Original Article

Bionomics of Phlebotomine Sand Flies in Different Climates of Leishmaniasis in Fars Province, Southern Iran

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Abstract

Background: *Phlebotomus* and *Sergentomya* are distributed in the old-world regions and transmit leishmaniases through mammalian and reptile hosts. Cutaneous leishmaniasis (CL) is one of the most important diseases in Iran. Iranian sand flies belong to three Oriental, Palearctic, and Afrotropical Regions. Fars Province is located in Palearctic, but southern parts are affected Oriental region situations on phglebotomine population variety. Therefore, a comprehensive study was required on the vectors of the disease in this Province.

Methods: Regarding the approved role of the environmental factors in creating the fauna and distribution of living creatures, the "de martonne climate method" was used, and the climate was noticed as an important environmental factor for the determination of vector distribution. Accordingly, 14 sampling sites were selected from 10 foci in different climates of Fars Province. 19648 sand flies were collected from the studied areas in this Province during 2016.

Results: *Phlebotomus papatasi* and *Se. antennata* were the most frequent species, which were caught from in/outdoor areas. *Phlebotomus sergenti* and *Ph. alexandri* were caught from both Palearctic and Oriental zones but were more prevalent in the cold semi-arid climate of the Palearctic zone. Moreover, they were not caught from the hot desert and summer Mediterranean climates of the Oriental zone.

Conclusions: It seems that *Ph. papatasi* as the main vector of CL could be well distributed in different climates in Fars. Moreover, some species like *Ph. sergenti* preferred especial climates in the Palearctic zone. Therefore, these data could be helpful to control leishmaniases more efficiently.

Keywords: Cutaneous leishmaniasis; Sand flies; *Phlebotomus papatasi*; Geographic Information System; Iran

Introduction

Leishmaniases, as one of the most common group of vector-borne diseases, are transmitted to humans through female sand flies' bites infected with *Leishmania* parasites in the world (1). There are more than 3,000 described Psychodid species distributed among six subfamilies of Phlebotominae, Bruchomyiinae, Sycoracinae, Trichomyiinae, Psychodinae, and Horaiellinae in the world (2). The last one, with its sole genus *Horaiella* and its four species, is restricted to the Himalayas, China, and Thailand (1). In addition, there are about 1000 species of phlebotominae sand flies in the world, of which 98 species are identified as the proven or suspected vectors of human leishmaniases (4). Sand flies have different breeding places in tropical and subtropical areas and their variations directly affect the prevalence, incidence, and transmission rates of the disease in different areas (5).

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Phlebotomus and *Sergentomya* (Diptera: Psychodidae) are distributed in the Old-World regions and transmit the parasite through humans, different mammalian, and reptile hosts (6). They have a wide range of mammalian hosts, such as canines, rodents, cats, hyrax, and humans. Consequently, human leishmaniases have different zoonotic and anthroponotic transmission patterns (7). Phlebotomine sand flies usually have different ecological habits and may tend to rest in or outdoors of residential places. Some sand fly species prefer to live within active rodents' burrows across plain or mountainous regions (8).

Cutaneous leishmaniasis (CL) is endemic in Iran and reported from different regions of the country (9). The study of Iranian sand flies began in the early years of this century, mainly by researchers such as Adler, Theodore, and Laurie. The first comprehensive study was carried out by Mesghali in 1961, which reported 12 species of *Phlebotomus* and 11 species of *Sergentomyia* from Iran (10). In continue, Javadian and Mesghali reported a total of 42 species in the country during 1975. Thereafter, 54 species of sand flies were reported from different regions of the country. Accordingly, 48 species were finally confirmed from two genera of *Phlebotomus* and *Sergentomyia* based on the studies carried out since 1930 (11). Species, such as *Ph. caucasicus*, *Ph. mongolensis*, *Ph. kazeruni*, *Ph. brevis*, *Ph. adlerius*, *Ph. papatasi*, *Ph. sergenti*, *Ph. alexandri*, *Se. sogdiana*, *Se. hodegson*, *Se. grekovi*, *Se. hodgsoni*, *Se. tiberiadis*, *Se. sintoni*, *Se. clydei*, *Se. dentata*, and *Se. antennata* have been usually reported from the northern zones of Iran. On the other hand, some species, such as *Ph. ansarii*, *Ph. longiductus*, *Ph. papatasi*, *Ph. sergenti*, *Ph. alexandri*, *Ph. bergeroti*, *Ph. major*, *Ph. mongolensis*, *Se. sintoni*, *Se. baghdadis*, *Se. theodori*, *Se. antennata*, *Se. clydei*, *Se. squamipleuris*, *Se. dentata*, and *Se. mervynae* have been more reported from the southern zones of the country including Fars Province. In this regard, *Ph. papatasi* and *Se. sintoni* species were reported as the dominant sand flies' species (9). *Phlebotomus papatasi* and *Ph. sergenti* have been reported as the suspected or proven vectors of CL. Moreover, *Ph. kandelakii*, *Ph. perfiliewi*, *Ph. sergenti*, *Ph. papatasi*, *Ph. ansarii*, *Ph. salehi*, and *Ph. caucasicus* have been reported as the suspected or proven vectors of visceral leishmaniasis (VL) in Iran (12, 13). Additionally, *Ph. papatasi*, *Ph. keshishiani*, *Ph. alexandri*, and *Ph. major* have been reported as the vectors of CL and VL in southern Provinces of the country including Fars and Bushehr (14).

