Control of the sandflies vector of leishmaniasis: Results of an entomological survey on the sandflies fauna in Morocco (El Hajeb)

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Abstract

Background

Sandflies are small midges that are the exclusive vectors of cutaneous and visceral leishmaniasis. These diseases are a public health problem in Morocco. Objective: To determine the composition of the phlebotomine fauna, to study the biodiversity and seasonal distribution of phlebotomine in the province of El Hajeb.

Methods

A total of six stations were surveyed, five of which had recorded cases of indigenous leishmaniasis in recent years. Bi-monthly captures from April to December 2019 were carried out using adhesive trapping in different biotopes.

Results

A total of 14590 sand flies were collected of which males represent 80.3%. The activity of the sand fly population started in April and ended at the end of November. The periods of high abundance were in July, September and November. Morphological identification of fasting males and females (14070 specimens) shows the presence of twelve species belonging to the genus *Phlebotomus* and *Sergentomyia*: *P. longicuspis* (79.4%), *P. sergenti* (7.2%), *S. minuta* (4.42%), *P. perniciosus* (2.7%), *P. papatasi* (2.05%), *P. bergeroti* (1.1%), *S. schwetzi* (1%), *P. alexandri* (0.7%), *S. fallax* (0.7%), *S. dreyfusi* (0.5%), *P. ariasi* (0.2%) and *S. antennata* (0.1%). The in-depth analysis shows that the leishmaniasis vector species predominate the population of collected sandflies and the risk periods are spread during the summer and autumn seasons.

Conclusion

The present study is the result of an entomological survey at the level of El Hajeb in central Morocco; it gives information on the captured species of sandflies. Therefore, it offers to the decision-makers an important tool to carry out effective vector control actions to limit the risk of bites and to limit the transmission of leishmaniasis.

Background

Sandflies (Diptera, Psychodidae, Phlebotominae) are widely distributed in temperate climates and are of great medical and veterinary importance. They are currently considered as the exclusive vectors of human and canine leishmaniasis [1]. These diseases are ranked second only to malaria as vector-borne diseases threatening global health [2]. They represent a complex group of parasitic diseases both clinically and eco-epidemiologically [1, 2].

In Morocco, leishmaniasis is a serious health problem and is endemic in several regions of the country, manifesting itself in two main clinical forms: cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL) [3, 4].

From the epidemiological point of view, the main foci of the visceral and cutaneous forms, which are caused by the *Leishmania infantum* (*L.infantum*) parasite, have been recorded essentially in the center and north of the country [5]. While, the outbreaks of wet cutaneous forms, which are caused by *Leishmania major* (*L. major*), were reported in the south of the country; and those of dry cutaneous forms, which are caused by *Leishmania tropica* (*L. tropica*), were reported mainly in the center [3, 6, 7].

Beyond this clinical and parasitic variety of leishmaniasis, their common feature is that they are transmitted in natural conditions by the bite of different species of an infected female sandfly [1]. These insects are called "Chniwla" in most regions of Morocco and are often confused with mosquitoes [8]

From an entomological point of view, only the vectorial capacity of phlebotomine of the genera *Phlebotomus* in the Old World and *Lutzomyia* in the New World has been demonstrated for the transmission of leishmaniasis but also of other viral or bacterial diseases [1, 9].

In contrast, in Morocco, five species of the genus *Phlebotomus* are involved in the transmission of leishmaniasis. The first one belongs to the subgenus *Phlebotomus* (*P.*), it is *P. papatasi* incriminated in the transmission of *L. major*, the second one belongs to the subgenus *Paraphlebotmus*, it is *P. sergenti* which is responsible for the transmission of *L. tropica*; the last three belong to the subgenus *Larroussius*: *P. perniciosus*, *P. ariasi* and *P. longicuspis*. These three species are involved in the transmission of *L. infantum*, the causative agent of sporadic cutaneous forms and the severe visceral form [10].

From a strategic point of view, a National Program for the Control of Leishmaniasis (NPCL) has been designed since 1997 to control the epidemiological situation of leishmaniasis in the country. Currently, its main objective is to eliminate all forms of leishmaniasis by 2030, based on a set of strategic axes: (a) free therapeutic case management; (b) control of the animal reservoir, especially dogs and rodents; (c) vector control; (d) continuous training of health personnel; (e) intersectoral collaboration and information, education and communication [3].

In addition, vector control is a very important strategic focus for limiting the spread of leishmaniasis. Studies have shown that improving the knowledge of the population and health professionals plays a crucial role in successful vector control activities [11, 12]. Also, Qualls et al. have shown the effectiveness of attractive sweet baits in controlling sandflies [13] However, the Ministry of Health confirms that vector control in Morocco is mainly based on the monitoring of

the sand fly population by identifying the localities at risk and with high abundance and the periods of transmission to implement targeted and precise control actions to limit the transmission of the disease to humans and animals [3].

Our study aims to determine the composition of the circulating phlebotomine fauna in El Hajeb province. To our knowledge, no entomological study has been conducted in this region. Their knowledge can lead to a better understanding of the vector species of these parasitic diseases and provide essential entomological data to promote effective vector control.

Methods

Study area

The entomological study was carried out in six localities of the province of El Hajeb: Aït Naaman (05°19'13.79" W; 33°43'15.38" N), Aït Rbaa (05°10'10.79" W; 33°51'04.67" N) and Aït Brahim (05°05'28.75" W; 33°49'48.46" N) stations located northwest of El Hajeb; the stations of Ain Taoujdate (05°13'07.78" W; 33°57'09.33" N), Sidi Mbarek (05°15'01.50" W; 33°57'59.60" N) and Aït Oufella (05°09'31.93" W; 33°56'20.51" N) located in the north of the province (Fig. 1).

The climate of El Hajeb is semi-humid of Mediterranean type; it is characterized by a hot and dry summer, and a mild and rainy winter. The average annual rainfall is about 572 mm and the average annual temperature is about 17C° with a maximum of 35 C in July and a minimum of 4C° in January [14].

The choice of trapping stations was based on epidemiological justifications. Indeed, we based on retrospective studies of the epidemiological situation of leishmaniasis in this province during the last five years [15]. These data allowed us to direct our survey efforts towards localities that were affected by these vector-borne diseases.

