Iranian Biomedical Journal 16 (2):113-120 (April 2012)
DOI: 10.6091/ibj.1038.2012

Distribution, Fauna and Seasonal Variation of Sandflies, Simultaneous Detection of Nuclear Internal Transcribed Spacer Ribosomal DNA Gene of *Leishmania major* in *Rhombomys opimus* and *Phlebotomus papatasi*, in Natanz District in Central Part of Iran

Parviz Parvizi*[1], Mohammad Akhoundi[1,2] and Hanieh Mirzaei[1,3]

[1]*Molecular Systematics Laboratory, Dept. of Parasitology, Pasteur Institute of Iran, Tehran, Iran.*
[2]*Université de Reims Champagne-Ardenne, Faculté de Pharmacie, Reims, France.* [3]*Dept. of Microbiology,*
Islamic Azad University of Lahidjan, Guilan, Iran

Received 12 November 2012; revised 18 January 2012; accepted 21 January 2012

ABSTRACT

Background: Zoonotic cutaneous leishmaniasis (ZCL) due to *Leishmania major* is increasing in many parts of Iran. This disease originally is a disease found in gerbils. *Leishmania* parasites are transmitted by sandflies that live and breed in gerbil burrows. Nested PCR amplified *Leishmania ITS1-5.8S rRNA* gene in both main reservoir host "*Rhombomys opimus"* and in the "*Phlebotomus papatasi*" main vector of ZCL, in Iran. Population differentiation and seasonal variation of sandflies were analyzed at a microgeographical level in order to identify any isolation by distance, habitat or seasons. **Methods:** Populations of sandflies were sampled from the edges of villages in Natanz, Isfahan province, Iran, using the Centers for Disease Control traps and sticky papers. Individual sandflies were identified based on external and internal morphological characters. Nested PCR protocols were used to amplify *Leishmania ITS1-5.8S rRNA* gene, which were shown to be species-specific via DNA sequence. **Results:** A total of 4500 sandflies were collected and identified. *P. papatasi*, *Phlebotomus sergenti* and *Phlebotomus jacusieli* from genus *Phlebotomus* and *Sergentomyia sintoni* and *Sergentomyia clydei* from genus *Sergentomyia* were identified in this region. *P. papatasi* was the most abundant sandfly in the collections. Ten out of 549 female *P. papatasi* and four out of 19 *R. opimus* were found to be infected with *L. major*. **Conclusion:** Seasonal activity of sandflies starts in June and ends in November. Abundance of *P. papatasi* was in September. Finding and molecular typing of *L. major* in *P. papatasi* and *R. opimus* confirmed the main vector and reservoir in this region. *Iran. Biomed. J. 16 (2): 113-120, 2012*

Keywords: Leishmania major, Sandflies, Leishmaniasis, Iran

INTRODUCTION

Leishmaniasis is caused by human infection with parasites of the genus *Leishmania*, which are transmitted by phlebotomine sandflies [1, 2]. *Rhombomys opimus* is believed to be the principal reservoirs and the phlebotomine sandfly *Phlebotomus (Phlebotomus) papatasi* (Scopoli) (Diptera: Psychodidae) is the proven vector of the parasitic protozoan *Leishmania major* Yakimoff and Schokhor (Kinetoplastida: Trypanosomatidae), the causative agent of zoonotic cutaneous leishmaniasis (ZCL) in Iran [3] the ex-USSR (Uzbekistan, Turkmenistan and Azerbaijan) [4], eastern Saudi Arabia [5], the Jordan Valley [6], central Tunisia [7], and southern Morocco [8]. In Iran, ZCL has great public health importance in rural regions of 15 out of 32 provinces [9]. This disease is generally restricted to the areas where are heavily infested by the sandflies, as the vector of this disease.

More than 46 species of sandflies have been reported in Iran [10], but only a few of them are common in ZCL foci and gerbils' bite and/or people. Many sandflies from the foci of rural ZCL have been dissected or screened by PCR and found to be infected with leptomonads [3, 11-13].

