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Seroprevalence of Visceral Leishmaniosis in Stray Dogs of Hamedan, West of Iran in 2018

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A B S T R A C T

Introduction: Canine zoonotic visceral leishmaniosis (VL) caused by *Leishmania infantum* has been reported from 20 out of 31 provinces in Iran. In 2013, 3.95% of dogs in Hamedan Province showed anti-*L. infantum* antibodies by indirect ELISA method. In 2018, we reevaluated *L. infantum* infection among stray dogs in the same region. **Methods:** From June to November 2018, blood sera from 180 stray dogs in Hamedan and the outskirts were examined for the presence of anti-*L. infantum* antibodies using the same ELISA kit employed in the previous study. **Results:** The ELISA detected anti-*L. infantum* antibodies in blood sera of 19 dogs (10.56%, 95% CI: 6.18–14.94). Infected dogs were significantly older than non-infected dogs (p=0.048) with an odds ratio of 1.02 (p=0.02) between age and infection, implying that the odds of infection increased 2% with each month of age. Seropositivity rate was 11.21% in females and 11.11% in males with no significant difference between the two sexes (p=1). The odds of infection in female dogs were 1.01 compared to that in males. **Conclusion:** The seroprevalence rate of *L. infantum* infection in stray dogs in this study showed an increase compared with 2013 (10.55% vs. 3.95%). An increase in antibodies against *L. infantum* in dogs of Hamedan and the presence of competent vectors for transmission of the parasite in the region makes the transmission of VL possible. Further PCR-based studies are required to confirm the infection in the infected dogs in sand flies.

INTRODUCTION

Canine leishmaniosis transmitted by phlebotomine sand flies is caused by at least 12 species of the genus *Leishmania* [1]. *Leishmania* (*L.*) *infantum* is the causative agent of zoonotic visceral leishmaniasis (VL), a neglected human disease in Central and South America, the Mediterranean region, the Middle East, and Central Asia [2]. Annually, the disease affects approximately 500,000 humans worldwide and may lead to death if left untreated [3]. In Iran, this parasite has been isolated from humans, domestic and wild canines, and rodents [4]. Visceral leishmaniasis (of humans) and leishmaniosis (of animals) is endemic in several regions of Iran including Ardabil and East-Azerbaijan (Northwest), Fars, Bushehr, Kerman (South), Qom (Center) and North-Khorasan (Northeast) Provinces [5] and the annual incidence of human VL reaches 100–300 new cases [4]. Over 50% of human VL cases in Iran occur in endemic areas of the northwest of the country [5]. In Hamedan, despite a report of seropositive dogs [6], only one VL human case was reported in the last nine years [personal communication with the office of Vice-Chancellor in Health Affairs, Hamedan University of Medical Sciences].

Infected domestic dogs are the principal reservoir hosts of VL [1]. However, in the absence of pathognomonic signs, and the presence of various clinical features, diagnosis of canine VL (CVL) is challenging [7]. Hence, enzyme-linked immunosorbent assay (ELISA), a powerful tool that is less susceptible to operator bias compared to immunofluorescence antibody test (IFAT), is widely used in the diagnosis of the CVL infection [8]. The prevalence rate of CVL in different areas of the country varies from 4–32% [5]. There are high genetic similarities between *L. infantum* isolates from humans and dogs in Iran [9]. A recent systematic analysis estimated that 16% of dogs in Iran had *L. infantum* infection [10]. In 2013, 3.9% of dogs in Hamedan Province showed antibodies against *L. infantum* using ID Screen® ELISA [6]. As changes in climate and populations of dogs and sand fly vectors affect the epidemiology of CVL, we reevaluated *L. infantum* infection among stray dogs in the same region.

MATERIAL AND METHODS

From June to November 2018, blood samples were taken from 180 stray mongrel dogs in Hamedan and outskirts. For all dogs except ten, metadata, including age and sex, were recorded systematically. Sera were examined for the presence of antibodies against *L. infantum* using the same commercial ELISA kit (ID Screen® Leishmaniasis indirect, ID-Vet, France) used in the previous study [6]. ID Screen® Leishmaniasis indirect is an indirect ELISA kit for the detection of anti-*L. infantum* antibodies in canine serum and plasma with *L. infantum* purified protein. ID Screen® has a 95.3% sensitivity, 100% specificity, and 97.5% accuracy [11]. According to the instructions by the manufacturer, a dog is defined as positive if S/P% (S=OD of sample-OD of negative control, and P=OD of positive control-OD of negative control) was ≥50%. Positive and negative controls are available in the kit. The positive control is the serum of a naturally infected dog with high titers of antibodies.

Description and analyses of data were performed using the Chi-square test (χ^2), Mann Whitney U test, and logistic regression model (SPSS, Version 16.0; SPSS Inc., Chicago, USA). *P*-value of ≤ 0.05 was considered significant.