Thus, the support of local authorities and the collaboration of community agents and inhabitants allowed us to agree to trap in six stations (five houses and one uncontrolled public dump) in families with a history of leishmaniasis. These are the Sidi Mbarek station and the Aït Oufella station in the rural district of Laqsir where autochthonous cases of LC and LV have been reported to the health authorities; the Aït Rbaa station in the rural district of Bitit where cases of LV and LC have also been reported. The station of Aït Brahim where the surveys were carried out in the public dump located near the district where infantile VL cases were notified. The station of Aïn Taoujdate where cases of LC were declared. The station of Aït Naaman in the district of El Hajeb where no case of leishmaniasis was reported was chosen as a control station.

Collection of specimens

The sampling of sandflies took place during the year 2019 in six stations in the province of El Hajeb in central Morocco. For the collection of sandflies, we used adhesive traps with white A4 papers, soaked in castor oil on both sides during the whole night [16]. During each capture session, 40 sheets were used and deposited in the afternoon at sunset and recovered the next morning at sunrise. These sticky traps were placed in the same location throughout the trapping period. Sandflies were captured in each locality on a bi-monthly basis throughout the survey period. The surveys were planned for one year from April 2019 to January 2020, the period of activity of sandflies in Morocco [17].

The main biotopes that were surveyed were: manure and animal burrows, chicken houses, stables, near vegetation and rocks, holes inside and outside the house. Thus, we have equipped, also, with a mini Mobile Microscope adapted to our Smartphone to photograph the sand flies captured in the field.

Work in the laboratory

The collected sandflies were transported to the laboratory where they were sorted, counted, sexed and preserved in the 95% ethanol solution. The sandflies were thinned in Marc-André solution and mounted between slide and coverslip and then placed under the microscope for morphological identification using the sand fly identification keys published by the Ministry of Health[3] based on the pharynx and external genitalia in males and the spermaceti and cibarium in females. Thus, the identification of sandflies of the subgenus *Larroussius* was also made by referring to Leger and Depaquit (2001) [18] and Boussaa (2008) [19] and those of the subgenus *Paraphlebotomus* by following Depaquit (2004, 2013) [20, 21].

Data analysis

The characteristics of the captured fauna were determined by calculating six parameters, namely:

(a) Abundance (A) represents the total collected count of each species.

(b) The relative abundance of each species (RA) is estimated by dividing the number collected of a given species by the total count of all species collected multiplied by 100:
$RA = n/N \times 100$

(c) Species richness (S) represents the total number of species in the stand.

(d) The diversity of the phlebotomine stand is expressed by the Shannon diversity index "H'" and the Evenns equitability index "E", where :
$H' = -\sum Pi \, Ln Pi$

Where Pi: the proportion of the total number of species total samples

$E = H'/Hmax$; Where Hmax : LnS

(e) Sex ratio (SR) is the ratio of males to females:

SR = M / F

(f) The density is represented by the number of individuals per unit area of oil paper. It is expressed as the number of sandflies per m2 per night:
D = ph/S/night

Where:

D: density

Ph: number of sandflies

S: surface

Night: night

Results

Diversity, species richness and sex ratio of captured sandflies

A total of 14950 sandflies were captured of which 80.3% (11715) were males. The abundance, diversity, species richness and sex ratio of the sand fly population captured at the six stations are presented in Table 1.

Twelve species of sandflies were identified with differences between stations and the sex ratio was in favor of males (4.07: 0.25). In fact, the ratio of males to females was similar in the station of Aït Taoujdate (2.8: 0.36), Sidi Mbarek (4.04: 0.25), Aït Oufella (8.23: 0.12), Aït Rbaa (2.85: 0.35), and Aït Naaman (1.95: 0.52). In contrast, in the locality of Aït Brahim, female sandflies were recorded more than males (0.73: 1.35).

The highest abundance was recorded at the station of Aït Oufella with 6833 specimens (46.8%), then that of Aïn Taoujdate with 3023 specimens (20. 7%) and station of Aït Rbaa with 2083 specimens (14.3%), then the station Aït Naaman with 1044 (9.6%) and Sidi M'barek with 1407 specimens (7.2%), and finally the station of Aït Brahim with 200 specimens (1.4%).

The diversity of the sand fly population in El Hajeb was calculated using the diversity index of Shannon "H'" and the equitability of Evenns "E" (Table 1). The phlebotomine fauna of the locality of Aït Rbaa and Sidi Mbarek showed the highest biodiversity (11 and 12 species out of 12 were collected). While that of Aït Brahim showed the least (7/12 species are collected).

The analysis of the diversity index of Shannon shows that the H' values in the stations of Aït Naaman and Aït Brahim are closer to each other, while they are different at the level of the maximum value of diversity.

Let us also note that the most diversified station was that of Aït Naaman with a value of E = 0.76. This is due to a rapprochement between its diversity value H and its maximum theoretical value H'. This explains why the reproductive potential of the phlebotomine population seems to be the same. While the lowest diversity value was recorded in the station of Aït Oufella with a value of E = 0.265.

Table 1
Species richness, diversity, abundance and sex ratio of the sand fly population captured at the six study sites

Station	Ain Taoujdate	Sidi Mbarek	Aït Oufella	Aït Rbaa	Aït Naaman	Aït Brahim
Environment	Peri-urban	Rural	Rural	Rural	Peri-urban	Rural
Altitude (m)	470	431	581	922	1150	704
Abundance	3023	1044	6833	2083	1407	200
Male	2227	837	6093	1542	931	85
Female	796	207	740	541	476	115
Sex-Ratio	2,8 : 0,36	4,04 : 0,25	8,23 : 0,12	2,85 : 0,35	1,95 : 0,52	0,73 : 1,35
Species richness	9	12	10	11	10	7
H'	0,596	1,173	0,611	0,894	1,751	1,539
Hmax	2,197	2,485	2,303	2,398	2,303	1,946
E	0,271	0,472	0,265	0,373	0,76	0,791
Biotopes	1- house and henhouse 2- cow and sheep pen 3- peri-domestic	1- domestic 2- stables 3- peri-domestic 4- rocks	1- manure 2- manure 3- pens for cows, hair and sheep 4- peri-domestic rock	1- domestic 2- sheep pen 3- peri-domestic	1- the hen house 2- pen for hair and sheep 3- domestic perimeter	1- Solid waste 2- Solid waste 3- Solid waste
Dominant species	*1-P. longicuspis* *2-P. perniciosus* *3-P. sergenti*	*1-P. longicuspis* *2-P. sergenti* *3-P. papatasi* *4-S. minuta*	*1-P. longicuspis* *2-P. sergenti* *3-P. perniciosus* *4-P. papatasi*	*1-P. longicuspis* *2-P. perniciosus* *3-P. sergenti*	*1-S. minuta* *2-P. longicuspis* *3-P. sergenti*	*1-P. papatasi* *2-P. sergenti* *3-P. bergeroti*

