

## ENTOMOLOGICAL INVESTIGATION OF AN EMERGING LEISHMANIASIS FOCUS IN AZILAL PROVINCE, MOROCCO

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### ABSTRACT

Cutaneous Leishmaniasis (CL) due to *Leishmania tropica* has become a serious health problem in Morocco during the last decade. The aim of study is to evaluate the risk of leishmaniasis transmission in the province of Azilal by determining the composition, density and the seasonal abundance of sand flies. In this entomological study, three localities, in Ouaouizaght, a rural district in the province of Azilal, have been investigated twice a month from April to November 2010. Sand flies were collected from stables and houses using sticky traps. A total of 2670 (2164 males and 506 females) sand flies were captured. Seven species of genus *Phlebotomus* and two of genus *Sergentomyia* were identified. *Phlebotomus perniciosus* and *P. sergenti* with 55% and 37% of collected specimens respectively, followed by *Sergentomyia munita* (4%), *P. longicuspis* (1.6%), *P. papatasi* (1%), *P. ariasi* (0.9%), *S. falax* (0.7%) and *P. chadlii* and *P. chabaudi* (0.1%). That will help for implementation of appropriate future control measures.

**Keywords:** Azilal, leishmaniasis, Morocco, Sand flies, seasonal abundance

### INTRODUCTION

In Morocco, leishmaniasis disease still presents an increasing public health problem. Nevertheless, the distribution area of different leishmaniasis forms in Morocco has been clearly lighted. It is endemic in many semi-arid and arid areas, especially in the center and the south of the country. In the center, it is mainly anthroponotic due to *L. tropica* and transmitted by *P. sergenti* Parrot (Guilvard *et al.*, 1991; Pralong *et al.*, 1991). However, in the south leishmaniasis is mainly zoonotic due to *L. major* and transmitted by *P. papatasi scopoli*. Cutaneous infections due to a dermatropic variant of *L. infantum* have also been reported in the center of the country, its vector is still unknown (Rioux *et al.*, 1996). Visceral leishmaniasis (VL) caused by *L. infantum* is sporadic in some areas and transmitted by *P. ariasi* and *P. perniciosus* (Dereure, 1986). The province of Azilal is the oldest *L. tropica* focus known in Morocco (Marty, 1989). This disease keeps standing and wide spreading in the province although all control programs deployed and new focus appears each year

like Ouaouizaght focus where this study was carried out to limit and anticipate the wide spearing of disease by monitoring its sand flies's vectors.

### MATERIALS AND METHODS

#### Study area

The study was conducted in three rural localities (Iamoumen, Ait Chribou and Ait Ouazik) situated in Ouaouizaght district, Azilal province, in the center of Morocco in the High-Atlas mountains (Fig.1). The study area is characterized by a semi-arid climate, hot and dry in the summer, cold and rainy in the winter, with a temperature ranging from 5 to 47 °C and total annual rainfall about 600 mm. The altitude is ranging from 1000 to 1200 m above sea level. The district covers an area of about 500 km<sup>2</sup> with 8940 inhabitants. They work mainly in agriculture and rural tourism. The houses are built of stone and mud.

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**Fig. 1**  
Map of Azilal province showing the study area

### Sand flies collection and identification

Sand flies were sampled bimonthly in five selected stations of each three localities from April to November 2010 using sticky traps (A4 blank sheets of papers coated with castor oil). The papers were attached indoor to the walls and roofs of human and animal's dwelling by nails. A total of about 50 traps were placed in each area and in each collection campaign at the same places, and left for one night. Sand flies caught were checked up using water color brushes and stored in tubes containing 96% ethanol. In laboratory, the head and genitalia of each sandfly were cut off in a drop of ethanol, cleared in Marc-Andre solution (Abonnenc, 1972), then mounted in microscopy slides for identification by examining the genitalia structure of males, spermathecae and pharynges of females. For identification the key proposed by (Lewis, 1982) and the activity guideline given by the Moroccan ministry of public health was used.

## RESULTS

### Sand flies biodiversity

Sand flies collected in the study area during the period of April-November 2010, which corresponds to the sand flies activity in Morocco, belong to two genera (*Phlebotomus* and *Sergentomyia*), four subgenera (*Larroussi*, *Phlebotomus Paraphlebotomus* and *Sergentomyia*) and nine species (Table I). These species were identified as: *P. (Larroussi) longicuspis* (Nitzulescu, 1930), *P. (Larroussi) perniciosus* (Newstead, 1911), *P. (Larroussi) ariasi* (Tonnoir, 1921), *P. (Larroussi) chadlii* (Rioux, 1966), *P. (Paraphlebotomus) sergenti* (Parrot, 1917), *P. (Paraphlebotomus) chabaudi* (Croset, Abonnec et Rioux, 1970), *P. (Phlebotomus) papatasi* (scopoli, 1786), *S. (Sergentomyia) munita* (Rodani, 1843) and *S. (Sergentomyia) fallax* (Parrot, 1921).

