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RESEARCH ARTICLE

Prevalence of Diroflariasis, Lyme, Anaplasmosis, and Ehrlichiosis in Dogs in Afyonkarahisar Province

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ABSTRACT

This study aimed to determine the prevalence of dirofilariasis, Lyme, anaplasmosis, and ehrlichiosis in sheltered dogs in Afyonkarahisar. The data were obtained and evaluated from 400 dogs (female, n = 246; male, n = 154) of different breeds and at different ages that were referred to Afyon Kocatepe University Animal Hospital.. Accordingly, ovariohysterectomy (n = 196), castration (n = 113), skin disease (n = 113), diarrhea, vomiting, fatigue and anorexia (n = 37), lameness (n = 13), transmissible venereal tumor (n = 9), ascites (n = 2), pregnancy diagnosis (n = 2), mammary tumor (n = 1) and conjunctivitis (n = 1) were determined in dogs. Blood samples of dogs were taken to hematology and biochemical analyses after the first clinical examination of dogs. SNAP 4Dx (Idexx) Quick test kit was used to diagnose dirofilariasis, Lyme, anaplasmosis, and ehrlichiosis. The prevalence of anaplasmosis, ehrlichiosis, dirofilariasis, and Lyme was 1.25%, 0.75%, 0.75%, and 0%, respectively. Although the prevalence of anaplasmosis, ehrlichiosis, and dirofilariasis is low, the results of the study suggested that these zoonotic diseases should still be evaluated as a risk factor for both humans and animals which live in this region as the disease spreads through vectors.

Keywords: Afyon region, Borrelliosis, heartworm, seroprevalence, zoonoses

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Afyonkarahisar Yöresi Köpeklerinde Diroflariozis, Lyme, Anaplazmozis ve Ehrlichiozisin Prevalansı

ÖZ

Bu çalışmada Afyonkarahisar'da yaşayan köpeklerde dirofilariazis, Lyme, anaplasmozis ve ehrlichiozis prevalansının belirlenmesi amaçlanmıştır. Veriler, Afyon Kocatepe Üniversitesi Hayvan Hastanesi 'ne sevk edilen farklı ırk ve yaştaki 400 köpekten (dişi, n=246; erkek, n=154) elde edildi ve değerlendirildi. Buna göre ovariohisterektomi (n=196), kastrasyon (n=113), deri hastalığı (n=113), ishal, kusma, yorgunluk ve anoreksi (n=37), topallık (n=13), bulaşıcı zührevi tümör (n= 9), asites (n=2), gebelik tanısı (n=2), meme tümörü (n=1) ve konjonktivitli (n=1) köpeklerde saptanmıştır. Köpeklerin ilk klinik muayenesinin ardından hematolojik ve biyokimyasal analizler için kan örnekleri alındı. SNAP 4Dx (Idexx) Hızlı test kiti, dirofilariasis, Lyme, anaplasmosis ve ehrlichiosis'i teşhis etmek için kullanıldı. Anaplazmoz, ehrlichiosis, dirofilariasis ve Lyme prevalansı sırasıyla %1.25, %0.75, %0.75 ve %0 olarak tespit edildi. Anaplazmoz, ehrlichiosis ve dirofilariazis prevalansı düşük olmasına rağmen, çalışmanın sonuçları, bu zoonotik hastalıkların, hastalık vektörler yoluyla yayıldığı için hem insanlar hem de bu bölgede yaşayan hayvanlar için bir risk faktörü olarak değerlendirilmesi gerektiğini göstermiştir.

Anahtar Kelimeler: Afyon yöresi, Borreliosis, kalp kurdu, seroprevalans, zoonoz

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INTRODUCTION

Vector-borne pathogens (VBPs) are globally distributed and rapidly spreading, which are transmitted by arthropods, including ticks, fleas, mosquitoes, and phlebotomine sandflies (Otranto et al., 2009). Bacteria such as *Anaplasma platys*, *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Borrelia burgdorferi* complex; protozoans such as *Babesia canis*, *Hepatozoon canis*, and *Leishmania infantum;* and the nematode *Dirofilaria immitis* are among the major VBPs in dogs (Day, 2011). Different serological methods, including immunofluorescent antibody test (IFAT), enzyme-linked immunosorbent assay, and SNAP4Dx, were used to assess the distribution of VBPs among European countries. Recent studies show that the prevalence of VBPs may vary depending on the method (Maggi et al., 2014; Sainz et al., 2015).

