



Serological survey of *Leishmania infantum* in wild rodents of Turkemen Sahra, Golestan province, North-East Iran

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ABSTRACT

Leishmania infantum, an etiologic agent of zoonotic visceral leishmaniasis (VL), is spreading in Iran. Dogs are the main reservoirs but *L.infantum* has been detected in jackals, foxes and wild rodents too. Despite high number of rodents in the world, the exact role of them in epidemiology of VL is not clear. In recent years the number of sporadic cases of dogs and humans VL has been increased in arid and semi-arid areas of Golestan province. The aim of this study was to detect *L.infantum* antibody in wild rodents of Turkemen Sahra district of Golestan province. 75 rodents were randomly trapped from 8 areas and after identification of the genus and species, blood was collected by cardiac puncture. Indirect immunofluorescence antibody test (IFAT) was performed on serum samples of captured rodents. % 8 of serum samples which belonged to *Mus musculus* population were positive. Results of current study highlight the possible role of wild rodents in the spreading of the *L.infantum* in ecosystem. More epidemiological and molecular study is recommended to imagine the exact role of wild rodents in life cycle of *L.infantum* in Golestan province.

1. Introduction

Visceral leishmaniasis (VL), an anthrozoosis, is caused by *Leishmania donovani* complex intracellular parasites, which includes *L. infantum*, whose life cycles involve two hosts a vertebrate (warm-blooded) and an insect (sand fly) (Baneth, 2006). VL is endemic in the Middle East and also in many tropical and subtropical areas of the world (Ozbel, 1995).

Dogs, red foxes (*Vulpes vulpes*), golden jackals (*Canis aureus*) and wolves (*Canis lupus*) have been introduced as the vertebrate reservoir hosts, also humans are the occasional hosts (Mohebbali, 2005).

Moreover, *L.infantum* has been isolated from some species of rodents such as *Meriones persicus* and *Mesocricetus auratus* and these species have been considered as secondary reservoirs for *L.infantum*, especially in the regions where sporadic cases of VL have been reported (Mohebbali, 1998).

So far, at least 7 endemic focal areas of VL have been detected in Iran which are: Fars province in the south, Ardabil province in the northwest, Azarbaijan-e-Sharghi province in the northwest, Khorramabad area (Lorestan province) in the west, Qom and Saveh areas in the central part, Khuzestan province in the southwest and Khorasan-Shomali province in the northeast of Iran. VL is spreading in

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Iran and it has been reported from non- endemic regions of Iran (Mohebbali, 2013).

Also in recent years, number of sporadic cases of dogs and humans VL has been increased in arid and semi-arid areas of Golestan province. Fakhhar and his colleagues have been detected VL infection in %30 of sampled dogs and %2.8 of sampled humans by PCR from Maraveh Tapeh district, Golestan province and they introduced Golestan province as a new endemic area for VL infection (Fakhhar, 2014). Occurrence of new cases of VL in non-endemic areas magnifies unsuccessful restriction of VL and also the risk of parasite introduction to these areas by wild and domestic canids or new vector species such as rodents in Iran (Fakhhar, 2011).

Rodents have the highest number of members among mammalian population and also wild rodents are known to inhabit in close proximity to human housing, especially where shelter and food are provided like those found in villages of Turkmen Sahra district in Golestan province which is consist of arid and semi-arid areas (Helhazar, 2013).

Turkemen Sahra district has arid and semi-arid climate where seems suitable for growth of sandflies which are the main vector of *Leishmania* Spp. (Ozbel, 1995).

Epidemiological investigations on rodents VL seems necessary to manage suitable strategies to control VL in Golestan province.

So to correlate the frequency of seropositive findings with possible risk factors for VL dispersing by wild rodents, present study has been investigated serological infection of *L.infantum* in wild rodents of Turkmen Sahra district, Golestan Province.

