E F
F E *Entomologie Faunistique – Faunistic Entomology* 2015 **68**, 201-211

Entomological and epidemiological investigations of an emerging focus of cutaneous leishmaniasis in Bzou, Morocco

Mohamed Zouirech*[(1)(2)], Chafika Faraj[(2)] & Driss Belghyti[(1)]

[(1)] Environment and Renewable Energy Laboratory, Faculty of Sciences, Ibn Tofail University, Kenitra 14000, Morocco.

[(2)] Laboratory of Medical Entomology, National Institute of Hygiene, 27 Ibn Batouta Avenue, Agdal, Rabat 10080, Morocco.

* E-mail: zouirech@gmail.com

Reçu le 15 janvier 2015, accepté le 17 avril 2015.

La leishmaniose cutanée (LC) à *Leishmania tropica* est très répandue au centre du Maroc où elle est endémique dans la province d'Azilal et ses régions. Au cours de ce travail, on a étudié l'écologie des phlébotomes, vecteurs de la maladie, et le profil épidémiologique des leishmanioses (2009-2012) dans le secteur de Bzou, qui renferme des foyers actifs de *L. tropica*. Ainsi, six localités appartenant au secteur de Bzou ont été prospectées de mars à novembre 2010. Un total de 488 spécimens a été capturé dans des biotopes domestiques et pré-domestiques en utilisant des pièges adhésifs et lumineux (CDC). 8 espèces ont été identifiées, dont 5 *Phlebotomus* spp. (96,31%) et 3 *Sergentomyia* spp. (3,69%). Pour le genre *Phlebotomus*, *P. sergenti* est le plus abondant (50,61%) suivi par *P. papatasi, P. perniciosus, P. alexandri* et *P. longicuspis* avec (23,16% ; 21,72% ; 0,61% et 0,20%) respectivement. Cependant, le genre *Sergentomyia* était moins fréquent, dominé par *S. minuta* (2,46%) suivi par *S. fallax* et *S. dreyfussi* avec (0,61%) chacune. A l'instar des autres foyers typiques de la LC au centre du Maroc, notre étude confirme l'implication de *P. sergenti* dans la transmission de *L. tropica* à Bzou.
Mots-clés: Phlébotome, abondance, tendance, leishmaniose cutanée, Bzou, Maroc.

Cutaneous Leishmaniasis (CL) due to *Leishmania tropica* has become widespread in the central part of Morocco over the High-Atlas Mountains, and it occurs as endemic disease in Azilal province. The aim of this study is to give an overview of the density, richness, abundance and equitability of sand flies in Bzou district, focus of *L. tropica,* and to undertake a retrospective epidemiological study of CL between 2009 and 2012. Therefore, in the entomological survey, six localities have been prospected from March to November 2010. A total of 488 sand flies were trapped from both animal and human dwellings using light and sticky traps. Five species were identified as *Phlebotomus* spp. (96.31%) and three as *Sergentomyia* spp. (3.69%). For the genus *Phlebotomus*, *P. sergenti* was the most dominant (50.61%), followed by *P. papatasi* (23.16%), and *P. perniciosus* (21.72%). The other species *P. alexandri* and *P. longicuspis* represented only 0.61% and 0.20% respectively. However, the genus *Sergentomyia* was less frequent. *S. minuta* represented only 2.46% followed by *S. fallax* and *S. dreyfussi* with 0.61% for each one. Obviously, like other foci of cutaneous leishmaniasis in Morocco, our survey supports the implication of *P. sergenti* as a potential vector of *L. tropica* in Bzou.
Keywords: Phlebotomine, abundance, trends, cutaneous leishmaniasis, Bzou, Morocco.

1 INTRODUCTION

Sand flies (Diptera: Psychodidae) are implicated in the transmission of many parasites that can cause diseases such as leishmaniasis. The World Health Organization has estimated that 12 million people are suffering from *Leishmania* and 350 million are at risk (Desjeux, 1996). In Morocco, the incidence of CL peaked up in 2010 with 2263 cases, and then remained stable in 2011 and 2012 with 2100 and 2137 cases, respectively (Anonymous, 2012). Cutaneous leishmaniasis due to *L. tropica* is mainly located in the center of the

country (Rioux *et al*., 1986a). It occurs as hypo-endemic disease in rural areas (Guilvard *et al.*, 1991; Rhajaoui, 2009). However, under certain circumstances of environmental changes, it may switch to an epidemic disease in suburban and urban areas. Thus, the continuous survey of the vectors and their control is required by health services to predict and prevent spread of leishmaniasis epidemic. This study aimed to survey sand flies species and to bring an overview about epidemiological situation in Bzou district, an emerging focus of CL.

2 MATERIAL AND METHODS

2.1 Study area

The study was carried out in six localities (Nzala, Bouaziar, Ouaourint, Tagount, Mazoz and Zengour) of Bzou district (32° 6' N; 7° 3' W) in Azilal Province, which located in the center of Morocco over the High-Atlas Mountains (**Figure 1**). The altitude ranges from 368 to 688 m above the sea level (asl). The study area is characterized by a semi-arid climate, hot and dry in the summer, cold and rainy in the winter. Temperature ranged from 2°C in January to 38°C in July. Total annual rainfall is about 336 mm. The region covers an area of about 9800 km² with 14550 inhabitants who work mainly in agriculture. Livestock farming is also an important source for income in the area. Animal settlement are located often in vicinity of households. Additionally, walls and floors of most houses in the region are made of mud leading to an ideal resting and breeding site for sand flies.

