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Spatial distribution of sand flies (Diptera: Psychodidae; *Larrousius* group), the vectors of visceral leishmaniasis in Northwest of IranEslam Moradi-Asl^{1,2}, Yavar Rassi²✉, Davoud Adham¹, Ahmad Ali Hanafi-Bojd²✉, Abedin Saghafipour³, Sayena Rafizadeh⁴¹Department of Public Health, School of Public Health, Ardabil University of Medical Sciences, Ardabil, Iran²Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran³Department of Public Health, Faculty of Health, Qom University of Medical Sciences, Qom, Iran⁴Ministry of Health, National Institute for Medical Research Development, Tehran, Iran

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ABSTRACT

Objective: To determine spatial distribution of sand flies (Diptera: Psychodidae; *Larrousius* group), the vectors of visceral leishmaniasis in Ardabil province, Northwest of Iran. **Methods:** Sand flies were collected using sticky traps from the 30 selected points in Ardabil province, during May–November 2017. The MaxEnt model in GIS software was used for modeling. **Results:** A total of 2 794 specimens of sand flies were collected, of which 33% were *Larrousius* subgenus sand flies. *Phlebotomus kandelakii* and *Phlebotomus wenyoni* were the highest and lowest collected species respectively. Based on the modeling, four areas in the province were identified with more than 70% probability of the presence of *Larrousius* group vectors which were at risk of visceral leishmaniasis disease transmission. **Conclusions:** The distribution of *Larrousius* subgenus sand flies was observed in all parts of Ardabil. But the northern parts of the province (Germi and Bilesavar counties) as well as central part (Ardabil and Meshkinshahr counties) were of great importance in terms of the presence of *Larrousius* subgenus sand flies and the possibility of transmission of the visceral leishmaniasis.

1. Introduction

Leishmaniasis is a group of systemic diseases that are caused by parasites of the *Leishmania* complex subgenus and transmitted to humans and other mammals by sand flies bites[1,2]. The mortality rate of leishmaniasis is estimated about 51 600 cases in 2010 in the world[3] and it also causes 3.3 million disabilities per year[4]. Leishmaniasis has been reported in 101 countries[5] and over

350 million people are living in at high-risk area of the world[6]. Visceral leishmaniasis (VL) or kala-azar is a severe type of disease that occurs in different parts of the world. Some species of two subgenera of *Phlebotomus* and *Lutzomyia* are the main vectors of diseases in the Old World and New World respectively[7,8]. The annual mortality rate for VL disease is calculated 0.2–0.4 million[6].

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More than 90% of VL disease cases are reported from five countries of Brazil, India, Nepal, Bangladesh and Sudan[9]. In Iran, the disease is sporadically reported from different regions, but the endemic areas have been located in rural parts of the northwest, northeast and southern Iran[10-13]. Annually, 100-300 new cases of VL are reported from Iran, especially in Ardabil province[14] and more than 89% of affected cases were children under 5 years old of age[15]. So far, in Iran, 44 species of the sand flies have been reported and the presence of other 10 species was doubtful[16]. Among them, six species of sand flies in *Larrousius* group and *Paraphlebotomus* subgenera have been identified as the main or probable vectors in the transmission of VL in Iran[13,17-22]. Three species of them including *Phlebotomus major* (*P. major*), *Phlebotomus keshishiani* and *Phlebotomus alexandri* in southern Iran and the rest including *Phlebotomus kandelakii* (*P. kandelakii*), *Phlebotomus perfiliewi* (*P. perfiliewi*) and *Phlebotomus tobbi* (*P. tobbi*) in northwest and northeast of Iran (Ardabil province) are reported as the vectors of the disease. The main aim of this study was to determine the distribution of sand flies in subgenus *Larrousius* as the vectors of VL in different regions of Ardabil province in northwest of Iran and to model their probability of the presence in the province to establish a VL disease surveillance system.