The Palearctic zone covers Eurasia, including Europe, Asia north of the Oriental Region, and northern Africa, and. The Oriental Region includes eastern Iran, China south of the Yangtze River, India and Sri Lanka, Southeast of Asia, the Philippines, the East Indies and Indo-Malayan Archipelago, and the large island regions of Taiwan (15). Iranian sand flies belong to three Oriental, Palearctic, and Afrotropical Regions (16). Environmental factors, such as the mean temperature of the wettest quarters, play an important role in sand flies' distribution (especially *Ph. papatasi*) in Iran and can be reported in the regions where biological situations are suitable (17). In fact, such factors as appropriate temperature and relative humidity are very important for their survival in different areas of the country (18). Moreover, rainfall, altitude from the sea level, land cover type, annual mean or minimum temperature in the coldest months, and mean temperature of the driest or wettest quarters have been reported to be effective in the distribution of different sand flies' species (19). Changes in the environment cause variations in the transmission pattern of communicable diseases. The consciousness of the relations between the environment changes and the incidence of vector-borne diseases can be helpful in planning effective control strategies (20, 21).

Due to the high incidence of leishmaniasis in Fars Province, a comprehensive study was required on the vectors of the disease in this Province. The precise control of cutaneous and/

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or visceral leishmaniasis helps to identify endemic vectors as well as reservoir hosts of the disease. The present study was designed to explore the fauna and the bio-ecology of sand flies in Fars Province, southern Iran, during 2016.

Materials and Methods

Study area

Fars Province includes 23 counties with an area of 122400km² in south of Iran (22). Shiraz as the capital city of the province is situated at 29° 59' 18" North, 52° 58' 37" East and about 1200m above the sea level. Recently, Fars has been the most important foci of CL in southern Iran. This Province has many different climates, but the most prevalent ones are cold semi-arid climate (in Shiraz, Kharameh, Marvdasht, Darian, and Sarvestan), hot desert climate (in Larestan, Zarin-Dasht, Hajiabad, Qir, Banaruiyeh, and Shahr-e Pir), hot semi-arid climate (in Farashband, Zahedshahr, Now Bandegan, Duzeh, Sheshdeh, and Sahrarud), hot-summer Mediterranean climate (in khan-e-Zenyan and Kazerun), cold desert climate (in Mehrdasht), and hot humid continental climate in southern parts. Shiraz has been considered to have a local steppe climate. The average annual temperature is 16.8 °C and there is little annual rainfall (the average rainfall is equal to 316mm) in Shiraz. Recently, Shiraz, Marvdasht, and Kharameh have been the most important foci of CL in Fars Province, southern Iran (Fig. 1).

Selection of Villages

Considering the approved role of the environmental factors in creating the fauna and distribution of living creatures, the "de martonne climate method" was used (23, 24), and climate was noticed as a key factor for determination of vector distribution affected by atmospheric precipitation, elevation, and vegetation. Accordingly, 14 sampling sites were selected from 10 foci in plain and mountainous areas regarding the contribution of different climates. Among these foci, eight were in endemic areas of ZCL and six belonged to non-endemic foci (Table 1).

