Original Article

Determination of Fauna and Seasonal Activity of Phlebotomine Sandflies (Diptera: Psychodidae: Phlebotominae) as Vectors of Disease Agents in Southwestern Iran

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Abstract

Aims: The current study determined the faunistic composition, sex ratio, seasonal abundance, and physiological status of sandflies as vectors of leishmaniasis in Abadan County, Khuzestan Province, Iran, during 2015–2016. **Materials and Methods:** Sandflies were captured using sticky paper traps installed at sunset and collected after sunrise at indoor and outdoor locations. Sandflies were mounted on slides in Puri's medium and identified using diagnostic keys. **Results:** Overall, 13 species (2 species of *Phlebotomus* Rondani and Bert 1840 and 11 species of *Sergentomyia* Franca and Parrot 1920) were identified among the 6173 sandflies collected including *Phlebotomus papatasi*, Scopoli, 1786 (45.64%); *Phlebotomus alexandri*, Sinton, 1928 (31.31%); and *Sergentomyia sintoni*, Pringle, 1953 (15.9%). Four species, including *Sergentomyia tiberiadis*, Adler, Theodor, and Lourie, 1930; *Sergentomyia clydei*, Sinton, 1928; *Sergentomyia antennata*, Newstead, 1912; and *Sergentomyia christophersi*, Sinton, 1927, are reported for the first time in Abadan. The sex ratios of the sandflies of genus *Phlebotomus* and *Sergentomyia* were 362.4 and 92.2 males per 100 females, respectively. The analysis of physiological status of sandflies in outdoors and indoors revealed 61.2% and 71.1% unfed, 2.9% and 4.8% blood-fed, 26.7% and 17.7% semi-gravid, and 9.2% and 6.4% gravid, respectively. **Conclusion:** *P. papatasi and P. alexandri* may play important as vectors of cutaneous leishmaniasis (CL) in Khuzestan Province, due to their high abundance and monthly prevalence in regions affected with CL.

Keywords: Ecology, fauna, Iran, leishmaniasis, Phlebotominae

Introduction

Leishmaniasis is a parasitic zoonotic disease, caused by various *Leishmania* species, with different clinical manifestations. The pathogen is a member of the Mastigophora class, Trypanosomatidae family, and the genus *Leishmania* and transmitted to healthy hosts by females of Phlebotomine sandflies during blood feeding from animal and human reservoirs.[1,2] The sandflies are known as the vectors of different types of leishmaniasis (visceral, cutaneous, and mucocutaneous) across the world. In the old world, the visceral leishmaniasis (VL) vectors mainly included the *Adlerius*, *Larrossius*, and *Euphlebotomus* subgenera and the cutaneous leishmaniasis (CL) vectors included the *Phlebotomus* and *Paraphlebotomus* subgenera.[3] However, a species of *Leishmania* found in Australia causes cutaneous lesions in kangaroo and is transmitted by day-biting midges.[4]

In many parts of the world, including Iran, leishmaniasis is a major health issue with socioeconomic importance. As a result, it is ranked as the top six important diseases in tropical and

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subtropical regions by the World Health Organization.[3,5] The disease has been reported in 88 countries in all five continents. A total of 14 million people are affected by different types of leishmaniasis throughout the world. Globally, the incidence of leishmaniasis is 2 million each year with 350 million at risk of acquiring the disease. Of 2 million new cases, 1.5 million have CL and 0.5 million have VL. The majority of CL cases (90%) occur in Syria, Afghanistan, Iraq, Saudi Arabia, Algeria, Brazil, Peru, and Iran. The majority of VL cases (90%) occur in Bangladesh, Nepal, India, Sudan, and Brazil. In addition, about 90% of mucocutaneous leishmaniasis cases occur in Brazil, Peru, and Bolivia.[6,7]

