https://doi.org/10.18502/jad.v18i3.18576

## Original Article

# *Leishmania* spp Infection in Patients and Great Gerbils (*Rhombomys opimus*) in a High-Risk Focus of Zoonotic Cutaneous Leishmaniasis in Central Iran: A Microscopic and Molecular Survey

**Narges Marvi-Moghadam[1], Mehdi Mohebali[2], Yavar Rassi[1], Ali Reza Zahraei-Ramazani[1], Mohammad Ali Oshaghi[1], Reza Jafari[3], Mahboubeh Fatemi[1], Mohammad Hossein Arandian[3], Hamid Abdoli[3], Nilufar Shareghi[3], Maryam Ghanei[3], Nilufar Jalali-Zand[3], Arshad Veysi[4], Javad Ramazanpoor[5], Kurosh Aminian[5], Ali Salehi[5], *Ali Khamesipour[6], *Amir Ahmad Akhavan[1]**

[1]Department of Vector Biology and Control of Diseases, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[3]Isfahan Health Research Station, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
[4]Research Institute for Health Development, Zoonoses Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran
[5]Isfahan Health Center, Isfahan University of Medical Sciences, Isfahan, Iran
[6]Center for Research and Training in Skin Diseases and Leprosy, Tehran University of Medical Sciences, Tehran, Iran

***Corresponding authors:** Prof Amir Ahmad Akhavan, E-mail: aaakhavan@tums.ac.ir, Prof Ali Khamesipour, E-mail: Ali.khamesipour@gmail.com

**(Received 22 June 2023; accepted 16 Jul 2023)**

## Abstract

**Background:** Zoonotic cutaneous leishmaniasis (ZCL) is an endemic disease in Varzaneh City where *Leishmania major* is the causative agent and the great gerbil, *Rhombomys opimus*, is the main reservoir host of the disease. Despite control efforts, ZCL outbreaks recur every few years. This study was conducted to revive information on the parasite/s species circulating between humans and the reservoirs in the region.

**Methods:** *Leishmania* infection in patients and *R. opimus* was studied using direct parasitological and molecular methods during 2019–2021. Nested-PCR and DNA sequencing were used for *Leishmania* parasite identification. Inter and intra-species variations in the *Leishmania* parasites were investigated using BLAST and MEGA7 software.

**Results:** All suspected patients (N=34) and 14 out of 36 great gerbils tested positive for *Leishmania* parasites via direct parasitological method. Nested-PCR method revealed all the patients were infected with *L. major* (94.1%) and mixed infection of *L. major* and *Leishmania turanica* (5.9%), and great gerbil specimens were infected with either *L. major* (44.4%), *L. turanica* (5.6%), or *Leishmania gerbilli* (5.6%) and also with mixed infection of *L. major* and *L. turanica* (30.5%), *L. major* and *L. gerbilli* (8.3%) and mix of all the three *Leishmania* species (5.6%).

**Conclusion**: The identical sequences of *L. major* in both human patients and rodents indicate that the great gerbils are the main reservoirs of *L. major* in Varzaneh City. The presence of *L. turanica* in patients would be of interest to carry out further studies to determine the role of this species in the persistence, signs, and treatment of ZCL in humans.

**Keywords:** Cutaneous leishmaniasis; Immune response; *Rhombomys opimus*; Salivary gland antigens; *Phlebotomus papatasi*

## Introduction

Leishmaniasis is still a public health issue in many parts of the world including Iran. In recent years the number of leishmaniasis foci has expanded in the country; several reasons have been proposed for this expansion including the migration of susceptible people to the disease foci, ecological or biological characteristic changes among vectors and reservoirs, and

reducing the use of insecticides (1–3). This disease causes remarkable side effects among people who are living in endemic areas such as long-term lesions and scars (4). *Leishmania major* is the causative agent of zoonotic cutaneous leishmaniasis (ZCL) in Iran and has been naturally isolated from patients (1, 5–6). Sand flies belonging to the subgenera *Phlebotomus* and *Paraphlebotomus* are the main and or secondary vectors of ZCL (7–9). *Phlebotomus* (*Phlebotomus*) *papatasi* is the main vector of ZCL and transfers the causative agent of the disease among rodent populations and also from rodents to humans. *Phlebotomus* (*Paraphlebotomus*) *caucasicus* is the main vector of the disease only among rodent populations (10). Rodents belonging to the Gerbillinae subfamily are the main reservoir hosts of ZCL in Iran and other countries of the Old World where cutaneous leishmaniasis due to *L. major* (CLM) is endemic (11–18). *Rhombomys opimus* (great gerbil) in central Asia, central and northern Iran), and Afghanistan (11, 13–15), *Meriones libycus* (Libyan Jird) in Central Asia, Iran, and the Arabian Peninsula; *Meriones hurrianae* in the southeast of Iran and India (12, 19–21) and *Tatera indica* in the southwest of Iran play as the main reservoir hosts of ZCL (12). It has been demonstrated that three species of *Leishmania* parasites, including *L. major*, *Leishmania turanica*, and *Leishmania gerbilli*, are widespread among *R. opimus* and *Ph. papatasi* populations at different ZCL foci in Iran (3, 11, 14, 20).