---

*Corresponding Author; Tel & Fax: (+98-21) 6649 6414; E-mail: parp@pasteur.ac.ir

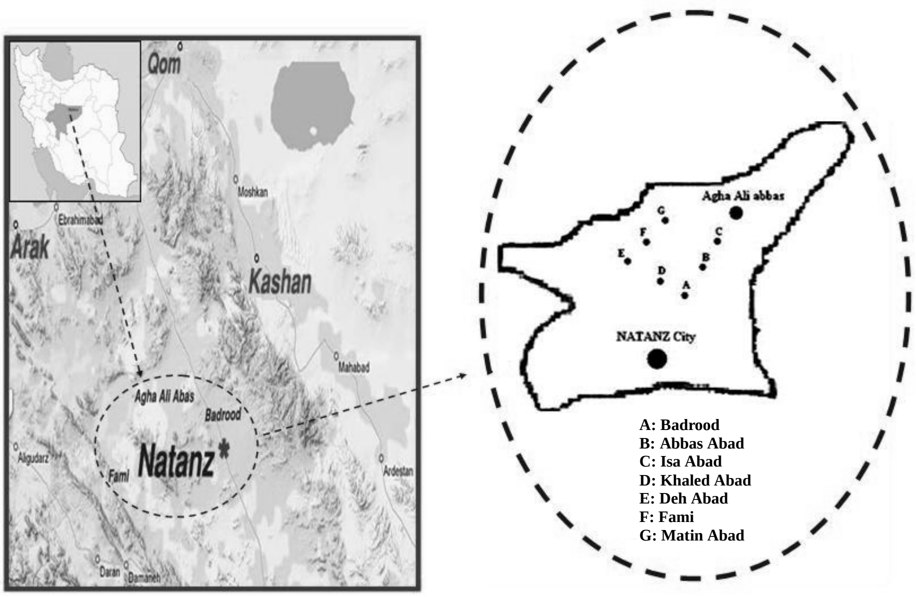

Fig. 1. Location of villages, Natanz, Isfahan province of Iran, where sandflies and *R. opimus* were sampled and screened for *Leishmania* infections.

In the present report, the population differentiation and seasonal variation of sandflies have been analyzed at a microgeographical level to identify any isolation by distance (among villages) or by habitat (between villages and colonies of the gerbil reservoir hosts of *L. major*) or by seasons (among different months of activity). Most of our collections were from sites at the edge of rural villages on a high-altitude cultivated plain (Fig. 1) within an important ZCL focus to the north of Natanz city, Isfahan province, in central part of Iran [11, 10].

Following the objectives of this report, we attempted to identify and type molecularly *L. major* in *P. papatasi* as the main vector and in *R. opimus* as the reservoir host of ZCL in Natanz district, in central part of Iran.

MATERIALS AND METHODS

The collections were carried out from the first half of June through the second half of November 2009 (during the season of activity of adult sandflies) in seven villages near Natanz city (Isfahan province, Iran) (Fig. 1). These areas are 3,397 km^2 with an altitude of 1600 meter above the sea level (33°40' N, 51°25' E). Also, the climate condition is very hot (up to +41°C) in the summer and cold (about -9°C) in the winter. The total annual rainfall is 270 mm.

Sand flies were collected on sticky papers (A4 sheets of white papers soaked in castor oil) placed overnight in ruined outhouses and at the entrances to gerbil burrows (1 paper per burrow; papers: 2-10 m apart, 30-40 per site, over a range of 120-200 m at each site). The Centers for Disease Control miniature light traps [14] (with the white-light bulb 1-2 m above ground level) were set overnight to sample sand flies in domestic animal shelters (1-2 traps per site), and a manual aspirator was sometimes used by a single collector to capture sand flies resting inside houses in the morning [15].

Sand flies captured in light traps and aspirators were narcotized with cigarette smoke, and those caught on sticky papers were removed with needles or fine brushes dipped in 70% ethanol. All specimens were then stored in analytical grade 80% ethanol, firstly at 4°C (in villages) and later at -20°C (in Tehran).

All sand flies were identified based on external and internal morphological characters of the head and abdominal Terminalia [4, 16], which were slide-mounted in Berlese's fluid [17], following dissection with sterilized forceps and micro-needles [18].

Rodents were caught with live traps by baiting cucumbers and dates. All rodents and sand flies from these collections were screened for infections of *Leishmania* species using nested PCR of *ITS-rDNA* gene [10, 19].

