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Evaluation of the ecological characteristics in the vector of anthroponotic cutaneous leishmaniasis in a new focus of Mohammad Abad, Kerman, southeast of Iran

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ABSTRACT

Objective: To evaluate the fauna, seasonal activity and the host preference of sand flies and to determine the main vectors of anthroponotic cutaneous leishmaniasis in Mohammad Abad, Kerman Province, southeast of Iran.

Methods: Sand flies were collected indoors and outdoors by sticky paper traps from May to November, 2012. Regarding the importance of host preference and its impact on leishmaniasis control, blood meal was analyzed by the restriction fragment length polymorphism method. The cytochrome b mitochondrial genomic regions (mitochondrial DNA) and enzymatic digestion of *Xho* I and *Hae* III were used for the diagnosis of human blood feeding. In the detection of leptomonad (promastigote) infection in sand flies, a nested PCR method and high resolution melt analysis were exploited.

Results: A total of 919 sand flies were identified as belonging to 14 species in two genera, namely, *Phlebotomus* spp. (5 species) and *Sergentomyia* spp. (9 species). The most frequently occurring species was *Phlebotomus sergenti* (*P. sergenti*) (67.46%), followed by *Phlebotomus papatasi* (19.37%). The highest indoor collection of *P. sergenti* was realized in late July, and the highest outdoor collection of *P. sergenti* ensued in early July. A total of 250 sand flies were collected for host preference analysis, and blood meals of 120 sand flies belonged to the species of *P. sergenti* were subjected to restriction fragment length polymorphism confirmation. A total of 39 *P. sergenti* sand flies (32.5%) were identified to have fed on human. Nested-PCR and high resolution melt analyses confirmed that these sand flies had been infected with *Leishmania tropica*.

Conclusions: The present study has confirmed *P. sergenti* as the main Phlebotomine sand fly vector for anthroponotic cutaneous leishmaniasis caused by *Leishmania tropica* in Southeastern Iran.

1. Introduction

Humans have always suffered from zoonotic diseases. Leishmaniasis is one of the most important zoonotic diseases with an unfortunate overwhelming increasing trend. Cutaneous leishmaniasis (CL) remains one of the serious health challenges in the world, and more than 70% of CL cases have been recognized in countries encompassing Iran, Algeria, Colombia, Brazil, Afghanistan, Syria, Ethiopia, North Sudan, Costa Rica and Peru[1].CL transmission in Iran is increasing to the extent that over 55% of the provinces in the country have currently been infected (17 out of 30 provinces)[2].

In Iran, *Leishmania major* (*L. major*) and *Leishmania tropica* (*L. tropica*) are the causative agents of CL, which are transmitted to human through the bites of female *Phlebotomus papatasi* (*P. papatasi*) and *Phlebotomus sergenti* (*P. sergenti*) sand flies, respectively. Rodents belonging to the Gerbilidae family are

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the main reservoirs of *L. major*, while human is the principal reservoir of *L. tropica*. Well-established foci of anthroponotic CL (ACL) are present in Tehran, Mashhad, Shiraz, Kerman, Yazd and Bam[3]. The disease occurs in almost all seasons of the year, and various risk factors including migration, climatic variations and environmental modification play an essential role in the distribution and development of the disease[1,4].

Following the massive earthquake of Bam in 2003, Mohammad Abad Village has become a new focus of ACL[5]. Mohammad Abad is located approximately 60 km away from the city of Bam, and due to its cool weather condition in the summer, it is considered as one of the summer resorts for Bam and Jiroft Districts. As a result of these migrations and recreational activities, Mohammad Abad has become a new focus of ACL transmission[5].

The main goals of this study were to evaluate the fauna, seasonal activity and host preference of sand flies in Mohammad Abad, and to determine the main vectors of ACL in this region.