H': Shannon-Wiener diversity index / E: Evenns

Inventory and relative abundance of identified sand fly species

Of the 14590 sandflies collected, 14070 were identified morphologically, the rest of the sandflies (520) pregnant and/or gorged females were retained for possible later molecular identification. The results of the identification of 14070 sandflies are presented in Table 2. In fact, among 14070 identified sandflies, 93.3% (13128) specimens belonged to the genus Phlebotomus and 6.7% (942) to the genus Sergentomyia.

The morphological identification of the collected specimens revealed the presence of 12 species: *Phlebotomus* (*Phlebotomus*) *papatasi* Scopoli; *P.* (*Phl.*) Parrot *bergeroti*; *P.* (*Paraphlebotmus*) *sergenti* Parrot; *P.* (*Par.*) *alexandri* Sinton; *P.* (*Lar.*) *longicuspis* Nitzelescu; *P.* (Lar.) perniciosus Newstead; *P.* (*Lar.*) ariasi; *Sergentomyia* (*Sergentomyia*) *minuta* Rodani; S. (*Ser.*) *schwetzi* Adler; *S.* (*Ser.*) *fallax* Parrot; S. (Ser.) *antennata*; and *S.* (*Grassomyia)* d*reyfusi* Parrot.

Of all the specimens identified, five species represent 95.7% of the phlebotomine population circulating in the El Hajeb region. These are *P. longicuspis*, *P. sergenti*, *S. minuta*, *P. perniciosus* and *P. papatasi*.

The results in terms of abundance revealed that the most abundant species in the province was *P. longicuspis* of the genus *Phlebotomus* with a relative abundance of 79. 7% among all the sandflies, it was encountered in all the stations, it was very abundant in the locality of Aït Oufella (89.8%), Ain Taoujdate (89.1%), Aït Rbaa (78.5%) and the locality of Sidi Mbarek (71.8%).

The species *P. Sergenti* was captured in all the sites surveyed with a relative abundance of 7.17% among all specimens. It represents 16.6% of the sandflies in the locality of Aït Rbaa, 14% in Aït Brahim, 13.3% in Sidi Mbarek.

The species *P. perniciosus* was detected at low relative abundance (2.44%) in almost all the stations, except in the locality of Aït Brahim where it was absent.

The species *P. papatasi* which was found with a relative abundance of 2.05% among all the sandflies collected was not detected in the station of Aït Naaman, but it was very abundant in the locality of Aït Brahim (54%).

The most abundant species of the genus *Sergentomyia* was *S. minuta*, it represents 65.9% of all sandflies caught in this genus. It was present in all sites, especially in the locality of Aït Naaman (42.3%).

Table 2
Inventory and relative abundance of phlebotomine species identified at the six study sites

Gender		*Phlebotomus*							*Sergentomyia*				
Subgenre		Phlebotomus		Paraphlebotmus		Larroussius			Sergentomyia				Gr
Species		P. papatasi	P. bergeroti	P. sergenti	P. alexandri	P. longicuspis	P. perniciosus	P. ariasi	S. minuta	S. schwetzi	S. fallax	S. antennata	S.
Ain Taoujdate	M	21	14	68	12	2000	105	0	3	3	1	0	0
	F	1	3	35	5	631	44	0	6	1	1	0	0
	Nb	22	17	103	17	2631	149	0	9	4	2	0	0
	RA%	0,74	0,58	3,49	0,58	89,1	5,04	0	0,3	0,14	0,07	0	0
Sidi Mbarek	M	35	16	85	5	651	9	2	18	1	4	5	6
	F	21	5	49	3	73	10	0	9	0	0	0	1
	Nb	56	21	134	8	724	19	2	27	1	4	5	7
	RA%	5,56	2,08	13,3	0,79	71,8	1,88	0,2	2,68	0,1	0,4	0,5	0,
Aït Oufella	M	62	63	222	22	5582	107	8	5	5	17	0	0
	F	46	2	82	5	373	18	1	4	2	6	0	0
	T	108	65	304	27	5955	125	9	9	7	23	0	0
	RA%	1,63	0,98	4,58	0,41	89,8	1,88	0,14	0,14	0,11	0,35	0	0
Aït Rbaa	M	3	8	284	29	1187	11	0	3	3	7	5	2
	F	6	0	44	6	365	6	0	7	0	0	1	1
	Nb	9	8	328	35	1552	17	0	10	3	7	6	3
	RA%	0,46	0,4	16,6	1,77	78,5	0,86	0	0,51	0,15	0,35	0,3	0,
Aït Naaman	M	0	0	97	7	267	31	15	335	82	44	5	48
	F	0	0	19	1	81	3	2	225	40	10	1	10
	Nb	0	0	116	8	348	34	17	560	122	54	6	58
	RA%	0	0	8,77	0,6	26,3	2,57	1,28	42,3	9,22	4,08	0,45	4,
Aït Brahim	M	33	26	13	0	0	0	0	4	4	5	0	0
	F	61	12	11	0	4	0	0	2	0	0	0	0
	Nb	94	38	24	0	4	0	0	6	4	5	0	0
	RA%	54	22	14	0	2,3	0	0	3,4	2,3	2,9	0	0
Total specimens		289	149	1009	95	11214	344	28	621	141	95	17	68

Seasonal density and monthly fluctuation of fauna at El Hajeb

Figures 2 and 3 present the results of the temporal evolution of the total density of the phlebotomine population. Indeed, the maximum total density was recorded in the summer and autumn seasons especially in July, August, September and November respectively 31 ph/m2/night, 53 ph/m2/night, 101 ph/m2/night, 21 ph/m2/night.

