

Canine visceral leishmaniasis: seroprevalence survey of asymptomatic dogs in an endemic area of northwestern Iran

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Abstract Canine visceral leishmaniasis is a major public health problem that is endemic in tropical and sub tropical countries and is fatal in humans and dogs. In addition to symptomatic dogs, asymptomatic ones seem as source of *Leishmania infantum* infection. Thus surveillance and control programs of reservoir hosts are essential. This study aimed to evaluate the sero-prevalence of visceral leishmaniasis in asymptomatic domestic dogs from in an endemic area of north west, Iran. A cross sectional study was carried out in Meshkin-Shahr district during 2011–2012. Blood samples collected from 508 asymptomatic domestic dogs were tested by direct agglutination test. In this study 508 dogs (397 males and 111 females, mean age, 3.24 years) from western and eastern parts of the Meshkin-Shahr were examined. A total of 508 dogs examined 119 dogs (23.4 %) had antibodies (titers of $\geq 1:320$)

against *L. infantum*. Statistically significance was occurred between male (25.4 %) and female (16.2) sero-prevalence ($P = 0.042$). No statistically significance was observed between age groups ($P = 0.22$). Compared with previous studies it seems to increase sero-prevalence of visceral leishmaniasis in dogs in the studied areas caused by ecological changes. High proportion of asymptomatic but seropositive dogs emphasizes the importance of dogs without clinical signs in the epidemiology of zoonotic leishmaniasis. Thus, the necessity of using serological tests in asymptomatic dogs is recommended for disease control strategy.

Keywords Visceral leishmaniasis · Domestic dog · Sero-prevalence · Direct agglutination test · Iran

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Introduction

Visceral leishmaniasis (VL) is a major public health problem in the worldwide. It is an important zoonotic disease, which is endemic in 62 countries, and the population at risk is 200 millions (Desjeux 2004).

In Iran, human visceral leishmaniasis (HVL) has been occurred sporadically, but, the northwestern and southern areas are the main endemic foci of the disease. It is estimated that around 100–300 new cases annually (Fakhar et al. 2011; Barati et al. 2008), mostly reported from Meshkin-Shahr district, where disease has been endemic for many years (Edrissian et al. 1988).

The dogs are considered as the most important reservoir hosts for human infection (Cortes et al. 2012; da Silva et al. 2009). In the endemic areas of Iran, ownership dogs are described as major risk factors for HVL (Gavvani et al. 2002).

In 1913 the first report of canine visceral leishmaniasis (CVL) in Iran was described from Tehran (Neligan 1913) and subsequently, another case was reported from other areas (Pouya 1950, 1949).

In addition to symptomatic dogs, asymptomatic ones are also as source of *Leishmania infantum* infection (Moshfe et al. 2009; Cortes et al. 2012). Thus surveillance and control programs of reservoir hosts are essential (Silva et al. 2012; Tesh 1995).

Serological methods are used widely for evaluation seroprevalence of CVL (Kalayou et al. 2011). Direct agglutination test (DAT) is the first line sero-diagnostic method in most of developing countries (Teran-Angel et al. 2007). This method has the advantages including, simply, high sensitivity, specificity and reproducibility, also easy to perform and it doesn't need complicated equipments (Teran-Angel et al. 2007; Sousa et al. 2011). So it is the most suitable method for using in field assays (Sundar et al. 1998).

The purpose of this study was to evaluate the seroprevalence of visceral leishmaniasis in dog population from in an endemic area of north west, Iran.

Materials and methods

Study area

The investigation was conducted in north west of Iran, where HVL is endemic. Meshkin-Shahr district in the central northern part of the Ardabil province located at an altitude of 1490 m above sea level between longitudes 47°19' and 48°17' east and latitudes 38°57' and 38°13' north. Forty-two percent of their populations settle in urban areas, 58 % live in rural areas and small population have nomadic living.

The study area has moderate mountainous weather. In this area there are a lot of dogs with a friendly relationship with the human population which used as guard and herd dogs and also the stray dog is found. In this region, the amount of animal manure can be seen in yards and alleys which is considered as the resting place of dogs.

Blood sampling

A descriptive cross sectional study was carried out during 2011–2012. The serum samples were collected from all domestic dogs in villages where human visceral leishmaniasis had been reported at least 5 cases during 3 years ago. After documented general information including age and gender from each dog by interviewing dog owners, whole blood sample collected from 508 ownership dogs into 10 ml polypropylene tubes. All the samples centrifuged at 800×g for 5–10 min, and then serum aliquots were stored at –20 °C until examined. All of the serum samples were transferred

leishmaniasis laboratory in the School of Public Health, Tehran University of Medical Sciences and were tested by DAT.

DAT test

The DAT antigen used in this study was prepared in the protozoology unit of the School of Public Health, Tehran University of Medical Sciences and stored at 4 °C until used. The principal phases of the procedure for preparing the DAT antigen were mass production of promastigotes of *L. infantum* (Iranian strain) in RPMI1640 medium supplemented with 10 % fetal bovine serum, trypsinization of the parasites, staining with Coomassie Brilliant blue and fixing with 2 % formaldehyde (Mohebalı et al. 2005; el Harith et al. 1989; Harith et al. 1986).

