

Bionomics of phlebotomine sand flies (Diptera: Psychodidae) as vectors of leishmaniasis in the County of Iranshahr, Sistan-Baluchistan Province, Southeast of Iran

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Abstract

Background: Leishmaniasis is one of the most important human's vector-borne diseases. This parasitic disease can cause by many species of *Leishmania* and transmitted by several species of sand fly species. In order to determine fauna and species combination of sand flies, an investigation was carried out in Iranshahr County, Sistan-Bluchistan Province, Southeast of Iran during 1997.

Materials and methods: Sand flies were collected by using sticky traps from selected villages (outdoors as well as indoors). Traps were fixed at sundown and sand flies were collected at sunrise. The indices of diversity, species richness and evenness of species were calculated using the appropriate formulas. The specimens were identified according to keys.

Results: Totally, 7794 sand flies comprising of *Phlebotomus* genus (32.9%) and *Sergentomyia* genus (67.1%) were collected. These sand flies consisted of: *P. papatasi*, *P. alexandri*, *P. sergenti*, *P. kazeruni*, *P. bergeroti*, *P. mesghali*, *P. salehi*, *P. eleanorae*, *P. keshishiani*, *S. clydei*, *S. tiberiadis*, *S. hodgsoni*, *S. sintoni*, *S. mervynae*, *S. baghdadis*, *S. christophersi*, *S. dentata*, *S. dreyfussi*, *S. iranica*, *S. palestinensis* and *S. sumbarica*. The predominant species were *S. clydei*, *S. tiberiadis* and *P. papatasi* respectively. During this study, *P. eleanorae*, *P. keshishiani*, *P. mesghali*, *S. christophersi*, *S. dreyfussi*, *S. hodgsoni*, *S. iranica*, *S. mervynae*, *S. palestinensis* and *S. sumbarica* are reported for the first time from Iranshahr County.

Conclusion: Based on the results, the vectors of cutaneous leishmaniasis include of *P. papatasi*, *P. salehi*, *P. sergenti* and moreover the vectors of visceral leishmaniasis include of *P. keshishiani* and *P. alexandri* have been found.

Keywords: Bionomics; Fauna; Sand fly, Leishmaniasis, Iranshahr, Iran.

Introduction

Leishmaniasis is one of the most important human's vector-borne diseases. This parasitic disease is transmitted by several sand fly species belonging to *Phlebotomus* and *Lutzomyia* genera, subfamily Phlebotominae, family Psychodidae. Leishmaniasis is endemic in 88 countries (mostly the developing countries), found in all continents except Australia and Antarctica. The overall prevalence of leishmaniasis is estimated at 12 million cases with 0.5 million new Visceral Leishmaniasis (VL) cases per year and 1-1.5 million new Cutaneous Leishmaniasis (CL) cases per year. In 1976, the WHO included it as one of the six items in the UNDP/World Bank/WHO Special Program for Research and Training in Tropical diseases (1, 2).

There are several reports indicating occurrence of Zoonotic Cutaneous Leishmaniasis (ZCL) in Iran (3, 4, 5, 6, 7, 8). The first important focus of disease located in central and northeast of Iran, where *Rhombomys opimus* and *P. papatasi* play an important role as a reservoir and vector respectively. The second focus of ZCL has been reported from west and southwest of Iran, where *Tatera indica* replaced with *R. opimus* as a reservoir and *P. papatasi* as a vector (1, 9, 10).

Sistan-Baluchistan Province, in southeast of Iran basically has not previously been known as an important endemic area for leishmaniasis (4, 11). Frequent reports of CL are only reported from a restricted rural area of

Chabahar Port in the southern part of province. Also an outbreak of CL occurred in a couple of villages of Mirjaveh City for the first time in 1996. Recently, this province is considered as a third focus of ZCL (3, 4, 5, 6). In this region *Meriones hurrianae* has been approved as a natural reservoir host for ZCL (12). The number of reported cases rose annually from a few in the early years to more than 400 in 2005, have caused a major public health problem in this area (4).

This paper gives the results of investigation to determine the species composition, sex ratio of each species and biodiversity of sand flies in Iranshahr County, Sistan-Bluchistan Province, Southeast of Iran.