Control of the disease in some areas is too difficult, impossible, or not successful due to unknown epidemiological factors. Despite significant developments in leishmaniasis research in recent years, such as biochemistry and molecular biology of the parasite(s), vector(s), reservoir host(s), and host immune responses, little information on practical disease management is available (22). Leishmanization has been successfully performed for ZCL prevention in Iran, but it has been stopped due to very rare unhealing lesion cases although it is still suggested for military personnel in high–risk areas (23). Until now, no vaccine against any type of human leishmaniasis has been registered (24). To control the disease, it is necessary to know the different epidemiological aspects of the disease, including the causative agents and reservoirs. Despite the implementation of control programs, ZCL epidemics occur in Varzaneh City every few years. In addition, there was no comprehensive investigation on the parasites and the reservoirs of the disease in the area for more than three decades. This study was conducted to up-to-date information on the disease causative agent(s) and reservoir(s) in this region and the role of *Leishmania* infection in humans and reservoir hosts to parasite maintenance and establishment of the natural transmission cycle of CL.

## Materials and Methods

### Study area

This study was conducted in Varzaneh City, Isfahan Province, center of Iran. This city is located 110km east of Isfahan and Zayandehrud River, which is located 30 km from Gavkhoni International Wetland and 7 km from sand dunes (Fig. 1). According to the last census report in 2016, its population was 17714 people.

This desert city has hot and dry weather in summer and cold weather in winter. In 2020–2021, the average maximum and minimum temperatures were 42.6 °C and 9.1 °C, respectively. The average minimum relative humidity was 12%, and the maximum was 46%. The drought period in Varzaneh City is from the first of March to the first of November. The average yearly wind speed in Varzaneh City is 3 m/s.

### Human cases and smear preparation

Thirty-four local persons suspected to be infected with the *Leishmania* parasite were selected for the study in 2018–2019. The demographic information of human cases such as

https://doi.org/10.18502/jad.v18i3.18576

age, sex, and site of lesion were recorded. A skin biopsy was taken from the border of active skin lesion/s of each patient under sterile conditions, fixed with methanol, and stained by Geimsa 10% for microscopic examinations. A smear was also prepared for molecular detection and identification. Finally, the patients received appropriate treatment.

**Reservoir host collection**

Live rodent specimens were collected using 10–20 Sherman traps baited with cucumbers in June, September, October, November, and December 2018 and 2019. The traps were placed in the mornings and collected before sunset. Caught rodents were identified by the valid morphological key (25).

**Direct microscopic examination**

The collected great gerbils were anesthetized in the laboratory by using Ketamine hydrochloride (60 mg/kg) and Xylazine (15 mg/kg) intramuscularly. Regardless of observing any obvious lesions, impression smears were prepared from the ear lobes of the rodents, stained using Giemsa, and directly examined under a light microscope (1000x) (26). After preparing direct smears, whole ear lobes were removed, disrupted by grinding with a pestle, and preserved in 96% ethanol at -20 °C for further molecular tests.

Prepared smears taken from human lesion/s were also stained using Giemsa 10% and examined for *Leishmania* amastigotes under the light microscope with high magnification (1000X).

**DNA extraction and nested-PCR amplification**

Detached ear lobes were grilled, crushed, and transferred to a microtube containing PBS (pH=7.2), then it was vortexed, and 300 ul of the solution was used for genomic DNA extraction according to the procedure recommended by the manufacturer (Gene All kit). Extracted DNA was resolved in 20 µl elution buffer and kept at -20 °C degrees till use.

Nested PCR was used to amplify the ITS2 region of the rRNA gene of *Leishmania* parasites based on the method already explained by Akhavan et al. 2010 (26) External primers include Leish out F (5 ′-AAA CTC CTC TCT GGT GCT TGC-3′) and Leish out R (5 ′-AAA CAA AGG TTG TCG GGG G-3 ′) and internal primers include Leish in F (5 ′-AAT TCA ACT TCG CGT TGG CC-3 ′) and Leish in R (5 ′-CCT CTC TTT TTT CTC TGT GC-3 ′). The initial PCR mixture consisted of 0.6 µM of each external primer, 12.5 µl Taq DNA polymerase, 2X Master Mix Red (Amplicon, Denmark), 1.5 µl of DNA template, and sterile distilled water to a final volume of 25 µl. The first round of PCR conditions was as follows: an initial denaturation step at 95 °C for 5min, followed by 30 cycles: denaturation at 94 °C for 30 seconds, annealing at 60 °C for 45 seconds, extension at 72 °C for 30 s, final extension at 72 °C for 5 minutes. The second round of nested PCR was performed in a final volume of 20 µl containing 1 µl of a 1:10 dilution in distilled water of the first round PCR product as a template, 0.3 µM of each internal forward (Leish in F) and reverse (Leish in R) primers, 10 µl of Taq DNA polymerase, 2X Master Mix Red (Amplicon, Denmark). The reactions were cycled under the following conditions: 95 °C for 2min, 25 cycles of 94 °C for 15 s, 62 °C for 30 s, and 72 °C for 45s followed by 72 °C for 5min. Reference strain of *L. major* (MRHO/IR/75/ER) and DDW were used as positive and negative controls, respectively. PCR products were separated by electrophoresis in a 1.5% agarose gel in TBE buffer (26).

