Abstract

Zoonotic cutaneous leishmaniasis (ZCL) is transmitted to humans by phlebotomine sand fly bites. ZCL is a major health problem in Iran, where basic knowledge gaps about sand fly species diversity persist in some ZCL-endemic areas. This paper describes the richness and spatial distribution of sand fly species, collected with sticky traps, in Qom province, a ZCL-endemic area in central Iran, where sand fly fauna has been poorly studied. Collected species were mapped on urban and rural digital maps based on a scale of 1/50,000. All analyses were undertaken with rural- and urban-level precision, i.e., rural and urban levels were our basic units of analysis. After identifying the sand flies, high-risk foci were determined. For spatial analysis of vector species population, the entomological sampling sites were geo-referenced using GPS. Arc GIS 9.3 software was used to determine the foci with leishmaniasis vector species. Following the analyses, two genera (Phlebotomus and Sergentomyia) and 14 species were identified. Based on the mapping and sand fly dispersion analysis, the rural districts were categorized into three groups—infected, uninfected, and no report. Based on Geographical Information System analyses, Kahak and Markazi districts were identified as high-risk foci with leishmaniasis vector species. These findings can act as a help guide to direct active control measures to the identified high-risk foci and, eventually, lead to reduction in incidence of the disease.

Key words: leishmaniasis, Phlebotomus, Sergentomyia, Geographical Information System, Qom, Iran

According to World Health Organization (WHO) reports, leishmaniasis is one of the six most important parasitic diseases in tropical regions of the world. Ninety-eight countries have reported some evidence of its transmission. After malaria, leishmaniasis is the most important tropical disease observed, mainly its cutaneous form. Prevalence of the disease is estimated to be about 12 million cases globally. There are around 2 million new cases diagnosed annually. Moreover, 350 million people are living in areas with risk of infection (WHO 1990). Cutaneous leishmaniasis (CL) is of the most important vector-borne diseases in Iran (Yaghoobi-Ershadi 2012). The Phlebotomus papatasi (Diptera: Psychodidae) is the main and proven vector and Leishmania major is the causative agent of zoonotic cutaneous leishmaniasis (ZCL) in Iran (Molyneux and Ashford 1983). The prevalence of cutaneous leishmaniasis in Iran is 0.028% (~20,000 cases per year); it is, however, estimated that the actual rate may be more than this estimate (Mohebali 1996). Although CL is not a fatal disease, it has always been under spatial attention due to patients' long-term involvement with acute wounds, treatment, and damages to facial esthetics, especially among women (Hewitt et al. 1998). Unfortunately, despite many efforts of national health authorities, this form of the disease has been increasing in the country in recent decades (Rassi and Hanafi-Bojd 2006). Some scientists have postulated that one reason for such an increasing trend can be due to discontinuation of malaria control programs (Killick-Kendrick et al. 1994). The geographical and climatic conditions of Qom Province provide a favorable breeding place for vectors of leishmaniasis. The first cases of leishmaniasis were observed in 1999 (incidence 2/1,000, 8 cases) in the province. The number of cases observed had been steadily increasing since then, amounting to 25 cases (8.8 per 1,000 people) in 2007. In 2008, with preventive measures, the incidence declined to 8.4 per 1,000 (24 cases), but it unexpectedly soared again afterwards so that there were 169 cases (32.7
per 1,000) in 2009 (Saghafipour et al. 2013). The sand flies residing in the province belong to order Diptera and Psychodidae family. These insects have a worldwide distribution and are more densely agglomerated in tropical and subtropical areas. So far, ~800 species or subspecies of phlebotominae sand flies have been identified (Zahraei-Ramazani and Leshan 2016) and classified into six genera of Phlebotomus, Sergentomyia, Chinius, Warelyia, Lutzomyia, and Brumptomyia (Rassi and Hanafi-Bojd 2006). The first comprehensive research on sand flies fauna of country was carried out by Mesghali in 1964. That study showed that 34 species of sand flies, including 20 species of Phlebotomus and 14 species of Sergentomyia genera, inhabited in Iran (Adler et al. 1930, Theodor and Mesghali 1964). According to recent sand flies fauna studies in Iran, there are, at least, 53 species of Phlebotomine sand flies, 34 species of genus Phlebotomus and 19 species of genus Sergentomyia, residing in the country (Zahraei-Ramazani et al. 2015). There is a direct relationship between public health issues and disease distributions with geography of a given local area. Therefore, Geographical Information System (GIS) can play an important role in management and planning for prevention and control of diseases. Capacity modeling, spatial analysis, and statistical capabilities used in discovery of spatial aggregation of diseases are the other advantages of GIS. In recent years, this software has been increasingly used in the field of health (Burrough 1986). Following studies are some examples of GIS in Iran—Hanafi Bojd et al. study for identification of sandflies fauna across the country (Karimi et al. 2014), Salahi Moghaddam et al. study for investigation of ecology of malaria vectors in Bandar Abbas (Pirmoradi et al. 2012), Hosseini Chagini’s study for parasitology and ecology of snails in Bandar Abbas city (Hosseini Chagini and Salahi-Moghaddam 2009), and Sedaghat et al. study for providing the map of rodents distribution in Iran (Sedaghat and Salahi Moghaddam 2010). Mapping and identification of dispersion of sand fly vector species can act as a help guide to direct active control measures to the identified high-risk foci and, eventually, lead to reduction in incidence of the disease. Therefore, considering importance of identification of sand fly species in vector control measures and CL management (e.g., health authorities need for surveillances and leishmaniasis control measures), the present study aimed to determine the fauna and geographical distribution of sand flies in Qom province by using GIS software.