Sampling

Phlebotomine sand flies were caught using sticky paper traps and aspirating tube from Zafar-Abad, Mahmood-Abad, Ahmad-Abad, Koh-Sabz, Tole-Mahtabi, Bahman, Deh-Dagh, and Khsoyeh villages from the Palearctic zones and Eslam-Abad, Bid-Karz, Baba-Monir, Hossein-Abad, Deh-Mian, and Ali-Abad from the climate zone of Oriental in Fars Province every month during 2016. Sand flies were sampled from indoors, such as bedrooms and bathrooms, and outdoors, such as rocks, rodent burrows, agricultural lands, and wall gaps. In each sampling round, 60 sticky paper traps (30 indoors and 30 outdoors) were fixed in the sunset and collected in the next morning before sunrise. Phlebotomine sand flies were collected, kept in ethanol (70%), mounted in the Puri's media, and taxonomically identified according to valid taxonomic criteria studies (25).

Pearson's correlation coefficient was used to find the relationship between the disease incidence rate which was reported from the Iranian Control Disease Center and the number of collected *P. papatasi* as the main vector of ZCL from different studied villages in 2016. The data were analyzed using the Arc Explorer software. This application is included three extensions of 3D Analyst, Spatial Analyst, and GeoStatistical Analyst. At first, topology data will be added to the geodatabase part, which is a feature originally available only with ArcInfo coverages. Then, these three extensions facilitate the ability to access data online, directly from the Geography Network site or other ArcIMS map services (26).

Results

In this study, a total of 19648 sand flies were collected from different villages of the studied areas in Fars Province during 2016.

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Among the collected sand flies, 11778 specimens (59.95%) were male and 7870 (40.05%) were female. In addition, 14030 (71.41%) and 5618 (28.59%) specimens were identified as the various species of *Phlebotomus* and *Sergentomyia*, respectively.

Phlebotomus species included 10654 *Ph. papatasi* (54.22%), 2052 *Ph. sergenti* (10.44%), 672 *Ph. alexandri* (3.42%), 548 *Ph. bergeroti* (2.79%), 80 *Ph. major* (0.41%), 10 *Ph. keshishiani* (0.05%), 8 *Ph. tobbi* (0.04%), and 6 *Ph. salehi* (0.03%). The most dominant species was *Ph. papatasi* caught from both indoor and outdoor places of all studied foci. On the other hand, among the 5618 collected specimens of *Sergentomyia*, 2250 were *Se. antennata* (11.45%), 1071 were *Se. sintoni* (5.45%), 884 were *Se. baghdadis* (4.5%), 662 were *Se. clydei* (3.37%), 473 were *Se. theodori* (2.41%), 118 were *Se. squamipleuris* (0.60%), 106 were *Se. mervynae* (0.54%), 47 were *Se. dentata* (0.24%), and 7 were *Se. palestinensis* (0.04). Besides, *Se. antennata* was the most frequent species caught from in/outdoors (Table 2).

In the Palearctic zone, all above-mentioned *Phlebotomus* species were caught, except for *Ph. keshishiani* that was only collected from Bid-Karz village in Mamasani focus in the Oriental zone (Fig. 2). Besides, among the collected *Sergentmyia* species, *Se. palestinensis* was not caught from the Palearctic zone and was only recorded from Eslam-Abad village of Kazerun in the Oriental climate region. In the current study, no *Ph. major*, *Ph. tobbi*, and *Ph. salehi* species were caught in the studied villages of the Oriental zone (Fig. 2). Indeed, the first two species were merely recorded from Koh-Sabz from Marvdasht focus and the last one was only collected from Tole-Mahtabi from Neyriz in the Palearctic zone (Table 2).

The results revealed significant variation ($p< 0.001$) between the incidence rates of CL reported from the Iranian Control Disease Center and the number of caught *Ph. papatasi* in different endemic and non-endemic foci of Fars Province during 2016. But it does not indicate a positive or negative relationship between CL cases and the number of *Ph. papatasi* sand flies caught in different foci. For example, in Niriz, where the number of CL cases was higher, the number of *Ph. papatasi* sand flies caught was less than in Shiraz with fewer CL cases (Table 3).

