A serological study of *Leishmania infantum* in dogs of Khorasan Razavi province, Iran

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Abstract *Leishmania infantum* is the principal agent of visceral leishmaniosis (kala-azar) in all areas of Iran. The disease is endemic in Fars, Ardabil, East Azerbaijan and Bushehr provinces while it is sporadic in other parts of the country. Dog as a member of canidae family is the main animal reservoir host of kala-azar. The present study was carried out to determine the sero-prevalence of canine leishmaniosis in Mashhad, the capital city of Khorasan Razavi Province, in the north east of Iran. Two-hundred and nine dogs in two different classes including stray and sheepdogs were selected for this cross-sectional study. The blood samples were collected and tested by indirect immunofluorescent antibody test for detection of anti-*Leishmania infantum* antibodies. The results showed that 16/209 (7.6 %) of blood samples were positive. The sero-prevalence rates in stray and sheepdogs were 11/94 (11.7 %) and 5/115 (4.3 %), respectively ($p < 0.05$). Based on the results obtained, it is concluded that visceral leishmaniosis is distributed among dogs of this area, also sero-prevalence of canine leishmaniosis in stray dogs higher than sheepdogs.

Keywords Leishmaniosis · Dog · Seroepidemiology · IFAT · Iran

Introduction

Visceral leishmaniosis is an infectious disease that is transmitted by sand flies. The various species of *Leishmania* parasites are responsible for the disease. They can infect human and animal in either the old or new world. Domestic dogs (*Canis familiaris*) are the major reservoir hosts of Mediterranean type of visceral leishmaniosis caused by *Leishmania infantum* (WHO 2010).

In 1908, Nicole and Comte (1908) discovered the canine leishmaniosis in Tunisia. Since then, the association of human visceral leishmaniosis foci with infected dogs has been reported in almost any patient in the Mediterranean area (Kirmse et al. 1987; Mancianti et al. 1986).

Among the members of canidae family, domestic dogs are efficient reservoir hosts because their dermal layer is a suitable place for the parasites of fixed macrophages to abundant and taken up by feeding sand flies (Ashford and Bettini 1987; Nash 1993), therefore dogs are considered as important risk factors for human infection in the endemic areas of the disease in Iran (Gavvani et al. 2002).

In 1913, the first case of canine leishmaniosis was reported from Tehran (Neligan 1913) and subsequently, in 1949 another case was reported from the Caspian area (Pouya 1950). Pouya (1950) also was the first who reported the first human case of visceral leishmaniosis in Iran. So far, visceral leishmaniosis have been reported in dogs from different parts of Iran (Mobedi et al. 1968; Hamidi et al. 1982; Mohebbi et al. 2001a; Bokai et al. 1998).

The wild carnivores such as jackals and foxes have been considered the main reservoirs of visceral leishmaniosis in Iran (Nadim et al. 1978; Hamidi et al. 1982; Mohebbi et al. 2001b). Mashhad, the capital city of the Khorasan Razavi Province and its surrounding areas are important nidus of urban leishmaniosis in Iran.

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Recently, some cases of visceral leishmaniosis have been reported in human in the Mashhad area. Since the infected dogs are the natural reservoirs of kala-azar in the endemic areas, the objective of this study was to determine the sero-prevalence of *L. infantum* as the cause of canine visceral leishmaniosis.

Materials and methods

Study area

The study has been done from February 2011 to February 2012 in Mashhad, the capital city of the Khorasan Razavi Province, northeast of Iran with northern temperature, a semi-arid climate with cold winters and moderate summer.

Blood sampling

Blood samples (2.5 ml) were taken from cephalic vein of dogs. The collected samples were properly labelled and transferred to parasitology laboratory of Faculty of Veterinary Medicine in the appropriate containers, under cold condition. The tubes of blood samples were centrifuged at $800 \times g$ for 5–10 min, the separated serum was stored into deep freeze at -20°C in freezing compartment.