Materials and Methods

The investigation was carried out in Iranshahr County (longitude: 42° 60' and latitude: 12° 27'), Sistan-Bluchistan Province, southeast of Iran. Its altitude is 591.1 meter above sea surface. The square of this county is 41730 km² with relative density of 5.8 people per km². Iranshahr County has 6 districts, 2 cities, 21 rural districts and 181 villages. With attention to geographical distribution of villages, totally 12 villages and city regions were selected for sand flies capturing in this study. The specimens were collected from Iranshahr city's district Abtar, Malekabad, Angori, Bandaman, Sarbaz, Rask, Bampor, Daman, Shamsabad, Kahiri and Pishin. Sand flies were collected with sticky traps from indoors (human and animal places) and outdoors (rodent borrows, mountain and cave rifts, wall rifts, roach rifts,...) in both of plain and mountainous areas. Totally, 1265 traps in 22 times have been installed after sunset and were collected before sunrise. Sand flies were removed from sticky traps with insect needle, rinsed

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in acetone and then conserved in 70% ethanol. All specimens were mounted as permanent microscopy slides, using in Puri's medium. The specimens were identified according to standard methods.

Faunistical analysis: The analysis of sand flies was based on abundance, diversity and sex ratio of each species. The indices of biodiversity refer to the relationship between the number of species and individuals of a community or ecosystem.

Measurement of diversity

The diversity index was calculated by using the Shannon – Wiener diversity index (1949).

$$H' = -\sum Pi \ln Pi$$

Where $Pi = S / N$

S = number of individuals of one species

N = total number of all individuals in the sample

ln = logarithm to base e

Measurement of species richness

Margalef's index was used as a simple measure of species richness (Margalef, 1958).

Margalef's index = $(S - 1) / \ln N$

S = total number of species

N = total number of individuals in the sample

ln = natural logarithm

Measurement of evenness

For calculating the evenness of species, the Pielou's Evenness Index (e) was used (Pielou, 1966). The

maximum diversity (H_{max}) which could possibly occur would be found in a situation where all species were equally abundant, in other words if $H' = H_{max} = \ln S$. The ratio of observed diversity to maximum diversity can therefore be taken as a measure of evenness (E).

$$e = H / \ln S$$

H = Shannon – Wiener diversity index

S = total number of species (13).

Results

Sandflies Fauna: A total of 7794 adult's sand flies belonging to *Phelobotomus* (32.9%) and *Sergentomyia* (67.1%) genus were collected by sticky traps from plain and mountainous areas. These consisted of 9 species of the genus *Phelobotomus* and 12 species of the genus *Sergentomyia*. 3347 and 4447 adult sand fly specimens were captured from mountainous and plain areas respectively. These sand flies consist of: *P. papatasi*, *P. alexandri*, *P. sergenti*, *P. kazeruni*, *P. bergeroti*, *P. mesghali*, *P. salehi*, *P. eleanorae*, *P. keshishiani*, *S. clydei*, *S. tiberiadis*, *S. hodgsoni*, *S. sintoni*, *S. mervynae*, *S. baghdadis*, *S. christophersi*, *S. dentata*, *S. dreyfussi*, *S. iranica*, *S. palestinensis* and *S. sumbarica*. Frequency of collected species has been showed in Table 1. *P. papatasi* (55.7%) and *P. alexandri* (25.6%) were the most abundant species comparison to other species of *Phelobotomus*. In *Sergentomyia* genus, *S. clydei* (51.3%) and *S. tiberiadis* (29.2%) were the most capturing species in comparison with other species of *Sergentomyia* (Table 1). During this study, *P. eleanorae*, *P. keshishiani*, *P. mesghali*, *S. christophersi*, *S. dreyfussi*, *S. hodgsoni*, *S. iranica*, *S.*

Table 1: Fauna, abundance and percentage of *Phlebotomus* and *Sergentomyia* sand flies collected from Iranshahr County, Sistan-Baluchistan Province, Iran (1997).

Species	No.	Total %	Male No.	%	Female No.	%	Sex Ratio: number of male/female×100
<i>P. alexandri</i>	658	25.6	591	89.8	67	10.2	882.1
<i>P. bergeroti</i>	37	1.4	37	100	-	-	-
<i>P. eleanorae</i>	6	0.2	4	66.7	2	33.3	200
<i>P. kazeruni</i>	141	5.5	131	92.8	10	7.2	1310
<i>P. keshishiani</i>	2	0.08	1	50	1	50	100
<i>P. mesghali</i>	27	1.05	21	77.8	6	22.2	350
<i>P. papatasi</i>	1429	55.7	1038	72.6	391	27.4	265.5
<i>P. salehi</i>	13	0.5	10	76.9	3	23.1	333.3
<i>P. sergenti</i>	253	9.8	221	87.6	32	12.4	690.6
Total	2565	100	2053	80	512	20	400.9
<i>S. baghdadis</i>	68	1.3	25	37.3	43	62.7	58.2
<i>S. christophersi</i>	63	1.2	42	66.7	21	33.3	200
<i>S. clydei</i>	2685	51.3	1654	61.6	1031	38.4	160
<i>S. dentata</i>	33	0.6	23	69.7	10	30.3	230
<i>S. dreyfussi</i>	17	0.3	2	11.8	15	88.2	13.3
<i>S. hodgsoni</i>	352	6.7	230	65.34	122	34.66	188.5
<i>S. iranica</i>	7	0.1	6	85.7	1	14.3	600
<i>S. mervynae</i>	125	2.4	32	25.6	93	74.4	34.4
<i>S. palestinensis</i>	1	0.02	-	-	1	100	0
<i>S. sintoni</i>	344	6.6	112	32.5	232	67.4	48.3
<i>S. sumbarica</i>	1	2.02	-	-	1	100	0
<i>S. tiberiadis</i>	1528	29.2	903	59.1	625	40.9	144.5
Total	5229	100	3029	57.9	2200	42.1	137.7

