Volume 13, Issue 4, Page 199-203, 2024 https://doi.org/10.46810/tdfd.1572258 Research Article

BİNGÖL UNIVERSITY

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

Investigation of Leishmaniasis Seroprevalence in Dogs in Bingöl Province of Turkey

Murat UZTİMÜR [1], Hakan KEÇECİ [1], Taylan TURAN [2], Cennet Nur ÜNAL [1*]

[1]Bingöl University, Faculty of Veterinary Medicine, Department of Internal Medicine, 12000, Bingöl, Turkey
[2] Gazi University, Faculty of Pharmacy, Department of Biochemistry, 06330, Ankara, Turkey.
Murat UZTİMÜR, ORCID No:0000-0001-9294-1825
Hakan KEÇECİ, ORCID No: 0000-0001-8236-100X
Taylan TURAN, ORCID No:0000-0001-7335-1213
Cennet Nur ÜNAL, ORCID No:0000-0002-8676-6490

**Corresponding author: cnaltunboga@gmail.com*

(Received: 23.10.2024, Accepted: 04.12.2024, Online Publication: 30.12.2024)

Keywords
Dog, ELISA, *Leishmania*, Prevalence

Abstract: Canine leishmaniasis is a lethal zoonotic disease caused by *Leishmania infantum* (*L. infantum*) and seen in Asia, Europe, America, and Africa. Dogs play an important role in the spread of the disease as they can be infected clinically and are reservoirs for other mammals and humans. The aim of the present study was to investigate the prevalence of leishmaniasis in asymptomatic dogs in Bingöl province. In this study, a total of 84 dogs of different breeds, ages, and sexes (male/female) were used. Leishmania antibody IgG levels in the serum of the dogs were measured using an ELISA Kit. It was determined that 5 (19.2%) of the dogs aged <2 years and 7 (12.1%) of the dogs aged ≥2 years were Leishmania seropositive. 7 (17.9%) of the female dogs and 5 (11.1%) of the male dogs were detected Leishmania seropositive. The prevalence of Leishmania seropositive was determined as 14.2% in this study. The effect of gender (p=0.562) and age (p=0.501) factors on the occurrence of Leishmania infection was not determined. In conclusion, in this study, the prevalence of leishmaniasis in asymptomatic dogs in Bingöl province, which is not an endemic region, was determined. This finding shows the presence of Leishmania infection in the region and poses a significant risk to public health.

Türkiye'nin Bingöl İli Köpeklerinde Leishmaniasis Seroprevalansının Araştırılması

Anahtar Kelimeler
Köpek, ELISA, *Leishmania*, Prevalans

Öz: Köpek leishmaniasisi, *Leishmania infantum*'un (*L. infantum*) yol açtığı Asya, Avrupa, Amerika ve Afrika kıtalarında görülen öldürücü zoonotik bir hastalıktır. Köpekler hem klinik olarak enfekte olabildikleri hem de diğer memelilere ve insanlara rezervuarları oldukları için hastalığın yayılmasında önemli bir rol oynamaktadır. Mevcut çalışma ile Bingöl ilindeki asemptomatik köpeklerde leishmaniasis prevalansının araştırılması amaçlanmıştır. Bu çalışmada farklı ırk, yaş ve cinsiyetten (erkek/dişi) oluşan toplam 84 adet köpek kullanıldı. Köpeklerin serumunda *leishmania* antikoru IgG seviyeleri bir ELISA Kiti kullanılarak ölçüldü. Yaşı <2 olanlardan 5 (%19.2), 2≥ olanlardan 7 (%12.1) adet köpeğin *leishmania* seropozitif olduğu belirlendi. Dişi köpeklerin 7 (%17.9), erkek köpeklerin 5 (%11.1) tanesinin *leishmania* seropozitif olduğu tespit edildi. Çalışmada *leishmania* prevalansı %14.2 olarak belirlendi. *Leishmania* enfeksiyonunun görülmesinde cinsiyet (p=0.562) ve yaş (p=0.501) faktörlerinin etkisi tespit edilmedi. Sonuç olarak, bu çalışmada endemik bölge olmayan Bingöl ilinde asemptomatik köpeklerde Leishmaniasis prevalansı tespit edilmiştir. Bu bulgu, bölgede *leishmania* enfeksiyonunun varlığını göstererek, halk sağlığı açısından önemli bir risk teşkil ettiğini ortaya koymaktadır.