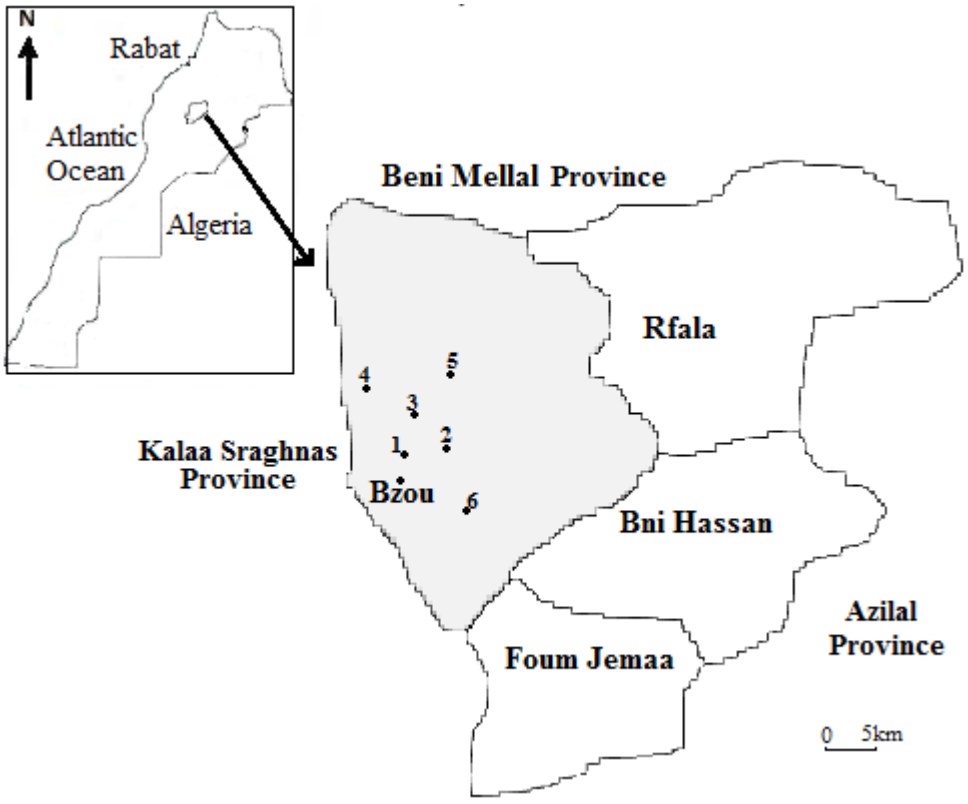

Figure 1: Map of study locations: 1) Tagount 2) Mazouz 3) Ouaourint 4) Nzala 5) Bouaziaire 6) Zen.

2.2 Epidemiological data

The diagnostic of CL is performed in laboratory of Azilal province then confirmed by National Institute of Hygiene. Epidemiological data and personal information concerning patients were collected by consulting the registers and annual reports of the local and national public health services. All the CL cases declared in Bzou district from 2009 to 2012 were compiled and were subject to a retrospective analysis

2.3 Sand flies collection and identification

Through the use of light and sticky traps, sand flies were collected in five houses that were

randomly selected in each of the six localities studied during the period of March-November 2010. The sticky traps (A4 blank sheets of papers coated with castor oil) were attached indoor to the walls and roofs of human and animal dwellings by nails. The light traps (CDC miniature battery-operated), were set in the resting room. About 50 sticky and three CDC tarps were set bimonthly, for one whole night, from sunset to sunrise, in the same place for every collection time and in each locality. Sand flies caught were retrieved using paintbrush then stored in tubes containing 96% ethanol. During analysis in the laboratory, sand flies were cleared in Marc-Andre solution then mounted in microscopic slides for identification by examining the genitalia structure of males, spermathecae and pharynges of female following morphological keys (Anonymous, 2010; Lewis, 1982).

2.4 Data analysis

Various parameters and ecological indexes were determined during the data analysis (Dajoz, 1985; Magurran, 1988).

Density (D) :	$D = \frac{\frac{Ph}{m^2}}{night}$	Ph/m², number of sand flies per m² of sticky traps
Degree of presence (d)* :	$d = \frac{k}{K} x100$	k, number of localities containing the species (x); K, total number of localities
Frequency (RA) :	$\% = \frac{n}{N} x100$	n, number of specimens of species (x); N, total number of specimens in the sample
Sex-ratio :	$Sr = \frac{F}{M} x100$	F, number of female; M, number of male
Specific richness (S) :		S, number of species in a given area
Shannon-Wiener index(H)	$H = -\sum_{i=1}^{s} P_i \, ln \, P_i$	Pi, the proportion of total number of species / total samples
Evenness (E)	$E = \frac{H'}{H'max}$	H′max = log2 S S, Specific richness

*Parameter calculated from mixed CDC and sticky traps captures.

