



# Molecular epidemiological study of cutaneous leishmaniasis in Beni Mellal and Fquih Ben Saleh provinces in Morocco



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## ABSTRACT

Leishmaniasis are parasitic diseases frequent in the Mediterranean Basin. Cutaneous leishmaniasis (CL) has been recently emerged in several new foci, causing a public health problem in Morocco. This study was performed to evaluate the epidemiological status of cutaneous leishmaniasis (CL) in Beni Mellal and Fquih Ben Saleh Provinces and to identify the causative agent. A total of 584 (56% female, 44% male) confirmed cases of CL were enrolled during the study period 2000–2012. Majority of cases (56%) were recorded in three sectors: Zaouiat Cheikh, Beni Mellal and Oulad Ayad. Fisher statistical test showed that there is a significant effect of the total cases of CL affected in the 185 localities. The age distribution of CL cases was relatively stable, with the majority of patients (62.34%) aged less than 9 years old with significant differences ( $p$ -value  $< 2.2e-16$ ). CL lesions were mostly located on the face (89%). The average number of lesions per patient was 1.38. *Leishmania tropica* was identified as the causative agent based on species-specific ITS1-PCR-RFLP assay.

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## 1. Introduction

Leishmaniasis is a protozoan parasitic disease caused by *Leishmania* species. About 100 countries and 350 million peoples are at risk of acquiring leishmaniasis. Twelve millions of persons are infected and an estimate of two millions of new cases occur annually (Alvar et al., 2012a,b). The two most common clinical forms of the disease, cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL), are mainly seen in 14 of the 22 countries of the Eastern Mediterranean Regional Office (EMRO) region, including Morocco (Ruiz Postigo, 2010). The lesions of CL may take several months to heal and, often, leave ugly scars on the face or other exposed parts of skin. The number of reported CL cases increased from 778 in 2000 to more than 4000 in 2011 and 2877 in 2012. Thereafter, the dis-

ease rate is increasing and new foci of CL are emerging in Morocco (Moroccan Ministry of Health, 2012). Beni Mellal and Fquih Ben Saleh provinces, located in central Morocco, are two emergent foci of CL since the last decade. Retrospective study, comparative sectorial distribution, molecular typing of *Leishmania* and identification of epidemiological characteristics of those foci are needed to establish effective control strategy in this area.

## 2. Materials and methods

### 2.1. Study area

This study was carried out in Beni Mellal and Fquih Ben Saleh provinces, which were separated administratively since 2011. These provinces (surface area of 7075 km<sup>2</sup>) are located in central Morocco, approximately between 32°20'22"N and 6°21'–39"W. Each province is constituted by districts (Fig. 1) subdivided into sectors themselves subdivided into localities. The climate is continental, the mean annual temperature is 18 °C and the average annual rainfall is between 300 mm and 400 mm. A very important

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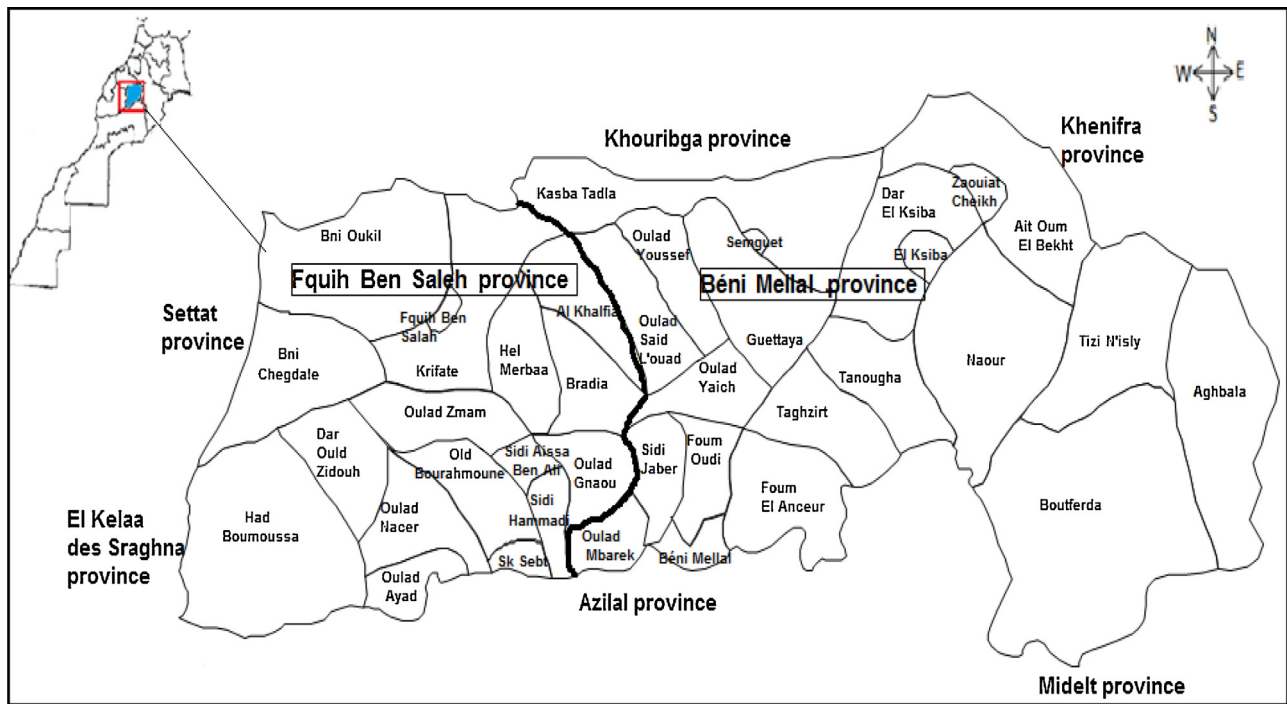


