

Entomological and epidemiological study of a new focus of cutaneous leishmaniasis in Morocco

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Abstract Cutaneous leishmaniasis (CL) due to *Leishmania tropica* Wright has become an increasingly important problem in Al-Haouz province, Morocco. The objective of this paper was to undertake a retrospective analysis of CL between 2000 and 2006 and to study the sandfly fauna for the first time in this focus. The consultation of bulletins, registers and monthly and annual reports published by local and national medical services permitted a global synthesis of CL in Al-Haouz between 2000 and 2006. Sandflies were collected using sticky-paper traps in five selected stations in two communes, Ourika and Sti Fadma, the most affected by CL in Al-Haouz. Overall, 2,648 sandflies, belonging to five species of *Phlebotomus* and three of *Sergentomyia*, were collected. *Phlebotomus sergenti* Parrot (14.5%) was found in all studied stations where it was associated with domestic and peridomestic habitats. Based on this first report on ecology of sandflies and epidemiology of CL, Al-Haouz seems to be a typical focus of *L. tropica* and *P. sergenti* like the other Moroccan foci. Two communes, Sti Fadma and Ourika, constituted an important CL micro-focus in this region. Considering their economical importance (trade and tourism), they contribute probably to the dissemination of *Leishmania* parasite in bordering regions.

Introduction

Cutaneous leishmaniasis (CL) and visceral leishmaniasis both occur in Morocco.

CL has become an increasingly important problem in Morocco. In 2006, the Ministry of Health reported 3,361 cases of human CL, and the disease was declared in some new areas.

In Morocco, CL is caused by *Leishmania major* Yakimoff and Schokhor and *Leishmania tropica* Wright (Rioux et al. 1986; Pralong et al. 1991). Infections due to a dermatropic variant of *Leishmania infantum* Nicolle have been reported (Rioux et al. 1996; Lemrani et al. 1999). CL caused by *L. tropica* is widespread in semiarid areas in central and southwestern Morocco, where it is transmitted anthroponotically by *Phlebotomus sergenti* Parrot (Guilvard et al. 1991). Despite the Ministry of Health's efforts, this form of disease continues to appear in non-endemic regions. In Al-Haouz, a province located in the High-Atlas mountains near Marrakech city, 354 cases of human CL due to *L. tropica* were recorded between 2000 and 2006. However, the epidemiological aspects of CL in this district had not been examined.

The goal of this study was to define the epidemiological pattern of CL and ecology of sandflies, for the first time, in Al-Haouz focus to help for implementation of future control measures.

Materials and methods

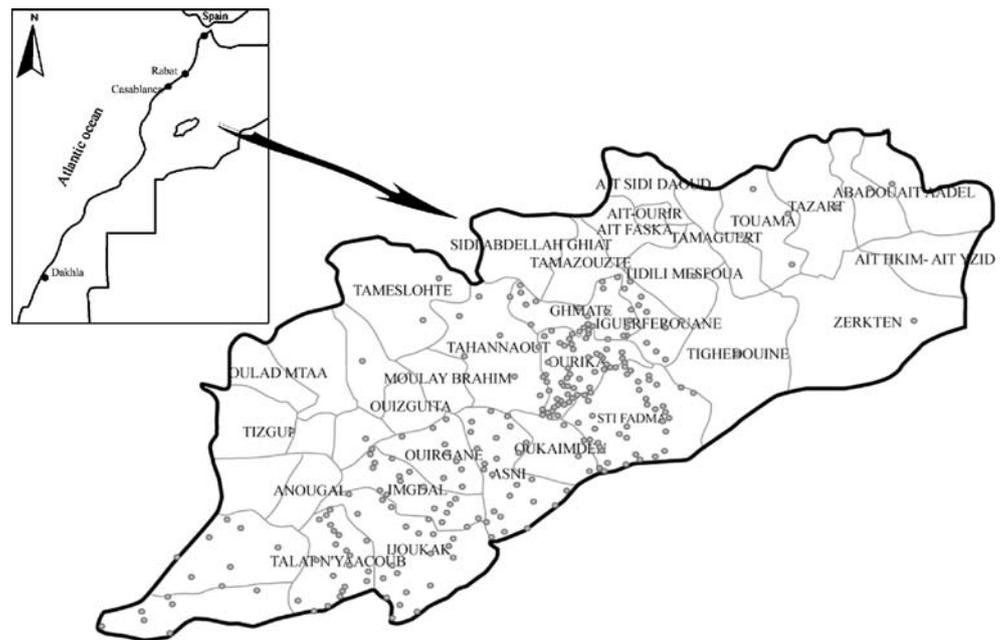
Study area

This study was carried out in Al-Haouz, a province (surface area of 6,231 km²) located in the western slope of the

