Surveillance of Leishmaniasis: Inventory and Seasonal Fluctuation of Phlebotomine Sandflies (Diptera: Psychodidae), at the Prefecture of Meknes (Center of Morocco)

Surveillance des leishmanioses : inventaire et fluctuation saisonnière des phlébotomes (Diptera : Psychodidae) à la préfecture de Meknès (centre du Maroc)

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Abstract Phlebotomine sandflies are bloodsucking insects responsible for the transmission of leishmaniasis. The average annual incidence in Morocco, of this pathology, was approximately estimated in 2014 as 7.8 per 100.000 inhabitants according to the Moroccan health information service records. The study of the composition of the Phlebotomian community and its seasonal dynamics are essential for determining the appropriate and adequate means of control at the prefecture of Meknes in Central Morocco. It is with this in mind that two capture sessions per month using sticky paper traps were conducted at the sites of sandflies. A total of 982 sandflies were captured. The identification of the specimens found via the Moroccan key revealed a percentage of 73.3% belonging to the subgenus *Paraphlebotomus*, 6.3% to the *Phlebotomus* subgenus, 16.2% to *Larroussius*, and 4.2% of *Sergentomyia* subgenus. *Phlebotomus sergenti*, vector of human cutaneous leishmaniasis, was found as the more abundant with 73.3% followed by *Larroussius longicuspis* with 8.2%. The largest number of species was harvested in July and September with, respectively, 44.8 ph/m²/night and 48.7 ph/m²/night. This study also unveiled that the two high-risk Leishmanian

months are July and September, hence, the need to strengthen efforts to monitor and control this disease during the vector activity period.

Mots clés Phlébotomes · *Phlebotomus sergenti* · *Larroussius longicuspis* · Dynamique saisonnière · Période de risque · Meknès (Maroc) · Maghreb · Afrique du Nord

Résumé Les phlébotomes sont des insectes hématophages vecteurs de la leishmaniose. L'incidence moyenne annuelle de cette affection au Maroc a été approximativement estimée en 2014 à 7,8 pour 100 000 habitants selon les registres du service de l'information sanitaire marocaine. L'étude de la composition du peuplement phlébotomienne et celle de la dynamique saisonnière des espèces locales sont indispensables pour déterminer les moyens de lutte contre cette pathologie. Dans cette optique, deux séances de capture par mois en utilisant des pièges adhésifs ont été effectuées pour déterminer les espèces phlébotomiennes et leurs fluctuations saisonnières dans la préfecture de Meknès au centre du Maroc. Neuf cent quatre-vingt-deux phlébotomes ont été identifiés, dont 73,3 % du sous-genre, *Paraphlebotomus*, 6,3 % du sous-genre *Phlebotomus*, 16,2 % du sous-genre *Larroussius* et 4,2 % du sous-genre de *Sergentomyia*. *Phlebotomus sergenti*, vecteur de la leishmaniose cutanée humaine, est le plus abondant avec 73,3 % suivi par *Larroussius longicuspis* avec 8,2 %. Le maximum d'espèces a été récolté aux mois de juillet et septembre avec respectivement 44,8 et 48,7 phlébotomes/m² par nuit (ph/m² par nuit). Cette étude montre que les deux mois à haut risque leishmanien se situent en juillet et septembre, d'où la nécessité de renforcer les efforts de lutte contre cette maladie durant ces périodes.

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Keywords Phlebotomine · *Phlebotomus sergenti* · *Larroussius longicuspis* · Seasonal dynamics · Risk period · Meknes (Morocco) · Maghreb · Northern Africa

Introduction

Phlebotomine sandflies (Diptera: Psychodidae) are Nematocera dipteran insects. They belong to the family Psychodidae and the subfamily Phlebotominae, including 700 to 800 species grouped within 13 genera [24,25].

Only sandflies of the *Phlebotomus* genus are of medical interest because they are vectors of flagellate protozoa of the *Leishmania* genus belonging to the Trypanosomatidae family [10].

Because of its clinical manifestations, leishmaniasis is considered an important public health problem, which is classified as one of the priority diseases to be eradicated worldwide.

Morocco is considered among the most threatened countries in North Africa by leishmaniasis. The first case was published by Remlinger in 1921 [33], but the situation only increased in the early 1990s with outbreaks of foci (Smimou, Alnif, Taza, Tanant, etc.) and worsening of incidence [29]. Leishmaniasis in these different forms are endemic in many areas and continue to pose a major public health problem [26], despite the efforts made by the state.

The control of this disease is mainly based on the treatment of detected human cases and vector control based primarily on environmental management, including solid waste disposal, net use, and residual spraying of insecticides.

However, few data are available on the seasonal fluctuation, abundance, and density of sandflies at the level of several regions of Morocco, among them Meknes prefecture.