mervynae, *S. palestinensis* and *S. sumbarica* are reported for the first time from Iranshahr County.

Twelve species of *Sergentomyia* and 8 species of *Phlebotomus* were collected from mountainous areas. Ten and 8 species of *Sergentomyia* and *Phlebotomus* were found in plain areas.

Although the number of the Phlebotominae species were further in the mountainous areas than plain areas but the number of total collected specimens of both genus were further in plain areas. 56.2% and 43.8% of *Phlebotomus*, also 57.2% and 43.8% of *Sergentomyia* specimens were collected from plain and mountainous areas respectively. *P. keshishiani*, *S. palestinensis*, *S. sumbarica* were only collected from mountainous areas and *P. salehi* was only collected from plain areas. (Table 2).

P. papatasi, *P. bergeroti*, *S. clydei* and *S. sintoni* were collected from plain areas frequently comparison to plain areas. *P. alexandri*, *P. kazeruni*, *P. sergenti*, *P. mesghali*, *P. eleanorae*, *S. baghdadis*, *S. christophersi*, *S. dentata*, *S. dryfussi*, *S. hodgsoni*, *S. iranica*, *S. mervynae* and *S. tiberiadis* were collected from mountainous area frequently comparison to plain areas (Table 2).

Table 2: Fauna, number and percentage of sand flies collected in plain and mountainous areas from Iranshahr County, Sistan-Baluchistan Province, Iran (1997).

Species	Plain areas		Mountainous areas		Total
	No.	%	No.	%	
<i>S. baghdadis</i>	10	14.9	58	85.1	68
<i>S. christophersi</i>	8	12.7	55	87.3	63
<i>S. clydei</i>	2570	95.7	115	4.3	2685
<i>S. dentata</i>	1	31	32	97	33
<i>S. dryfussi</i>	1	6.2	16	93.8	17
<i>S. hodgsoni</i>	26	7.4	326	92.6	352
<i>S. iranica</i>	1	14.3	6	85.7	7
<i>S. mervynae</i>	3	2.4	122	97.6	125
<i>S. palestinensis</i>	0	0	1	100	1
<i>S. sintoni</i>	285	82.8	59	17.2	344
<i>S. sumbarica</i>	0	0	1	100	1
<i>S. tiberiadis</i>	100	0.06	1428	93.4	1528
Total	3005	57.5	2224	42.5	5229
<i>P. alexandri</i>	26	3.9	632	96.1	658
<i>P. bergeroti</i>	26	70.3	11	29.7	37
<i>P. eleanorae</i>	2	33.3	4	66.7	6
<i>P. kazeruni</i>	9	6.5	131	93.5	140
<i>P. keshishiani</i>	0	0	2	100	2
<i>P. mesghali</i>	3	11.1	24	88.9	27
<i>P. papatasi</i>	1353	94.7	76	5.3	1429
<i>P. salehi</i>	13	100	0	0	13
<i>P. sergenti</i>	10	4	243	96	253
Total	1442	56.2	1123	43.8	2565

The predominant species in plain areas were *S. clydei* (57.8%) and *P. papatasi* (30.4%) respectively. In mountainous areas *S. tiberiadis* (42.7%), *S. hodgsoni* (9.8%), *P. alexandri* (18.9%) and *P. sergenti* (7.3%) were predominant species respectively (Tables 4 and 5).

The CL vectors include of *P. papatasi*, *P. sergenti*, *P. salehi* have been found in this area. The sex ratio (number of male/female×100) of these species were 265.5, 333.3 and 690.6 respectively (Table 1). Moreover the probable vectors of VL include of *P. alexandri* and *P. keshishiani* have been found with sex ratio of 882.1 and 100. The proportion of captured males was higher in all species of *Phlebotomus* and *Sergentomyia* than females (Table 1).

