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# Vectorial-transmission Risk Assessment of Leishmaniasis due to the Presence of Sand Flies in Northwest Morocco

**Aziz El Aasri[1*], Youssef El Madhi[1,2], Nizar Shawket[1], Arwa AL Khali[1], Khadija El Karrim[1], Driss Belghyti[1]**

[1]Laboratory of Biotechnology, Environment and Quality, Department of Biology, Faculty of Science, Kenitra, Morocco

[2]Research team Right of Transport and Distribution (TRADIS), Mohammed V University in Rabat, Morocco

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***Corresponding Author**: Aziz El Aasri, Professor of Life Sciences and the Earth (second cycle). Ministry of National Education and Vocational Training, Morocco.Tel: +212611681480. E-mail: elaasri.aziz@uit.ac.ma



## Introduction

Leishmaniasis are parasitic diseases occur in tropical and subtropical areas of 98 countries, including 72 developing countries. The endemic areas are southern Europe and many countries in East Africa, South Asia and South America. The global prevalence is estimated at 12 million cases. The incidence of leishmaniasis is increasing worldwide. There are between 1.5 and 2 million new cases of leishmaniasis each year worldwide and about 40000 deaths, due to visceral leishmaniasis [1,2]. Leishmaniasis are transmitted by the bite of the hematophagous female sand fly of the genus Plebotomus (diptera psychodidae) [1].

In Morocco, as in most countries around the Mediterranean, leishmaniasis are also a serious public health problem because of the increasing number of cases detected each year and the spread of these parasitic diseases to other previously free areas [3,4]. The aim of this entomological survey was to determine the structure of phlebotomine sand flies fauna in order to evaluate the vectorial-transmission risk of leishmaniasis in the GCBH area in north-west Morocco.

## Material and Methods

### Study area

The GCBH area occupies the northern parity of the greater region of Rabat-Sale-Kenitra. It includes 3 provinces (Kénitra, Sidi Kacem and Sidi Slimane), 10 urban communes and 53 rural communes. It is bounded on the west by the Atlantic Ocean, on the north by the Tangier-Tetouan-El Hoceima region, on the east by the Fes-Mekness region and on the south by the two provinces of Khemisset and Salé. It covers

about 7900 km² km², or nearly 1.08% of the area of Morocco. Its population is 1,859,540 hab, or 6.22% of the national population. The average density is 211 inhabitants / km2, five times higher than the national density (40 hab / Km2).

### Sand Fly Collection

We used three methods to capture sand flies:

#### (1) Sticky traps

This technique is particularly best suited to the inventory of sand flies resting sites in the extended region. These adhesive traps are made of matt white paper -format 15 x 20 cm- largely soaked purified castor oil. They are placed at the entrance of sand flies lodgings in the evening around 6pm, and picked up until the next morning around 7am. They allowed us to study the spatial distribution and compare stocking densities in different habitats [5].

#### (2) Mouth aspirator

The Standard Mouth Aspirator features a 12 inch long tube made of polycarbonate plastic is used for aspirating live shadflies in different natural biotopes. This method is not very interesting at the harvest but it makes it possible to obtain the shadflies in good condition [6].

#### (3) Garland

The Garland consists of a rectangular wooden box whose front is divided into two rectangular windows (29 cm of languor and 21 cm of width). Behind each window, a torch attached to a nail attached to the back wall of the case illuminates the oiled paper. This trap is placed on a support less than one meter above the ground and allows inventorying the fauna of the litter and herbaceous vegetation [7].

### Treatment

All the collected samples were preserved in alcohol (70%) until the microscopic mounting. They have been clarified by 10% potash for 4 hours and Marc-André liquid for 2 hours. Then they were mounted between slide and cover slip in the Marc Andre liquid. The head is placed on its underside, its dorsal face in the upper position (for the Phlebotomus genus) to have an easy observation of pharyngeal armatures. Male genitalia and the distal portion of female abdomen are deposited on their side faces to observe the different elements necessary for this specific diagnosis [5, 8].

## Results

### Provincial distribution of prospected stations

During the period from April 2006 to June 2015, More than 109 sites were surveyed in the GCBH area (Table 1). Among all the prospected stations, 1, 44.6% are positive compared to 55.4% negative. Sidi Kacem Province ranks first in the number of positive stations (22.02%), Followed by Kenitra (11.93%) and finally the province of Sidi Slimane with 11% of stations with presence of sand flies.

### Global fauna inventory

The overall results of the entomological investigations carried out between 2006 and 2015 are shown in (Table 2). The sandflies identified belong to 7 species and two genera. The genus Phlebotomus represented by four subgenera and six species is dominant (79.42%) on the genus Sergentomyia (20.58%) that is represented by the single species Sergentomyia minuta.

