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Molecular characterization of *Leishmania* spp. in reservoir hosts in endemic foci of zoonotic cutaneous leishmaniasis in Iran

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Abstract: Zoonotic cutaneous leishmaniasis (ZCL) is an expanding disease and a public health issue in Iran. In the present study, rate of natural infection of rodent populations with *Leishmania* was investigated in six endemic foci including 28 villages in Golestan, Esfahan, Yazd, Fars, Khuzestan and Ilam provinces. A total of 593 rodents were captured and identified as *Rhombomys opimus* (n = 325), *Meriones libycus* (n = 171), *Meriones persicus* (n = 27), *Tatera indica* (n = 37), *Nesokia indica* (n = 12), *Rattus rattus* (n = 13) and *Mus musculus* (n = 8). Microscopic examinations of Giemsa-stained smears showed that 108 out of 593 (18.2%) rodents were infected with *Leishmania* spp., whereas infection of 186 out of 593 (31.4%) rodents with *Leishmania* was then confirmed by ITS1-PCR. The highest rate of infection was found in *R. opimus* (prevalence of 35%) and *M. libycus* (31%). Based on Restriction Fragment Length Polymorphism (RFLP), 145 (78%) of 186 samples detected as *Leishmania* DNA were identified as *L. major*, 8 (4%) samples as *L. turanica* and 33 (18%) as mixed infection (*L. major* and *L. turanica*). Samples from infected rodents were inoculated subcutaneously at tail base of BALB/c mice. In 35 of them, nodules and ulcers containing amastigotes appeared at the inoculation site. The samples prepared from infected rodents were cultured in NNN medium and only two samples were positive. *Rhombomys opimus*, *M. libycus*, *M. persicus*, *T. indica* and *N. indica* were confirmed as reservoir hosts of ZCL in the studied regions. *Leishmania major* infection was usually accompanied *L. turanica* in naturally infected gerbils (*R. opimus* and *M. libycus*) in Golestan, Esfahan and Fars provinces.

Keywords: *Leishmania major*, *Leishmania turanica*, reservoir hosts, rodents, gerbils, PCR-RFLP, Asia

Cutaneous leishmaniasis due to *Leishmania major* Yakimoff et Schokhor, 1914 is a public health problem in some areas of the Old World (Desjeux 2004). *Leishmania major* is widely distributed in various populations of rodents in arid and savannah regions (Gramiccia and Gradoni 2005). Rodents play an important role in natural transmission cycle and epidemiology of zoonotic cutaneous leishmaniasis (ZCL), and were already demonstrated as principal vertebrate reservoirs by Hertig et al. (1957).

Rodents belonging to the subfamily Gerbillinae Gray are the main reservoir hosts of ZCL in Iran and other countries, where ZCL due to *L. major* is endemic (Strelkova 1996, Mohebali et al. 2004, Gramiccia and Gradoni 2005). The disease is endemic in many rural districts of 17 out of 31 provinces of Iran (Yaghoobi-Ershadi 2012). Identification of the natural hosts of *Leishmania* Ross, 1903 is crucial to determine the natural cycle of the parasite and to understand the epidemiology of the disease.

In Iran, the first study on the reservoir hosts of cutaneous leishmaniasis was carried out in Turkemen-Sahara region by Ansari and Mofidi (1950) and later by Ansari and Faghih (1953) in Sarakhs district. Several reports indicated occurrence of cutaneous leishmaniasis due to *L. major* (see Mohebali et al. 2004, Rassi et al. 2006).

Considering the importance of rodents in maintenance of *L. major* in nature, identification of reservoir hosts of the disease is an important step in the control of ZCL. Four important foci of the disease based on rodent reservoir hosts have been reported in Iran: (i) central and north-east of Iran, where *Rhombomys opimus* Lichtenstein (Rodentia:

