

Seroprevalence of Leishmaniosis Among Healthy Dogs in Istanbul

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ABSTRACT

Canine leishmaniosis (CanL), is one of the important zoonotic health problems of dogs, however it is not a notifiable disease in veterinary medicine in Turkey as yet, due to insufficient seroprevalence studies. Istanbul is a crossroad between Europe and Asia, which is of importance in order to evaluate the prevalence of CanL for better control policies. For this purpose, blood was taken from 171 clinically healthy dogs. Serum samples were examined using the Immunofluorescence antibody (IFA) serological method in a leishmania reference laboratory of the Italian government. Serum samples were diluted 1:40-1:5,120, where the dilution of 1:40 was considered positive. Five dogs were found positive (2.92%) at 1:160 and 1:320 dilutions and three dogs were positive at a 1:40 dilution. The prevalence rate was found to be similar to previous studies made in İstanbul. Due to the importance of the geographical location of İstanbul within Turkey, there is an elevated risk for distribution of CanL. For this reason, more seroprevalence studies are warranted for the control of the disease.

Key words: Leishmaniosis; Canine; IFA; Seroprevalence.

INTRODUCTION

Vector-borne diseases are a group of infections transmitted by the bite of arthropod species such as fleas, mosquitoes, ticks and sand flies that are infected with a pathological agent. The domestic dogs are the main reservoir of many zoonotic pathogens including leishmaniasis (1-3).

Leishmania infantum, is an important zoonotic protozoon that causes visceral and cutaneous leishmaniosis. Dogs are the main reservoirs of the parasite in most urban areas of the Mediterranean region (4). The parasite is transmitted through phlebotomine sand-fly bites (4). Dogs with visceral leishmaniosis often experience varied symptoms such as weight loss, local or generalized lymphadenopathy, chronic diarrhea, ophthalmic disorders, kidney disorders, hepatic diseases, epistaxis and limping (5-6). In addition to these symptoms there are some dogs which do not show any clinical signs. When these asymptomatic dogs are ignored and not

treated, 30-70% of the infected dogs from endemic regions may develop clinical disease within 2-3 years of diagnosis (7). In addition, these dogs play an active role in the transmission of the disease acting as a source of infection for phlebotomine vectors (8-9). Therefore, identification of asymptomatic dogs is of great importance for controlling the disease.

Leishmaniosis is endemic Asia, southern Europe, South and Central America, Africa, and America in more than 70 countries (2, 10). The rate of the disease is between 1% and 37% in the studies carried out on the Mediterranean coasts (4). In addition, seropositive animals have been detected in Bursa, İzmir, İstanbul, Karabük, Kocaeli, Konya, Manisa, Muğla, Ankara, Sivas, and Şanlıurfa in Turkey (11-13). The first leishmania case in İstanbul was reported by Gönül *et al.* in 2000 (12).

Leishmania is predictably present all over Turkey due to the presence of the transmitting fly species all over our

country (14). Additionally, imported sick or infected dogs constitute a veterinary and public health problem in non-endemic countries. In addition to this, it is sufficient for maintaining the infection even with a low proportion of sand flies with *L. infantum* (10). İstanbul is a crossroad between Europe and Asia which makes it important to evaluate the prevalence of CanL within the city for designing control policies all over the Turkey.

The aim of this study was to investigate the prevalence rates of *Leishmania spp.* in healthy household dogs living in İstanbul, using serological methods.

MATERIALS AND METHODS

A total of 171 dogs of different breeds, ages and genders were included in the present study for a period of a year. All animals were presented to the clinics of İstanbul University-Cerrahpaşa, Internal Medicine department for health checks. A full medical record was kept for each individual dog and recorded. Clinical signs at presentation were noted. Blood was collected from all animals by cephalic venipuncture in EDTA for hematology and clot tubes for serum. Complete blood cell count (CBC) (Mindray BC Vet China), plasma concentrations of glucose, urea, creatinine, AST, ALT, total protein, albumin, globulin, albumin/globulin ratio (calculated) and cholesterol were assayed (Tokyo Boeki TMS 1024, Japan).