By season, the evolution of the total density of the sand fly fauna in the region shows that the periods of risk coincide with the summer and autumn seasons. Indeed, the total captures of sandflies collected during the year showed a tri-phase evolution with three peaks, the first in early June-July, the second peak which is most important in September and the third in November.

On the other hand, the evolution of the specific monthly activity for the five most abundant species in El Hajeb Province: *P. longicuspis* (79.4%), *P. sergenti* (7.2%), *S. minuta* (4.4%), *P. perniciosus* (2.7%) and *P. Papatasi* (2%) (Fig. 4) reveals that the highest periods of activity were recorded during July (12.8%), August (21.7%), September (42%) and November (8.5%). The lowest rates were in May (2.7%) and June (3.9%).

By station (Fig. 5), the evolution of the specific monthly seasonal activity of the sandflies identified at the station of Aït Oufella (Fig. 5, a) shows that the species of the genus *Phlebotomus* were the most encountered especially *P. longicuspis* presented a bimodal variation, the first peak was in July and the second in September and *P. sergenti*, *P. papatasi* and *P. perniciosus* which recorded only one peak in September. While in the station of Ain Taoujdate (Fig. 5, b), it is the species of the genus *Phlebotomus* that were found most abundant so *P. longicuspis* recorded three peaks, the first in June, the second who is the most important was in August-September and the third in November. On the other hand, at the station Aït Rbaa (Fig. 5,c), it is the species of *P. longicuspis*, *P.*

sergenti, *P. alexandri* and *P. perniciosus* that were most encountered where *P. longicuspis* recorded four peaks, first in May, then in July, then in September and finally in November. However, for the station Aït Naaman (Fig. 5, d), the species of *S. minuta*, *P. longicuspis*, *S. schwetzi* and *P. sergenti* which were found successively abundant whose fluctuation of these species was marked by two peaks, the first one was in the months of July-August and the second one was in November. At the level of the station Sidi M'barek (Fig. 5, e), the species of *P. longicuspis*, *P. sergenti*, *P. papatasi* and *S. minuta* were the most encountered. The seasonal activity of these species was marked by two peaks, the first was in the months of July-August and the second was in November. Thus, at the level of the station of Aït Brahim (Fig. 5, f), the species of *P. papatasi*, *P. bergeroti*, *P. sergenti* and *S. minuta* were found most abundant. The fluctuation of these species was marked by two peaks, the first was in May and the second was in the months of July-August.

By species, the specific monthly evolution of *Phlebotomus longicuspis* shows that its activity extends from April to November with three peaks, the first begins in June, and the number of sandflies continues to increase to reach its maximum in September where it will mark its second most important, then the number decreased steadily in October, and then increased slightly to record a third less important peak in November (Fig. 6). This species was found in all the stations and during all the periods of study but with a very important abundance in the two localities Aïn Taoujdate and Aït Oufella. While, *P. perniciosus* which showed a similar trend but with two peaks, the first one July-August and November, was not found in the station of Aït Brahim although it was collected during the whole study period in the other stations. However, morphological identification of this species showed that almost all the males captured were in typical form (Fig. 7).

The monthly activity of *S. minuta* showed a bimodal peak pattern, the first in August and the second in September. This species was most abundant in the locality of Aït Naaman where we collected 90.2% of the total number of this species (Fig. 8).

P. sergenti and *P. Papatasi* showed a bi-phasic pattern with two peaks, one in June-July and the other in September. *P. sergenti* was caught in all stations and during the whole study period with a very important abundance in the localities of Aït Oufella and Aït Rbaa. Whereas, P. Papatasi was absent in the locality of Aït Naaman but it was met in the stations of Aït Oufella and Aït Brahim with abundance (Fig. 9 **and** Fig. 10).

Thus, it should be noted that despite the abundance of the other species being low, the morphological identification of the species *P. bergeroti* showed that most of the males captured had a three-lobed paramere with a larger upper lobe and a lateral lobe with 3 spatulate terminal spines instead of 2 spines (Fig. 11).

Discussion

From an eco-epidemiological point of view, the province of El Hajeb constitutes a very interesting field of study because of its geographical proximity to the epidemic foci of cutaneous leishmaniasis in the provinces of Sefrou [22], Moulay Yacoub [23] and Fes-Boulomane [24]. Similarly, no entomological study has been carried out to determine the composition of the existing phlebotomine fauna despite the presence of indigenous cases of leishmaniasis in this region [15].

It is within this framework that the present study was carried out to identify the species of sandflies circulating in the province and those vectors of leishmaniasis. Thus, our results offer for the first time the inventory of the sand fly fauna in the region and give information on its biodiversity and its periods of activity during the year. These data are necessary to guide the actions and periods of vector control.

In Morocco, 24 species of sandflies have been described, five of which are known to transmit leishmaniasis, a public health problem [25]. These are *P. papatasi*, the vector of cutaneous leishmaniasis caused by *L. major* in the South and South-East of the country [3, 26, 27]. The *P. sergenti* is responsible for the transmission of cutaneous leishmaniasis to *L. tropica* where most of the recorded cases are notified in the center of the kingdom [27, 28]. The three species are *P. perniciosus*, *P. ariasi* and *P. longicuspis* which have been proven to be vectors of severe forms of the disease including visceral leishmaniasis with *L. infantum* where most cases have been reported in the north [3, 29].

In our study, 12 species were identified which represents 50% of the Moroccan sand fly species. Indeed, the five vector species of the disease were found in this study, they represent 91.6% of the sandflies identified in 83% of the sites surveyed (5/6). Also, the analysis of the results obtained showed that there is cohabitation between 3 species: *P. papatasi*, *P. sergenti* and *P. longicuspis*. Although the province of El Hajeb is located in the center of Morocco, this coexistence was also found by Ouanaimi and colleagues [30] in their study conducted in the south and north of the country.

In terms of individuals collected, the abundance of sandflies was very high compared to the low incidence of leishmaniasis recorded in the said province [15]. This finding has been revealed by other studies in other regions of Morocco [30, 31, 32, 33]. This can be explained by the words of Ouanaimi et al, [30] who state that leishmaniasis in Morocco is determined by the ecology of the parasite rather than the distribution of the vector.

