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ORIGINAL ARTICLE

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Seroprevalence of canine visceral leishmaniasis in southeast of Iran

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Abstract Visceral leishmaniasis is an endemic disease in many parts of Iran and infected dogs constitute the main domestic reservoirs that play a key role in transmission to humans. The objective of this study was to assess the seroprevalence of canine visceral leishmaniasis (CVL) by enzyme linked immunosorbent assay (ELISA) in southeast of Iran. This survey was carried out from 2009 to 2011 in Kerman, Bam and Baft districts in Kerman province and Zabol in Sistan-Baluchestan province. Blood samples were taken from 201 dogs after complete clinical examination. Following hematological evaluation; collected sera were tested by indirect ELISA method for the presence of anti *Leishmania infantum* antibodies. Overall seroprevalence was 15.4 %, including 6.4, 3.5, 3 and 2.4 % in Bam, Zabol, Baft and Kerman, respectively. However, seroprevalence of disease was not significantly related to age, gender, presence of clinical signs and hematological disorders. Based to the results of the present study, CVL is endemic in southeastern Iran. Delayed diagnosis and euthanasia of potentially infectious animals may occur with an increased transmission risk to sand flies and subsequently to humans. Implementation of potent screening tests with high validity is essential for rapid detection and successful dog elimination programs in endemic parts of Iran.

Keywords Canine visceral leishmaniasis · Seroprevalence · ELISA · Iran

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Introduction

Leishmaniasis is a spectrum of disease condition with considerable health impacts, caused by different species of *Leishmania*. This disease is currently endemic in 98 countries and territories in the world. Overall, annual prevalence is 12 million and the population at risk is approximately 350 million (WHO 2010). It is estimated that about 1.5 million new cases of cutaneous leishmaniasis (CL) and 500,000 cases of visceral leishmaniasis (VL) occur each year, in various parts of the world. Global warming caused an incensement in leishmaniasis outbreaks due to increase in the number of vectors (Adil et al. 2011; Desjeux 2004).

Visceral leishmaniasis is the most severe form of leishmaniasis (Desjeux 2001). Mediterranean visceral leishmaniasis (MVL) is a common parasitic disease in Iran. In Iran, the seroprevalence of *Leishmania infantum*, the most prevalent VL species has been ranged between 10 and 37 % in different geographical areas (Gavgani et al. 2002; Moshfe et al. 2008). *Leishmania infantum* is transmitted by various species of female phlebotomine sand flies (Moncaz et al. 2012). The parasite migrates to the reticuloendotelial organs such as liver, spleen, lymph nodes and bone marrow and if left untreated it will always result in the death in host (Desjeux 2004).

In Iran, VL is endemic in Ardabil, East Azerbaijan, Fars, Boushehr, Kerman and recently in Qom and northern Khorasan provinces (Edrissian et al. 1998; Mahmoudvand et al. 2011; Mohebali et al. 2011a). Sporadic cases of VL are reported from other parts of the country (Mohebali et al. 2006).

Domestic dogs (*Canis familiaris*) are considered as major reservoir for *L. infantum* which play a key role in transmission of infection to humans (Gavgani et al. 2002). Unfortunately, host factors that determine clinical outcome are poorly understood in dogs (Hamarsheh et al. 2012; Jamshidi et al. 2011). In the infected dogs, clinical manifestations may appear 3 months to 7 years post-infection and may include alopecia, lymphadenopathy, splenomegaly, hepatomegali, dermatitis, anorexia, cachexia, ocular lesions, onychogriposis and cutaneous ulcerations (Cardoso et al. 2004; Desjeux 2004; WHO 2010).

Canine visceral leishmaniasis is partly resistant to common therapeutic regimes, which are used, in human medicine. Whereas depopulation of symptomatic seropositive dogs is sometimes unacceptable for the owners, it is ineffective because symptomless and even seronegative dogs may be the sources of parasite transmission (Slappendel and Ferrer 2005). As a high proportion of infected dogs are asymptomatic, detection of specific antibodies remain the method of choice for mass screening in epidemiological researches (França-Silva et al. 2003; Quinnell et al. 2001; Reithinger et al. 2002).

Serological methods are highly sensitive and non-invasive; hence, they are best suited for use in field conditions (Sharifi and Daneshvar 1998; Maria Costa et al. 2012). Several diagnostic tests are available to detect anti-*Leishmania* antibodies in canine sera. Indirect fluorescent antibody, ElISA, direct agglutination test, and western blotting are the well-known methods and more recently the purified recombinant antigen for ELISA; rk39 has been used for serodiagnosis in human and dogs (Green 2006).

