Prevalence of anti-*Leishmania infantum* antibodies in dogs from West of Iran

Jamal Gharekhani · Heidar Heidari ·
Hassan Hajian-Bidar · Ehsan Abbasi-Doulatschahi ·
Hossein Edalati-Shokat

Received: 12 October 2014 / Accepted: 6 November 2014 / Published online: 26 November 2014
© Indian Society for Parasitology 2014

Abstract The main aim of present study was to determine the sero-prevalence of canine visceral leishmaniasis (CVL) among stray and owner shepherd dogs in Hamedan province, West of Iran. A cross-sectional study was performed in 2013 using enzyme-linked immuno sorbent assay (ELISA). Blood samples ($n = 380$) were collected randomly from 170 stray and 210 owner shepherd dogs in different regions of Hamedan province. All of animals were asymptomatic. Antibodies to *Leishmania infantum* were found 3.95 % (0.95 CI: 2–5.9 %). The sero-prevalence rate of infection in stray dogs (6.47 %) was reported higher than owner shepherd dogs (1.9 %) ($P = 0.023$). There was significant differences among age groups ($P = 0.003$); unlike to different gender ($P = 0.11$). This is the first report of CVL in western Iran. Further comprehensive studies in infection rate and predominant *Leishmania* species in humans, sand flies and animals hosts (wild canines and rodents) is recommended in this region.

Keywords *Leishmania infantum* ·
Canine visceral leishmaniasis (CVL) · Dog · ELISA ·
Hamedan · Western Iran

Introduction

Canine visceral leishmaniasis (CVL), caused by the protozoan parasite *Leishmania infantum* (Kinetoplastida: Trypanosomatidae), is one of the important and endemic zoonotic diseases in the Mediterranean basin and Middle-East including Iran (Khanmohammadi et al. 2010; Hosseini-ninejad et al. 2012; Haddadzade et al. 2013).

L. infantum is the principal agent visceral leishmaniasis (VL) in humans and canine in Iran (Mohebbi 2013). Domestic dogs (*Canis familiaris*) and wild canines are the main reservoir hosts of CVL in all regions of Iran; which is transmitted among canines and to humans by sandflies of the genera *Phlebotomus* and *Lutzomyia* (Diptera: Psychodidae) (Moshfe et al. 2008).

Lymphadenopathy, dermatitis, alopecia, cutaneous ulcerations, onychogriposis, lameness, anorexia, weight loss, cachexia, ocular lesions, epistaxis, anaemia, diarrhea and renal failure are the main clinical signs of CVL (Haddadzade et al. 2013). Dermatological alterations are very frequent (Heidarpour et al. 2012). It appears in dogs in acute, chronic and sub clinical forms and in a variety of cases without any clinical symptoms leading to the death of animal (Khanmohammadi et al. 2008).

The incidence of leishmaniasis depends on a variety of ecological and biological factors, such as the presence of various *Leishmania* species, specific reservoirs and vector (Khanmohammadi et al. 2010).

The difference of methods are available for diagnosis of CVL including microscopy, culture, serology and molecular techniques (Mohebbi 2013). Several serological techniques such as direct agglutination test (DAT), indirect immuno-fluorescence antibody test (IFAT) and enzyme-linked immuno sorbent assay (ELISA) are available to detection of anti-*Leishmania* antibodies. The sensitivity

J. Gharekhani · H. Hajian-Bidar
Department of Parasitology, Central Veterinary Laboratory,
Iranian Veterinary Organization, Hamedan, Iran

H. Heidari (✉)
Department of Parasitology, Paraveterinary Faculty of Bu-Ali
Sina University, Janbazan Street, Postal Box: 65167, Hamedan,
Iran
e-mail: Heidari346@basu.ac.ir

E. Abbasi-Doulatschahi · H. Edalati-Shokat
Central Veterinary Laboratory, Department of Microbiology,
Iranian Veterinary Organization, Hamedan, Iran

and specificity of ELISA is higher than other techniques (Khanmohammadi et al. 2008; Moshfe et al. 2008; Haddadzade et al. 2013). In epidemiological studies, determine of specific antibodies remains the choice method for mass screening in asymptomatic hosts (Moshfe et al. 2008).

Information about CVL is essential and highly important for control of human VL in each area. Some studies on CVL done in different part of Iran (Mohebbali et al. 2005; Mohebbali 2013). However, there is no published information of epidemiology on leishmaniasis in the dogs from West of Iran. The main purpose of the present study was to investigate the sero-prevalence of CVL among stray and owner shepherd dogs in different regions of Hamedan province, West of Iran using ELISA.

Materials and methods

Study area

Hamedan province by mountainous and mild climate is located in west part of Iran (34.77°N and 48.58°E). It covers an area of 19,546 km² and average annual temperature is 11.3 °C. This province is economically important for crops and animal husbandry.