**Sequencing and molecular phylogenetic analysis**

For reconfirming the nested-PCR results and also comparing the parasite sequences of *L. major* isolated from humans and great gerbils, 13 ITS2 nested-PCR products from the *Leishmania*-infected humans and rodents were randomly selected and sequenced using the same primers used for PCR amplification by Codon


http://jad.tums.ac.ir
Published Online: September 30, 2024

https://doi.org/10.18502/jad.v18i3.18576

Genetic Group, Tehran, Iran.

DNA sequences of humans and rodents were compared to the available sequences in the GenBank Database by using the Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih).

A molecular phylogeny tree was constructed using *L. major* ITS2-rRNA gene sequences detected in humans (H) and *R. opimus* (R) in combination with some representative ITS2 sequences available in GenBank. The *Leishmania turanica* sequence was used as an outgroup for phylogenetic analysis. The tree was computed by maximum likelihood based on the Tamura-Nei model embedded in MEGA 7.0 software (27–28).

## Results

Fourteen cases (38.9%) of 36 *R. opimus* (32 females and 4 males) specimens were positive by microscopic examination, while all 36 (100%) specimens were positive by Nested PCR.

The results showed that 44.4 % of the great gerbils were infected with *L. major*, 5.6% with *L. turanica*, 5.6% with *L. gerbilli*, 30.5% with mixed infection of *L. major* and *L. turanica*, 8.3% with mixed infection of *L. major* and *L. gerbilli*, and 5.6% with mixed infection of all three species (Table 1, Fig. 2).

Infection with *L. major* and mixed infection with *L. major* and *L. turanica* were observed in all seasons. Natural infection with *L. turanica* and mixed infection with *L. major* and *L. gerbili* were detected in the fall and winter while mixed infection with *L. major*, *L. turanica*, and *L. gerbili* was only observed in the fall. The maximum *L. major* infection rate was observed in winter (61.50%) and the minimum infection rate was observed in spring and summer (25%). A statistically significant difference was observed in *Leishmania* infection rates among different seasons ($P< 0.0001$) (Fig. 3) but no significant difference was observed in *Leishmania* spp infection rates between male and female great gerbils ($P> 0.05$).

Table 1 represents seasonal *Leishmania* species infection rates detected in *R. opimms* specimens tested by nested-PCR in Varzaneh, 2019-2020. The most prevalent *Leishmania* species in rodents was *L. major* (47.2%) and its mixed infection with *L. turanica* (25%).

In the current study four *Meriones libycus* (2 males and 2 females) specimens were also trapped and investigated for *Leishmania* infection. Two out of four jirds were infected with *L. major* and two of them were infected with mixed of *L. major* and *L. turanica*.

The *Leishmania* parasites were also identified by using the nested PCR method in 34 human specimens which were positive by microscopic direct observation. Of 34 positive human cases, 94.1% were infected with only *L. major* and 5.9% with mixed of *L. major* and *L. turanica.* Identification interpretation of the nested PCR products was based on fragment size polymorphisms which are 245 or 233 bp for *L. major*, 206bp for *L. gerbilli*, and 141 bp for *L. turanica* (Fig. 4).

https://doi.org/10.18502/jad.v18i3.18576

**Table 1.** Seasonal *Leishmania* species infection in *Rhombomys opimus* specimens tested by ITS2 nested PCR, Varzaneh City, Isfahan Province, Iran, 2019–2020

| Season | | *L. major* | *L. gerbilli* | *L. turanica* | *L. major* and *L. gerbilli* | *L. major* and *L. turanica* | *L. major*, *L gerbili* and *L. turanica* |
|---|---|---|---|---|---|---|---|
| | No. of samples tested | No. of Positive (%) | No. of positive (%) | No. of positive (%) | No. of positive (%) | No. of positive (%) | No. of positive (%) |
| **Spring** | 4 | 1(25) | 2 (50) | 0 | 0 | 1(25) | 0 |
| **Summer** | 4 | 1(25) | 0 | 0 | 2(50) | 1(25) | 0 |
| **Fall** | 15 | 6(40) | 0 | 1(6.7) | 0 | 6(40) | 2(13.3) |
| **Winter** | 13 | 8(61.5) | 0 | 1(7.7) | 1(7.7) | 3(23.1) | 0 |
| **Total** | 36 | 16(44.4) | 2 (5.6) | 2(5.6) | 3(8.3) | 11(30.5) | 2(5.6) |