This study aimed to determine seroprevalence of canine visceral leishmaniasis in the southeast of Iran by ELISA.

Materials and methods

Study area

This study was conducted in Kerman and Sistan-Baluchestan provinces. Kerman is located in the southeast of Iran; it is the second largest province of the country with an area of 180,726 km^2. The population of the province is about 3 million. Sistan-Baluchestan province is in the southeast of the country, bordering Pakistan and Afghanistan. The province is the largest in Iran, with an area of 181,785 km^2 and a population of 2.3 million.

Sampling

The survey was carried out during a 2-year period from 2009 to 2011. Blood samples were taken from 201 randomly selected dogs in southeast of Iran, including Kerman and Sistan-Baluchestan provinces. In Kerman province, the samples were taken from three districts including, Kerman, Bam and Baft. In Sistan-Baluchestan, the samples were taken from Zabol district. A questionnaire was completed for each dog, recording sex, age, raising place, and pathognomonic clinical manifestations of VL including presence of skin lesions, alopecia, nose hyperkeratosis and cachexia.

Hematology

5 ml blood samples were taken and 2.5 ml injected into evacuated EDTA tubes, stored at +4 °C and analyzed within 8 h. Complete blood count were performed using a fully automated hematology analyzer Sysmex Kx-21 (Sysmex Corporation, Japan). The remaining blood portion was kept in refrigerator for at least 30 min. Serum samples were separated by centrifugation at 3,000 rpm for 3–5 min and stored at −20 °C for serological examination.

ELISA test

All serum samples were tested by an indirect ELISA kit (ID Screen Canine Leishmaniasis, ID-Vet Company, France) according to the manufacturer's manual and the samples were read at 450 nm by an ELISA reader (ELX800).

The proportion rate of each sample over positive control were calculated by the following formula $\{S/P = \frac{OD(sample) - OD(NC)}{\overline{OD(PC)} - \overline{OD(NC)}} \times 100\}$ and the sample was interpreted as positive if the rate was greater than or equal to 50 %. The ratio greater than 40 % and inferior to 50 % was considered doubtful and less than or equal to 40 % was recorded as negative.

Pathology

Four positive symptomatic cases were euthanized after obtaining their owner's consent. At necropsy, suspected dogs were inspected for enlargement of RES such as spleen and liver and lymphadenomegaly. In these cases, 1 × 1 × 1 cm dimensions slices were preserved in 10 % formalin for sectioning by microtome for further histopathology staining by haematoxylin and eosin (H&E).

Data analysis

Analyses were performed using SPSS software ver. 15 and positive ELISA test was set as an outcome variable.

Independent variables were sex, age and health status of each animal. A primary screening was performed using two K contingency tables (cross-tab) of exposure variables by Chi square and fisher exact tests and $P < 0.05$ was defined significant.

Results

In this study, anti-*Leishmania* antibody was detected in 15.4 % of 201 studied dogs. No significant difference between VL infection and gender was found ($P = 0.41$).There was no association between seropositivity and age ($P = 0.47$), but the frequency of sampled dogs was not equal in different age groups and this could be a confounding factor (Table 1). Bam was the most infected district (6.4 %), followed by Zabol (3.5 %), Baft (3 %), and Kerman (2.4 %), (Table 1). Only 4 of 31 seropositive cases showed clinical manifestations including cachexia, skin lesion, alopecia and nose hyperkeratosis, and others showed hepatosplenomegaly and generalized lymphadenopathy at necropsy but no statistical significance was observed between presence of clinical sign and seropositivity ($P = 0.22$). Neither there was relation between the seroprevalence rate of VL and hematological disorders ($P = 0.27$), (Table 1).

Table 1 *Leishmania infantum* infection among studied dogs regarding to age, sex clinical status and raising place

Parameter	Seropositive no. (%)	Seronegative no. (%)	Total no. (%)
Age (years)			
>3	7 (3.4)	40 (19.9)	47 (23.4)
3–6	15 (7.4)	113 (56.2)	128 (63.9)
<6	9 (4.4)	17 (8.4)	26 (13)
Sex			
Male	21 (10.4)	100 (49.8)	121 (60.2)
Female	10 (5)	70 (34.8)	80 (39.8)
Clinical status			
Symptomatic	4 (2)	8 (4)	12 (6)
Non-symptomatic	27 (13.5)	162 (80.5)	89 (94)
CBC test			
Normal	5 (2.5)	84 (41.8)	89 (44.3)
Lukocytosis	15 (7.5)	43 (21.4)	58 (28.9)
Leucopenia	1 (0.5)	13 (6.4)	14 (6.9)
Anemia	10 (5)	30 (14.9)	40 (19.9)
Location			
Bam	13 (6.4)	44 (21.9)	57 (28.3)
Baft	6 (3)	26 (13)	32 (16)
Kerman	5 (2.4)	62 (30.8)	67 (33.2)
Zabol	7 (3.5)	38 (19)	45 (22.5)
Total	31 (15.4)	170 (84.6)	201 (100)