Fig. 1. Geographic location of Beni Mellal and Fquih ben Saleh provinces and its districts.

geographical richness of cultivated vegetation exists in the target provinces where shall we find olives-trees and fruit-trees. Landscape is mainly constituted by plains crossed by Oum Errabiaa river. Forests surface occupy about 69.7% of the area, the population is estimated to 973,000 inhabitants in 2012 (50.2% in rural area and 49.8% in urban sectors) with an overall density of 136.1 inhabitants per square kilometer (High Commission for planning, 2012).

## 2.2. Sampling and diagnosis

Tissue samples were taken from patients with CL, who had consulted the healthcare facilities in Beni Mellal and Fquih Ben Saleh provinces during 2000–2012. An individual form was completed for each patient: date, age, gender, address, number and localization of lesions. All slides were examined by the provincial laboratory and sent to the national laboratory of leishmaniasis in National Institute of Hygiene (NIH Morocco) for control and confirmation of the results, and molecular exam for some slides selected from the most affected localities.

## 2.3. DNA extraction and PCR-RFLP analysis

This test was performed on 28 positives slides. DNA was prepared from stained slides of CL and the ribosomal internal transcribed spacer (ITS1) region was amplified, followed with endonuclease *HaeIII* digestion, then the entire product was loaded and analysed on agarose gel as previously described (Schönian et al., 2003; Al Jawabreh et al., 2004).

Distilled water was used as negative control and a DNA of *L. infantum* (MHOM/MA/1998/LVTA), *L. tropica* (MHOM/MA/2010/LCTIOK-4) and *L. major* (MHOM/MA/2009/LCER19-09) were used as positive controls during PCR to ensure reliability, validity and to check for possible contaminations of the amplification reactions. The RFLP analysis using enzyme *HaeIII* revealed two bands with size of 185 and 57 bp for *L. tropica*; 232 and 132 bp for *L. major*; 184 and 72 bp for *L. infantum*.

## 3. Results

### 3.1. Temporal distribution of CL

During the period 2000–2012, the Moroccan Ministry of Health noted 584 human cutaneous leishmaniasis cases in Beni Mellal and Fquih Ben Saleh provinces. The first case of human CL in Beni Mellal province was recorded in 1998. Over the 2000–2012, the temporal evolution of the number of new CL cases was unstable in these provinces. There are two epidemiological phases: an hypoendemic phase from 2001 to 2006 with only 54 cases and an average of 9 cases per year and a highly endemic phase from 2007 to 2012 with a significant increase and an average of 87 cases per year. The maximum number of cases was recorded in 2012 with 135 cases in the two provinces (Fig. 2).

### 3.2. Geographical distribution of CL

The study showed that in Beni Mellal province, 13 districts were affected by CL. Zaouiati Cheikh district, composed of a single sector,

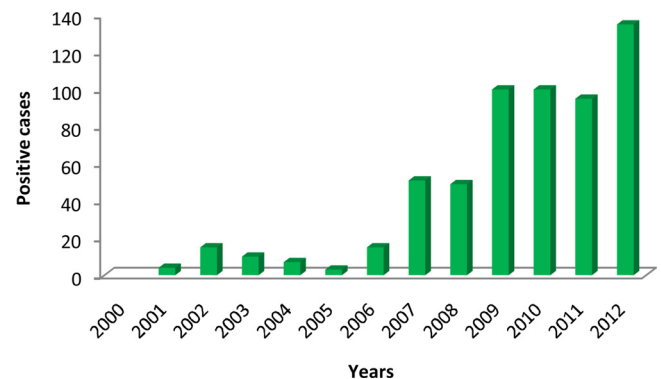


Fig. 2. Evolution of CL in Beni Mellal and Fquih Ben Saleh provinces from 1997 to 2012 (MMH, 2012).

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