2. Materials and methods

2.1. Study sites

Ardabil province is one of 31 provinces of Iran located on the northwest of the country with a longitude of 38.2514 and latitude of 48.2973. The population of province was 1 270 420 people in 2017. This province has 10 counties and has a cold region in the northwest of Plateau with an area of 17 952.5 Sq.km. It is part of the triangular Plateau of Iran in the east of Azerbaijan Plateau, about 2/3 of which has mountainous texture with high altitude and the rest is formed by the flat and low areas. The climate of Ardabil province is largely dependent on four factors of altitude, latitudes, water resources and Migratory Lows. Other factors such as vegetation, industrial and mining agricultural activities affect in small (Figure 1).

2.2. Sand flies collection

Sand flies were collected from 30 areas of 10 counties in the province during May-November 2017. The studied areas were identified and selected based on the prevalence of human cases of VL during the last 15 years. Six urban areas and twenty-four villages were selected and their geographic coordinates and altitude were recorded by GPS (Figure 1). Sand flies were collected using caster oiled papers (60 papers for indoors and outdoors of each area). They were installed before sunset and collected the following morning before sunrise. All collected sand flies were transferred to acetone and then stored in the 70% alcohol. All specimens were mounted a single drop of Puris' medium and were identified using relevant identification keys[23,24].

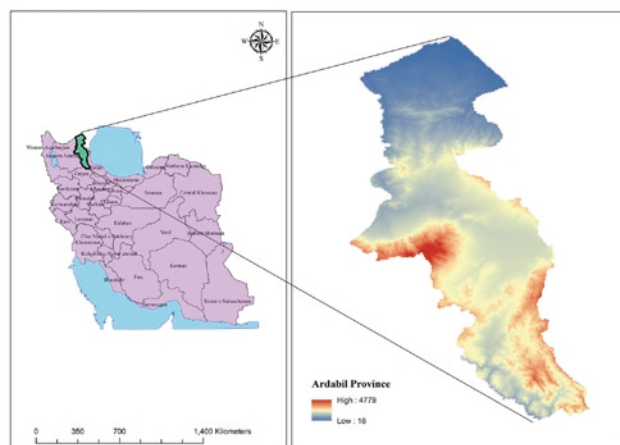


Figure 1. Study area in Ardabil province, Iran 2017.

2.3. Data analysis

Excel and SPSS version 21 were used to analyze the collected data. Poisson's regression analysis was conducted to investigate the relationship between sand fly frequency with average monthly temperature and relative humidity ($P < 0.05$).

2.4. Modeling

The Maxent version 3.3.3 was used to predict the presence of subgenus *Larrousius* sand flies in order to prepare the distribution map at 52 locations (22 locations from previous published studies and 30 locations from the finding of present studies) in Ardabil province northwest of Iran[13,21,25-28]. Jackknife test was used to analyze the relationship between weather variables and distribution of sand flies and the relevant variables were identified with percentages and non-relevant variables were assigned zero. The output of the Maxent model was included in the ArcMap software and the probability of the presence of *Larrousius* subgenus members was divided to 5 categories of 0%-10%, 11%-30%, 31%-50%, 51%-70% and more than 70%. The output map of the ASCII format was converted into the raster format in the GIS software version 10.3.

3. Results

A total of 2 794 sand flies were collected and identified, of which 33.1% belonged to the *Larrousius*, 8.5% *Adlerius*, 20% *Paraphlebotomus*, 23.7% *Phlebotomus*, 0.2% *Synphlebotomus* and 14.5% *Sergentomyia* subgenera. From the *Larrousius* subgenus five species of *P. kandelakii*, *P. perfiliewi transcausicus*, *P. tobbi*, *P. major* and *Phlebotomus wenyoni* (*P. wenyoni*) were observed. The most frequency was related to *P. kandelakii* (39.3%) and the least frequency was related to *P. wenyoni* (0.4%) (Table 1). Considering sex, 72% of the sand flies were males and 28% of them were females. The sex ratio for the total of collected sand flies, the *Larrousius* sand flies and the *P. kandelakii* as the dominant species were calculated as 256.83, 258.14 and 185.82, respectively.