1. INTRODUCTION

Canine leishmaniasis is a lethal zoonotic disease caused by *Leishmania infantum* (*L. infantum*) and seen in Asia, Europe, America and Africa. Domestic dogs are known to be the main reservoir of human infection and phlebothumus sand flies are known to be the vectors of the disease [1]. Dogs play an important role in the spread of the disease as they can both be clinically infected and act as reservoirs for other mammals and humans [2]. In

addition, it can be transmitted non-vectorally between dogs through blood transfusion, placenta, and mating [3]. *Leishmania* species are defined as diheteroxenous parasites because they complete their life cycle in the bodies of both a vertebral and an invertebral vector [1]. *Leishmania* parasites exist in two main morphological forms, amastigote and promastigote. Sand flies ingest infected macrophages containing the amastigote form while sucking blood from infected animals [1]. In the vector's organism, it turns into a promastigote form within 4–25 days and multiplies by simple division. Then, this form is transferred to the host while the vector sucks blood. In the host, the parasite infects macrophages and initiates the disease with the amastigote form [3].

Leishmaniasis disease is seen in four main clinical forms: visceral, cutaneous, mucocutaneous, and diffuse cutaneous forms. In Turkey, two forms caused by different *leishmania* species are seen as cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) [3, 4]. Although the most common form of the disease is the cutaneous form, the visceral form is the most serious as it clinically affects vital organs [3]. In dogs affected by the disease, showing more than three clinical signs are classified as symptomatic, dogs showing one to three clinical signs are classified as oligosymptomatic, and dogs showing no clinical signs are classified as asymptomatic [4, 5]. Common clinical symptoms of leishmaniasis include local or generalized lymphadenopathy, weight loss, anorexia, dermatological lesions, swelling in the joints and legs, and onychogryposis, while less common clinical symptoms include eye lesions, epistaxis, renal failure, lameness, diarrhea, and meningitis [1, 4]. Although the prevalence of leishmaniasis may vary depending on the diagnostic method used, it is reported to be between 0% and 27.5% in Turkey [3, 6, 7], percent 16.6 in the Mediterranean basin with 2.5 million infected dogs, and 15.2% globally [8]. The prevalence of leishmaniasis in asymptomatic dogs in Bingöl province is unknown. Based on this, the aim of the present study was to investigate the prevalence of leishmaniasis in asymptomatic dogs in Bingöl province.

2. MATERIAL AND METHOD

The study was conducted with the approval of the Bingöl University Animal Experiments Local Ethics Committee (B.Ü HADYEK Date: 2024/01 Decision No:01/08).

2.1. Collection of Blood Samples

In this study, a total of 84 dogs of different breeds, ages and genders were used. Breeds, ages and genders of dogs are presented in Table 1. The study was conducted in Bingöl province, which is located between 41º 20 and 39º - 56º east longitudes and 39º - 31 and 36º - 28º north latitudes of Turkey. The animals were brought to the Internal Medicine Department of the Animal Hospital of the Faculty of Veterinary Medicine of Bingol University. As a result of the systematic clinical examination, age, gender and clinical examination findings were recorded. Five ml blood samples were taken from the vena cephalica antebrachi of the animals into gel serum tubes (BD Vacutainer, Plymouth, UK). Blood samples were centrifuged at 5000 rpm for 5 minutes and the sera were transferred to Eppendorf tubes and stored at -20 oC for up to 3 months until analyzed.

