Short Paper

Seroprevalence of canine visceral leishmaniasis in asymptomatic dogs in Iran

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Summary

Clinically infected dogs have been identified as the main reservoir hosts of visceral leishmaniasis (VL) caused by *Leishmania infantum*. Recently asymptomatic infected dogs were regarded to be as important as clinically ill dogs. This study was aimed to determine the seroprevalence of *L. infantum* infection among asymptomatic dogs in the southwest and central parts of Iran and to investigate possible risk factors associated with this infection. 548 serum samples were collected from dogs in three Iranian provinces and subjected to direct agglutination test (DAT) in dilutions of 1:80 to 1:20480. Fifty three (9.67%) of the dogs had detectable anti-*L. infantum* antibodies at dilutions of ≥1:80. Living status of the dogs (household or free roaming) was a potential risk factor for the infection; seroprevalence was significantly higher in free roaming dogs (P<0.001). Dogs of more than 2-year-old had a significantly higher infection rate in comparison with younger dogs (P<0.001). No significant statistical differences were seen between seroprevalences of the male and female dogs. The results of this study show relatively high sero-prevalence of *L. infantum* infection in evaluated regions and higher seroprevalence in old and free roaming dogs, which shows the importance of environmental contamination and access of the dogs to the other reservoir hosts.

Key words: Visceral leishmaniasis, *Leishmania infantum*, Dogs, Iran

Introduction

Visceral leishmaniasis (VL) is one of the important parasitic diseases. Leishmaniasis is endemic in 88 countries in the world and 350 million people are considered at risk (WHO, 2006). Dog is considered the reservoir host for *Leishmania infantum* and phlebotomine sand flies are the biological vectors whose hosts are animals or human beings (Nekouie *et al*., 2006). Leishmaniasis is a highly focal disease with widely scattered foci. Canine visceral leishmaniasis (CVL) caused by *L. infantum* is endemic in most Mediterranean basin. CVL is especially endemic in the north-west and southern parts of Iran (Mohebali *et al*., 2005).

Canine leishmaniasis was firstly described in Iran in Tehran province (Neligan, 1913) followed by the northern provinces (Pouya, 1949). Three dogs were also reported with clinical leishmaniasis in Tehran province in 1968 (Mobedi *et al*., 1968). Epidemiologic aspects of CVL in Iran were described in 2005 (Mohebali *et al*., 2005). Clinically infected dogs were regarded as the main reservoir hosts of CVL, although a recent investigation showed that clinically healthy dogs with *L. infantum* infection can harbor this pathogen in their peripheral blood and skin as well (Moshfe *et al*., 2009).

The aim of this study was to determine the seroprevalence of anti-*Leishmania* antibodies among asymptomatic dogs from three Iranian provinces (Chaharmahal-va-Bakhtiary, Khoozestan and Tehran) to

evaluate potential hygienic hazard associated with them and to investigate possible risk factors for infection.

Materials and Methods

Blood samples were collected from saphenous vein of 548 asymptomatic dogs during an 18 month period from July 2007 to February 2009 in three Iranian provinces including: Chaharmahal-va-Bakhtiari, Khoozestan and Tehran. All of the dogs were referred to veterinary clinics for annual vaccination and check up or were shepherd or guard dogs. A comprehensive data collection form focused on possible risk factors including age, sex and living status (household or free roaming) was prepared when sampling was carried out.

The *L. infantum* antigen for this study was prepared in the protozoology unit of the school of public health in the Tehran University of Medical Sciences. DAT antigens were made using mass production of promastigotes of *L. infantum* (MCAN/IR/07/Moheb-gh) in RPMI1640 plus 10% fetal bovine serum, tripsinization of the parasites, staining with coomassie brilliant blue and fixing with formaldehyde 1.2% (El Harith *et al.*, 1989; Edrissian *et al.*, 1996).

Initially, for screening purposes, serum dilutions were made from 1:80. Samples with titers of 1:80 were diluted further to give the end-point dilution of 1:20480. DAT Ag control wells (antigen only; on each plate) and known negative and positive controls were tested in each plate. The titer was defined as the highest dilution at which agglutination was still visible, as blue dot, compared with negative control wells, which had clear blue dots. The positive control sera were obtained from dogs with confirmed *L. infantum* infection using microscopy, culture and animal inoculation (Mohebali *et al.*, 2005). Positive serum controls had detectable antibodies in titer of 1:20480. After 12-18 h incubation at room temperature, the results were read (El Harith *et al.*, 1989). Test results were read by two persons separately.

The prevalence was estimated from the ratio of positive results to the total number of dogs examined. Assessment of association between seroprevalence of anti-*L. Infantum* antibodies in dogs and selected risk factors were made by Chi-square test with confidence interval of 0.95 using SPSS 16 (SPSS Inc. Headquarters USA) software.