Scientists and public health officials have reported an alarming increase in infectious diseases (Woolhouse & Gowtage, 2005). The possible occurrence of zoonotic pathogens is approximately 1.9 times higher than the non-zoonotic pathogens among the infectious pathogens. In comparison, the possible occurrence of VBPs is approximately 2.3 times higher than non-VBPs (Taylor et al., 2001). Several factors can contribute to the increase in the incidence of diseases caused by VBPs. These factors include changing climate leading to warmer winters (Eisen et al., 2015), increasing the ways people come in contact with nature, suburbanization bringing pets and pathogens together, migratory birds carrying ticks or pathogens to new areas, reducing insecticide use (Loh et al., 2015), increasing the import of different breed dogs from foreign countries and the travel of dogs from Europe or other continents to the tropics along with their owners has increased the prevalence of diseases (Özata, 2012; Unvar et al., 2006). Human-canine bonding that provides several benefits to people has led to some problems. Among these, some risks, such as the transmission of zoonotic diseases seen in dogs to humans, can be included (Doğanay & Şahal, 1987).

The expansion of international tourism and increase in travel activities may have increased tick-borne infections in dogs, which is known as 'emerging infectious disease' (Düzlü et al., 2014). Pets are known as reservoirs in the transmission of some VBPs, even when they do not show any clinical signs (Skotarczak, 2018).

A total of 107 zoonotic infections (37 bacteria, 13 fungi, 29 viral, and 28 parasites) have been reported in Turkey. Of these infections, 19 are transmitted by arthropod vectors. Therefore, 21 of these zoonotic infections are considered a high-priority disease in Europe (Düzlü et al., 2020). Afyonkarahisar is located in the migration routes of migratory birds passing through Turkey from different areas and is a junction point between big cities and holiday destinations in Turkey. The millions of visitor birds use shelters or nests in Turkey during their annual migration, and this leads to a constant threat of spreading new infectious agents. Despite the high endemicity of tick-borne pathogens and the availability of suitable tick habitats in Turkey, no in-depth epidemiological studies and research are available on tick-borne diseases (Inci et al., 2016).

The study aimed to reveal the relationship between the prevalence of zoonosis and common vector-borne diseases in Afyonkarahisar, as well as the predisposing reasons that may cause the presence of these diseases in Afyonkarahisar.

MATERIAL AND METHODS

Animals

The data were evaluated in 400 dogs (female, n = 246; male, n = 154) of different breeds and at different ages that were taken to the xx University Animal Hospital. The age of dogs was as follows: 281 dogs were 1–3 years old, 93 were 4–6 years old and 26 were older than 7 years old. Accordingly, ovariohysterectomy in dogs (n = 196); castration (n = 113); skin problem (n = 113); diarrhea, vomiting, weakness and anorexia (n = 37); lameness (n = 13); transmissible venereal tumor (n = 9); ascites (n = 2); pregnancy examination (n = 2); breast tumor (n = 1); and conjunctivitis (n = 1) complaints were determined.

Rapid Test Kit

We used rapid test kits (IDEXX Snap 4Dx, Westbrook, Maine 04092 United State). The kit functions based on the principle of antigen testing for *D. immitis* and antibody testing for other factors. Three drops of sample and four drops of conjugate were dropped separately into the Eppendorf tubes, and the tube was mixed gently 3–5 times. The final mixture was dropped into the sample compartment, and when the mixture reached the activation section of the kit, the activator part was pressed and waited for 8–10 min to read the result. Lastly, the results were read and recorded (Figure 1).

