

A Faunistic Study of Sand Flies of Musian District, Southwestern of Iran

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Abstract.- Cutaneous leishmaniasis is endemic in many parts of Iran including Ilam Province. Sand flies are biological vectors of *Leishmania* species in human and between human and animals in the old world and new world. The special objectives of the present study regarding to the sand flies were to determine the species diversity, relative population density and sex ratio of sand flies in Musian as a part of Ilam province. The entomological studies were conducted in the four zoonotic cutaneous leishmaniasis (ZCL) infected villages, from May 2008 - October 2008. Sticky traps were used to collect sand flies from indoor and outdoor places during the present study. In this faunistic entomological study, totally 1335 sand flies, including 17.5% females and 82.5% males, were collected from indoor and outdoor places, 857 (62.2%) and 478 (37.8%), respectively. Totally 10 species of sand flies were recognized, 3 belonging to the *Phlebotomus* (*P. alexandri*, *P. papatasi* and *P. mongolensis*) and 7 belonging to *Sergentomyia* (*S. sintoni*, *S. antennata*, *S. mervynae*, *S. theodori*, *S. clydei*, *S. tiberiadis* and *S. palestinesis*) genera. Finally, it is concluded that the composition of species in Mousian is almost similar to the other parts of Iran with dominance of *P. papatasi*.

Keyword: Sand flies, faunistic study, Simpson Index, Musian County, Iran.

INTRODUCTION

Sand flies are biological vectors of *Leishmania* species in human and between human and animals in the old world and new world. Different species of *Leishmania* cause leishmaniasis in man and animals. Three forms of leishmaniasis including: Zoonotic Cutaneous Leishmaniasis (ZCL), Anthroponotic Cutaneous Leishmaniasis (ACL) and Visceral Leishmaniasis (VL) have been causing some health and medical problems related to humans in Iran and its adjoining countries like Iraq, Afghanistan and Pakistan. The different species of *Phlebotomus* are the vectors of ZCL among humans, rodents and from rodents to humans; however, *P. papatasi* is the main vector to humans in the old world (Rassi *et al.*, 2004;

Jahanifard *et al.*, 2009; Yaghoobi-Ershadi, 2012).

The study of sand flies of Iran has been started since early of 20 century mainly based on foreign entomologist works: Adeler, Teodor and Louri. The first comprehensive entomological study on Iranian phlebotomine fauna had been done in 1960 by Mesghali (Seyedi-Rashti and Nadim, 1992) who reported 12 species of *Phlebotomus* and 11 of *Sergentomyia*. Javadian and Nadim (1975) have reported 42 species of phlebotomine of Iran. In addition, Kasiri *et al.* (2000) have published a checklist of Iran sand flies (*Phlebotomus* and *Sergentomyia*) included 54 species; however, Rassi and Hanafi-bojd (2006) has explained that the fauna of Iran is included 44 confirmed species and 10 unconfirmed, as Iran latest phlebotomine faunistic study (Kakarsulemankhel, 2010; Yaghoobi-Ershadi, 2012).

ZCL is the most frequent and endemic form of leishmaniasis in Iran (about 80% of cases reported in Iran), including west and south-west foci

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of Iran. Ilam Province has been concerned as one of ZCL foci in west and south west of Iran. Residents of Musian County and its suburbs, as a part of Ilam province are endangered of this disease (Yaghoobi-Ershadi and Javadian, 1996; Yaghoobi-Ershadi, 2012). Cutaneous leishmaniasis has made a lot of problems in Dehloran, special in Mousian area among the residents and armed forces (Yaghoobi-Ershadi and Javadian, 1996; Kavarizadeh, 2005; Yaghoobi-Ershadi, 2012).

Estimations are showing an increasing in the rate of ZCL cases in the region among the human population during 2000-2005 (Kavarizadeh, 2005). In addition, Mousian is almost near to Khuzestan province as one of the most important foci of ZCL in southwest of Iran (Kavarizadeh *et al.*, 2011).

Characterizing the distribution and ecology of these vector species would be valuable in better understanding the epidemiology of leishmaniasis. Therefore, a phlebotomine faunistic study was conducted in Musian. The special objective of the present study was to determine the species diversity, relative population density and sex ratio of sand flies in Musian County. These factors provide basic epidemiological information to make vector control programs to reduce the incidence of ZCL in the region.