2.2. ELISA Analysis

Leishmania antibody IgG levels in the serum of dogs were measured using a commercially available ELISA Kit (Shanghai Coon Koon Biotech Co., Ltd, China, CK-bio-24415) according to the manufacturer's instructions. The method relies on the ability of biotinylated detection antibodies to capture the *leishmania* antibody IgG present in serum. Before 50 µL of positive and negative control were placed into the positive and negative wells, while 10 µL serum samples were diluted with 40 µL sample diluent and added to the remaining wells. Next, 100 µL HRP conjugate reagent was added to each well and incubated at 37 oC for 60 min. After the incubation step, the plate was inverted to empty all contents and were washed 5 times with 400 µL of wash solution. Subsequently, 50 µL of chromogen solution A and then 50 µL of chromogen solution B were added to each well respectively and incubated at 37 oC for 15 min. After that, 50 µL stop solution was added to each well. Absorbances were measured in the microplate reader at a wavelength of 450 nm within 15 min after having added the stop solution. The ELISA test was considered valid, if the mean absorbance of the positive control was greater than 1.00 and the mean absorbance of the negative control was less than 0.15. The evaluation of the test result was based on the critical cut off value. The critical cut off value was calculated by adding 0.15 to the average absorbance value obtained in the negative control wells. The absorbance of the sample was considered negative if less than this cut-off value and positive if greater than or equal to this cut-off value.

2.3. Statistical Analysis

Statistical analysis of the data was performed using SPSS 26 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Gender and age variables were compared using the Chi-square test. Differences with a value of <0.05 were considered statistically significant as a result of the analysis.

3. RESULTS

In the study, 84 dogs consisting of Kangal, Golden Retriever, Malinois, and mixed breeds were used. Information was obtained from the patient owners that all of the dogs lived in a home and went outside at certain times of the day. When the dogs were categorized according to age, 26 were <2 years old, 58 were 2≥ years old, and when classified according to gender, 45 dogs were male and 39 dogs were female. It was determined that 5 (19.2%) dogs aged <2 years and 7 (12.1%) dogs aged ≥2 years were seropositive for *leishmania*. 7 (17.9%) female dogs and 5 (11.1%) male dogs were found to be seropositive for *leishmania*. The prevalence of *leishmania* seropositive was found to be 14.2% in the study. Gender ($p = 0.562$) and age ($p = 0.501$) factors had no effect on the occurrence of *leishmania* infection. The obtained

epidemiological data, animal number information, percentage expressions, and Chi-square test results are presented in Table 1.

Table 1. Prevalence of *leishmania* infection and epidemiological data based on sex, age and race and Chi-square test results

Epidemiological Data	Number and Rate of Dogs Tested	Number and Rate of Positive Dogs	Chi-square test P values
Breed			
Kangal	15 (%17.8)	5 (%33.3)	
Golden Retreiver	2 (%2.3)	-	
Malinois	23 (%27.3)	1 (%4.3)	
Mix Breed	44 (%52.3)	6 (%13.6)	
Sex			0.562
Male	45 (%53.5)	5 (%11.1)	
Female	39 (%46.4)	7 (%17.9)	
Age			0.501
<2	26 (%30.9)	5 (%19.2)	
2≥	58 (%69.04)	7 (%12.1)	
Total	84	12(%14.2)	

4. DISCUSSION AND CONCLUSION

Leishmaniasis is considered a neglected tropical zoonotic disease that poses a potentially fatal risk to humans and causes serious public health problems in developing countries. Dogs that show clinical signs of disease can be diagnosed and managed. However, asymptomatic dogs are potential sources of *leishmania* vector infection and facilitate the transmission cycle of the disease [9]. In order to adequately implement the necessary control measures for *leishmania* disease and reduce transmission, its prevalence in a region must be known. In this study, the prevalence of *leishmania* in asymptomatic dogs in Bingöl province is reported for the first time.

Various serological diagnostic tests, including indirect immunofluorescence tests, direct agglutination tests, ELISA tests, and cross immunoelectrophoresis, are used in the diagnosis of leishmaniasis [10]. Molecular tests can go beyond the limitations found in serological tests, but it is stated that they are not fully applicable in field studies due to high cost and laboratory environment requirements [11]. Immunochromatographic tests and direct agglutination tests are frequently used in the field and their confirmation is done with ELISA tests that allow the evaluation of more animals [12]. In this direction, in the presented study, it was preferred to investigate the prevalence of leishmaniasis in dogs in Bingöl province with the ELISA method, similar to the studies conducted by Zerpa et al. [13] and Arslan et al. [14].