Table 1

Sand flies (*Larrousius* subgenus) fauna in the studied area, Northwest of Iran, 2017.

County	Collection site	<i>P.</i>					Total
		<i>kandelakii</i>	<i>tobbi</i>	<i>perfiliewi</i>	<i>major</i>	<i>wenyoni</i>	
Meshkinshahr	Gasabe	34	5	12	12	0	63
	Kangarlu	27	3	6	9	1	46
	Parikhan	55	14	15	30	0	114
Germi	Daryaman	14	3	23	12	0	52
	Shahrak	24	2	24	22	0	72
	Ibishabad	7	2	15	6	0	30
Bilehsavar	Rohkandi	7	2	18	3	0	30
	Injirlu	22	2	20	10	0	54
	Foladlu	7	2	18	2	0	29
Parsabad	Bodje	2	1	4	1	0	8
	Aslanduz	10	2	12	6	0	30
	Iran abad	5	0	6	2	0	13
Khalkhal	Khangah	6	2	8	3	0	19
	Tolash	4	0	4	4	0	12
	Andabil	10	2	18	3	0	33
Sareyn	Sareyn	4	1	4	3	0	12
	Viladarag	7	2	16	1	0	26
	Aldashin	0	0	14	5	0	19
Ardabil	Talebgeshlagi	30	2	10	9	0	51
	Divlag	32	1	5	8	1	47
	Hamlabad	18	2	4	6	0	30
Kowsar	Garegeshlag	0	0	0	4	0	4
	Chalgarod	5	2	5	4	2	18
	Gorgabad	0	1	9	1	0	11
Namin	Namin	21	0	12	6	0	39
	Nanekaran	0	0	2	1	0	3
	Anbaran	0	2	5	3	0	10
	Geynarje	0	4	6	7	0	17
Nir	Borjlu	9	0	0	7	0	16
	Nir	3	0	4	9	0	16
Total		363	59	299	199	4	924

Two main vectors of *P. perfiliewi transcaucasicus* and *P. kandelakii* (*Larrousius* group species) were collected from 93% and 82% of the studied areas. The monthly activity of the *Larrousius* sand flies is started from end of May and is finished in late November with one peak in July (Figure 2).

The results showed that 43% of the sand flies were collected from indoor and the rest from outdoor sites. The highest number of sand flies (15%) was collected from the stables (indoors) and the yards (24.5%) as outdoors places (Table 2).

Using *Chi-square* analysis, the relationship between the species collection in the indoor and outdoor spaces was significant ($P < 0.05$). The frequency of all species in indoor areas was less than outdoor sites, except for *P. kandelakii* which is collected with frequency of 59% in indoor sites.

Table 2

Collection of *Larrousius* group sand flies in different sites, Northwest of Iran, 2017.

<i>Larrousius</i> group	Indoor						Outdoor				
	Bedroom	Bathroom	Toilet	Animal shelters	Chicken coops	Warehouse	Yard	Wall	Rock and mountain gap	Ruined places	Fox and rodent nest
<i>P. perfiliewi</i>	14	15	40	21	6	17	62	8	19	34	63
<i>P. tobbi</i>	1	0	2	5	1	1	2	0	22	0	25
<i>P. wenyoni</i>	0	0	0	0	0	0	2	0	0	0	2
<i>P. major</i>	19	0	11	6	12	11	74	4	43	4	15
<i>P. kandelakii</i>	51	13	30	107	6	7	86	12	33	11	7
Total	85	28	83	139	25	36	226	24	117	49	112

The results of linear regression analysis for each temperature, humidity and rainfall variables indicated that there is a significant relationship between temperature and humidity with monthly activity of sand flies ($P < 0.05$), but there is no significant relationship between rainfall and seasonal activity of sand flies ($P > 0.05$). The result of the Maxent model showed that the sub-curve surfaces (AUC) for *P. kandelakii* and *Larrousius* group were 0.809 and 0.783, respectively.