Leishmaniasis is the second important parasitic disease, next to malaria, transmitted by vectors in Iran.[8] Iran is one of the most important foci of CL in the world with the rural CL (wet sore) caused by *Leishmania major* and the urban CL (dry sore) caused by *Leishmania tropica*. Different species of Gerbillinae subfamily and human are the reservoirs of rural and urban CLs, respectively, and *Phlebotomus papatasi*, Scopoli, 1786, and *Phlebotomus sergenti*, Parrot, 1917, are their major vectors. Dogs are the minor reservoir of urban CL, and it can transmit from humans to humans, respectively.[8,9]

A study in Iran reported 54 species of sandflies.[10] Another study reported 44 species of sandflies in Iran (26 *Phlebotomus* species and 18 *Sergentomyia* species).[11] Between 1968 and 1971, 21 species of sandflies (11 *Phlebotomus* species and 10 *Sergentomyia* species) were identified in the coastal, plain, and mountainous regions of Khuzestan Province, Southwestern Iran.[12] Another study (1962) in Khuzestan Province (Shush, Dezful, Abadan, Ahvaz, and Izeh) reported a total of 20 species of sandflies, including 10 *Phlebotomus* species and 10 *Sergentomyia* species.[13] A study (1975) conducted in Ahvaz and Dezful added more sandfly species, namely *Sergentomyia clydei*, Sinton, 1927, and *Sergentomyia pawlowskyi*, Perfiliew, 1933, to its fauna in Khuzestan Province.[14] A study in 2009 reported three sandfly species in Shadegan and Hoveyzeh wetlands in Khuzestan Province.[15] A study (2012) in Shush and Khorramshahr Counties in Khuzestan Province reported 11 sandflies species.[16] In a study in Sistan- Baluchistan Province in 1997 and according to a published paper in 2020 about the check-list of Phlebotomine sandflies in Iran[10], Kassiri *et al*. reported *P. kabulensis* (Artemiev, 1978), *P. salengensis* (Artemiev, 1978), *P. similis* (Perfiliew, 1963), *S. indica* (Theodor, 1931), *S.dreyfussi turkestanica* (Theodor and Mesghali , 1964), and *S. (Rondanomyia)* sp. for the first time in Iran. Studies in the northwest provinces of Iran showed the presence of 27 sandfly species including 18 *Phlebotomus* species and 9 *Sergentomyia* species.[17] A faunistic study in Rofayyeh in Khuzestan Province collected and identified five species of phlebotomine sandflies.[18] A study in Asalouyeh (Bushehr Province, southern Iran) reported a total of five sandfly species (4 *Phlebotomus* species and 1 *Sergentomyia* species).[19] The prevalence of CL in different provinces has been estimated between 1.8% and 37.9%. The highest prevalence of CL has been observed in Bushehr, Khorasan, Yazd, Fars, Khuzestan, Ilam, and Isfahan, with the mean incidence rate of 166 cases per 100,000 populations. In contrast, Western provinces had the lowest incidence rate with 10 cases per 100,000 populations.[20] A molecular study on 100 CL cases in Ahvaz County, Southwestern Iran, showed that 93% were affected by *L. major* and 3% by *L. tropica*.[21] Natural leptomonad infection was observed in *P. papatasi*; *Phlebotomus alexandri*, Sinton, 1928; and *Sergentomyia sintoni*, Pringle, 1953, from rodent burrows in Ahvaz, Shush, and Shushtar Counties of Khuzestan Province.[22] A study by Coleman *et al.* (2003–2004) in Iraq used a light trap to collect sandflies and reported three predominant species, namely *P. papatasi*, *P. sergenti*, and *P. alexandri*; in addition, *P. papatasi* was introduced as the major CL vector.[23]

This study was conducted to determine the species composition, frequency, monthly population variations, sex ratio, sex percentage, and abdominal status of phlebotomine sandflies in indoor and outdoor places in Abadan County in order to use its findings in control programs against the CL vectors.

Materials and Methods

The study was approved by the Committee of Ethics in Research, Ahvaz Jundishapur University of Medical Sciences, and registered as IR.AJUMS.REC.1395.205.