Sampling

A cross-sectional study was performed in 2013. Blood samples (3 ml by cephalic or saphenous venipuncture) were collected randomly from 170 stray and 210 owner shepherd dogs (total = 380) in around of villages in different regions of Hamedan province. All of animals were asymptomatic and mix breed. Information about age (≤ 2 and > 2 year) and gender in animals were taken from physical examination (Table 1).

Ethical approval

The permission was accorded by the Institutional Animal Ethics Committee (Iranian Veterinary Organization) to collect the sample from live animals.

Serology

All sera were removed after centrifugation at 1,000×g for 10 min and stored at −20 °C until laboratory testing (Haddadzade et al. 2013). Anti-*L. infantum* antibodies were detected using a commercially available indirect ELISA kit (ID Screen® Canine Leishmaniasis, ID-VET company, France). The kit was used according to the manufacturer's instructions. The presence of antibody was determined by calculating of sample to positive ratio (S/P) according to

Table 1 Prevalence of anti-*Leishmania infantum* antibodies in different variables in Hamedan province, West of Iran

Variables	No. of sample	No. of positive	Prevalence rate (%)	P-value
Life style				
Stray dogs	170	11	6.47	0.023
Owner shepherd dogs	210	4	1.9	
Gender				
Male	178	4	2.25	0.11
Female	202	11	5.45	
Age groups (year)				
≤ 2	166	1	0.6	0.003
> 2	214	14	6.54	
Total	380	15	3.95	

the manual of kit. The S/P of ≥ 50 % was considered positive.

Statistical analysis

Statistical analysis was performed by using the software package SPSS version 16.0 for windows. Odds ratios (OR), confidence interval (CI), χ^2 and P value were calculated separately for each variable. A P-value ≤ 0.05 was considered statistically significant.

Results

Antibodies to *L. infantum* were found in 15 out 380 samples (3.95 %; 0.95 CI: 2–5.9 %). The sero-prevalence rate of CVL in stray dogs (6.47 %) was detected higher than owner shepherd dogs (1.9 %) ($\chi^2 = 5.165$, $P = 0.023$, OR = 3.5). There was significant differences among age groups ($\chi^2 = 8.698$, $P = 0.003$, OR = 11.5); unlike to different gender ($\chi^2 = 2.552$, $P = 0.11$) (Table 1).

Discussion

CVL was described for first time in 1908 (Nicole and Comte 1908). Currently, VL is a great concern in the Mediterranean and Middle-East countries (Tsachev et al. 2008). The prevalence of CVL is varies in worldwide; this rate has been reported 1.7–10 % in Cyprus (Deplazes et al. 1998), 25 % in Venezuela (Zerpa et al. 2003), 22.1–30.3 % in Italy (Zaffaroni et al. 1999), 9–76 % in Turkey (Ozbel et al. 2000; Atasoy et al. 2010), 10–36 % in Brazil (Ashford et al. 1998) and zero to 81 % in Bulgaria (Tsachev et al. 2007, 2008). Also, Leishmaniasis is encountered in

non-endemic regions such as Austria, Belgium, Germany, Switzerland, Canada and USA (Tsachev et al. 2008).

In previous studies from Iran, sero-prevalence rate of CVL was reported 4.1 % in North (Fakhar et al. 2011), zero to 23.4 % in Northwest (Moshfe et al. 2008; Khanmohammadi et al. 2008, 2010; Sharifdini et al. 2011; Barati et al. 2013; Mohebbali 2013), 7.9 and 8.67 % in Northeast (Heidarpour et al. 2012; Mohebbali 2013), 3.6–8.92 % in Central (Hosseininejad et al. 2012; Razzaghi Maensh et al. 2012; Haddadzade et al. 2013; Mohebbali 2013; Malmasi et al. 2014), 7.7–16 % in Southwest (Avizeh et al. 2007; Hosseininejad et al. 2012; Moshfe et al. 2008; Mohebbali 2013), and 23 % in Southeast (Mahmoudvand et al. 2011).

The zoonotic form of VL caused by *L. infantum* occurs sporadically in all geographical zones of Iran, but the disease is endemic in Northwest and South regions (Mohebbali 2013). Meshkin-Shahr district (Ardabil province, Northwest of Iran) is the most important endemic area of VL in humans and animals in Iran (Mohebbali et al. 2005). The sero-prevalence of CVL was reported 17.4 % (Moshfe et al. 2008), 15.8 % (Sharifdini et al. 2011), and 23.4 % (Barati et al. 2013) in this region. In our study, this rate was determined 3.95 %; similar to Haddadzade et al. (2013) investigation in Alborz province, Central of Iran (3.6 %), and Sideris et al. (1999) in Greece (3.7 %).