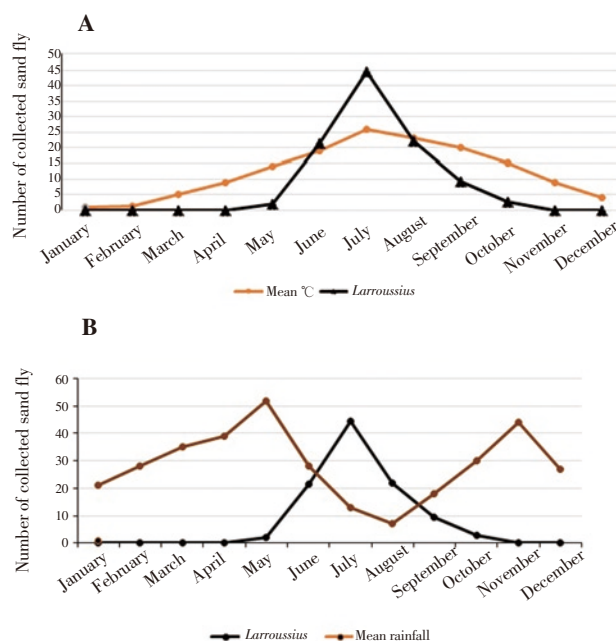


Figure 2. Correlation of seasonal activity of sand flies with temperature (A) and rainfall (B), 2017.

3.1. Jackknife analysis for *Larrousius* subgenus sand flies

In Figure 3 (Right), the predicted potential locations for the presence of *Larrousius* subgenus sand flies are shown. In the study, the red color revealed the probability of presence of the subgenus *Larrousius* which is more than 70%, and the probability of transmission of the VL in these areas was very high. The results of the jackknife test showed that among the environmental and climate variables, the ground slope, NDVI, slope and Bio-15 (seasonal precipitation) have the greatest effect on the distribution of *Larrousius* subgenus in Ardabil province (Figure 4).

3.2. Jackknife analysis for *P. kandelakii* (main vector)

P. kandelakii is the main vector in the Northwest of Iran and the

province of Ardabil. The probability of presence of this species with more than 70% was determined in three counties of Meshkinshahr, Germe and Ardabil (Figure 3 Left). The most significant climate variables that affected the distribution of the species were NDVI, Bio-15 (Seasonal precipitation), slope, and Bio-7 (annual temperature range) (Figure 5). The relationship between environmental and climate variables was direct which coincides with the peak activity of the sand flies, especially for *Larrousius* sand flies and the NDVI of the region was maximum in June and July. According to seasonal rainfall and temperature, the most frequency of sand flies was observed in July and August when the air temperature was increased and seasonal rainfall decreased (Figure 2).

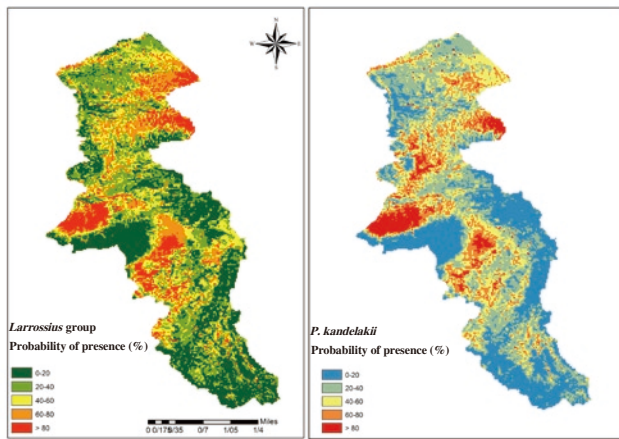


Figure 3. Probability of presence areas for the sand flies vectors of visceral leishmaniasis (*Larrousius* subgenus) in Northwest of Iran, 2017.