Concerning the species identified, they belong to two genera: Phlebotomus and Sergentomyia. The species of the first genus belong to three subgenera: Phlebotomus (*P. papatasi* and *P. bergeroti*), *Paraphlebotmus* (*P. sergenti* and *P. alexandri*) and *Larroussius* (*P. longicuspis*, *P. ariasi* and *P. perniciosus*). While the species of the second genus belong to two sub-genus: *Sergentomyia* (*S. minuta*, *S. schwetzi*, *S. fallax* and *S. antennata*) and *Grassomyia* (*S. dreyfusi*).

For species of the genus *Phlebotomus*, 79.7% of the identified sand flies were *P. longicuspis*, this species was the most abundant in our area, it was collected during the whole period and in the six localities studied with a pattern of three peaks, the first in July, the second in September and the third in November. On the other hand, the long period of activity and high abundance of this confirmed VL vector species is threatening and indicates the potential risk of transmission of the visceral form in the said province. These results corroborate with the study of Al-Koleeby et al, (2021) [34] and Guernaoui et al, (2005) [35] in one part where the most important peak is located in September and diverges in the other part where this species can show a pattern of a single peak as the case of Chichaoua (35) or with a pattern of two peaks as the case of Zagora province [34]. And even, it can show a pattern of three peaks as in our study. This difference can be explained by the ability of *P. longicuspis* to adapt to the environmental conditions of each region. In addition, 53% of this species were

captured in the locality of Aït Oufella whose altitude is 581 m. In this context, Guernaoui et al (2006) [33] showed that this species is very abundant between the altitudes of 600 m and 799 m.

The species *P. sergenti* was encountered in all stations and its period of activity extends from April to November with a bimodal pattern, the first in June-July and the other in September. Our results confirm those found in the province of Fez [36] which revealed that the seasonal activity of this species is bimodal. This can be explained by the climatic and geographical conditions of the province of El Hajeb which are similar to its neighbor Fez.

Concerning *P. perniciosus*, it should be noted that the majority of specimens identified were found in their typical form. This species was captured from April to November and its seasonal trend reflects a bimodal pattern with two peaks. This corroborates with the results of Talbi in Sefrou [22].

As for the species *P. Papatasi* as a proven vector of *L. major* especially in southern Morocco and which has long been considered adapted to the arid climate [27], also proves to be adapted to the temperate climate that prevails in the center of the country since our results show its presence from April to November with two peaks and in the majority of the stations (5/6). This result was also revealed by studies carried out in the neighboring provinces of our study site of El Hajeb where it was also collected from April to November [22].

From another perspective, sandflies of the genus *Sergentomyia* prefer, according to Boussaa et al, [37], altitudes between 800 m and 1000 m. Similarly, Guernaoui et al, (2006) [33] state that only the species *S. minuta* persists at altitudes of 1200 m to 2000 m. This was confirmed in our study, particularly in the station of Aït Naaman which is located at an altitude of 1150 m and whose inventory of sand flies captured in this station revealed that the most abundant species belonging to the genus Sergentomyia notably *S. minuta* which represents 65.9%. Its period of activity extends from April to November with a bimodal pattern in two peaks, the most important in August. In this framework, it is very useful to point out that the selection of the station of Aït Naaman was carried out to compare the fauna of this station where no case of leishmaniasis was declared with the other stations. These results confirm, therefore, the words of experts who argue that the species of the genus Sergentomyia are not yet proven to be involved in the transmission of leishmaniasis [1, 3, 6]. Nevertheless, the absence of leishmaniasis cases should not eliminate the potential risk of transmission since the vector species of *L. infantum* (*P. longicuspis* and *P. sergenti*) were also found.

In summary, this entomological survey around the sand fly carried out in the province of El Hajeb in central Morocco has provided both information on the circulating sand fly fauna in the region and an opportunity to enrich the Moroccan inventory. The sandfly is popularly known by the name "Chniwla" [11, 38]. However, despite the abundance of this insect on the national territory and the recrudescence of cutaneous and visceral leishmaniasis cases declared each year, sand flies are poorly known and their vectorial capacities to transmit these diseases are underestimated by citizens and even health professionals [12]. Vector control strategies must be implemented with emphasis on improving the knowledge of the population and health professionals on sand flies and the means of protection and prevention of health risks.

Conclusion

In summary, this study presents for the first time the circulating phlebotomine fauna in the El Hajeb region of central Morocco. It provides basic data on the abundance, biodiversity, seasonality of sand flies and risk periods. It was shown that the species of sand flies involved in the transmission of leishmaniasis (*P. papatasi*, *P. sergenti*, *P. longicuspis* and *P. perniciosus*) occupy the first place in terms of predominance. These results serve as a very important tool for the decision-makers of the National Leishmaniasis Control Program to orient their vector control actions towards the periods of high risk of transmission which coincide with the periods of peak density especially in the summer and autumn seasons. Thus, entomological surveillance must be accompanied by awareness and information actions for the inhabitants to avoid the health threats of sand flies and prevent the spread of leishmaniasis.

Declarations

Competing interests

None

Authors' contributions

KEM & SM conceived and designed the study for the collection of sand flies. KEM and CF did the processing and identification of phlebotomine. KEM, SB, MF, SM, CF and AC analyzed the data and drafted the manuscript. All authors reviewed and approved the final manuscript.