2. Materials and Methods

2.1. Sample collection

From 2013 to 2014, wild rodents were captured in 8 villages throughout Turkmen Sahra district (six villages from Gonbad and two villages from Maraveh Tapeh towns), Golestan province (36° 50' 21.48" N, 54° 26' 39.84" E). First trapping locations were chosen, and then in each site, 20 handmade cages were used during nights to catch animals alive. Geographical coordinates of the trapping places were recorded. Animals were collected early in the morning and transferred to a field laboratory, where blood samples were collected by cardiac

puncture and serum sample separated by centrifugation at 3000 rpm for 10 min. The genus and species of the rodents were determined by external characteristics including color, body measurements, ears, tail, feet, teeth and cranium (Boitani and Bartoli, 1980).

2.2 Indirect fluorescent antibody test (IFAT)

The assay was carried out according to the procedures previously described by Camargo, 1969. The samples showing titer of 1:40> and 1:80 ≤ were considered negative and positive, respectively (Khanmohammadi, 2011).

2.3. Statistical analysis

The obtained data were correlated with the frequency of seropositive results and analyzed by the chi-square test using the SPSS 20 statistical analysis software, with a significance level of P< 0.05.

3. Result

75 rodents were collected during the field study, including 46 (61%) *Mus musculus* and 22(29%) *Rattus norvegicus* and 7 (10%) *Meriones libycus*. 6(8%) of 75 sampled rodent which belonged to *Mus musculus* population (46) were positive (Table 1).

Table 1. Visceral leishmaniasis seropositivity of sampled rodents according to the species.

Name of species	S/C	P	F
<i>Mus musculus</i>	46/75	6/46	6/75(8%)
<i>Rattus norvegicus</i>	22/75	0/22	0/75(0%)
<i>Meriones libycus</i>	7/75	0/7	0/75(0%)

S: Number of species, C: Number of captured rodents, P: Number of positive samples; F: Frequency of seropositivity

4. Discussion

Since 1949 when the first case of VL was reported in humans and also dogs in Mazandaran province, VL infection has been reported from many districts of Iran (Pouya, 1949).

Identification of reservoir hosts as well as detection of probable new vectors is the main problems which epidemiologists have been faced to control VL. Despite considerable research on VL, the main reservoir hosts and the species of sand-flies

which are active in life cycle of the parasite have still not been identified in many areas.

Serofrequency of *L.infantum* in sampled rodents was %8. Compare to *L.infantum* infection in rodents of Azar-Shahr (%5.3) which has been shown as one of the endemic area in Iran, obtained result in this study seems high enough to consider probability of VL outbreak in the sampled areas (Fallah, 2006). Although Mohebbali *et al.*, 1998 has been detected *L.infantum* infection by microscopical examination in %16.5 of sampled rodents in another endemic area of Iran, Meshkin-Shahr (Mohebbali, 1998).

L.infantum infection has been detected in *Cricetulus migratorius*, *Meriones persicus* and *Mesocricetus auratus* from northwestern Iran, but we couldn't find any report about *L.infantum* infection of *Mus musculus* from Iran, so it is the first report of *L.infantum* infection of *Mus musculus* from Iran (Fallh, 2006; Mohebbali, 1998).

Natural infection of *Mus musculus* by *L.infantum* has been previously detected in Italy and Portugal too (Di Bella, 2003; Helhazar, 2013). *L.infantum* has been detected in *R. norvegicus* in Italy, Greece, Portugal and Yugoslavia, but in this study all the serum samples that belonged to this rodent species were negative. This result may be due to low numbers of this species in our study (Di Bella, 2003; Helhazar, 2013; Petrovic, 1975; Papadogiannakis, 2010).

Despite high number of rodents' species in the world, there is not enough survey on VL infection of rodents to clarify the exact role of rodent as vertebrate reservoirs in life cycle of *L.infantum*. Further studies on rodents VL is recommended for clarification of the exact role of rodents as vertebrate reservoirs in life cycle of *L.infantum*.

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