In order to prevent and to understand this zoonosis, we conducted, for the first time in the city of Meknes central Morocco, a taxonomic inventory of the phlebotomine community and a study of its seasonal dynamics.

This study could also allow the design of surveillance strategies with reasonable use of insecticides taking into account the months of risk of leishmaniasis.

Material and Methods

Study Area

The study area in the prefecture of Meknes (Fig. 1) is part of the Sais basin, located at about 500 m asl. This region is located in the Mediterranean bioclimatic stage subject to continental influences during the summer and winter seasons. The climate of the region is semiarid Mediterranean with a moderate winter and an average rainfall of 660 mm/year.

The urban township of Meknes is one of the municipalities of the prefecture of Meknes; it is located above the northern end of the Saïs Plateau, north of Mjjat, covering an area of 370 km².

Six different biotopes distributed in this township of Meknes have been chosen. They are adapted to the development of sandflies (laying and/or resting medium). These are mainly cowsheds (of cows, sheep, and donkeys) rich in organic matter (waste of any kind), unsanitary housing, and ruined house as well as caves. They were chosen according to environmental factors, proximity to urban agglomerations, and especially for their epidemiological history [14].

Capture of Sandflies

Two monthly capture sessions of sandflies in selected sites were carried out during 1 year: from April 2016 to March 2017. Depending on the trapping site, castor oil impregnated papers (sticky paper traps) were arranged either in cornets and introduced into the wall crevices or vertically on a wall or nailed to a support. It is a technique based on the enticing and attractive properties of castor oil. It is particularly adapted to the qualitative and quantitative inventory of phlebotomine fauna in the Mediterranean region [9,31].

Forty-eight traps were placed (eight traps for each site) in the evening at dusk, and then recovered at dawn the next

Fig. 1 The prefecture of Meknes in the center of Morocco / *La préfecture de Meknès au centre du Maroc*

morning. The captured sandflies were kept in plastic tubes containing 70% ethyl alcohol.

Identification of Sandflies

The captured sandflies were treated at the Laboratory of Medical Entomology at the National Institute of Hygiene (NIH). They were softened, thinned, and then mounted between slides and lamellae in a solution of lactophenol [1], before their identification using the key of determination of the sandflies of Morocco [3], based:

- In males, on the external morphology (style) and internal morphology (penis sheath) of the genitals and on the anatomy the cibarial and pharynges.
- In females, on the anatomy of the spermathecae, the cibarial, and pharynges.

Data Analysis

Four characteristics of sandfly fauna have been determined:

- Relative abundance (RA): relative percentage of individuals of each species in relation to all collected sandflies;
- Species richness: number of species in the stand;
- Density: expressed in number of sandflies per paper m² per night;
- D = ph/S/night;
- Frequency: expressed in percentage F = (p * 100)/P, with:
 - p, the number of months that a given species was collected;
 - P, the number of months of the study;
 - According to the value of F, we distinguish species as follows:
 - (F ≥ 50%) very common species;
 - (25% ≤ F < 50%) common species;
 - (10% ≤ F < 25%) rare species, and
 - (F < 10%) very rare species.

For the statistical processing of the data, we used the chi-square test accompanied by the calculation of the p-value

which will enable us to conclude on the significance of the result. The confidence interval is set at 95%, and the significance is taken if p-value < 0.05. The calculations of chi-square (χ^2) and the p-value are carried out using the software R.

Results

Inventory of Sandflies

The results of the morphotaxonomic study of sandflies (Table 1) showed that the sandfly fauna consisted of two genera: the genus *Phlebotomus* with 96% and the genus *Sergentomyia* with only 4% (Table 1).

Three subgenera within the *Phlebotomus* genus were encountered during the study period: *Paraphlebotomus*, *Phlebotomus*, and *Larrousius* with a relative abundance, respectively, of 6.3%, 73.3%, and 16.2%; however, only one subgenus of the genus *Sergentomyia* was caught (4.2%).

The chi-square test confirmed that the relationship between the two genera was statistically not significant ($\chi^2 = 44$, $P > 0.005$).

With the exception of *Ph. papatasi* and *S. minuta*, the other species were found present in all the stations studied: *Ph. papatasi* and *S. minuta* were absent in the locality of Sidi Bouzakri (Table 2).

Sex Ratio

We noticed during our captures by the adhesive traps that there were more males than females. The statistical analysis shows the existence of a statistically significant relationship between the two sexes ($\chi^2 = 84$, $P < 0.05$).

The sex ratio comparison by species (Table 1) shows that males outnumbered females in all species of the *Phlebotomus* and *Sergentomyia* genera.

