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Epidemiologic study and molecular detection of *Leishmania* and sand fly species responsible of cutaneous leishmaniasis in Foum Jamâa (Azilal, Atlas of Morocco)

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ABSTRACT

The region of Foum Jamâa (province of Azilal) has become endemic for cutaneous leishmaniasis (CL) since 2006. The objective of this study was to investigate molecular identification of the etiological agent of CL in this region; we also carried out an entomological survey of Phlebotomine sand flies (Diptera: Psychodidae) in this focus to study the sand fly fauna, species composition, and the monthly prevalence of sand flies during 1 year. In the period between 2009 and 2010, skin scrapings spotted on glass slides were collected from 119 patients, aged from 9 months to 70 years old, who came from 43 localities distributed in 3 sectors in Foum Jamâa (FJ). The ITS1 PCR-RFLP was used to identify the *Leishmania* parasite responsible for the recent cases of CL in FJ. Our results revealed that the disease is caused by *L. tropica*. No significant association was observed between gender and the rate of CL in presenting patients, while the highest rate of positive lesions was found in the age group of 9 years old or under (86.67%). In this study, we found also that *L. tropica* infection mostly caused single lesions (67.90%) that were located in the face (96.30%). Morphological identification was performed on a total of 1152 sand flies (23% females and 77% males) collected by sticky paper traps. 57% of the total collected flies were identified as *Phlebotomus (Paraphlebotomus) sergenti* (*Parrot*).

1. Introduction

In Morocco, three species of *Leishmania* are endemic, causing human cutaneous leishmaniasis (CL). *L. major* is responsible of zoonotic CL and is localized in areas south of the Atlas Mountains (Rioux et al., 1986) where regular epidemics, with more than 2000 cases are reported (Ministry of Health, 2008). In the North of the country, some sporadic cases of CL due to *L. infantum* were observed (Lemrani et al., 1999; Rhajaoui, 2009). *L. tropica* has the largest geographic distribution and is considered a major public health threat (Ministry of Health, 2008); this form was reported for the first time in 1989 (Marty et al., 1989), since this first case a large eco-epidemiological study led to the detection of a large focus in central and southern areas of the country. Recently, Morocco is known for the emergence of several new foci in the north Guessous-Idrissi (Guessous-Idrissi et al., 1997; Rhajaoui et al., 2004, 2007) and *L. tropica* has been found in some regions previously known for transmission of *L. major* (Rhajaoui, 2009). *L. tropica* is considered to be purely anthroponotic, however, more recently it has become clear that in some cases *L. tropica* could be zoonotic (Guessous-Idrissi et al., 1997).

In the province of Azilal, FJ region has become an epidemic focal point for CL.

It seems obvious that FJ was free from CL before 1990; the first cases recorded by the provincial delegation of health go back to 1986 and 1987 in Tanant, at 16 km from FJ. Early in 2000, several provincial centers declared some scattered cases in FJ. During the period 2006–2009, we registered about 500 cases of CL in this region (Arroub et al., 2012), the efforts done by the Ministry of Health between 2006 and 2009 have stabilized the number of cases in the region, nevertheless, the disease is still persistent and the number of cases can increase at any time.

In our previous work focused on the eco-epidemiological and socioeconomic study, we have described CL in FJ as a rural domestic form, characterized by affecting the whole family nucleus, due to the fact that dwellings are located near the natural focus of transmission; thus propitiating the vector's arrival in the house (Arroub et al., 2012). Based on clinical symptoms, we suggested that the