PCR products were directly sequenced to identify *Leishmania* haplotypes infecting individual rodent and sandfly, and all haplotypes were identified by species using phylogenetic analysis. DNA sequences were edited and aligned using Sequencher^{Tm} 3.1.1 software (Gene Codes Corporation), and the multiple alignments of new DNA haplotypes and homologous GenBank sequences were exported into PAUP* software [20] for phylogenetic analysis.

RESULTS

Approximately, 4500 sandflies were collected from various habitats in different regions from Natanz. A number of 1646 sandflies were dissected from outdoors (ruined outhouses, yards and at the entrances to gerbil burrows), and indoors (domestic animal shelters, resting houses) by sticky paper and the Centers for Disease Control light traps.

Overall, *P. papatasi* was the most abundant sandfly in the collections (34.9%), followed by *P. sergenti* (26.9%), *Sergentomyia (Sergentomyia) sintoni* Pringle (22.4%), *S. clydei* (8.1%) and *P. jacusieli* (7.7%) (Tables 1 and 2).

Ten out of 549 female *P. papatasi* were found to be infected with *L. major* using nested primers ITS1F and ITS2R4. Fifty one (9.2%) out of 549 sandflies, collected from gerbil burrow, were screened for *Leishmania* infection and only one (0.18%) was found to be infected with *L. major.* In addition, 498 (90.7%) out of 549 sandflies, collected from animal shelters inside houses and yards, were screened for *Leishmania* infection and nine were found to be infected with *L. major*. *L. major* was also detected in 4 out of 19 *R. opimus* captured in two villages.

The analyses of *L. major ITS-rDNA* gene were based on the 211 bp of the *ITS1* gene and 168 bp of *5.8S* gene, followed by 15 bp of the *ITS2* gene (total 394 bp).

At least 14 males and 14 females of each sandfly species were taken from each collection sample sites for identification, seasonal variation, fauna and analysis of population. Morphological identification was based on external and internal morphological characters of the head and abdominal Terminalia. Only undamaged sandflies were used, but selection was otherwise made at random.

The proportion of male and female of sandfly population in different locations of Natanz has been shown in Table 1. In our previous report [11] after female's gonotrophic stage analyzing, we showed that total *Leishmania* infection rates were higher for females with large eggs (semi-gravid and gravid). Also in *P. papatasi* with or without blood meal remains, we found low *Leishmania* infections (Table 1).

Prevalence of sandfly species in different habitats of Natanz villages has been shown in Table 2. Also, the numbers, frequency rate and seasonal activity of sandfly species collected from various villages of Natanz have been presented in Figure 2.

Among dissected sandflies, *P. papatasi* with high density (34.9%) was collected during activity season of sandflies from outdoors (ruined outhouses, yard and at the entrances to gerbil burrows) and indoors (domestic animal shelters, resting inside houses).

DISCUSSION

Sequences and homologous of *ITS1-5.8S rRNA* gene of *L. major* fragment from *P. papatasi* and *R. opimus* were searched in GenBank and 3 haplotypes were identified (Fig. 3). After phylogenetic analysis, the sequences from Iranian sandflies and rodents were found to be diagnostic for *L. major.* Three haplotypes of *L. major* were identified. The common haplotype of *L. major* was found to be identical to that of isolates of *L. major* from Iran and Sudan (GenBank accession no. EF413075 and AJ300481, respectively), and it predominated Iranian sandflies and rodents infected with this species (10/12 infections). Also, this common haplotype (GenBank accession no. EF413075) differs pair wise by only one nucleotide position from a haplotype of *L. major* from Central Asia and Sudan (GenBank accession no. AJ000310 and AJ272383, respectively); also by two nucleotide