MATERIALS AND METHODS

Study area

The investigation was carried out in 2009 in Musian District (32°31'20"N 47°22'31"E) (Fig. 1) with 119 meters above mean sea level, with a warm and dry climate is located in Ilam Province, southwest of Iran. Musian area is about 3051 squared kilometer and its population was 15933 on 2008. The lowest temperature in winter is 5°C and the highest temperature in summer is more than 50°C (I.R. Iran Meterological Organization, 2012).

The majority of people's occupations in this region are agriculture and livestock farming (Mansoori *et al.*, 2009).

Sand fly collection and identification

The entomological studies were conducted from May 2008 - October 2008 in the four ZCL infected villages, Nahr Anbar, Borom, Nasr and



Fig. 1. Map of Musian Districts in the southwest of Iran.

Cham Hendi in Musian District. Sand flies were collected using 720 sticky traps (castor oil-coated white papers 20 cm × 30 cm) from indoors (30 traps/village/time) and outdoors (30 traps/ village/time) of 3 replicates. Climatological condition of Musian during sampling attempts is presented in Table I. The traps were set at dusk and collected at dawn. Sand flies were removed from the traps, rinsed in acetone and then conserved in 70% ethanol. All specimens were mounted as permanent microscopy slides, using Puri's medium (Smart *et al.*, 1965). The sex of all specimens were determined and identified by using keys of Nadim

and Javadian (1976), Lewis (1982) and Seyedi-Rashti and Nadim (1992). In addition, sex ratios of the most abundant species of sand flies were determined in Musian. Simpson's Diversity Index was applied to determine richness of biodiversity of sandfly

$$D = \sum (n/N)^2$$

where n represents the total number of organisms of a particular species and N represents the total number of organisms of all species (Iffwell Woodland and Wildlife Trust, 2000).

Table I.- Climatological condition of Mousian County during sampling time in 2008.

Month	Day	Temperature (°C)		Relative humidity (%)	
		Max	Min	Max	Min
May	13	40.4	28.0	31	10
	14	36.8	25.4	44	21
	15	34.2	25.0	51	23
	16	35.0	23.6	48	19
July	25	47.2	33.8	29	13
	26	48.0	32.0	25	13
	27	48.0	34.8	27	10
	28	49.0	34.0	27	06
October	26	34.4	21.0	30	17
	27	34.0	20.4	33	16
	28	34.6	21.2	32	14
	29	34.0	20.6	34	19

RESULTS

In this study, 1335 sand flies (17.5% females and 82.5% males) were collected of which 857 from indoor (62.2%) and 478 from outdoor (37.8%) places.

Ten species of sand flies were recognized, 3 belonging to *Phlebotomus* and 7 belonging to *Sergentomyia* genera (Tables II and III). The most frequent *Phlebotomus* species was *P. papatasi* (77.6%) followed by *P. alexandri* (11%). *S. sintoni* with 9.5% frequency of sandflies was the most frequent *Sergentomyia* sandflies. *P. papatasi* was recognized as the dominant species of the region that formed 73.8% and 79.6% of total sand flies of indoor and outdoor traps, respectively. It is followed by *P. alexandri* and *S. sintoni* with 11.5% and 7.6% in the indoor places and 9.6% and 13% in the outdoor places, respectively.

The sex ratios (number of males/females × 100) of *P. papatasi*, *P. alexandri* and *S. sintoni* were 650.55, 800 and 124.14, in the indoor places, respectively and 508.62, 2200 and 87.88 in the outdoor places, respectively.

The geography distribution of collected sandflies according to species are presented in Figure 1 and Table IV. Biodiversity Indices of sandflies in Musian using Simpson's indices showed that the regions of Cham Hendi and Nasr with $D = 0.17$ and $D = 1$ were the greatest and lowest sandfly biodiversity in the current study, respectively (Table V).

DISCUSSION

In the current study 3 species of *Phlebotomus* and 7 species of *Sergentomyia* have been collected. This is the first report of some ecological aspects of sand flies in Musian rural District.

