	25^{1}	8_{a}	34_{b}	21_{a}	41^{1}		
	+	0^{1}	3_{a}	6_{a}	$5_{a,b}$	3_{a}	$1_{a,b}$	5_{a}	0^{1}	4_{a}	2_{b}	8_{a}	0^{1}	0.000	52.064

*Different letters in the same line indicate statistically significant difference.

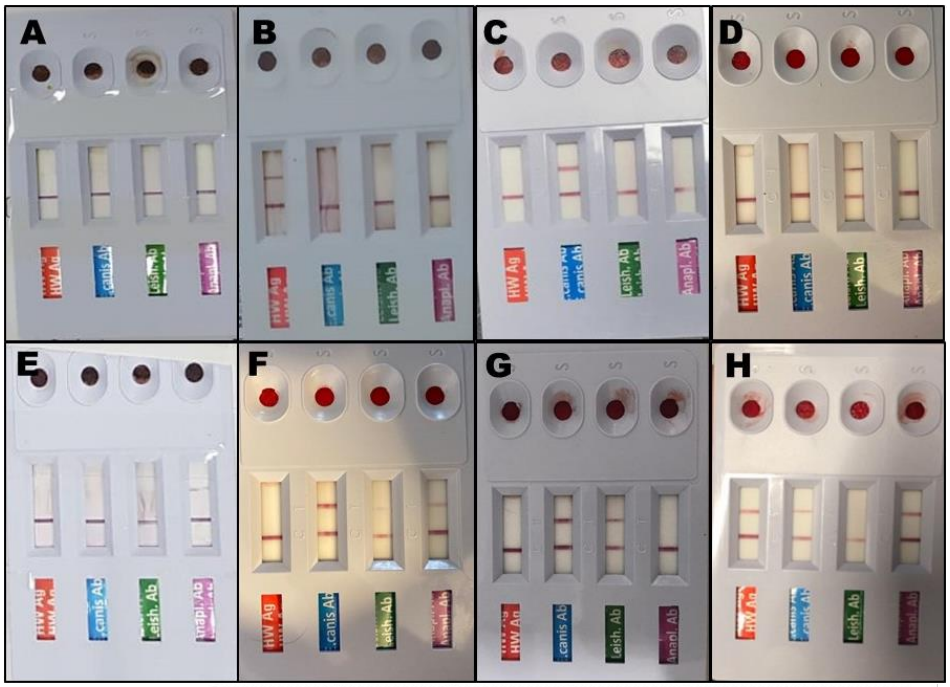

Fig. 3: Bands showing negative and positive reactions on the immunochromatographic test kit; Negative test results (A), Positive reactions for *Dirofilaria immitis* (B), *Ehrlichia canis* (C), *Leishmania infantum* (D), *Anaplasma* spp. (E), dua co-infection of *Ehrlichia canis* and *Anaplasma* spp. (F), dual co-infection of *Ehrlichia canis* and *Leishmania infantum* (G), triple co-infection of *Dirofilaria immitis, Ehrlichia canis* and *Anaplasma* spp.