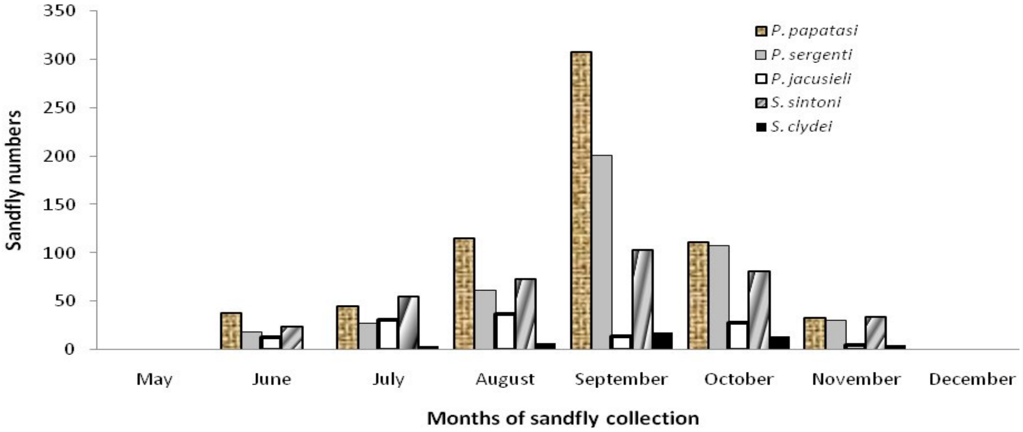

Fig. 2. Seasonal activities of different adult sandflies species in villages of Natanz district, Isfahan province, Iran from May to December 2009.

[DOR: 20.1001.1.1028852.2012.16.2.5.8]
[DOI: 10.6091/ibj.1038.2012]

Table 1. Proportion of male and female of sandfly population in different locations of Natanz.

Villages	**No.**	**Species**																								
		P. papatasi					***P. sergenti***					***P. jacusieli***					***S. sintoni***					***S. clydei***				
		M	**F**				**M**	**F**				**M**	**F**				**M**	**F**				**M**	**F**			
			FF	**SG**	**G**	**UF**		**FF**	**SG**	**G**	**UF**		**FF**	**SG**	**G**	**UF**		**FF**	**SG**	**G**	**UF**		**FF**	**SG**	**G**	**UF**
Seyed Abad	87	9	5	7	9	15	4	1	2	4	6	1	_	_	_	1	4	2	4	5	7	_	_	_	_	1
Matin Abad	178	21	15	19	14	31	11	5	9	7	15	1	_	_	1	1	8	1	4	7	5	1	_	1	_	1
Khaled Abad	262	27	11	15	6	41	15	5	8	14	25	5	1	3	5	9	14	7	4	13	24	2	_	1	3	4
Isa Abad	237	15	14	14	11	43	11	4	6	8	18	4	2	6	5	11	19	10	8	9	13	2	_	1	1	2
Fami	157	10	7	5	8	14	13	4	3	8	11	7	_	3	5	13	8	2	5	9	15	2	_	2	1	2
Deh Abad	167	21	6	14	11	18	15	5	7	8	10	5	2	3	1	8	5	9	4	1	6	2	_	1	2	3
Chane Abad	83	7	3	5	3	9	6	2	7	5	6	1	_	_	_	2	4	2	1	3	14	1	_	_	1	1
Chahe Arabha	124	9	5	11	7	12	9	3	5	14	18	2	_	_	1	1	9	1	5	3	8	_	_	_	_	1
Badrood	77	5	_	2	4	6	4	1	2	5	11	3	_	1	1	3	9	_	2	5	10	1	_	_	_	2
Agha Ali Abas	150	20	3	6	11	16	11	2	7	5	21	2	_	_	1	2	10	3	9	4	15	1	_	_	_	1
Abas Abad	124	18	5	8	3	16	7	4	8	7	12	2	_	_	3	_	11	2	7	2	4	2	_	1	2	_
Total	**1646**	**162**	**74**	**106**	**87**	**221**	**106**	**36**	**64**	**85**	**153**	**33**	**5**	**16**	**23**	**51**	**101**	**39**	**53**	**61**	**121**	**14**	**0**	**7**	**10**	**18**
Percent	**100**	**9.8**	**4.5**	**6.4**	**5.3**	**13**	**6.4**	**2.19**	**3.9**	**5.2**	**9.3**	**2**	**0.3**	**1**	**1**	**3.1**	**6.1**	**2.37**	**3.2**	**3.7**	**7.35**	**0.9**	**0**	**0.43**	**0.6**	**1.09**

M, male; F, female; FF, fresh fed; SG, semi-gravid; G, gravid; UF, un-fed

Table 2. Prevalence of sandflies in different habitats of Natanz's villages.

