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Research Article

ENTOMOLOGICAL INVESTIGATIONS IN MOULAY YACOUB, LEISHMANIASIS FOCUS IN THE CENTER OF MOROCCO

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ABSTRACT

Introduction: In Morocco leishmaniasis represent entities of great clinical and epidemiological diversity, and therefore, a public health problem. Sandflies are the only known vectors of such diseases.

Objective: The objective of this study is to investigate sandflies of the province of Moulay Yaâcoub to inventory the species that run and know their distribution in all municipalities of the province. This aspect has never been studied and so the phlebotomy fauna of the region remains unknown until now.

Methods: Trapping was done using sticky traps once a month during the period of activity of sandflies from April to October.

Results: A total of 3287 specimens of sandflies were collected, 8 species belonging to two genera *Phlebotomus* (94.3%) and *Sergentomyia* (5.7%). The study of sex ratio showed an advantage of males to females for all species. However, for the species *Sergentomyia falax, Sergentomyia antennata,* and *Sergentomyia minuta,* we have not collected females. The highest sex ratio was determined for *Phlebotomus perniciosus* and lowest among *Sergentomyia dreyfussi*. The results show a difference in the distribution of species between different localities.

Conclusion: It was possible to distinguish two major entities; a dominant subgenre *Phlebotomus and Paraphlébotomus*, responsible for the cutaneous form in the center and northwest of the province, and a subgenre dominated *Larroussius* entity responsible for the visceral form the northeast of the province with a small entity in the southeastern province. The results of this study will help undoubtedly health authorities to establish appropriate monitoring and antivectorial control measures.

Keywords: Leishmaniasis, Phlebotomes, Entomological investigations, Distribution, Moulay Yaâcoub, Morocco.

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INTRODUCTION

Sandflies are the only known vectors of leishmaniasis so far. In Morocco, leishmaniasis represents the entities of a great clinical and epidemiological diversity with all the combinesd forms and thus constitutes a serious problem threatening public health. As such, this identified issue is currently integrated in the priorities of the Ministry of Health [1]. Indeed, in 2010, 8846 cases were reported including 8707 of cutaneous leishmaniasis (CL) cases and 139 cases were of visceral leishmaniasis (VL) [2]. We also note that even a small decrease in number of cases (2641 in 2014) according to studies of the Moroccan Ministry of Health [3], thus, the situation constitutes a real health problem. Globally, leishmaniasis affects between 1.5 and 2 million people each year [4]. In 2013, 72,026 cases of leishmaniasis have been reported worldwide [5]. Despite a number of remedies available, leishmaniasis is still a speedy migrating and deadly infection due to the resistance of the parasite to the drugs as well as their toxicity [6]. Recent studies have been conducted on the effects of medicinal plants on the leishmania parasites [7].

The transmission of anthroponotic CL is held by *Infantum, Phlebotomus (Paraphlebotomus) sergenti (Phlebotomus sergenti)* Parot 1917, vector of *Leishmania tropica* Wright [8,9] or *Leishmania infantum* zoonotic coetaneous form is transmitted by *Phlebotomus papatasi* Scopoli 1786 vector of *Leishmania major* Yakimoff Schokhor [10-12], while VL is caused by *L. infantum* Nicolle, transmitted through three species namely *Phlebotomus ariasi*, *Phlebotomus perniciosus*, and *Phlebotomus (Larroussius) longicuspis* Nitzulescu 1930 [13]. Until 1999, this form was mainly limited to rural areas with hypoendemic transmission, since then the disease has spread gradually from south to north and from rural to urban areas [14]. *P. longicuspis* is also responsible for sporadic CL caused by *Leishmania* in the semi-arid belt [15] and even in the arid belt [13].

The area of Moulay Yaâcoub has been reported as a micro focus of CL [16]. In 2001, an epidemic was triggered and increased the number of cases to 1600 cases. 205 cases were recorded from 2005 to 2015, however, the phlebotomy fauna of the province remains unclear with the exception of a few fragmentary works especially parasitology and our work in two areas [17,18]. The objective of this study is to inventory the province sand fly species and to know their spatial distribution in all municipalities of the study area, to understand the epidemiological situation to prevent any risk of outbreak of epidemic. This aspect has never been studied and so the phlebotomy fauna of the region remains unknown until now. The results of this study will help health authorities to establish appropriate and antivectorial control measures.