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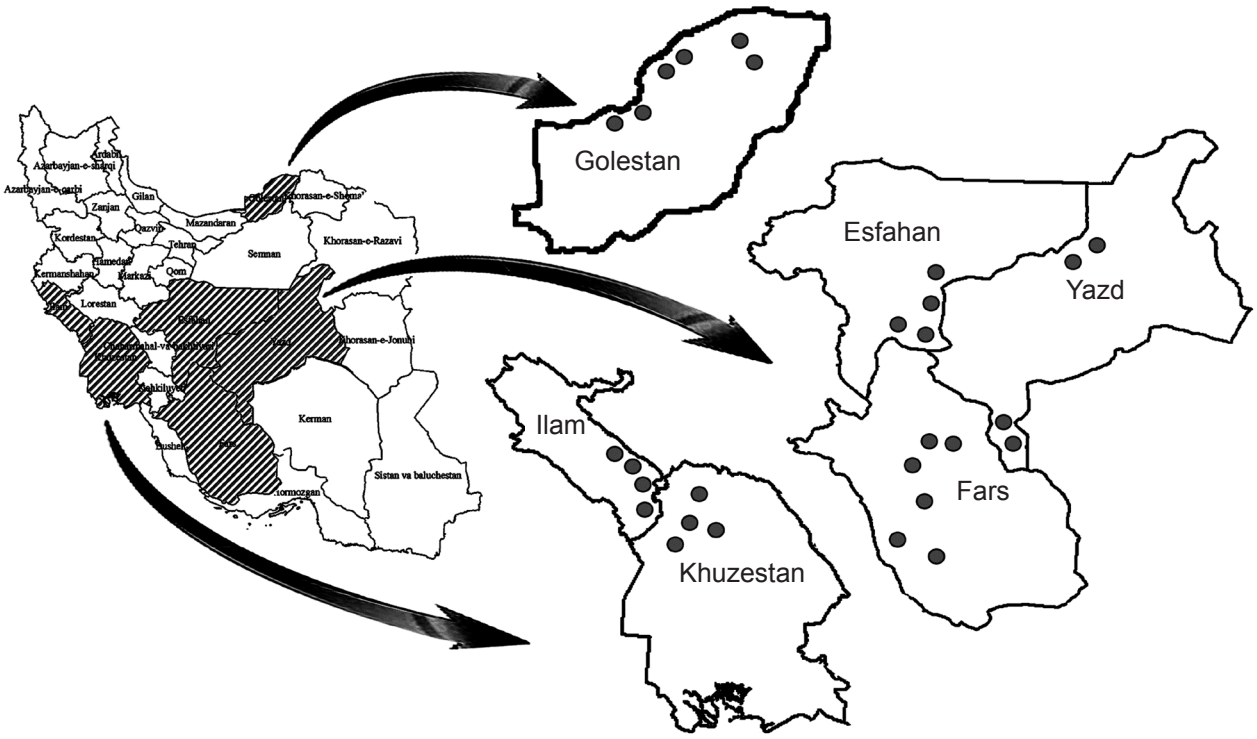

Fig. 1. Sampled rural regions with six endemic foci of zoonotic cutaneous leishmaniasis (ZCL) in Iran.

Gerbillinae) is the main reservoir of the disease (Javadian et al. 1976, Gramiccia and Gradoni 2005, Mirzaei et al. 2011); (ii) west and south-west of Iran, where *Tatera indica* Hardwicke (Rodentia: Gerbillinae) (Indian jird) replaced *R. opimus* and plays an important role as reservoir (Javadian et al. 1988); (iii) south-eastern Iran, where *Meriones hurrianae* Jerdon (Rodentia: Gerbillinae) was reported as a natural reservoir host (Kassiri et al. 2011), and (iv) southern Iran, where *Meriones libycus erythrourus* Lichtenstein (Rodentia: Gerbillinae) is considered the primary and main reservoir host of the disease, whereas *R. opimus* and *T. indica* are absent (Rassi et al. 2006, 2007).

Rhombomys opimus is reported as the main reservoir host of *L. major* in the vast territory of the Turan lowland (Central Asia, Afghanistan, Pakistan, Mongolia and some provinces of China) (Strelkova 1996, Shar et al. 2008). In Iran, *R. opimus* is the main reservoir of ZCL in the centre and north-east of the country. Several *Leishmania* infections due to *L. major* have been reported from *R. opimus* in endemic and non-endemic foci of ZCL throughout the country (Yaghoobi-Ershadi et al. 1996, 2001, Mohebali et al. 2004, Mirzaei et al. 2011).

Meriones libycus erythrourus is the primary reservoir of ZCL in some areas of the central and southern Iran (Rassi et al. 2001, 2006, 2011a, Moemenbellah-Fard et al. 2003, Mohebali et al. 2004). It has also been found infected in Turkemen-Sahara, Lotfabad and Esfahan, but it was found in localities where only infected *R. opimus* is present (Nadim and Seyedi-Rashti 1971).

Moreover, several investigations have also been carried out into reservoir hosts in other endemic foci of ZCL. *Tatera indica*, *N. indica* Gray et Hardwicke (Rodentia: Muridae) and *M. hurrianae* were reported as the main reservoirs infected with *L. major* in the west, south-west and south-east of Iran, respectively (Javadian 1988, Javadian et al. 1988, Yaghoobi-Ershadi et al. 1996, Rassi et al. 2001, Kassiri et al. 2011).

One of the major problems to control ZCL is the lack of information on the dynamics of *Leishmania* infection in rodent populations serving as reservoir hosts. Therefore, the aim of the present study is to identify the reservoir hosts and to characterize *Leishmania* infection in rodents in six important foci of ZCL in Iran.

MATERIALS AND METHODS

The investigation was conducted from August 2008 to October 2011 in 28 villages belonging to provinces of Golestan (Dashboron, Daneshmand, East Ghare Gol, Ozbak Abad, Okhey Tapeh and Shurdegesh), Esfahan (Sajzi, Yelengi, Timiart and Fasaran), Yazd (Tork Abad, Chahak, Chah Afzal and Fath Abad), Fars (Lapoui, Dolat Abad, Gol Dasht, Pol-e-Fasa, Ghir [Band-e-Bast] and Farashband), Khuzestan (Safi Abad, Deh Iji, Ghaale Mokhtar and Ali Abad) and Ilam (Vahdat Abad [Majhin], Badreh, Kolm and Sheikh Makan) (Fig. 1).