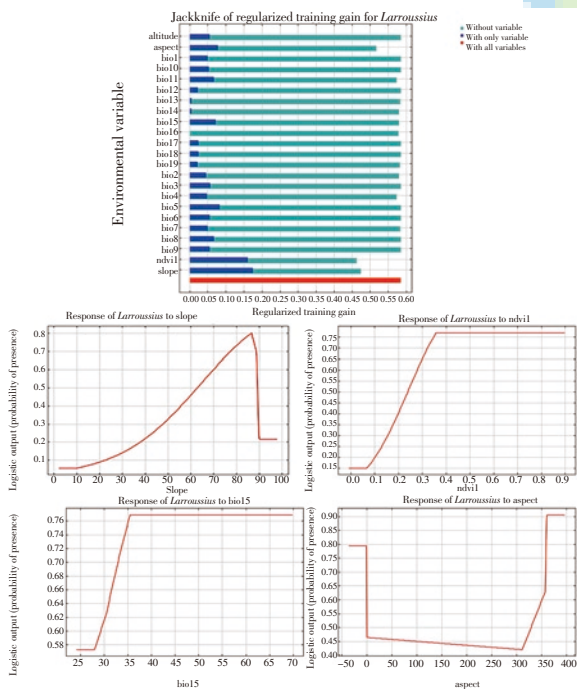


Figure 4. Jackknife test in MaxEnt model for *Larrousius* subgenus sand flies and response to the most influencing factors.

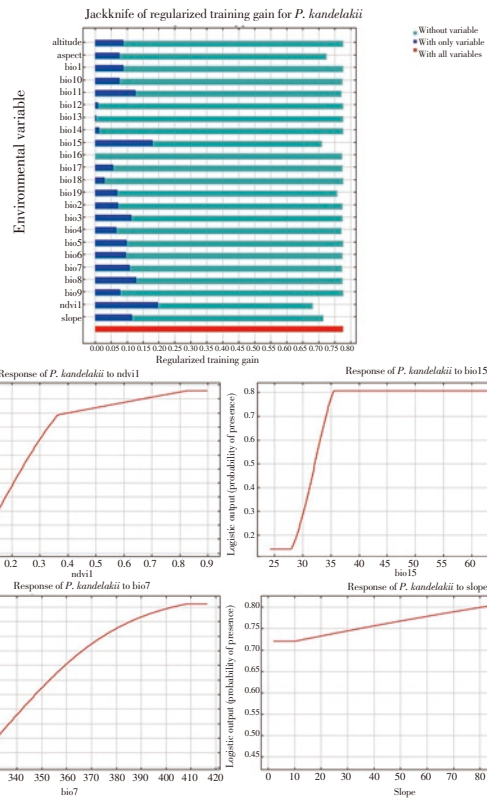


Figure 5. Jackknife test in MaxEnt model for *P. kandelakii* and response to the most influencing factors.