	Habitats	Species																			
		P. papatasi				***P. sergenti***				***P. jacusieli***				***S. sintoni***				***S. clydei***			
		Home	**A.Sh**	**R.B**	**Yard**	**Home**	**A.Sh**	**R.B**	**Yard**	**Home**	**A.Sh**	**R.B**	**Yard**	**Home**	**A.Sh**	**R.B**	**Yard**	**Home**	**A.Sh**	**R.B**	**Yard**
Villages	Seyed Abad	11	18	9	7	7	5	4	1	_	2	_	_	7	4	6	5	_	1	_	_
	Matin Abad	27	37	21	15	17	12	11	7	_	_	2	1	8	10	7	_	_	1	2	_
	Khaled Abad	18	38	27	17	16	23	15	13	4	6	8	5	15	18	18	11	_	4	6	_
	Isa abad	21	34	28	14	13	14	9	11	6	11	9	2	18	14	19	8	1	3	2	_
	Fami	11	10	13	10	8	11	13	7	2	9	13	4	4	12	10	8	1	2	4	_
	Deh Abad	10	26	23	11	6	16	14	9	_	2	14	3	3	9	7	6	2	2	3	1
	Chane Abad	7	9	6	5	5	10	7	4	_	2	1	_	1	11	9	3	1	_	2	_
	Chahe Arabha	9	16	13	6	9	19	14	7	_	1	3	_	3	10	11	2	_	1	_	_
	Badrood	3	11	3	_	2	9	7	5	2	3	2	1	4	9	6	7	_	2	1	_
	Agha Ali Abas	17	15	17	7	8	18	15	5	1	2	2	_	8	16	11	6	_	1	1	_
	Abas Abad	8	16	15	11	17	10	7	4	1	3	1	_	3	8	11	4	1	2	1	1
Total		**142**	**230**	**175**	**103**	**108**	**147**	**116**	**73**	**16**	**41**	**55**	**16**	**74**	**121**	**115**	**60**	**6**	**19**	**22**	**2**
Percent		**8.63**	**13.97**	**10.63**	**6.26**	**6.56**	**8.93**	**7.05**	**4.43**	**0.97**	**2.49**	**3.34**	**0.97**	**4.5**	**7.35**	**6.99**	**3.65**	**0.36**	**1.15**	**1.34**	**0.12**