Among the species of genus *Phlebotomus*, *P. perniciosus* was the most abundant species with 54.9% of flies trapped, followed by *P. sergenti* 36.6%. The five remaining species were less frequent, they represented less than 8.5% of captures; *P. longicuspis* (1.6%); *P. papatasi* (1%); *P. ariasi* (0.9%) and finally *P. chadlii* and *P. chabaudi* (0.1%). Concerning the species of *Sergentomyia* genus, *S. munita* and *S. fallax* exhibited a low abundance of 4.2 % and 0.7% respectively.

Table 1 shows the number and relative abundance of these species in the three prospected localities. *P. chadlii*, was not found in Ait Ouazik and *P. chabaudi*, was not recorded in two localities (Ait Ouazik and Iamoumen). The other species were largely widespread but with a varied frequency.

### Seasonal abundance and dynamics

Only the five most abundant species (*P. perniciosus*, *P. sergenti*, *P. longicuspis*, *P. papatasi* and *P. ariasi*), which played a role in the transmission of leishmaniasis disease in Morocco, were analyzed according to their dynamic (Fig. 2). The density of other species was very low so it was delicate to determine the monthly density. June represented the month of peak activity, when the population of flies increased to a maximum and then decreased to a minimum during November, except for *P. papatasi* and *P. ariasi*. The first one disappeared in august-September and reappeared in October-November, whereas the activity of the second one started at the end of July then rose to its highest level in October. While there were two peaks in the density of *P. papatasi* and *P. perniciosus*: the first and the more significant one in June and the second one in October-November.

### Sex ratio

Obviously, like others studies, (Boussaa *et al.*, 2005; Guernaoui *et al.*, 2005) more adult males than females sand flies were captured by sticky traps. The mean sex ratio (number of males/females) was 4.07.

## DISCUSSION

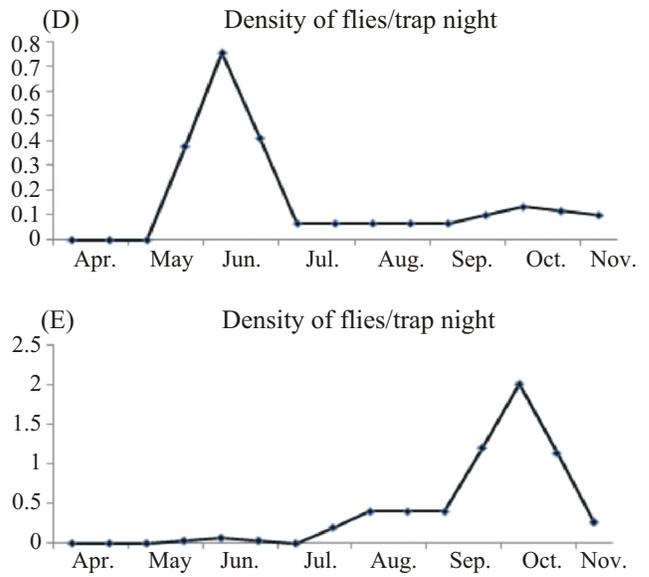
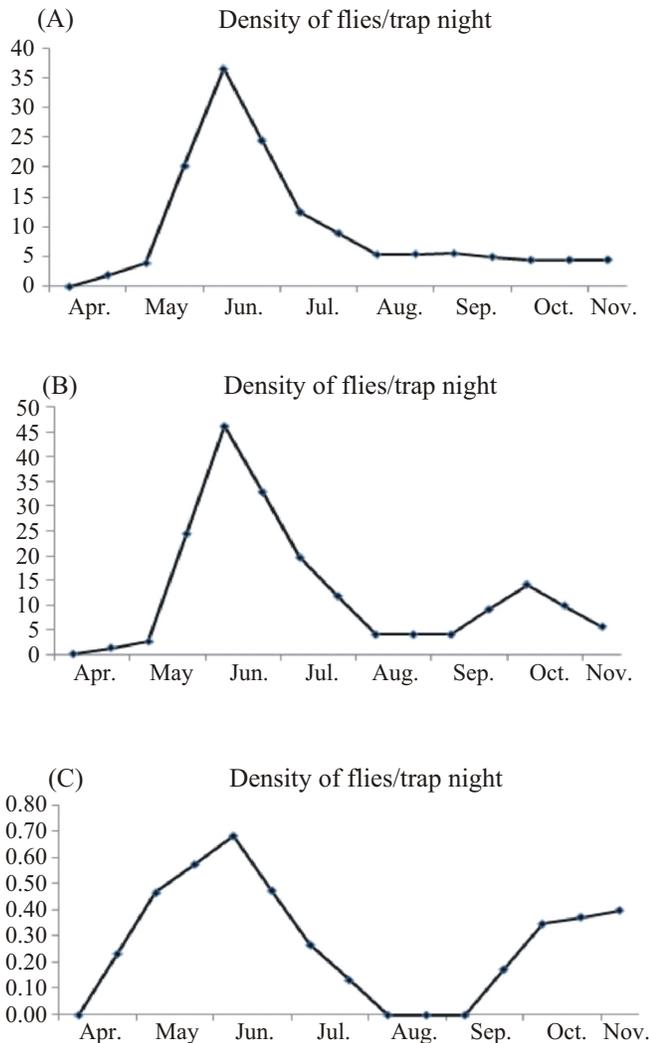
We have conducted a survey of phlebotomine sand flies in Ouauizaght during the transmission season of 2010. It was the first one in this focus. Azilal is an area where leishmaniasis has been present for a long time. Besides, the first case of *L. tropica* in Morocco was notified in Azilal (Marty, 1989). Among nine species found in these areas, five are involved or suspected as vectors of disease; *P. sergenti* is proven vector of *L. Tropica*, *P. papatasi* is proven vector of *L. major*, *P. longicuspis*, *P. perniciosus* and *P. ariasi* are involved in the transmission of *L. Infantum* (Rioux *et al.*, 1984). This contributes to the persistence of the leishmaniasis transmission cycle in Azilal. Previous studies in Morocco revealed that these species are the most common flies in high mountains areas (Guernaoui *et al.*, 2006; Boussaa *et al.*, 2010; Rioux *et al.*, 2001), and frequently trapped inside houses and in the vicinity of human dwellings (Ramaoui *et al.*, 2008; Boussaa *et al.*, 2009).