Table 1. The geographic coordinates of the studied sites

Climate Zone	Climate type	Topographical type	Foci	Village	X_long altitude	Y_lat altitude	ZCL cases
Palearctic	Cold semi-arid	Plain	Shiraz	Zafar-Abad	653501.1	3254461	864
		Mountainous	Shiraz	Mahmood-Abad	659075.2	3249343	
		Plain	Kharameh	Ahmad-Abad	708846.3	3259576	224
		Mountainous	Marvdasht	Koh-Sabz	664812.1	3310268	901
		Mountainous	Neyriz	Tole-Mahtabi	823232.7	3237904	95
		Plain	Abadeh	Bahman	641681.6	3450615	29
		Plain	Abadeh	Deh-Dagh	662026	3445878	
	Hot desert	Plain	Zarin-Dasht	Khosoyeh	831592.2	3161876	135
Oriental	Hot desert	Mountainous	Larestan	Deh-Mian	803992.9	3048333	240
		Plain	Larestan	Ali-Abad	864440.2	3067707	
	Hot semi-arid	Plain	Farashband	Hossein-Abad	612280.1	3186581	69
	Hot-summer Mediterranean	Plain	Kazerun	Eslam-Abad	596084.8	3244258	44
		Mountainous	Mamasani	Bid-Karz	501180.5	3311488	11
		Plain	Mamasani	Baba-Monir	519969	3326872	

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Table 2. Sand fly species collected from different zone and climate regions of Fars Province during 2016

Zones	Climates	Foci	Villages	No. of collected Phlebotomine sand flies																	
				Phlebotomus papatasi	*Phlebotomus sergenti*	*Phlebotomus alexandri*	*Phlebotomus bergeroti*	*Phlebotomus major*	*Phlebotomus keshishiani*	*Phlebotomus tobbi*	*Phlebotomus salehi*	*Sergentomyia antenata*	*Sergentomyia sintoni*	*Sergentomyia clydei*	*Sergentomyia dentata*	*Sergentomyia mervynae*	*Sergentomyia theodori*	*Sergentomyia baghdadis*	*Sergentomyia squamipleuris*	*Sergentomyia palestinensis*	Total
Palearctic	Cold semi-arid	A	a	1473	654	480	0	0	0	0	0	0	88	0	9	9	213	492	0	0	3418
		B	b	652	321	0	0	0	0	0	0	0	53	0	7	8	92	161	0	0	1294
		C	c	828	99	29	164	0	0	0	0	320	24	25	0	0	21	0	19	0	1529
		D	d	1839	217	0	112	80	0	8	0	78	0	0	0	60	8	0	0	0	2402
		E	e	1818	0	0	102	0	0	0	6	492	85	0	0	0	6	0	0	0	2509
			f	19	78	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	97
			g	9	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11
		F		1038	106	0	24	0	0	0	0	536	364	344	0	7	64	0	0	0	2483
Subtotal																					**13743**
The Oriental	HD	G	i	428	0	0	51	0	0	0	0	337	369	144	0	0	49	0	0	0	1341
			j	763	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	800
	**HSA	H	k	275	0	57	0	0	0	0	0	60	0	36	31	0	7	91	51	0	608
	***HSM	I	**l**	864	0	0	92	0	0	0	0	95	0	27	0	0	0	140	44	7	1269
		J	**m**	2	249	106	3	0	10	0	0	332	76	81	0	22	13	0	4	0	898
			n	646	326	0	0	0	0	0	0	0	12	5	0	0	0	0	0	0	989
Subtotal																					**5905**
Total				10654	2052	672	548	80	10	8	6	2250	1071	662	47	106	473	884	118	7	19648
%				54.22	10.44	3.42	2.79	0.41	0.05	0.04	0.03	11.45	5.45	3.37	0.24	0.54	2.41	4.5	0.6	0.04	100

A, Shiraz; B, Kharameh; C, Marvdasht; D, Neyriz; E, Abadeh; F, Zarin-Dasht; G, Larestan; H, Farashband; I, Kazerun; J, Mamasani

a, Zafar-Abad; b, Mahmood-Abad; c, Ahmad-Abad; d, Koh-Sabz; e, Tole-Mahtabi; f, Bahman; g, Deh-Dagh; h, Kho-soyeh; i, Deh-Mian; j, Ali-Abad; k, Hossein-Abad; l, Eslam-Abad; m, Bid-Karz; n, Baba-Monir. *Hot desert; ** Hot semi-arid; ***Hot summer Mediterranean

Table 3. Comparison of the number of collected sand flies and incidence rates of CL in different endemic and non-endemic foci of CL in Fars Province during 2016

Foci	CL cases	Incidence per 100000	No. of caught *Phlebotomus papatasi*	P value
Marvdasht	901	150.1	1839	
Shiraz	864	57.6	2125	
Kharameh	224	373.3	828	
Neyriz	95	135.7	1818	
Zarin-Dasht	135	184.4	1038	
Abadeh	29	49.1	28	<0.001
Mamasani	11	9.4	648	
Larestan	240	386.8	1191	

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Table 3. Continued ...