METHODS

Study area

Our study was conducted in the area of Moulay Yaâcoub, North central of Morocco, a rural area with an urbanization rate of 2% (85.5% of the population are living in rural areas) (Census 2014).

The province is located at an average altitude of 563 m above sea level; it is characterized by rugged terrain composed of plateaus and hills whose altitude varies from 50 to 600 m, and mountains whose highest top reaches about 910 m (Zalagh and Tghat). The population of the region is estimated at about 174,079 inhabitants according to Census 2014.

There are two geographical units in the province: The Saiss Plain in south and west (Sbaa Rouadi, Ain Chkef, and part of Ain Kansara) and the Pre-Rif hills throughout the northern area.

Collection and identification of sandflies

The collection of sandflies was performed during the period of activity of sand fly from April to October [18]. The catch was performed using adhesive traps (A4 paper coated with castor oil) [19] and miniature light traps with type the Centers for Disease Control [20].

Traps were set up dusk and recovered the next day at dawn once a month. After collection, the captured specimens were removed from the traps using a brush dipped in alcohol, and preserved in ethanol at 95% [21]. Sandflies captured by light traps were necessary to be put in the refrigerator for about 30 minutes before being removed.

The specimens were thinned by Marc-André solution (acetic acid chloral hydrate) and were mounted in Canada balsam for identification [22].

The identification was made by the identification key of Morocco sandflies based on the examination of the morphology of the male's genital organs, spermatic and throat for identifying the females [23].

Required parameters

1. Relative frequency $= \frac{\text{Number of specimens of the species}}{\text{Total number of collected specimens}}$
2. Abundance: The number of specimens collected for each species.
3. Sex ratio: The representativeness of the males' sandflies compared to females for each species $R = \frac{Nm}{Nf}$

   Nm: Number of males

   Nf: Number of females.
4. Specific richness: The number of species that account settlement and for each the locality is studied.

Topographic coordinates

Geographic coordinates and altitudes at each site were determined using a GPS.

Statistical analysis

Statistical analysis includes comparison of proportions test Chi-square Pearson at the 95% using the Statistical Package for the Social Sciences software (version 20).

RESULTS

Topographic coordinates and altitude of 11 sites have been determined (Table 1).

Relative frequency and abundance of species

In this study, a total of 3287 specimens of sandflies were collected. The morphological identification has revealed the presence of eight species belonging to two genera *Phlebotomus* (94.3%) and *Sergentomyia* (5.7%). The difference between genders is statistically significant (χ^2=3287, p<0.001).

P. papatasi (Scopoli 1786) is the dominant species with 35.11%±2.86, followed by *Ph.longicuspis* Nitzulescu 1930 (24.46%±2.07), *P. perniciosus* Newstead 1911 (18.02%±2.59), *P. sergenti* Parrot 1917 (16.27%±0.68), *Sergentomyia falax* (3.67%±0.7), *Sergentomyia antennata* (0.79%±0.12), *Sergentomyia minuta* (Rondani 1843) (0.49%±0.13), *Sergentomyia dreyfussi* (0.26%±0.1) (Table 2). The difference between the species collected is statistically significant (χ^2=2929.85, p<0.001).

Sex ratio

The males are the most represented with 82.36%, while females represent only 17.64% of the collected total in 11 localities. The difference between the sexes is statistically significant (χ^2=1806.80, p<0.001). The study of sex ratio showed an advantage of males to females, for all species. However, for species such as *S. antennata* and *S. minuta* we have not collected females and for *S. falax* we have only collected females. The highest sex ratio was determined at the species *P. perniciosus* with a rate of 10.77, followed by *P. longicupis* with 7.49, *P. sergenti* 5.89, *P. papatasi* 3.85 and the sex ratio lower was determined at the *S. dreyfussi* species with 1.66 (Fig. 1). The Chi-square test confirmed the existence of a statistically significant relationship between the species and the observed sex in each of them at the 95% confidence level (χ^2=1137.81, p<0.001).

Species

Species richness

The morphological identification has revealed the presence of eight species belonging to two genres: *Phlebotomus* with (94.3%) and *Sergentomyia* with (5.7%). The highest species richness was recorded at two resorts Ain Kansara and Ain Chkef where we identified the eight species mentioned above followed by the center of Moulay Yaâcoub and Louadain resort with seven species, Ain Bouali and Sebt Loudaya with six species, Sidi Daoud and Mikkes with five species, Laajajra and Sbaa Rouadi with four species. However, it appears that the location Ouled Mimoun is poorer where we have only identified three species (*P. sergenti*, *P. papatasi*, *P. perniciosus*) (Table 3).