Analysis was performed using SPSS Software packages.

3 RESULTS

3.1 Epidemiological study

During the 4 years (2009-2012), 1263 cases of CL have been reported by the Moroccan health services in Bzou. As (**Figure 2**) shows, the number of CL rose steadily at first in 2009 with 318 cases, peaked up in 2010 with 442 cases then decreased in 2011 and 2012.

The number of CL cases rose gradually from September to February (**Figure 3**), and then there was a marked peak in March (over 297 cases). The number of cases dropped then in April and bottomed out in September (14 cases).

Cutaneous leishmaniasis affected both young and old people but those under 5 years old were most vulnerable (60% of cases being recorded) (**Figure 4**). In addition, Females were slightly more affected than males with 53% of total declared cases.

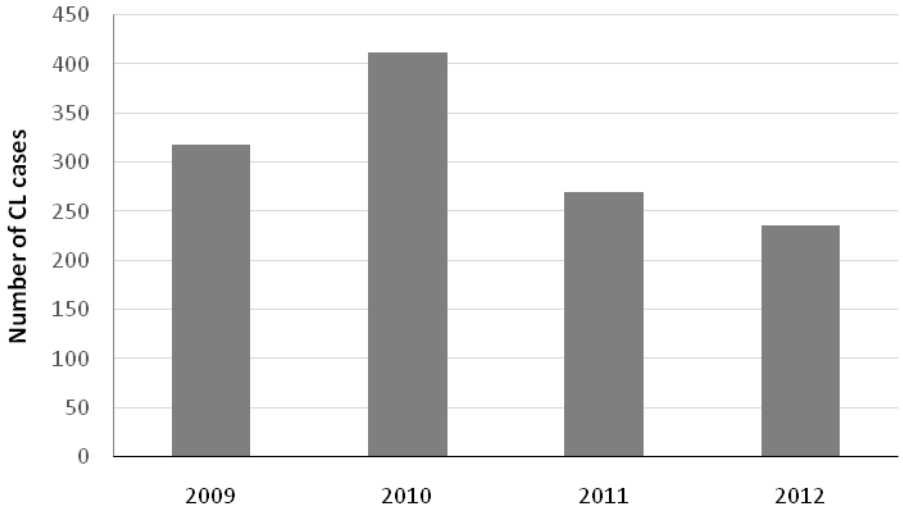

Figure 2: Evolution of the number of cutaneous leishmaniasis cases in Bzou (2009-2012).

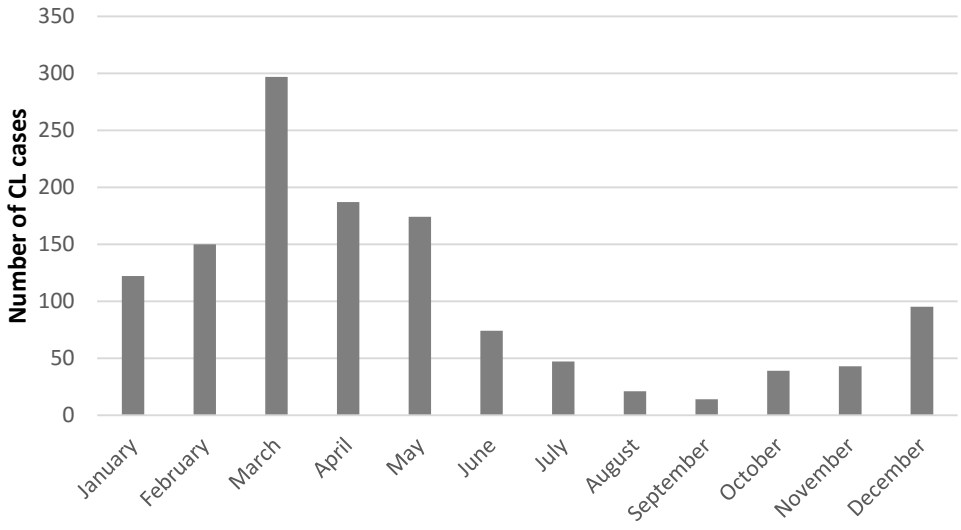

Figure 3: Numbers of cutaneous leishmaniasis per month in Bzou (2009-2012).

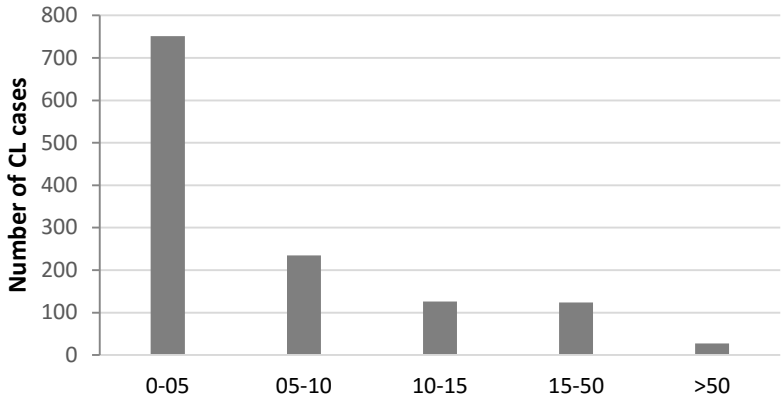

Figure 4: Distribution of cutaneous leishmaniasis cases by age in Bzou (2009-2012).

