



## Nocturnal Activity, Monthly Leptomonad Infection, Parity Rate and Physiological Status Vectors of Zoonotic Cutaneous Leishmaniasis (Diptera: Psychodidae) in Southeastern Iran

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### ABSTRACT

**Background:** *Phlebotomus papatasi* and *P. salehi* are reported as vectors of cutaneous leishmaniasis in southeast of Iran. Seasonal activity of these species is different in zoogeographical zones and must be known well for better management of the disease.

**Objectives:** To determine the nocturnal activity of sand flies and their leptomonad infection rates.

**Materials and Methods:** A cross-sectional study was carried out in Chabahar county, where sand flies were collected using sticky-paper traps at 2 - hours intervals from rodent burrows during May to October. Females of *Phlebotomus* spp. were dissected for leptomonad infection.

**Results:** A total of 9367 sand flies from four species of *Phlebotomus* and *Sergentomyia* genera were collected. The most sand flies were collected in the first third of the night (7:00-11:00 PM), although there was seasonal variation in the nocturnal activity of different species. A total of 1132 female sand flies from two species of *P. papatasi* and *P. salehi* were dissected. Totally 2.1% of *P. papatasi* and 1.07% of *P. salehi* were found to have leptomonad infection in their midgut, pharynx or head. The infection was observed in September and October in *P. salehi*, but June, August, September and October for *P. papatasi*.

**Conclusions:** Knowledge about nocturnal activity and biting rhythms of sand flies and therefore the risk of disease transmission is important for planning control programs for cutaneous leishmaniasis (CL).

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### ► Implication for health policy/practice/research/medical education:

Decision makers in health system can do their best for control of vector-borne diseases, when different particles of transmission puzzle are defined. An important particle in the case of cutaneous leishmaniasis in the study area is clarified which helps for better managing control programs.

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## 1. Background

Leishmaniasis is an important public health problem in the world. The disease have cutaneous and visceral forms in Iran (1). Phlebotomine sand flies (Diptera: Psychodidae) are introduced as vectors of leishmaniasis (2). There is a list of 54 reported species from the country (3), some of them have been found to be infected with *Leishmania* spp. *Phlebotomus papatasi*, *P. salehi*, *P. sergenti* and *P. caucasicus* group are reported as vectors of cutaneous leishmaniasis in Iran, although the last one is responsible to circulate the infection in the rodent population (4-13).

Chabahar county is a focus of cutaneous leishmaniasis in southeast of Iran. There are some studies on the disease and sand flies fauna of this area (14, 15). Because the nocturnal activity of sand flies and therefore their biting times has a critical role in the disease transmission and planning the control programs as well, this study was conducted to answer some questions in this field.

## 2. Objectives

This study aimed to determine the seasonal fluctuation of leptomonads infection in sand flies, their nocturnal activity in the study area, their physiological age during the study period and the seasonal changes in age groups of sand flies in Chabahar county, Sistan Va Baluchistan province, Southeastern of Iran.

## 3. Materials and Methods

### 3.1. Study Area

Chabahar county (25° 17' 31" N, 60° 38' 35" E) is situated on the Makran coast of the Sistan Va Baluchistan province of Iran, 9m above the sea level, and is officially designated as a Free Trade and Industrial Zone by Iran's government. The county has a warm humid weather in the summer and a temperate weather in the winter (Statistical Center of Iran and Iran Meteorological Organization). It has an average maximum temperature of 34 °C and an average minimum temperature of 10.5 °C. Chabahar County is subdivided into three districts, has two cities and 592 inhabited villages (Statistical Center of Iran and Iran Meteorological Organization)

### 3.2. Nocturnal Activity of Sand flies

Considering the importance of biting times of sand flies and therefore the probable times of the parasite transmission, sand flies were collected by sticky paper traps from the rodent burrows during May to October in Polan village (Figure 1). Trapping was conducted once a month at two hours intervals, from 7:00 PM till 7:00 AM of the next day. The number of traps for each interval was 15 and they were changed every 2 hours. The attached sand flies were removed by needle, washed in acetone and then conserved in ethanol 70%. In the laboratory the

permanent mounts were prepared using Puri's medium and identified by the relevant keys of sand flies (16). Sex ratio, (i.e. the rate of males per 100 females) was calculated for all species.