The Dynamic Evolution of Sandflies Species

The evolution of the total average monthly density of the species collected (Fig. 2) shows that the period of activity

Table 1 Diversity and relative abundance of species in Meknes urban commune / *Effectif et abondance relative des espèces dans la commune urbaine Meknès*

Genus	Subgenus	Species	Male	Female	Total	RA	Frequency
<i>Phlebotomus</i>	<i>Larrousius</i>	<i>Ph. perniciosus</i>	61	17	78	7.94%	50%
		<i>Ph. longicuspis</i>	68	13	81	8.25%	50%
	<i>Paraphlebotomus</i>	<i>Ph. sergenti</i>	560	173	720	73.32%	66.66%
	<i>Phlebotomus</i>	<i>Ph. papatasi</i>	50	12	62	6.31%	58.33%
<i>Sergentomyia</i>	<i>Sergentomyia</i>	<i>S. minuta</i>	36	5	41	4.18%	41.66%
		Total	775	220	982	100%	

Table 2 Diversity and relative abundance of sandflies species at studied stations / <i>Effectif et abondance relative des espèces phlébotomiennes dans les stations étudiées</i>												
Species	Ras Aghil		Sidi Amer		Sidi Bouzakri		Sidi Baba		Bab RhA		Zahoua	
	Nb	RA%	Nb	RA%	Nb	AR%	Nb	RA%	Nb	RA%	Nb	RA%
<i>Ph. sergenti</i>	138	13.76%	111	11.07%	95	9.47%	148	15%	75	7.5%	153	15.3%
<i>Ph. papatasi</i>	12	1.20%	7	0.70%	0	0.00%	17	1.7%	4	0.4%	22	2.19%
<i>Ph. perniciosus</i>	12	1.20%	7	0.70%	8	0.80%	26	2.6%	9	0.9%	19	1.89%
<i>Ph. longicuspis</i>	10	1.00%	12	1.20%	6	0.60%	24	2.4%	6	0.6%	20	1.99%
<i>S. minuta</i>	4	0.40%	4	0.40%	0	0.00%	10	1.0%	8	0.8%	15	1.50%
TOTAL	176	17.55%	141	14.06%	109	10.9%	225	22%	102	10%	229	22.8%
Nb: Number; RA: Relative Abundance												

of sandflies extends between the months of April and November and that the highest densities were recorded between the month of June and the month of October.

We also note a maximum density during the 2 months of July and September which were, respectively, 44.8 ph/m²/

night and 48.7ph/m²/night, while it is null during the months of December, January, February, and March.

The evolution of the monthly specific density of the insects collected (Fig. 3) shows that *Ph. sergenti* extends from April to November with two peaks of activity, one in

Fig. 2 Temporal evolution of the total density of sandflies fauna / *Évolution temporelle de la densité totale de la faune phlébotomienne*

Fig. 3 Temporal evolution of the monthly specific density of the different sand fly species / *Évolution temporelle de la densité spécifique mensuelle de la faune phlébotomienne*

July with 30.3 ph/m²/night and the other in September with 33 ph/m²/night.

Ph. papatasi and *Ph. longicuspis* are present from May to November with a biphasic evolution, the largest of which is September with 3.54 ph/m²/night for *Ph. papatasi* and 5.21 ph/m²/night for *Ph. longicuspis*.

The period of activity of *Ph. perniciosus* extends from April to October with a biphasic evolution with two peaks, the most important of which is the one of September (4.2 ph/m²/night); however, *S. munita* has a monophasic distribution with a single peak in September.

Discussion

This study presents a set of data on the Phlebotomian fauna, its seasonal fluctuations, and its periods of activity, which makes possible to determine the means and the period of adequate control in this province.

Inventory of Sandflies

During the year of study, the inventory of sandflies shows the existence of two genera: the genus *Phlebotomus* which groups together the mammalian species vectors of *Leishmania* [28], and the genus *Sergentomyia* which feeds on reptiles, amphibians, and birds and rarely bites the man [27]. The latter is not considered as a vector of human leishmaniasis [20]. The difference between the two genera is not statistically significant ($\chi^2 = 44$, $P > 0.005$).

The majority of the sandflies captured were of the genus *Phlebotomus*; these results could be due to the type of prospected biotopes and also by the adaptation of this genus with the anthroponotic environment. The exhaustive list of exophilic species, and particularly of whose resting places are in wild biotopes, is not the aim of our survey [4,5].

These results are consistent with the research of Lahouiti, in the region of Moulay Yakoub in Fez (Morocco) [21] which showed the dominance of the genus *Phlebotomus* by comparison to the genus *Sergentomyia*.

A total of five species have been identified. The most abundant species was *Ph. sergenti* (73.3%), followed by *Ph. perniciosus* (7.9%), *Ph. longicuspis* (8.2%), *Ph. papatasi* (6.3%), and *S. minuta* (4.2%).