3.2 Entomological survey

A total of 488 sand flies were captured by the both type of traps, the maximum was trapped by sticky traps (92.21%), and the results are shown in the **Table 1**. Five species were identified as *Phlebotomus* genus (96.31%) and three as *Sergentomyia* genus (3.69%). For the genus of *Phlebotomus*, *P.* (*Paraphlebotomus) sergenti* (Parrot 1917) was the most dominant by 50.61% followed by *P.* (*Phlebotomus) papatasi* (Scopoli 1786) with 23.16% and *P. (Larroussius) perniciosus* (Newstead 1911) with 21.72%. While, *P. (Paraphlebotomus*) *alexandri* (Sinton 1928) and *P.* (*Larroussius*) *longicuspis* (Nitzelescu 1930) were weakly represented with 0.61% and 0.20% respectively. The genus *Sergentomyia* was even less frequent. Therefore, the predominant specie was *S. (Sergentomyia) minuta* (Rodani 1843) which represented 2.46% of the total sand flies collected, followed by *S. (Sergentomyia) fallax* (Parrot 1921) and *S. (Grassomyia) dreyfussi* (Parrot 1933) each with 0.61%. The sex ratio for total collection species determined to be 23.29%.

Phlebotomus sergenti and *P. perniciosus* were common species and showed the largest distribution as indicated by the high degree of presence (d) although a varied prevalence in each locality. They were collected from the six prospected localities d=100%. *P. papatasi* and *S. minuta* were also common species with a large distribution d=80%. However, *P. longicuspis*, *P. alexandri* and *S. fallax* were rarely represented d=20% (**Table 1).**

Sand fly communities showed differences in biodiversity among different sampling localities. The biodiversity indexes reached their maximum in the locality of Bouaziar (H=1.33; E=0.64), the relatively low evenness in Zengour and Nzala (E=0.28; E=0.22 respectively) could be linked to the predominance of *P. sergenti*. The high richness occurred in Bouaziar with eight species, followed by Mazoz with five species. Tagount, Ouaourint and Nzala had the same richness with four species. The lowest richness was observed in Zengour with only two species (**Table 2**).

The mean density of sand flies differs among the sampled localities (**Figure 5**). It was found to be relatively high in Bouaziar (32.7 Ph/m²/night), moderate in Nzala (22.4 Ph/m²/night) and low in the rest of the sites: Ouaourint, Tagount, Mazoz and Zengour with (17.4; 14.3; 12.3 and 4.2 Ph/m²/night respectively). As the (**Figure 6**) shows, sand flies were dynamic from April through September. The mean density of sand flies started at 23.6 Ph/m²/night in April, peaked up in May 46.3 Ph/m²/night, then dropped remarkably in June and July, followed by a significant increase in August (22.1 Ph/m²/night), and decreased again in September to disappear in October.

4 DISCUSSION

Since the first case declared in Tanant in the Azilal province (Marty *et al.*, 1989), the epidemiological situation became a serious concern to public health services where the number of recorded cases kept increasing during the last decade. The epidemiological data shows clearly that Bzou is a re-emerging focus of CL.
The high number of CL cases might be related to the efficiency and accessibility to health services.
However, the decrease shown in the last two years, 2011-2012, could be attributed to

Table 1: Species composition and frequency of sand flies caught by sticky traps. *ST: Sticky traps.

	ST* (m²)	*P. sergenti*			*P. longicuspis*			*P. papatasi*			*S. minuta*			*S. fallax*			*P. perniciosis*			*S. dreyfussi*			*P. alexandri*			**Total**		
		M	F	%	M	F	%	M	F	%	M	F	%	M	F	%	M	F	%	M	F	%	M	F	%	M	F	%
Ouaourint	25.3	16	0	7.2	0	0	0	25	3	25	1	2	27	0	0	0	16	0	17	0	0	0	0	0	0	58	5	14.0
Bouaziar	34.7	48	7	24.8	1	0	100	41	3	39	0	1	9	0	3	100	44	10	57	0	1	33	3	0	100	137	25	36.0
Mazoz	29.7	20	3	10.4	0	0	0	18	5	20	0	2	18	0	0	0	2	0	2	2	0	67	0	0	0	42	10	11.6
Nzala	25.4	58	27	38.3	0	0	0	4	0	4	3	1	36	0	0	0	2	0	2	0	0	0	0	0	0	67	28	21.1
Tagount	24.7	21	8	13.1	0	0	0	14	0	12	1	0	9	0	0	0	13	2	16	0	0	0	0	0	0	49	10	13.1
Zengour	13.7	8	6	6.3	0	0	0	0	0	0	0	0	0	0	0	0	4	1	5	0	0	0	0	0	0	12	7	4.2
Total	153.6	171	51	100	1	0	100	102	11	100	5	6	100	0	3	100	81	13	100	2	1	100	3	0	100	365	85	100

Table 2: Species richness, Shannon-Wiener diversity index, and evenness.