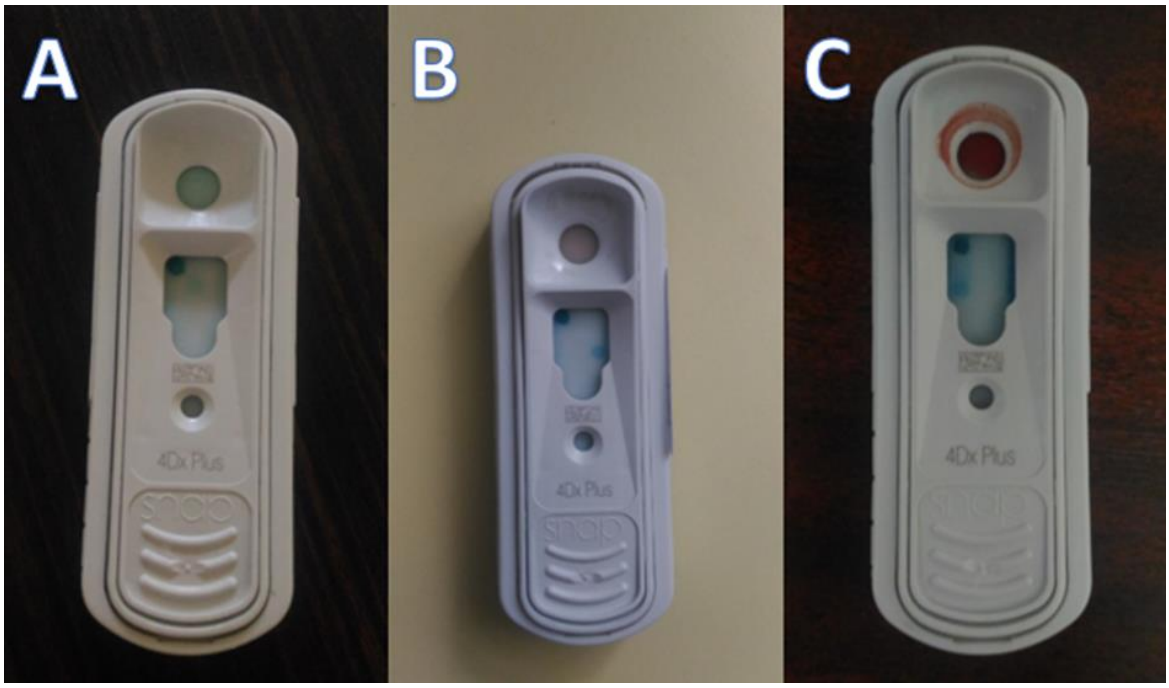

Figure 1: Examples of positive kits (A: A. Phagocytophilum, B: D. immitis, C: E. canis)

Hematological Examination

Blood samples were taken into tubes with K3EDTA, and simultaneously measured using the full automatic hematology device (Mindray BC-2800 Vet, Mindray, Shenzhen, China) in the internal medicine laboratory of our hospital for hematological examinations.

Statistical Analysis

Dogs are classified according to different ages, sexes, and housing patterns. The data obtained from the dogs included in the study were evaluated by the percentage analysis method. Descriptive evaluation of hematological findings in dogs were analyzed with the Windows SPSS 16.0 Package Program (SPSS Inc., Chicago, IL, USA).

RESULTS

The ages and diagnoses of the dogs are presented in Table 1. A total of 400 dogs were included in the study; 33 were indoor fed, and 367 were outdoor fed. Three dogs diagnosed with *D. immitis* infection and five dogs diagnosed with *A. phagocytophilum* infection were all females. In the case of *E. canis* infection, two dogs were female, and one was male. No dog was diagnosed with *B. burgorferi* infection in our study. The prevalences of *D. immitis* was 0.75% (indoor 0%, outdoor 0.82%), *E. canis* was 0.75% (indoor 9.1%, outdoor 0%), *A. phagocytophilum* was 1.25% (indoor 0%, outdoor 1.36%) and *B. burgorferi* was 0% (Table 1 and 2). Two dogs diagnosed with *Ehrlichia* were of Rottweiller (indoor), and one was of Setter (outdoor) breeds. One of the dogs had fainting problems, the other had epistaxis and respiratory problems, and the third one was brought to the obstetrics clinic for pregnancy examination. Leukocytosis and anemia were detected in two of the three dogs. No hematologically abnormal result was seen in the third dog.