This descriptive cross-sectional study was conducted to determine the bioecology of phlebotomine sandflies in Abadan County, Khuzestan Province. Abadan is located in Southwest Iran (longitude: 48°17’ and latitude: 30°20’) and covers an area of 2796 km^2.

Sandflies were caught using the sticky traps soaked with castor oil. The sandflies were removed from the traps using the entomological needle and placed in an acetone-containing glass container for cleaning the oil. Then, the sandflies were placed in glass vials containing 70% alcohol and labeled with the village name, catching date, collector name, and habitat (indoors or outdoors). First, the desired pathways and villages were selected by drawing a detailed and accurate map of the region and consulting with the specialists in the Health Medical Services Center of Abadan County. Since it is a CL-endemic region, ten villages with different geographical positions and higher prevalence of CL were selected for catching sandflies: Albuebadi, Sadat, Seydaviyeh, Fayyaziyeh, Shalheh Haj Hossein, Tangeh, and Tarreh Bakhakh (from the central district), Sadoni and Nahr-e Hamid (from the Arvandkenar district), and Chauhbedeh (from the Chauhbedeh district), as well as the Abadan City.

Sampling was repeated three times in each village and city of Abadan in each season. Three houses were selected in each village and ten sticky traps were set up in each house. One house was selected from the marginal area of the village, one in the center, and one between the marginal and central areas. It was tried to select those houses which had a place for keeping livestock and poultry (sheep, cow, goat, chicken, rooster, and pigeon). The

traps were placed in bedrooms, living rooms, places for keeping livestock and poultry, storerooms, straw stores, corridors, toilets, and bathrooms. In addition, 30 sticky traps were placed in outdoors, including rodent's burrows, ruined places, mud holes, cracks in garden walls, cracks in house walls, wild animal dens, rock cracks, rock masses, riversides, and irrigation canals. In each sampling stage, 30 sticky traps were placed in indoors and 30 sticky traps in outdoors. Sandflies then were removed from the 70% ethyl alcohol and dried by contact with filter paper. Afterward, the head of sandflies separated from the body on slides. Puri's medium and lactophenol used for permanent mounting and temporary mounting, respectively. The prepared slides were dried out for 1 week on wooden rails at room temperature. After this period, the body of sandflies became completely transparent. Sandfly species was determined under a microscope by investigating male genitalia, spermatheca, pharyngeal armature, buccal armature, and other important morphological features, based on the diagnostic keys of sandflies.[24,25] Data related to sandflies were inputted into SPSS (IBM SPSS software/ Singapore, version 22.0) and presented in descriptive tables, and their diagrams were drawn.

Results

In total, 6173 sandflies were collected from indoor and outdoor places by sticky traps, and their species were determined. Of these sandflies, 1953 sandflies (31.6%) were collected from indoor places and 4220 sandflies (68.4%) from outdoor places. In total, 4749 sandflies (76.9%) were *Phlebotomus*, Rondani and Bert, 1980, and 23.1% were *Sergentomyia*, Franca and Parrot, 1920. This study identified 13 sandfly species: *P. papatasi*, Scopoli, 1786 (45.64%); *P. alexandri*, Sinton, 1928 (31.31%); *S. sintoni*, Pringle, 1953 (15.9%); *Sergentomyia dentata*, Sinton, 1933 (5%); *Sergentomyia baghdadis*, Adler and Theodor, 1929 (0.65%); *Sergentomyia tiberiadis*, Adler, Theodor, and Lourie, 1930 (0.54%); *Sergentomyia iranica*, Lewis and Mesghali, 1961 (0.3%); *Sergentomyia theodori*, Parrot, 1942 (0.24%); *S. clydei*, Sinton, 1928 (0.15%); *Sergentomyia antennata*, Newstead, 1912 (0.11%); *Sergentomyia mervinae*, Pringle, 1953 (0.08%); *Sergentomyia squamipleuris*, Grassomyia, 1942 (0.05%); and *Sergentomyia christophersi*, Sinton, 1927 (0.03%) [Table 1].

