

Seroprevalence of Canine Visceral Leishmaniasis Around the Aegean Cost of Turkey ^{[1][2]}

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[1] *This study was financially supported by Adnan Menderes University Research Fund (BAP-Project Number VTF-04002)*

[2] *This study was approved by Adnan Menderes University Ethics Committee with no. B.30.2.ADU.0.00.00/124-HEK/2005/0011*

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Makale Kodu (Article Code): KVFD-2009-186

Summary

Visceral leishmaniasis (VL) is an infectious and zoonotic disease of people, wild and domestic mammals residing in tropical and subtropical climate zone. VL is a potential risk for human health and regarding the economical loss as a result of the disease, endemic areas should be recognized the effective implementation of control measures, and detailed epidemiological surveys should be performed in an attempt to obtain information about the incidence and prevalence of the disease in locations where the disease is diagnosed. Besides, in our country, most of the traveling has been drifted to areas dominating Mediterranean climate increasing the importance of investigations on visceral leishmaniasis in Aegean region. In this study, it was aimed to determine the seroprevalence of the infection from Selçuk/İzmir, Turgutlu/Manisa, Aydın city province, Kuşadası/Aydın, Marmaris/Muğla, Bodrum/Muğla in stray dogs that are regarded as the main reservoirs for Visceral Leishmaniasis. For this purpose, anti-*leishmania* antibodies were determined with immunofluorescence antibody diagnostic procedure in serum samples obtained from untreated and unprotected 300 stray dogs located in these settlements. According to the results of the study 27 (9%) out of a total 300 dogs were infected with canine visceral leishmaniasis (CanL). It was considered that the result of this study may be used as a reference for further studies regarding human and dogs residing in Turkey.

Keywords: *Leishmaniasis, Dog, Seroprevalence, Turkey*

Kıyı Ege Bölgesindeki Köpeklerde Visseral Leishmaniasis'in Seroprevalansı

Özet

Visceral leishmaniasis (VL) tropikal ve subtropikal iklim kuşağında yaşayan insanların, vahşi ve evcil memeli hayvanların infeksiyöz ve zoonotik bir hastalığıdır. VL'nin insan sağlığı için potansiyel bir risk oluşturması ve bu hastalığın ulusal ekonomideki kayıplar açısından düşünüldüğünde hastalığın saptandığı bölgelerde gerekli korunma önlemlerinin alınması ve bu bölgelerde prevalans ve insidansı hakkında detaylı epidemiyolojik araştırmaların yapılması gerekmektedir. Ülkemizde, seyahatlerin özellikle Akdeniz ikliminin hakim olduğu bölgelere kayması, visceral leishmaniasis'in son yıllarda Ege Bölgesinde araştırılmasının önemini arttırmaktadır. Bu çalışmada, Ege bölgesinde İzmir/Selçuk, Aydın/Merkez, Aydın/Kuşadası, Manisa/Turgutlu, Muğla/Bodrum, Muğla/Marmaris'te hastalığın en önemli rezervuarı olan sokak köpeklerinde köpek visceral leishmaniasis'in (CanL) seroprevalansının belirlenmesi amaçlanmıştır. Bu amaçla, bu yerleşim alanlarında tedavi edilmemiş ve sahihsiz 300 adet sokak köpeğinden toplanan serum örneklerinde immun floresan antikor tekniği ile anti-*leishmania* antikorları belirlenmiştir. Çalışmanın sonuçlarına göre, toplam 300 köpeğin 27'sinin (%9) enfekte olduğu tespit edilmiştir. Ülkemizde bu çalışmanın sonuçlarının insanlarda ve köpeklerde gelecekte yapılacak çalışmalarda referans olarak kullanılabileceği kanısındayız.

Anahtar sözcükler: *Leishmaniasis, Köpek, Seroprevalans, Türkiye*



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INTRODUCTION

Leishmaniasis is a zoonotic disease caused by parasites of the genus *Leishmania* parasites infecting numerous mammal species, including humans ¹. These parasites are transmitted by the bite of female sandflies - *Phlebotomus* in the old world and *Lutzomyia* in the new world. Visceral leishmaniasis (VL) is considered by far the most severe form of leishmaniasis and is often fatal if left untreated. Over the last 10 years, endemic region have been widened and there has been a sharp increase in the number of recorded cases ². According to WHO (2005) and available data, leishmaniasis is a major global health problem and the dogs are the most important reservoir of *Leishmania infantum* for human infection. The disease is endemic in the world with new 500.000 VL cases per year.