RESULTS

The examined dogs included 107 female (64.44%, age 19.12 ± 26.07 months) and 63 male (35.55%, age 24.60 ± 25.67 months). According to the age, dogs were classified in three groups: G1 [63/170, 37.05%, 9.96 ± 2.2 months old], G2 [51/170, 30%, 21.41 ± 3.15 months old] and G3 [56/170, 32.94%, 48 ± 23.80 months old].

ELISA detected anti-*L. infantum* antibodies in sera of 19 dogs (10.56%, 95%CI: 6.18–14.94).

Infected dogs were significantly older (39.26±33.19 months old) than non-infected dogs (24.65±21.25 months old) (p=0.01) (Fig. 1). Statistical analysis showed that the infection was associated with age and increased with aging (p=0.048). Univariate logistic regression showed that the odds of infection between age in month and infection was 1.02 (p=0.02), implying that the odds of infection increased 2% with each month of age. Moreover, 5.7% of fluctuation in infection was justified by age. As for age groups, the infection was more common in dogs of G3 (19.64%) in comparison with G2 (7.84%) and G1 (6.35%) (Table 1, Fig. 2).

Seropositivity rate was 11.21% in females (12/107, 95%CI: 5.33–17.09) and 11.11% in males (7/63, 95%CI: 3.27–18.95) with no significant difference between two sexes (OR=1.01, p=1) (Table 1). The odds of infection in female dogs compared to that in males was 1.01. The 0.01% of fluctuation in infection rate was justified by gender.

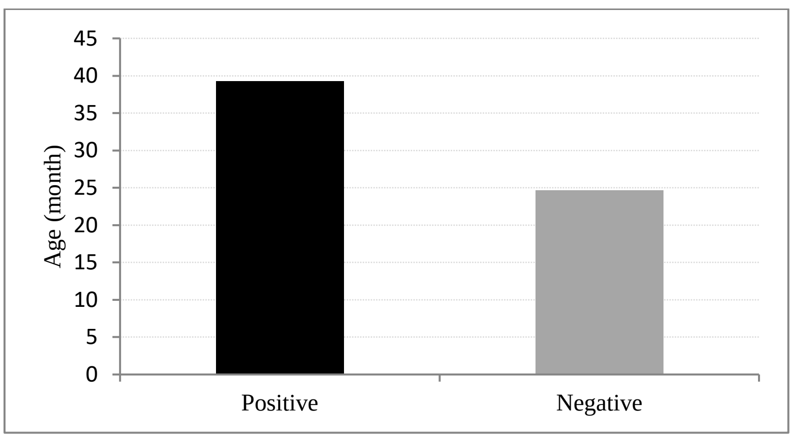

Fig. 1. The average age of infected and non-infected dogs: 39.26±33.19 vs. 24.65±21.25 months old, respectively (p=0.01)

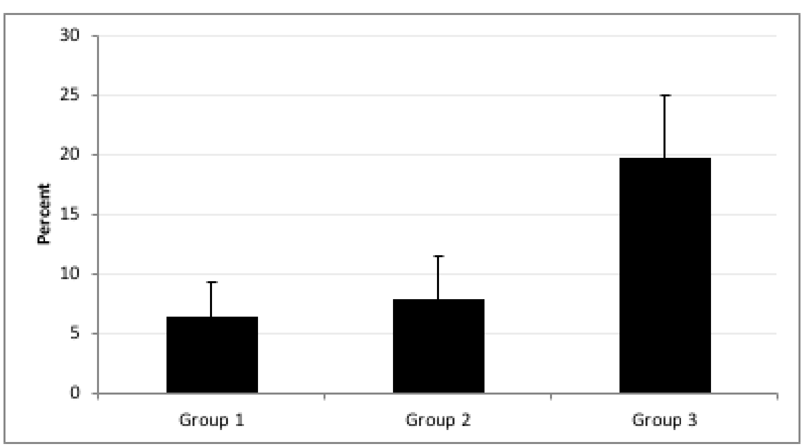

Fig. 2. Infection of dogs based on age groups: Group 1 (6.35%), Group 2 (7.84%), Group 3 (19.64%). Group 1: ≤12 months old, Group 2: 13–24 months old, Group 3: ≥25 months old

Table 1. Seroprevalence rate of *L. infantum* infection in stray dogs from Hamedan according to their sex and age

Variables	Number of samples examined (%)	No. of Positive samples (%)	95% CI [a]	*P* value[b]	OR[b] (95% CI[c])
Age				0.048	
Puppy (≤12 months; G1)	63 (37.06)	4 (6.35)	0.47–12.23		1
Young (13-24 months; G2)	51 (30)	4 (7.84)	0.51–15.17		1.26 (0.3–5.29)
Adult (≥25 months; G3)	56 (32.94)	11 (19.64)	9.27–30.01		3.61 (1.08–12.07)
No info	10	0 (0)			
Sex				1	
Male	63 (37.06)	7 (11.11)	3.27–18.95		1
Female	107 (62.94)	12 (11.21)	5.33–17.09		1.01 (0.38–2.72)
No info	10	0 (0)			