They are located in the endemic regions of ZCL in the north-east, centre, south and south-west of Iran with altitude between 580 m to 900 m. The temperature ranged between 34–40 °C and the humidity ratio was between 25–55% during our samplings in the studied areas.

Active colonies of gerbils in the studied districts were identified and rodents were caught alive using 60 Sherman traps. The traps were placed at gerbil burrows and checked regularly. The identification of the specimens was based on external characteristics: colour, body measurements, ears, tail, feet, teeth, cranium and other specific taxonomic criteria (Boitani and Bartoli 1980, Ziaei 1996).

In the laboratory, the rodents were anesthetized using ether. To confirm infections of rodents, regardless of having any obvious lesions, their ears, tails and the foot pads were examined and impression smears were made (Edrissian et al. 1982). The smears were fixed in methanol, stained with Giemsa and directly examined under a light microscope at high magnification (1 000×) to search for *Leishmania* parasites.

Samples from infected rodents were inoculated subcutaneously at the tail base of BALB/c mice. Parasites were also re-isolated from infected mice and cultured in Novy-MacNeal-Nicolle (NNN) culture plus liver infusion broth tryptose (LIT) medium containing 200 IU penicillin per ml, incubated at 20–21 °C and monitored periodically for *Leishmania* growth. All positive cultures were subcultured every seven days. Genomic DNA was extracted and purified using a conventional phenol-chloroform protocol (Sambrook and Russel 2001).

All prepared smears from ears, tails and the foot pads of rodents were washed with absolute ethanol to eliminate immersion oil on the smears and covered with 300 µl lysis buffer (50 mM Nacl, 50 mM Tris, 10 mM EDTA, pH 7.4, 1% v/v Triton x-100 and 100 µg of proteinase K per ml in pH 7.4). After a short time, the smeared material was transferred to a 1.5 ml reaction tube. Then, 15 µl proteinease K (20 mg/ml) was added to each tube. Cell lysis was accomplished after incubation for 4–6 h at 56 °C. The lysate was extracted by phenol-chloroform followed by ethanol precipitation. The DNA was resuspended in 30 µl TE 1X and stored at -20 °C.

Leishmania DNA was amplified by targeting Internal Transcribed Spacer 1 (ITS1) using the primers LITSR (forward: 5'-CTGGATCATTTTCCGATG-3') and L5.8S (reverse: 5'-TGATACCACTTATCGCACTT-3') under conditions described by El Tai et al. (2000) and Schönian et al. (2003). The length of amplicons was about 300–350 bp. Negative and positive controls were used for each batch of PCR. Amplicons were analysed using electrophoresis in agarose gel 1.5% containing ethidium bromide. *Hae*III enzyme was selected with cut site GG↓CC was selected as the best enzyme for RFLP diagnosis was chosen using CLC DNA Workbench 5.2 software (CLC bio A/S, Aarhus, Denmark). Reference strains of *L. major* (MRHO/IR/75/ER) and *L. turanica* Strelkova, Peters et Evans, 1990 (MRHO/SU/1983/MARZ-051) were used as control.

Endonuclease digestion was performed in a volume of 30 µl, which included 10 µl of PCR product, 2 µl of *Hae*III enzyme (Fermentas), 2 µl of 10× buffer and 16 µl of distilled water for four hours at 37 °C (El Tai et al. 2000, Schönian et al. 2003). Two negative controls were used, one without restriction enzyme and the other with no PCR product. The fragments were analysed using electrophoresis on agarose gel 3% containing ethidium bromide versus DNA ladder 50 bp (Fermentas). The fragments of 203 bp and 132 bp were observed for *L. major*. Concerning *L. turanica*, the expected length fragments of according to the software analysis was 203, 57, 53 and 24 bp. Due to the small size of the fragment of 24 bp and overlapping of two fragments with a similar molecular weight of 52 bp and 54 bp, the observed fragment length on the gel were about 200 bp and 50 bp (Fig. 2). To confirm the results, several PCR products were sequenced directly in both directions using the L5.8S and LITSR primers.

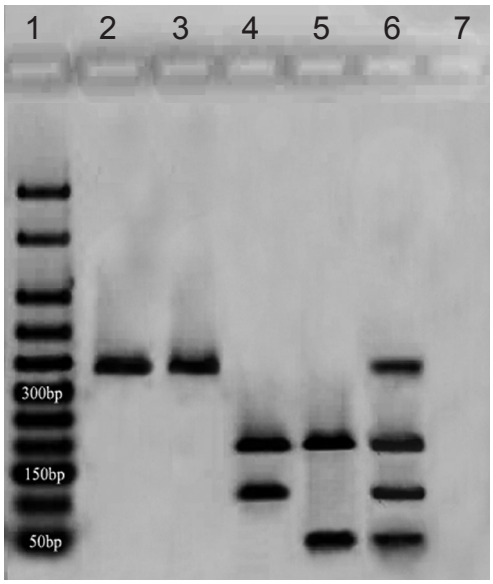

Fig. 2. Ethidium bromide-stained agarose gel of *HaeIII* digested PCR products of *Leishmania* species extracted from Giemsa-stained smears. Lane 1 – molecular marker (50 bp), Lanes 2,3 – undigested *L. major* and *L. turanica*; Lanes 4–6 – digested *L. major*, *L. turanica* and mixed infection (natural co-infection); Lane 7 – negative control.