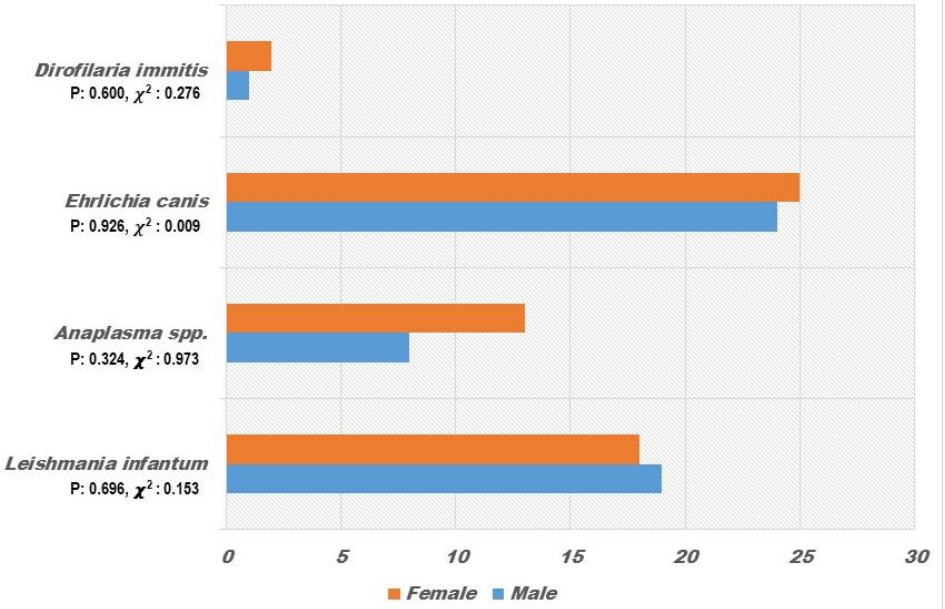

Fig. 4: Infection numbers according to the gender of dogs, and some parameters indicating no statistically significant difference between the gender and the prevalence of each pathogen.

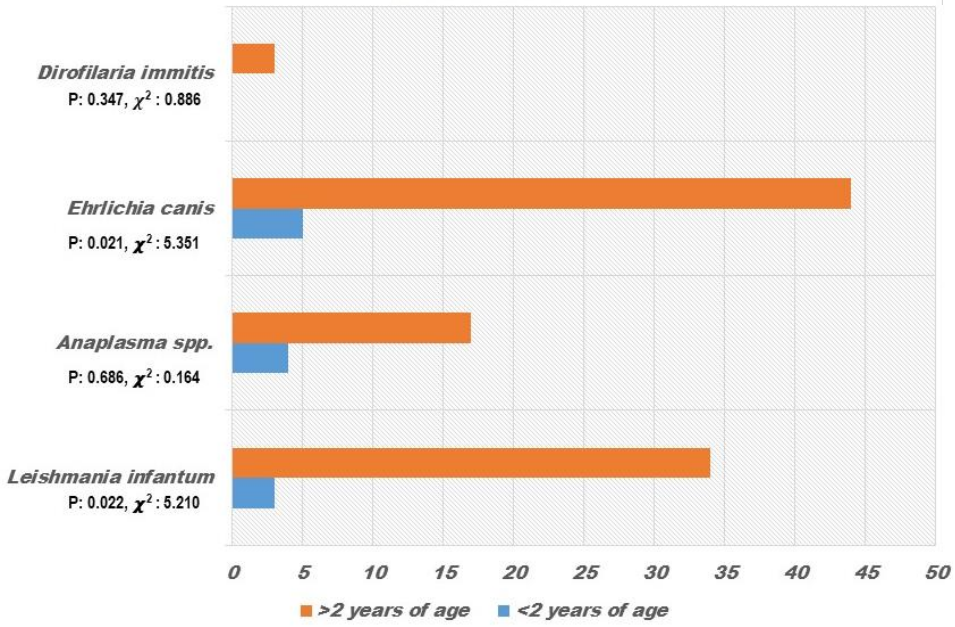

Fig. 5: Infection numbers according to the age of dogs, and some parameters indicating statistically significant or insignificant differences between the age and the prevalence of each pathogen.

Dirofilaria species are filarioid helminthic parasites causing dirofilariasis. The main species causing this disease are *D. repens* and *D. immitis*, and mosquitoes are the vectors of these nematodes. Although both species have zoonotic significance, *D. immitis* is more important because of the life-threatening disease it causes. Adult *D. immitis* filariae cause canine and feline cardiopulmonary dirofilariasis. The disease is particularly severe in dogs, and specific findings are rarely encountered in cats (Fuehrer *et al*., 2016; Inci *et al*., 2018; Zaman *et al*., 2020). Canine dirofilariasis is a common nematode infection worldwide, and the disease has been reported in humans and dogs in Turkey (Inci *et al*., 2018). In the studies conducted in different provinces of Turkey, the prevalence of *D. immitis* was revealed by microscopic, serological and molecular techniques (Atas *et al*., 2018). As a result of serological studies, the seroprevalence of *D. immitis* in Turkey was determined as 0-40% (Simsek *et al*., 2008; Kose and Erdogan, 2012; Sari *et al*., 2013; Ural *et al*., 2014; Aslan Celik *et al*., 2020, Ceylan, 2020; Demir and Aktas, 2020). The seroprevalence of *D. immitis* was determined as 1.2% in this study. This result was found to be consistent with the results of other seroepidemiological studies conducted in Turkey. In the study, *D. immitis* infection was detected in Aydın, Balıkesir and Kocaeli provinces. It was previously reported that *D. immitis* was detected in dogs from Aydın and Kocaeli provinces (Simsek *et al*., 2008; Ural *et al*., 2014). To the best of our knowledge, the infection has been reported for the first time from Balıkesir province in the present study. It should not be forgotten that dogs infected with *D. immitis* pose a risk to other healthy dogs and humans in the presence of vector mosquitoes, and necessary precautions should be taken in this direction.