Results

From 548 sampled dogs, 300 (54.74%) were males and 248 (45.26) were females. Information about age revealed that 113, 200 and 235 of the dogs belonged to age groups of <12, 12-23 and ≥24 months of age, respectively. 317 dogs were free roaming (guard, shepherd or stray) and 231 were household dogs.
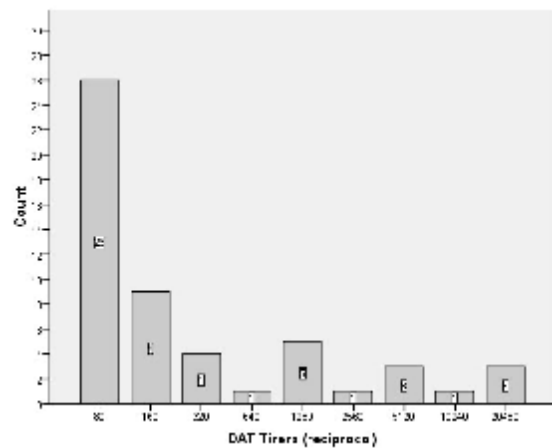
Fig. 1: The number of dogs with different direct agglutination test titers (reciprocal) in evaluated serum samples

Among 548 evaluated samples, 53 (9.67%) had detectable anti-*L. infantum* antibodies in evaluated serum samples diluted from 1:80 to 1:20480 (Fig. 1). Regarding sampling regions, seroprevalences of 8.94%, 10% and 16% were found for Tehran, Khoozestan and Chaharmahal-va-Bakhtiari provinces, respectively.

Regarding the sampling regions, seroprevalences of 8.94%, 10% and 16% were found for Tehran, Khoozestan and Chaharmahal-va-Bakhtiari provinces, respectively. From 53 serum samples with detectable antibodies, 30 (56.6%) were males and 23 (43.4%) were females. Statistical analysis using Chi-square test showed no correlation between *Leismania*

infection and gender (P=0.77).

Evaluation of results between different age groups revealed a significantly higher chance for infection in dogs older than 3 years in comparison to the dogs younger than 12 months (P<0.001) and also dogs of 12-23 months of age (P<0.001). No significant differences were seen between results of the dogs younger than 12 months and dogs of 12-23 months (P=0.60). To investigate the role of living place in occurrence of infection, positively reacted serum samples were compared between household and other dogs. Infection rate was significantly higher in free roaming dogs compared to household dogs (P<0.001) (Table 1).

Discussion

Measurement of canine *Leishmania* infection rate is necessary to control zoonotic visceral leishmaniasis in animals and human populations (Tesh, 1995). Direct Agglutination test is an excellent and reliable test for sero-diagnosis of *Leishmania* infection, both in human and dogs (Boelaert *et al*., 1999). Different cut off titers were used for DAT in different studies (Cortada *et al*., 2004). A previously done study showed a cut off point of 1:320 in clinically infected dogs with *L. infantum* in Iran (Mohebali *et al*., 2005), although a lower cut off point of 1:80 was used to detect any infection in clinically healthy dogs.

Seroprevalence of 9.67% for *L. infantum* infection was found in asymptomatic dogs in investigated parts of Iran. Seroprevalence is higher in cold climates of Chaharmahal-va-Bakhtiari province compared to Khoozestan with a warm and humid climate.

Seroprevalence of 18.2, 12.3 and 4.4% were found for three distinct established zones of the northwest, intermediate and southwest of Iran, respectively in 2005. In that investigation, seroprevalence was higher in cold climates of the northwest regions and lower in the warm climate of the southwest (Mohebali *et al*., 2005). This finding is probably due to different activities of sandflies in these regions.

Sero-epidemiologic study of canine visceral leishmaniasis in an endemic focus of this infection in Iran; Meshkin-Shahr showed higher seroprevalence of 17.4% with titers of 1:320 and higher (Moshfe *et al*., 2008).

Comparison of infection rate between two sexes showed no significant differences between males and females i.e. sex was excluded from possible risk factors of *L. infantum* infection. This is a finding in concordance with the other investigators (Pozio *et al*., 1981; Abranches *et al*., 1983; Sideris *et al*., 1996; Mohebali *et al*., 2005). Our finding that the chance of having anti-*L. infantum* antibodies increases with age of the dogs is in agreement with previous investigations (Abranches, 1983; Cardoso *et al*., 2004; Mohebali *et al*., 2005). This finding is probably due to a higher rate of exposure to parasite in older dogs.

A positive association was seen between *L. infantum* seropositivity and being free roaming; either in owned guard and shepherd dogs or in stray ones. Lower rate of infection in household dogs is probably due to the lower chance of exposure i.e. free roaming is a risk factor for infection. Presence of infected dogs without clinical signs (even with high titers up to 20480 seen in our study) emphasizes the importance of these dogs as possible carriers of the

Table 1: Seroprevalence of anti-*L. infantum* antibodies in different groups (dillution ≥1:80)

Groups		Sample No.	DAT positive No.[*]	DAT positive samples (%)
Living status	Free roaming	317	48	90.56
	Household	231	5	9.44
Sex	Male	300	30	56.6
	Female	248	23	43.4
Age group	<12 months	113	5	9.43
	12-23 months	200	9	17
	≥24 months	235	39	73.6

organism (Moshfe *et al.*, 2009). The unspecificity of clinical signs (Ciaramella *et al.*, 1997) and a high proportion of asymptomatic among the sero-positive animals (Mohebali *et al.*, 2005) emphasizes the importance of serology as a diagnostic tool.

In conclusion, this study shows the importance and necessity of serologic screening of *Leishmania* infection in clinically healthy dogs other than symptomatic dogs and possible risk factors to provide control strategies.

Acknowledgement

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