The abundance of collected *P. papatasi* and *P. salehi* clear that these species can be the probable vectors of CL in plain area, while in mountainous areas *P. sergenti* can be the vector of the disease.

Calculation of Shannon-Weaver diversity index, species richness and species evenness in plain and mountainous areas showed that although the number of individuals belonging to sand flies in plain areas was more than mountainous areas but the biodiversity indices (diversity index, species evenness and species richness) of the latter were more suggesting more species diversity than that of plain areas. Shannon-Weaver index of diversity of mountainous areas was 1.7 times higher than what was obtained in plain areas.

In mountainous areas diversity index (H') is: $H' = 1.91$ (Table 3)

Table 3: Shannon-Weaver diversity index of sand flies fauna in mountainous areas of Iranshahr County, Sistan-Baluchistan Province, Iran (1997).

Species	No.	P_i (%)	$\ln P_i$	$P_i \ln P_i$
<i>S. baghdadis</i>	58	0.017	-4.05	-0.07
<i>S. christophersi</i>	55	0.016	-4.11	-0.07
<i>S. clydei</i>	115	0.034	-3.37	-0.12
<i>S. dentata</i>	32	0.010	-4.65	-0.04
<i>S. dryfussi</i>	16	0.005	-5.34	-0.03
<i>S. hodgsoni</i>	326	0.098	-2.33	-0.23
<i>S. iranica</i>	6	0.002	-6.32	-0.01
<i>S. mervynae</i>	122	0.037	-3.31	-0.12
<i>S. palestinensis</i>	1	0.0003	-8.11	-0.00
<i>S. sintoni</i>	59	0.018	-4.04	-0.07
<i>S. sumbarica</i>	1	0.0003	-8.11	-0.00
<i>S. tiberiadis</i>	1428	0.427	-0.85	-0.36
<i>P. alexandri</i>	632	0.189	-1.67	-0.31
<i>P. bergeroti</i>	11	0.003	-5.72	-0.02
<i>P. eleanorae</i>	4	0.001	-6.73	-0.01
<i>P. kazeruni</i>	131	0.039	-3.24	-0.13
<i>P. keshishiani</i>	2	0.001	-7.42	-0.00
<i>P. mesghali</i>	24	0.007	-4.94	-0.04
<i>P. papatasi</i>	76	0.023	-3.78	-0.09
<i>P. sergenti</i>	243	0.073	-2.62	-0.19
Total	3347			$H' = 1.91$

Species richness: $D_{Mg} = 2.34$

Species evenness index:

$E = H' / H_{max} = H' / \ln S$

$S = 20 \rightarrow E = 1.91 / 2.993 = 0.67 \rightarrow E = 0.67$

In plain areas diversity index (H') is:

$$H' = 1.12 \text{ (Table 4)}$$

Specis richness: $D_{Mg} = 2.02$

Species evenness index:

$$E = H'/H'_{max} = H'/\ln S$$

$$S = 18 \rightarrow E = 1.91 / 2.89 = 0.38 \rightarrow E = 0.38$$

Table 4: Shannon-Weaver diversity index of sand flies fauna in plain areas of Iranshahr County, Sistan-Baluchistan province, Iran (1997).

Species	No.	P _i (%)	lnP _i	P _i lnP _i
<i>S. baghdadis</i>	10	0.002	-6.1	-0.014
<i>S. christophersi</i>	8	0.002	-6.3	-0.011
<i>S. clydei</i>	2570	0.578	-0.5	-0.317
<i>S. dentata</i>	1	0.0002	-8.4	-0.002
<i>S. dryfussi</i>	1	0.0002	-8.4	-0.002
<i>S. hodgsoni</i>	26	0.006	-5.1	-0.030
<i>S. iranica</i>	1	0.0002	-8.4	-0.002
<i>S. mervynae</i>	3	0.001	-7.3	-0.005
<i>S. sintoni</i>	285	0.064	-2.7	-0.176
<i>S. tiberiadis</i>	100	0.022	-3.8	-0.085
<i>P. alexandri</i>	26	0.006	-5.1	-0.030
<i>P. bergeroti</i>	26	0.006	-5.1	-0.030
<i>P. eleanorae</i>	2	0.0004	-7.7	-0.003
<i>P. kazeruni</i>	9	0.002	-6.2	-0.013
<i>P. mesghali</i>	3	0.001	-7.3	-0.005
<i>P. papatasi</i>	1353	0.304	-1.2	-0.362
<i>P. salehi</i>	13	0.003	-5.8	-0.017
<i>P. sergenti</i>	10	0.002	-6.2	-0.014
Total	4447			H'=1.12

DISCUSSION

Study of the sand flies fauna and leishmaniasis vectors is necessary for all epidemiological and ecological studies. Although Iranshahr County is not an important endemic focus of CL, but because of the prevalence of the disease in Chabahar and Mirjaveh Counties from Sistan-Baluchistan province as two endemic foci of CL, it was necessary to collect information about sand flies fauna of this area.