2. Materials and methods

2.1. Study area

Mohammad Abad is located 60 km southwest of the city of Bam and 45 km northeast of the city of Jiroft. The topography of the region is mostly mountainous, and the village contains nearly 4 000 inhabitants. However, due to the natural scenery, coupled with the pleasant and favorable weather conditions during summer, the village has served as a habitation for temporary residents of Jiroft and Bam where summers are very hot. The village experiences a fairly moderate climate, with an altitude of 2 423 m above sea level.

2.2. Sand fly collection and monitoring

In the investigation of the fauna and seasonal activities of sand flies in Mohammad Abad, sand flies were collected indoors and outdoors by 60 sticky paper traps from May to November, 2012. The aspirator procedure for sand fly collection was employed, prior to the molecular identification and host preference analysis of dominant sand fly species. All collected samples were identified using valid identification keys[6-8].

2.3. Host preference

Considering the importance of host preference and its impact on leishmaniasis control, blood meals were examined by the restriction fragment length polymorphism method. The blood-fed female sand flies were collected, identified to the species level by removing the head and the three terminal segments of the abdomen, and the remaining bodies were transferred into microtubes. The cytochrome b mitochondrial genomic regions (mitochondrial DNA) were used for the diagnosis of human blood feeding. The PCR analysis revealed a band of 358 bp of mitochondrial DNA *cytb* gene [Genet Bio Master Mix (Korea)]. The restriction enzyme, *Xho* I, was used for enzymatic digestion which produced two bands (215 bp and 143 bp) to distinguish human DNA in blood-fed sand flies. The resulting mixes were incubated at 37 ˚C for 16 h, and were finally separated by electrophoresis on 2% agarose gel[9]. An additional enzymatic digestion by *Hae* III was used for the differentiation of DNA of the blood of non-human vertebrates on the 623 bp region of the *cytb* gene[10].

2.4. Molecular study

In this study, a nested PCR was performed for the detection of leptomonad in sand flies, by following the protocol employed by Noyes *et al.*[11]. The primers were designed based on the minicircles of kDNA, and the sizes of bands created were 680 bp for *Leishmania infantum* and *Leishmania donovani*, and 750 bp and 560 bp for *L. tropica* and *L. major*, respectively. Primers were selected from ITS1 genomic sequences of *Leishmania* [5'-AGCTGGATCATTTTCCGATG-3' (forward) and 5'-ATCGCGA CACGTTATGTGAG-3' (reverse)]. Additionally, high resolution melting analysis was used to confirm the identification of *L. tropica*[12].

3. Results

In this entomological survey, a total of 919 sand flies were identified as belonging to 14 species in two genera [*Phlebotomus* spp. (5 species) and *Sergentomyia* spp. (9 species)]. The most frequently occurring species was *P. sergenti* (67.46%), followed by *P. papatasi* (19.37%) (Table 1). Overall, 34.34% of the sand flies were collected indoors, and 65.66% outdoors. Eventually, the highest indoor collection of *P. sergenti* occurred in late July, and the period of early July recorded the highest outdoor collection of *P. sergenti* (Figure 1).

A total of 250 sand flies collected for host preference analysis in the summer were transported to the laboratory and preserved in alcohol. Of these, 120 sand flies identified as *P. sergenti* were selected for molecular studies. Restriction fragment length polymorphism analysis showed that 39 of these sand flies (32.5%) had fed on humans (Figure 2), and the remaining had fed on other vertebrates.

Table 1

Frequency and fauna of sand flies species collected from Mohammad Abad, Jiroft County, Kerman Province, Iran, 2012.