Materials and Methods

Study Area

The Qom Province is bounded by Tehran Province in the north, Isfahan Province in the south, Semnan Province in the east, and Markazi Province in the west, with an area of ~11,240 square kilometers (0.68% of total area of Iran; Fig. 1). This study was performed from April to November 2015 in Qom city and 15 villages (Khor abad, Sarm, Ghobadbezan, Mir abad, Malek Ghaleh, Qomrood, Zavarian, Rahjerd, Khadijeh Khatoon, Tagharoood, Pachian, Mahmood Abad, Chahak, Kohandan, and Mehrzamin) belonging to districts of Kahak, Markazi, Salafchegan, Jafar Abad, and Khalajestan, covering all corners of Qom Province (34° 09'–35° 11' N latitude and 50° 06'–51° 58' E longitude, with the elevation of almost 1,500 m above sea level). Average annual minimum and maximum temperature is 16.5 °C and 49 °C in January and July, respectively. Total annual rainfall is about 150 mm. Relative humidity is about 84% and 28% in December and June in the region, respectively (Iran Meteorological Organization 2015).

Specimen and Data Collection

In terms of geography and topography, the province was divided into a city and five districts. Considering previous research in the province and also available data about topography and meteorological factors, ecological factors, sand fly abundance in previous years, and cutaneous leishmaniasis incidence rate, the required villages

Fig. 1. Study locations. Each dot shows the sampling locations. Distance ranged from 56 km (Markazi) to 160 km (Markazi and Khalajestan).
were chosen from each district. Some of the chosen villages are located in desert and some in mountainous areas. Three villages were randomly selected from each district. In each village, three houses were randomly chosen from the entrance, the middle, and the end-point of the village. In each house, 10 indoor (bedroom, bathroom, toilets, hall, and stables) and outdoor (rodent burrows) traps were set. These traps were also set in three predetermined points within the Qom city. Sand flies were collected biweekly from all those rural and urban places using 30 sticky paper traps (castor oil-coated white paper 20 by 32 cm). The collection process continued from the first half of April 2015 to the first half of November 2015. Distance between the districts and Qom city ranged from 56 km (Markazi district) to 160 km (Markazi and Khalajestan districts; Map 1).

The caught sand flies were transferred to a laboratory in Qom Health Center. For identification of species, the head and last two abdominal segments of the sand flies were mounted in Paris medium (Smart et al. 1965) and identified after 24–72 h, using the morphological characters (Theodor and Mesghali 1964). Molecular tests were used to diagnose infection in the collected sand flies.

The global positioning system (GPS) is used to minimize uncertainty in geographical studies. Calculation of spatial features allows data to be matched to appropriate resolution of remote sensing data and also for modeling or other spatial analyses (Foley et al. 2009). In the present study, trapping sites were at first located precisely by GPS in all rural districts of Qom province villages and their coordinates were recorded. Based on GPS calculations, 15 villages were selected from five districts and sand flies were collected from those villages. Then, species were mapped on urban and rural digital maps based on a scale of 1/50,000. All analyses were undertaken with rural- and urban-level precision, i.e. rural and urban levels were our basic units of analysis. After identification of sand flies, the high-risk foci of leishmaniasis were determined. For data analyses, ArcGIS 9.3 software was used to determine the foci with leishmaniasis vector species.