RESULTS

A total of 593 rodents of seven species, namely *Rhombomys opimus* (325 samples; i.e. 54.8%), *Meriones libycus* (171 samples; 28.8%), *Meriones persicus* Blanford (Rodentia: Gerbillinae) (27 samples; 4.5%), *Tatera indica* (37 samples; 6.3%), *Nesokia indica* (12 samples; 2%), *Rattus rattus* Linnaeus (Rodentia: Muridae) (13 samples; 2.2%) and *Mus musculus* Linnaeus (Rodentia: Muridae) (8 samples; 1.4%), were caught in the present study (Table 1).

Of these, 186 (31.4%) were positive for *Leishmania* as detected by ITS1-PCR. Amastigotes were demonstrated in 108 (18.2 %) of total smears (Fig. 3), whereas promastigotes in 2 only (1.1 %) cultured rodent samples. A total of 114 out of 325 (35%) *R. opimus* and 53 out of 171 (31%) *M. libycus* were found to be infected with *Leishmania* using DNA amplification. No infection was observed in PCR assessments of *R. rattus* and *M. musculus* (Fig. 4).

Of 186 infected samples, 23 (12.3%) showed *Leishmania* spp. infection on one ear and 7 (3.7%) on both ears. The others had no cutaneous leishmaniasis lesion on their ear lobes.

Based on RFLP results, 145 (78%) of 186 samples detected as *Leishmania* DNA were identified as *L. major*, 8 (4%) samples as *L. turanica* and 33 (18%) were

Table 1. Characterisation of rodent hosts of zoonotic cutaneous leishmaniasis caught in six endemic foci located in the north-east, centre, south and south-west of Iran.

Province		Golestan	Esfahan	Yazd	Fars	Khuzestan	Ilam
Rhombomys opimus		227	40	54	-	4	-
Microscopic (+ve)		32	4	12	-	-	-
NNN culture (+ve)		-	-	-	-	-	-
Balb/c inoculation (+ve)		29	-	1	-	-	-
ITS1-5.8C rRNAgene (+ve)	*L. major*	59	13	14	-	-	-
	L. turanica	8	-	-	-	-	-
	Mix	14	6	-	-	-	-
Meriones libycus		19	10	10	121	4	7
Microscopic (+ve)		5	2	2	36	-	-
NNN culture (+ve)		-	-	-	2	-	-
Balb/c inoculation (+ve)		-	-	-	5	-	-
ITS1-5.8C rRNAgene (+ve)	*L. major*	7	4	2	27	-	-
	L. turanica	-	-	-	-	-	-
	Mix	-	-	-	13	-	-
Meriones persicus		-	-	2	25	-	-
Microscopic (+ve)		-	-	-	7	-	-
NNN culture (+ve)		-	-	-	-	-	-
Balb/c inoculation (+ve)		-	-	-	-	-	-
ITS1-5.8C rRNAgene (+ve)	*L. major*	-	-	-	9	-	-
	L. turanica	-	-	-	-	-	-
	Mix	-	-	-	-	-	-
Tatera indica		-	-	-	-	19	18
Microscopic (+ve)		-	-	-	-	3	4
NNN culture (+ve)		-	-	-	-	-	-
Balb/c inoculation (+ve)		-	-	-	-	-	-
ITS1-5.8C rRNAgene (+ve)	*L. major*	-	-	-	-	4	5
	L. turanica	-	-	-	-	-	-
	Mix	-	-	-	-	-	-
Nesokia indica		-	-	-	-	6	6
Microscopic (+ve)		-	-	-	-	1	-
NNN culture (+ve)		-	-	-	-	-	-
Balb/c inoculation (+ve)		-	-	-	-	-	-
ITS1-5.8C rRNAgene (+ve)	*L. major*	-	-	-	-	1	-
	L. turanica	-	-	-	-	-	-
	Mix	-	-	-	-	-	-

mixed infections. *Leishmania turanica* was found only in *R. opimus*, whereas mixed infections were detected in *R. opimus* and *M. libycus*. These mixed infections were detected in samples from Golestan, Esfahan and Fars provinces.

The samples prepared from infected rodents were inoculated subcutaneously at the tail base of BALB/c mice. Thirty five of 186 (19%) mice developed nodules and ulcers containing numerous amastigotes appeared at the site of inoculation 40–50 days after injection.