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Fig. 1 Map delimiting Al-Haouz province in Morocco and showing distribution of cutaneous leishmaniasis cases (grey dots) in different communes. Sandfly trapping was performed in two communes, Ourika and Sti Fadma



Central High-Atlas mountains, at 30 km at the south-east of Marrakech city, Morocco (Fig. 1).

The climate is semi-arid to sub-humid. The annual average pluviometry is of 475 mm.

Precipitations are characterized by their space–time inequality and by stormy rains in summer. The average monthly temperature varies from 11.2°C in January to 27.3°C in July, with daily, seasonal and annual important variations.

The monthly averages of the relative humidity at 18 and 21 h are 49% and 55%, successively. The mean monthly wind velocity does not exceed 1 m/s.

The vegetation is characterized by thermophilous species (*Olea europae*, *Ceratonia siliqua*, *Pistacia lentiscus*, *Chamaerops humis*, *Ephedra fragilis*) and a very important cultivated vegetation (fodder plants, olive-trees, almond trees and other fruit-bearing trees). The forest surface accounts for 45.6% of the area.

The population of this province is of 435,090 inhabitants, and 41.3% have the age lower than 15 years. The general density is 80 inhabitants per square kilometre. Eighty-eight

per cent of the population resides in the rural zone. Agriculture and tourism are the most important sectors in the economy in this region.

The complex mountainous topography (altitude varies from 600 to 4,165 m above sea level) exposes the population to problems of accessibility and enclosure.

Epidemiology

This is a retrospective analysis of the human CL cases declared in Al-Haouz focus between 2000 and 2006. Consultation of the bulletins, registers and monthly and annual reports published by the local and national medical services enabled us to make a global synthesis of these data.

Entomology

Our entomological survey was carried out twice monthly between July and September 2006. According to previous

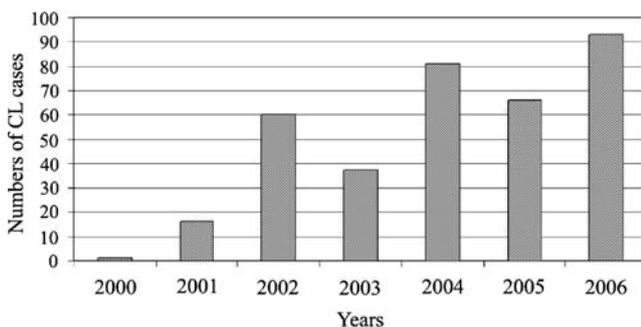


Fig. 2 Evolution of number of cutaneous leishmaniasis cases in Al-Haouz focus from 2000 to 2006

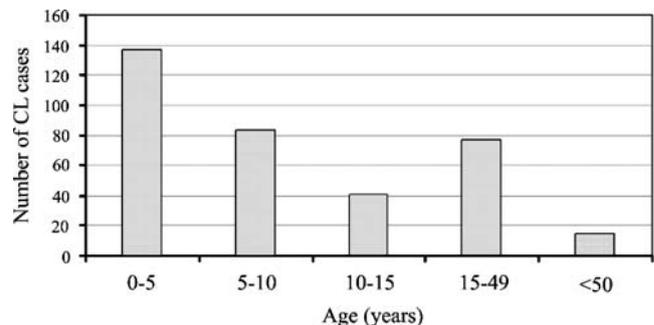
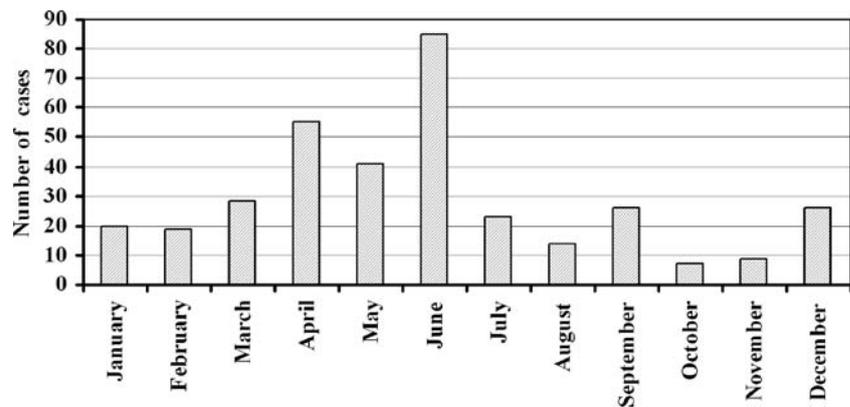