A.Sh, animal shelter; R.B, rodent burrow

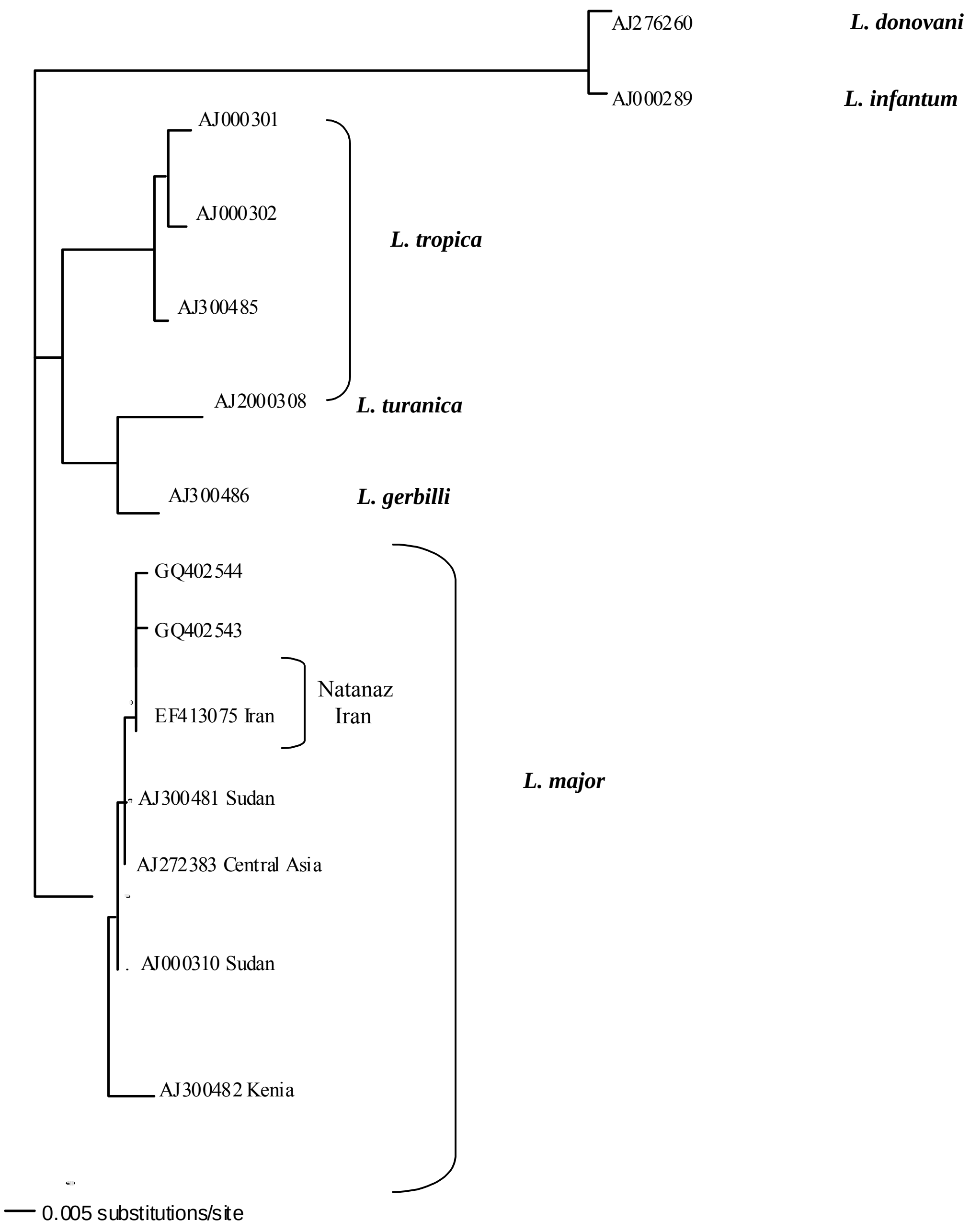

Fig. 3. Unrooted neighbour-joining tree showing the relationships of the haplotypes of the *ITS1-5.8S rDNA* for the isolates of *L. major* as the species infecting sandflies and rodents, mentioned in text using PAUP* software [31], and it relates *ITS1-5.8S rDNA* haplotypes in GenBank.

[DOR: 20.1001.1.1028852.2012.16.2.5.8]

[DOI: 10.6091/ibj.1038.2012]

positions from a haplotype from Kenya (GenBank accession no. AJ300482). Two new haplotypes of *L. major* (GenBank accession no. GQ402543 and GQ402544) were also found in single infections with only one nucleotide position from a common haplotype (GenBank accession no. EF413075) of *L. major* (Fig. 3).

No great differences were found in different species, locations or habitats. Regarding monthly distribution, there are no sandflies in May and before that and also in December 2006. In August, September and October, abundance of sandflies is much higher, especially abundance of *P. papatasi* in September is very high.

Among sandfly vectors of *Leishmania, P. papatasi* may acquire *L. major* from rodent reservoir hosts living in the peridomestic habitats. Immigration of vectors from more distant colonies of *R. opimus* was less likely. Because mark-release-recapture experiments had shown that *P. papatasi* rarely disperses more than 1.5 km, with the extent of dispersal depending on the availability of resting and breeding sites (for both sexes) and of blood meal sources (females) [21].

The same results were reported for *P. papatasi* living in similar habitats in the Jordan Valley [22]. Schlein *et al.* [22] showed that non-gravid female flies were found to travel at least 800 m between colonies of the local reservoir host (*Psammomys obesus)* of *L. major*, and fed of turkeys, which provided many blood meals for female sandflies. Using exit-entrance traps in gerbil burrows [23] and light traps without bulbs in nearby fields [24] males and gravid females were shown to be active mostly between sunset and midnight, when they left burrows to find sugar meals from nearby plants and then sought resting sites in nearby burrows [25]. In contrast, non-gravid females were active throughout the night, sometimes dispersing across fields to seek blood meals and often between midnight and sunrise, entering burrows far from their starting points [23, 25, 26].

Our results demonstrate that there has been no isolation of sandflies within different villages (Table 1) and habitats (Table 2) in Natanz, Isfahan province, Iran.

Within the ZCL focus, *P. papatasi* has been frequently collected in and around houses in villages, where it was shown to be the predominant *Phlebotomus* species and to obtain many of its blood meals from humans, birds and large domestic mammals [27, 28]. It could be concluded that most peridomestic populations of *P. papatasi* are isolated from those in gerbil colonies, with peridomestic female sandflies rarely feeding on gerbils; therefore, rarely becoming infected with *L. major*.