The species causing anaplasmosis in dogs are *A. phagocytophilum* and *A. platys*. Among these species, *A. phagocytophilum* causes canine granulocytic anaplasmosis mainly characterized by fever, lethargy, lymphadenomegaly, anorexia, arthritis, splenomegaly, weight loss, and vomiting. On the other hand, *A. platys* causes canine cyclic thrombocytopenia, which usually shows an asymptomatic course (Sykes and Foley, 2014; Ceylan *et al*., 2021). These diseases have a large distribution worldwide, including in Turkey. It was determined that the seroprevalence of *A. phagocytophilum*/*A. platys* varied between 0-30.1% in Turkey (Gunes *et al*., 2011; Ural *et al*., 2014; Guven Gokmen *et al*., 2019; Ceylan, 2020; Demir and Aktas, 2020; Aslan Celik *et al*., 2020; Ceylan *et al*., 2021). In this study, anti-*Anaplasma* spp. antibodies have been detected totally in 21 dogs (8.5%) from Adana (n: 5), Aydın (n: 3), Izmir (n: 1), Istanbul (n: 2), Kocaeli (n: 5), Muğla (n: 1) and Sakarya (n: 4). The seroprevalence rate obtained in the present study is in line with the results of previous studies conducted in Turkey. *Anaplasma* infection was detected for the first time in dogs in Adana and Sakarya provinces in this study. It is thought that this situation may be related to the distribution of *Ixodes ricinus*, which is known as the main vector of *A. phagocytophilum* (Skotarczak, 2018). Aydin and Bakirci (2007) studied the geographical distribution of this tick species and stated that this tick species is found in the Marmara, Aegean, Mediterranean, Black Sea and East Anatolia regions of Turkey. In this study, *Anaplasma* infection was also detected in various provinces of the Marmara, Aegean and Mediterranean regions of Turkey. Canine *Anaplasma* infections are predicted to be detected in different provinces or regions of Turkey due to the increased distribution of vector ticks in the future. Therefore, it is thought that more comprehensive studies to be conducted in different parts of Turkey are of importance in terms of the epidemiology of *Anaplasma* infections.

Ehrlichiosis is one of the most important tick-borne rickettsial infections affecting dogs, and severe infections are mainly associated with *E. canis*, and the disease caused by this rickettsial microorganism is known as canine monocytic ehrlichiosis (CME), which is characterized by anorexia, depression, fever, lymphadenomegaly, lethargy, mucosal pallor and splenomegaly (Little, 2010; Mylonakis *et al*., 2019; Ceylan *et al*., 2021). The disease is common in dogs in Turkey (Ceylan *et al*., 2021). Mylonakis *et al*. (2019) stated that CME might be one of the leading causes of life-threatening pancytopenia in dogs in endemic countries as well as Turkey. As a result of studies conducted in Turkey, it was reported that the only species detected in canine ehrlichiosis cases was *E. canis* with a seroprevalence rate ranging from 0 to 74% (Sancak *et al*., 2002; Erdeger *et al*., 2003; Icen *et al*., 2011; Altas *et al*., 2013; Sari *et al*., 2013; Ural *et al*., 2014; Cetinkaya *et al*., 2016; Guven Gokmen *et al*., 2019; Aslan Celik *et al*., 2020; Demir and Aktas, 2020). The canine vector-borne pathogen with the highest seroprevalence rate was determined as *E. canis* (19.8%) in the present study. *Ehrlichia canis* specific antibodies could not be detected only in blood samples obtained from Antalya and Denizli provinces. It is thought that this may be due to the low number of samples taken from these provinces. On the other hand, the detection of this pathogen in all other provinces can be associated with the geographical distribution of the primary vector tick *Rh. sanguineus* in Turkey. Aydin and Bakirci (2007) reported that this tick species could be detected in all regions of Turkey. In addition, it is thought that the prevalences of infections in some provinces show statistically significant differences depending on the distribution of the tick vector ($P< 0.05$).