Phlebotomus papatasi was the most predominant sand fly collected in this study. This result is similar to the other studies performed in the regions near to Musian of Iran. Yaghoobi-Ershadi (2012) has reported 5.6% of *P. papatasi*, infected with *Leishmania major* of Iran. *P. papatasi* which normally prefers to live in plains area rather than in mountains (Rassi and Hanafi-bojd, 2006), has been collected from all parts of Iran including Musian District (119 m above level of sea) from both the indoor and outdoor places. Cross *et al.* (1996) have reported that *P. papatasi* is the most abundant in areas with mean minimum temperature of 16°C and maximum temperature of 44°C from May to October. In the current study this species was collected from Musian villages where the maximum and minimum of 49°C in July and 14°C in October (May-October), respectively. It is concluded that this species has a wider ecological valance. Belen *et al.* (2004) have also reported that this species can be found at elevations ranging from near sea level to over 1100 m. In the present study this species was collected from Musian villages which are 119 m above sea level. This species was abundantly collected from Borom village, which is the largest water resource of Musian District and provides good niches for *P. papatasi* and agricultural activities.

Table II.- Frequency of *Phlebotomus* and *Sergentomyia* species of Musian, 2008.

Species	Males		Females		Frequency of total sand fly population
	Number	%	Number	%	
<i>P. papatasi</i> (Scopoli, 1786)	887	86	149	14	77.60
<i>P. alexandri</i> (Sinton, 1928)	132	91	13	9	11
<i>P. mongolensis</i> (Sinton, 1928)	2	100	-	-	0.15
<i>S. sintoni</i> (Pringle, 1953)	65	51	62	49	9.50
<i>S. antennata</i> (Newstead, 1912)	3	33	6	67	0.67
<i>S. mervynae</i> (Pringle, 1953)	-	-	1	-	0.70
<i>S. theodori</i> (Parrot, 1942)	3	100	-	-	0.22
<i>S. clydei</i> (Sinton, 1926)	6	75	2	25	0.60
<i>S. tiberiadis</i> (Adler, Theodor and Lourie, 1930)	3	100	-	-	0.22
<i>S. palestinensis</i> (Adler and Theodor, 1927)	1	100	-	-	0.07
Total	1021	86.31	162	13.69	100

Table III.- Frequency of *Phlebotomus* and *Sergentomyia* species of Musian according to sex and places of trapping, 2008.

Species	Indoor						Outdoor					
	Males		Females		Total		Males		Females		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
<i>P. papatasi</i>	592	86.6	91	13.4	683	79.7	295	83.6	58	16.4	353	73.8
<i>P. alexandri</i>	88	88.9	11	11.1	99	11.5	44	95.6	2	4.4	46	9.6
<i>P. mongolensis</i>	2	100	-	-	2	0.2	-	-	-	-	-	-
<i>S. sintoni</i>	36	55.4	29	44.6	65	7.6	29	46.8	33	53.3	62	13
<i>S. antennata</i>	1	33.3	2	66.7	3	0.4	2	33.3	4	66.7	6	1.2
<i>S. mervynae</i>	-	-	-	-	-	-	-	-	1	0.01	1	0.2
<i>S. theodori</i>	-	-	-	-	-	-	-	-	3	0.03	3	0.6
<i>S. clydei</i>	2	100	-	-	2	0.2	2	33.3	4	66.7	6	1.3
<i>S. tiberiadis</i>	3	100	-	-	3	0.4	-	-	-	-	-	-
<i>S. palestinensis</i>	-	-	-	-	3	0.4	1	100	-	-	1	0.2
Total	724	84.6	133	15.4	857	64.2	373	78	105	32	478	35.8

Table IV.- Geography distribution of collected sandflies according to species Musian, 2008.

Coordinates of studied Districts of Musian 32°31'20"N 47°22'31"E	<i>Pp</i> *	<i>Pa</i> *	<i>Pm</i> *	<i>Ss</i> *	<i>Sa</i> *	<i>Sm</i> *	<i>St</i> *	<i>Sc</i> *	<i>Sti</i> *	<i>Sp</i> *	Total
Cham Hendi 32°18'54"N 47°35'59"E	176	144	2	93	-	-	1	2	-	1	419
Nahr Anbar 32°27'24"N 47°27'12"E	175	-	-	26	9	1	1	3	1	-	216
Borom 32°28'15"N 47°47'10"E	441	1	-	8	-	-	1	2	2	-	455
Nasr 32°20'14"N 47°50'56"E	244	-	-	-	-	-	-	1	-	-	245

* Abbreviation of sandflies of Musian: *Pp*, *P. papatasi*; *Pa*, *P. alexandri*; *Pm*, *P. mongolensis*; *Ss*, *S. sintoni*; *Sa*, *S. antennata*; *Sm*, *S. mervynae*; *St*, *S. theodori*; *Sc*, *S. clydei*; *Sti*, *S. tiberiadis*; *Sp*, *S. palestinesis*.

