



Burçak ASLAN ÇELİK^{1, a}
Özgür Yaşar ÇELİK^{2, b}
Tekin ŞAHİN^{2, c}
Kıvanç İRAK^{3, d}
Memiş BOLACALI^{4, e}
Özlem BIÇİCİ^{2, f}
Vedat BALDAZ^{2, g}

¹ University of Siirt,
Faculty of Veterinary,
Department of Parasitology,
Siirt, TURKEY

² University of Siirt,
Faculty of Veterinary,
Department of Internal
Medicine,
Siirt, TURKEY

³ University of Siirt,
Faculty of Veterinary,
Department of Biochemistry,
Siirt, TURKEY

⁴ University of Siirt,
Faculty of Veterinary,
Department of Animal
Breeding and Husbandry,
Siirt, TURKEY

^a ORCID: 0000-0002-0130-970X

^b ORCID: 0000-0001-6365-2688

^c ORCID: 0000-0002-1164-3429

^d ORCID: 0000-0001-9765-0330

^e ORCID: 0000-0002-4196-2359

^f ORCID: 0000-0001-9210-4744

^g ORCID: 0000-0001-6799-1716

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Correspondence Yazışma Adresi

Özgür Yaşar ÇELİK
University of Siirt,
Faculty of Veterinary,
Department of Internal
Medicine
Siirt – TURKEY

oyc@siirt.edu.tr

A Serologic Survey of Dirofilariasis, Leishmaniasis, Ehrlichiosis, and Anaplasmosis in Dogs in Siirt Province

Canine vector-borne diseases are caused by a wide range of pathogens transmitted by arthropods. The aim of the present study was to determine the prevalence of *Dirofilaria immitis* antigen and *Ehrlichia canis*, *Leishmania infantum*, and *Anaplasma phagocytophilum/platys* antibodies using a Rapid test (Anigen Rapid Caniv-4 Leish, Bionote, South Korea). The study was conducted with a total of 50 mixed-breed dogs (26 males and 24 females). *Anaplasma phagocytophilum/platys* antibodies were detected in 5 of the dogs (10%). None of the tested dogs were positive for *D. immitis* antigen or *L. infantum* and *E.canis* antibodies. As a result; it was concluded that protection and control measures regarding anaplasmosis should be taken.

Key Words: Anaplasmosis, dirofilariasis, ehrlichiosis, leishmaniosis, Siirt

Siirt İli Köpeklerinde Dirofilariasis, Leishmaniasis, Ehrlichiosis ve Anaplasmosis Üzerine Serolojik Bir Çalışma

Köpeklerin vektör kaynaklı hastalıklarına arthropodlar tarafından nakledilen oldukça geniş çeşitlilikte patojenler neden olmaktadır. Bu çalışmanın amacı Siirt ili köpeklerinde *Dirofilaria immitis* antijeni ile *Ehrlichia canis*, *Leishmania infantum* ve *Anaplasma phagocytophilum/platys* antikorlarının hızlı tanı testi (Anigen Rapid Caniv-4 Leish, Bionote, Güney Kore) kullanılarak belirlenmesiydi. Çalışmanın materyalini farklı ırklardan 26 erkek ve 24 dişi olmak üzere toplam 50 köpek oluşturdu. Çalışma sonucunda örneklerin 5 (%10)'ünde *Anaplasma phagocytophilum/platys* antikorları tespit edilirken *D. immitis* antijeni, *L. infantum* ve *E.canis* antikorları tespit edilemedi. Sonuç olarak; anaplazmozis ile ilgili koruma ve kontrol tedbirlerinin alınması gerektiği kanısına varılmıştır.

Anahtar Kelimeler: Anaplazmozis, dirofilariyozis, erlişiyozis, layşmanyozis, Siirt

Introduction

Canine vector-borne diseases (CVBD) are caused by a wide range of pathogens transmitted by arthropods. While the CVBD is very important in terms of veterinary practice, they also pose a significant risk for human health due to their zoonotic potentials (1-3). Amongst the diseases transmitted to dogs by the vectors, Dirofilariasis, Leishmaniasis, Ehrlichiosis, and Anaplasmosis are particularly of importance. Anaplasmosis and Ehrlichiosis are transmitted by ticks, while Dirofilariasis is transmitted by mosquitos and Leishmaniasis is transmitted by the biting midges. Presence of all of these diseases in Turkey have been reported (4).