Figure 1. A View of Rodent Burrows in the Study Area, Southeast of Iran

### 3.3. Physiological Age, Leptomonad Infection Rates and Parity Status

During June to October, sand flies were collected from rodent burrows of Negor, Polan and Nobandian villages by using sticky paper traps. Females were dissected for leptomonad infection in their head and midgut. Giemsa staining was used for staining of the positive slides, and then they were examined under light-microscope using 40X and 100X objectives. During the dissection, accessory glands were observed for parity status of sand flies, while abdominal status was also considered and recorded as unfed, blood-fed, semi-gravid and gravid.

## 4. Results

A total of 9367 sand flies were collected from the Polan village. Males (55%) were more than females (45%). Four species were identified as *P. papatasi* (47.9%), *P. salehi* (6.8%), *Sergentomyia clydei* (40%) and *S. sintoni* (5.3%). The total peak of activity was found to be at 7:00 to 9:00 PM, while only in August it was at 1:00 to 3:00 AM (Figure 2).

Sex ratio was 157.5, 110.3, 112.2 and 23.7 for *P. papatasi*, *P.*

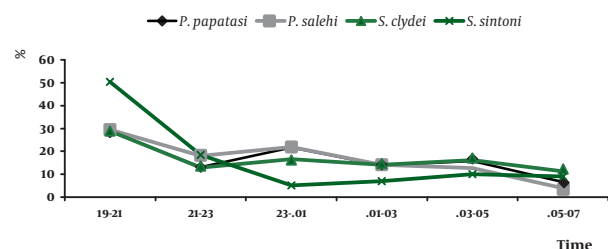


Figure 2. Nocturnal Activity of Collected Sand flies in Polan Village, Chabahar district, Southeastern Iran

Table 1. Seasonality of Leptomonad Infection Rates and Other Physiological Aspects of *P. papatasi*, Chabahar County

	Infected, No.	Infection rate,%	Dissected, No.	Age Group			Physiological status				Place of leptomonad infection		
				Parous	Nulli-parous	Unclear	Unfed	Blood-fed	Semi-gravid	Gravid	Midgut	Pharynx	Mouthpart
2 <sup>nd</sup> June	1	3.1	32	11	19	2	16	5	3	8	1	0	0
1 <sup>st</sup> July	0	0	49	14	34	1	28	7	5	9	0	0	0
2 <sup>nd</sup> July	0	0	79	21	52	6	38	16	12	13	0	0	0
1 <sup>st</sup> Aug	0	0	76	19	54	3	43	10	9	14	0	0	0
2 <sup>nd</sup> Aug	4	6.9	58	26	21	11	25	14	7	12	4	1	1
1 <sup>st</sup> Sep	0	0	50	25	21	4	13	11	10	16	0	0	0
2 <sup>nd</sup> Sep	3	2.5	121	70	39	12	37	17	22	45	3	1	1
1 <sup>st</sup> Oct	6	3	202	131	60	11	56	28	45	73	6	3	2
<b>Total</b>	<b>14</b>	<b>2.1</b>	<b>667</b>	<b>317</b>	<b>300</b>	<b>50</b>	<b>256</b>	<b>108</b>	<b>113</b>	<b>190</b>	<b>14*</b>	<b>5</b>	<b>4</b>

\* In some specimens we found the leptomonad in different parts of the body

*salehi*, *S. clydei* and *S. sintoni*, respectively. During the first third of the night (7:00 to 11:00 PM) 43.7% of sand flies were collected (57.5% male, 42.5% female). This value was 35.5% (56.2% male, 43.8% female) for the second third and 23.8% (48.8% male, 51.2% female) for the last third of the night. The majority of sand flies for each species were collected in the first third of the night (7:00 to 11:00 PM), followed by the second (11:00 PM-3:00 AM) and last thirds (3:00-7:00 AM). Only one exception observed about *S. sintoni*, where the number of collected specimens in the last third of the night was higher than the second one.