In the 11 studied localities three species are still present, *P. papatasi*, *P. Sergenti,* and *P. pernciosus. P. Longicuspis* is present in 10 locations;

Table 1: Topographic coordinates and altitude of 11 sites

Localities	X	Y	Altitude (m)
Ain Kansara	559,480	388,438	345
Ain Chkef	534,207	373,873	500
Sbaâ Rouadi	524,471	381,632	347
Ouled Mimoun	532,415	401,514	470
Moulay Yaâcoub	520,209	387,603	308
Louadaine	542,593	400,398	152
Ain Bouali	533,944	395,768	150
Laâjajra	522,765	399,405	452
Sidi Daoud	523,789	411,457	478
Sebt Loudaya	509,505	402,816	55
Mikkes	506,132.3	392,754.8	190

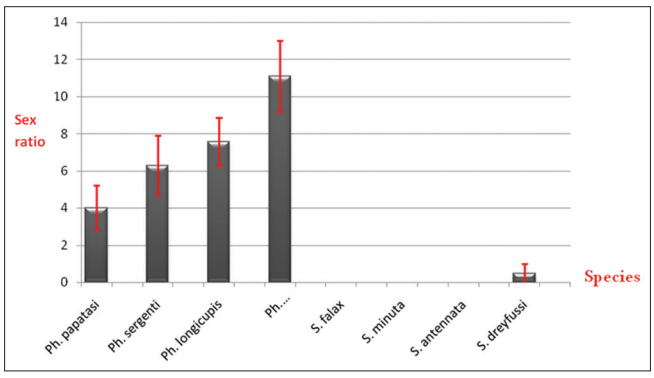

Fig. 1: Sex-ratio of collected species

Table 2: Frequency and abundance of sandflies collected by species

Genus	Subgenus	Espece	Abundance*	Frequency %*
Phlebotomus	*Phlebotomus*	*P. papatasi*	420±44.8	35.11±2.86
	Paraphlebotomus	*P. sergenti*	191±11.53	16.27±0.68
	Larroussius	*P. longicuspis*	287±27.87	24.46±2.07
	Larroussius	*P. perniciosus*	212±23.06	18.02±2.59
Sergentomyia	*Sergentomyia*	*S. falax*	42.33±8.02	3.67±0.7
	Sergentomyia	*S. antennata*	9.33±1.52	0.79±0.12
	Sergentomyia	*S. minuta*	5.66±1.52	0.49±0.13
	Grassomyia	*S. dreyfussi*	2.66±1.15	0.26±0.1

*Values were expressed as mean±SD of three catches (n=3287). SD: Standard deviation, *S. falax: Sergentomyia falax, S. antennata: Sergentomyia antennata, S. minuta: Sergentomyia minuta, S. dreyfussi: Sergentomyia dreyfussi, P. papatasi: Phlebotomus papatasi, P. sergenti: Phlebotomus sergenti, P. longicuspis: Phlebotomus longicuspis, P. perniciosus: Phlebotomus perniciosus*

Table 3: Specific richness by localities

Localities	Specific richness
Ain Kansara	8
Sbaâ Rouadi	4
Ain Chkef	8
Ouled Mimoun	3
Moulay Yaâcoub	7
Sidi Daoud	5
Laâjajra	4
Mikess	5
Sebt Loudaya	6
Ain Bouali	6
Louadaîn	7

it is not present at the locality "Ouled Mimoun." *S. falax* is the most abundant of the four identified species of *Sergentomyia*, it was collected in eight localities, followed by *S. minuta* in 6 localities and *S. antennata* who is present at 4 localities. However, *S. dreyfussi* can be considered as the very rare species in our collection, it was only identified at two locations Ain Kansara and Ain Chkef (Table 4).

Table 4 shows the abundance of each species and species richness of 11 localities studied. To investigate the relationship between the studied stations and the species we used Chi-square test which confirmed that there is a statistically significant relationship between the stations and the presence of species in each locality at a 95% confidence level (χ^2=1832.10, p<0.001).

Hence, we can distinguish between two main entities, one with a dominant subgenus *Phlebotomus* and *Paraphlebotomus* which responsible for cutaneous form in the center and northeast of the province, and an entity with a dominant subgenre of *Larroussius* which responsible for the visceral form in northwest of the province with a small entity in the southeastern province (Fig. 2).