Dirofilariasis is a zoonotic disease caused by *Dirofilaria spp.*, a filarial nematode, and is transmitted by mosquitos (5-7). The most common species that can cause the disease are *Dirofilaria immitis* and *Dirofilaria repens*. The adult form of *D. immitis* mostly locates in the right ventricle or right atrium of the heart and in the pulmonary arteries, while it can less commonly be found in the vena cava, camera oculi anterior, and peritoneal cavity as well (8-10). Clinical symptoms depend on the severity of the disease and the duration of infection. Weight loss, drowsiness, cough, respiratory distress, and acidosis can be seen in sick animals (4). Practical antigenic tests based on the detection of parasite-specific antibodies, native examination, and the modified Knott technique can be used in the clinical diagnosis of dirofilariasis (9).

Ehrlichiosis is a disease encountered in dogs living in tropical and sub-tropical regions and is caused by the strict-intracellular pathogen *Ehrlichia canis* (11, 12). The disease may have an acute, subclinical, or chronic course. Fever, weight loss, anorexia, nasal and ocular discharges, dyspnea, hemorrhagic disorders, anemia, thrombocytopenia, and leukocytosis or leukopenia can be seen in the acute phase of the disease. With treatment in the acute phase, the dogs may recover, may become subclinically infected, or may progress to the chronic phase (13, 14). Blood smears, Indirect fluorescence antibody (IFA), Western blot, and Enzyme-Linked Immuno Sorbent Assay (ELISA) techniques can be used in the diagnosis of the disease (15).

Leishmaniasis is a zoonotic protozoan disease commonly encountered all around the world and is caused by the *Leishmania* species (16-18). In Turkey, two clinical forms of the disease known as the cutaneous leishmaniasis and the visceral leishmaniasis can

be seen, which are caused by different *Leishmania* species (19-21). Dogs infected with the disease may not show symptoms or may have one or more of the nine major clinical symptoms like skin lesions, weight loss or loss of appetite, local or general lymphadenopathy, ocular lesions, epistaxis, lameness, anemia, renal failure and diarrhea (22, 23).

The anaplasma are small (0.2–0.9 µm) gram-negative, non-motile, unencapsulated, asporogenic, coccoid, ring-shaped, strictly intracellular bacteria that have a zoonotic nature (24). The species that cause disease in the dogs are the *Anaplasma phagocytophilum* (*A. phagocytophilum*) and the *Anaplasma platys* (*A. platys*). *A. phagocytophilum* locates itself especially to neutrophils and eosinophils, while *A. platys* is settled in the platelets (12). Common clinical findings in animals infected with *A. phagocytophilum* are high fever, anorexia, depression, and lethargy, all of which occur after 1-2 weeks of incubation. Reluctance to move and lameness are widely detected muscle and skeletal system symptoms (25, 26). Vomiting, diarrhea, coughing, and hemorrhage can be seen in some animals (24, 27). In the infections caused by *A. platys*, fever, anorexia, lethargy, mild anemia, and lenfoadenomegaly can be encountered (12). IFAT, ELISA, and Polymerase chain reaction (PCR) tests are used to diagnose anaplasmosis.

Seroprevalence studies are important in the determination of the geographical, regional, national, and universal distribution of diseases, and preparation and execution of control and eradication programs for them (4). The aim of this study, therefore, was to determine the seroprevalences of *D. immitis* antigen, *L. infantum*, *E. canis*, and *A. phagocytophilum/platys* antibodies in the dogs of Siirt province.

Materials and Methods

Study Area: This study was carried out between October 2019 and December 2019 in the Siirt province located in the Southeastern Anatolia Region of Turkey (Figure 1). Siirt province is in the semi-arid climate region. The average, the highest, and the lowest temperatures are 36.9 °C and 18.9 °C in summer, and 8.7 °C and –0.5 °C in winter, respectively. Water shortages and droughts are frequent during the summer (28).

Sample Collection and Preparation: The animal material of the study consisted of 26 male and 24 female totals of 50 stray dogs between the ages of 3 months and 6 years.

Blood samples were collected from the vena cephalica antibrachii of the animals into the non-anticoagulant tubes. The obtained samples were kept in room temperature for 30 minutes and then centrifuged in 3000 rpm for 10 minutes to separate the serum, after which they were kept in –20 °C until they were analyzed.

Test Procedure: A rapid diagnosis test kit (Anigen Rapid Caniv-4-Leish, Bionote, South Korea) prepared for the simultaneous detection of canine vector-borne

diseases (*D. immitis* antigen, *L. infantum* antibody, *E. canis* antibody, and *A. phagocytophilum/platys* antibody) was used in the study. The specifications of the test report the sensitivity of the test as 94.4%, 97.6%, 95.6% and 88.5% for *Dirofilaria*, Ehrlichia, Leishmania, and Anaplasma, respectively, while the corresponding specificities are reported as 100%, 99%, 98.0%, and 97.1%, respectively. A micropipette was used to drop 10 µl of serum samples into the sample holes of the rapid test kit. All holes were then added 3 drops of assay diluent solution. Test results were interpreted after 15 minutes (Figure 2).