A total of 1132 female sand flies belonged to genus *Phlebotomus* were collected from Negor, Polan and Nobandi-an villages, and dissected for leptomonad infection; 41.1% were *P. salehi* and 58.9% *P. papatasi* (Figure 3). In the case of *P. papatasi* 47.5% were parous, 44.9% nulliparous, also the physiological age of 7.6% was not clear (Table 1). The analysis of physiological status of dissected *P. papatasi* revealed 38.4% unfed, 16.2% blood-fed, 16.9% semi-gravid and 28.4% gravid. The highest rate of gravid and parous *P. papatasi* was collected in first half of October (Table 1).

In *P. salehi* the parity rate was as follow: 53.3% parous, 39.4% nulliparous and 7.3% unclear. The physiological status of this species showed 50.9% unfed, 9.9% blood-fed, 8.4% semi-gravid and 30.8% gravid (Table 2). About the *P. salehi* gravidity and parity rates were found to be highest in first half of October (Table 2).

Totally 2.1% of 667 dissected *P. papatasi* were found to be infected with leptomonad flagellates of *Leishmania* spp (Table 1). This rate was 1.07% out of 456 for *P. salehi* (Table 2). The infection rate was observed in first and second halves of September (1.8%, 3.3%) and also October (0.6%) in *P. salehi*, but about *P. papatasi* were 3.1%, 6.9%, 2.5% and 3%, in June, August, September and October respectively.

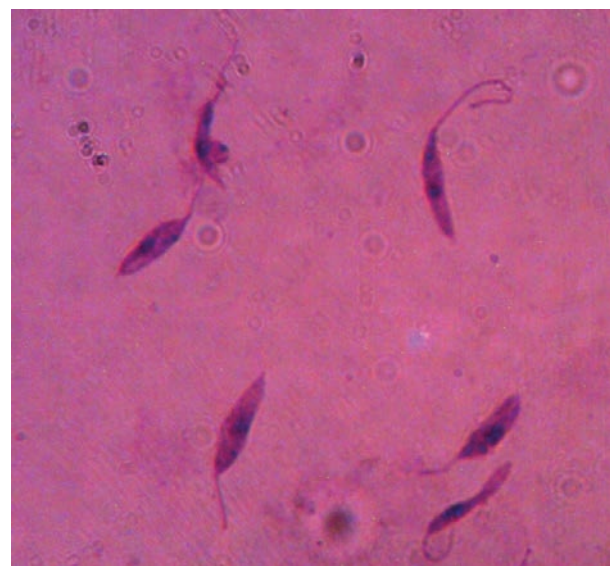


Figure 3. Leptomonad Infection in Sand flies of Chabahr District, South-east of Iran

Table 2. Seasonality of Leptomonad Infection Rates and Other Physiological Aspects of *P. salehi*, Chabahar County<sup>a</sup>

	Infected, No.	Infection rate, %	Dissected, No.			Age Group				Physiological status				Place of leptomonad infection		
			Parous	Nuli-parous	Unclear	Unfed	Blood-fed	Semi-gravid	Gravid	Midgut	Pharynx	Mouthpart				
2 <sup>nd</sup> June	0	0	17	6	11	0	10	1	2	4	0	0	0	0	0	
1 <sup>st</sup> July	0	0	30	8	19	3	19	3	3	5	0	0	0	0		
2 <sup>nd</sup> July	0	0	50	15	32	3	31	4	7	8	0	0	0	0		
1 <sup>st</sup> Aug	0	0	48	13	33	2	28	6	6	8	0	0	0	0		
2 <sup>nd</sup> Aug	0	0	17	7	9	1	8	3	3	3	0	0	0	0		
1 <sup>st</sup> Sep	1	1.8	54	30	16	8	26	5	4	19	1	0	0	0		
2 <sup>nd</sup> Sep	3	3.3	91	60	28	3	37	13	6	35	3	0	0	0		
1 <sup>st</sup> Oct	1	0.6	158	109	35	14	78	11	8	61	1	1	1	1		
<b>Total</b>	<b>5</b>	<b>1.07</b>	<b>465</b>	<b>248</b>	<b>183</b>	<b>34</b>	<b>237</b>	<b>46</b>	<b>39</b>	<b>143</b>	<b>5*</b>	<b>1</b>	<b>1</b>	<b>1</b>		

