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Spatial distribution and infection rate of leishmaniasis vectors (Diptera: Psychodidae) in Ardabil Province, Northwest of Iran

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ABSTRACT

Objective: To determine the spatial distribution and infection rate of sand flies as vectors of *Leishmania* parasite in Ardabil province, northwest of Iran.

Methods: This was a descriptive cross-sectional study. The sand flies were collected from 30 areas in all 10 districts of Ardabil province during 2017. The specimens were caught using the sticky traps. The head and genitalia of sand flies were separated and mounted in Berlese solution for microscopic identification. The Geographical Information System ArcMap10.4.1 software was used to provide the spatial maps.

Results: A total of 2 794 sand flies specimens were collected and 22 species of sand flies were identified from the two genera: *Phlebotomus* and *Sergentomyia* from Ardabil province. The highest frequency was found in *Phlebotomus papatasi* (23.7%) followed by *Phlebotomus kandelakii* (13.0%). The promastigote form of *Leishmania infantum* parasite has been reported from the three main vectors of visceral leishmaniasis (*Phlebotomus kandelakii*, *Phlebotomus perfiliewi* and *Phlebotomus tobbi*) from Ardabil province, where the spatial distribution map of these visceral leishmaniasis vectors was prepared. Some important species of sand flies such as *Phlebotomus kandelakii*, *Phlebotomus perfiliewi* and *Phlebotomus tobbi* were reported and identified as main and probable vectors of visceral leishmaniasis in Ardabil.

Conclusions: According to the Geographic Information System based maps, the frequency of the sand flies as leishmaniasis vectors, the leishmania parasite infection rate and the prevalence of the disease in the central areas of Ardabil province are higher than in other areas in Ardabil province.

1. Introduction

Despite considerable advances in diseases control, communicable diseases still continue to threaten people's health around the world[1]. Leishmaniasis is a group of parasitic, communicable and vector-borne diseases, and is the second most protozoan disease after malaria that affects numerous populations in tropical and subtropical regions of the world[2,3]. Classically, human leishmaniasis is classified into three groups: visceral leishmaniasis

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(VL) or kala-azar, cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL). Almost 90% of CL cases have been reported from 8 countries, and Iran is one of those countries. Leishmaniasis, especially CL in Iran has a long history[4,5]. In Iran, there are two types of leishmaniosis: visceral and cutaneous forms[6]. More than 20 000 cases of CL are reported annually from all provinces of Iran especially in 17 out of the 31 provinces of the country as the endemic foci. Also, about 100-300 new cases of VL have been reported from the endemic areas of the northwestern and southern Iran annually. In addition to these foci, the foci of the disease in west, center, and northeast of the country should also be mentioned[6-8]. *Leishmania* parasites are observed in two forms: amastigote and promastigote in vertebrate hosts and sand fly vectors respectively[5]. In New World, the sand flies of genus *Lutzomyia* and in Old World the genus of *Phlebotomus* are vectors of the leishmaniasis. In Old World, including Iran, *Phlebotomus* sand flies species have been identified as vectors of leishmaniasis in humans due to blood feeding of vertebrates[9]. So far, approximately 1 000 species of sand flies have been identified in the world, of which 50 species have been confirmed as vectors of leishmaniasis[10]. According to results of previous studies on sand flies in Iran, there are 50 species of sand flies in the country, 30 species from genus *Phlebotomus* and 18 species from genus *Sergentomyia*[11-13]. In different areas of Iran, natural promastigote infection of *Leishmania* parasite has been isolated from 13 species of sand flies[13]. Ardabil province is one of the endemic foci of VL in the northwest of Iran[14]. The incidence of disease in the province is rising from 2.9 to 9.2 per 100 000 people population from 2009 to 2017[15]. It seems that climate change has a major impact on the incidence of disease and vectors activity in the past decade[16]. Recently, the geographical distribution of vectors and the mapping of the prevalence of disease have been applied to understand the epidemiology of these diseases. In recent years, studies have been conducted in Iran on the geographical distribution of vector-borne diseases[17,18]. The aim of the present study was to determine the spatial distribution of leishmaniasis vectors (Diptera: Psychodidae) in Ardabil Province, northwest of Iran.