Fig. 3 Distribution of the cutaneous leishmaniasis cases according to age in Al-Haouz focus

Fig. 4 Mean monthly distribution of the cutaneous leishmaniasis cases in Al-Haouz focus



works in the neighbouring areas, this period is marked by a maximum activity of sandflies (Boussaa et al. 2005; Guernaoui et al. 2005a).

Sandflies were collected from five stations. Three, Amkhlij (31°22' N, 7°51' W, 839 m), Akhlij (31°22' N, 7°46' W, 893 m) and Lakhmis (31°23' N, 7°46' W, 895 m) have a purely rural character, and two others, Laagureb (31°22' N, 7°48' W; 881 m) and Tnin Ourika (31°16' N, 7°43' W, 1000 m), are located in the per-urban perimeter.

The choice of these stations was made on the basis of several criteria. Indeed, they are localities with strong concentration of human population with the presence of various potential lodgings of phlebotomine sandflies. In addition, they constituted a micro-foci with high prevalence of leishmaniasis. They are situated in the communes of Ourika and Sti Fadma, where the maximum of the human cases of CL was detected (more than 46%; Fig. 1).

Sandflies were collected using sticky traps, sheets of papers (20×20 cm) coated with castor oil. We used 60 traps in each station and in each trapping campaign. Several biotopes were prospected: inside houses, interior of rooms, cattle sheds, stables, outside of the dwellings in dumps, close to vegetation, under the heaps of stones, ruins and weepholes.

Table 1 Sandfly species collected in Al-Haouz focus and their relative abundance (%)

Subgenus	Species	Male	Female	Total	(%)
<i>Phlebotomus</i>	<i>P. papatasi</i>	357	45	402	15.18
<i>Paraphlebotomus</i>	<i>P. sergenti</i>	278	106	384	14.5
	<i>P. alexandri</i>	13	2	15	0.57
<i>Larroussius</i>	<i>P. perniciosus</i>	200	14	214	8.08
	<i>P. longicuspis</i>	177	13	190	7.18
<i>Sergentomyia</i>	<i>S. minuta</i>	231	421	652	24.62
	<i>S. fallax</i>	249	532	781	29.49
<i>Grassomyia</i>	<i>S. dreyfussi</i>	7	3	10	0.38
Total		1,512	1,136	2,648	100

Data are presented as the number of sandflies per square metre of sticky trap per night.

Collected sandflies were stored in 70% ethanol. Species identification was made by examining the morphology of the pharyngeal armatures and spermathecae of female flies and the external genitalia of males. In this area, the male of *Phlebotomus perniciosus* Newstead did not present the typical morphology (Guernaoui et al. 2005b). To differentiate it from that of *Phlebotomus longicuspis* Nitzulescu, we examined both the form of copulatory valves and the number of coxite hairs.

Results

Epidemiology

During the last 7 years (2000–2006), the Moroccan Ministry for Health noted 354 human cutaneous leishmaniasis cases due to *L. tropica* in this focus. All these declared cases were confirmed by the local health services by isolating and identifying the parasite in their cutaneous ulcers.