[a] *P* values are generated based on the Chi-Square test.
[b] OR (odds ratio) quantifies the association between exposure and an outcome.
[c] CI (confidence interval) is a range of plausible values that is likely to include a population value with a certain degree of confidence

DISCUSSION

In the present study, we detected anti-*L. infantum* antibodies in blood sera of 10.55% of dogs in Hamedan. In a recent article that reviewed 57 studies in Iran, out of 23450 dogs surveyed from 1982 to 2017, 12.52% were positive for CVL [5]. It worth mentioning that in the majority of studies on dogs in Iran, a direct agglutination test (DAT, a cost-effective diagnostic method) was employed, which has a lower sensitivity compared with commercial ELISA [10]. Hamedan, with the lowest infection rate among other provinces, is considered a non-endemic area for CVL [5]. However, the results of the present study showed a noticeable increase in seropositivity, *i.e.*, from 3.95% in 2013 to 10.55% in 2018. Meshkin-Shahr district of Ardebil displayed the same pattern with an increase in CVL from 14.8% in 1992 to 23.4% in 2011–2012, and 32.7% in 2014 employing one diagnostic test (DAT) and cut-off point (1:320) [12-14]. Ecological changes might contribute to increase antibody titers in dogs; however, additional surveys that can show the specific factors related to the increase of CVL in Iran are necessary.

In this study, the infection rate increased by age. Similarly, a meta-regression analysis from Iran revealed that the infection rate increased by age, and the rates in dogs >7 years old were almost two times more than dogs with 3–5 years old [10]. Moreover, the logistic regression model revealed that the odds ratio (OR) of infection increased by 2%, with each month of age. The increased infection in older dogs is explained with more probability of exposure to infected sand fly bite [15].

The seropositivity rate was 11.21% and 11.11% in female and male dogs, respectively, with no significant differences between sexes, which is in agreement with other studies [15]. Although in many studies, no difference in the prevalence of CVL between male and female dogs was observed, higher prevalence in males might be due to their roaming behavior [15].

In Iran, CVL is mainly attributed to bites of infected sand flies; however, fleas and ticks have been suspected as potential vectors of *L. infantum* [16]. It will be valuable to find out the role of these arthropods in the transmission of *Leishmania* species in endemic areas of the country. Also, factors such as length of hair coat, weight, breed, breed size, and the number of dogs living in groups [15, 17] have not received adequate attention in epidemiological studies in Iran.

Consecutive faunistic studies from Hamedan between 1993 to 2013, reported the competent vectors for *L. infantum*, including *Phlebotomus major* sensu lato, *P. kandelakii*, *P. halpensis*, *P. longiductus* and *P. alexandri* [18-22]. However, no data on the identification of *Leishmania*-infected sand flies is available. In the last 8 years in Hamedan, only one VL human case was reported. In summer 2012, a 7–10 years old boy with signs of fever, weakness, inappetence, splenomegaly, weight loss, and anemia was diagnosed infected by microscopic observation of Leishman-Donovan bodies in the bone marrow biopsy. Serological diagnostic tests, including DAT, indirect immunofluorescent assay (IFA), ELISA, and anti-rk39 IgG, were negative. He had marked anemia, leukocytopenia, and thrombocytopenia with atypical lymph nodes. The patient completely recovered with meglumine antimoniate (Glucantime®) treatment for 15–21 days [personal communication with the office of Vice-Chancellor in Health Affairs of Hamedan University of Medical Sciences]. Although no other VL case has occurred in the last 8 years, the increased seropositivity among stray dogs suggests a higher possibility of contracting the disease. Since local physicians in non-endemic areas do not regularly visit VL individuals, diagnosis and management of the diseases in Hamedan might be challenging.

An increase in antibodies against *L. infantum* in dogs of Hamedan and the presence of competent vectors for transmission of the parasite in the region makes the spread of VL possible. Further molecular-based study for the identification of the parasite in infected dogs, *Leishmania*-infected sand flies, and other associated risk factors can bring more insight into the epidemiology of the disease in the area. Identification of putative reservoir hosts by the diagnosis of vertebrate host blood in sandflies [23] will help towards the understanding of other animal roles such as cats in the epidemiology of VL as well [24]. Since the effectiveness of the culling of seropositive dogs in endemic areas as a control strategy has no scientific evidence and efficacy of available vaccines is below 90% [2], insecticide-impregnated dog collars [25] could be suggested to prevent and control spreading of VL in Hamedan.

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ETHICS APPROVAL

Cephalic or saphenous blood samples were taken from dogs after getting official permission and under the supervision of the Institutional Animal Ethics and Research Committee of Iranian Veterinary Organization (IVO, Iran), Hamedan Office (Certificate No. 32/1397.4.1).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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