Dogs are clinically affected by CanL and pose a risk for human health by acting as reservoirs of the infection (Ica, 2004). Although several *Leishmania* species infect dogs, the most important species is *L. infantum*, which is the causative agent of viscerocutaneous leishmaniosis. CanL is predominantly encountered in dogs living in the Mediterranean and Aegean regions of Turkey (Inci *et al*., 2018). It has been determined that the seroprevalence of CanL in Turkey is between 0 and 37.4%. (Ozbel *et al*., 2000; Utuk *et al*., 2018; Guven Gokmen *et al*., 2019; Bolukbas *et al*., 2016; Balcioglu *et al*., 2009; Ozensoy Toz *et al*., 2009; Ceylan, 2020; Aslan Celik *et al*., 2020; Bakirci and Topcuoglu, 2021). In this study, the seroprevalence of CanL (14.9%) was found to be compatible with the prevalences determined in the previous studies. It has been determined that the majority of the infected dogs belong to the provinces of Muğla, Aydın, Denizli and İzmir, located in the Aegean region, where the infection is endemic. A low seropositivity rate

was also detected in Balıkesir, Bursa and Kocaeli provinces, located in the Marmara region where the infection is endemic. Seropositivity was also detected in three dogs from Ankara province, located in the Central Anatolia region, where leishmaniosis is non-endemic. *Leishmania infantum* seropositivity (0.4%) was previously reported from the Yenimahalle district of Ankara province in only one study (Kocak, 2010). This study indicates that the number of CanL cases in dogs tends to increase in Ankara, located in the non-endemic region. It is thought that this may be due to the travels of dogs from endemic regions to non-endemic regions. In such cases, vector sandflies in the non-endemic regions increase the risk for the entire region.

In the present study, dual (6.4%) and triple (0.4%) coinfections were detected in dogs in different combinations. Seventeen (6.8%) dogs were seropositive to two (*E. canis* + *Anaplasma* spp. and *E. canis* + *L. infantum*) or three (*D. immitis* + *E. canis* + *Anaplasma* spp.) different pathogens. It has been reported that the detected coinfections may be caused by the immunosuppression status of dogs or common transmission routes or vectors (Diaz-Reganon *et al*., 2020). *Ehrlichia canis* came into prominence as the most common pathogen among all coinfections. *Ehrlichia canis* was detected in 16 (94.1%) of the 17 coinfected dogs in the study. This may be related to the widespread distribution of *Rh. sanguineus*, the primary vector of *E. canis*, in Turkey.

Conclusions: This study presents data on the serological prevalence of *D. immitis*, *E. canis*, *A. phagocytophilum*/*A. platys* and *L. infantum* detected in dogs in some western provinces of Turkey. The provincial differences in seroprevalence can be elucidated by many factors, including climate changes, regional variations, sampling sizes and vectors' distribution. The study's positive serological results may indicate a previous infection and do not always mean an acute infection. However, this situation reveals that veterinarians should make further evaluations at diagnosing subclinical infections in dogs that require treatment. In addition, taking protective measures against arthropod vectors transmitting infections to humans and dogs is essential for animal welfare and human health.

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Authors contribution: OC and AU conceived and designed the research. OC, AU and OO executed the research under the supervision of FS. OC and FS analyzed the data. OC wrote the manuscript. OC, AU, OO and FS reviewed the manuscript.

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