<sup>a</sup> In some specimens we found the leptomonad in different parts of the body

## 5. Discussion

There is a global estimate of 12 million leishmaniasis cases with 2 million new annual events and a population of 350 million at risk of the disease (17). Cutaneous leishmaniasis is a major public health problem in arid and semi-arid zones of the Middle East, northern Africa and Central Asia (18). Although four types of leishmaniasis including cutaneous and visceral forms exist in the Middle East, whereas the cutaneous form (CL) is the most common. Middle East and Maghreb countries harbour are attributed to approximately 15% of the global leishmaniasis burden, which is almost exclusively attributable to CL (19).

Unfortunately the cases of CL, especially zoonotic form of the disease caused by *Leishmania major* have reported from 17 out of 31 provinces of the country (7). Based on the reports of leishmaniasis unit of CDC, ministry of health, during the previous decade (2001-2010) a total of 228725 cases of CL were reported from different foci of the disease in Iran, out of them 2.5% was discovered in Sistan and Baluchistan province (Personal communication). Besides the cessation of indoor residual spraying for malaria control in most parts of Iran, other reasons for the increasing trend of CL in Iran, seems to be the unplanned growth and development of urban areas as well as human made changes in ecosystem, which has resulted in close contacts of human to zoonotic cycle of the disease (i.e. gerbil colonies) (20).

Cutaneous leishmaniasis has been emerged during recent years in rural areas of Mirjaveh district, Sistan and Baluchistan province in Southeast of Iran (21). This area is close to boundaries of Iran and Afghanistan, although the disease has been reported from Chabahar and Konarak as well (13). Therefore the study on biology and ecology of sand flies, as the vectors of cutaneous leishmaniasis, is important.

During this study two proven vectors of zoonotic cutaneous leishmaniasis (ZCL) in Iran, i.e. *P. papatasi* and *P. salehi* were collected, which were infected with leptomonad. Parous, gravid and semi-gravid of these species were increased toward the end of seasonal activity during September and October for *P. papatasi* with total infection rate of 2.1%. During these months the condition of temperature and relative humidity is appropriate for the sand flies which also increases their longevity. Therefore the probability of leptomonad infection and disease transmission increase among the old sand flies. The same trend of infection is reported from north Siani in Egypt (22, 23). *P. papatasi* is introduced as ZCL vector in Chabahar county (13, 24). The same trend of parity and physiological status was observed for *P. salehi* with a total infection rate of 1.07%. This species is also introduced as ZCL vector in Chabahar county (13, 24).

The most nocturnal activity for all species occurred during 19:00 to 21:00, while *P. papatasi* and *P. salehi* had another peak of activity in midnight (23:00 to 00:01). The

pattern reported from Turkey had an increasing trend from sunset to midnight for this species (25), while study in Morocco represented a decreasing trend for *P. papatasi* from 19:00 to next morning (26). Some studies reported two peaks of activity, it means for instance, in Saudi Arabia the first peak observed in 21:00 to 22:00 and the second in 06:00 to 07:00 (27), while in Palestine the first and second peaks recorded at 22:00 to 24:00 and 00:02 to 00:04, respectively (28).

In conclusion, inhabitants of the study area are in a great risk of sand flies bites. Understanding the nocturnal activity of these insects and their biting habits improves the ability of health authorities to plan for reducing contacts between sand flies and human that will result in better prevention and control of CL transmission. Based on all mentioned studies and our findings as well, sand flies usually start their host seeking behavior soon after sunset. During this time people are mostly active and therefore prevention of contact with sand flies is possible only by using repellents. But in the second peak of nocturnal activity, most of people are sleep and so long-lasting insecticide treated nets (LLINs) can help to better prevention and control of cutaneous leishmaniasis.

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## Authors' Contribution

None declared.

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