2. Materials and methods

2.1. Study site

Ardabil province is located in the northwest of Iran, bordering the Republic of Azerbaijan on the north side, between 38.2514ºN 48.2973ºE, with an area of 17 800 km^2 (Figure 1). Based on the most recent census in 2016, the province has a population of approximately 1 270 000 people. The Ardabil province is divided into 10 counties: Ardabil, Meshkinshahr, Germi, Bilasavar, Parsabad, Khalkhal, Sarein, Nir, Namin and Kowsar. This study was conducted in 30 regions of all 10 counties from the beginning to end of seasonal activity of sand flies (May to Oct 2017).

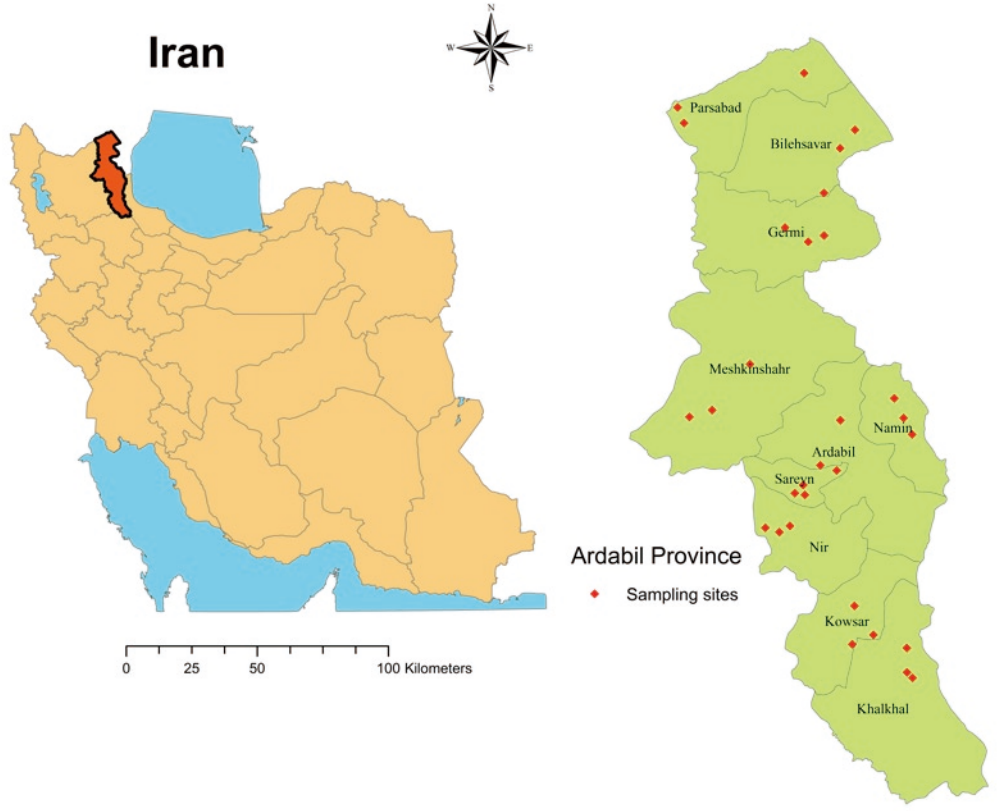

Figure 1. Location of the study area; Ardabil province, northwest of Iran.

2.2. Sand fly sampling

This descriptive cross-sectional study was carried out on sand flies fauna (Vectors of leishmaniasis) in 5 urban fixed and 25 rural variable areas (30 areas of all 10 counties) from the beginning to the end of seasonal activities of sand flies (May to Oct 2017) every 15 days for 14 times.