DISCUSSION

Distribution of *Leishmania major*, the causative agent of ZCL, has been found to coincide with *Rhombomys opimus* (see Strelkova 1996). Therefore, it is important to accurately assess the rate of *Leishmania* spp. infections in

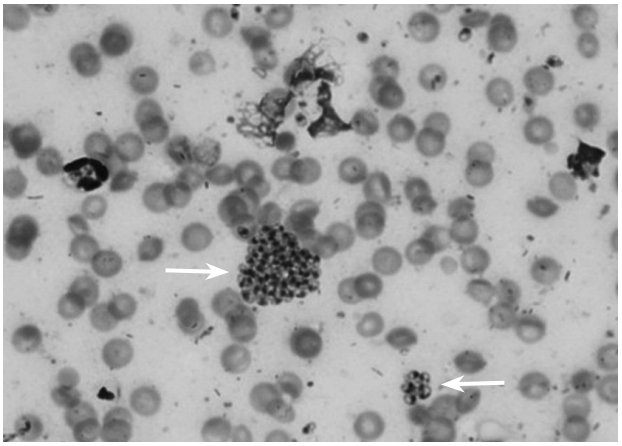

Fig. 3. Giemsa-stained smear prepared from infected *Rhombomys opimus*, positive for *Leishmania* amastigotes.

R. opimus and other important rodents that may serve as reservoir hosts.

Rhombomys opimus is the main reservoir host of ZCL in Iran and in some other countries (Javadian et al. 1976, Gramiccia and Gradoni 2005, Mirzaei et al. 2011). In agreement with previous studies, it is the principal reservoir host of ZCL in the central and north-eastern Iran. This great gerbil represented a little more than half (55%) of rodents caught in the present study. Moreover, 35% of infected rodents belonged to this species.

The infection of *R. opimus* with *L. major* was previously reported from Kalaleh (37.5% – Rassi et al. 2008a), Shahroud (91.9% – Rassi et al. 2008b), Damghan (40% – Rassi et al. 2011b) and Natanz (55.8% – Akhavan et al. 2010) counties in Iran. In our study, we have found this gerbil in Golestan, Esfahan, Yazd and Khuzestan provinces. It had been previously reported from Khorasan district, Semnan, Kerman and Sistan va Baluchestan provinces (Nadim and Seyedi-Rashti 1971, Mohebali et al. 2004, Sedaghat and Salahi-Moghadam 2010).

Meriones libycus was the second frequent species (28.8%), which was caught abundantly in the present study. It was previously found to be infected with *Leishmania* and can act as a secondary reservoir host in the absence of *R. opimus* in some endemic foci of north-east and centre of the country (Rassi et al. 2007). This role is in agreement with results of this study. Based on our findings, *M. libycus* can be considered an abundant species among rodents caught in Fars province (81.2%). As many as 33% of processed *M. libycus* were infected with *L. major* in prospected villages in this province. This species also acts as the main reservoir in southern Iran (Rassi et al. 2001, 2006, 2007, Momenbellah-Fard et al. 2003).

Meriones persicus was only caught in two out of six studied foci in the present study, namely in the Yazd and Fars provinces. Nine out of 25 (36%) *M. persicus* were infected with *L. major* in Fars province. In fact, this rodent

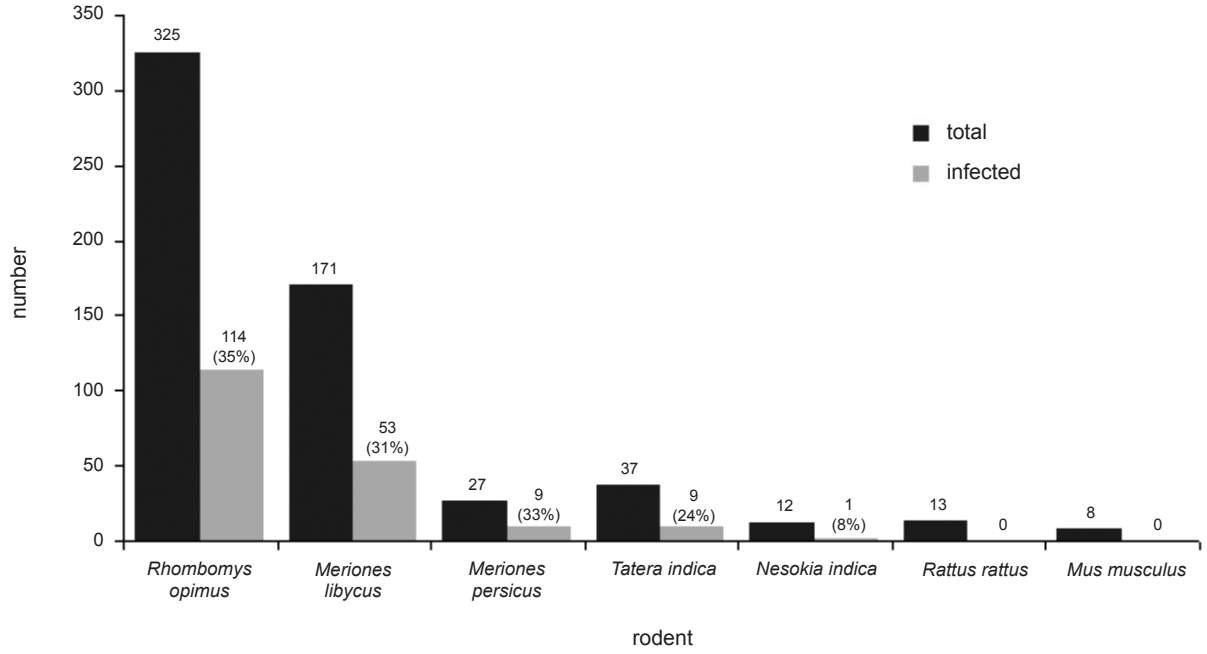

Fig. 4. Graph showing the number of rodent species trapped and infected in the studied counties. Prevalence of infection of individual species of rodents is provided in parentheses.