Canine visceral leishmaniasis (CanL) causes various clinical signs, such as skin lesions, weight loss, local or generalized lymphadenopathy, ocular lesions, epistaxis, anaemia, wasting, swollen limbs and joints, lameness, renal failure and chronic diarrhoea. Because of the variable clinical symptoms and adversity of detection of the parasite, a presumptive diagnosis involving various serological tests (indirect immunofluorescence assays, enzyme-linked immunosorbent assay, western blotting and direct agglutination) and polymerase chain reaction should be performed ³⁻⁵.

Seroprevalence of CanL in Mediterranean countries ranges from 1% to 37% ⁶. As the dog is being considered to be the reservoir of VL and is responsible for subsequent transmission of disease to human, the attention on the number of seropositive dogs is increased in some countries. Previous reports indicated that the sero-

prevalence of CanL ranges between 2.58% and 28.26% in different regions of Turkey ⁷.

The aim of the present study was to determine the seroprevalence of the anti- *Leishmania* antibodies among stray dogs and observation on the disease situation in Aegean region in Turkey.

MATERIAL and METHODS

Study Area and Animals

Three hundred (300) untreated and protected mongrel stray dogs, aged between 2-8 years, captured and kept in municipal kennels located in Aydın Province, Kusadasi/Aydın, Selçuk/Izmir, Turgutlu/Manisa, Bodrum /Mugla and Marmaris/Mugla (Fig. 1) were used in the present study. The dogs were examined for clinical signs of CanL including weight loss, pale mucous membranes, skin and hair coat alterations, enlarged lymph nodes, onychogryphosis, epistaxis and ocular lesions. One hundred ninety-four (64.6%) of the dogs were female and 106 (35.4%) were male. The breed, age, and gender of each dog were recorded before sampling.

Sample and Data Collection

Blood samples (5 ml) were collected by cephalic vein. The samples were centrifuged at 3000 rpm for 5 min. Then, the serum was separated and stored at -20°C until serological diagnosis.

Serological Test

Serum titers of specific anti-*Leishmania* antibody were determined by immunofluorescence antibody test (IFAT). The IFAT was performed using standard procedures. Titres ≥ 128 were considered as seropositive ⁸.



Fig 1. Localization of samplings

Şekil 1. Örnekleme yapıldığı bölgeler

Parasitological Examination

Popliteal lymph node puncture was performed in 16 seropositive dogs. Lymph node aspirates were stained with Giemsa and examined by microscopy for detection of *L. infantum* amastigotes in dogs.

Statistical Analysis

Breed, age, gender and the proportion of positive animals were compared using the non parametric Chi-square test. Correlation between parameters was estimated using Pearson's correlation test. The differences were considered statistically significant with a probability (P) value of <0.05. The statistical package SPSS (Version 10.0) was used for analyses.

RESULTS

Twenty seven (9%) of the 300 dogs were found to be positive by IFAT. The seropositivity ratios among dogs were found to be 14.1% (11/78) in Kuşadası, 4.6% (3/65) in Selçuk, 3.8% (1/26) in Turgutlu, 2.0% (1/50) in Marmaris and 22% (11/50) in Bodrum (Table 1). All dogs from Aydın Province were found to be negative.

Table 2. Evaluation of seropositive dogs naturally infected with *Leishmania* spp

Table 2. Doğal olarak *Leishmania* spp. ile enfekte seropositif köpeklerin değerlendirilmesi