Ethical Approval: Ethical approval for this study was obtained from the Siirt University Local Ethics Committee for Animal Experiments (Decision number: 2019/02/05).

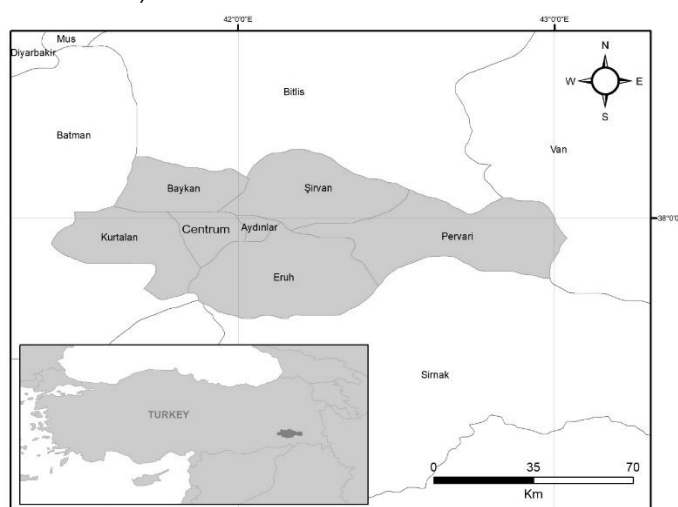


Figure 1. The map of Siirt province, in which the study was performed

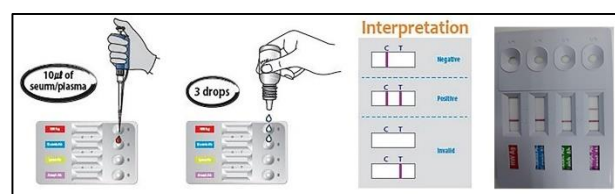


Figure 2. Test procedure

Results

Of the 50 dogs examined in the study, 5 (10%) were detected to have the *A. phagocytophilum/Platys* antibodies, while *Dirofilaria* antigens, Leishmania and Ehrlichia antibodies were not detected at all. The gender and age distributions of the findings are presented in Table 1.

As it can be seen in Table 1, 3 of the 26 male dogs (11.54%) and 2 of the 24 female dogs (8.33%) were found to have positive indicators. When the dogs are grouped based on their ages, the Anaplasma infection was found to be most common in the over 3 years of age group, followed by the 1-3 years of age group. No infection was found in the under-one-age group.

Table 1. Distribution of seropositive and seronegative animals by gender and age groups

Parameters	Examined	D.immitis		E.canis		L.infantum		A. phagocytophilum	
		Positive		Positive		Positive		Positive	
		n	%	n	%	n	%	n	%
Sex									
Male	26	-	-	-	-	-	-	3	11.54
Female	24	-	-	-	-	-	-	2	8.33
Age Group									
<1	24	-	-	-	-	-	-	0	0.00
1-3	12	-	-	-	-	-	-	2	16.67
>3	14	-	-	-	-	-	-	3	21.43
Overall	50	0	0.0	0	0.0	0	0.0	5	10.00

Discussion

Besides the environmental factors like temperature, humidity, and the presence of mosquitoes, age and shelter conditions also play significant roles in the development of *D. immitis* infections (9). As a result of the study conducted by Öncel and Vural (29) to determine the presence of antigens in the stray dogs of İstanbul, the prevalence of *D. immitis* was found as 1.52%, while the same researchers reported that no positivity was encountered in the stray dogs in İzmir. While no positivity was found by Civelek et al. (8) in their study in Bursa performed by native and modified Knott methods, their study which used the ELISA method reports 2% prevalence for *D. immitis*. Kozan et al. (30) conducted a study using the modified Knott method to determine the prevalence of *Dirofilaria* sp. in Afyonkarahisar and Eskişehir provinces, for which they determined positivities of 3.6% and 1.4%, respectively. In a study conducted in Diyarbakır with the ELISA method, *D. immitis* seroprevalence was reported as 2.4% (5) while the prevalence was 1.5% in Erzurum (31). The Rapid test method was used in a study in Antalya province, in which no *D. immitis* antigens were encountered (32).

Similar to the findings of researchers (8, 29, 32), all samples in this study were found to be seronegative in terms of *D. immitis*. The efficient insecticide applications performed in the Siirt province might be the reason why the dogs had no infection with the disease.

