IDENTIFICATION OF COLLECTED SAND FLIES FROM ROFAYYEH AREA, SOUTH WEST OF IRAN

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ABSTRACT: An assay was conducted to determine the sand fly fauna in Rofayyeh city, where is situated in common border line with Iraq country, in 2009. Totally, 1420 Phlebotomous and 117 Sergentomyia were collected using sticky traps from outdoor and indoor resting places. In present study P. papatasi, P. alexandri, P. caucasicus, P. mongolensis and S. sintoni were identified. P. papatasi (69.68%) and P. alexandri (12.36%) were dominant species, respectively. The highest sex ratio was found in P. caucasicus which was collected in outdoor resting place. Further epidemiological research should be done to investigate vectors and reservoirs of Zoonotic Cutaneous Leishmaniasis (ZCL) in this area for prevention of disease outbreak.

Key words: Sand fly fauna-Zoonotic Cutaneous Leishmaniasis-Khuzestan, Iran.

INTRODUCTION

Sand flies are biological vectors of Leishmania species in human and between human and animals in the old world and new world. Different species of Leishmania, a Protozoa, cause leishmaniasis in man and animals. Three forms of leishmaniasis including: Zoonotic Cutaneous Lieshmaniasis (ZCL), Anthroponotic Cutaneous Lieshmaniasis (ACL) and Visceral Lieshmaniasis (VL) have been causing some health and medical problems related to humans and animals in Iran and its adjoining countries: Iraq, Afghanistan, Pakistan and Turkey. Cutaneous leishmaniasis (CL) due to Leishmania major is endemic in Iran and causes a major public health problem in 17 of 31 provinces of Iran including Khuzestan, a south west province (Rassi et al. 2004, Shakila et al. 2006, Akhavan 2007, Simsek 2007, Azizi and Fekri 2010, Saki and Khademvatan 2011, Yaghoobi-Ershadi 2012). Vazirianzadeh et al. (2013) as the latest molecular Leishmaniasis study in Khuzestan have isolated L.major from both reservoir and vector, Tatera indica (Rodentia: Gerbillidae) and P.papatasi at the same time from Roffaye area.

The status of sand flies in the different parts of Khuzestan province has been published by Nadim et al. (1974), Javadian and Nadim (1975), and Yaghoobi-Ershadi (2012). In their research 20 species of sand flies have been reported of two genera Phlebotomus and Sergentomyia. Javadian and Nadim have added two more species of Sergentomyia to the list of sand flies in this province in their study (Javadian and Nadim 1975). In another study, natural leptomond infection was found in P. papatasi, P. alexandri and S. sintoni which were collected from rodent burrows in some parts of this region (Javadian and Mesghali 1974). In a research study, three species of Phlebotomus were identified in two large marshlands of Khuzestan province (Jahanifard et al. 2009). In the latest study of sand flies in Khuzestan, Kavarizadeh et al. (2011) have recognized four species of Phlebotomus sand flies from Maleh area, Shoush County in the north of Khuzestan province. All sand fly species in Kavarizadeh et al. (2011) and (Jahanifard et al. 2009) studies have already been reported from the other parts of Iran.

Estimations are showing an increasing in the rate of ZCL cases in the region among the human population including army staff in border line with Iraq during 2005-2008 (Jahanifard, 2010). Therefore, in respect to importance of ZCL, abundance of sand flies, high density of rodent (reservoir) and heavy infection (Nadim 1994) in this area we arranged a new study on sand fly identification in Rofayyeh. In fact, there has been little survey about species composition of Phlebotominae in this border line of Rofayyeh area. Therefore, the purpose
of this research was to determine the composition of sand flies in Rofayyeh area, west part of Khuzestan province, Iran. The special objectives of the present study regarding to the sand flies were to determine the species diversity, relative population density and sex ratio of sand flies in Rofayyeh area. These factors provide basic epidemiologic information to make vector control programs to reduce the incidence of ZCL in the region.