No	City/Village	Bred	Age (Year)	Sex	IFAT 1/	Smear (Amastigotes)	Clinical Signs
1	Kusadasi	Mongreal	5	Female	256	POS	WL, SI, LA, PM, ONG
2	Kusadasi	Mongreal	4	Female	256	POS	LA, OCL
3	Kusadasi	Mongreal	6	Male	128	Nd	PM, ONG
4	Kusadasi	Mongreal	3	Male	256	NEG	SI, LA
5	Kusadasi	Mongreal	4	Male	256	POS	WL, SI, LA, PM, ONG
6	Kusadasi	Mongreal	3	Female	128	NEG	LA, PM
7	Kusadasi	Mongreal	6	Female	256	POS	-
8	Kusadasi	Mongreal	5	Female	256	POS	EP, LA
9	Kusadasi	Mongreal	3	Female	512	POS	WL, SI, LA, PM, ONG
10	Kusadasi	Mongreal	3	Female	512	POS	SI
11	Kusadasi	Mongreal	4	Male	256	NEG	WL, SI, LA, PM
12	Selcuk	Mongreal	3	Male	128	Nd	WL, SI, LA,
13	Selcuk	Mongreal	4	Female	128	Nd	LA
14	Selcuk	Mongreal	2	Male	128	NEG	SI, LA, PM
15	Turgutlu	Mongreal	5	Female	256	POS	WL, SI, ONG
16	Marmaris	Mongreal	6	Male	128	Nd	LA
17	Bodrum	Mongreal	4	Male	128	Nd	LA, PM
18	Bodrum	Mongreal	3	Male	128	NEG	WL, LA, OCL
19	Bodrum	Mongreal	3	Female	128	Nd	SI, ONG
20	Bodrum	Mongreal	4	Male	512	POS	SI, LA
21	Bodrum	Mongreal	6	Male	128	Nd	LA, PM
22	Bodrum	Mongreal	7	Male	128	Nd	SI, LA, PM
23	Bodrum	Mongreal	3	Male	128	Nd	WL, SI, LA, PM
24	Bodrum	Mongreal	5	Female	512	POS	PM, ONG
25	Bodrum	Mongreal	4	Male	128	Nd	WL, LA
26	Bodrum	Mongreal	3	Female	128	Nd	-
27	Bodrum	Mongreal	4	Male	4096	POS	WL, SI, LA, PM, EP

WL, weight loss; **SI**, skin involvement; **LA**, lymphadenopathy; **PM**, pale mucosae; **OCL**, ocular lesions; **EP**, epistaxis; **ONG**, onychogryphosis; **POS**, positive; **NEG**, negative; **Nd**, not done

Table 1. Distribution of CanL in 6 locations in aegean region

Table 1. Ege bölgesindeki 6 bölgede CanL'in dağılımı

City	Dogs sampled		Seropositive	
	n		n	%
Kusadasi/Aydin	78		11	14.1
Selcuk /Izmir	65		3	4.6
Turgutlu/Manisa	26		1	3.8
Marmaris/Mugla	50		1	2
Bodrum/Mugla	50		11	22
Aydin City Province	31		-	-
Total	300		27	9

Popliteal lymph node aspiration was performed in 16 seropositive dogs with lymphadenopathy. Only 11 dogs were found to be positive.

Twenty five of 27 seropositive dogs showed two or more clinical signs of CanL and two of the seropositive dogs were asymptomatic. Weight loss, skin disorders, pale mucousal membranes and lymphadenopathy were the most commonly observed clinical signs in seropositive dogs (Table 2).

One hundred ninety-four (64.6%) of dogs were

female and 106 (35.4%) were male. Infections ratio in female dogs were 6.2% (12/194), in male dogs were 14.2% (15/106). Twelve (44.4%) of the seropositive dogs (12/27) were female, while fifteen (15/27) were male. Seropositivity of the male animals was significantly ($P < 0.05$) different than that of the females one.

DISCUSSION

Because of dogs are the most important reservoir of *L. infantum* for human VL and are one of the main risk factor for immunosuppressive patients, determining the infection rate and the distribution of active foci are essential. Epidemiological surveys on leishmaniasis are basic research to understand the situation and to set up control strategies in endemic regions ⁹.

The determination of infection levels for canine populations depends on the evaluation of several parameters including disease symptoms, anti-leishmanial antibody titres, and microscopical detection of the parasite ¹⁰. In a huge dog population, serological diagnostic procedures have been used frequently for investigation of seroprevalence of CanL, because of the variable clinical signs, the presence of asymptomatic dogs and low sensitivity of parasitological methods ^{3,4}. The evidence provided in the present study confirms previous observations demonstrating that CanL can be diagnosed by IFAT because of its higher sensitivity ⁸. In this study, blood samples were collected from 300 stray dogs and the disease was determined by IFAT.

Although CanL is known to exist in Turkey ⁵, for epidemiological request, more investigation need to be put forward. Previous studies indicated that the seroprevalence of CanL ranged between 2.58% - 28.26% in different parts of Turkey ^{5,11,12}. Result of several studies conducted in dogs suggested that CanL infection has been widespread in Turkey. In agreement with the findings of previous studies, seroprevalance of CanL obtained in the present study is 9% ^{5,10-14}.