was previously reported as a probable reservoir of ZCL in Iran (Edrisian et al. 1975).

Tatera indica is the main reservoir host of ZCL in south-western Iran. We found this species in two foci in Khuzestan and Ilam provinces. More than half (54% and 58%) of rodents that we caught in these provinces belonged to *T. indica* and 21% and 27% of them were infected with *L. major*, respectively. Our findings are in accordance with previous investigations, which have been carried out in the above mentioned regions (Nadim and Faghih 1968, Javadian et al. 1988, Mohebali et al. 2001).

Nesokia indica has been considered a secondary reservoir in the absence of *T. indica* in south-west of the country (Nadim and Faghih 1968, Javadian et al. 1988, Mohebali et al. 2001). This species represented 18% (6 out of 33) and 19% (6 out of 31) of rodents captured in Khuzestan and Ilam provinces, respectively. Among processed *N. indica* in these two ZCL foci, one out of 6 (17%) *N. indica* was infected with *L. major* in Khuzestan province.

In vast territories of Central Asia, mixed infections of wild rodents with *L. major* (pathogenic to human beings) and *L. turanica* (non-pathogenic to man) are typical. *Leishmania turanica* was reported to be the dominant strain in *R. opimus* populations located in endemic foci of ZCL in Turkmenistan and Uzbekistan (Strelkova et al. 2001).

Based on our findings, it can be assumed that *L. major* and *L. turanica* circulate in *R. opimus* populations of Golestan and Esfahan provinces. Eight out of 88 (9%) of *Leishmania* DNA positive samples in PCR-RFLP assessment were identified as *L. turanica* in Golestan province. In Esfahan province, no case of *L. turanica* has been identified in processed *R. opimus*, whereas we found it as a mixed infection with *L. major* in 32% of *Leishmania* DNA positive isolates in our molecular assessment.

In most of the studies, identification of *Leishmania* species was done after isolation of parasites from culture media, which usually resulted in growth of only one species (Yaghoobi-Ershadi et al. 1996, Mohebali et al. 2004). In Iran, *L. turanica* was reported from *R. opimus* for the first time by Mohebali et al. (2004). Later, the infection of rodent reservoirs with *L. turanica* was reported from Kermanshah (Hajjaran et al. 2009), Esfahan (Akhavan et al. 2010) and Damghan (Rassi et al. 2011b) counties. In the present study, *L. major* infection was accompanied by *L. turanica* in 20 (17.5%) *R. opimus.*

The close association of *Phlebotomus papatasi* Scopoli as the vector of ZCL and infected rodents, e.g. *R. opimus,* with human population facilitates transmission of the disease. Moreover, urbanization changes in these endemic foci may have contributed to cutaneous leishmaniasis in this concomitant infection. Migration of refugees from Afghanistan and Iraq has also provided suitable conditions for further spreading of the disease in some endemic foci of ZCL in Golestan, Khuzestan and Ilam provinces.

The occurrence of ZCL in some endemic foci, e.g. Golestan, Ilam and Khuzestan provinces, seems to be the result of rapid urbanization, construction of buildings in farms near the colonies of rodents, poor sanitary condition, particularly among populations of illegal immigrants, storage of waste materials around towns, which are suitable for building nests by rodents, etc. Moreover, some

agricultural projects close to rural houses and existence of some animal shelters among old mud houses together with urbanization changes could increase the number of wild rodents and sand flies to facilitate disease transmission.

In conclusion, this study shows the species composition of rodents that may serve as reservoirs of *Leishmania* spp. in some regions of Iran. It has also revealed the infection rate of *R. opimus*, *M. libycus*, *M. persicus*, *T. indica* and *N. indica*, which may all serve as the main or secondary reservoirs ZCL in the studied regions. Further ecological and biological studies of rodents, sand flies and human cases in endemic foci of ZCL are necessary to better clarify the circulation of the disease.

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REFERENCES

Akhavan A.A., Yaghoobi-Ershadi M.R., Khamesipour A., Mirhendi H., Alimohammadian M.H., Rassi Y., Arandian M.H., Jafari R., Abdoli H., Shareghi N., Ghanei M., Jalali-zand N. 2010: Dynamics of *Leishmania* infection rates in *Rhombomys opimus* (Rodentia: Gerbillinae) population of an endemic focus of zoonotic cutaneous leishmaniasis in Iran. Bull. Soc. Pathol. Exot. 103: 84–89.