MATERIALS AND METHODS

Study area

Dasht-e-Azadegan County is located in North West of Ahvaz near to Iran-Iraq border grounds. Latitude and longitude of this county are 31Ú 33' N and 48Ú 10' E, respectively. Altitude from sea level is 10 m. It involves three regions: central, Hoveyzeh and Boustan. Hoveyzeh region has two cities: Rofayyeh and Hoveyzeh. Rofayyeh (31Ú 55' N, 47Ú 40') is situated in 25 km from west of Hoveyzeh and 50 km from southwest of Susangerd city, the center of Dasht-e-Azadegan, in border line between Iran and Iraq (Fig 1). There is not any synoptic meteorology station in Roffayeh, therefore month average of environmental temperature and relative humidity of Boustan and Ahvaz and are considered as meteorology information rather than Roffayeh area, in the present study. The month average of temperature(ºC, June-September) in Boustan (1986-2005) and Ahvaz(1957-2005) have been recorded : 33.30±1.26, 35.50±1.37,35.10±1.43 , 31.20 ±0.87 and 35.10±1.01,37.1 ±1.11,36.5±1.28 and 32.8 ±1.01,respectiveley. The month average of relative humidity(%, June-September) in Boustan (1986-2005) and Ahvaz (1957-2005) have been recorded : 28, 29, 30,31and 23, 24,28 and 29, respectiveley (I.R OF IRAN METEOROLOGICAL ORGANIZATION (IRIMO,2013).

Sand flies sampling and identifying

Sand flies were captured using 580 of sticky traps (papers 20 cm x 30 cm coated with castor-oil) from outdoor (rodent burrows) and indoors (stables and trench) once a month during June till September 2009. Trapping was conducted in two different rural areas of Rofayyeh including two indoor places per area (30 sticky traps per place) and 10 sticky traps for outdoors in each areas. The sticky traps were set before sunset and gathered in the next morning before sunrise. All traps were transferred to Razi Vaccine and Serum Research Institute in Ahvaz. The specimens were removed by needle and washed in acetone then they reserved in ethanol 70%. For preparation of permanent microscopy slides, the samples were mounted by the use of Puri’s medium. The sand flies were identified using the external and internal morphological characteristics in females and males (Theodor and Mesghali 1964). First, sex of all specimens was determined. Then, they were identified by species level using the keys of Lewis (1982), Nadim and Javadian (1976) and Nadim et al. (1994). Finally, sex ratios of the predominant species were determined by formula of number of males / females × 100 (Kakarsulemankhel 2010).Sex ratios are calculated based on the female population, therefore, the larger size of vector sand fly female population provides more probability of leishmania transmission in a region. It should be mentioned that because females two species of P. mongolensis and P. caucasicus are not morphologically distinguishable from each other, therefore when from one site only males of P. mongolensis or P. caucasicus were collected, the collected females were indentified as same as male species.

RESULTS

In this entomofaunistic study, totally 1537 sand flies (31.75% males and 68.25% females) were collected during four months from Rofayyeh area. In the current research study five species of sand flies were recognized, four belonging to Phlebotomus and one belonging to Sergentomyia genera. The most frequent Phlebotomos species was P. papatasi Scopoli 1786, followed by P. alexandri Sinton 1928, P. caucasicus Marzinowsky 1917, P. mongolensis Sinton; Theodor, 1958 and S. sintoni Pringle 1953 with 69.68%, 12.36%, 7.35%, 2.99% and 7.62%, respectively. Table 1 presents the sex ratio of the sandfly specimens in outdoor and indoor resting places, Rofayyeh area of Khuzestan province. The highest sex ratio was observed in P. caucasicus which was collected in outdoor resting place. P. papatasi was recognized as dominant species of the region that formed 57.61% and 42.39% of indoor and outdoor traps, respectively. The results are presented in details in Figure 2.

Table 1 shows the sex ratio of the sandfly specimens in outdoor and indoor resting places, Rofayyeh area of Khuzestan province. Figure 2 illustrates the abundance of Phlebotomus species in outdoor and indoor resting places.

DISCUSSION

Khuzestan is an endemic focus of ZCL (Saki and Khademvatan 2011). Forty seven years ago, a survey was carried out for determining human infection rate in Dezful, Susangerd and Dasht-e-Mishan. These rate was calculated 25%,10%, and 5-10% for mentioned areas, respectively. Furthermore, in Mussian area, north of Dasht-e-Mishan, most of soldiers who were in military service infected by Leishmania major (Nadim et al. 1974).
Nadim et al. (1974) published a paper about sand flies in southern Iran in which they showed the majority of the captured sand flies were *P. papatasi*. It is interesting that similar result was found in the current study regarding *P. papatasi* density. Jahanifard et al. (2009) have pointed to three species (*P. papatasi, P. alexandri* and *P. mongolensis*) in their study which were collected using insect collecting net from Hovizeh and Shadegan Marshlands of Khuzestan province.