This is the second report of sand flies fauna in this part of Sistan-Baluchistan province. The first study was conducted by Seyedi-Rashti *et al.* They reported 12 species of Phlebotominae consisting of 6 species of *Sergentomyia* and 6 species of *Phlebotomus* from Iranshahr for the first time (11). In present research 21 species consisting of 12 species of *Sergentomyia* and 9 species of *Phlebotomus* were collected from plain and mountainous areas. *P. eleanorae*, *P. keshishiani*, *P. mesghali*, *S. christophersi*, *S. dreyfussi*, *S. hodgsoni*, *S. iranica*, *S. mervynae*, *S. palestinensis* and *S. sumbarica* are reported for the first time from Iranshahr County. We didn't collect *S. sintoni*, *S. squamipleuralis* and *S. pawlowskyi* in faunistical survey but these 3 species were reported by Seyedi-Rashti *et al.* from Iranshahr. Six

species of *Phlebotomus* in plain areas and 11 species in mountainous areas were reported by Abdolli *et al.* from Kohpayeh district, Esfahan province, Iran (14).

The proportion of captured males was higher in *Phlebotomus* (80%) and *Sergentomyia* (58%) than females. This overall percentage of male sand flies recorded during the study period is consistent with other published data. Kaspas *et al.* reported that sand flies male composed of 80% of their collections conducted in an endemic CL focus (15). Morrison *et al.* reported that 60% and 83% of *Lutzomyia longipalpis* population density conducted at the pigpen and cattle corral was male (16). Reza and Mansour collected significantly more males than females of *P. papatasi* (85%) and *P. sergenti* (70%). According to the results of Dinesh *et al.* males and non-fed females of *P. argentipes* comprised >60% of total collections (17, 18). It is known that male sand flies arrive on their host first, from an aggregation and wait for the females for mating. This "lekking" behavior of the males is believed to allow them to disperse high levels of sex pheromone to attract females and increase their chances to mate (15, 16).

S. clydei is the most abundant species in *Sergentomyia* genus, so that 51.3% of all *Sergentomyia* collected, belong to this species. In the *Phlebotomus* genus, the highest population density was represented by the species *P. papatasi* with 55.7% of all specimens in Iranshahr during the period studied. Such species has been described in several endemic areas of ZCL in Iran (3, 7, 8, 9, 10, 11, 14,).

Based on the results, the vectors of CL include of *P. papatasi*, *P. sergenti*, *P. salehi* and moreover the vectors of VL include of *P. alexandri* and *P. keshishiani* (19) have been found in Iranshahr County while CL prevalence is scarce and VL hasn't yet reported from Iranshahr.

P. alexandri as probable VL vector were frequently found in mountainous areas, so the people who live in this region, probably are more exposing to VL than who are in plain areas.

In the plain areas, *P. papatasi* as the main and proven vector of ZCL in Iran is dominant and it is probable that residents of plain areas are exposing to ZCL rather than other forms of leishmaniasis.

When species composition of sand flies collected in this survey was compared in plain and mountainous areas, remarkable differences were observed, probably due to the climate and floristic composition, peculiar to each biome. The number of collected species in mountainous areas was more than plain areas, but the number of all captured specimens in plain areas was larger. So, the diversity of sand flies in mountainous areas was more than plain areas and this result confirm by the other researchers (8, 14,19). In the plain areas, for example, *S. clydei* and *P. papatasi* were the very abundant and dominant species, while in mountainous areas *S. tiberiadis*, *S. hodgsoni*, *P. alexandri* and *P. sergenti* were predominant species respectively.

Diversity indices used in environmental assessment, mainly to monitor changes in the diversity of organisms,

could be applied to monitor sand flies species (13). Diversity indices should be used to monitor sand flies vector species at many sites in relation to habitat type, latitude and land use and the databases generated throughout monitoring time should be used to forecast the effects of environmental change in sand flies populations. Our results demonstrate the existence of important vector species of sand flies in the study area. *P. papatasi* and *S. clydei* were the highest recorded species. Although the abundance of sand fly species is not by itself sufficient to incriminate it as a vector, it is clear that *P. papatasi* could potentially transmit CL in this area. Further studies should focus on *P. papatasi* to confirm if the sand flies species transmitting CL in this region.

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