The studied areas were selected based on the incidence of VL cases in the last 10 years. Their geographical coordinates and elevations were recorded using Global Positioning System (Figure 1). The samples were collected based on the World Health Organization guidelines using 60 sticky traps, 30 traps in indoors and 30 traps in outdoors. The captured sand flies were isolated from the sticky traps using insulin syringes. They were washed in acetone and transferred to 70% ethanol to remove castor oil coated sand flies. Then microscopic slides were prepared with head and genitalia of each sample[19]. After that, the samples were identified using valid identification keys[9,14,20].

2.3. Data analysis

To determine the parasitic infestation of sand flies, previous studies were carried out in Ardabil province[21-24]. The GISArcMap10.4.1 software was used for mapping the spatial distribution of leishmaniasis vectors and their leishmania parasite infection.

2.4. Ethical approval

The study was approved by the Ethical Committee of Ardabil University of Medical Sciences, Iran (Code of ethics: IR.ARUMS.REC.1397.112).

3. Results

A total of 2 794 (1 947 males and 847 females) sand flies specimens were collected from the beginning to the end of seasonal activities of sand flies. Totally, 22 species of sand flies were identified from the two genera: 20 species of *Phlebotomus* (86%) and two species of *Sergentomyia* (14%) from this study area.

The most abundant species was *Phlebotomus papatasi* (*P. papatasi*), accounting for 23.7% of all sand flies captured from all studied areas; while the lowest species was *Phlebotomus simisi* that was only collected from Ardabil and Germi counties (0.1% of all collected samples). The other species were presented in Table 1, Figures 2 and 3.

Ardabil County showed the most species diversity (22 species sand flies) and Nir County showed the lowest (12 species). In addition, in terms of abundance, the highest 761 (27.2%) and lowest 87 (3.1%) of collected sand flies were observed in Meshkinshahr and Kowsar counties respectively (Table 1).

The most frequency of sand flies as proven vectors of CL in Iran was found in *P. papatasi* (23.7%) and *Phlebotomus sergenti* (*P. sergenti*) (12.1%); while *Phlebotomus kandelakii* (*P. kandelakii*) and *Phlebotomus perfiliewi* (*P. perfiliewi*) as probable vectors of VL in Iran was 13.0% and 10.7% respectively. Seasonal activates of these four important sand flies species was presented in Figures 4 and 5.

In term of capture place, 1 029 (36.83%) and 1 765 (63.17%) sand flies were trapped from indoor and outdoor places, respectively. Sand flies were collected mostly from animal shelters (16.75%) and rock and mountain gaps (24.13%) respectively (Figure 6). We found that ratio of collected sand flies per number of used sticky traps was 0.31 sand flies per trap in all counties of the province. Out of all counties, the highest and lowest of sand flies per trap abundance ratio was found in Meshkinshahr (0.84) and Kowsar counties, respectively (0.10).

Table 1. Fauna and number of collected sand flies from the endemic area of ZVL in Ardabil province, northwest Iran, 2017 [*n*(%)].