Localities	Altitude (m)	Species richness	Shannon-Wiener diversity index	Evenness
Ouaourint	368	4	1.20	0.58
Bouaziar	404	8	1.33	0.64
Mazoz	406	5	1.10	0.53
Nzala	580	4	0.45	0.22
Tagount	381	4	1.11	0.53
Zengour	688	2	0.58	0.28

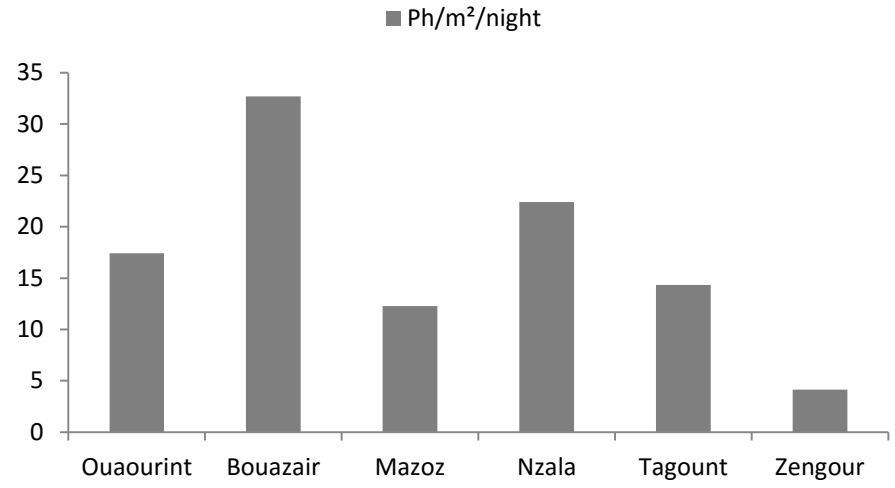

Figure 5: Density of sand flies by prospected locality.

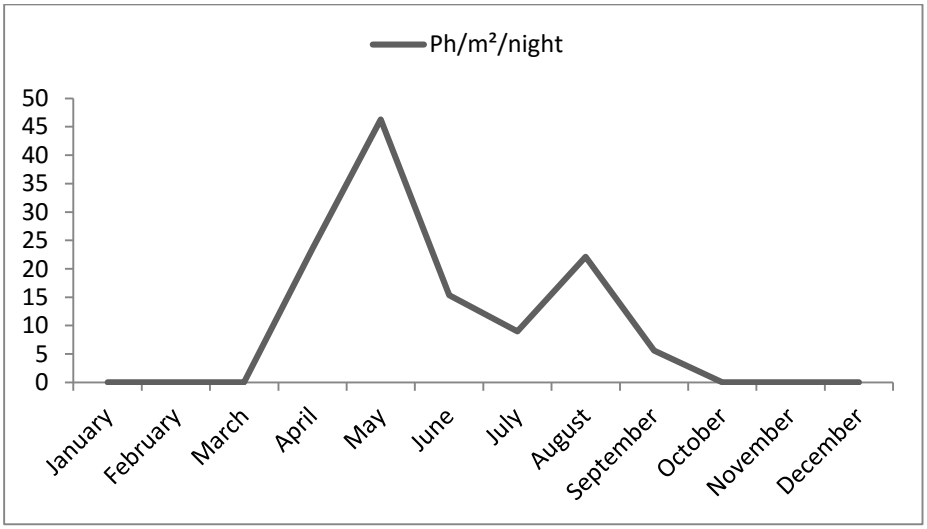

Figure 6: Trend of mean density of sand flies per month in all prospected localities of Bzou.

insecticide spray in some focus of CL and the rise of awareness about disease in these areas.

Youth were found to be the most affected by the leishmaniasis because they have not yet developed protective immunity against leishmaniasis. The high density and widespread of *P. sergenti* in Bzou inside human houses and surrounding areas had created an efficient cycle for the transmission of the disease. Our analyses are in good agreement with previous studies in similar foci of CL (Guessous-Idrissi *et al.*, 1997a; Guernaoui *et al.*, 2005b; Ramaoui *et al.*, 2008; Zouaghi *et al.*, 2011). Generally, numbers of declared cases vary within the months, the maximum cases of CL were reported in March, which is in harmony with the dynamic of the vector (*P. sergenti*) combined with the latent stage of the disease. The highest record in March could be linked, as well, to the efficiency of active cases detection by medical staff in the schools and the remote areas during this period of the year. Ramaoui *et al.* (2008) reported the maximum cases in June at Al-Haouz province. This disagreement is due, not only to the difference in seasonal dynamic trends of the vector between the different considered areas, but to the scheduling of active cases detected in different periods of the year as well.

