

Cutaneous leishmaniasis in Ouazzane and Sidi Kacem provinces, Morocco (1997-2012)

Leishmaniose cutanée dans les provinces d'Ouazzane et Sidi Kacem, au Maroc (1997-2012)

H. El Miri · C. Faraj · O. Himmi · A. Hmamouch · S. Maniar · T. Laaroussi · M. Rhajaoui · F. Sebti · A. Benhoussa

Reçu le 18 août 2015 ; accepté le 14 juin 2016
© Société de pathologie exotique et Lavoisier SAS 2016

Abstract Cutaneous leishmaniasis (CL) is a major public health problem in Morocco. Three distinct parasites are involved; *Leishmania tropica*, *Leishmania major* and *Leishmania infantum*. The objective of this study is to investigate the epidemiological and the clinical features of endemic foci of CL in Sidi Kacem and Ouazzane provinces in the north of Morocco including molecular identification of parasites. We studied the evolution and the distribution of 1,656 CL cases coming from 39 sectors in these provinces between 1997 and 2012. The causative agents of CL in these areas were identified by using the ITS1-PCR-RFLP method. A tendency of seasonality in incidence was observed, showing a peak in April. Most infected patients were from Ouazzane province. The patients' ages ranged from 6 months to 85 years; 54% of them were females. The highest rate lesions were found in the age group of 9 years or less and most lesions were localized in the face (79.6%). The movement of populations

from neighboring endemic areas and establishment of habitation in areas where housing conditions are unfavorable favored the emergence of the disease.

Keywords Cutaneous leishmaniasis · *Leishmania tropica* · *Leishmania infantum* · molecular identification · Sidi Kacem · Ouazzane · Morocco · Maghreb · Northern Africa

Résumé La leishmaniose cutanée (LC) est un problème majeur de santé publique au Maroc. Trois parasites distincts sont impliqués : *Leishmania tropica*, *Leishmania major* et *Leishmania infantum*. L'objectif de cette étude est d'étudier l'épidémiologie et les caractéristiques cliniques des foyers endémiques de LC dans les provinces Sidi Kacem et Ouazzane au nord du Maroc, y compris l'identification moléculaire des parasites. Nous avons étudié l'évolution et la répartition de 1656 cas de LC provenant de 39 secteurs dans ces provinces entre 1997 et 2012. Les parasites responsables de la LC dans ces régions ont été identifiés en utilisant la méthode ITS1-PCR-RFLP. Une tendance à la saisonnalité de l'incidence a été observée, montrant un pic en avril. La plupart des patients infectés étaient de la province d'Ouazzane. L'âge des patients variait de 6 mois à 85 ans ; 54 % d'entre eux étaient des femmes. Les taux les plus élevés des lésions ont été trouvés dans le groupe d'âge de 0 à 9 ans et la plupart des lésions étaient localisées sur le visage (79,6 %). Le mouvement des populations des régions endémiques voisines et l'installation des habitations dans les zones où les conditions de logement sont défavorables ont favorisé l'émergence de la maladie.

Mots clés Leishmaniose cutanée · *Leishmania tropica* · *Leishmania infantum* · identification moléculaire · Sidi Kacem · Ouazzane · Maroc · Maghreb · Afrique du Nord

H. El Miri (✉) · C. Faraj · M. Rhajaoui · F. Sebti
Department of Parasitology, National Institute of Hygiene,
27 av. Ibn Battuta, Agdal, 11400 Rabat, Morocco
e-mail : hichamelmiri@gmail.com

O. Himmi
Laboratory of Zoology and Animal Ecology, Scientific Institute,
Mohammed V Agdal University, Rabat, Morocco

A. Hmamouch
Laboratory of Microbial Biotechnology, Department of Biology,
Faculty of Sciences and Technology, Sidi Mohammed Ben
Abdellah University, Fes, Morocco

S. Maniar
Regional Observatory of the Health Fes-Boulemane,
Hospital Al Ghassani, Fes, Morocco

T. Laaroussi
Provincial Delegation of the Ministry of Health, Sidi Kacem,
Morocco

H. El Miri · A. Benhoussa
Biology of Populations Laboratory, Faculty of sciences,
Mohammed V Agdal University, Rabat, Morocco.