Sera samples were centrifuged at 1000 RPM for 15 minutes and stored at -20°C until serological analyses were performed. The samples were sent to the leishmania reference laboratory of the Italian government for serological investigation. Samples were transported to the laboratory in coolers with ice packs. The Immunofluorescence antibody (IFA) method on dogs was performed according to OIE Terrestrial Manual chapter 2.1.11. (10, 15). The IPT1 ZMON1 strain was used as antigen fixed on multispot microscope slides (Bio-Merieux, Marcy L'Etoile, France) in an acetone bath. The canine sera were serially diluted (1/40 to 1/5120) in phosphate-buffered saline (PBS), pH 7.2, and added to the antigen-coated wells. The slides were incubated for 30 minutes at 37°C. Positive and negative controls were included in each series of analyzed samples. Fluorescent staining was performed using an anti-dog IgG labelled with fluorescein isothiocyanate (Sigma-Aldrich, Saint Louis, MO, USA) diluted 1:200 in PBS. The slides were examined by a Leica

Table 1: The breeds of the dogs used in the study

Breed	Healthy Dogs	Dogs with Leishmania
Golden Retriever	57	–
Terrier	30	1
German Shephard	25	–
Rottweiler	24	–
Mixed-Breed	20	–
Kangal	5	2
Boxer	2	2
Pomerian	1	–
Beagle	1	–
Chow chow	1	–

Table 2: The ages of the dogs used in the study

Ages	Healthy Dogs	Dogs with Leishmaniosis
10-12 months	17	–
1-7 years old	97	4
7-14 years old	42	1
>14 years-old	10	–

DM 4000B fluorescence microscope (Leica, Heerbrugg, Switzerland). The cut off value, according to OIE Terrestrial Manual chapter 2.1.8., for a positive result was established at a serum dilution of 1:40 (15). The positive control consisted of a known titer serum of a dog with positive cultured isolation. The negative control consisted of serum from a dog with negative results on culture isolation attempts (3, 16).

RESULTS

All animals were healthy according to physical examination, CBC, and biochemical profile. Ninety-six the dogs were female. The breeds were as follows: Golden Retriever (n=57), Terrier (n=31), German Shephard (n=25), Rottweiler (n=24), and other breeds or mixed-breed dogs (n=34) (Table 1). Ages of the dogs ranged from 10-months old to 17 years old (the median age was nine-years). Seventeen of the dogs were between 10 month-12 month, 101 of them between 1-7 years old, 43 of them between 7-14 years-old, and 10 of them were older than 14 years-old (Table 2).

Of 171 samples, 5 (2.92%) were found seropositive. Antibody titres for these patients were 1/40 (3 dogs), 1/160 and 1/320. The dogs included four females and a male dog. Four of them were 1 year old and the other was 3-years-old.

Breeds included West Highland Terrier, Kangal and Boxer (Table 3).

DISCUSSION

Dogs with/without clinical signs compatible with leishmaniosis are the two common situations that the practitioners are confronted in the diagnosis of leishmaniosis. The reasons for the investigation of healthy dogs in this study was for an epidemiological survey, to evaluate the use of dogs as blood donors and the movement of the dogs from endemic to non-endemic areas (8, 17). Guarga *et al.* (17) determined that phlebotomine flies are more attracted to the healthy skin of the infected dogs. Disruption of the skin structure as the disease progresses reduces its attraction to the female sand flies. This behavior may give good reason for the increased risk for infection in asymptomatic dogs in *Leishmania* endemic areas (17).

Culture, cytology, histopathology, immunohistochemistry, direct immunofluorescence and PCR can be used in the diagnosis (9). Serological diagnosis is frequently used to evaluate the prevalence of the disease, as approximately 50% of infected dogs may not have any clinical signs (3, 10). In addition in a study of Fakhar in 2012, 88% of the PCR-positive and 67% of the seropositive dogs were asymptomatic (7).