Species	Meshkinshahr	Germi	Bilehsavar	Parsabad	Khalkhal	Sareyn	Ardabil	Namin	Nir	Kowsar	Total
Phlebotomus											
P. perfiliewi	33 (4.3)	62 (15.9)	56 (18.4)	22 (14.5)	30 (16.5)	34 (17.2)	19 (5.9)	19 (11.4)	10 (4.2)	14 (16.1)	299 (10.7)
P. papatasi	175 (22.9)	75 (19.2)	62 (20.4)	40 (26.5)	44 (24.3)	70 (35.5)	52 (16.2)	46 (27.7)	74 (31.3)	24 (27.5)	662 (23.7)
P. sergenti	132 (17.3)	43 (10.5)	28 (9.2)	20 (11.9)	20 (11.0)	21 (10.6)	17 (5.3)	18 (10.8)	35 (14.8)	6 (6.9)	340 (12.2)
P. tobbi	22 (2.9)	7 (1.7)	6 (1.9)	3 (2.0)	4 (2.2)	3 (1.3)	5 (1.5)	2 (1.2)	4 (1.7)	3 (3.4)	59 (2.1)
P. major group	39 (5.1)	28 (7.1)	15 (4.9)	5 (3.3)	5 (2.7)	5 (2.5)	14 (4.3)	3 (1.8)	10 (4.2)	7 (8.0)	131 (4.7)
P. caucasicus	22 (2.9)	2 (0.5)	3 (0.9)	2 (1.3)	0 (0)	0 (0)	1 (0.3)	0 (0)	0 (0)	0 (0)	30 (1.1)
P. weyoni	1 (0.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.3)	0 (0)	0 (0)	2 (2.3)	4 (0.1)
P. elenorae	0 (0)	0 (0)	0 (0)	2 (1.3)	0 (0)	0 (0)	2 (0.6)	0 (0)	0 (0)	1 (1.1)	5 (0.2)
P. major	12 (1.5)	12 (3.1)	0 (0)	4 (2.6)	5 (2.7)	4 (2.0)	9 (2.8)	7 (4.2)	13 (5.5)	2 (2.3)	68 (2.4)
P. mongolensis	13 (1.7)	6 (1.5)	9 (2.9)	2 (1.3)	2 (1.1)	2 (1.0)	6 (1.8)	3 (1.8)	3 (1.2)	3 (3.4)	49 (1.8)
P. kandelakii	116 (15.2)	45 (11.5)	36 (11.8)	17 (11.2)	20 (11.0)	11 (5.5)	80 (25.0)	21 (12.6)	12 (5.1)	5 (5.7)	363 (13.0)
P. caucasicus group	38 (5.0)	16 (4.1)	11 (3.6)	5 (3.3)	7 (3.8)	5 (2.5)	14 (4.3)	4 (2.4)	3 (1.2)	2 (2.3)	105 (3.8)
P. andrejevi	13 (1.7)	3 (0.7)	8 (2.6)	0 (0)	0 (0)	0 (0)	9 (2.8)	0 (0)	0 (0)	0 (0)	33 (1.2)
P. halepensis	25 (3.2)	2 (0.5)	10 (3.3)	1 (0.6)	1 (0.5)	1 (0.5)	6 (1.8)	2 (1.2)	0 (0)	1 (1.1)	49 (1.8)
P. longidnctus	25 (3.2)	1 (0.2)	3 (0.9)	1 (0.6)	2 (1.1)	3 (1.5)	5 (1.5)	4 (2.4)	0 (0)	1 (1.1)	45 (1.6)
P. balcanicus	12 (1.5)	4 (1.0)	3 (0.9)	2 (1.3)	3 (1.6)	0 (0)	5 (1.5)	3 (1.8)	0 (0)	1 (1.1)	33 (1.2)
P. brevis	5 (0.6)	6 (1.5)	8 (2.6)	0 (0)	1 (0.5)	1 (0.5)	1 (0.3)	3 (1.8)	0 (0)	2 (2.3)	27 (1.0)
P. chinansis group	18 (2.3)	17 (4.1)	11 (3.6)	3 (2.0)	5 (2.7)	4 (2.0)	14 (4.3)	2 (1.2)	4 (1.7)	2 (2.3)	80 (2.9)
P. simici	0 (0)	1 (0.2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.3)	0 (0)	0 (0)	0 (0)	2 (0.1)
P. jacusieli	0 (0)	0 (0)	2 (0.6)	0 (0)	1 (0.5)	0 (0)	3 (0.9)	0 (0)	0 (0)	0 (0)	6 (0.2)
Sergentomyia											
S. sintoni	26 (3.4)	23 (5.9)	11 (3.6)	6 (3.9)	7 (3.8)	6 (3.0)	15 (4.6)	7 (4.2)	12 (5.1)	2 (2.3)	115 (4.1)
S. dentata	34 (4.4)	37 (9.5)	22 (7.2)	17 (11.2)	24 (13.2)	27 (13.7)	41 (12.8)	22 (13.2)	56 (23.7)	9 (10.3)	289 (10.3)
Total	761 (27.2)	390 (14.0)	304 (10.9)	152 (5.4)	181 (6.5)	197 (7.1)	320 (11.5)	166 (5.9)	236 (8.4)	87 (3.1)	2 794 (100)