In current study, *L. infantum* infection rate in stray dogs (6.47 %) was 3.5-fold higher than owner shepherd dogs (1.9 %); the significant difference was found between sero-prevalence and dogs' life style similar to Hosseininejad et al. (2012) investigation. In Haddadzade et al. (2013) study, infection rate in owned and stray dogs were 10 and 1.6 %, respectively ($P = 0.01$). Also, in similar research from Northeast of Iran, this rate in owned dogs (9.5 %) was higher than stray dogs (7 %) (Heidarpour et al. 2012). The stray dogs are life in outside, therefore they will be exposed to sand flies bites and higher chance of infection.

In our investigation, the sero-prevalence of CVL in >2 age group (6.54 %) was higher than ≤ 2 (0.6 %); the significant difference was detected among different age group ($P = 0.003$, OR = 11.5). Anti *L. infantum* antibodies increases with age of the dogs (Hosseininejad et al. 2012; Haddadzade et al. 2013); this is parallel to our finding. In Moshfe et al. (2008) and Malmasi et al. (2014) studies, there was a direct correlation between sero-prevalence of CVL and increase of dogs age; but no significant difference was detected. In a study from Northeast of Iran, the higher rate was reported in dogs less than 1 year old ($P = 0.03$) (Heidarpour et al. 2012). Atasoy et al. (2010) believe that CVL has not been depended on age predisposition factor. High infection has been attributed to a greater probability for exposure to the bite of sand flies infected over time, increasing the susceptibility in older dogs (Haddadzade et al. 2013).

There was no significant difference between sero-prevalence of CVL and different gender; this is consistent to other Iranian investigators (Avizeh et al. 2007; Khanmohammadi et al. 2010; Moshfe et al. 2008; Hosseininejad et al. 2012; Heidarpour et al. 2012; Haddadzade et al. 2013; Malmasi et al. 2014) and in conflict with Barati et al. (2013) finding. In Atasoy et al. (2010) study in Turkey, this rate in male dogs was significantly higher than females ($P < 0.05$). Farmers in Iran mostly reared male dogs in their farms. Therefore, the male dogs might have been infected more than females.

As per our knowledge, the different of serological assays, experimental strategies, climatic variations and frequency of vectors are the main causes of varied results.

Seropositive dogs without clinical signs are very important for distribution of zoonotic leishmaniasis. Thus, using of serological tests may be helpful to design the control strategy in asymptomatic dogs.

In conclusion, this is the first report of CVL in western Iran. The comprehensive epidemiological studies in humans, sand flies and animal hosts (wild canines and rodents) and determine the predominant *Leishmania* species is recommended in this region.

Acknowledgments This study has not been supported by any foundation. The authors declare that there is no conflict of interests.

References

- Ashford DA, David JR, Freire M, David R, Sherlock I, Conceic M, Sampaio DP, Badaro R (1998) Studies on control of visceral leishmaniasis: impact of dog control on canine and human visceral leishmaniasis in Jacobina, Bahia, Brazil. *Am J Trop Med Hyg* 59(1):53–57
- Atasoy A, Pasa S, Ozensoy-Toz S, Ertabaklar H (2010) Seroprevalence of canine visceral leishmaniasis around the Aegean Coast of Turkey. *Kafkas Univ Vet Fak Derg* 16(1):1–6
- Avizeh R, Mohebbali M, Sheikholeslami M (2007) Seroepidemiological investigation of visceral leishmaniasis in dogs of Ahvaz district Iran. *Arch Razi Inst* 62(1):31–37
- Barati M, Mohebbali M, Alimohammadian MH, Khamesipour A, Akhoundi B, Zarei Z (2013) Canine visceral leishmaniasis: seroprevalence survey of asymptomatic dogs in an endemic area of northwestern Iran. *J Parasit Dis*. doi:10.1007/s12639-01303252
- Deplazes P, Grimm F, Papaprodromou M, Cavaliero T, Gramiccia M, Christofi G, Christofi N, Economides P, Eckert J (1998) Canine leishmaniosis in Cyprus due to *Leishmania infantum* MON 1. *Acta Trop* 71:169–178
- Fakhar M, Rahmati B, Gohardehi S, Mohebbali M, Akhoundi B, Sharif M, Mahdavi SA (2011) Molecular and seroepidemiological survey of visceral leishmaniasis among humans and domestic dogs in Mazandaran province North of Iran. *Iran J Parasitol* 6(4):51–59
- Haddadzade HR, Fattahi R, Mohebbali M, Akhoundi B, Ebrahimzade E (2013) Seroepidemiological investigation of visceral leishmaniasis in stray and owned dogs in Alborz province, Central Iran using direct agglutination test. *Iran J Parasitol* 8(1):152–157