Results

A total of 10,461 sand flies were collected using sticky paper traps—6,979 (66.71%) from outdoors and 3,482 (33.29%) from indoor resting places. Two genera (Phlebotomus and Sergentomyia) and 14 species were identified, including Phlebotomus (Phlebotomus) papatasi (52.38%), P. (Paraphlebotomus) sergenti (7.59%), P. (Paraphlebotomus) alexandri (1.25%), P. (Paraphlebotomus) caucasicus (1.01%), P. (Paraphlebotomus) caucasicus group (Females) (7.50%), P. (Larroussius) kandelaki (4.90%), P. (Larroussius) tobbi (0.11%), P. (Larroussius) major (3.89%), P. (Adleriuss) halepensis (0.33%), P. (Adleriuss) brevis (0.04%), P. adleriuss group (Females) (0.13%), Sergentomyia (Sergentomyia) sintoni (20.06%), S. (Sergentomyia) theodori (0.77%), and S. (Rondanomyia) pauloweski (0.03%) (Table 1). Details about the number and monthly activity of P. papatasi and S. sintoni are illustrated in Figs. 1 and 2. Interestingly, P. halepensis was not captured from outdoors and S. pauloweski from indoors. The month-wise density of P. papatasi and S. sintoni in study areas are shown in Figs. 2 and 3. The sex ratio of P. papatasi was 81.42% and 63.27% in outdoors and indoors, respectively. The rate was 102.1% in outdoors for S. sintoni. In this region of Iran, the P. papatasi monthly activity started from April to November (when density of sand flies reach to zero). Also its’ activity had two peaks in early Jun and late July (Fig. 2). Monthly activity of S. sintoni in Qom Province started in April and ended in November (when density of sand flies reach to zero). Also, it had two peaks in late May and late July (Fig. 3). Also sand fly species presence/absence and their infection with Leishmania parasite by district in Qom province was displayed (Table 2). As maps show, P. papatasi was reported from almost all study areas of the province except for Khalajestan district (Fig. 4). L. major was identified from these species in Markazi and Kahak districts by using molecular methods in previous studies (Saghafipour et al. 2016). The green color in the all illustrated maps points to no report of a sand fly species from those districts, the yellow points to reports of collection, and the red color represents the areas where sand fly species were found to be positive to the Leishmania parasite. Phlebotomus sergenti was collected from all areas of Qom Province. But leishmania infection was not found in this species (Fig. 4). Phlebotomus alexandri, P. caucasicus, and P. caucasicus group (Females) were collected from all studied areas, but no Leishmania infection was found in these species across province (Figs. 5 and 6). Phlebotomus kandelaki and P. tobbi were collected from Kahak and Khalajestan districts, but P. major was only collected from Khalajestan district (Figs. 6 and 7). Phlebotomus halepensis, P. brevis, and P. (Adleriuss) group

Table 1. The fauna and the number of collected sand flies from the endemic area of ZCL in Qom province, central Iran, 2015.

<table>
<thead>
<tr>
<th>Site</th>
<th>Male No. (%)</th>
<th>Female No. (%)</th>
<th>Total No. (%)</th>
<th>Male No. (%)</th>
<th>Female No. (%)</th>
<th>Total No. (%)</th>
<th>Male No. (%)</th>
<th>Female No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. papatasi</td>
<td>1675(45.79) 1983(54.21) 3658 (100)</td>
<td>816 (44.81) 1005 (55.19) 1821 (100)</td>
<td>23.82</td>
<td>2491</td>
<td>2988 (25.86) 5479 (52.38)</td>
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<tr>
<td>P. sergenti</td>
<td>121 (39.16) 188 (60.84) 309 (100)</td>
<td>382 (78.76) 103 (21.24) 485 (100)</td>
<td>503 (4.81)</td>
<td>291 (2.78) 794 (7.59)</td>
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</tr>
<tr>
<td>P. alexandri</td>
<td>22 (62.86) 13 (37.14) 35 (100)</td>
<td>27 (28.13) 69 (71.87) 96 (100)</td>
<td>49 (0.47)</td>
<td>82 (0.78) 131 (1.25)</td>
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<tr>
<td>P. brevis</td>
<td>45 (100)</td>
<td>61 (100)</td>
<td>106 (1.01)</td>
<td>106 (1.01)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. major</td>
<td>3 (100)</td>
<td>0 (0)</td>
<td>3 (100)</td>
<td>3 (100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. major</td>
<td>15 (28.30) 38 (71.70) 53 (100)</td>
<td>105 (29.66) 249 (70.34) 354 (100)</td>
<td>408 (3.90)</td>
<td>105 (1) 513 (4.90)</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>P. kandelaki</td>
<td>173 (80.46) 42 (19.54) 215 (100)</td>
<td>235 (78.86) 63 (21.14) 298 (100)</td>
<td>3 (0.03)</td>
<td>9 (0.09) 12 (0.12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. tobbi</td>
<td>15 (28.30)</td>
<td>249 (71.70)</td>
<td>304 (100)</td>
<td>120 (1.15)</td>
<td>128 (1.01)</td>
<td>248 (2.06)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. sintoni</td>
<td>1007 (51.12) 963 (48.88) 1970 (100)</td>
<td>50 (39.06) 78 (60.94) 128 (100)</td>
<td>1057 (10.10)</td>
<td>1041 (9.96) 2098 (20.06)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. theodori</td>
<td>30 (56.60) 23 (43.40) 53 (100)</td>
<td>19 (67.86) 9 (32.14) 28 (100)</td>
<td>49 (0.46)</td>
<td>32 (0.31) 81 (0.77)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. pauloweski</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3092 (44.29) 3857 (55.71) 6979 (100)</td>
<td>1732 (49.74) 1730 (50.26) 3482 (100)</td>
<td>4824 (46.11)</td>
<td>5637 (53.89) 10461 (100)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
were only collected from Khalajestan district. However, the infection of these sand flies with *Leishmania* was not detected (Figs. 8 and 9). Although *S. sintoni*, *S. theodori*, and *S. pawlowski* were collected from all districts, *Leishmania* infection was not observed in this region (Figs. 9 and 10).