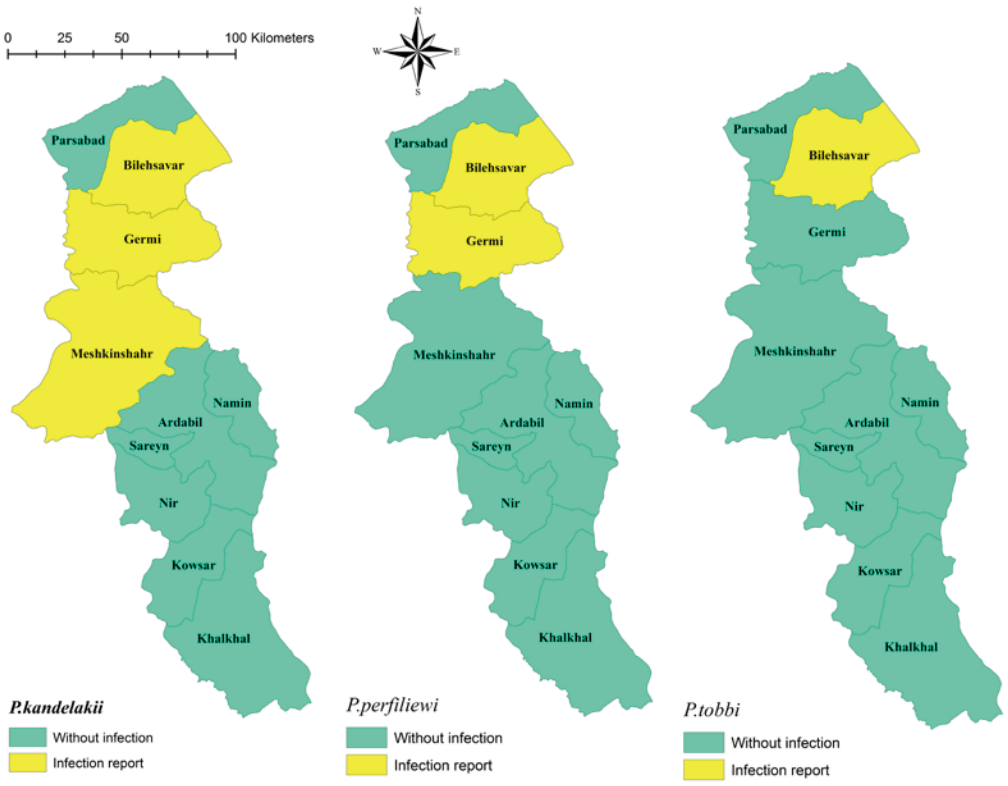

Figure 2. Spatial distribution of *P. kandelakii* (left), *P. perfiliewi* (center), *P. tobbi* (right) and their infection with *Leishmania* parasites in Ardabil Province, northwest Iran, 2017.