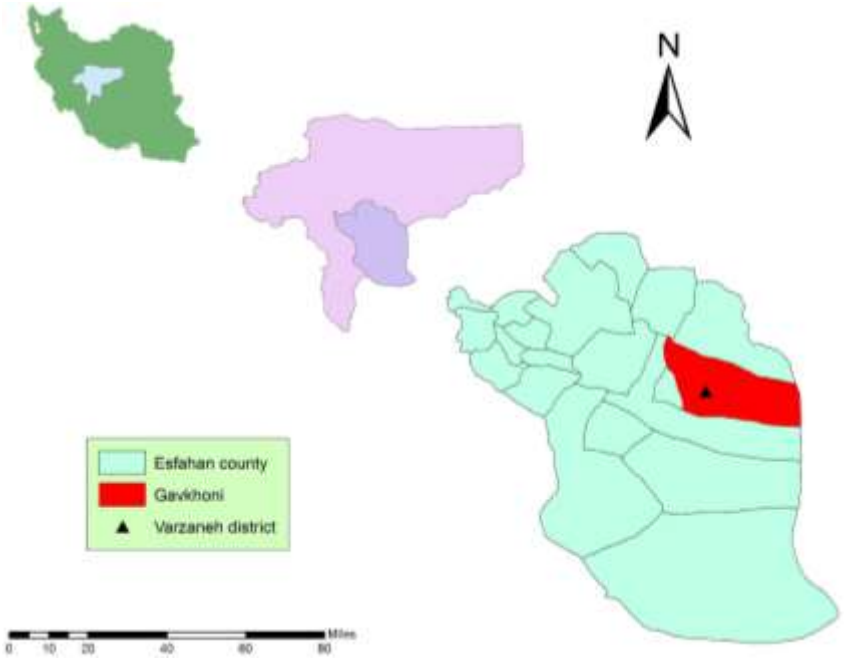


**Fig. 1.** Map of the study area in the hyperendemic zoonotic cutaneous leishmaniasis focus of Isfahan Province, central Iran

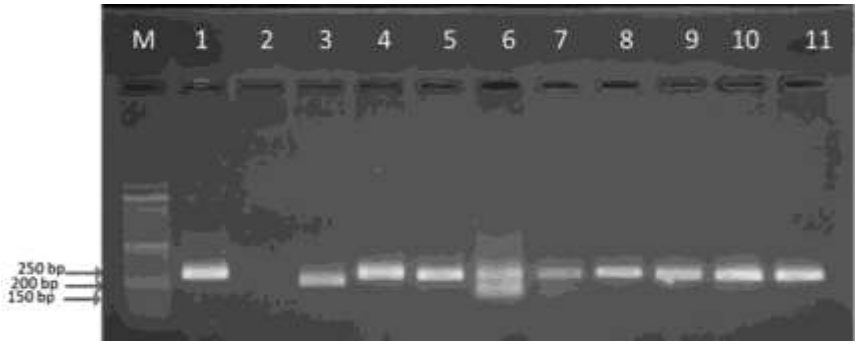


**Fig. 2.** Results of ITS2 nested PCR for detection *Leishmania* parasites in *Rhombomys opimus* from Varzaneh City, Isfahan 2019–2020. M: 50 bp ladder (SinaClon, Iran), 1: *L. major* positive control, 2: negative control (DDW), 3: *L. gerbilli*, 4 and 5: *L. major*, 6: mixed infections of *L. major* and *L. turanica*, 7–11: *L. major*


http://jad.tums.ac.ir
Published Online: September 30, 2024

https://doi.org/10.18502/jad.v18i3.18576

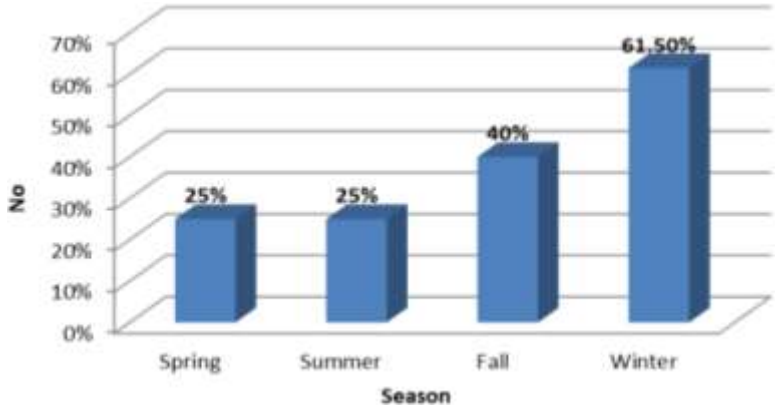


**Fig. 3.** Seasonal fluctuation of *Leishmania major* infection rate detected by ITS2 nested-PCR in the great gerbils (*Rhombomys opimus*) specimens from Varzaneh City, Isfahan Province, Iran, 2019–2020