Our finding determined that the density of *P. alexandri, P. mongolensis, P. caucasicus, P. sintoni* and *S. sintoni* were high in rodent burrows. This result was accordance to this fact that *P. papatasi* is the main vector of ZCL to human, whilst *P. alexandri, P. andrejevi, P. caucasicus, P. mongolensis* and *P. ansari* are vectors among rodents in rural areas (Nadim and Faghih 1968, Javadan et al. 1977, Nadim et al. 1994, Yaghoobi-Ershadi et al. 1994, 1995).

It is important that *P. papatasi* is the main vector of Cutaneous Leishmaniasis among countries in neighboring or near to Iran such as Afghanistan (Nadim et al. 1979), Saudi Arabia (Killick-Kendrick et al. 1985), Kuwait (Lane and Al-Taql 1983) and Iraq (Coleman et al. 2007). In addition, it has known as the main vector of ZCL in the old world (Gramiccia and Gradoni 2005). The highest collected specimens in this essay were *P. papatasi*.
Findings of the current study is supported by that of Jahanifard et al. (2009), Kavarizadeh et al. (2011) and Kavarizadeh et al (2013) who found these two species as the most frequent samples in two large marshlands of Khuzestan province, Maleh area of Shoush (Khuzestan province) and Musian district of Ilam province (South west of Iran), respectively.

P. papatasi which normally prefers to live in plains area rather than in mountains (Belen et al., 2004, Rassi and Hanafi-bojd, 2006), has been collected from all parts of Iran including Rofayyeh (10 m above level of sea) from both indoor and outdoor places. In the current study this species was collected from Rofayyeh area where the maximum and minimum temperature were recorded 44.3°C in July and 31.7°C in September, respectively (I.R OF IRAN METEOROLOGICAL ORGANIZATION (IRIMO, 2013). From both ecologic points including sea level and temperature of environment, it is concluded that this species has a wide ecological valance.

However, Rassi and Hanafi-bojd (2006) have appointed P. alexandri as a rare species in different areas of Iran including plain and highlands, our findings of the current study showed that it was the most frequent species after P. papatasi in Rofayae. Studies of Jahanifard et al. (2009), Kavarizadeh et al. (2011) and Kavarizadeh et al (2013) support the related result of our study in Rofayye. It is assumed that the presence P. alexandri in the present study is accordance to the second explanations of Rassi and Hanafi-bojd (2006) which indicated this species was fitted to live in the regions with high percentage of relative humidity and warmer niches, because Rofayye meets both mentioned environmental conditions.

*Sergentomyia sintoni* has been reported from different parts of Iran (Azizi et al, 2010). However, they were with many other species of *Sergentomyia*, but in the current study only this species has been reported. It is assumed that this finding has come from small sampling of the current study and restriction of seasonal sampling.

Moreover, *P. papatasi* was dominant sand flies which were collected in indoor resting places during the present study. It shows that *P. papatasi* is a predomestic, endophilic and endophagic species. Therefore, elimination of *P. papatasi* population in this area is a recommended health protocol as Khuzestan is recognized as an endemic focus of ZCL. However, control important of this species is not only due to transmission of zcl, but also referred to transmission of sandfly fever as an arbovirus disease in middle east (Colacicco-Mayhugh et al, 2010). In this term control population of *ph. alexandri* is also important because this sandfly is recognized as vector of VL (Colacicco-Mayhugh et al, 2010).

Finally, it is requested with respect to two forms of cutaneous and visceral leishmaniasis in Iraq, existence of *P. papatasi* in this country, having border line and similar weather to the south west of Iran and reporting natural leptomonad infection in *P. papatasi* and *P. alexandri* (Javidian and Mesghali 1974), more epidemiological investigation is needed to determine vectors and reservoirs of disease in this area, using molecular method.

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REFERENCES


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Table 1: The sex ratio of the sandfly specimens in outdoor and indoor resting places, Rofayyeh area of Khuzestan province.

<table>
<thead>
<tr>
<th>Species</th>
<th>No.</th>
<th>Sex ratio</th>
<th>Outdoor</th>
<th>Indoor</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. papatasi</td>
<td>1071</td>
<td>45.98</td>
<td>28.27</td>
<td></td>
</tr>
<tr>
<td>P. caucasicus</td>
<td>113</td>
<td>196.55</td>
<td>not calculated</td>
<td></td>
</tr>
<tr>
<td>P. mongolensis</td>
<td>46</td>
<td>not calculated</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>P. alexandri</td>
<td>190</td>
<td>26.47</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>S. sintoni</td>
<td>117</td>
<td>40.96</td>
<td>not calculated</td>
<td></td>
</tr>
</tbody>
</table>