S. iranica, *S. theodori*, *S. clydei*, *S. antennata*, *S. mervinae*, *S. squamipleuris*, and *S. christophersi* were captured only in outdoor places. *P. papatasi*, *P. alexandri*, *S. sintoni*, *S. dentata*, *S. baghdadis*, and *S. tiberiadis* were collected from both indoor and outdoor places [Tables 2 and 3]. Abundance of phlebotomine sandfly species of human dwellings was as follows: *P. papatasi* (50%), *P. alexandri* (41.2%), *S. sintoni* (7.6%), *S. dentata* (0.6%), *S. baghdadis* (0.3%), and *S. tiberiadis* (0.3%). Meanwhile, abundance of phlebotomine sandfly species of stables was as follows: *P. papatasi* (52.9%), *P. alexandri* (40.4%), *S. sintoni* (5.2%), *S. dentata* (0.8%), *S. baghdadis* (0.5%), and *S. tiberiadis* (0.2%). *P. papatasi* and *P. alexandri* accounted for the majority of sandflies in human habitats and stables. With respect to abdominal status, the majority of captured sandflies collected from indoor and outdoor places had an unfed stomach. Abdominal status of phlebotomine sandflies of indoor places was as follows: empty (71.1%), semi-gravid (17.7%), gravid (6.4%), and blood-fed (4.8%). Meantime, abdominal status of phlebotomine sandflies of outdoor places was as follows: empty (61.2%), semi-gravid (26.7%), gravid (9.2%), and blood-fed (2.9%).

In total, 4405 sandflies (71.4%) were male and 1768 sandflies (28.6%) were female. Moreover, 78.4% of *Phlebotomus* species were male and 21.6% were female [Table 1]. On the other hand, 48% and 52% of *Sergentomyia* species were male and female, respectively. In general, the majority of sandflies were male. Studies on the determination of sex percent and sex ratio (number of males per 100 females) showed that the highest of *P. papatasi, P. alexandri, S. dentata, S. tiberiadis, S. iranica, S. theodori, S. clydei, S. antennata, S. mervinae,* and *S. christophersi* were

Table 1: Total abundance of phlebotomine sandflies species of Abadan County

Species	Males, *n* (%)	Females, *n* (%)	Frequency of *Phlebotomus* or *Sergentomyia* population, *n* (%)	Frequency of total sandfly population (%)
Phlebotomus papatasi	2162 (76.8)	654 (23.2)	2816 (59.3)	45.64
Phlebotomus alexandri	1560 (80.7)	373 (19.3)	1933 (40.7)	31.31
Sergentomyia sintoni	432 (44)	550 (56)	982 (69)	15.9
Sergentomyia dentata	173 (56)	136 (44)	309 (21.7)	5
Sergentomyia baghdadis	9 (22.5)	31 (77.5)	40 (2.8)	0.65
Sergentomyia tiberiadis	25 (73.5)	9 (26.5)	34 (2.4)	0.54
Sergentomyia iranica	14 (77.8)	4 (22.2)	18 (1.3)	0.3
Sergentomyia theodori	12 (80)	3 (20)	15 (1)	0.24
Sergentomyia clydei	6 (66.7)	3 (33.3)	9 (0.7)	0.15
Sergentomyia antennata	6 (85.7)	1 (14.3)	7 (0.5)	0.11
Sergentomyia mervinae	4 (80)	1 (20)	5 (0.3)	0.08
Sergentomyia squamipleuris	-	3 (100)	3 (0.2)	0.05
Sergentomyia christophersi	2 (100)	-	2 (0.1)	0.03
Total	4405	1768	6173	100

Table 2: Abundance of phlebotomine sandflies species of outdoor places in Abadan County