The significance of any phlebotomine species to be as a disease vector and rodent to be as a disease reservoir can be dependent on many factors; therefore, finding *L. major* in *P. papatasi* and in *R. opimus* is not sufficient evidence for considering them as a vector or reservoir [29]. Of the sandflies recorded from Iran, only *P. papatasi* was judged to be a proven vector of *L. major* [30].

ACKNOWLEDGEMENTS

The work was supported by Pasteur Institute of Iran (grant no. 263), awarded to Dr. Parviz Parvizi. The collection of sandflies and rodents were made possible by the assistance of the Centre of Health Service in Natanz, Isfahan province, Iran. We thank Mehdi Baghban for his help with the field work and Elnaz AlaeeNovin for her help in Molecular Systematics Laboratory, Pasteur Institute of Iran. A part of this research was assigned by Mr. Mohammad Akhondi, Ph.D. studentship at Pasteur Institute of Iran, Tehran, and registered for Université de Reims Champagne-Ardenne, Faculté de Pharmacie, 51 rue Cognacq-Jay, 51096 Reims cedex, France and also by Hanieh Mirzaei a M.Sc. studentship at Pasteur Institute of Iran, Tehran, and registered for Islamic Azad University of Lahidjan.

REFERENCES

1. Nadim A, Mesghali A, Amini H. Epidemiology of cutaneous leishmaniasis in the Isfahan province of Iran, III: The vector. *Trans R Soc Trop Med Hyg.1968 Jun; 62(4):543-9.*
2. World Health Organization. Control of the leishmaniasis: report of a WHO expert committee. *Geneva: WHO, Tech Rep Ser. 1990; no.793.*
3. Nadim A, Seyedi-Rashti M.A. A brief review of the epidemiology of various types of leishmaniasis in Iran. *Acta Med Iran.1971 Oct; 14: 99-106.*
4. Perfil'ev, PP. Fauna of the U.S.S.R. diptera. *Academy of Sciences of U.S.S.R., Zoological Institute.New Series.1966;93 (3) 382.*
5. Killick-Kendrick R, Leaney AJ, Peters W, Rioux JA, Bray RS. Zoonotic cutaneous leishmaniasis in Saudi Arabia: the incrimination of *Phlebotomus papatasi* as the vector in the Al-Hassa oasis. *Trans R Soc Trop Med Hyg.1985;79(2):252-5.*
6. Schlein Y, Warburg A, Schnur LF, Gunders AE. Leishmaniasis in the Jordan Valley, II. Sandflies and transmission in the central endemic area. *Trans R Soc Trop Med hyg.1982;76(5):582-6.*
7. Ben-Ismail R, Helal H, Bach-Hamba D, Ben Rachid MS. Infestation naturelle de *Phlebotomus papatasi* dans un foyer de leishmaniose cutanée zoonotique en Tunisie. *Bull Soc Pathol Exot.1987;80(4):613-4.*