Our entomological survey in Bzou, confirmed the occurrence of eight species of sand flies in six prospected localities, throughout April-September (2010). According to many authors this period is characterized by the highest activity of sand flies (Boussa *et al.*, 2005; Guernaoui *et al.*, 2005; Ghrab *et al.*, 2006; Ramaoui *et al.*, 2008). Among eight trapped species, five belonged to the *Phlebotomus* genus and three to the *Sergentomyia* genus The *Phlebotomus* genus includes three different subgenera: *Larroussius*, *Phlebotomus* and *Paraphlebotomus:* The species of *Larroussius*, *P. perniciosus*, *P. longicuspis* and *P. ariasi* are known to be the proven vectors of *L. infantum* in the Mediterranean countries (Izri *et al.*, 1990; Killick-Kendrick, 1990; Rioux & Lanotte, 1990; Harrat & Belkaid, 2003). In the north of Morocco, Hamdani (1999) proved the role of *P. ariasi* in the transmission of *L. infantum* in visceral leishmaniasis foci. Recently *L. infantum* DNA was detected in *P. longicuspis* from a visceral leishmaniasis endemic focus in Sefrou province (Es-Sette *et al.*, 2014). For the *Paraphlebotomus* subgenus, we have found *P. sergenti* and *P. alexandri*. The first one is confirmed as vectors of *L. tropica* in this area (Guilvard *et al.*, 1999; Rioux *et al.*, 2001; Yahia *et al.*, 2004). However, *P. alexandri* remains a suspect vector of leishmaniasis in Morocco. The last species belongs to *Phlebotomus* subgenus and was identified as *P. papatasi*, which is the sole species responsible for the transmission of *L. major* in Morocco (Rioux *et al*., 1986b; Izri *et al*., 1992). The species of *Sergentomyia* genus were very rare in human and animal dwellings.

Phlebotomus sergenti was found to be the most dominant species in Bzou as it was reported in previous studies in Morocco (Bailly-Choumara *et al*., 1971; Geussous *et al*., 1997; Boussa *et al*., 2010; Faraj *et al.*, 2013; Zouirech *et al*., 2013). We paid particular attention to this species because it is considered as one of the most vectors of *L. tropica* in these areas. Therefore, the close contact between the vector and human has contributed to the widespread transmission of the disease to no endemic villages around Bzou, where CL has been regularly reported by the Ministry of Health (Anonymous, 2012).

Phlebotomus papatasi was the second most abundant species in the field study, followed by *P. perniciosus*. Those species have been reported in nearly all the previous studies carried out in Morocco with varied prevalence (Bailly-Choumara *et al.*, 1971; Geussous *et al.*, 1997; Boussa *et al.*, 2010; Faraj *et al.*, 2013). The first one is considered as Saharan species because it is more prevalent in the southern part of the country (Rioux *et al.*, 1986b). *Phlebotomus perniciosus* belongs to humid areas (Guessous-Idrissi *et al.*, 1997b) where the highest cases of visceral leishmaniasis were noticed in the north of Morocco (Anonymous, 2012).

The high frequency of *P. papatasi* was found at Mazoz followed by Ouaourint. However, this frequency remains very low in comparison with other *L.* major endemic areas (Ramaoui *et al.* 2008; Boussaa *et al.*, 2006; Guernaoui *et al.*, 2005). Besides, it is important to consider the absence of *Meriones shawi*, the reservoir hosts for *L. major* infection, in these areas. This may explain the absence of *L. major* in Bzou.

Sand flies were active in Bzou district from April to September displaying a bimodal annual abundance pattern corresponding to two generations with peaks in May and August. These results meet with the finding of Boussaa *et al.* (2005) in Marrakech and are in good agreement with Faraj *et al.* (2013) in several provinces

(Azilal, Essaouira, Boulmane, Sefrou and Tinghir). However, they do not meet completely with the results of Guessous-Idrissi *et al.* (1997a) in Taza, and El Miri *et al.* (2013) in Sidi-Kacem. This discordance in the results with studies elsewhere might be explained by the divergence between bioclimatic areas (Rioux *et al.*, 2001).

The altitude of prospected localities did not diverge much. It ranged from 368 at Ouaourint to 688 m (a.s.l.) at Zengour. That might explain the difference in sand fly richness and diversity. Previous studies linked the richness and the density to altitude (Guernaoui *et al.*, 2006). However, our finding did not concord with those studies. We have noticed difference in richness and diversity among Bouaziar, Mazoz and Tagount even if they share a similar altitude. Furthermore, our results confirm results of Faraj *et al.* (2013) which suggested that the altitude alone seems not to be responsible of the distribution of sand flies. The occurrence and the distribution of species in Morocco seem more affected by the climate and the abiotic factors (vegetation, type of ground), more than altitude.

5 CONCLUSION

Our survey showed the Bzou region as typical focus of CL due to *L. tropica* with *P. sergenti* as a suspected vector, active from April to September. Hence, the control of sand fly populations, in addition to raising awareness and promoting earlier access to health services for case management could be an effective control method against this disease.

6 ACKNOWLEDGMENTS

We are grateful for National Institute of Hygiene, Department of Epidemiology and Disease Control (DELM) and for Azilal medical delegation staff for their kind and valuable technical assistance in collection of sand flies.

BIBLIOGRAPHY

Anonymous (2012). Situation épidémiologique des leishmanioses, *Rapport annuel des maladies parasitaires. Ministère de la santé*, p. 7-9.