Species	Males, *n* (%)	Females, *n* (%)	Frequency of *Phlebotomus* or *Sergentomyia* population, *n* (%)	Frequency of total sandfly population (%)
Phlebotomus papatasi	1323 (73.8)	470 (26.2)	1793 (61.1)	42.5
Phlebotomus alexandri	859 (75.3)	282 (24.7)	1141 (38.9)	27
Sergentomyia sintoni	398 (45.6)	475 (54.4)	873 (67.9)	20.7
Sergentomyia dentata	163 (55.4)	131 (44.6)	294 (22.9)	7
Sergentomyia baghdadis	7 (22.6)	24 (77.4)	31 (2.4)	0.7
Sergentomyia tiberiadis	21 (72.4)	8 (27.6)	29 (2.2)	0.7
Sergentomyia iranica	14 (77.8)	4 (22.2)	18 (1.4)	0.4
Sergentomyia theodori	12 (80)	3 (20)	15 (1.2)	0.35
Sergentomyia clydei	6 (66.7)	3 (33.3)	9 (0.7)	0.22
Sergentomyia antennata	6 (85.7)	1 (14.3)	7 (0.54)	0.17
Sergentomyia mervinae	1 (20)	4 (80)	5 (0.4)	0.13
Sergentomyia squamipleuris	-	3 (100)	3 (0.22)	0.08
Sergentomyia christophersi	2 (100)	-	2 (0.14)	0.05
Total	2812	1408	4220	100

Table 3: Abundance of phlebotomine sandflies species of indoor places in Abadan County

Species	Males, *n* (%)	Females, *n* (%)	Frequency of *Phlebotomus* population, *n* (%)	Frequency of total sandfly population (%)
Phlebotomus papatasi	839 (82)	184 (18)	1023 (56.4)	52.4
Phlebotomus alexandri	701 (88.5)	91 (11.5)	792 (43.6)	40.6
Sergentomyia sintoni	34 (31.2)	75 (68.8)	109 (79)	5.6
Sergentomyia dentata	10 (66.7)	5 (33.3)	15 (10.9)	0.7
Sergentomyia baghdadis	2 (22.2)	7 (77.8)	9 (6.5)	0.4
Sergentomyia tiberiadis	4 (80)	1 (20)	5 (3.6)	0.3
Total	1590	363	1953	100

male, whereas the majority of *S. sintoni, S. baghdadis,* and *S. squamipleuris* species were female. The sex ratio of the sandflies of genus *Phlebotomus* and *Sergentomyia* was 362.4 and 92.2 males per 100 females, respectively. Furthermore, the sex ratio of phlebotomine species was 249.1. The sex ratio for *P. papatasi, P. alexandri, S. sintoni, S. dentata, S. baghdadis, S. tiberiadis, S. iranica, S. theodori, S. clydei, S. antennata, S. mervinae, S. squamipleuris,* and *S. christophersi* was 330.6, 418.2, 78.5, 127.2, 29, 277.8, 350, 400, 200, 600, 400, 0, and 200, respectively. The sandflies were more active in June and September in Abadan County [Figure 1].

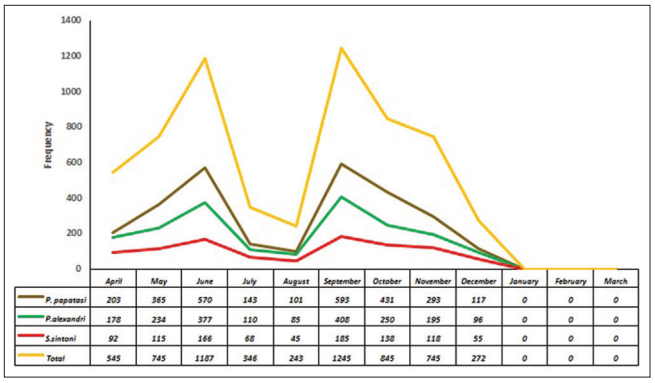

Figure 1: Monthly activity of phlebotomine sandflies in Abadan County

Discussion

In total, 6173 sandflies were collected from indoor and outdoor places to determine their bioecology and fauna in Abadan County. In this study, 13 sandfly species (2 *Phlebotomus* and 11 *Sergentomyia*) were collected and identified. Some of them, such as *P. papatasi* and *P. alexandri*, were introduced as potential vectors and known as proven leishmaniasis vectors in Iran and the world.[26,27] Moreover, *S. clydei, S. sintoni,* and *S. dentata* were reported as the vectors of lizard leishmaniasis.[28-30]