Since the recording of the first case in 1994, the epidemiologic situation remained stable until the year

Table 2 Density (number of specimens per square metre of sticky trap per night) of sandfly species at the prospected stations in Al-Haouz focus

Species	Amkhlij	Lakhmis	Laagureb	Akhlij	Tnin Ourika
<i>P. papatasi</i>	10.45	6.36	7.28	1.82	12.20
<i>P. sergenti</i>	15.82	3.87	5.04	1.26	4.16
<i>P. alexandri</i>	0.17	0.79	0	0	0
<i>P. longicuspis</i>	8.16	1.49	0.65	0.16	6.13
<i>P. perniciosus</i>	10.42	3.26	0	0	1.51
<i>S. minuta</i>	9.94	17.25	20.97	5.24	10.69
<i>S. fallax</i>	18.84	18.22	12.35	3.09	1.09
<i>S. dreyfussi</i>	0.06	0.08	0	0	0.68

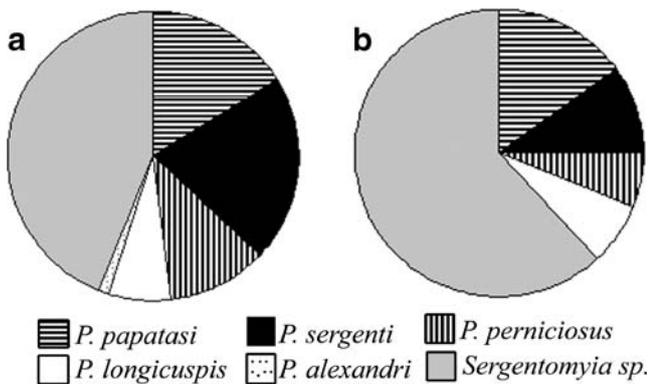


Fig. 5 Mean relative abundance of sandfly species in domestic (a) and peridomestic (b) environments in Al-Haouz focus

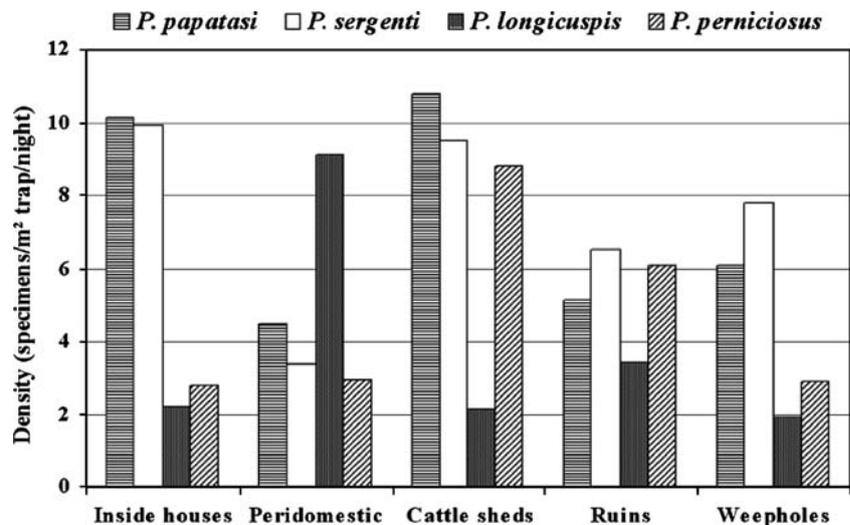
2000. After that, the disease had an exponential increase in the cases recorded during the 6 years. Figure 2 shows the evolution of the number of cutaneous leishmaniasis cases in Al-Haouz focus from 2000 to 2006.

In a general way, all ages are affected by the disease (Fig. 3) with a high prevalence for ages less than 10 years. They represented more than 62% of the declared cases. In the same way, females were more affected (57% of the total cases).

The appearance of the cases in this focus was different according to the season and month (Fig. 4). More than 51% of the cases were declared between April and June of each year. The monthly maximum of the cases was recorded in June.

In total, 28 communes (72% of the communes of the province) are affected by the disease (Fig. 1). However, two communes, Ourika and Sti Fadma, the most touched, recorded 46% of the total number of the detected cases (Fig. 1).

Fig. 6 Distribution of sandfly species in the prospected biotopes in Al-Haouz focus



Entomology

During June–September 2006, a total of 2,648 sandflies were collected and identified (Table 1). Among the species of the genus *Phlebotomus*, *Phlebotomus (Phlebotomus) papatasi* (Scopoli) (15%) and *P. (Paraphlebotomus) sergenti* (Parrot) (15%) were the most prevalent species, followed by *P. perniciosus* (8.08%) and *P. longicuspis* (7.18%). *Phlebotomus (Paraphlebotomus) alexandri* Sinton was less frequent (0.57%). Among the species of the genus *Sergentomyia*, *Sergentomyia (Sergentomyia) fallax* Parrot (29.49%) and *Sergentomyia (Sergentomyia) munita* Adler et Theodor (24.62%) were predominant, while *Sergentomyia (Grassomyia) dreyfussi* Parrot (0.38%) was rare. The number and relative abundance of these species are given in Table 1.