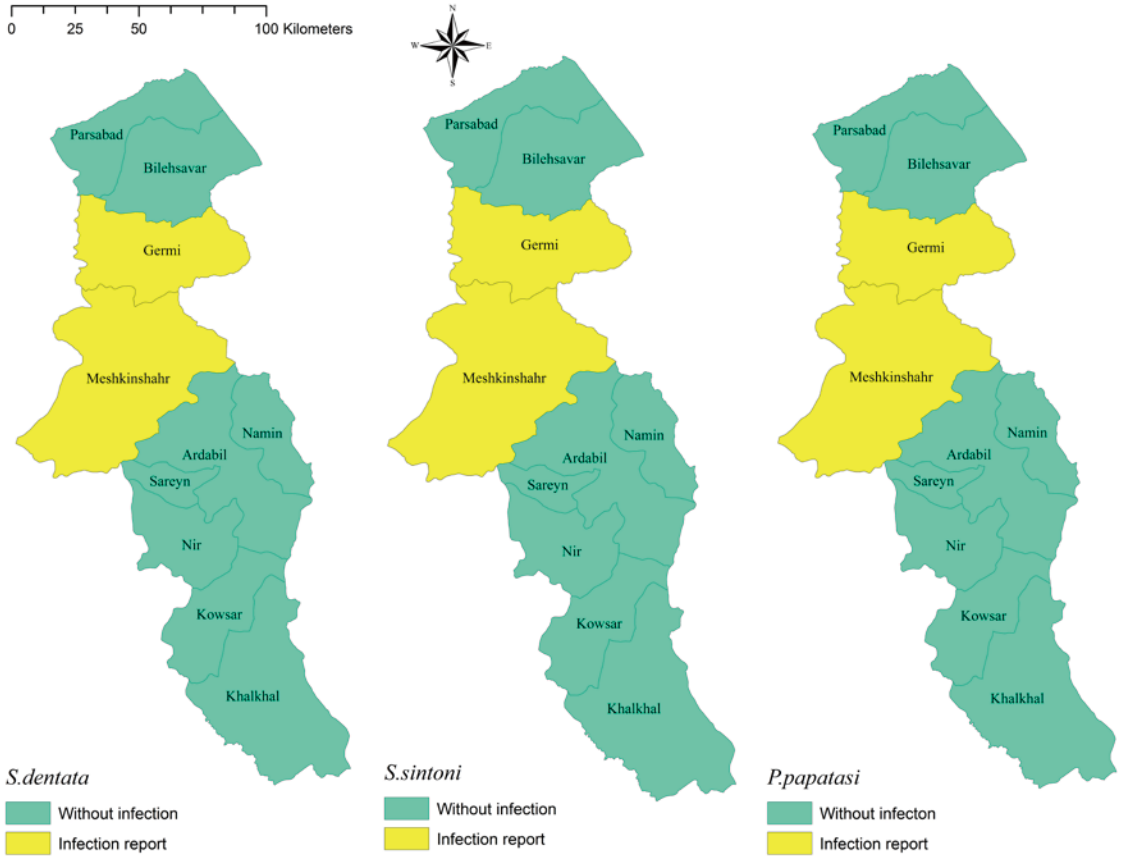

Figure 3. Spatial distribution of *S. dentata* (left), *S. sintoni* (center), *P. papatasi* (right) and their infection with *Leishmania* parasites in Ardabil Province, northwest Iran, 2017.

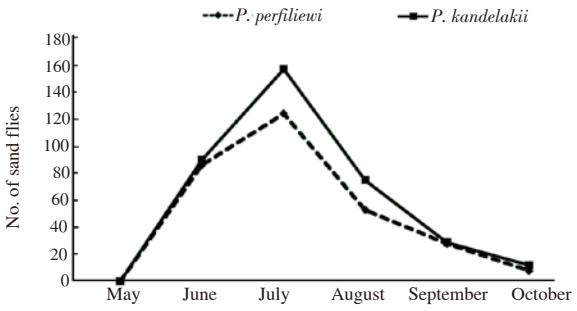

Figure 4. Seasonal activity of *P. kandelakii* and *P. perfiliewi* collected in Ardabil Province, northwest Iran, 2017.