Data Availability

The data used in this study are included in the article

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Figures

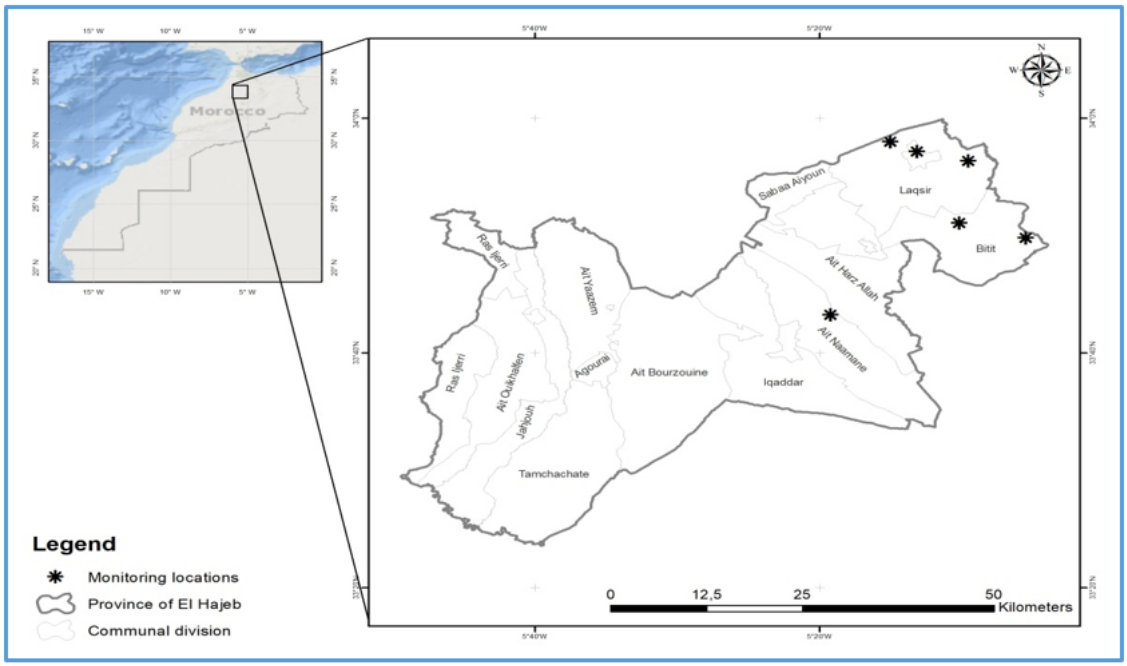

Figure 1

Map showing the sand fly collection stations in El Hajeb, Morocco

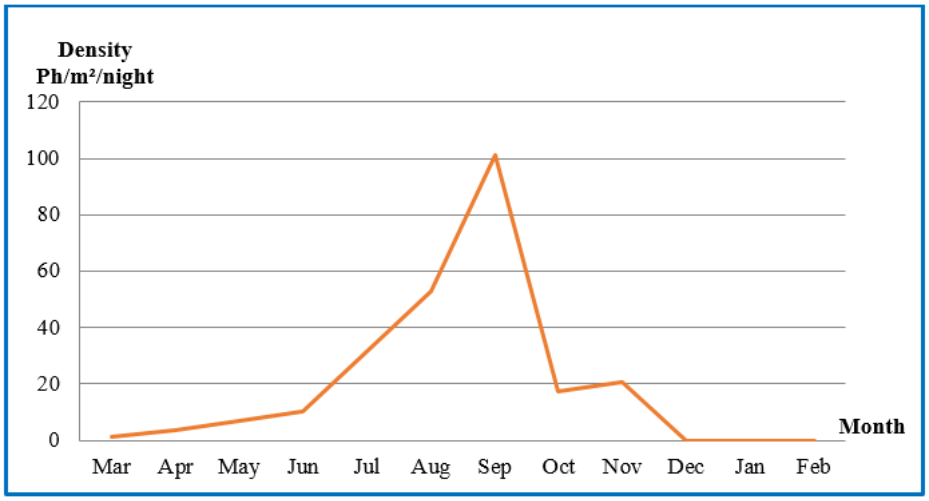

Figure 2

Monthly evolution of the total density of the phlebotomine fauna in El Hajeb, Morocco

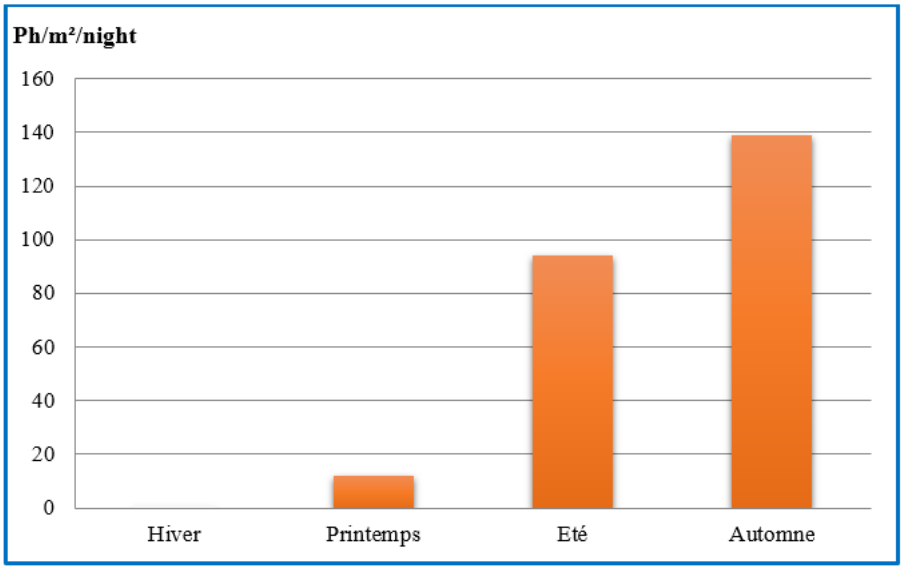

Figure 3

Seasonal evolution of the total density of the phlebotomine fauna in El Hajeb, Morocco