Our results showed that the study region is very rich in terms of sandfly species. In a study between 1968 and 1971in Abadan and Khorramshahr Counties, Javadian *et al.* reported 11 sandfly species, namely *P. papatasi, P. alexandri, S. sintoni, S. dentata, S. theodori, S. antennata, S. baghdadis, Sergentomyia palestinensis, S. iranica, S. squamipleuris,* and *S. mervinae*.[12] The present study reported four new sandfly records in Abadan: *S. clydei*, *S. antennata*, *S. christophersi*, and *S. tiberiadis*. However, *S. palestinensis*, reported in the past in this county, was not found among the collected species.

A study on sandflies in Rafsanjan County (Southeast Iran) reported the presence of *P. papatasi, P. sergenti, Phlebotomus*

mongoliensis, S. clydei, S. tiberiadis, S. sintoni, S. dentata, and *S. baghdadis*.[31] A study on the fauna of sandflies in Baft County (South Iran) reported the presence of *P. papatasi, P. alexandri, P. sergenti, Phlebotomus major, Phlebotomus halepensis, P. mongoliensis, Phlebotomus elenorae, Phlebotomus caucasicus, S. sintoni, S. theodori, Sergentomyia africana, S. baghdadis, S. dentata, Sergentomyia sumbarica, S. mervinae,* and *S. tiberiadis*.[32]

In the present study, *P. papatasi, P. alexandri,* and *S. sintoni* accounted for the majority of captured species with an abundance of 45.64%, 31.31%, and 14.3%, respectively. Different studies have shown that *P. papatasi* has a major role in transmission of rural CL. This sandfly was observed domestically in Iran and the whole Palearctic region and even in a large region of Ethiopia (Sub-Saharan Africa) and the Oriental region (the Indian subcontinent). It can be said that no other sandfly species so far could adapt itself to reproduce in the range of human dwellings.[33]

Among the important findings of this study was the relatively high abundance of *P. alexandri* in Abadan. This species has been reported as the proven VL vector in some countries, such as China.[34] Moreover, infection of *P. alexandri* with *Leishmania infantum* (the causative agent of Mediterranean VL) has been proven.[35] In the present study, *S. sintoni, S. dentata,* and *S. clydei* accounted for the majority of captured sandflies with a relative frequency of 15.9%, 5%, and 0.15%, respectively. The natural leptomonad infection was observed from these three species in Iran.[30]

In this study, 11 sandfly species were captured in outdoor places and only 6 species were captured in indoor places. A broader variety of sandfly species was observed in outdoor places than indoor places. The most frequent indoor species were *P. papatasi* (52.4%), *P. alexandri* (40.6%), and *S. sintoni* (5.6%). Moreover, the most frequent outdoor species were *P. papatasi* (42.5%), *P. alexandri* (27%), and *S. sintoni* (20.7%). Jahanifard *et al.* reported 6 species in indoors and 7 species in outdoors in Khorramshahr County, Khuzestan Province, southwestern Iran.[16]

The sex frequency results showed that 71.4% of the captured phlebotomine species in Abadan were male and 28.6% were female. The sex ratio of phlebotomine species (the number of males compared to 100 females) was 249.1. The obtained sex ratios for *P. papatasi, P. alexandri,* and *S. sintoni* were 330.6, 418.2, and 78.5, respectively. The sex ratio of captured indoor and outdoor phlebotomine species was 438 and 50.1, respectively. The sex ratio studies showed the domination of male sandflies.[36,37]

Conclusion

According to the results, the sandfly vector species of CL (*P. papatasi* and *P. alexandri*) were highly frequent in Abadan; in addition, since it is close to CL foci in Iraq and Iran, regional authorities are recommended to prevent the formation of new foci through taking appropriate measures.

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Conflicts of interest

There are no conflicts of interest.

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