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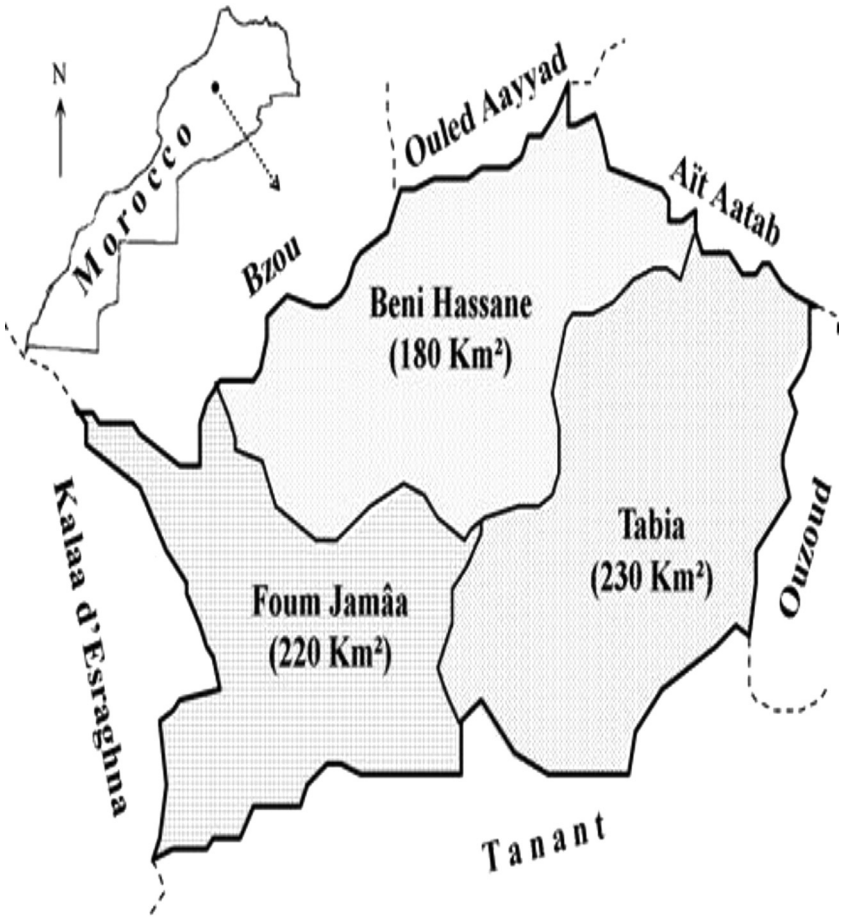

Fig. 1. The location of sampling zones, the surfaces of the three sectors (Beni Hssan, Foum Jamâa, Tabia) are indicated in between bracket below each corresponding sectors name.

disease may be caused by *L. tropica*; however, accurate identification of parasite and sand fly species is crucial to enable adequate treatment and appropriate public health control measures. Accepted standard diagnosis involves isolation of parasites either microscopically, or by culture, but *Leishmania* species are similar and their morphological species identification is not possible (Shahbazi et al., 2008). Currently, multilocus enzyme electrophoresis (MLEE) is the gold standard technique for the identification and classification of isolates of *Leishmania*; however, this method is quite slow, laborious, and costly because it requires culturing and obtaining the profile of 10–20 different enzymes. Lately, several PCR-based methods have proven to be highly sensitive and specific, as compared to standard methods, and are considered valuable for diagnosis (Akhavan et al., 2010; Azizi et al., 2006; Parvizi and Ready, 2008). These molecular methods are particularly useful for identification of *Leishmania* species directly on clinical samples without the need for prior cultivation (Schonian et al., 2003; Vega-Lopez, 2003). The PCR targets include the kDNA minicircles, the small subunit rRNA, the spliced leader sequence, or the miniexon gene. Typing methods, such as restriction analysis of the internal transcribed spacer (ITS) region (Cupolillo et al., 1995), PCR using non-specific primers (Noyes et al., 1996), and direct DNA sequencing and single-strand conformation polymorphism analysis, have been employed.

ITS1 sequences have been extensively studied in *Leishmania* because of their high sensitivity to detect *Leishmania* in clinical samples and their adequately polymorphic to differentiate strains at least to the species level (Schonian et al., 2001). In the present study, we selected the ITS1-PCR method for detecting *Leishmania* in skin scrapings spotted on glass slides; the ITS1 PCR-RFLP was used to identify the *Leishmania* parasite responsible for the recent cases of CL in FJ. Considering the importance of the vector in the transmission of the CL cycle, an entomological survey was carried out in this focus to study the sand fly fauna, species composition, and the monthly prevalence of sand flies during 1 year.

2. Materials and methods

2.1. Study sites

Studies were conducted in 3 adjacent sectors in FJ region, the province of Azilal, High Atlas of Morocco (Fig. 1). The three sectors are Beni Hassan, Foum Jamâa and Tabia, they comprise 43 localities with a total area of about 63,000 ha. It has a population of 30,000 persons. This region is located near the Western High Atlas National Park (32°08′ N, −6°60′ W) at different altitude from 523 to 1086 m. The maximum and minimum mean monthly temperatures were respectively 45 °C (August) and 10 °C (December), and the rainfall is relatively low with two peaks; one in autumn (November to December) and the second in spring (March and April). The vegetation is rare and mainly dominated by cactus, jujube plant, lentils, olive, and almond trees.

2.2. Collection and identification of sand flies

The collections were carried out during 1 year on 17 stations, representing 8 main different biotopes in the region of FJ (henhouse, stable, stable henhouse, cave, ruined house, dwelling, bridge, windows, outdoor) and regularly distributed to cover all