Leishmaniasis is more common seen in Mediterranean regions with tropical and subtropical climates due to the long lifespan and breeding season of sandflies and is considered endemic in these areas [15]. Among the regions of Turkey considered endemic for leishmaniasis, seropositivity rates have been reported as 14.1% in Aydın/Kuşadası, 4.6% in İzmir/Selçuk, 3.8% in Manisa/Turgutlu, 22% in Muğla/Bodrum [5], 27.18% in Adana [16], and 18.5% in Mersin [17]. Similarly, high prevalence rates have been detected abroad, including 18.6% in South America [8] and 33.1% in Venezuela [13] where it is considered endemic. However, as a result of changing climatic conditions and animal movements, the disease is also encountered in non-endemic regions. When the prevalence in non-endemic regions is evaluated, it has been detected as 58.1% in Germany [18], 2.72% in valley villages and 11.32% in villages on the foothills of mountains in France [19], 4.7% in northern Spain, 3% [20], in Çankırı province in Turkey [21], 2.92% in Istanbul province [22], and 0% in Diyarbakır province [7]. In this study, it was determined that the seroprevalence of leishmaniasis in dogs in the non-endemic Bingöl province was 14.2%, and this finding was higher than in the non-endemic regions of Çankırı, İstanbul, Diyarbakır, France, and lower than in the non-endemic region of Germany. The differences in the seropositivity prevalence rates of leishmaniasis in non-endemic regions may be related to the results of climate conditions, animal movements, types of analysis, and variable protective measures.

Age is considered a risk factor for leishmaniasis, for which no consensus has been reached [20,23]. Dantas-Torres et al. [24] and Gálvez et al. [25] reported higher *leishmania* seropositivity rates in young dogs, while Miró et al. [20] and Selim et al. [26] reported higher seropositivity rates in older dogs. In contrast to these, in the study conducted by Miranda et al. [23] and Almeida et al. [27], age was not seen as a risk factor, while in the study conducted by Gálvez et al. [28], it was reported that a bimodal age distribution could be formed, with one peak in young dogs (1-2 years old) and the second peak in old dogs (7-8 years old). The reason for the variability in the age factor among leishmaniasis seropositive studies is explained as the immaturity of the immune system in young people or the fact that resistant animals are infected at a young age and the immune system weakens in older ages and as a result of various diseases [23]. In this study, according to the Chi-square test result, age factor was found to be a factor that did not affect leishmaniasis seropositivity ($p = 0.501$). The results obtained in the present study are consistent with those determined by Almeida et al. [27] and Miró et al. [20]. It is thought that this variability between studies is related to race, categorization of age range, environmental factors, and population size.

The gender factor is considered a risk factor in some studies [5, 28], while it is considered insignificant in some studies [14, 29]. While a study by Dantas Torres et al. [24] reported higher seropositivity in male dogs, studies by Almeida et al. [27] and Cortes et al. [30] found no difference between the genders. In studies with higher prevalence in males, this was attributed to the fact that male dogs exhibit more roaming behaviour [26]. Although the number of male dogs was relatively higher in this study, the Chi-square test between male and female dogs did not reveal a significant difference in leishmaniasis seropositivity ($p=0.562$). The results obtained in the present study are similar to those of Almeida et al. [27], Selim et al. [26], and Cortes et al. [30]

but not in agreement with those found by Dantas-Torres et al. [24].

In conclusion, in this study, the prevalence of leishmaniasis was detected in %14.2 asymptomatic dogs in Bingöl province, which is not an endemic region. This finding shows the presence of *leishmania* infection in the region and poses a significant risk to public health. The data obtained may form an important basis for the development of effective control strategies and public health measures in our country. In further studies, it will be useful to evaluate different risk factors in the prevalence of *leishmania*, to test various diagnostic methods and to conduct research in a larger study population.