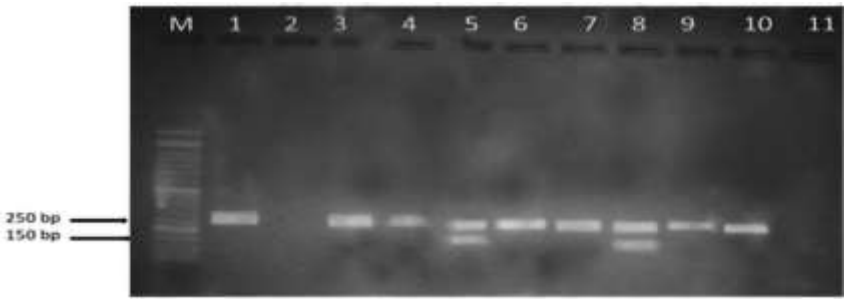


**Fig. 4.** Results of ITS2 nested PCR for *Leishmania* parasites detection and identification in patients from Varzaneh City, Isfahan. M: 50 bp ladder (SinaClon, Iran); 1: *L. major* positive control, 2: negative control (DDW), 3–4: *L. major*, 5: mixed infections of *L. major* and *L. turanica*, 6–7: *L. major*, 8: mixed infection of *L. major* and *L. turanica*, 9–10: *L. major*

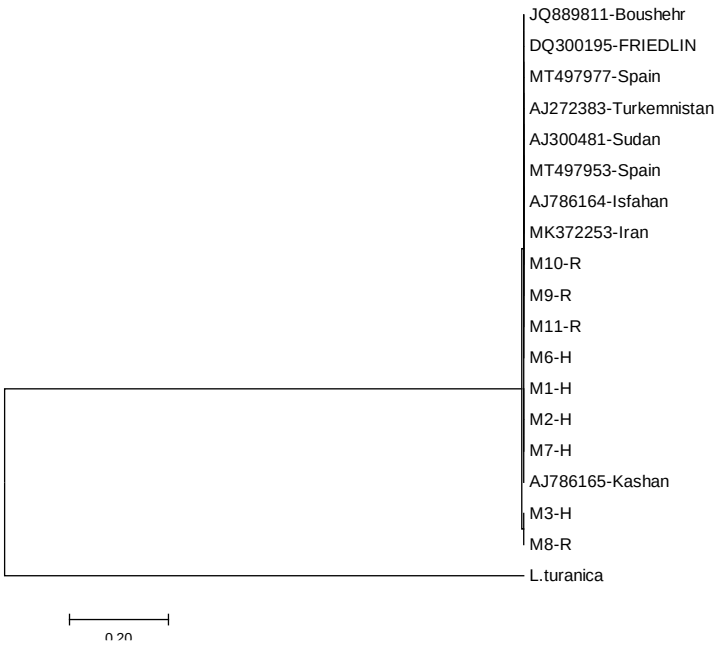


**Fig. 5.** Phylogenetic tree inferred from *Leishmania major* ITS2 sequences originated from humans (Mn-H) and great gerbil rodents (Mn-R), Varzaneh City, Isfahan Province, Iran. The scale shows the genetic distance between sequences

https://doi.org/10.18502/jad.v18i3.18576

## Discussion

Due to an unexpected increase in new cases of cutaneous leishmaniasis in Varzaneh City, Isfahan Province in recent years, this field parasitological study was conducted to investigate the causative agents of the diseases among humans and rodent reservoir hosts.

In this study, we found a high infection rate for *Leishmania* parasites, particularly *L. major*, and a high abundance and widespread distribution of great gerbils in the Varzaneh City of Isfahan Province. The same DNA sequences were found in both patients and rodents. These findings, together with the rodents' long life span and survival during the sand fly active season, make them appropriate candidates as reservoir hosts for *Leishmania* infection, including *L. major*.

As a result, it can be hypothesized that *R. opimus* populations play the main reservoir and can maintain the permanent circulation of the parasite in the Varzaneh endemic area.

The results of the present study show that *L. major*, *L. gerbilli*, and *L. turanica* are circulating among *R. opimus* populations in central Iran.

The results of the present study are similar to another recent study that showed *R. opimus* and *M. libycus* were the main reservoir hosts of ZCL in some areas of central Iran. In the latter study *L. major*, *L. turanica*, and *L. gerbilli* were reported from rodents belonging to the subfamily Gerbillinae in some parts of Iran (20).