Species	Frequency [*n* (%)]
P. sergenti	620 (67.46)
P. papatasi	178 (19.37)
Phlebotomus caucasicus	18 (1.96)
Phlebotomus major	3 (0.33)
Phlebotomus longiductus	1 (0.11)
Sergentomyia baghdadis	26 (2.83)
Sergentomyia sumbarica	12 (1.31)
Sergentomyia africana	15 (1.63)
Sergentomyia sintoni	13 (1.41)
Sergentomyia squamipleuris	9 (0.98)
Sergentomyia dentata	7 (0.76)
Sergentomyia grekovi	8 (0.87)
Sergentomyia mervynae	6 (0.65)
Sergentomyia antennata	3 (0.33)
Total	919 (100)

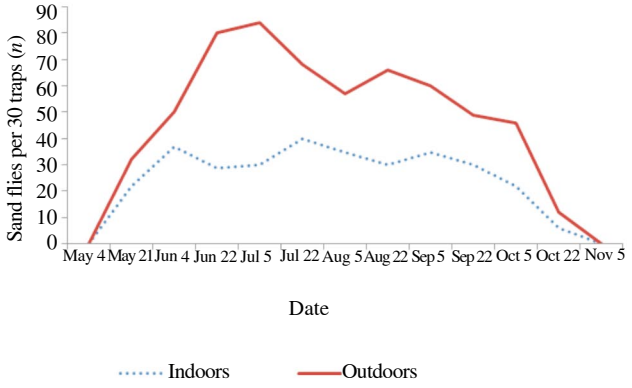

Figure 1. Monthly collection of *P. sergenti* indoors and outdoors in Mohammad Abad, Jiroft County, Kerman Province, 2012.

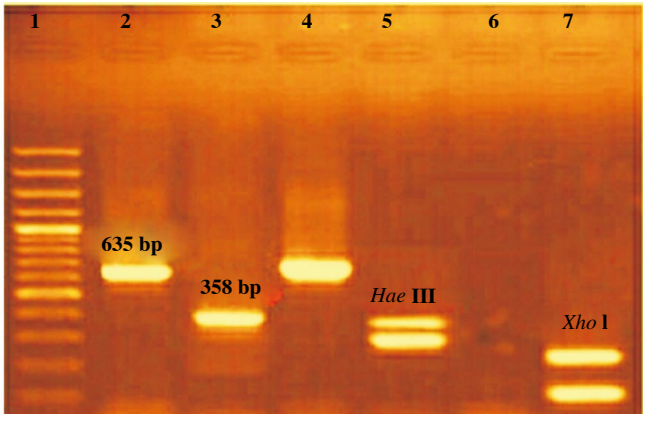

Figure 2. Electrophoresis of mitochondrial DNA cytochrome b gene fragments from blood meals of *P. sergenti* digested with *Xho* I and *Hae* III. Lane 1: Molecular weight marker (100 bp, CinnaGen, Iran); Lanes 2 and 4: Other vertebrates blood other than human; Lane 3: Human blood; Lane 5: Digested with *Hae* III in other vertebrates blood other than human; Lane 6: Negative control; Lane 7: Digested with *Xho* I in the human blood.

In the detection of natural leptomonad infection in sand flies by the use of nested PCR method, 129 sand flies were collected, in which 75 sand flies were trapped from outdoor areas and 54 from indoor dwellings. Correspondingly, of the 129 collected sand flies, 90 *P. sergenti* and 39 *P. papatasi* sand flies were identified. Two *P. sergenti* (2.2%) sand flies were leptomonad-infected, and their bands (750 bp) were observed after electrophoresis, which was indicative of *L. tropica* infection (Figure 3). The two *P. sergenti* sand flies, following the high resolution melt method, were confirmed to be naturally infected with *L. tropica*.