*Phlebotomus perniciosus*, the proven vector of visceral leishmaniasis in the Mediterranean areas (Boussaa *et al.*, 2009) is apparently the most abundant species in Ouauizaght (55%), which suggest that it is well adapted to mountainous climate (Bailly-Choumara *et al.*, 1971). Our results agreed with already published studies in high atlas mountains by Guernaoui *et al.* (2006) and Boussaa *et al.* (2010). As found by the other scientists, this species was trapped only in its a typical form *P. perniciosus* was active from May to November and reaches its peak of activity in June. That is in conflict with the observations of Guernaoui *et al.* (2006) which mentioned its peak of activity in September-October.

**Table 1**  
Density; distribution; abundance and prevalence of sand flies in each prospected locality.

Species	Ait Chribou				Iamoumen				Ait Ouazik				Total			
	Abundance		Frequency	Density	Abundance		Frequency	Density	Abundance		Frequency	Density	Abundance		Frequency	Density
	M	F	%	D	M	F	%	D	M	F	%	D	M	F	%	D
<i>P. perniciosus</i>	554	84	48.67	17.80	502	78	55.72	17.70	170	22	60.38	5.70	1226	184	54.9	41.20
<i>P. sergenti</i>	406	137	41.42	15.10	319	69	37.27	11.90	78	21	31.13	2.90	803	227	36.6	29.90
<i>P. longicuspis</i>	11	4	1.14	0.40	14	16	2.88	0.90	2	0	0.63	0.10	27	20	1.6	1.40
<i>P. papatasi</i>	22	1	1.75	0.60	6	1	0.67	0.20	2	0	0.63	0.10	30	2	1.0	0.90
<i>P. ariasi</i>	6	7	0.99	0.40	9	3	1.15	0.40	2	0	0.63	0.10	17	10	0.9	0.90
<i>P. chadlii</i>	1	0	0.08	0.00	1	0	0.10	0.00	0	0	0.00	0.00	2	0	0.1	0.00
<i>P. chabaudi</i>	2	0	0.15	0.10	0	0	0.00	0.00	0	0	0.00	0.00	2	0	0.1	0.10
<i>S. munita</i>	25	29	42.1	1.50	10	12	2.11	0.70	14	6	6.29	0.60	49	47	4.2	2.80
<i>S. falax</i>	8	14	1.68	0.60	0	1	0.10	0.00	0	1	0.31	0.00	8	16	0.7	0.60
Total	1035	276	100	36.50	861	180	100	31.80	268	50	100	9.50	2164	506	100.0	77.80

M: Male F: Female



**Fig. 2**  
Seasonal abundance of *Phlebotomus* sand flies in Ouauizaght, A: *P. sergenti*; B: *P. perniciosus*; C: *P. papatasi*; D: *P. longicuspis*; and E: *P. Ariasi*.