REFERENCES

[1] Bañuls AL, Hide M, Prugnolle F. *Leishmania* and the leishmaniases: a parasite genetic update and advances in taxonomy, epidemiology and pathogenicity in humans. Adv Parasitol. 2007;64:455-458.

[2] Bolukbas CS, Pekmezci GZ, Gurler AT, Pekmezci D, Guzel M, Hokelek M, et al. Evidence of *Leishmania spp*. antibodies and DNA in dogs in the Middle Black Sea Region of Turkey. Ankara Üniv Vet Fak Derg. 2016;63(2):111-114.

[3] Çelik BA, Çelik ÖY, Şahin T. Retrospective Evaluation of Canine Leishmaniasis in Turkey. FÜ Sağ Bil Vet Derg. 2019;33(2):123-130.

[4] Bilgin Z, Turan N, Yilmaz H, Ferroglio E, Tuzer E. Prevalence of leishmaniosis in dogs in Istanbul, Turkey determined by using PCR. J Hellenic Vet Med Soc. 2015;66(2):106-112.

[5] Atasoy A, Pasa S, Ertabaklar H. Seroprevalence of canine visceral leishmaniasis around the Aegean cost of Turkey. Kafkas Üniv Vet Fak Derg. 2010:16(1);1-6.

[6] Dogan N, Ozbel Y, Ozensoy Toz S, Dinleyici EÇ, Bor O. Sero-epidemological survey on canine visceral leishmaniasis and the distribution of sandfly vectors in Northwestern Turkey: Prevention strategies for childhood visceral leishmaniasis. J Trop Pediatr. 2006;52(3):212-217.

[7] İçen H, Babür C, Bademkıran S, Çelebi B, Şimşek A, Özyurtlu N, et al. Diyarbakır bölgesindeki sahipsiz köpeklerde toxoplasmosis, leishmaniasis ve listeriozisin seroprevalansı. Türkiye Parazitol Derg. 2010;34(1):6-10.

[8] Priolo V, Ippolito D, Rivas-Estanga K, De Waure C, Martínez-Orellana P. Canine leishmaniosis global prevalence over the last three decades: a meta-analysis and systematic review. Comp Immunol Microbiol Infect Dis. 2024;112;102211.

[9] Lopes JV, Michalsky ÉM, Pereira NCL, Paula AJVD, Souza AGM, Pinheiro LC, et al. Canine visceral leishmaniasis in area with recent *Leishmania* transmission: prevalence, diagnosis, and molecular identification of the infecting species. Rev Soc Bras Med Trop. 2020;53:e20200141.

[10] Pozio E, Gradoni L, Bettini S, Gramiccia M. Leishmaniasis in Tuscany (Italy): VI. Canine leishmaniasis in the focus of Monte Argentario (Grosseto). Acta Trop. 1981;38(4):383-393.

[11] Pessoa-e-Silva R, Vaitkevicius-Antão V, de Andrade, TAS, de Oliveira Silva, AC, de Oliveira GA, Trajano-Silva LAM, et al. The diagnosis of canine visceral leishmaniasis in Brazil: Confronting old problems. Exp Parasitol. 2019;199:9-16.

[12] Travi BL, Cordeiro-da-Silva A, Dantas-Torres F, Miró G. Canine visceral leishmaniasis: Diagnosis and management of the reservoir living among us. PLOS Negl Trop Dis. 2018:12(1);e0006082.

[13] Zerpa O, Ulrich M, Negrón E, Rodríguez N, Centeno M, Rodríguez V. Canine visceral leishmaniasis on Margarita Island (Nueva Esparta, Venezuela). Trans R Soc Trop Med Hyg. 2000:94(5), 484-487.

[14] Arslan S, Öncel T, Yenilmez K, Turan N. Detection of *Leishmania* infantum seropositivity in dogs by ELISA technique in Thrace region of Turkey. Eurasian J Vet Sci. 2019;35(3);165-169.