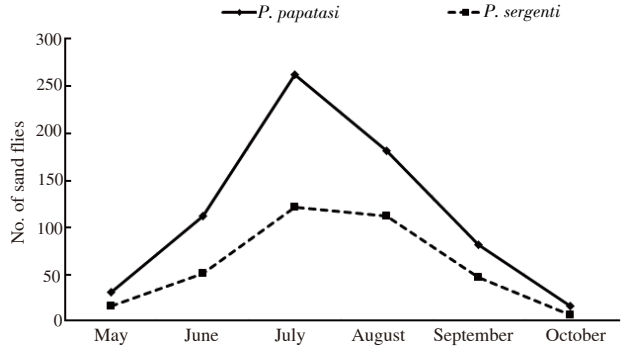

Figure 5. Seasonal activity of *P. papatasi* and *P. sergenti* collected in Ardabil Province, northwest Iran, 2017.

4. Discussion

This study investigated the fauna of sand flies in the entire Counties of Ardabil province for the first time, and 22 species of the two genera; *Phlebotomus* and *Sergentomyia* were collected and identified. In this study, the most species of sand flies were reported in comparison to previous studies in the northwest of Iran. In previous studies that have been carried out in some areas of Ardabil province on sand flies and their leishmania infection, it was reported that there were 20 species of sand flies in Ghorbani *et al* in Meshkinshahr, 6 species in Dehkordi *et al* in Bilehsavar, 13 species in Oshaghi *et al* in Germi and 5 species in Sadeghi *et al* in Meshkinshahr, respectively[21,25-27]. Furthermore, it founded that by using parasitological methods the promastigote form of the *Leishmania infantum* (*L. infantum*) parasite has been detected in three probable vectors of VL [*P. kandelakii*, *P. perfiliewi* and *Phlebotomus tobbi* (*P. tobbi*)] from Meshkinshahr, Germi and Bilehsavar counties of Ardabil province, where the spatial distribution map of these VL vectors was prepared[22-24]. Also, promastigote form of the *Leishmania* parasite has been reported from *Sergentomyia dentata* and *Sergentomyia sintoni* in Ardabil province[13]. In addition, *L. infantum/donovani* infection has been isolated from *Phlebotomus perfiliewi* and *P. kandelakii* using semi-nested PCR[25,26]. Leishmania infection was also reported from *P. papatasi* by using molecular method[13]. In recent years, faunistic studies of sand flies in different areas of Iran are carried out regularly. For instance, Hazratian *et al*. has reported 17 species from different regions of East Azerbaijan, which is in cooperation with the Ardabil province[28]; while Saghafipour and colleagues in Qom province have identified 14 species of sand flies[17]. According to previous studies, *P. papatasi* is the main vector of zoonotic cutaneous leishmaniasis (ZCL) in Iran, and its parasitic infection in Iran has been reported to be between 0.2%-10.9%. Also, *P. sergenti* is the main vector of anthroponotic cutaneous leishmaniasis (ACL) in Iran, and its parasitic infection rate is reported to be 0.1%-1.5% in different regions of Iran[22,29-31]. In present study, *P. papatasi* and *P. sergenti* were caught with high infection rate in different areas of Ardabil province, especially in the