Discussion

Zoonotic visceral leishmaniasis (ZVL) caused by *L. infantum* is an important emerging parasitic disease found in countries around the Mediterranean basin in the Middle east, and in Latin America. In these areas, domestic dogs are the principal rural reservoir hosts and wild canids constitute major sylvatic reservoirs (Ashford 2000).World Health Organization (WHO) considered, visceral leishmaniasis is one of the most important parasitic diseases, which is endemic in different parts of Iran (WHO 2010).

In this context, sensitive diagnostic tests, applicable to field conditions, are becoming increasingly necessary to facilitate and improve the control of disease.

The common serological methods for screening of VL are indirect fluorescent antibody test (IFAT), ELISA and direct agglutination test (DAT) (Rajasekariah et al. 2001; Mikaeili et al. 2007).

ELISA has been used as a potential serodiagnostic tool, with high sensitivity, but its specificity depends upon the antigen. In this study the specificity and sensitivity of the ELISA test which was used for detection of CVL was reported to be 99.1 and 98.5 % in endemic areas, respectively (Pourquier et al. 2007), more valid than DAT method which is used for VL screening in endemic parts of Iran.

In the present study, the overall seroprevalence of disease was 15.4 % which seems to be higher than south west parts. The rate of anti-*Leishmania* antibody in dogs from the north west, south west, and central part of Iran was reported 43.4, 3.1 and 22.8 %, respectively (Sarkari et al. 2010; Fakhar et al. 2012). In another study, seroprevalence of VL in Baft with IFA and ELISA methods was estimated 18 and 14.5 %, respectively (Sharifi and Daneshvar 1998). In the current study, among 32 studied animals, six dogs were seropositive which represent similar prevalence in this area. Seroprevalence of VL was not related to gender which is consistent with reported findings by Bokai et al. (1998) and Mohebali et al. (2005) in Iran. Contrary to Mohebali et al. (2005) findings who reported the relation between age and disease prevalence in many parts of Iran, this association was not significant in the present study.

Symptomatic dogs showed a higher prevalence of disease in Meshkinshahr (13.6 and 25.4 %) (Bokai et al. 1998; Moshfe et al. 2008) and in Fars province (8.1 %) (Fakhar et al. 2012) but in our study only one-third of the symptomatic dogs were seropositive indicating the importance of differential diagnosis in endemic areas. Zinc responsive alopecia, discoid lupus erythromatosis and bullous pemphigoid were the most common skin disorders which diagnosed in suspected seronegative cases in present study. Furthermore, *L. tropica* was proposed as a causing factor for symptomatic visceral leishmaniasis in Iran (Mohebali et al. 2011b).

On the other hand, 27 of 31 seropositive dogs (84.3 %) were asymptomatic which showed that asymptomatic dogs could play an important role in epidemiology and transmission of VL to human beings. Dogs with no clinical symptoms have the potential to transmit VL to phelebotomin vectors and to human. In a study by Molina et al. (1994) in Spain, asymptomatic dogs reported to play the same role as symptomatic dogs in epidemiology of leishmaniasis because the ability of sand flies to pick up infection is not dependent upon clinical manifestations.

Undoubtedly, the seropositivity indicates previous contact with the parasite, but we do not know whether these dogs are immune resistant animals or whether they will subsequently develop the disease (Cabral et al. 1998). Thus, detection of infection in animals with variable magnitude range of anti-*Leishmania* antibody titers, regardless of their clinical status, is critical for diagnosis and control of VL. The failure to detect infection in these animals may contribute to the maintenance of parasite and transmission of agent in both canine and human populations.

The resurgence of leishmaniasis and its emergence in newer geographical areas and in newer hosts, besides changing the clinical profile of infected patients, has put forward newer challenges in the areas of diagnosis, treatment, and disease control. Based on the cases of kala-azar reported in children in southeast of Iran (Barati et al. 2008; Nik-nafs et al. 1994), and the result of present study, which indicate the role of domestic dogs in disease epidemiology, there is a need for application of a field work monitoring system in dogs and vectors population. Successful elimination of infected dogs and implementation of potent rapid screening tests is essential for planning of future control programs.

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