Kazerun	44	45.5	864
Farashband	69	339.6	275
Total	2612	94.9	10654

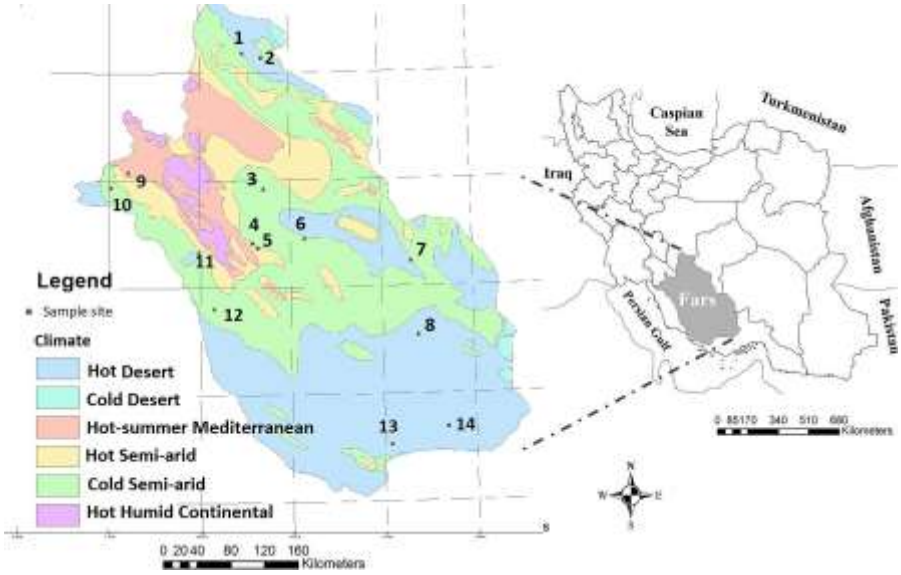

Fig. 1. Map of Iran showing the sample sites in different locations of Fars Province, southern Iran during 2016; Abadeh (Nos. 1 and 2), Marvdasht (No. 3), Shiraz (Nos. 4 and 5), Kharameh (No. 6), Neyriz (No. 7), Farashband (No. 8), Mamasani (Nos. 9 and 10), Kazerun (Nos. 11 and 12), and Larestan (Nos. 13 and 14)

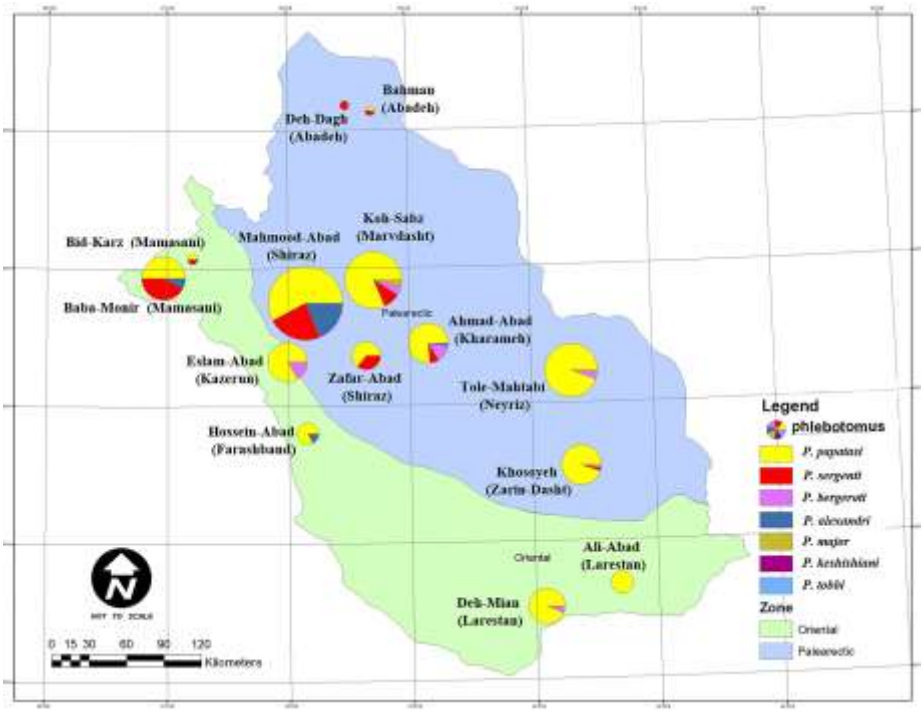

Fig. 2. Map of the geographic distribution of *Phlebotomus* species in the Palearctic and influenced Oriental zones of Fars Province during 2016