Five dogs included in our study were diagnosed with *Anaplasma phagocytophilum*. All five dogs were fed outdoors. One of the dogs was of Terrier breed, one of them was of Kangal breed, and the other three were cross breeds. Four out of the five dogs were brought to our hospital for ovariohysterectomy, and a positive diagnosis was made, although no clinical findings were detected. One dog was brought to our hospital with complaints of loss of appetite and chronic weight loss.

D. immitis was diagnosed in three dogs brought to our hospital for ovariohysterectomy. No abnormal findings were found in the clinical examination of the dogs. The hematological examination results revealed anemia in one dog and leukocytosis in the other dog.

As a result of the statistical evaluation of the blood samples taken from the dogs included in the study, there was no statistically significant difference between *D. immitis, Anaplasma phagocytophilum*, *E. canis* and dogs without disease (Table 3).

Table 1. Prevalence and seroprevalences using the 4DX SNAP test (IDEXX Laboratories, Inc., Westbrook, Maine, USA) for 400 dogs analyzed for *Dirofilaria immitis* antigens and *Ehrlichia* spp., *Anaplasma* spp., and *Borrelia burgdorferi* antibodies in the Afyonkarahisar region of Turkey, according to age and sex. (%) percentage of dogs; (+) positive dogs; (N) number of dogs sampled.

	D. immitis %(+/N)	**E. canis %(+/N)**	**A. phagocytophilum %(+/N)**	**B. burgorferi %(+/N)**	**Total %(+/N)**
Age					
1–3	0,71% (2/281)	0% (0/281)	0,71% (2/281)	0% (0/281)	1,42% (4/281)
4–6	1,08% (1/93)	3,23% (3/93)	3,23% (3/93)	0% (0/93)	7,5% (7/93)
> 7	0% (0/26)	0% (0/26)	0% (0/26)	0% (0/26)	0% (0/26)
Sex					
Male	0% (0/154)	0,65% (1/154)	0% (0/154)	0% (0/154)	0,65% (1/154)
Female	1,22% (3/246)	0,81% (2/246)	2,03% (5/246)	0% (0/246)	4,07% (10/246)
Total	0,75% (3/400)	0,75% (3/400)	1,25% (5/400)	0% (0/400)	2,75% (11/400)

Table 2. Prevalence and seroprevalences using the 4DX SNAP test (IDEXX Laboratories, Inc., Westbrook, Maine, USA) for 400 dogs analyzed for *Dirofilaria immitis* antigens and *Ehrlichia* spp., *Anaplasma* spp., and *Borrelia burgdorferi* antibodies in the Afyonkarahisar region of Turkey, according to habitus. (%) percentage of dogs; (+) positive dogs; (N) number of dogs sampled.

	D. immitis %(+/N)	**E. canis %(+/N)**	**A. phagocytophilum %(+/N)**	**B. burgorferi %(+/N)**	**Total %(+/N)**
Habitus					
İndoor	0% (0/33)	9,1% (3/33)	0% (0/33)	0% (0/33)	9,1% (3/33)
Outdoor	0,82% (3/367)	0% (0/367)	1,36% (5/367)	0% (0/367)	2,18% (8/367)
Total	0,75% (3/400)	0,75% (3/400)	1,25% (5/400)	0% (0/400)	2,75% (11/400)

Table 3. Descriptive analysis of the hematological findings of the dogs included in the study (Mean ± SD)