In Turkmen Sahra, northeast of Iran, *L. major* and *L. turanica* were identified using molecular methods (15, 29). In the present study, we showed that the highest natural *L. major* infection of *R. opimus* was in winter and the lowest infection was in spring and summer. A previous study showed that the highest and lowest infection rates of *R. opimus* by *Leishmania* were seen in fall and summer respectively (14). In other studies, it was shown that three species of *Leishmania* parasites were seen as single-species infections or, in some cases, as mixed infections in gerbil populations (14, 30, 31).

Evidence has shown that in Varzaneh, *L. major* and *L. turanica* are the main circulating species, but the prevalence of *L. gerbilli* infection was low.

Our study showed in direct microscopic tests, that 38.9% of the great gerbils were positive whereas this rate raised to 100% when Nested-PCR method was used. In similar studies, it was shown that Nested-PCR is remarkably more sensitive than microscopic examination for *Leishmania* detection.

(20, 26). In the present study, more than 94% of human cases were infected with *L. major* and two cases had mixed infection of *L. major* and *L. turanica*. These results are similar to the results of another study conducted in Turkmen Sahra, Golestan Province in northeast Iran which more than 98% of human leishmaniasis cases were due to *L. major* and less than 2% was due to *L. turanica* (32). In wide territories of central Asia, mixed infections of rodents with *L. major* (pathogenic to humans) and *L. turanica* (nonpathogenic to humans) are usual. In these territories, it has been proved that the rodents are sensitive to *L. major*, *L. turanica*, and *L. gerbilli*. Rarely, a single infection with *L. major* has been seen in *R. opimus. Leishmania major* infection is usually associated with *L. turanica* in naturally infected gerbils and *L. turanica* boosts the durability of *L. major* infection in the great gerbils (33).

Among sequenced human samples, we found 2 genotypes; the main difference between them was related to the TA satellite, genotype 1 was 12 bp longer than genotype 2. In rodent samples, the sequences showed the presence of five haplotypes among the rodents which were different only in two positions that seemed to be non-informative. Only one genotype was detected among the gerbils which was similar to

genotype 2 of human cases. The phylogenetic tree showed that all samples in the study area are clustered in a single main clade and the test sequences shared common ancestry with *L. major* strains that existed in Iran.

The infection of rodents to *Leishmania* parasites in all seasons of the year, their prevalence, distribution, and identical DNA sequences of *L. major* in both patients and rodents indicate that the great gerbils are the main reservoir of the endemic parasite *L. major* in Varzaneh and Libyan jirds act as the secondary proven reservoir of ZCL.

Moreover, the presence of *L. turanica* in human patients would be interesting for future research to determine the role of this species in the persistence, duration, drug resistance, and symptoms of ZCL in humans.

## Conclusion

This study identifies *R. opimus* (great gerbils) as the main reservoir for *L. major* in Varzaneh City, Isfahan Province, Iran. High rates of *Leishmania* infection were detected in rodent populations using nested PCR, highlighting the limitations of microscopic examination. The highest infection rates occurred in winter, indicating environmental influence on transmission dynamics. Molecular analysis revealed identical *L. major* DNA sequences in both rodents and humans, supporting *R. opimus* as a main reservoir. Mixed infections involving *L. major*, *L. turanica*, and *L. gerbilli* were also found, suggesting *L. turanica* may enhance *L. major* persistence in gerbils. The rare detection of *L. turanica* in humans raises questions about its role in cutaneous leishmaniasis (CL), requiring further study.

In conclusion, *R. opimus* is the primary reservoir for *L. major*, with *M. libycus* as a secondary reservoir. Rodent control and ongoing monitoring are crucial to reducing ZCL transmission, and further research is needed on the clinical implications of *L. turanica* in human infections.

## Acknowledgements

This study was conducted as a part of a PhD dissertation of Narges Marvi Moghadam, financially supported by the School of Public Health (SPH), Tehran University of Medical Sciences (TUMS), code 99-1-99-43776. The authors are grateful to all colleagues at the Isfahan Research Station, SPH, TUMS, and the staff of Isfahan Health Center and Varzaneh Health Center, Isfahan University of Medical Sciences for their kind help.

## Ethical considerations

This study has been reviewed and approved by the School of Public Health (SPH), Tehran University of Medical Sciences (TUMS) ethics committee and has been registered with the code IR.TUMS.SPH.REC.1399.081.

## Conflict of interest statement

The authors declare there is no conflict of interest.