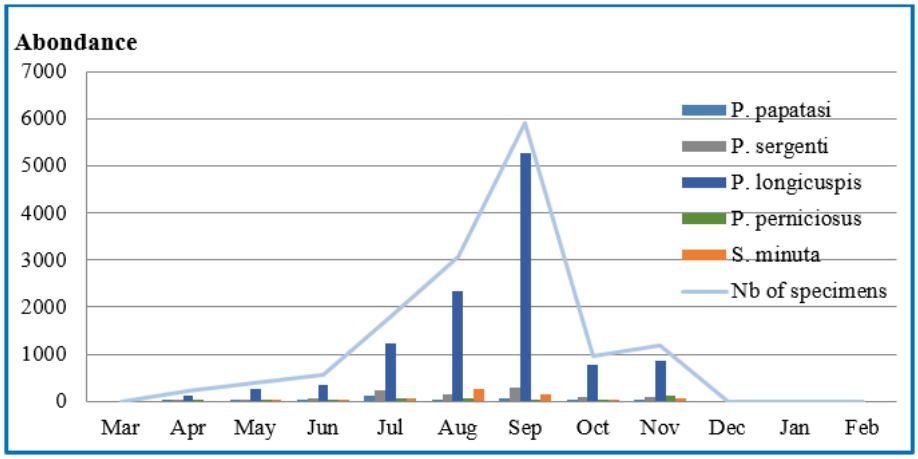

Figure 4

Monthly fluctuation of sandflies in the province of El Hajeb, Morocco

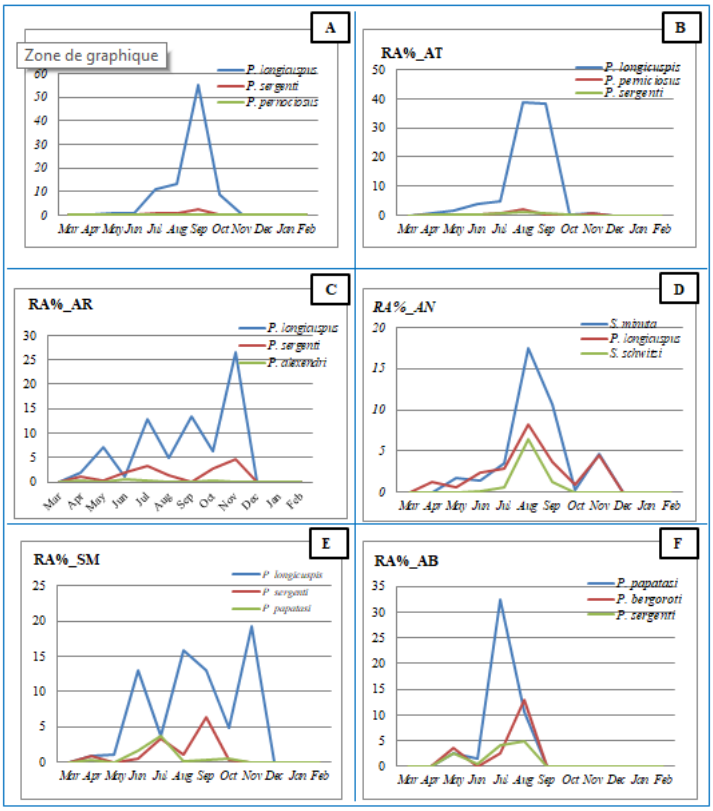

Figure 5

Monthly fluctuation and relative abundance (RA) of the three most frequent sandfly species in the study sites. *A*: Aït Ouflah (AO); *B*: Aïn Taoujdate (AT); *C*: Aït Rbaa (AR); *D*: Aït Naaman (AN); *E*: Sidi Mbarek (SM) and *F*: Aït Brahim (AB)

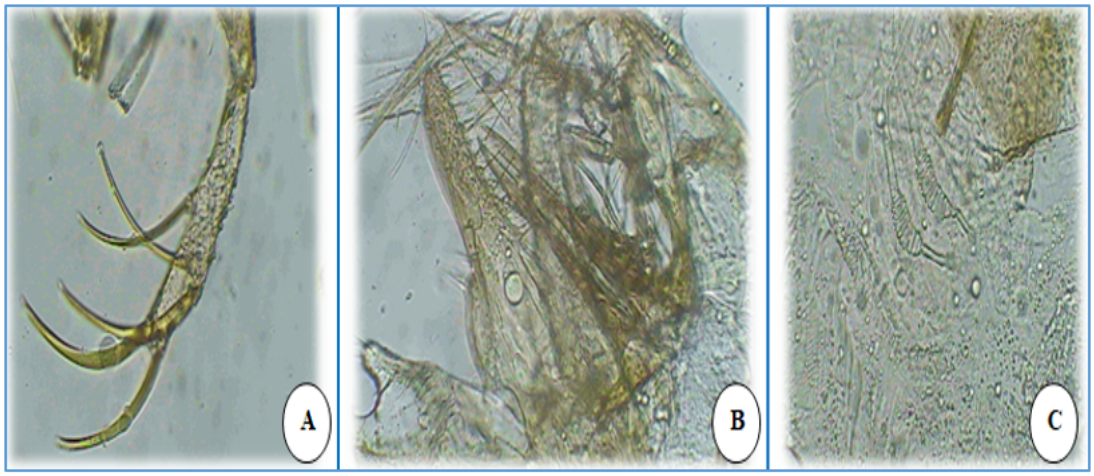

Figure 6

Phlebotomus longicuspis (A and B: style and pointed copulatory valves of male; C: spermathecae of female)