Seroprevalance of CanL varies to vary in endemic regions, in relationship with the presence of vector population, ecological conditions such as moisture and climate and immunological response of the reservoir population ^{4,15}. In the present study, the numbers of infected dogs were eleven out of seventy eight (14.1%) in Kuşadası, three out of sixtyfive (4.6%) in Selcuk, one out of twenty six (3.8%) in Turgutlu. These results have been supported by the previous studies on the source of the disease and the presence of *Phlebotomus* sand flies ⁵. We were unable to detect any positive dog from Aydın Province. This result was also similar with previous

results ⁵. This could be explained by one or more referred factors that are not predisposing the disease in this region.

This is the first study on the investigation of the prevalence of CanL in Marmaris and Bodrum. One dog (2%) in Marmaris and eleven dogs (22%) in Bodrum had been infected with *Leishmania spp.* suggesting that *Phlebotomus* sand flies are apparently close to the reservoir and source of the disease. Intensified travelling through these places increased the importance of this study's findings.

Studies have been notified that seroprevalence of CanL has been indicated different ratios in nearby countries and the Mediterranean basin. Seroprevalence of CanL was notified as 0.7-8.5% in Portugal ¹⁵. Moreno and Alvar ² have also shown that seroprevalence of the disease was 14.5% in Apulia Region and it was found to be 24% in Tuscany Region in Italy ². In another study carried out in Italy, 326 dogs kept from to the Santa.

Anastasia were screened serologically using IFAT, seropositivity ratio was also found to be 40.4% ¹⁶. It was determined as 44.9% in Sicily ¹⁵. In Greece, the disease is endemic ¹⁷ with a 22.4% seroprevalence ratio ¹⁸. The seroprevalence of CanL is 6% in Tunisia ¹⁹, 37.5% in Algeria ²⁰, 17.3% in Malta ²¹, 10% in Cyprus ²² and 11.5% in Israel ²³. In the present study, the seroprevalence of infection found in Aegean region (9%) in Turkey is similar with Israel, Tunisia, Cyprus; but showing lower ratios compared to the other countries when nearby countries and the Mediterranean basin taking into consideration. These variable prevalence ratios could have been related with warming climate in the South-eastern Mediterranean countries that enhances the propagation of the sandfly population, changes in agricultural pesticide practices, increase in imported dog populations, and ineffective chemotherapeutic management of symptomatic dogs ⁷.

CanL has not been depended on age predisposition factor ²; however, the disease has rarely been seen in young (≤ 2 old) and elderly (especially older than 8 years) dogs. Similarly to the other authors' reports, we have found the life range of infected dogs between 3 to 7 years ³. We should consider the fact that CanL has a long incubation period and untreated infected dogs should have short life span.

In this study, we have determined that 15 of 27 (55.6%) seropositive dogs were male and twelve were female (44.4%). Twelve (6.2%) seropositive dogs have been determined among 194 female dogs and fifteen (14.1%) in 106 male dogs. Male seropositive dog number was significantly higher ($P < 0.05$) from females

in respect with some author reports ^{2,3,5,10}.

Skin lesions, weight loss, local or generalised lymphadenopathy and pale mucous membranes are reported as major clinical signs in CanL ¹³. In our study, clinical symptoms could not be observed in two of twenty seven seropositive dogs. Leishmaniasis had a prolonged asymptomatic period and unprotected dogs had severe clinical symptoms. Increased symptomatic dog numbers should possibly have been related with unsuitable conditions in kennel, insufficient immune response and severity of disease, untreated dogs and concurrent disorders.

Definitive diagnosis of CanL is based on detection of amastigotes in stained smears of aspirates of bone marrow or lymph node aspirates ¹⁴. However, amastigotes form could not always been detected ^{13,17}. In respect with some author's reports, we didn't determine the amastigote form in all of the examined aspirates (11/16). We think that, it could have probably been related with severity and stage of the disease.

In this study, we have determined the seroprevalence of CanL in Selçuk/İzmir, Turgutlu/Manisa, Aydın Province, Kusadası/Aydın, Marmaris/Muğla and Bodrum/Muğla settlements in stray dogs. It was considered that the result of this study may be used as a reference to further studies on human and dogs. In Turkey, there is need for sufficient information about the disease for the veterinary and human practitioners so that, necessary protection, precautions should be made in the regions. Considering the facts that CanL is a potential risk for human disease and may cause important economical losses. According to our opinion, more seroprevalence studies should be performed regularly in order to detect symptomatic and asymptomatic dog populations and in human beings.

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