[15] Espejo LA, Costard S, Zagmutt FJ. Modelling canine leishmaniasis spread to non-endemic areas of Europe. Epidemiol Infect. 2015;143(9):1936-1949.

[16] Karakuş M, Töz S, Ertabaklar H, Paşa S, Atasoy A, Arserim SK, et al. Evaluation of conjunctival swab sampling in the diagnosis of canine leishmaniasis: A two-year follow-up study in Çukurova Plain, Turkey. Vet Parasitol. 2015;214(3-4):295-302.

[17] Utuk A, Gokmen G, Bolacalı M, Balkaya I, Simsek S. A serologic survey on canine leishmaniasis in Kocaeli, Sakarya, Mersin and Elazığ provinces of Turkey. Isr J Vet Med. 2018; 4: 3-7.

[18] Geisweid K, Weber K, Sauter-Louis C, Hartmann K. Evaluation of a conjunctival swab polymerase chain reaction for the detection of *Leishmania* infantum in dogs in a non-endemic area. Vet J. 2013;198(1):187-192.

[19] Dereure J, Vanwambeke SO, Malé P, Martinez S, Pratlong F, Balard Y, et al. The potential effects of global warming on changes in canine leishmaniasis in a focus outside the classical area of the disease in southern France. Vector Borne Zoonotic Dis. 2009;9(6):687-694.

[20] Miró G, Checa R, Montoya A, Hernández L, Dado D, Gálvez R. Current situation of *Leishmania* infantum infection in shelter dogs in northern Spain. Parasit Vectors. 2012;5:1-7.

[21] Yucesan B, Ozkan O, Tuncer S, Ocal Z. Molecular Detectıon of Canine Leishmaniasis in Northern Anatolia, Turkiye. Iran J Parasitol. 2023;18(4);483.

[22] Koenhemsi L, Fabrizio V, Mariella P, Antonella M, Or E. Seroprevalence of leishmaniosis among healthy dogs in Istanbul. Isr J Vet Med. 2020;75(1):31-34.

[23] Miranda S, Roura X, Picado A, Ferrer L, Ramis A. Characterization of sex, age, and breed for a population of canine leishmaniosis diseased dogs. Res Vet Sci. 2008;85(1):35-38.

[24] Dantas-Torres F, de Brito MEF, Brandão-Filho SP. Seroepidemiological survey on canine leishmaniasis among dogs from an urban area of Brazil. Vet Parasitol. 2006;140(1-2):54-60.

[25] Gálvez, R., Montoya, A., Cruz, I., Fernández, C., Martín, O., Checa, R., et al. Latest trends in

Leishmania infantum infection in dogs in Spain, Part I: Mapped seroprevalence and sand fly distributions. Parasit Vectors, 2020;13:1-12.

[26] Selim A, Shoulah S, Abdelhady A, Alouffi A, Alraey Y, Al-Salem WS. Seroprevalence and risk factors associated with canine leishmaniasis in Egypt Vet Sci. 2021;8(10):236.

[27] Almeida ADBPFD, Sousa VRF, Cruz FACSD, Dahroug MAA, Figueiredo FB, Madeira MDF. Canine visceral leishmaniasis: seroprevalence and risk factors in Cuiabá, Mato Grosso, Brazil. Rev Bras Parasitol Vet. 2012;21:359-365.

[28] Gálvez R, Miró G, Descalzo MA, Nieto J, Dado D, Martín, O, et al. Emerging trends in the seroprevalence of canine leishmaniosis in the Madrid region (central Spain). Vet Parasitol. 2010;169(3-4):327-334.

[29] Bakirci S, Bilgiç HB, Köse O, Aksulu A, Hacılarlıoğlu S, Erdoğan H, et al. Molecular and seroprevalence of canine visceral leishmaniasis in West Anatolia, Turkey. Turk J Vet Anim Sci. 2016;40(5):637-644.

[30] Cortes S, Vaz Y, Neves R, Maia C, Cardoso L, Campino L. Risk factors for canine leishmaniasis in an endemic Mediterranean region. Vet Parasitol. 2012;189(2-4):189-196.