DISCUSSION

The interest of this study is the importance of the data presented on the biodiversity of sandflies in the province of Moulay Yaâcoub, their spatial distribution, abundance and richness of localities with species of sandflies. Indeed these data are very interesting for the establishment of a fighting strategy against sandflies in this province.

Relative frequency and abundance of species

The results showed the coexistence of two kinds of sandflies, the statistical test (Chi-square) revealed that there is a difference between the two statistically significant kinds (χ^2=3287, p<0.001). The genus *Phlebotomus* is the kind shown in the transmission of leishmaniasis, in the new world, the kind *Sergentomyia* can be in some cases offending case of transmission of leishmaniasis [24,25]. The abundance and dominance of collected species seem like varied from one locality to another, and sandflies are unevenly distributed. *P. papatasi* dominates in four low-lying areas of the west-central Moulay Yaâcoub Province Ain Kansara, Urban Centre Moulay Yaâcoub, Ain Bouali and Loudaine, with respectively, 63.62%, 62.02%, 47.47%, and 39.33%. At both locations Ouled Mimoun and Sbaa Rouadi the species *P. sergenti* dominates with 93.34% and 66.66%, respectively. *P. papatasi* a proven vector of *L. major* in Morocco [26] is also present in our study area, although it is considered more suited to the arid climate, some authors [27] consider that *P. papatasi* has its maximum density at arid and periarid areas and becomes scarcer in the stages subhumid, humid and semiarid. Guernaoui *et al*. [28] negatively correlated the abundance of this species with altitude and found that it is so abundant in low altitude resorts between 300 and 400 m, which is consistent with the results of our work.

P. sergenti was confirmed as the responsible of the dry CL [26,29,30] at *L. tropica* in Morocco [8]. It dominates in arid and semiarid [26,31]. In this field of study, *P. sergenti* is dominant in two localities Ouled Mimoun and Sbaa Rouadi with 93.34% and 66.66%, respectively. In Taza, a semi-arid area of northern Morocco, the study of the dynamics of this species showed two density peaks [32]. An entomological study in the province of Sidi Kacem, located in northwest border of the province of Moulay Yaâcoub, highlighted the dominance of *P. sergenti* and determine its responsibility in the transmission of CL at *L. tropica* [33].

In the northeastern part the species *P. longicuspis* dominates at three locations, plus a southeastern town in the Province Sidi Daoud, Mikkes, Ain Chkef and Sebt Loudaya with 51.6%, 50%, 49.34% and 32.17%, respectively. The species *P. perniciosus* only dominates at a single location: Laâjajra with 45.72% and constitutes a continuation of *P. longicuspis* with the same subgenus, *P. longicuspis* species is frequent and widespread in Morocco [31,34] considers that this is a species which is subservient to semiarid and subhumid. It is known for its role in the transmission of VL in *L. infantum* in the Mediterranean basin [35] and in Morocco [27].

El Miri *et al*. [33] have suggested that the *P. longicuspis* is responsible for the transmission of VL in *L. infantum* in the province of SidiKacem. *P. perniciosus*, one of the most competent vectors of *L. infantum* in the homes of the Mediterranean [36], was present for the duration of our study, this is consistent with the results obtained by Guernaoui *et al*. [37] in the province of Chichaoua, in the south-west of Morocco. In our study area and the view of the low dominance of *P. perniciosus* we can affirm that the transmission of VL is always assured by *P. longicuspis*.

Sex ratio

The study of sex ratio showed that phlebotomy wildlife area is rather represented by males than females with 4.66 males to one female. The Chi-square test confirmed the existence of a statistically significant relationship between the species and the observed sex in each of them at the 95% confidence level (χ^2=1137.81, p<0.001). The work of El Miri *et al*. [33] in SidiKasem, in the west of Province Moulay Yaâcoub has found a sex ratio of 4 males to one female, which is very close to our results. However, the work of Talbi *et al*. [38] on the province of Sefrou in the south of our study area, found that males are 7.54 times more abundant than females. For all species sex ratio is >1, however, for the