**Table (1)**: Regional distribution of prospected sites

| Province Province | Number of stations surveyed | | | % Positive citations |
|---|---|---|---|---|
| | Positive | Negative | Total | |
| **Kenitra** | 13 | 31 | 44 | 11,92 |
| **Sidi Slimane** | 12 | 17 | 29 | 11 |
| **Sidi Kacem** | 24 | 12 | 36 | 16,51 |
| **Total** | 49 | 60 | 109 | 44,6 |

It also appears from our results that Phlebotomus sergenti is the most abundant and most frequently encountered species in our catches (52.18%) followed by Sergentomyia minuta (20.58%) and Phlebotomus longicuspis with 20.46%. The other species are less represented; Phlebotomus papatasi represents only 5.62%, Phlebotomus perniciosus (1.08%) and Phlebotomus ariasi with only 0.06%. The latter species was harvested for the first time in the GCBH area.

**Table (2)**: Numbers and abundance of species caught (2006-2015)

| Genus | Subgenus | species | Number | Abundance (%) |
|---|---|---|---|---|
| **Phlebotomus** | Larrousius | *p longicuspis* | 2512 | 20,46 |
| | | *p perniciosus* | 133 | 1,08 |
| | | *p ariasi* | 7 | 0,06 |
| | Paraphlebotomus | *P.sergenti* | 6404 | 52,18 |
| | | *p chabaudi* | 2 | 0,02 |
| | Phlebotomus | *p papatasi* | 689 | 5,62 |
| **Sergentomya** | Sergentomyia | *s minuta* | 2525 | 20,58 |
| **Total** | | **7** | **12272** | **100** |

According to Table 3, a dominance of male specimens by contribution to the females (84.4% males against 15.6% females). Indeed, the proportions by species show the dominance of males on females in 6 species among the 7 inventoried in this study. Female dominance is observed in the only species of Phlebotomus ariasi (3 females and 1 male).

**Table (3)**: Proportion of species by sex

| Species | Male | | Female | | Total | |
|---|---|---|---|---|---|---|
| | Nb | % | Nb | % | Nb | % |
| ***p. longicuspis*** | 2162 | 17,61 | 350 | 2,85 | 2512 | 20,46 |
| ***p. perniciosus*** | 116 | 0,95 | 17 | 0,14 | 133 | 1,08 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***p. ariasi*** | 3 | 0,027 | 4 | 0,033 | 7 | 0,06 |
| ***P.sergenti*** | 5471 | 44,58 | 933 | 7,60 | 6404 | 52,18 |
| ***p. chabaudi*** | 2 | 0,016 | 0 | 0 | 2 | 0,02 |
| ***p. papatasi*** | 509 | 4,15 | 180 | 1,47 | 689 | 5,62 |
| ***s. minuta*** | 2094 | 17,06 | 431 | 3,51 | 2525 | 20,58 |
| **Total** | 10357 | 84,4 | 1915 | 15,6 | 12272 | 100 |

## Distribution of species according to the types of traps

The distribution of identified spaces according to the Sampling Techniques is recorded in (Table 4).

**Table (4)**: Distribution of species according to the types of traps

| **Species** | **Sticky traps** | | **Garland** | | **Mouth aspirator** | | **Total** | |
|---|---|---|---|---|---|---|---|---|
| | **Nb** | **%** | **Nb** | **%** | **Nb** | **%** | **Nb** | **%** |
| ***P. longicuspis*** | 2376 | 19,36 | 139 | 1,13 | 0 | 0 | 2515 | 20,49 |
| ***P. perniciosus*** | 133 | 5,85 | 0 | 0 | 0 | 0 | 133 | 1,08 |
| ***P. ariasi*** | 3 | 0,024 | 1 | 0,008 | 0 | 0 | 4 | 0,03 |
| ***P. sergenti*** | 5775 | 47,05 | 611 | 4,98 | 18 | 0,15 | 6404 | 52,18 |
| ***P. chabaudi*** | 2 | 0,016 | 0 | 0 | 0 | 0 | 2 | 0,02 |
| ***P. papatasi*** | 672 | 5,47 | 15 | 0,12 | 2 | 0,016 | 689 | 5,62 |
| ***S. minuta*** | 21555 | 17,56 | 359 | 2,92 | 11 | 0,09 | 25225 | 20,58 |
| **Total** | 11116 | 90,58 | 1125 | 9,17 | 31 | 0,25 | 12272 | 100 |

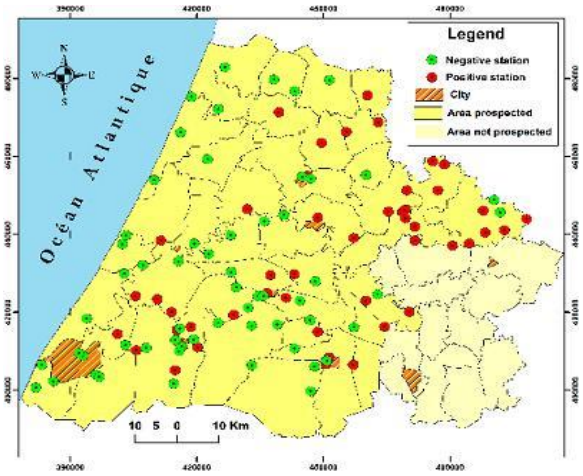
In total, the adhesive traps allowed us to catch 11116 sandflies representing 90.58% of the captured insects. The "Garland" collects 1125 specimens representing 9.17% of the total fauna. The Mouth Capturator captured only 31 specimens or 0.25% of the total fauna. The use of adhesive traps is the only method that allowed collection of all species

encountered in our study area. The "garland" allows the capture of 5 species, whereas, the Mouth aspirator made it possible to collect 3 species among the 7 identified.