Introduction

Human leishmaniasis constitute an important public health problem in Morocco. They are endemic throughout many

areas of the country with three distinct parasites and two disease forms; cutaneous and visceral. Anthroponotic cutaneous leishmaniasis (ACL) caused by *L. tropica* and zoonotic cutaneous leishmaniasis (ZCL) caused by *L. major* are the most widespread manifestations of the disease. Cutaneous lesions due to *L. infantum* has also been sporadically demonstrated, mainly in the North of the country [1-3]. Visceral leishmaniasis is mostly localized in the North, but some sporadic cases were also described in the Center and the South of the country [4].

The provinces of Sidi Kacem and Ouazzane in the North, known to be endemics for VL, housed also many former and emerging CL foci, while epidemiological data on these foci are scarce and fragmentary. For this reason, we conducted this study to determine the epidemiological characteristics of these foci and to identify the causative parasite species.

Materials and Methods

Study area

Sidi Kacem and Ouazzane provinces are located in the North of Morocco (Fig. 1). They are bounded, on the East by Taounate province and in the South by Moulay Yacoub province.

They are experiencing a Mediterranean climate. The maximum and minimum mean monthly temperatures are respectively (36-40°C) between July and August and (4-7°C) in January. Rainfalls range from 500 mm to 600 mm. Agriculture and tourism are the most important sectors in the economy of these provinces [5].

Epidemiological data

Epidemiological data were collected by consulting the registers and annual reports of the local and national public health services. All CL cases reported in Sidi Kacem and Ouazzane provinces from 1997 to 2012 were compiled and retrospectively analysed. Clinical symptoms and personal information for each patient (location, number of lesions, sex, age and geographic origin) were reported.

DNA extraction and identification of *Leishmania* species

Parasite DNA was extracted from 18 positive microscopic slides collected between 2012 and 2013 in Sidi Kacem (7 slides from three sectors) and Ouazzane (11 slides from six sectors). DNA was purified by QIAamp Tissue kit QIAGEN following the manufacturer's instructions. A PCR-restriction fragment length polymorphism (RFLP) approach was