## References

1. Sharifi I, Khosravi A, Mohammad Reza Aflatoonian MR, Salarkia E, Bamorovat M, Karamoozian A, Nekoei Moghadam M, Sharifi F, Aghaei Afshar A, AghaKuchak Afshari S, Gharachorloo F, Shirzadi MR, Amiri B, Zainali M, Doosti S, Zamani O, Gouya MM (2023) Cutaneous leishmaniasis situation analysis in the Islamic Republic of Iran in preparation for an elimination plan. Front Public Health. 11: 1091709.
2. Razavi MR, Mohammad Reza Shirzadi MR, Mohebali M (2021) Human Cutaneous Leishmaniosis in Iran, Up to Date-2019. J Arthropod-Borne Dis. 15(2): 143–151.
3. Karimi A, Jahanifard E, Abai MR, Rassi Y, Veysi A, Hanafi-Bojd AA, Hosseini M,

https://doi.org/10.18502/jad.v18i3.18576

Saeidi Z, Tavakoli M, Maleki M, Jafari R, Hejazi AR, Yaghoobi-Ershadi MR, Akhavan AA (2020) Epidemiological survey on Cutaneous Leishmaniasis in southwestern Iran. J Vector Borne Dis. 57: 121–127.

4. Firooz A, Mortazavi H, Khamesipour A, Ghiasi M (2020) Old world cutaneous leishmaniasis in Iran: clinical variants and treatments. J Dermatolog Treat. 32(7): 673–683.
5. Rassi Y, Javadian E, Jalali M, Motazedian M, Vatndoost H (2004) Investigation on zoonotic cutaneous leishmaniasis, Southern Iran. Iranian J Publ Health. 33(1): 31–35.
6. Fatemi M, Yaghoobi-Ershadi MR, Mohebali M, Saeidi Z, Veysi A, Gholampour F, Akhondi B, Karimi A, Arandian MH, Amin Mohammadi AM, Rassi Y, Zahraei-Ramazani A, Khamesipour A, Akhavan AA (2018) The potential role of humans in the transmission cycle of *Leishmania major* (Kinetoplastida: Trypanosomatidae), the causative agent of the Old World zoonotic cutaneous leishmaniasis. J Med Entomol. 55(6): 1588–1593.
7. Jafari R, Najafzadeh N, Sedaghat M, Parvizi P (2013) Molecular characterization of sand flies and *Leishmania* detection in main vector of zoonotic cutaneous leishmaniasis in Abarkouh District of Yazd Province, Iran. Asian Pac J Trop Med. 6 (10): 792–797.
8. Yaghoobi-Ershadi M (2012) Phlebotomine sand flies (Diptera: Psychodidae) in Iran and their role on *Leishmania* transmission. J Arthropod Borne Dis. 6(1): 1–17.
9. Yaghoobi-Ershadi MR, Akhavan AA (1999) Entomological survey of sandflies (Diptera: Psychodidae) in a new focus of zoonotic cutaneous leishmaniosis in Iran. Acta Trop. 73(3): 321–326.
10. Yaghoobi-Ershadi MR, Javadian E, Tahvildare-Bidruni GH (1994) The isolation of *Leishmania major* from Phlebotomus Paraphlebotomus) caucasicus in Isfahan Province, Islamic Republic of Iran. Trans R Soc Trop Med Hyg. 88: 518–519.
11. Akhavan AA, Yaghoobi-Ershadi MR, Mirhendi H, Alimohammadian M, Rassi Y, Shareghi N, Jafari R, Arandian MH, Abdoli H, Ghanei M, Jalali-Zand N (2010) Molecular epizootiology of rodent leishmaniasis in a hyperendemic area of Iran. Iran J Public Health. 39(1): 1–7.
12. 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 Stud Biol. 3(6): 247–256.
13. Dubrovsky Y (1979) Biology of great gerbil-the principal carrier of zoonotic cutaneous leishmaniasis. WHO Traveling Seminar on leishmaniasis control ex-USSR Ministry of Health.
14. Akhavan AA, Yaghoobi-Ershadi MR, Khamesipour A, Mirhendi H, Alimohammadian M, Rassi Y, Arandian MH, 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(2): 84–89.
15. Hajjaran H, Mohebali M, Abai MR, Oshaghi MA, Zarei Z, Charehdar S, Mirjalali H, Sharifdini M, Teimouri A (2013) Natural infection and phylogenetic classification of *Leishmania* spp. infecting *Rhombomys opimus*, a primary reservoir host of zoonotic cutaneous leishmaniasis in northeast Iran. Trans R Soc Trop Med Hyg. 107(9): 550–557.
16. Bakhshi H, Oshaghi MA, Abai MR, Rassi Y, Akhavan AA, Mohebali M, Hajaran H, Mohtarami F, Mirzajani H, Maleki-Ravasan N (2013) MtDNA cytb structure of *Rhombomys opimus* (Rodentia: Gerbellidae), the main reservoir of cutaneous leishmaniasis in the borderline of Iran-Turkmen-