Ansari N., Faghih M. 1953: Leishmaniose cutane/*L. tropica* chez *Rhombomys opimus*. Ann. Parasitol. Hum. Comp. 25: 24–26.

Ansari N., Mofidi S. 1950: Contribution l'étude des formes humides de leishmaniose cutané. Bull. Soc. Path. Exot. 43: 601–607.

Boitani L., Bartoli S. 1980: Macdonald Encyclopedia of Mammals. Macdonald and Co., London, 512 pp.

Desjeux P. 2004: Leishmaniasis: current situation and new perspectives. Comp. Immunol. Microbiol. Infect. Dis. 27: 305–318.

Edrissian G.H., Ghorbani M., Tahvildar-Biruni G. 1975: *Meriones persicus*, another probable reservoir of zoonotic cutaneous leishmaniasis in Iran. Trans. R. Soc. Trop. Med. Hyg. 69: 517–519.

Edrissian G.H., Zovein H., Nadim A. 1982: A simple technique for preparation of smears from the ear of *Rhombomys opimus* for the detection of leishmaniasis infection. Trans. R. Soc. Trop. Med. Hyg. 76: 706–707.

El Tai N.O., Osman O.F., El Fari M., Presber W., Schönian G. 2000: Genetic heterogeneity of ribosomal internal transcribed spacer (ITS) in clinical samples of *Leishmania donovani* spotted on filter paper as revealed by single-strand conformation polymorphisms (SSCP) and sequencing. Trans. R. Soc. Trop. Med. Hyg. 94: 1–5.

Gramiccia M., Gradoni L. 2005: The current status of zoonotic leishmaniasis and approaches to disease control. Int. J. Parasitol. 35: 1169–1180.

Hajjaran H., Mohebali M., Alimoradi S., Abaei M.R., Edrissian G.H. 2009: Isolation and characterization of pathogenic *Leishmania turanica* from *Nesokia indica* (Rodentia, Muridae) by PCR-RFLP and ITS1 sequencing in Iran. Trans. R. Soc. Trop. Med. Hyg. 103: 1177–1179.

Hertig M., Fairchild E.B., Joihnson C.M. 1957: Leishmaniasis transmission-reservoir project. Ann. Rep. Gorgas. Mem. Lab. 9: 9–11.

Javadian E., Dehestani M., Nadim A., Rassi Y., Tahvildare-Biruni G.H., Seyedi-Rashti M.A., Shadmehr A. 1988: Confirmation of *Tatera indica* (Rodentia:Gerbillidae) as the main reservoir host of zoonotic cutaneous leishmaniasis in the west of Iran. Iran. J. Publ. Hlth. 27: 55–60.

Javadian E., Nadim A., Tahvildare-Biruni G.H., Assefi V. 1976: Epidemiology of cutaneaous leishmaniasis in Iran. B: Khorassanarea, part V; report on a focus of zoonotic cutaneaous leishmaniasis in Esferayen. Bull. Soc. Pathol. Exot. 69: 140–143.

Kassiri H., Javadian E., Abdigoudarzi M. 2011: Natural *Leishmania* infection in *Meriones hurrianae* and *Tatera indica* (Rodentia: Cricetidae: Gerbillinae) in Sistan-Baluchistan Province, South-Eastern of Iran. Adv. Studies Biol. 6: 247–256.

Lichtenstein M. 1823: Beschreibung viele bisher unbekannter Arten von Säugetieren, Vögeln, Amphibien und Fischen. Naturh. Anh. Eversmann's Reiser. 1: 123.

Mirzaei A., Rouhani S., Taherkhani H., Farahmand M., Kazemi B., Hedayati M., Baghaei A., Davari B., Parvizi P. 2011: Isolation and detection of *Leishmania* species among naturally infected *Rhombomys opimus*, a reservoir host of zoonotic cutaneous leishmaniasis in Turkemen Sahara, North East of Iran. Exp. Parasitol. 129: 375–380.

Moemenbellah-Fard MD., Kalantari M., Rassi Y., Javadian E. 2003: The PCR-based detection of *Leishmania major* infections in *Meriones libycus* (Rodentia: Muridae) from southern Iran. Ann. Trop. Med. Parasitol. 97: 811–816.

Mohebali M., Hamzavi Y., Edrissian G.H., Forouzani A. 2001: Sero-epidemiological study of visceral leishmaniasis among humans and animal reservoirs in Bushehr province, Islamic Republic of Iran. East Med. Hlth. J. 7: 912–917.

Mohebali M., Javadian E., Yaghoobi-Ershadi M.R., Akhavan A.A., Hajjaran H., Abaei M.R. 2004: Characterization of *Leishmania* infection in rodents from endemic areas of the islamic republic of Iran. East Med. Hlth. J. 10: 591–599.

Nadim A., Faghih M. 1968: Epidemiology of cutaneous leishmaniasis in the Isfahan province of Iran: I. The reservoir, II. Human infection, Trans. R. Soc. Trop. Med. Hyg. 62: 534–542.