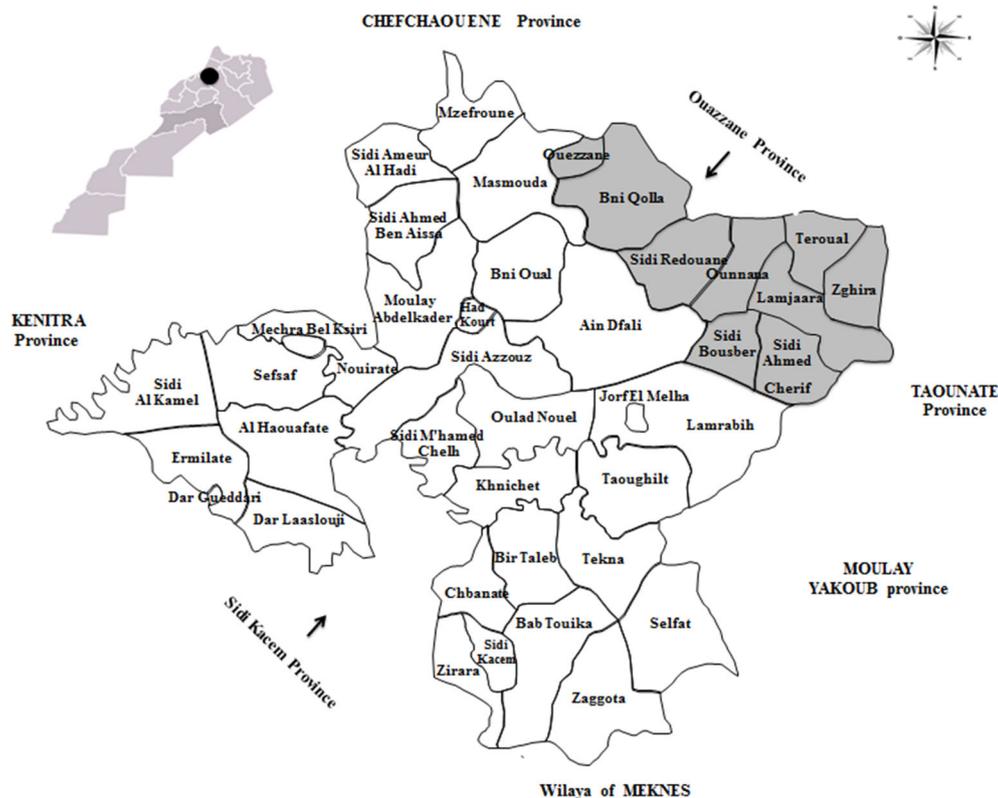


Fig. 1 Map of study areas / Carte des zones d'étude

applied for the detection and identification of the *Leishmania* parasites. PCR product was digested with the restriction endonuclease MnlI.

Data analysis

The software R. χ^2 of Pearson test was used to compare percentages and to perform statistical analysis. For all tests, the significance level was 0.05.

Results

Epidemiological study

Cutaneous Leishmaniasis is seen throughout the year with an average of 138 cases per month and a peak in April-May. The distribution of the yearly incidence of the disease per

sector between 1997 and 2012 (Fig. 2) revealed that at the beginning, cases emerged mainly in eastern parts of the region in the sectors of Teroual, Lamjaâra, Sidi Ahmed Chrif, Ounnana and Aïn Dfali. These areas are adjacent to the provinces of Taounate, an endemic focus of visceral and cutaneous leishmaniasis. Then later on, we observed a tendency of high incidence rates to occur in the northeastern part of the region as time evolves and cases were reported in the central and western parts. After 2006, endemicity spread in the whole area except for a few sectors in the South.

Clinical aspects

Analysis for patients' age showed that all age groups were affected. However, the class 0-9 years was significantly the most infected with 26.2% of all cases.