https://doi.org/10.18502/jad.v18i3.18576

istan. J Arthropod-Borne Dis. 7(2): 173–184.
17. Oshaghi MA, Rassi Y, Tajedin L, Abai MR, Akhavan AA, Enayati A, Mohtarami F (2011) Mitochondrial DNA diversity in the populations of great gerbils, *Rhombomys opimus*, the main reservoir of cutaneous leishmaniasis. Acta Trop. 119(2–3): 165–171.
18. Gholamrezaei M, Mohebali M, Hanafi-Bojd AA, Sedaghat MM, Shirzadi MR (2016) Ecological Niche Modeling of main reservoir hosts of zoonotic cutaneous leishmaniasis in Iran. Acta Trop. 160: 44–52.
19. Yaghoobi-Ershadi MR, Akhavan AA, Mohebali M (1996) *Meriones libycus* and *Rhombomys opimus* (Rodentia: gerbillidae) are the main reservoir hosts in a newfocus of zoonotic cutaneous leishmaniasisin Iran. Trans R Soc Trop Med Hyg. 90: 503–504.
20. Golshani Asl F, Mohebali M, Jafari R, Akhavan AA, Shirzadi MR, Zarei Z, Fadaei R, Ramezanpour J, Hassanpour Gh, Izadi Sh, Hajjaran H, Elikaee S (2022) *Leishmania* spp. infection in *Rhombomys opimus* and *Meriones libycus* as main reservoirs of zoonotic cutaneous leishmaniasis in central parts of Iran: Progress and implications in health policy. Acta Trop. 226: 106267.
21. Darvish J (2011) Morphological comparison of fourteen species of the genus *Meriones* Illiger (Rodentia: Gerbillinae) from Asia and North Africa. Iran J Anim Biosyst. 7(1).
22. Veysi A, Vatandoost H, Yaghoobi-Ershadi MR, Arandian M, Jafari R, Hosseini M, Rassi Y, Heidari K, Sadjadi A, Fadaei R, Ramazanpour J, Aminian K, Mr Shirzadi, Akhavan AA (2012) Comparative study on the effectiveness of Coumavec® and zinc phosphide in controlling zoonotic cutaneous leishmaniasis in a hyperendemic focus in central Iran. J Arthropod-Borne Dis. 6(1): 18–27.
23. Mohebali M, Nadim A, Khamesipour A (2019) An overview of leishmanization experience: A successful control measure and a tool to evaluate candidate vaccines. Acta Trop. 200: 105173.
24. Alvar J, Croft SL, Kaye P, Khamesipour A, Sundar S, Reed SG (2010) Case study for a vaccine against leishmaniasis. Vaccine. 31: B244-B249.
25. Etemad E (1978) Mammals of Iran, Vol. 1. Rodents and their Identification Keys. National Society of Guardianship of Natural Resources and Human Environment, Tehran.
26. Akhavan AA, Mirhendi H, Khamesipour A, Alimohammadian MH, Rassi Y, Bates P, Kamhawi S, Valenzuela JG, Arandian MH, Abdoli H, Jalali-zand N, Jafari R, Shareghi N, Ghanei M, Yaghoobi-Ershadi MR (2010) *Leishmania* species: detection and identification by nested PCR assay from skin samples of rodent reservoirs. Exp Parasitol. 126(4): 552–556.
27. Kumar S, Stecher G, Tamura K (2016) Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 33(7): 1870–1874.
28. Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol. 10(3): 512–526.
29. Bakhshi H, Oshaghi MA, Abai MR, Rassi Y, Akhavan AA, Sheikh Z, Mohtarami F, Saidi Z, Mirzajani H, Anjomruz M (2013) Molecular detection of *Leishmania* infection in sand flies in border line of Iran-Turkmenistan: restricted and permissive vectors. Exp Parasitol. 135(2): 382–387.
30. Nezamzadeh-Ezhiyeh H, Mirhendi H, Jafari R, Veysi A, Rassi Y, Oshaghi MA, Arandian MH, Abdoli H, Bahrami S, Zahraei Ramazani AR, Fadaei R, Ramazanpoor J, Farsi M, Aminian K, Saeidi Z (2021) An eco-epidemiological study on zoonotic cutaneous leishmaniasis in central Iran. Iran J Public Health. 50(2): 350–359.

https://doi.org/10.18502/jad.v18i3.18576

31. Rassi Y, Oshaghi MA, Azani SM, Abai MR, Rafizadeh S, Mohebai M, Mohtarami F, Zeinali Mk (2011) Molecular detection of *Leishmania* infection due to *Leishmania major* and *Leishmania turanica* in the vectors and reservoir host in Iran. Vector Borne Zoonotic Dis. 11(2): 145–150.
32. Bordbar A, Parvizi P (2014) High infection frequency, low diversity of *Leishmania major* and first detection of *Leishmania turanica* in human in northern Iran. Acta Trop. 133: 69–72.
33. Strelkova MV (1996) Progress in studies on central asian foci of zoonotic cutaneous leishmaniasis: a review. Folia Parasitol (Praha). 43(1): 1–6.