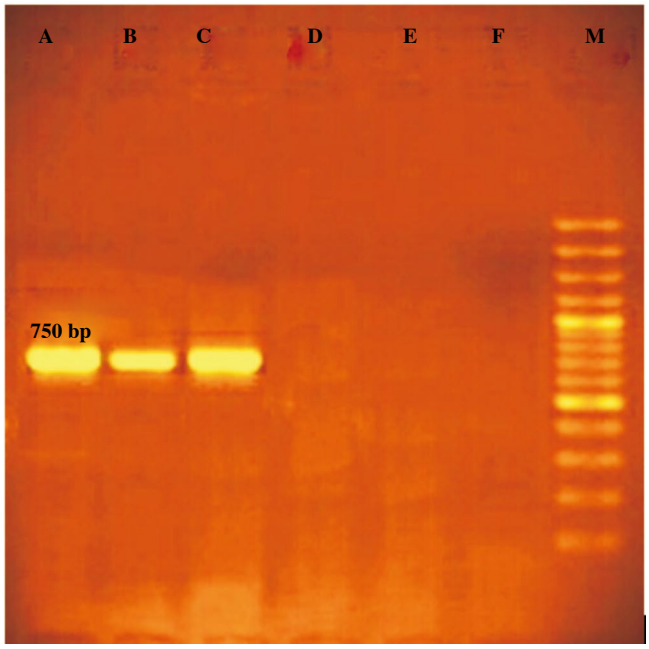

Figure 3. PCR amplifications of *L. tropica* kDNA in the mid gut of *P. sergenti* (750 bp).
Lane A: Positive control; Lanes B and C: *L. tropica* in *P. sergenti*; Lanes D, E and F: Negative control; Lane M: Molecular weight marker.

4. Discussion

Since 2010, when Mohammad Abad was presented as a new focus of ACL, the identification and evaluation of some ecological characteristics of vector(s) has become necessary. In the present study, *P. sergenti*, a proven vector of *L. tropica* in Iran[13], is the most abundant species in this area. *P. sergenti* has one activity peak each indoors and outdoors, so that one generation is observed in its activity period. Therefore, if necessary, the insecticide spraying against sand flies should be done in July in this region[14]. In contrast, the period of sand flies activity in the city of Bam is longer than that observed in this area, and *P. sergenti* has two activity peaks which is attributed to the differences in altitude and temperature between the two regions[15]. In Iran, *P. sergenti* has a wider distribution range in many areas of the country where *L. tropica* has been isolated from *P. sergenti*[13].

The results of blood meal assay showed that more than 80% of blood-fed female *P. sergenti* sand flies were collected from indoor areas. Remarkably, this particular sand fly species seems to be highly anthropophilic, and this confirms a strong dependence of sand flies on human which helps us understand the role of human

in the transmission of *L. tropica* in this focus.

Two molecular techniques were used for the detection of natural leptomonad infection in sand flies, namely, nested PCR and high resolution melt method. Two female *P. sergenti* sand flies were infected with *L. tropica*. Hence, the high frequency of *P. sergenti* indoors and their infection with *L. tropica* show that this species is the main vector of ACL in this locality. This finding is consistent with the results reported from North Africa, Middle East and Central Asia; *P. sergenti* is the principal vector of *L. tropica* in these countries[16-20]. In conclusion, present study has confirmed *P. sergenti* as the main Phlebotomine sand fly vector for ACL infection caused by *L. tropica* in Southeastern Iran.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Alvar J, Vélez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One* 2012; **7**(5): e35671.

[2] Shirzadi MR, Gouya MM. National guidelines for cutaneous leishmaniasis surveillance in Iran. Tehran: Zoonoses Control Department, Ministry of Health and Medical Education; 2012, p. 1-78.

[3] Sharifi I, Aflatoonian MR, Fekri AR, Hakimi Parizi M, Aghaei Afshar A, Khosravi A, et al. A comprehensive review of cutaneous leishmaniasis in Kerman Province, Southeastern Iran – narrative review article. *Iran J Public Health* 2015; **44**(3): 299-307.

[4] Sharifi I, Nakhaei N, Aflatoonian M, Hakimi Parizi M, Fekri A, Safizadeh H, et al. Cutaneous leishmaniasis in Bam: a comparative evaluation of pre- and post-earthquake years (1999–2008). *Iran J Public Health* 2011; **40**(2): 49-56.

[5] Mirzaei M, Sharifi I, Poursmaelian S. A new focus of anthroponotic cutaneous leishmaniasis and identification of parasite species by nested PCR in Jiroft, Iran. *Comp Clin Pathol* 2012; **21**(5): 1071-5.