	D. immitis	A. phagocytophilum	E. canis	Negative
WBC (10^9/l)	16,95±8,13	16,98±6,32	20,57±7,45	19,88±8,45
Lym (10^9/l)	4,70±5,23	1,78±0,95	2,57±0,49	3,16±2,86
Mon (10^9/l)	0,55±0,35	0,50±0,16	0,37±0,32	0,55±0,28
Gran (10^9/l)	11,70±2,55	14,70±5,47	17,43±7,05	16,18±6,75
Lymp %	22,75±20,01	10,28±2,12	12,93±2,59	15,41±8,73
Mon %	3,15±0,49	3,43±2,07	3,13±1,11	2,99±1,15
Gran %	74,10±20,51	86,30±3,33	83,93±3,44	81,84±8,42
EOS %	8,40±10,32	3,10±1,00	1,90±0,53	7,56±10,79
RBC 10^{12}/l	5,46±2,91	5,73±0,72	4,48±2,13	6,34±1,20
Hb g/dl	12,70±7,21	13,15±2,46	10,07±6,13	14,37±2,79
HCT (%)	39,70±22,20	38,53±6,00	30,37±18,05	43,61±8,52
MCV (fl)	72,20±2,26	67,22±4,39	65,20±9,97	68,93±4,54
MCH (pg)	22,95±0,92	22,85±3,04	21,40±3,54	22,65±1,81
MCHC (g/dl)	31,85±0,35	34,00±2,50	32,90±0,52	32,93±1,83
RDW (fl)	10,90±0,28	14,95±0,89	14,83±3,15	14,27±4,21
PLT (10^9/l)	313,00±45,25	404,00±159,61	199,33±114,61	379,14±220,06
MPV (fl)	9,60±0,28	8,15±0,79	8,60±1,10	8,58±1,16
PDW (fl)	16,00±0,00	15,80±0,24	16,30±0,30	15,98±0,39
PCT (10^9/l)	0,30±0,03	0,33±0,13	0,17±0,09	0,31±0,12

WBC: White Blood Cell, Lym: Lymphocyte, MID: Monocyte, Gra: Granulocyte, Hb: Hemoglobin, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RBC: Red Blood Cell, RDW: Red Cell Distribution Width, HCT: Hematocrit, PLT: Platelet, PCT: Procalcitonin, PDW: Platelet Distribution Width, MPV: Mean Platelet Volume

DISCUSSION

Although Afyonkarahisar is located in the Aegean region, the annual average temperature values are much lower than in the coastal Aegean cities. During the meteorological data examination, the temperature decreases to −27 °C in the winter months, with an annual average of 11.3 °C. Similarly, yearly precipitation is at the lowest level with 443.6 mm compared to other Aegean cities. Vectors are adversely affected by low-temperature values and dryness (Aydın et al., 2021).

There is increasing evidence that changes in land use affect the microclimate conditions and the habitat suitability for both ticks and their hosts (Asghar et al., 2016; Jones et al., 2011; Estrada- Peña, 2001). For example, the abandonment of farmland and regrowth of native vegetation may provide a suitable environment for ticks (Hrkľová et al., 2008; Medlock et al., 2013); however, intensive agricultural activities, loss of native vegetation, and reduced rodent host diversity combined with lack of leaf litter may lead to unsuitable conditions for tick development (Michel et al., 2006; Perez et al., 2016). Sheltering of the majority of stray animals in the settlements, the regular spraying of the landscape areas of the settlements by the boroughs to combat ticks and mosquitoes, and the regular removal of leaf litter where ticks can live may reduce vector-borne diseases.

Researchers in different countries and Turkey have used different test kits and methods to determine the prevalence of vector-mediated zoonotic diseases such as *D. immitis, B. burgdorferi, E. canis, and A. phogocytophilum* (Bell & Patel, 2005; Küçüker, 2016; Gokmen, 2019). In a study in the Aegean region, 307 dogs were screened with Snap 4Dx. The prevalences of the diseases were as follows: *E. canis,* 24.42%; *E. canis* + *A. phagocytophilum* co-infection, 10–42; *A. Phagocytophilum,* 7.49%; and *D. immitis,* 2.28% (Ural K et al., 2014). In a study on 100 dogs in Igdır province, *D. immitis* (40%) and *E. canis* (1%) were reported (Sarı et al., 2013). *D. immitis* (2.4%) and *E. canis* (4.8%) were found in a study with 82 dogs in Diyarbakır (İçen et al., 2011). In a study conducted on 100 dogs

in the Osmaniye region, it was found that E.canis / E. ewingii was 3%, D.immitis was 1%, *A.platys* / *A.phagocytophilum* and Lyme were 0% (26). In this study, the prevalence of *D. immitis* was 0.75%, *E. canis* was 0.75%, *A. phagocytophilum* was 1.25%, and *B. burgforferi* was 0%. Although Afyonkarahisar province is located in the Aegean region, we found that the prevalences are significantly lower than the studies performed in the other areas of the Aegean region, which may be because Afyonkarahisar is in the Central Anatolia continental climate region.