Table 2 shows the distribution of sandfly species in each prospected station. *P. papatasi*, *P. sergenti*, *P. longicuspis*, *Sergentomyia minuta* and *S. fallax* are largely widespread, while *P. alexandri*, *P. perniciosus* and *S. dreyfussi* had a limited distribution.

Sandfly densities recorded in Akhlj were low, which could be due to an insecticide spraying operation organised by the health services in the village.

The study of domestic and peridomestic faunas (Fig. 5) demonstrated the anthropophilic behaviour of *P. sergenti*. It accounts for 21% and 10% of sandflies collected inside and in the vicinity of the human dwellings, respectively. *P. papatasi* accounted for 16% and 15%, respectively.

Figure 6 presents sandfly distribution in various prospected biotopes. Generally, all these biotopes were favourable for sandflies. Species of the genus *Phlebotomus* preferred domestic habitat, while *Sergentomyia* species preferred extradomestic habitats.

Discussion

CL is a serious and increasing public health problem in Al-Haouz area. The parasite was isolated from patients in this region by local health services and was typed as *L. tropica* (Ministry of Health 2007). After the first case was reported in 2000, the number of the declared cases had a significant increase from 2001 to 2006 (354 cases). These data show clearly that Al-Haouz is an emerging focus of CL. The relative decrease recorded in 2003 and 2005 is justified by the inefficiency of passive detection of cases, physical inaccessibility to the health structures as well as total ignorance and lack of awareness campaign of the population. Moreover, among the 28 communes affected by CL, 46% of cases were notified in two communes only (Ourika and Sti Fadma), the most accessible and equipped in medical infrastructure in the province. In this context, several cases pass implicitly without notification, and incidence rate is far from reflecting reality.

The infantile population remains the most affected by CL. The occurrence of the disease in both sexes, with large numbers of women and children infected, indicates that *Leishmania* transmission may have occurred in the peridomestic habitat.

The manifestation of ulcers showed a seasonal variation in relation with the seasonal dynamics of the vector and latent period of the disease. The monthly maximum of CL cases was recorded in June.

Our entomological survey, the first one in this focus, allowed collecting 2,648 specimens belonging to eight species of sandflies. *P. papatasi* and *P. sergenti*, two common vectors of CL (Killick-Kendrick 1990), were largely widespread. In this region of the High-Atlas mountains, recent investigations (Guernaoui et al. 2006) showed that, from 1,000 m altitude, *P. papatasi* was less frequent and less abundant, while *P. sergenti* was collected in all altitudinal zones until 1,400 m altitude. *P. sergenti*, a proven vector of *L. tropica* (Killick-Kendrick 1990), was suspected recently to be the vector in Chichaoua, a neighbouring province of Al-Haouz (Guernaoui et al. 2005a). In Al-Haouz area, *P. sergenti* is an anthropophilic species, collected in abundance in domestic and peridomestic habitats. The study of species affinity for different biotopes sampled demonstrated, generally, that species of the genus *Phlebotomus* are pledged to domestic area and its vicinity. It confirmed also the strong anthropophilic behaviour of *P. sergenti*, which abounded in-house.

In Akhlij, we recorded very low sandfly species densities. This is due to an insecticide spraying operation organised by the health services in the village. It means that a controlled spraying with residual insecticides inside and around houses could help to control vector populations.

In conclusion, based on this first report on ecology of sandflies and epidemiology of CL, Al-Haouz seems to be a typical focus of *L. tropica* and *P. sergenti* like Chichaoua and the other Moroccan foci. Other investigations are in process to prove the vectorial role of *P. sergenti* and to elucidate other aspects of the transmission cycle essential for planning a control program.

Two communes, Sti Fadma and Ourika, constituted an important micro-focus of CL in this region. Considering their economical importance (trade and tourism), they probably contribute to the dissemination of *Leishmania* parasite in bordering regions.

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