Nadim A., Seyedi-Rashti M.A. 1971: A brief review of the epidemiology of various types of leishmaniasis in Iran. Acta Med. Iran 14: 99–106.

Rassi Y., Abaei M.R., Javadian E., Rafizadeh S., Imamian H., Mohebali M., Fateh M., Hajjaran H., Ismaili K. 2008b: Molecular data on vectors and reservoir hosts of zoonotic cutaneous leishmaniasis in central Iran. Bull. Soc. Pathol. Exot. 101: 425–428.

Rassi Y., Gassemi M.M., Javadian E., Rafizadeh S., Motazedian H., Vatandoost H. 2007: Vectors and reservoirs of cutaneous leishmaniasis in Marvdasht district, southern Islamic Republic of Iran. East Med. Hlth. J. 13: 686–693.

Rassi Y., Jalali M., Javadian E., Motazedian M. 2001: Confirmation of *Meriones libycus* (Rodentia, Gerbillidae) as the main reservoir host of zoonotic cutaneous leishmaniasis in Arsanjan, Fars Province, South of Iran (1999–2000). Iranian J. Publ. Hlth. 30: 143–144.

Rassi Y., Javadian E., Amin M., Rafizadeh S., Vatandoost H., Motazedian H. 2006: *Meriones libycus* is the main reservoir of zoonotic cutaneous leishmaniasis in south Islamic Republic of Iran. East Med. Hlth. 12: 475–477.

Rassi Y., Oshaghi M.A., Mohammadi Azni S., Abaei M.R., Rafizadeh S., Mohebali M., Mohtarami F., Zeinali M.K. 2011b: Molecular detection of *Leishmania* infection due to *Leishmania major* and *Leishmania turanica* in the vectors and reservoir host in Iran. Vector-Borne Zoon. Dis. 11: 145–150.

Rassi Y., Saghafipour A., Abaei M.R., Oshaghi M.A., Rafizadeh S., Mohebali M., Yaghoobi-Ershadi M.R., Mohtarami F., Farzin Nia B. 2011a: *Phlebotomus papatasi* and *Meriones libycus* as the vector and reservoir host of cutaneous leishmaniasis in Qomrood District, Qom Province, central Iran. Asian Pac. J. Trop. Med. 4: 97–100.

Rassi Y., Sofizadeh A., Abaei M.R., Oshaghi M.A., Rafizadeh S., Mohebali M., Mohtarami F., Salahi R. 2008a: Molecular detection of *Leishmania major* in the vectors and reservoir hosts of cutaneous leishmaniasis in Kalaleh District, Golestan Province, Iran. Iran. J. Arthropod Borne Dis. 2: 21–27.

Sambrook J., Russel D.W. 2001: Molecular Cloning. Third Edition. Cold Spring Harbor Laboratory Press, New York, 2344 pp.

Schönian G., Nasereddin A., Dinse N., Schweynoh C., Schalling H.D., Presber W., Jaffe C. 2003: PCR diagnosis and characterization of *Leishmania* in local and imported clinical samples. Diag. Microbiol. Infect. 47: 349–458.

Sedaghat M.M., Salahi-Moghadam A. 2010: [Mapping the distribution of the important rodent reservoirs in Iran.] J. Army Univ. Med. Sci. 8: 210–223. (In Persian.)

Shar S., Lkhagvasuren D., Molur S. 2010: *Rhombomys opimus*. IUCN Red List of Threatened Species – www.iucnredlist.org.,4/2010.

Strelkova M.V. 1996: Progress in studies on Central Asian foci of zoonotic cutaneous leishmaniasis: a review. Folia Parasitol. 43: 1–6.

Strelkova M.V., Eliseev L.N., Ponirovsky E.N., Dergacheva T.L., Annacharyeva D.K., Erokhin P.I., Evans D.A. 2001: Mixed leishmanial infection in *Rhombomys opimus*: a key to the persistence of *Leishmannia major* from one transmission season to the next. Ann. Trop. Med. Parasitol. 95: 811–819.

Yaghoobi-Ershadi M.R. 2012: Phlebotomine sand flies (Diptera: Psychodidae) in Iran and their role on *Leishmania* transmission. J. Arthropod-Borne Dis. 6: 1–17.

Yaghoobi-Ershadi M.R., Akhavan A.A., Mohebali M. 1996: *Meriones libycus* and *Rhombomys opimus* (Rodentia: Gerbillidae) are the main reservoir hosts in a new focus of zoonotic cutaneous leishmaniasis in Iran. Trans. R. Soc. Trop. Med. Hyg. 90: 503–504.

Yaghoobi-Ershadi M.R., Hanafi-Bojd A.A., Akhavan A.A., Zahraei-Ramazani A.R., Mohebali M. 2001: Epidemiological study in a new focus of cutaneous leishmaniasis due to *Leishmania major* in Ardestan town, Central Iran. Acta Trop. 79: 115–121.

Ziaei H. 1996: A field guide for identifying of Iranian desert mamalians. In: Rodents of Iran. Press Association Familiarity with Wildlife. Vol. 1 Iranian Environment Organization. Tehran, pp. 129–187.

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