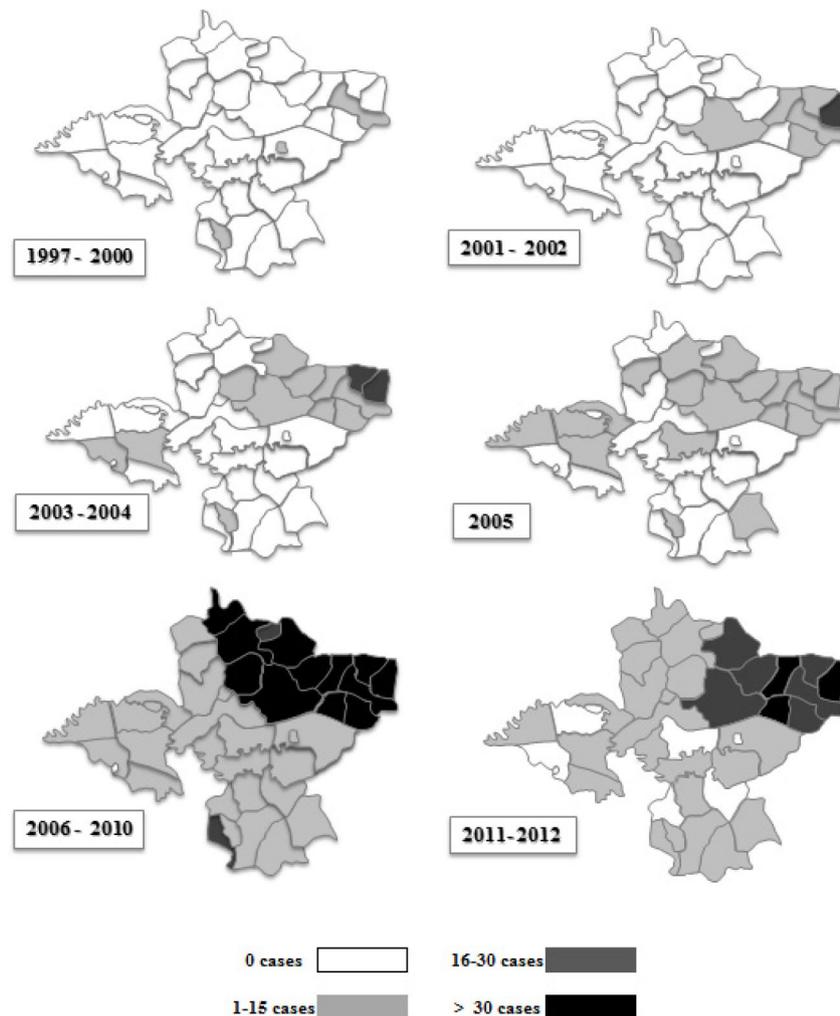


Fig. 2 Spatiotemporal distribution of CL cases in Sidi Kacem and Ouazzane provinces between 1997 and 2012 / Répartition spatio-temporelle des cas de LC dans les provinces de Sidi Kacem et Ouazzane (1997-2012)

Cutaneous leishmaniasis infection was significantly more prevalent among females (54%) than males (46%) ($\text{Khi}^2 = 10.9986$, $p\text{-value} = 0.0009118$).

Most lesions were observed on the face with 79.6% of cases, followed by upper limbs, lower limbs and trunk with 17.1%, 2.5% and 0.8% respectively. The number of lesions per patient ranged from 1 to 4; 953 of the patients had a single lesion (92.9%).

Identification of parasites species

Parasite species identification reveals the presence of two species in both provinces; *L. tropica* and *L. infantum* (Table 1).

Discussion

Cutaneous leishmaniasis has been endemic in Sidi Kacem and Ouazzane provinces since 1997. Since 2000, an epidemic has emerged in the East and expanded to the whole parts of the region with a peak incidence in 2010. The largest numbers of cases is recorded in the eastern and northeastern sectors of the region.

As obvious from experience around the world, the impact of human activities such as environmental modifications, resettlement of non-immune populations, urbanization or development of agricultural and water resources projects increased risk of CL transmission and introduced the parasite to new areas. Indeed, the beginning of the expansion of CL in the study area is coincident in time with the construction, in 1996, of Al Wahda dam in the east of the region, in Ouazzane province. Construction of dams can modify the tempe-

rate and humidity of the soil and vegetation, which may result in changes in the density of sandfly species. The occurrence in different parts of Morocco of potential sandfly vectors such *Phlebotomus sergenti* and *Larrousius* group has been known for a long time. Thus, the intense migration of workers, most of them from the CL endemic region (Taounate province), could explain the outbreak of the disease in this region. The new settlements of non-immune populations, inadequate housing and poor sanitations facilitate later outbreaks of leishmaniasis. Moreover, the increased number of cases diagnosed in the studied area during the last three years can be also awarded to the strengthening in the disease surveillance within the framework of the national leishmaniasis control program and to the evolution of biological techniques of diagnosis.

CL is affecting both sexes in these provinces. The infection rate among females was slightly higher than on males. Such result has been previously reported in different endemic areas [3,6]. The possible explanation is that women are consulting more than men because of the unsightly lesions [7].

Similar to the findings of previous studies in Morocco [3,7], the most affected part of the body was the face followed by the upper limb, then the lower limb and the trunk. Other studies in Iran [8] and Tunis [9] showed that upper limbs were the most affected.

The molecular identification of the *Leishmania* species responsible for the CL in the study area revealed the presence of two co-existing species *L. infantum* and *L. tropica*.