[6] Rassi Y, Hanafi-Bojd AA. [*Phlebotomine sandflies, vectors of leishmaniases: morphology, biology, ecology, and field and laboratory methods with pictorial key of Iranian sandflies*]. Tehran: Noavaran-Elm Publication; 2006. Persian.

[7] Lewis DJ. A taxonomic review of the genus *Phlebotomus* (Diptera: Psychodidae). *Bull Br Mus Nat Hist (Ent)* 1982; **45**: 121-209.

[8] Theodor O, Mesghali A. On the Phlebotominae of Iran. *J Med Entomol* 1964; **1**: 285-300.

[9] Oshaghi MA, Chavshin AR, Vatandoost H. Analysis of mosquito bloodmeals using RFLP markers. *Exp Parasitol* 2006; **114**(4): 259-64.

[10] Maleki-Ravasan N, Oshaghi M, Javadian E, Rassi Y, Sadraei J, Mohtarami F. Blood meal identification in field-captured sand flies: comparison of PCR-RFLP and ELISA assays. *Iran J Arthropod Borne Dis* 2009; **3**(1): 8-18.

[11] Noyes HA, Reyburn H, Bailey JW, Smith D. A nested-PCR-based schizodeme method for identifying *Leishmania* kinetoplast minicircle classes directly from clinical samples and its application to the study of the epidemiology of *Leishmania tropica* in Pakistan. *J Clin Microbiol* 1998; **36**(10): 2877-81.

[12] Aghaei AA, Rassi Y, Sharifi I, Vatandoost H, Mollaie H, Oshaghi MA, et al. First report on natural *Leishmania* infection of *Phlebotomus sergenti* due *Leishmania tropica* by high resolution melting curve method in South-eastern Iran. *Asian Pac J Trop Med* 2014; **7**(2): 93-6.

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

[14] Afshar AA, Rassi Y, Sharifi I, Abai M, Oshaghi M, Yaghoobi-Ershadi M, et al. Susceptibility status of *Phlebotomus papatasi* and *P. sergenti* (Diptera: Psychodidae) to DDT and deltamethrin in a focus of cutaneous leishmaniasis after earthquake strike in Bam, Iran. *Iran J Arthropod Borne Dis* 2011; **5**(2): 32-41.

[15] Aghasi M, Sharifi I. Survey of the fauna and monthly activity of the sand fly as the vectors of the cutaneous leishmaniasis in the city of Bam. *J Kerman Univ Med Sci* 2003; **10**: 85-91.

[16] Ajaoud M, Es-Sette N, Charrel RN, Laamrani-Idrissi A, Nhammi H, Riyad M, et al. *Phlebotomus sergenti* in a cutaneous leishmaniasis focus in Azilal Province (High Atlas, Morocco): molecular detection and genotyping of *Leishmania tropica*, and feeding behavior. *PLoS Negl Trop Dis* 2015; **9**: e0003687.

[17] Ready PD. Biology of Phlebotomine sand flies as vectors of disease agents. *Annu Rev Entomol* 2013; **58**: 227-50.

[18] Mondragon-Shem K, Al-Salem WS, Kelly-Hope L, Abdeladhim M, Al-Zahrani MH, Valenzuela JG, et al. Severity of old world cutaneous leishmaniasis is influenced by previous exposure to sandfly bites in Saudi Arabia. *PLoS Negl Trop Dis* 2015; **9**: e0003449.

[19] Boubidi SC, Benallal K, Boudrissa A, Bouiba L, Bouchareb B, Garni R, et al. *Phlebotomus sergenti* (Parrot, 1917) identified as *Leishmania killicki* host in Ghardaia, South Algeria. *Microbes Infect* 2011; **13**(7): 691-6.

[20] Tabbabi A, Bousslimi N, Rhim A, Aoun K, Bouratbine A. First report on natural infection of *Phlebotomus sergenti* with *Leishmania promastigotes* in the cutaneous leishmaniasis focus in Southeastern Tunisia. *Am J Trop Med Hyg* 2011; **85**(4): 646-7.