*Phlebotomus ariasi* the proven vector of visceral leishmaniasis in northern Morocco is following the same evolution of *P. perniciosus*, but it was less abundant in this biotope. That could be the result of climatic conditions. Moreover, similar study (Chafika *et al.*, 2013) have reported that these species was present only at high altitude (>1000m) and shows more preference for sub-humid and humid climates. *Phlebotomus longicuspis* is considered as a suspected vector of *L. infantum* in Morocco (Guessous *et al.*, 1997) with a wide distribution predominately in arid and Saharan bioclimate (Bally-choumara *et al.*, 1971; Rioux *et al.*, 1984). It was less

frequent through this investigation (1.6%). Our results are in harmony with the observations in Chichaoua (Guernaoui *et al.*, 2006), a neighboring region of Azilal, which indicate that the density of *P. longicuspis* was very high between 600-799 m of altitude and turn down outside this range. But it was in conflict with other results (Chafika *et al.*, 2013), which mentioned that the altitude alone cannot explain the sand flies distribution in Morocco and *P. longicuspis* was abundant outside this range in arid and Saharan areas.

*Phlebotomus sergenti* the main vector of *L. tropica* (Rioux *et al.*, 1984) has a wide distribution from sub-Saharan Sahel to the centre of Asia through Middle East and India and also is the most adapted to Mediterranean climate (Al-zahrani *et al.*, 1988) especially for semi-arid habitats (Bally-choumara *et al.*, 1997; Chafika *et al.*, 2013; Rioux *et al.*, 1984, 1997). We gave a particular attention to this fly because it is the main concern of the Leishmania disease in Ouaouizaght. Unfortunately, it was not only active throughout over eight months (April-November) but the second abundant species with high density in Ait Chribou and Iamoumen (15.10 and 11.87), while it has a low density in Ait Ouazik (2.93). This may be due to the difference in community structure and the nature of the habitats sampled.

*Phlebotomus papatasi* is well known as vector of *L. major* in Mediterranean countries and especially in arid-area (Bally-choumara *et al.*, 1971; Boussaa *et al.*, 2005; Chafika *et al.*, 2013; Rioux *et al.*, 1984). However, it is not susceptible to *L. tropica* (Killick-Kendrick *et al.*, 1994). Janini *et al.* (1995) and Kamal *et al.* (2003) found *P. papatasi* widely distributed in predomestic and domestic habitats in arid area, but it was less frequent in current report. This species is more pledged for an arid and Saharan environment (Bally-choumara *et al.*, 1971; Rioux *et al.*, 1984; Boussaa *et al.*, 2005) and less adapted to the semi-arid and humid environment.

The dynamic of the sand flies population in Ouaouizaght was analyzed. The activity periods of species varied with seasonality. Two seasonal patterns were observed; a monophasic model for *P. sergenti* and *P. perniciosus*, and a biphasic model for *P. papatasi* and *P. longicuspis*. All species were active from May to November, except for *P. papatasi* which disappeared in August-September. Indeed, this may be due to the high temperature. In fact, the hot temperature (Singh *et al.*, 1999), biotic factors such as the abundance of vertebrate hosts (Cross *et al.*, 1996) and abiotic factors like habitat availability are considered as a limiting factor for *P. papatasi* activity. June and October were the months of peak density which corresponds to the wettest months during the dry season. Our results were in good agreement with the findings in Marrakech city (Boussaa *et al.*, 2005), Chichaoua city (Guernaoui *et al.*, 2005) and Al-Bahah province of Saudi Arabia (Doha and Samy *et al.*, 2010).

## CONCLUSION

This study was conducted especially in vicinity and houses by public health services to evaluate the density and faunistic diversity of sand flies associated with cutaneous leishmaniasis endemic areas. This is to implement the most adapted control measures. Presence of five species of sand flies involved in transmission of Leishmaniasis diseases in Azilal makes the central part of Morocco an area of high risk

of leishmaniasis: Sanitation in localities with a low density of flies, pesticide impregnated bed nets in areas with a high density of *P. sergenti* and insecticide spraying in the localities with a high density of leishmaniasis vector.

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