Table 4: Species collected, their abundances in different localities studied

Station	*P. papatasi*	*P. sergenti*	*P. longicuspis*	*P. perniciosus*	*S. falax*	*S. antenata*	*S. minuta*	*S. dreyfussi*
Ain Kansara	208±26.3	24±7.9	9±2.6	29.33±8.3	6.66±1.5	1.33±0.5	3.66±1.5	1.66±1.52
Ain Chkef	8.66±1.52	10.66±2	24.66±4.9	1±1	2±1	1.33±1.5	1±1	1±1
Sbaâ Rouadi	2±1	8.66±1.5	1.66±0.5	0.66±0.5	0	0	0	0
Ouled Mimoun	0.66±0.5	18.66±1.1	0	0.66±0.5	0	0	0	0
Centre Moulay Yaâcoub	69.66±11.9	3.66±1.15	6.3±0.57	29.66±4.5	0.66±1.15	2±1	0.33±0.57	0
Louadain	39.33±4.7	23±6	21±6.5	9.66±2.5	4.66±2	1.66±0.5	0.66±0.57	0
Ain Bouali	59.33±7	16.66±4.9	16±3	21±2.6	9±2	3±1	0	0
Sidi Daoud	2.66±0.5	33.33±12	86±12	36.33±11.7	8.33±2.5	0	0	0
Laâjajra	5±1	15±2.6	18±2	32±4.3	0	0	0	0
Mikkes	11.66±4.5	36±10.5	81.66±21.6	30.33±11.2	3.66±1.15	0	0	0
Sebt Loudaya	13.33±3	1±1	22.33±7.5	21.33±5.1	7.33±2.5	0	0	0

Values were expressed as mean±SD of three catches (n=3287). SD: Standard deviation, *P. papatasi: Phlebotomus papatasi, P. sergenti: Phlebotomus sergenti, P. longicuspis: Phlebotomus longicuspis, P. perniciosus: Phlebotomus perniciosus*

Fig. 2: Dominance of subgenus collected in the province of Moulay Yaâcoub in the center of Morocco

species. *S. antennata*, *S. minuta* we have not collected females and for *S. falax* we have only collected females. Therefore, we were unable to determine the sex ratio. The highest sex ratio was determined at the species *P. perniciosus* with a rate of 10.77, followed by *P. longicuspis* with 7.49, *P. sergenti* 5.89, *P. papatasi* 3.85 and the lowest sex-ratio was determined in species *S. dreyfussi* with 1.66. The work of Kahim *et al*. [39] in the plain of Al Haouz in Marrakech and in the mountains of the High Atlas, for each species the sex ratio is >4, and for *S. minuta* it is >1. However, for *S. falax* the sex ratio is 0.67, while for *S. dreyfussi* it is with equal distribution.

Females *P. sergeni*, *S. dreyfussi,* and *S. falax* were most abundant in the work of Boussaa [40], while the sex ratio is in favor of males to *Sergentomyia africana* and *S. minuta*.

Species richness

The results of the study of species richness showed that the spatial distribution of sandflies species varies in different localities, and some localities are richer in species than others. This distribution may be related to variations in altitude, vegetation, and can also be linked to the type of soil because the female sand fly are laying their eggs in the soil. Abonnec [41] connects the appearance of sandflies, their density, their term of office and their disappearance with latitude, altitude, season, and species.

The species *P. ariasi* was absent in our investigation. Guernaoui *et al*. [37] have raised *P. ariasi* between 1000 and 1400 m, whereas in our study area elevations range from 47 m in the northwest and 867 m on the hills in the center of the territory which explains the absence of this species.

Notwithstanding that the morphological identification showed that the species *P. papatasi* is most common in the province with (31.45%), the calculation of the relative frequency of species in each studied locality showed that dominance of species varies from one locality to another, as the dominant species in a locality may be the least represented, while they are absent in another locality.

CONCLUSION

Leishmaniasis is a disease that constitutes a real threat to public health. Our investigation in the province of Moulay Yaâcoub allowed collecting 3287 samples with two genres and eight species. One can conclude that the province of Moulay Yaâcoub presents a diversity of phlebotomies fauna, this diversity varies from one locality to another, and some localities are richer in species than others.

The species are distributed in a more or less logical way, and we were able to determine two major entities, one with a dominant subgenre *Phlehotomus* and *Paraphlebotomus* responsible for the cutaneous form in the center and northeast of the province, and an entity with *Larroussius* dominance responsible for the visceral form in the northwest province. Species diversity and abundance of sandflies make the province more vulnerable in the face of leishmanian risk.

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