P. alexandri is present in different ecologic areas ranging from sea level to 1500 m above sea level and including coastal plain, inland plateau, and highland valleys from Spain and Morocco east to the mountains in northwestern China and as far

south as southern Ethiopia and Djibouti (Fryauff *et al.*, 1995; Depaquit, 1997; Maroli *et al.*, 2001; Kamal *et al.*, 2003). This species is a rare species in different areas of Iran including plain and highlands (Rassi and Hanafi-bojd, 2006). In the current study,

Table V.- Biodiversity indices of sandflies in Musian, SW of Iran, 2008.

Region	Biodiversity indices		
	D ¹	(1-D) ²	(1/D) ³
Cham Hendi	0.17	0.83	5.9
Nahr Anbar	0.67	0.33	1.49
Borom	0.94	0.06	1.06
Nasr	1	0	1

¹ Simpson's Index (the greater the value of D, the lower the diversity)

² Simpson's Index of Diversity (the greater the value, the greater the sample diversity)

³ Simpson's Reciprocal Index (The higher the value, the greater the diversity)

this species has been found in outdoor and indoor areas with 9.6% and 11.5% all of the collected sandflies. This species was collected only from Cham Hendi Village which was recognized as the area of richest biodiversity of sandflies. *P. alexandri* prefers regions with high percentage of relative humidity and warmer niches (Rassi and Hanafi-bojd, 2006). It is assumed that Doirej River near this village provides relative humidity suitable for agriculture activities.

Javadian *et al.* (1997) have collected 11 species from the genus *Phlebotomus* and 11 species from the genus *Sergentomyia* in Ilam which is nearest to our study area in the west of Iran. *P. papatasi*, *P. alexandri*, *S. sintoni*, *S. mervynae*, *S. tiberiadis* and *S. theodori* have been collected in both the studies.

Javadian and Nadim (1975) have reported 10 species of *Phlebotomus* from Khuzestan, of these *P. papatasi*, *P. alexandri*, *P. mongolensis* and *P. sergenti* have been reported in the current study in the Musian area.

The sand flies reported from Musian County have also been reported from other regions near to Musian District. Kavarizadeh *et al.* (2011) have reported *P. papatasi* followed by *P. alexandri*, *P. caucasicus*, *P. mongolensis* and *P. sergenti* of Maleh area in Shoush. They also showed similar pattern of sex ratio, however, the difference of sex ratio between two areas is reflected by the different ecological conditions in both areas.

In a study in Arsanjan, south of Iran, Rassi *et al.* (2004) collected 8 species of *Phlebotomus* (*P.*

papatasi, *P. sergenti*, *P. alexandri*, *P. mongolensis*, *P. andrejevi*, *P. tobbi*, *P. keshishiani*, *P. halepensis*), and 4 of *Sergentomyia* (*S. sintoni*, *S. dentata*, *S. theodori* and *S. clydei*). Among the 12 species identified in Rassi *et al.* (2004) study, 6 species have been reported in the current study. The most common species in their study was *P. papatasi*, which is similar to the current study results. Motazedian *et al.* (2006) have collected only *P. papatasi* from Larestan, south of Iran. In a faunistic study in Jask, south of Iran, 8 species (3 *Phlebotomus* and 5 *Sergentomyia*) were reported by Azizi and Fekri (2010). *P. papatasi*, *S. sintoni*, *S. theodori*, *S. clydei* and *S. tiberiadis* were reported in the current study too.

In Shiraz, south of Iran, Rasoolian *et al.* (2007) have reported 10 species consisting of 3 species from genus *Phlebotomus* and 7 species from *Sergentomyia*. *P. papatasi*, *S. sintoni*, *S. theodori*, *S. clydei*, *S. palestinensis* and *S. mervynae* were the same as in the current study.

In the present study Doirej river which passes through the village is the reason for this great biodiversity providing suitable breeding places including high relative humidity for egg laying by the female sand flies (Rassi and Hanafi-bojd, 2006).

Finally, it is concluded that globally, the sand flies species reported from Musian County have already being reported from other regions of Iran. Agriculture activities are important factors to promote ecologic conditions for sandfly activities.

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