IFAT

In the present study, the indirect immunofluorescent antibody test (IFAT) was used as sero-diagnostic tool. The *L. infantum* antigen (Megacor Co, Austria MegaScreen FLUOLEISH) has been used for this study. For each reaction, $10 \mu\text{l}$ of serum dilution (1:50) in PBS were added over the slide holes. The slides were incubated in a humid home at 37°C for 30 min. The slides were washed for 5 min in PBS. The conjugate was diluted to 1:200 in PBS with 0.025 % Evan's Blue and then $15 \mu\text{l}$ of this solution was placed over the slide holes. The incubation and washing steps were repeated once, as outlined above. Slides were mounted with buffered glycerin, covered with a cover slip and read under an Olympus BX-FLA fluorescent microscope equipped with a 100 W mercury lamp with $400\times$ magnifying power. The serum dilution showing an evident yellow-green fluorescent signal upon microscopic examination was accepted to be positive IFAT titre.

A Chi-square test was used to determine significant differences between proportions. The probability less than 0.05 was considered to be statistically significant (Minitab Statistical Software, 2000).

Results

The results of this study showed that 7.6 % ($n = 16$) of all 209 blood samples were positive. The sero-prevalence of leishmania infection in stray dogs was significantly higher than sheepdogs ($p < 0.05$) (Table 1). There was not observed differences between the sero-prevalence of male and female dogs ($p > 0.05$) (Table 2).

Discussion

Dogs and wild canines are the main domestic reservoirs of *L. infantum* in the old and new worlds. Determination of the prevalence of leishmania infection in dogs is necessary to define control measures for zoonotic visceral leishmaniosis (Tesh 1995).

Serological methods are highly sensitive and being non-invasive. They are comparatively more suited for diagnosing visceral leishmaniosis in endemic regions (Alborzi et al. 2007). We used IFAT for serological test in dogs' serum as a practical and sensitive test in seroepidemiological studies (Harith et al. 1989; Mohebbi et al. 2006).

The seropositive rate of visceral leishmaniosis was 7.6 % in dogs of Mashhad and surrounding areas. The investigations of canine leishmaniosis in Iran have been carried out in Northwest and Southwest, but no study has been done in Mashhad that is located in Northeast of Iran.

In Iran, the geographical regions with various climates have been shown different sero-prevalence rates of *L. infantum* in dogs. (Mohebbi et al. 2005). The highest sero-prevalences rates of canine leishmaniosis have been observed in the Northwest region with cold climate. The reported sero-prevalence rates were 17.4 % in 2008, (Moshfe et al. 2008), 18.2 % in 2005 (Mohebbi et al. 2005) and 21.6 % in 2002 (Mazloumi Gavvani et al. 2002). While, the reported Leishmania infection rate in dogs in the Southwest region with hot climate was 4.4 % in 2005 (Mohebbi et al. 2005).

In the present study, The obtained results show the effect of climatic factor on the prevalence of canine visceral leishmaniosis.

We found that there were no significant differences between the sero-prevalence of Leishmania infection in

Table 1 Sero-prevalence of Leishmania infection in different kinds of dogs in Mashhad area, Iran

Kind of dog	Positive no (%)	Negative	Total
Stray	11 (11.7)	83	94
Sheepdogs	5 (4.3)	110	115
Total	16 (7.6)	193	209

Table 2 Sero-prevalence of canine Leishmania infection by gender in Mashhad area, Iran

Gender	Positive no (%)	Negative	Total
Male	9 (6.1)	138	147
Female	7 (11.2)	55	62
Total	16	193	209

dogs regarding to their gender. Similar results were found by Pozio et al. (1981), Sideris et al. (1996), Bokai et al. (1998) and Mohebbali et al. (2005).

However, a study has reported a predominance of leishmaniosis in male humans, (Brabin and Brabin 1992), probably due to the more exposure of males to the sand fly bites (Amusatogui et al. 2003).

Our study showed the higher sero-prevalence *L. infantum* in stray dogs in compared to sheepdogs. In a similar seroepidemiological study in Algeria, Four hundred and sixty-two dogs were involved and divided in four groups according to their functional type: stray dogs, farm dogs, national guard dogs and pet dogs. The stray dog group showed the highest prevalence of leishmaniosis (11.7 %), followed by the national guard dogs (9.7 %) and the farm dogs (5.9 %) (Adela et al. 2010).

In conclusion, the most important result was a high proportion of seropositivity for leishmaniosis among stray and sheepdogs without clinical signs of canine leishmaniosis. Therefore, control methods such as culling of seropositive dogs and chemical control against sand fly vector are needed.

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