8. Rioux JA, Guilvard E, Dereure J, Lanotte G, Denial M, Pratlong F, et al. Infestation naturelle de *Phlebotomus papatasi* (Scopoli, 1786) par *Leishmania major* MON-25. *Leishmania, Taxonomie et Phylogenèse. Applications Eco-épidemiologiques. 1986;439-4.*
9. Yaghoobi-Ershadi MR, Akhavan AA, Zahraei-Ramazani AR, Jalali-Zand AR, Piazak N. Bionomics of *Phlebotomus papasasi* (Diptera: Psychodidae) in an endemic focus of zoonotic cutaneous leishmaniasis in central Iran. *J Vector Ecol.2005 Jun;30(1):115-8.*
10. Seccombe AK, Ready PD, Huddleston LM. A catalogue of old world phlebotomine sandflies (Diptera: Psychodidae, Phlebotominae). *Occas Pap Syst Entomol. 1993;8:1-57.*
11. Parvizi P, Ready PD, Nested PCRs and sequencing of nuclear ITS-rDNA fragments detect three *Leishmania* species of gerbils in sandflies from Iranian foci of zoonotic cutaneous leishmaniasis. *Trop Med Int Health. 2008 Sep;13(9):1159-71.*
12. Nadim A, Seyedi-Rashti MA, Mesgali A. On the nature of leptomonads found in *Sergentomyia sintoni* in Khorassan, Iran and their relation to Lizard leishmaniasis. *J Trop Med Hyg.1968;71:240-2.*
13. Javadian E, Seyedi-Rasti MA. Sandflies and their leptomonad infection in Iran. *Parassitologia.1991;33:5.*
14. Sudia WD, Chamberland RW, Battery-operated light trap, an improved model. *J Am Mosq* Control Assoc.*1988 Dec;*4(4)*:536-8.*
15. Parvizi P, Benlarbi M, Ready PD. Mitochondrial and *Wolbachia* markers for the sandfly *Phlebotomus papatasi*: little population differentiation between peridomestic sites and gerbil burrows in Isfahan province, Iran. *Med. Vet. Entomol.2003 Dec;17(4):351-62.*
16. Nadim A, Javadian E. Key for species identification of sandflies (Phlebotominae; Diptera) of Iran. *Iran J Public Health 1976;5(1):35-44.*
17. Lewis DJ. A taxonomic review of the genus *Phlebotomus* (Diptera: Psychodidae). *Bull Br Mus Nat Hist Entomol.1982 Jun;45:121-209.*
18. Testa JM, Montoya-Lerma J, Cadena H, Oviedo M, Ready P.D. Molecular identification of vectors of *Leishmania* in Colombia: mitochondrial introgression in the *Lutzomyia townsendi* series. *Acta Trop.2002 Dec;84(3):205-18.*
19. Mirzaei A, Rouhani S, Taherkhani H, Farahmand M, Kazemi B, Hedayati M et al. Isolation and detection of *Leishmania* species among naturally infected *Rhombomis opimus*, a reservoir host of zoonotic cutaneous leishmaniasis in Turkemen Sahara, North East of Iran. *Exp Parasitol.2011 Dec;129(4):375-80.*
20. Swofford DL. PAUP: Phylogenetic Analysis Using Parsimony, version 4.0.*,* Washington DC: Smithsonian Institution Press. 2002.
21. Petrischeva PO. Migration of sandflies. Voprosy Kraevoy Obschei. *Experimentalnoi Parasitologi. 1949;4:96-109.*
22. Schlein Y, Gunders AE, Warburg A. Leishmaniasis in the Jordan Valley, I: Attraction of *Phlebotomus papatasi* (Psychodidae) to turkeys. *Ann Trop Med Parasitol.1982 Oct;76(5):517-20.*
23. Yuval B, Schlein Y. Leishmaniasis in the Jordan Valley, III: Nocturnal activity of *Phlebotomus papatasi* (Diptera: Psychodidae) in relation to nutrition and ovarian development. *J Med Vet Entomol.1986 Jul;23(4):411-5.*
24. Schlein Y, Yuval B. Leishmaniasis in the Jordan Valley. IV: Attraction of *Phlebotomus papatasi* (Diptera: Psychodidae) to plants in the field. *J Med Entomol.1987Jan;24(1):87-90.*
25. Yuval B, Warburg A, Schlein Y. Leishmaniasis in the Jordan Valley: V. Dispersal characteristics of the sandfly *Phlebotomus papatasi*. *Med Vet Entomol.1988 Oct;2(4):391-5.*
26. Schlein Y, Yuval B, Jacobson RL. Leishmaniasis in the Jordan Valley: differential attraction of dispersing and breeding site populations of *Phlebotomus papatasi* (Diptera: Psychodidae) to manure and water. *J Med Entomol.1989 Sep;26(5):411-3.*
27. Javadian E, Tesh R, Saidi S, Nadim A. Studies on the epidemiology of sandfly fever in Iran. III: Host-feeding patterns of *Phlebotomus papatasi* in an endemic area of the disease. *Am J Trop Med Hyg.1977Mar;26(2):294-8.*
28. Yaghoobi-Ershadi MR, Javadian E, Tahvildare-Bidruni GH. *Leishmania major* MON-26 isolated from naturally infected *Phlebotomus papatasi* (Diptera: Psychodidae) in Isfahan Province, Iran. *Acta Trop.1995Aug;59(4): 279-82.*
29. Killick-Kendrick R, Ward R.D. Ecology of *Leishmania*. *Parasitology. 1981; 82: 143- 152.*
30. Killick-Kendrick R. Phlebotomine vectors of the leishmaniases: A review. *Med Vet Entomol.1990 Jan;4(1):1-24.*