CanL was first detected in the early 1950's in Turkey, however epidemiological studies were carried out only after 1993 (18). Serological leishmaniosis in dogs has a higher prevalence rates than human infection in Turkey (5). Rates of leishmaniosis in dogs differs between 1.45 and 27.5%, and the overall prevalence is 11.32% in Turkey according to previous studies (5-17).

Promastigotes of a local strain, MCAN/TR/95/EP17 with zymodem MON1, were used as antigen in the study of Coşkun *et al.* (6). In addition Aysul *et al.* used as the antigen *Leishmania infantum* (MON-1) strain, previously isolated from a dog. Nevertheless, Handemir *et al.* (11) used the same strain as in our study.

In our study 2.92% of the dogs were found seropositive. The prevalence rate was similar to those reported in previous studies conducted in İstanbul by the IFA serological method. Coşkun *et al.* (6) conducted a study in the western parts of Turkey and found all dogs sero-negative. All dogs were negative in another study in Kavacık, İstanbul (13). In addition, Handemir *et al.* (11) reported 3 stray dogs with 1/64 titers,

Table 3: Breed, age, gender and titer of the dogs with *leishmaniosis*

Breed	Age (years)	Gender	Titer
Kangal	1	Male	1/40
West Highland terrier	3	Female	1/160
Boxer	1	Female	1/320
Boxer	1	Female	1/40
Kangal	1	Female	1/40

however according to the cut-off levels, all of the 152 dogs were seronegative. Contrary to these studies Aysul *et al.* in 2012 have found 1.96% of the dogs above 1:128 titer and 5.88% of them at 1:64 (16).

In the battle against vector-borne diseases, it is important to keep in mind the potential of reservoirs spreading the disease. Therefore, any kind of disease occurrence that may occur in the İstanbul is important in terms of both human and animal health. İstanbul creates a bridge between two continents so that it is important to carry out prevalence studies from time to time to determine control policies. Serology results determined low-positive in clinically healthy dogs. When the serology of a dog is found positive, there is an indication that the dog has been exposed and may develop the disease in the near future and therefore close monitoring of the dog is required (9). Three dogs with low-positive results were determined in our study.

Molecular techniques such as polymerase chain reaction (PCR), nested PCR and real-time PCR are frequently used to detect the species of the pathogen. Although their performance depends on the methodology, molecular techniques have a high sensitivity and specificity (9,21). Fakhar *et al.* (21) determined higher rates with PCR than serology in their study. In a study carried out by Bilgin *et al.* (22), the percentage of positive dogs in İstanbul was determined 12.99% by using PCR. Only serological tests were used in the present study however if PCR were to be used higher rates of disease may have been detected.

CanL is mainly seen in the western and southern coasts of the Aegean and Mediterranean sea (3). However climatic factors and movement of the dogs can change the distribution (8, 19). In the present seroprevalance study, rates were lower than those in the Mediterranean coasts of Turkey due to the climatic conditions of the area shortening the sand fly's active season (3). This may have been the reasons of the low seroprevalance rates in İstanbul.

Breed, age and genetic background were found to be predisposing factors that may play a role in the development of the disease according to some studies. Susceptible breeds were Boxer, Cocker Spaniel, Rottweiler, and German Shepherd according to a study of Solano-Gallego *et al.* (10). Too few dogs were detected positive in our study making it difficult to conclude definitively regarding predisposition.

The most frequent CBC and blood biochemistry findings in leishmaniosis are normochromic, normocytic, non-regenerative anemia, increased protein and globulin levels and decreased albumin and albumin/globulin ratio (9-10). Our study group consisted of clinically healthy dogs and all CBC and serum biochemistry levels within normal limits.

In conclusion, the seroprevalence of leishmaniosis in İstanbul is similar based on previous published data in this study. Due to increased migration and travelling, ongoing climate changes as well as various aspects of globalization, there is an elevated risk for the introduction and spreading of CanL (19). For these reasons, the attention to leishmaniosis in dogs needs to be continued in order to prevent public health risks.

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