The relationship between vector-borne diseases and gender is still being investigated. Although no effect of gender on *D. immitis* infection has been stated in dogs, some researchers have reported that this parasite is more common in male dogs than females (Theis et al., 2001; Fan et al., 2001; Aranda et al., 1998; Montoya et al., 1998 ; Martin & Collins, 1985; Selby et al., 1980; Graham, 1974). This may be because females are more stable and less active than males. In contrast to other studies, we determined that the number of female animals was higher in the groups with positivity. We believe that gender has no role in predisposition to vector-borne diseases.

Dogs infected by *A. phagocytophilum* typically present with sluggishness, fever, and loss of appetite. Furthermore, lameness and reluctance to move because of neutrophilic polyarthritis may be observed. In addition to these, vomiting, diarrhea, and bleeding disorders such as epistaxis have been reported. However, the majority of dogs appear healthy. In the endemic regions of the disease, 60% of the dogs were seropositive, and no clinical findings were found (Özcel, 2013). Similar to other studies, cachexia and stagnation were observed in only one of the five reported dogs with anaplasmosis in this study. In contrast, no clinical abnormality was found in the other four dogs.

Coagulation disorders may occur in the chronic form of ehrlichiosis because of thrombocytopenia and thrombocytopathy. As a result of the coagulation disorders, epistaxis, melena, petechiae, hematuria, intraocular, brain, and lung hemorrhages can be observed (Aytuğ, 2012). Similarly, in this study, one of the dogs diagnosed with *Ehrlichia* was fainting, the other had epistaxis, and the third one had no clinical pathology. The disease spreads widely in Turkey, and the prevalence of the disease in different regions has been reported to be between 0.06% and 20% (Coşkun et al., 1992; Zeybek, 1989; Alkan & Sarınç, 1986). The prevalence of the disease is higher in village dogs than in both sheltered and pet dogs. This may be because of the presence of reservoir dogs in villages and anti-parasitic pesticides in shelters (Voyvoda et al, 2004). Similarly, in this study, all positive dogs were fed outdoors. The high prevalence of these dogs can be explained by the fact that they may be more in contact with the vector.

Although Lyme disease in dogs is zoonotic, very few studies have been found on the presence and prevalence of the disease in Turkey. In Turkey, Gulanber et al. (Gulanber et al., 2007) reported the first clinical case in dogs which was a St. Bernard breed in Istanbul. Esendal et al. (Esendal et al., 1996) found that the seroprevalence of Lyme disease was 78.4% in 74 dogs using the IFAT method in Ankara. Satir (Satir, 2006) performed polymerase chain reaction in 96 dogs in Istanbul, and no positivity was detected in any dogs. Bhide et al. (Bhide et al., 2008) determined that the disease was seropositive in 93 dogs (23.2%) using the enzyme-linked protein A/G assay test in 400 dogs that were taken to Bursa Uludag University Veterinary Faculty Internal Medicine Clinic. No ticks of the genus *Ixodes*, which is the vector of *B. burgdorferi*, were found in 10,303 ticks collected from the Sivas region (Gunes et. al., 2005). A study conducted in the Aydın region reported that the seroprevalence of the disease was 40.8% (Uslu, 2008). *B. burgdorferi* factor was not found in the study performed on 100 dogs in Osmaniye region (Gokmen, 2019). *B. burgdorferi* was not detected in 400 dogs examined in this study.

CONCLUSIONS

As a result, the prevalence of vector-borne diseases in the Afyonkarahisar region is similar to areas with similar climatic conditions. Even if the prevalence is low, the disease poses a risk to both dogs and humans. It needs attention by veterinarians and researchers not only for regions with tropical climates but also for other regions.

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Authors Contribution Rate: The authors declare that they have contributed equally to the article.

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