Anonymous (2010). *Lutte contre les leishmanioses, guide des activités*, Ministère de la santé, Rabat, 143 p.

Bailly-Choumara H., Abonnec E. & Pastre J. (1971). Contribution to the study of sand flies of Morocco (Diptera, Psychodidae). Faunistic and ecological data. *Cahiers - Office de la recherche scientifique et technique outr-mer. Service Entomology and Medical Parasitology* **IX**(4), p. 431-460.

Boussaa S., Neffa M., Pesson B. & Boumezzough A. (2010). Phlebotomine sandflies (Diptera: Psychodidae) of southern Morocco. Results of entomological surveys along the Marrakech–Ouarzazat and Marrakech–Azilal roads. *Annals of Tropical Medicine and Parasitology* **104**, p. 163-170

Boussaa S., Guernaoui S., Pesson B. & Boumezzough A. (2005). Seasonal fluctuations of phlebotomine sand fly populations (Diptera: Psychodidae) in the urban area of Marrakech, Morocco. *Acta Tropica* **95**, p. 86-91.

Dajoz R. (1985). *Précis d'écologie*. Dunod, Paris, 505 p.

Desjeux P. (1996). Leishmaniasis - public health aspects and control. *Clinics in Dermatology* **14**, p. 417- 423.

El-Miri H., Rhajaoui M., Himmi O., Ouahabi S., Benhoussa A. & Faraj C. (2013). Etude entomologique de cinq foyers de leishmaniose cutanée dans la province de Sidi Kacem au nord du Maroc. *Annales de la Société entomologique de France (N.S.) : International Journal of Entomology* **49**, p. 154-159.

Es-Sette N., Ajaoud M., Laamrani-Idrissi A., Mellouki F. & Lemrani M. (2014). Molecular detection and identification of *Leishmania* infection in naturally infected sand flies in a focus of cutaneous leishmaniasis in northern Morocco. *Parasites & Vectors* **7**, p. 305.

Faraj C., Adlaoui E.B., Ouahabi S., El Kohli M., El Rhazi M., Lakraa L. & Ameur B. (2013). Distribution and bionomic of sand flies in five ecologically different cutaneous leishmaniasis foci in Morocco. *Parasites & Vectors* **6**, p. 334-342.

Fellah H., Rhajaoui M., Ouahabi S., Belghiti D. & Lyagoubi M. (2007). Occurrence of Human Cutaneous Leishmaniasis in Zouagha My Yacoub Province (Morocco). *International Journal of Agriculture & Biology* **1**, p. 197-198.

Ghrab J., Him A., Bach-Hamba D., Chahed M.K., Aoun K., Aouira S. & Bouratbine A. (2006). Phlebotominae (Diptera: Psychodidae) of human leishmaniasis sites in Tunisia. *Parasite* **13**, p. 23-33.

Guernaoui S., Boumezzough A. & Laamrani A. (2006). Altitudinal structuring of sand flies (Diptera: Psychodidae) in the High-Atlas mountains (Morocco) and its relation to risk of leishmaniasis transmission. *Acta Tropica* **97**, p. 346-351.

Guernaoui S., Pesson B., Boumezzough A. & Pichon G. (2005). Distribution of phlebotomine sandflies, of the subgenus *Larroussius*, in Morocco. *Medical and Veterinary Entomology* **19**, p. 111-115.

Guernaoui S., Boumezzough A., Pesson B. & Pichon G. (2005b). Entomological investigations in Chichaoua: an emerging epidemic focus of cutaneous leishmaniasis in Morocco. *Journal of Medical Entomology* **42**, p. 697-701.

Guessous-Idrissi N., Chiheb S., Hamdani A., Riyad M., Bichichi A. & Krimech A. (1997a). Cutaneous leishmaniasis: an emerging epidemic focus of *Leishmania tropica* in north Morocco. *Transactions of the Royal Society of Tropical Medicine & Hygiene* **91**, p. 660-663.

Guessous-Idrissi N., Hamdani A., Rhalem A., Riyad M., Sahibi H., Dehbi F., Bichichi M., Essari A. & Berrag B. (1997b). Epidemiology of human visceral leishmaniasis in Taounate, a northern province of Morocco. *Parasite* **2**, p. 181-185.

Guilvard E., Rioux J.A., Gallego M., Pratlong F., Mahjour J., Martinez-Ortega E., Dereure J., Saddiki A. & Martini A. (1991). *Leishmania tropica* au Maroc, III-Rôle de *Phlebotomus sergenti*. A propos de 89 isolats. *Annales de Parasitologie Humaine et Comparée* **66**, p. 96-99.

Hamdani A. (1999). *Etude de la faune phlébotomienne dans trois foyers de leishmanioses au Nord du Maroc : Espèces, Abondance, Saisonnalité et incrimination du vecteur*. Thèse de 3ème cycle, Université Cadi Ayyad, Marrakech, Maroc, 57 p.

Harrat Z. & Belkaid M. (2003). Leishmaniasis in Algiers: epidemiologic data. *Bulletin de la Société de pathologie exotique* **96**, p. 212-214.