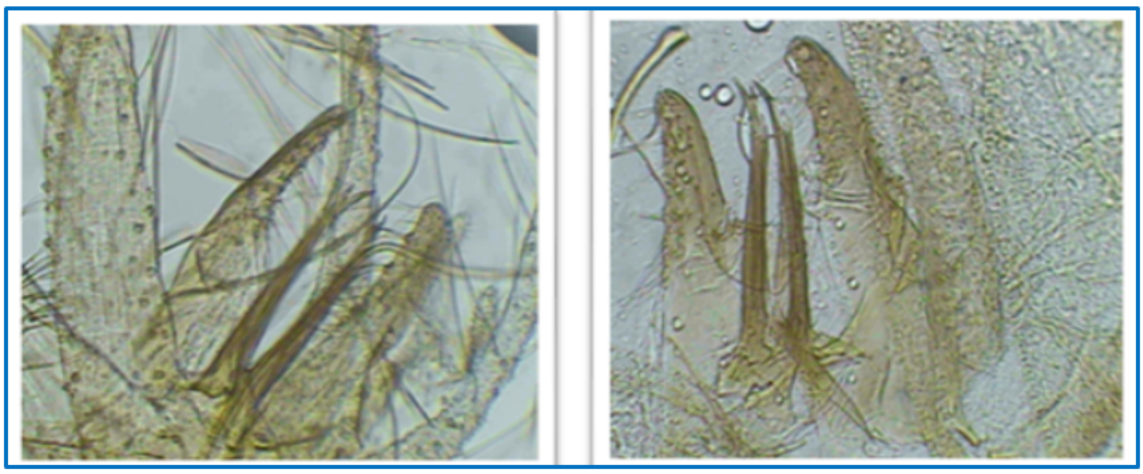

Figure 7

P. perniciosus of typical form identified at the studied stations

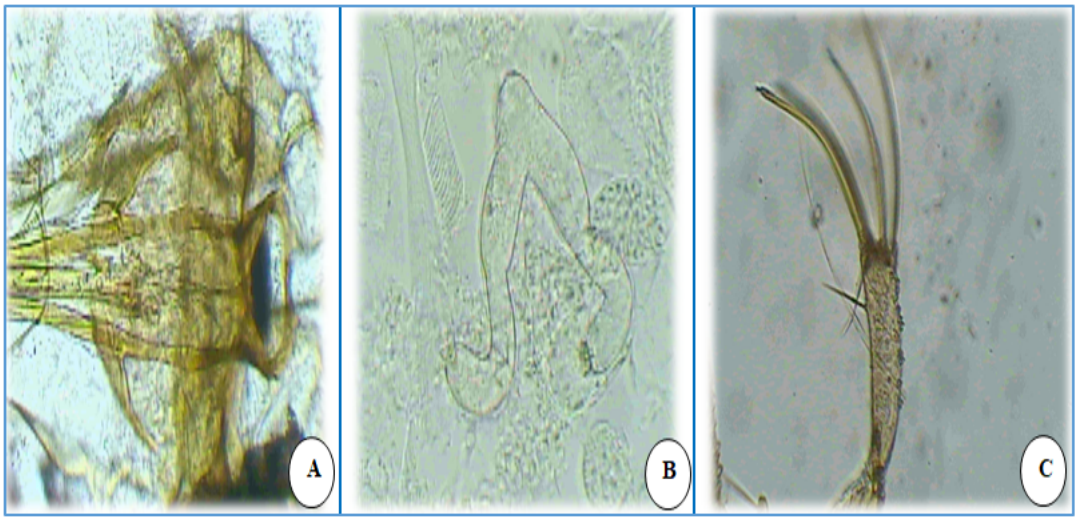

Figure 8

Sergentomyia minuta (A: cibarium of female; B: spermathecae of female; C: style with non-deciduous silk of male)

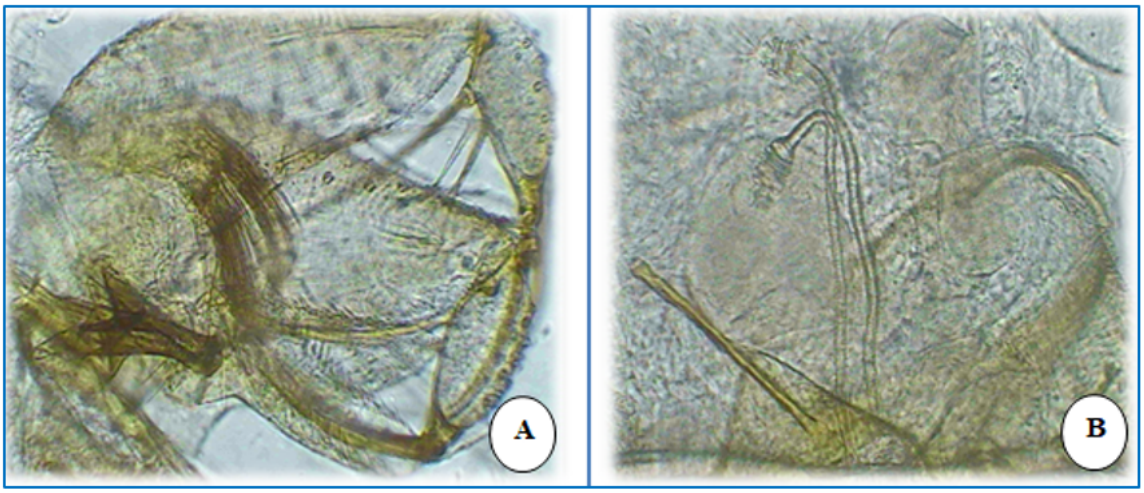

Figure 9

Phlebotomus sergenti (A: male paramere; B: female spermathecae)

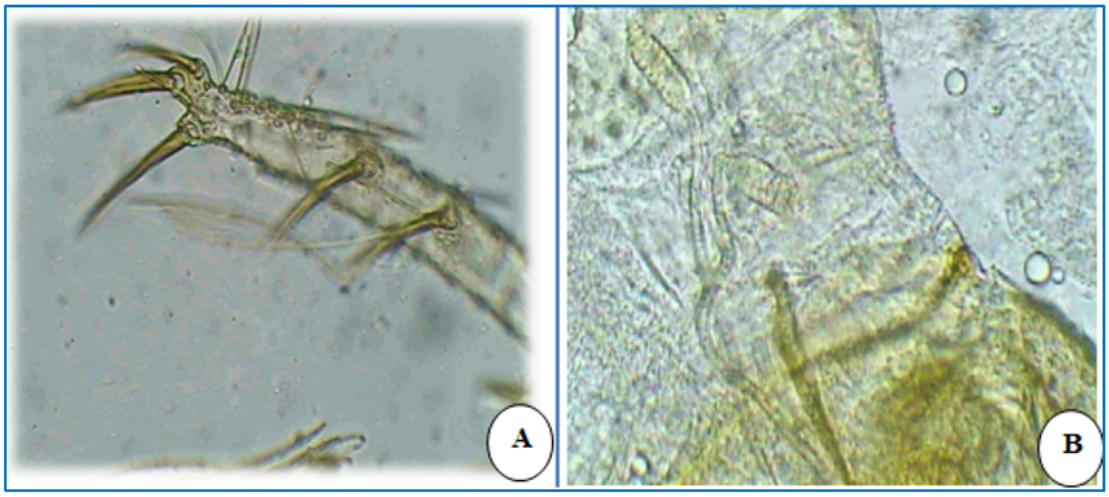

Figure 10

Phlebotomus papatasi (A: slender style with five terminal spines of male; B: spermathecae of female)

Figure 11

P. bergeroti identified at the surveyed stations