The dog serum samples were tested by DAT according to the methods described by (el Harith et al. 1989; Mohebalı et al. 2011; Mohebalı et al. 2006). For initial screening purposes, two fold dilutions were prepared from 1:80 and 1:320 in dog samples. Sera with titers 1:80 were diluted further to give maximum serum dilution of 1:20480. Negative control antigen (antigen only) and known negative and positive controls were tested in each plate.

In the present study we considered antibody titers $\geq 1:320$ as cut off point for canine according to the previous studies (Bokaie et al. 1998; Mohebalı et al. 2005; Edrissian et al. 1996).

Data analysis

Sero-prevalence values of anti-*L. infantum* antibodies relative to gender, age groups were statistically compared using the Chi squared (χ^2) or Fisher's exact tests. Analyses were carried out using SPSS software version 18 with a probability (*P*) value of <0.05 as statistically significant and confidence interval of 0.95.

Results

In this study, were examined 508 ownership dogs, 397 males and 111 females with mean ages, 3.24 years. A total of 508 dogs examined in this study, anti *Leishmania* antibody have been shown in 256 dogs (50.4 %). It has ranged from 1:80 to 1:20480 titers (Table 1).

Statistically significant was occurred between male and female (*P* = 0.042). No statistically significant was observed between age groups (*P* = 0.22) (Table 2).

Discussion

CVL is a major zoonotic disease that is endemic in tropical and sub tropical countries and is fatal in humans and dogs

Table 1 Distribution of titers of anti *Leishmania infantum* antibodies in asymptomatic dogs in Meshkin-Shahr district, northwest of Iran (2011–2012)

Titer of antibodies	No.	Percentage
<1:80	251	49.4
1:80	109	21.5
1:160	29	5.7
1:320	34	6.7
1:640	42	8.3
1:1280	12	2.4
1:2560	7	1.4
1:5120	2	0.4
1:10240	2	0.4
1:20480	20	3.9
Total	508	100

Table 2 Sero-prevalence of canine visceral leishmaniasis by gender and age group in Meshkin-Shahr district, northwest of Iran (2011–2012)

	No. of examined (%)	DAT test positive ($\geq 1:320$)	
		No.	Sero-prevalence (%)
Gender			
Male	397 (78.1)	101	25.4
Female	111 (21.9)	18	16.2
Total	508 (100)	119	23.4
Age group (years)			
<2	164 (32.3)	29	17.7
2–5	264 (52)	67	25.4
≥ 6	80 (15.7)	23	28.7
Total	508 (100)	119	23.4

Statistically significant was occurred between male and female ($P < 0.05$)

(Baneth et al. 2008). Because the clinical manifestations of infected dogs are variable and nonspecific, the diagnosis may be difficult to manage disease (Ferreira Ede et al. 2007). There are several methods for the diagnosis of CVL, among the serological method, DAT is the reliable, practical, affordable and excellent test for diagnosis of HVL and CVL (Boelaert et al. 1999).

Although there are limitations such as, needs to cold chain and various temperatures, but this test is capable to detect anti *L. infantum* antibodies by glycerol-preserved (GP)-DAT antigen (Akhoundi et al. 2012).

Therefore this method is the best reference test in the most of sero-prevalence surveys (Sousa et al. 2011). For this reason, we used DAT method for detection of *L. infantum* antibodies in canine.

In the current study, we have determined the sero-prevalence of CVL, 23.4 % with titers of 1:320 and higher

which increase in antibody titer in dogs can be related ecological changes during recent years in these studied areas. Other sero-prevalence study of CVL in Meshkin-Shahr district showed that of 14.8 and 17.4 % with titers of $\geq 1:320$ (Moshfe et al. 2008; Bokaie et al. 1998) which increased compared to previous study likely related to surveillance actions in these areas.

The present study area has been found as endemic foci for HVL and large population of dogs can be considered as a risk factor for the disease (Bokaie et al. 1998).

Asymptomatic dogs composed of more than fifty percent of the infected animals that were capable to transmission of disease by vector (Solano-Gallego et al. 2001; Sousa et al. 2011). Based on previous studies in endemic areas of CVL in Iran, 13–24 % of infected dogs had clinical manifestations thus, almost 75 % of infected dogs were asymptomatic (Mohebbali et al. 2005; Mohebbali et al. 2011). Based on study carried out by (Moshfe et al. 2009), asymptomatic dogs had potential role of transmission of *L. infantum* to susceptible hosts.

Therefore it can be public health problem and without identification and detection of asymptomatic dogs, control of disease is unsuccessful.

This survey observed that dogs of <2 years of age had low infection, while the high prevalence was from ≥ 6 - years. The reason for this pattern probably related to more time exposure of older dogs to transmission by sand flies (Kalayou et al. 2011; Sousa et al. 2011; Mohebbali et al. 2005).

In the present study, significant difference was found between male and female dogs, with higher prevalence in males, which could be due to higher maintenance of male dogs. This finding is in agreement with other author (Miranda et al. 2008).

Since the sero-prevalence rates of CVL and HVL associated with weather conditions and humidity in endemic areas of Iran (Mohebbali et al. 2011) and also cold climate can influence in increase of the rate of infection (Mohebbali et al. 2011; Mohebbali et al. 2005), so in current study high level of titer of infected dogs can be related various weather in this part.

In conclusion high proportion of asymptomatic infected domestic dogs emphasizes the importance of the animals for maintenance of *L. infantum* infection in endemic areas of zoonotic visceral leishmaniasis. Using serological tests such as DAT can be helped us for the detection of asymptomatic dogs and control program of visceral leishmaniasis.

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