**Discussion**

In this entomological study of phlebotomine sand flies, two genera (*Phlebotomus* and *Sergentomyia*) and 14 species of these sand flies were identified. Sand flies fauna had many similarities with obtained results by Hesam–Mohammadi et al. in Kashan city, eastern neighbor of Qom Province (Hesam-Mohammadi et al. 2014). Similarity of the sand flies population composition between Qom province and the neighboring cities can be due to climate conditions similarities. Monthly activity of sand flies in Qom Province starts in April and ends in November when density of sand flies reaches to zero. This issue resembles with many areas of central Iran such as Isfahan (Zahraei-Ramazani and Leshan 2016), Yazd (Jafari et al. 2013), Kashan (Hesam-Mohammadi et al. 2014) and so on. In our study, geographical and digital database distribution of sand fly species was provided by GIS software. In recent years, GIS was used in medical studies to determine special distribution of some diseases, to identify breeding places of vectors, to determine reservoir of some zoonotic diseases, and to map trend of diseases and their effective factors in Iran (Pirmoradi et al. 2012, Hosseini Chagini and Salahi-Moghaddam 2009, Sedaghat and Salahi Moghaddam 2010). The following studies are examples of entomological studies conducted in Iran using GIS: modeling the distribution of cutaneous leishmaniasis vectors in Iran during 1990–2013 (Hanafi-Bojd et al. 2015a); mapping spatial and temporal distributions of phlebotomine sand
flies in Iran during 1930–2012 (Karimi et al. 2014); and prediction of distribution of visceral leishmaniasis vectors in Iran (Hanafi-Bojd et al. 2015b). The use of GIS and generation of objective maps can greatly help health staffs to effectively use health data for planning control programs, interventional measures, and for evaluation of these programs (Mott et al. 1995, Haghdoost et al. 2007). Humbly put, the most important vantage point of this study was to collect and classify decentralized information about sand fly species as digital database in Qom Province, which accelerates and eases the data searching. Based on geographical distribution maps, although P. papatasi was collected and identified from almost all studied districts of Qom Province except for Khalajestan district, Leishmania infection was only observed in P. papatasi that was collected from Markazi and Kahak districts by using molecular methods in previous studies (Rassi et al. 2011, Saghafipour et al. 2016). The P. papatasi species is the main sand fly vector of ZCL in Iran (Yaghoobi-Ershadi 2012). Phlebotomus sergenti was collected from all areas of Qom Province, but leishmani infection was not detected in this species. It was previously shown that P. sergenti acts as the vector of Anthroponotic Cutaneous Leishmaniasis (ACL) in some areas of Iran. Parasitological surveys have reported of infection of this sand fly in some areas of the country (Parvizi and Ready 2008, Oshaghi et al. 2010). Phlebotomus alexandri, P. caucasicus, and P. caucasicus group were collected from all studied areas, but Leishmania infection has not been reported among these species in this province so far. Previous studies have shown that P. caucasicus group has a role in zoonotic cycle of cutaneous leishmaniasis among rodents in Iran (Yaghoobi-Ershadi 2012). Phlebotomus kandelakii