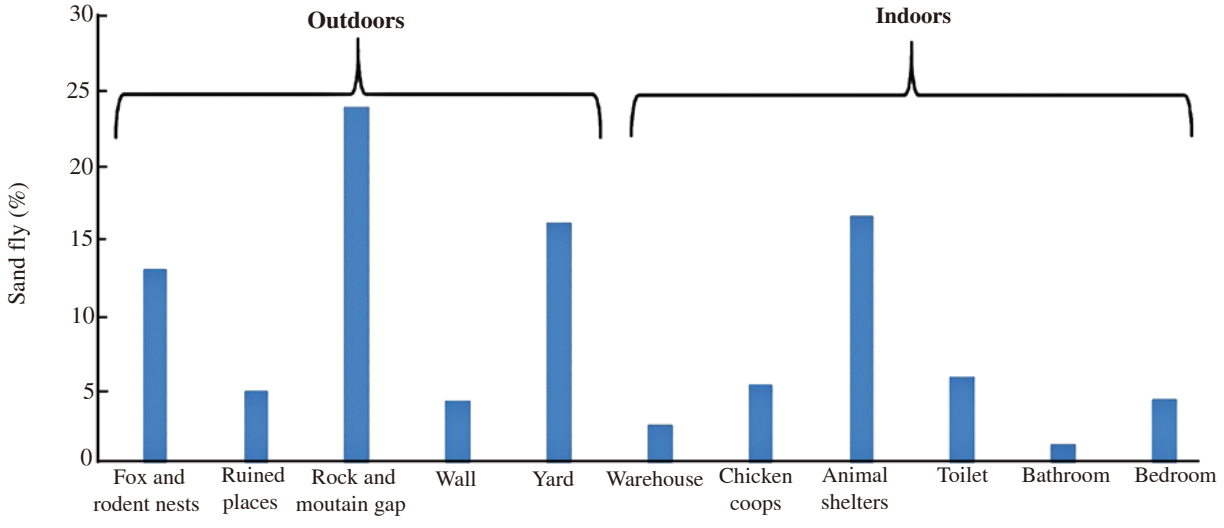

Figure 6. Sand fly collection from indoors and outdoors in Ardabil province, 2017.

Balehsavar and Germi counties. According to the Prevention and Diseases Control Department of Ardabil Province Health Center, the number of CL cases in this province is 30 people in 2017. In 2009, *P. papatasi* has been reported as proven vector of leishmania parasite in Ardabil province, by using the molecular method, but *P. sergenti* parasitic infection of *Leishmania* has not been reported until now[23]. In addition, in this study, out of VL vectors *P. kandelakii*, *P. perfiliewi* and *P. tobbi* were identified. Also, we observed that the infection rate of the *P. kandelakii* is higher in the central regions of the Province (Ardabil, Meshkinshahr) and the infection rate of *P. perfiliewi* and *P. tobbi* in the northern areas of the province (Garmi, Bilehsavar and Parsabad) is higher. *L. infantum* as a causative agent of VL has been reported by using parasitological and molecular methods of this three species of sand flies (*P. kandelakii*, *P. perfiliewi* and *P. tobbi*) in Ardabil Province[22-24,32,33]. In 2012, Rassi *et al*. reported the *L. infantum* parasite infection in *P. kandelakii* in different regions, including the northeast of Iran for the first time[34]. In addition to these three vectors, three other sand flies have been reported as the main vector of VL in different regions of Iran, and the infection of *L. infantum* has been confirmed, including *Phlebotomus alexandri*, *Phlebotomus major* and *Phlebotomus keshishiani* in southern areas of Iran using by PCR method[35-37]. According to this study, the main vectors of VL has been collected from all areas of Ardabil province. Furthermore, central areas of this province have high-abundance of sand flies, which is consistent with the places where the incidence of the disease has been high in recent years[15]. In addition, we founded that CL vectors were prevalent in all areas with high incidence of the disease. According to the Ardabil Health Center's report, the incidence of CL is 20-30 people each year, therefore studies on leishmania infection rate of vectors are needed to find out types of CL, ZCL or ACL. According to the results of this study, species diversity of sand flies in Ardabil province is higher than other areas of Iran. Probable vectors of all three important types of diseases: VL, ZCL, and ACL are more abundant in most areas of Ardabil province. The high risk and most important areas for the occurrence of all three types of leishmaniasis are central areas of the province according to high species diversity of sand flies in central areas of this province. The low-risk area of VL occurrence and main vectors in Ardabil province are identified in southern province.

Conflict of interest statement

Authors declare that there are no competing interests.

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