Human CL due to *L. infantum* was identified in almost all the infected sectors, in the two studied provinces. This finding showed that this parasite species is more prevalent than indicated in literature [1,4] and requires more investigations to clarify its transmission cycle and to identify the implicated strains.

The clinical picture of CL due to *L. tropica* and *L. infantum* observed in Sidi Kacem and Ouazzane was the same, since most patients had a single lesion, mostly on the face. This result demonstrates the need for parasite species identification to better-characterized CL foci.

Conclusion

This study reported a continuous transmission of the CL parasite (1997-2012), affecting almost all sectors of the two studied provinces. Two species of *Leishmania* are responsible for CL in these foci, *L. tropica* and *L. infantum*, causing diseases with similar clinical pictures. These results must be considered by the sanitary authorities for the implementation of control strategies more adapted to such foci mainly by strengthening indirect control measures on dogs, the main reservoir of *L. infantum*.

Table 1 Distribution of parasite species causing CL in Sidi Kacem and Ouazzane provinces (1997-2012) / Répartition des espèces de parasite causant la LC dans les provinces de Sidi Kacem et Ouazzane (1997-2012).			
Provinces	Sectors	<i>Leishmania</i> Species	Number of Slides
Ouazzane	Teroual	<i>L. infantum</i>	3
		<i>L. tropica</i>	2
	Sidi Bousber	<i>L. infantum</i>	1
	Sidi Ahmed Chrif	<i>L. infantum</i>	1
	Had Kourt	<i>L. tropica</i>	3
Sidi Kacem	Khnichat	<i>L. infantum</i>	1
	Zirara	<i>L. infantum</i>	2
	Bab Touika	<i>L. tropica</i>	1
	Sidi Kacem	<i>L. infantum</i>	2
	Ain Dfali	<i>L. infantum</i>	1
<i>L. tropica</i>		1	

Conflict of interest: The authors do not have any conflict of interest.

References

1. Al-Jawabreh A1, Schnur LF, Nasereddin A, et al (2004) The recent emergence of *Leishmania tropica* in Jericho (A'riha) and its environs, a classical focus of *L. major*. *Trop Med Int Health* 9 (7):812–6
2. Aoun K, Bouratbine A (2014) Cutaneous Leishmaniasis in North Africa: A review. *Parasite*, 21:14
3. Aoun K, Ben Abda I, Bousslimi N, et al (2012) Caractérisation comparative des trois formes de leishmaniose cutanée endémiques en Tunisie. *Ann Dermatol Vénéreol* 139(6-7):452–8
4. Arroub H, Alaoui A, Lemrani M, Abbari K (2012) Cutaneous Leishmaniasis in Foum Jamâa (Azilal, Morocco): Micro-environmental and Socio-Economical Risk Factors. *J Agric Soc Sci* 1:10–16
5. Chaara D, Haouas N, Dedet JP, et al (2014) Leishmaniasis in Maghreb: An endemic neglected disease. *Acta Trop* 132:80–93
6. Hamdi S, Faouzi A, Ejghal R, et al (2012) Socio-economic and environmental factors associated with Montenegro skin test positivity in an endemic area of visceral leishmaniasis in northern Morocco. *Microbiol Res (Pavia)* 3:e7
7. Haouas N, Garrab S, Gorcii M, et al (2011) Development of a polymerase chain reaction-restriction fragment length polymorphism assay for *Leishmania major*/*Leishmania killicki*/*Leishmania infantum* discrimination from clinical samples, application in a Tunisian focus. *Diagn Microbiol Infect Dis* 68(2):152–8
8. Kimutai A, Kamau NP, Kiprotich TW, et al (2009) Leishmaniasis in northern and western Africa: A Review. *Afr J Infect Dis* 3(1):26–30
9. Lemrani M, Nejjar N, Benslimane A (1999) A new focus of cutaneous leishmaniasis due to *Leishmania infantum* in Northern Morocco. *G Ital Med Trop* 4:3–4