<table>
<thead>
<tr>
<th>Site</th>
<th>Species</th>
<th>Presence/absence</th>
<th>Infection/ no infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qom county</td>
<td>P. papatasi</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>Markazi district</td>
<td>P. tobbi</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>Kahak district</td>
<td>P. major</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Jafarabad district</td>
<td>P. brevis</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Salafchegan district</td>
<td>P. (adlerius) group</td>
<td>○</td>
<td>●</td>
</tr>
<tr>
<td>Khalajestan district</td>
<td>P. sintoni</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>Qom county</td>
<td>P. sergenti</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Markazi district</td>
<td>P. sergenti</td>
<td>●</td>
<td>○</td>
</tr>
<tr>
<td>Kahak district</td>
<td>P. sergenti</td>
<td>○</td>
<td>●</td>
</tr>
<tr>
<td>Jafarabad district</td>
<td>P. sergenti</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Salafchegan district</td>
<td>P. sergenti</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>Khalajestan district</td>
<td>P. sergenti</td>
<td>○</td>
<td>○</td>
</tr>
</tbody>
</table>

Presence=●, absence=○.
Infection=●, no infection=○.

The presence of the sand flies in this study was depicted by determination of the fauna of sand flies in Qom province. The infection with Leishmania parasites was detected in two districts of Markazi and Kahak districts. Previous molecular studies reported Leishmania parasites infection in Markazi district (Rassi et al. 2011). In a molecular study (PCR-ITS1), Leishmania parasites infection was detected in Kahak district (Saghafipour et al. 2016). Leishmania infection has not been reported in other districts of Qom province so far.
Fig. 5. The richness and spatial distribution of *P. alexandri* (left) and *P. caucasicus* (right) and their infection with *Leishmania* parasites in Qom Province, central Iran, 2015.

Fig. 6. The richness and spatial distribution of *P. caucasicus* group (Females) (left) and *P. kandelakii* (right) and their infection with *Leishmania* parasites in Qom Province, central Iran, 2015.

Fig. 7. The richness and spatial distribution of *P. tobbi* (left) and *P. major* (right) and their infection with *Leishmania* parasites in Qom Province, central Iran, 2015.
Fig. 8. The richness and spatial distribution of *P. halepensis* (left) and *P. brevis* (right) and their infection with *Leishmania* parasites in Qom Province, central Iran, 2015.

Fig. 9. The richness and spatial distribution of *P. (Adlerius)* group (left) and *S. sintoni* (right) and their infection with *Leishmania* parasites in Qom Province, central Iran, 2015.

Fig. 10. The richness and spatial distribution of *S. theodori* (left) and *S. pawlowski* (right) and their infection with *Leishmania* parasites in Qom Province, central Iran, 2015.
and *P. tobbi* were collected from Kahak and Khalajestan districts, and *P. major*, *P. halepensis*, *P. brevis*, and *P. (adlerius)* group were only collected from Khalajestan district. The infections of these sand flies with *Leishmania* were also not detected. *Phlebotomus kandeli-kii* and *P. major* have been reported as probable vectors of visceral leishmaniasis in some areas of Iran (Seyedi-Rashki et al. 1995, Azizi et al. 2008, Sanei Dehkordi et al. 2011, Rassi et al. 2012). Some species of genus *Sergentomyia* sand flies are capable of transmitting lizarid leishmaniasis agents. There are reports of infection in three sand fly species of *S. sintoni*, *S. dentata*, and *S. clyde* (Karimi et al. 2014). Moreover, *S. sintoni*, *S. dentata*, and *S. clydes* have been reported as vectors of lizarid leishmaniasis in Iran (Parvizi and Ready 2008; Rassi et al. 1997, 2011; Oshaghi et al. 2009). Although *S. sintoni*, *S. theodori*, and *S. paulovoskii* were collected from all districts in this study, *Leishmania* infection was not detected in this province. This study showed that by using GIS, one can integrate descriptive information of sand flies with geo-spatial information. Moreover, it was shown that by providing digital maps we can easily determine geographical distribution of sand flies in different districts of a province. In addition, by categorizing the rural districts and identifying their species, not only can we detect the high-risk areas for disease transmission, but also we can predict formation of new foci by classification and detection of inhabitation of each species in different district. All these processes can finally show us the areas of high priority for intervention. As the analyses revealed in this study, rural districts in Qom province can be categorized into three groups—infestation report, no infection, and no report. Based on GIS analyses, Kahak and Markazi districts were identified as high-risk foci for leishmaniasis vector species in Qom province. Considering the present situation, disease control plans and health education for people is necessary.

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