Ph. sergenti was the only species present in all the studied stations with important values; this could be explained by the best adaptation of this species to the ecological and climatic conditions of the region. All species of the *Phlebotomus* genus had a frequency greater than or equal to 50% (Table 1), so they were very common species, whereas *S. minuta* was found with a frequency between 25% and 50% so it was a common species.

The latest works conducted in Morocco in the region of Marrakech, Sefrou, and those of Azilal [8,34,35] confirmed the presence of the five species found by our traps.

Sex Ratio

The sex ratio comparison shows that males outnumbered females, with 3.52 male for a female. These results are statistically significant because the p -value < 0.05 with $\chi^2 = 84$ at the 95% confidence level.

This could be explained by the fact that the sticky paper traps used in our study are more attractive to males [19] and that females generally go in search of a blood meal or spawning lodge [31].

The works of Lahouiti in Moulay Yacoub [22] and those of El Miri in Sidi Kacem [13] confirm the dominance of the males by comparison to the females with a sex ratio, respectively, of 4.7 and four males for one female. These results are consistent with those found in our study.

The Dynamic Evolution of Sandflies Species

The study of the dynamics of sandflies makes it possible to have preliminary knowledge on vectors likely involved in the transmission which could facilitate the determination of the period (or the) period(s) of intervention against these vectors.

The variation in density of phlebotomine fauna during the study period showed that the period of activity of sandflies extends between the months of April and November with the high densities between the month of June and the month of October. That could be explained by the weather disturbances in the area: humidity, wind speed, temperature, and precipitation.

Indeed, the activity of this fauna is from April to November, which corresponds to the dry season where there is no rain. These results confirm the data obtained by Galvez in Spain [16].

The evolution of the monthly specific density of the insects collected showed that *Ph. sergenti*, vector of *L. tropica* cutaneous leishmaniasis in Morocco [2], was the most dominant in our study. The period of its activity extended from April to November with two peaks. Our result confirms the work of El Aasri in Sidi Yahya [11], who showed that the annual cycle of *Ph. sergenti* is of biphasic type, while the work of Boussa in the Marrakesh region [8] has revealed that the cycle of this insect is monophasic. This difference in the seasonal activity of *Ph. sergenti* could be explained by the geographical position, especially climate, of each region.

Ph. papatasi, a vector of *L. major*, responsible for cutaneous leishmaniasis [6,18], which increases density with aridity [30], is present from May to November with a biphasic

evolution; these results are similar with those found in the region of Fez [8] and in Marrakech [34].

In view of its major role in the transmission of *Leishmania infantum*, the causal agent of human visceral leishmaniasis [7], *Ph. perniciosus* is considered one of the most important species. The analysis of the seasonal dynamics of populations of *Ph. perniciosus* revealed that the period of activity extended from April to October with a biphasic evolution with two peaks, the most important of which being that of September (4.2 ph/m²/night). These results confirm those found in the Chichaoua region [17].

Ph. longicuspis species is confirmed to be responsible for the transmission of *L. infantum* that is responsible for visceral leishmaniasis in Morocco [32]. This species activity extends from May to November with two peaks, the largest of which was the month of September with 5.2 ph/m²/night. The results corroborate with those of Faraj et al. [15] concerning the seasonal dynamics with two peaks of activity. However, there is a difference in the months when these peaks of activity were recorded, which could be explained by the climatic and geographical characteristics of each region.

S. minuta has a single peak in September, but it has no epidemiological significance in Morocco [23] and rarely bites the human beings [27].

Our study showed that the activity period of sandflies coincides with the dry period of the year. Indeed, it is expected to have an extension of this period of activity as Morocco is a country known for its climatic hazards that can generate successive periods of drought and the aridity of a large part of its territory.

The distribution of sandflies is due to several factors not only climatic but also environmental, in particular, solid waste, which are a major constraint for local communities and a growing threat to the environment [12] and which favors the multiplication of insects.

Conclusion

Seasonal fluctuations of sandflies in the Meknes region are characterized by a monophasic evolution for *S. minuta* populations and a biphasic evolution for *Ph. perniciosus*, *Ph. longicuspis*, *Ph. papatasi*, and *Ph. sergenti*. Indeed, for this second category, the first peak is often observed in July and the second is in September.

As a dominant species (73.3%), *Ph. sergenti*, vector of *L. tropica*, shows two density peaks that correspond well to the two peaks of total density.

These results reveal two high-risk leishmaniasis annual periods in Meknes marked by a high total density of sandflies which can reach up to 48.75 ph per m² of trap per night and coincides with the hot and dry period.

An in-depth study of the environmental and climatic factors responsible for the proliferation of sandflies would be essential for a better surveillance of this disease.

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