- Heidarpour M, Pourtaghi M, Khoshnegah J (2012) Prevalence and risk factors for canine leishmaniasis in Mashhad, North-East of Iran. *Iran J Vet Sci Technol* 4(1):37–46
- Hosseinienejad M, Mohebbali M, Hosseini F, Karimi S, Sharifzad S, Akhoundi B (2012) Seroprevalence of canine visceral leishmaniasis in asymptomatic dogs in Iran. *Iran J Vet Res* 13(1):54–57
- Khanmohammadi M, Sadaghian M, Babaey-neghad F, Zakaria M (2008) Study on seroprevalence of visceral leishmaniasis in stray dogs of Marand (East Azerbaijan) with indirect immunofluorescence antibody test (IFAT) and its health importance in 2007–2008. *Int J Parasit Dis* 4(1):1–3
- Khanmohammadi M, Fallah E, Rahbari S, Sohrabi I, Farshchian M, Hamzavi F, Mohammadpourasl A (2010) Seroepidemiological survey of canine visceral leishmaniasis in Sarab district, East Azerbaijan province, Northwest of Iran in 2009. *Afr J Microbiol Res* 4(19):2022–2028
- Mahmoudvand M, Mohebbali M, Sharifi I, Keshavarz H, Hajjaran H, Akhoundi B, Jahanbakhsh S, Zarean M, Javadi A (2011) Epidemiological aspects of visceral leishmaniasis in Baft district, Kerman province, southeast of Iran. *Iran J Parasitol* 6(1):1–11
- Malmasi A, Janitabar S, Mohebbali M, Akhoundi B, Maazi N, Aramoon M, Khorrami N, Seifi HA (2014) Seroepidemiologic survey of canine visceral leishmaniasis in Tehran and Alborz Provinces of Iran. *J Arthropod-Borne Dis* 8(2):132–138
- Mohebbali M (2013) Visceral leishmaniasis in Iran: review of the epidemiological and clinical features. *Iran J Parasitol* 8(3):348–358
- Mohebbali M, Hajjaran H, Hamzavi Y, Mobedi I, Arshi S, Zarei Z, Akhoundi B, Naeini KM, Avizeh R, Fakhar M (2005) Epidemiological aspects of canine visceral leishmaniasis in the Islamic Republic of Iran. *Vet Parasitol* 129:243–251
- Moshfe A, Mohebbali M, Edrissian G, Zarei Z, Akhoundi B, Kazemi B, Jamshidi S, Mahmoodi M (2008) Seroepidemiological study on canine visceral leishmaniasis in Meshkin-Shahr district, Ardabil province, Northwest of Iran during 2006–2007. *Iran J Parasitol* 3(3):1–10
- Nicole CH, Comte CH (1908) Recherche sur le kala-azar enterprises a l'Institut Pasteur de Tunis. IV: origine canine kala-azar. *Arch Inst Pasteur Tunis* 3:59–62
- Ozbel Y, Oskam L, Ozensoya S, Turgay N, Alkan MZ, Jaffe CL, Ozcel MA (2000) A survey on canine leishmaniasis in western Turkey by parasite, DNA and antibody detection assays. *Acta Trop* 74:1–6
- Razzaghi Maensh M, Mahabadi S, Ghamesi A, Namjoo AR (2012) Prevalence of canine leishmaniasis in dogs at Ardestan district detected by PCR. *Vet Res* 5(2):22–25
- Sharifdini M, Mohebbali M, Keshavarz H, Hosseinienejad M, Hajjaran H, Akhondi B, Rahimi-Foroushani A, Zarei Z, Charehdar S (2011) Neospora caninum and *Leishmania infantum* co-infection in domestic dogs (*Canis familiaris*) in Meshkin-shahr District Northwestern Iran. *Iran J Arthropod-Born Dis* 5(2):60–68
- Sideris V, Papadopoulou G, Dotsika E, Karagouni E (1999) Asymptomatic canine leishmaniasis in Greater Athens area, Greece. *Eur J Epidemiol* 6(15):271
- Tsachev I, Papadogiannakis E, Kontos V, Ivanov A, Chakarova B, Stojanchev K, Peshev R (2007) Seroepidemiology of *Leishmania* among healthy dogs in Bulgaria. *Turk J Vet Anim Sci* 31(1):73–74
- Tsachev I, Papadogiannakis E, Harizanov R, Zarkov I (2008) Canine visceral leishmaniasis: current situation. *Trakia J Sci* 6(1):106–115
- Zaffaroni E, Rubaudo P, Anfranchi L, Mignone W (1999) Epidemiological patterns of canine leishmaniasis in Western Liguria (Italy). *Vet Parasitol* 81:11–19
- Zerpa O, Ulrich M, Borges R, Rodríguez V, Centeno M, Negrón E, Belizario D, Convit J (2003) Epidemiological aspects of human and canine visceral leishmaniasis in Venezuela. *Rev Panam Salud Publica* 13(4):20–23