Canine leishmaniasis represents a significant problem for both animal and human health due to its zoonotic nature (33). In a study conducted in Manisa, the sera of 490 dogs were investigated with the IFAT method and the seroprevalence of the leishmaniasis was found as 5.3% (34). In the study conducted by Voyvoda et al. (35) in Antalya, the prevalence of *L. infantum* was determined as 3.63%, while the same researchers reported the prevalence of the disease for the province of İzmir as 2.5%. In a study conducted in Ankara by Aslantaş et al. (36), 116 dog serum samples were analyzed with the IFAT, and the seroprevalence of the disease was reported as 2.58%. In the study conducted

by Kilic et al. (37) in Sivas, the sera of 50 dogs were analyzed with the IFAT and the seroprevalence of the disease was determined as 2%. Atasoy et al. (38) conducted research that included the provinces of Aydın, Manisa, Muğla, and İzmir, and the seroprevalence of the disease were determined as 14.1%, 3.8%, 12%, and 4.6%, respectively.

A study was conducted by Handemir et al. (39) in various locations of İstanbul, and the researchers reported that no seropositivity was encountered. Babür et al. (40) conducted a study in Şanlıurfa, in which they reported all of the samples collected from 80 dogs were seronegative. In a study conducted by İca et al. (41) in Kayseri, all of the 300 dog serum samples were found to be seronegative. A study was performed by Tok et al. (42) in Çanakkale, and all of the 27 dog serum samples were seronegative. Celik and Sekin (17) used IFAT method in their research they conducted in the Dicle and Hani districts of the Diyarbakır province, in which they report no seropositivity was encountered in the 120 samples they inspected.

Similar to the findings and reports of researchers (17, 39-42), all samples in this study were seronegative in terms of *L. infantum*. Within the "cause network" that cause the development of Canine visceral leishmaniasis, the presence of Leishmania species, presence of the biting midges, biting midges stinging the infected host, a susceptible reservoir host being present nearby, the immune system reaction of the host, and environmental factors (humidity, air movements, light) are all present (35). It is possible that all samples were found to be negative in this regard due to no leishmania species being present in the environment, or that the insecticide applications against the mosquitoes also affected the phlebotomus and caused a lack of vectors for the disease.

Canine monocytic ehrlichiosis is amongst the most infectious diseases of dogs worldwide (43). It is reported that a Bull terrier breed dog brought to the Veterinary Faculty of the İstanbul University was diagnosed with ehrlichiosis using the IFAT method (15). In the study conducted by İcen et al. (5) in Diyarbakır using the

ELISA, the prevalence of *E. canis* was found as 4.8%. In a study conducted by Güneş et al. (44) in the Sinop province using the ELISA, the seroprevalence of *E. canis* was reported as 18.28%. Sari et al. (45) conducted a study in the Iğdır province, in which they reported the seroprevalence of 1% for *E. canis*. Elitok and Ungur (46), on the other hand, conducted a study in Uşak and reported a prevalence of 7% for *E. canis*.

All the samples analyzed in the present study were found to be seronegative in terms of *E. canis*. The seropositivity rates of Ehrlichia infection can be dependent on the target population, climate, and the diagnosis method used (47). On the other hand, the number of dogs infected with the parasite is reported to be higher in summer and spring months, compared to winter periods (48). It is possible that no seropositivity was encountered in this study for the reasons specified by Ansari-Mood et al. (47), or the fact that the disease is less frequently encountered in the period the study was conducted, or that the presence of the vector for the disease (*Rhipicephalus sanguineas*) in the region was not reported in any literature study.

Anaplasma phagocytophilum and *A. platys* are the species that cause Anaplasmosis in dogs (49). In a study conducted in Erzurum, the rate of *Anaplasma spp.* antibodies obtained was reported as 0.8% (4). The

seropositivity of *A. phagocytophilum* was determined as 30.1% in Sinop (50), 7.8% in Kayseri (51), and as 7.49% in a study that involved different provinces in the Aegean region (52).

In the current study, seropositivity was detected in five dogs (10%) against *Anaplasma spp.* It has been reported that the results of studies performed to determine the seroprevalence of *Anaplasma spp.* in dogs might be dependent on the number of samples used, the analysis method, and the density of the ticks in the region (53, 54). The seropositivity rate determined in the present study is in line with the studies conducted in Kayseri (51) and the Aegean region (52).

As a result, this is the first study that investigated the seroprevalence of Dirofilariosis, Ehrlichiosis, Leishmaniasis, and Anaplasmosis in the dogs in Siirt province. It was concluded that protection and control measures regarding anaplasmosis should be taken and more detailed studies on vector-borne infections are needed.

Conflicts of interest

The authors declare that there is no conflict of interest.

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