4. Discussion

The main purpose of this study was to determine the spatial distribution of *Larrousius* group sand flies in Ardabil province as one of the main foci of VL in the Iran[18]. The evaluation of distribution pattern of the sand flies is very important in this area because since the year 1949, when the first case of the VL was reported in Iran, by the end of 1997, about 5 244 cases of the VL were reported in Iran with 43.5% of them from Ardabil province[29,30]. In this study, most diversity of subgenus *Larrousius* sand flies including *P. kandelakii*, *P. perfiliewi transcaucasicus*, *P. major*, *P. tobbi*, and *P. wenyoni* was found compared to previous studies[21,26-28]. In the center of Europe, among the vectors of *Leishmania infantum*, five species including *Phlebotomus ariasi*, *Phlebotomus mascittii*, *Phlebotomus perniciosus*, *Phlebotomus neglectus* and *P. perfiliewi* were identified and reported[31]. Our study showed that among the specimens of subgenus *Larrousius*, the two species of *P. kandelakii* and *P. perfiliewi transcaucasicus* were more prevalent in the Ardabil province which was compatible with previous studies[21,26-28]. Also the natural infection of these species with *Leishmania infantum* and *Leishmania donovani* was reported 1%-1.5% from different areas of the Ardabil province which confirmed that they play the main role in transmission of VL to human in the province. According to these studies, the abundance of *P. kandelakii* in the Meshkinshahr and Ardabil and *P. perfiliewi transcaucasicus* in Germe was high, which were in consistent with earlier studies[13,18]. The collection rates of *Larrousius* subgenus sand flies in outdoor and indoor sites were found 57% and 43% respectively, indicating the high abundance of sand flies in outdoor places, which were similar to the results

of another study conducted in central Iran[32]. The male sand flies were collected 2.5 times more than the female sand flies, which indicated the higher abundance of males against females. In most studies, the male to female ratio was 3-2 times confirming the same results[33-35]. In this study, the sticky trap technique was used to indicate that males are more attracted to this type of trap while, if the light trap was used, females were mostly absorbed. A study in India reported *Phlebotomus argentipes* was more attracted to light traps[36]. Modeling for one or more of the vectors of diseases helps to determine the potential of the distribution of vectors species in a region, thereby detecting the pattern of distribution of diseases transmitted by vectors[37,38]. Many studies in the world and Iran are done on modeling of CL[38-41]. About vectors of VL, an earlier study in Iran modeled the three important vector species of *P. major*, *Phlebotomus alexandri* and *P. kandelakii*[42]. The results of modeling for subgenus *Larrousius* sand flies in the province of Ardabil for the first time showed that four counties were important in terms of the probability of presence of subgenus *Larrousius* sand flies, two areas in the north part (Germi and Bilehsavar), which are located across the borders of the Republic of Azerbaijan, and two areas (Ardabil and Meshkinshahr) in the center part of that are located to the borders of East Azerbaijan Province. In these four areas, about 64 838 people were at risk of transmitting the VL. In addition, Meshkinshahr has been a tourist destination and the Bilehsavar has a border terminal with the Republic of Azerbaijan, which is visited by a large number of travelers and tourists from these two regions and provinces every month, and they are also at risk in this regard. In this study the most variables that affected the distribution of subgenus *Larrousius* were slope, NDVI, seasonal rainfall and slope direction. In a study conducted in Brazil, NDVI index was the one of the most influential environmental factors on distribution of *Lutzomyia longipalpis*[43]. Hanafi-Bojd et al. conducted modeling for three main vectors of VL in Iran and revealed that the important factor was isothermality which had the greatest effect on the distribution for all three species[42]. The results of the study of Camila Gonzalez in North America, which modeled on *Lutzomyia* sand flies, showed that the greatest effect on the distribution of *Lutzomyia* sand flies were the average temperature in the cold and hot months of the year as well as the annual average temperature[44]. In modeling for *Phlebotomus perniciosus* using Maxent, the NDVI had a direct effect on the distribution of this vector in Europe[31], and in another study in Central Europe, the variables of climate, mean temperatures in hot and cold months and annual rainfall were more effective on the vectors of VL[45]. So, climate change and environmental factors have a significant impact on the development of vectors in the world. These changes and their effects on vectors distribution and VL should be regularly monitored. However, the cold weather in the studied area and being mountainous were limitations of present study.

In conclusion, the findings of this study showed that the vectors of VL were dispersed in most parts of Ardabil province and four important foci have been identified in province with more than 70% of the probability of presence of *Larrousius* subgenus sand flies, where the VL could be transmitted in these regions. More comprehensive studies are needed to identify other vectors with novel methods, such as molecular methods which are recommended for next studies.

Conflict of interest statement

We declare no conflict of interest.

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