Izri M.A., Belazzoug S., Pratlong F. & Rioux J.A. (1992). Isolement de *L. major* chez *Phlebotomus papatasi* (Scopoli, 1786b) à Biskra (Algérie). *Annales de Parasitologie Humaine et Comparée* **67**, p. 31-32.

Izri M.A., Belazzoug S. & Boudjebla A. (1990). *Leishmania infantum* MON-1 isolé de *Phlebotomus perniciosus* en Kabylie (Algérie). *Annales de Parasitologie Humaine et Comparée* **3**, p. 151-152.

Killick-Kendrick R. (1990). Phlebotomine vectors of leishmaniasis - a review. *Medical and Veterinary Entomology* **4**, p. 1-24.

Lemrani M., Nejjar N. & Benslimane A. (1999). A new focus of cutaneous leishmaniasis due to *Leishmania infantum* in Northern Morocco. *Giornale Italiano di Medicina Tropicale* **4**, p. 3-4.

Lewis D.J. (1982). A taxonomic review of the genus *Phlebotomus* (Diptera: Psychodidae). *Bulletin of the Natural History Museum Entomology* **45**, p. 121-209.

Magurran A.E. (1988). *Ecological diversity and its measurement*. Princeton University, Press, Priceton, New Jersey, 179 p.

Marty P., Fichoux Y., Pratlong F., Lanotte G., Rioux J.A. & Lacour J.P. (1989). Cutaneous leishmaniasis due to *Leishmania tropica* in young Moroccan child observed in Nice France. *Transactions of the Royal Society of Tropical Medicine & Hygiene* **83**, p. 5-10.

Pratlong F., Rioux, J.A., Dereure J., Mahjour J., Gallego M., Guilvard E., Lanotte G., Perieres J., Martini A. & Saddiki A. (1991). *Leishmania tropica* au Maroc. IV Diversité isozymique intrafocale. *Annales de Parasitologie Humaine et Comparée* **66**, p. 100-104.

Ramaoui K., Guernaoui S. & Boumezzough A. (2008). Entomological and epidemiological study of a new focus of cutaneous leishmaniasis in Morocco. *Parasitology Research* **103**, p. 859- 863.

Rhajaoui M. (2011). Les leishmanioses humaines au Maroc : une diversité nosogéographique. *Pathologie Biologie* **59**(4), p. 226-229.

Rioux J.A. (2001). Thirty years of Franco-Moroccan cooperation on Leishmaniasis: Detection and analysis of homes, risk factors, climate change and dynamic nosocomial Geographic. *Alumni Association of the Pasteur Institute* **168**, p. 90-101.

Rioux J.A. & Lanotte G. (1990). *Leishmania infantum* as a cause of cutaneous leishmaniasis. *Transactions of the Royal Society of Tropical Medicine & Hygiene* **84**(6), p. 898.

Rioux J.A., Lanotte G., Petter F., Dereure J., Akalay O., Pratlong F., Velez I.D., Fikri N.B., Maazoum R., Denial M., Jarry D.M., Zahaf A., Ashford R.W., Cadi-Soussi M., Killick-Kendrick R., Benmansour N., Moreno G., Perieres J., Guilvard E., Zribi M., Kennou M.F., Rispail P., Knechtli E. & Serres E. (1986a). Les leishmanioses cutanées du bassin Méditerranéen occidental, de l'identification enzymatique à l'analyse éco-épidémiologique. L'exemple de trois «foyers», tunisien, marocain et français. *In* Rioux J.A. (ed.) *Leishmania. Taxonomie et Phylogenèse. Applications éco-épidémiologiques - International Colloquium CNRS/INSERM) : Institut Méditerranéen d'Etudes Épidémiologiques et Ecologiques, Montpellier, France*, 1984, p. 365-395.

Rioux J.A., Guilvard E., Dereure J., Lanotte G., Denial M., Pratlong F., Serres E. & Belmonte A. (1986b). *Infestation naturelle de Phlebotomus papatasi (Scopoli, 1786b) par Leishmania major MON-25. A propos de 28 souches isolées dans un foyer du Sud Marocain. In* Rioux J.A. (ed.) *Leishmania. Taxinomie et phylogenèse. Applications éco-épidémiologiques. (International Colloquium*

CNRS/INSERM) : Institut Méditerranéen d'Etudes Épidémiologiques et Ecologiques. Montpellier, France, 1984, p. 471-480.

Yahia H., Ready P.D., Hamdani A., Testa J.M. & Guessous-Idrissi N. (2004). Regional genetic differentiation of *Phlebotomus sergenti* in three Moroccan foci of cutaneous leishmaniasis caused by *Leishmania tropica*. *Parasite* **11**, p. 189-199.

Zougaghi L., Bouskraoui M., Amine M., Akhdari N. & Amal A. (2011). Leishmaniose cutanée à *Leishmania tropica* dans la région de Marrakech (Maroc), un foyer rebelle ! *Revue Francophone Des Laboratoires* **429**, p. 35-39.

Zouirech M., Belghyti D., El-Kohli M. & Faraj C. (2013). Entomological investigation of an emerging leishmaniasis focus in Azilal province, Morocco. *Pakistan Entomologist* **35**, p. 11-15.

(**37 réf.)**