Discussion

In this study, phlebotomines were caught from various climates in the Palearctic and affected zones of Oriental in Fars Province. Among the 19648 sand flies caught, 14030 (71.41%) and 5618 (28.59%) specimens were identified as various species of *Phlebotomus* and *Sergentomyia*, respectively. *Phlebotomus papatasi* was the most frequent species and was collected from all in/outdoors of people's residual places in both Palearctic and affected zones of Oriental in Fars Province. Indeed, wall gaps of old rooms and agricultural lands were the most frequent breeding places of these species. Resting sites of this species is naturally infected with *L. major* and reported from different parts of Iran, including Fars Province (27).

The high biodiversity of the Iranian fauna is the result of its area and the influences of four ecozones from Palaearctic and old periodical connections with the Nearctic by Bering Strait in north, Afrotropical from the Arabian Peninsula in south, and Oriental in southeast includes Fars (28, 29). Fars Province includes a wide variety of sand fly fauna. Species such as *Ph. papatasi, Ph. alexandri, Ph. keshishiani*, and *Ph. major* were formerly reported as the vectors of ZCL and VL in this province. But *Ph. papatasi* has been noticed as the main proven vector of ZCL in all endemic foci of Fars. Also, this species was the main frequent species (54.22%) in the current study in both geographical zones. In a similar study conducted in southern foci of this Province in Palearctic zone, it seems that relative humidity was the main environmental factor affecting phlebotomine activity and spatial distribution. Accordingly, their activity increased significantly when the average humidity was induced to more than 10% and reduced rapidly when the average humidity average was over 50% (30). These findings are in contract with those who believe that low humidity which followed by low wind speed and high temperature were the main factors affecting phlebotomine activities (31). However, some other studies indicated that low wind velocity, light intensity, temperature, and low relative humidity (Maximum wind speed of 3m/s, humidity of 10%, and minimum temperature of 11 °C) were the main factors affecting their activity and distribution (32).

Softwares such as Geographic Information System (GIS) and Ecological Niche Models (ENMs) have been used to generate the distribution map for Phlebotomine sand flies to find effective environmental factors on these vectors on the prevalence of Leishmaniasis (21). Therefore, more research are needed about the seasonal variations and abiotic situations (cloud cover, lunar cycle, wind speed and so forth) in order to improve our knowledge of these epidemiologically important subjects.

However, species such as *Ph. sergenti* were formerly recorded as the sole proven vector of *Leishmania tropica* in Iran (33). Also, *Ph. sergenti* has been found positive with *Leishmania* parasite in Shiraz and some other important cities in the country (34). In addition, *Ph. alexandri* was previously recorded as the probable vector of *Leishmania infantum* in southern regions (35-37). In the current study, *Ph. sergenti* and *Ph. alexandri* were caught from both Palearctic and affected zones of Oriental in the Province, but were more prevalent in the cold semiarid climate of the Palearctic zone. Moreover, they were not caught from the selected sites of Larestan and Kazerun in hot desert and hot summer Mediterranean climates of the zones influenced by Oriental zone.

Phlebotomus major has been reported from northern and southern regions of Iran and has been naturally infected with *L. infantum* in Fars (38). However, in the current study, this species was only collected from Koh-Sabz village from Marvdasht in the Palearctic zone. The incidence and distribution of leishmaniasis are both influenced by environmental variables affecting the Phlebotomine sand flies (as vectors) and reservoirs populations and human behaviors (37). Due to the high incidence of leishmaniasis in Fars Province (39, 40), a comprehensive

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study was required on the vectors of the disease in this Province. The precise control of cutaneous and/or visceral leishmaniasis helps identify the endemic vectors as well as the reservoir hosts of the disease (40). Moreover, careful monitoring of environmental variables in relation to biology of vector and leishmaniasis is important for designing and implementation of control plans.

Conclusion

Phlebotomus papatasi as the main vector of CL could be well distributed in different climates in Fars. Moreover, some species like *Ph. sergenti* prefered especial climates in the Palearctic zone. It seems that environmental factors play an important role in distribution of phlebotomine sand flies and can be reported in the regions where biological situations are suitable. Therefore, the obtained data could be helpful to control leishmaniases more efficiently.

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Ethical considerations

Ethical approval for this study was obtained from the Ethics Committee at Shiraz University of Medical Sciences (Iran).

Conflict of interest statement

Authors declare that there is no conflict of interest.

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