## Discussion

The entomological investigations carried out between 2006 and 2015 show that the majority of positive sites are located mainly in the East and in the center of the GCBH area, while the western coastal strip has no positive station. This geographical distribution coincides with that of cutaneous leishmaniasis with L.tropica reported in this region (Figure 1) [5]. The overall faunistic inventory of these entomological investigations was 12,272 specimens divided into two genera, four subgenera and seven species of the 25 identified to date in Morocco [9,10].

**Figure (1)**: Geographical location of different stations survey




### *Phlebotomus sergenti*

It is the dominant species of our captures, it is a proven vector of cutaneous leishmaniasis caused by Leishmania tropica and it would have its ecological optimum on the sub-humid and semi-arid [11,12]. In this study Phlebotomus sergenti represents half of our catches (52.18%), this predominance confirms its role in the transmission of cutaneous leishmaniasis in the north-west of Morocco [13].

### *Sergentomyia minuta*

Is the second most abundant species in our study area with the proportion of 20.58%. Its high densities are more localized on the sub-humid and humid stages than the Saharan stages [14]. It does not intervene in the transmission of leishmaniasis [15].

### *Phlebotomus longicuspis*

Occupies the third position by its abundance which is of 20.49%, it is a suspected vector in an active focus of visceral leishmaniasis in Morocco [16]. The ethology of this species, which is also an arbovirus vector, is often close to that of Phlebotomus perniciosus [17].

### *Phlebotomus papatasi*

Is a confirmed vector of L. major [11,18], it is the most studied species because of its frequency and the importance of its geographical range [19]. In our catches it only represents 5.62% of the total fauna captured, this may be explained by the fact that we did not capture in their biotopes.

### *Phlebotomus perniciosus*

This species that lives in sympathy with Phlebotomus longicuspis is less abundant in our crop with only 1.08% of the total fauna [15]. This proportion does not reflect its ubiquitous character especially in northern Morocco [2]. These poor results can be explained by the difficulties encountered in the identification of this species. Indeed, the existence of atypical morphs which are at the origin of misidentifications and consequently of confusion with Phlebotomus longicuspis [15].

### *Phlebotomus ariasi*

Species reported as vector of visceral leishmaniasis in Mediterranean countries [20]. It represents only 1.08% of our catch in the GCBH area. This low percentage confirms its presence in too small a quantity in North Africa [20,21].

### *Phlebotomus chabaudi*

It is a species that has never been recognized as a proven vector of leishmaniasis even though Killik-Kendrik (1990) suspects that it transmitted L. tropica [11,20]. It was found during our investigation only twice, or 0.02% of the total fauna. This weak representation does not allow us to make the epidemiological interpretations.

The presence of two proven vector species of cutaneous leishmaniasis (Phlebotomus sergenti, papatasi) and one suspected (Phlebotomus chabaudi) confirms the endemo-epidemic situation of cutaneous leishmaniasis in Morocco and its northwest part in particular [5]. Thus, the identification of three species incriminated in the transmission of visceral leishmaniasis (Phlebotomus longicuspis, P. perniciosus and P. ariasi) is a worrying sign of the situation. Indeed, these results show that the area of GCBH is seriously threatened to be endemic area of this Leishmanian form.

The sex ratio of our catches is in favor of males for most species. Male dominance is observed in six of the 7 inventoried species, it can be explained by the fact that our catches were made especially in the places of activity of the males. In fact, the majority of catches were made outside dwellings and in animal shelters. The existence of males near the host can also be explained by the attraction of females for reproduction [22].

Regarding the abundance of species according to the capture techniques, we observed that the adhesive traps are the most profitable with 90.58% of the total fauna, followed by the Garland (9.17%), then the Mouth aspirator with less than 0,25%. This is explained by the fact that the use of adhesive traps was considered as a basic technique for the capture of sandflies during this study. This is the most suitable method for the qualitative and quantitative inventory of specimens [11,23]. The use of other techniques has been used only occasionally because they require special conditions which are not always appropriate. The use of other techniques has been used only occasionally because they require special conditions which are not always appropriate.

## Conclusion

Our sporadic and irregular surveys carried out between 2006 and 2015 remain insufficient to to establish a precise table of species and to draw maps for the geographic distribution of sandflies in the GCBH area. But, the presence of 7 species of which 5 are incriminated and one suspected in the transmission of leishmaniasis, constituting a risk that can confirm a rather endemic situation presenting precisely the growing cutaneous form than the visceral form in this part of the Moroccan North-West.

## Abbreviations

GCBH: Garb Chrarda Beni Hcen; P: Phlebotomus; L: leishmania; CL: Coetaneous leishmaniasis; VL: Visceral leishmaniasis.

### Competing interests

The authors declare that they have no competing interests.

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