



## Epidemiological characteristics of visceral leishmaniasis in Morocco (1990–2014): an update



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### ABSTRACT

Leishmaniasis are parasitic diseases frequent in the Mediterranean Basin. Visceral leishmaniasis (VL) is a notifiable parasitic disease that increased in incidence in Morocco over the past few years and has recently emerged in several new foci, causing a public health problem in Morocco. The aim of this study is to describe the spatio-temporal distribution of VL in Morocco between 1990 and 2014 period in order to highlight important features and trends of VL and its epidemiology and to assess whether the activity of the unit reflects the situation of the disease at the national level and whether it could constitute an indicator of public health relevance. Two thousand four hundred and twenty one cases were reported in Morocco between 1990 and 2014 with an average annual reported incidence rate of 0.4 cases per 100,000 inhabitants. Before 1996 the average annual incidence of VL was 50 cases on average. After this date the number of cases increased and then remained stable with around 100–150 cases per year. Children whose age varies between 1 and 4 years old are the most affected with 1327 (74%) of total cases; nevertheless the adult starts to be affected by the disease. In 2000, 65% of positive cases of VL are concentrated at both northern regions: Taza-Al Hoceima- Taounate with 45% of cases, Tanger- Tetouan mainly represented by Chefchaoun with 20% of cases. The Fez-Boulemane region located in the center recorded 12% of cases. Throughout the years the map VL distribution has been progressively changed and spatial spread of the disease to the center is noted in 2007. 2014 has been marked by an even greater extension of the disease to the center and south of Morocco. Nationally in 2014, 34 of 75 provinces and prefectures are affected compared to 2000, when 22 out of 82 provinces and prefectures were affected. *Leishmania infantum* was identified the causative agent based on species- specific PCR-Lei70 assay. VL remains a sporadically endemic parasitic disease in Morocco with a progressive extension of its range of distribution. Such a situation would relate to the geographical succession of Phlebotomine sand fly vectors, the difficulty of actions against the canine population reservoirs of *L. infantum* and unfavorable socio-economic factors.

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### 1. Introduction

Visceral leishmaniasis (VL) is a systemic vector-borne parasitic disease caused by obligate intracellular protozoa of the genus

*Leishmania* (*L.*) and transmitted by the bite of an infected female Phlebotomine sand flies of the genus *Phlebotomus*. The disease VL remains a major and serious public health problem in 62 countries (Guerin et al., 2002); more than 90% of global VL cases occur in just six countries: India, Bangladesh, Sudan, South Sudan, Brazil and Ethiopia (Alvar et al., 2012a,b). The worldwide incidence of VL is approximately 0.5 million cases per year (Desjeux, 2004) and a total of 200 million people are at risk of infection. The clinical manifestations of the disease vary from asymptomatic, self-limiting infections to fatal visceral leishmaniasis. The incubation period of VL ranges from a few months to several years depending on

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parasite virulence as well as genetic susceptibility of the host (Michel et al., 2011).

In Morocco, VL has been known since 1921 in the Tanger province of Morocco (Remilinger, 1926; Rhajaoui, 2011); however, it hasn't been ranked among the notifiable diseases until January 1995 (Guessous-Idrissi et al., 1996). It was localized in the north and caused by *L. infantum* zymodeme MON 1, predominantly affecting children under the age of 10 years (89%) and the case fatality rate in hospitals is estimated at 2.6% (Moroccan Ministry of Health, 2000). Dogs constitute the main host reservoir of *L. infantum* in Morocco as in all the Mediterranean basin (Guessous-Idrissi et al., 1997; Rami et al., 2003) and, hence, play an important role in the transmission cycle of VL. The Phlebotomine sand fly vectors of *L. infantum* in this country are: *P. perniciosus*, *P. longicuspis* and *P. ariasi* (Faraj et al., 2012).

However, in recent years, our exploration of epidemiological data showed that several new changes of epidemiological characteristics of the disease are observed as an increase in incidence, emergence of new leishmanian foci, alarming spread of the disease from the North to the south of the Morocco, the disease's affecting adults and an increased resistance of *Leishmania* to treatment in Morocco. This study aimed at describing the spatio-temporal distribution of VL in Morocco between 1990 and 2014 period in order to highlight important features and trends of VL and its epidemiology and to assess whether the activity of the unit reflects the situation of the disease at the national level and whether it could constitute an indicator of public health relevance.

## 2. Materials and methods

### 2.1. Study area

Morocco is approximately 710,850 sq km. It is extended on five large geographical units: 1- the Rif Mountain extending in the north of the Morocco; 2- the Atlas Mountains corresponding to central Morocco. They include the Middle, the High, and the Anti-Atlas Mountains; 3- the maritime plains located between the Atlantic Ocean and the western sides of the Atlas Mountains; 4- the pre-Saharan region at the south and the south east of the Atlas Mountains; 5- the Sahara extending on the south part of the Morocco. The large latitudinal extension of Morocco, the importance of its maritime facades and its relief involve a large geographical variation in its climate. So, the annual means of precipitation vary from less than 25 mm in Sahara to about 2000 mm in the Rif. The analysis of the climatic map (Sauvage, 1963) showed five ecological zones, the Saharan bioclimate in the south and east gradually turning to arid, semiarid, subhumid, and humid in high altitudes of Atlas and Rif mountains.

### 2.2. Data collection

This is a retrospective study collecting data of incidence VL annual reports of parasitic diseases from the Epidemiological direction of fight against diseases of the Ministry of Health from 1990 to 2014. The data are the result of passive monitoring, based on the notifications registered by all medical staff of University Hospitals (UH) of Morocco that declared each VL cases to the epidemiology department of the Ministry of Health. Database contains all patients data including gender, age, residence and time of hospitalisation. A standard form was used in this study to extract epidemiological data between January 1990–February 2015. Data on the distribution of cases of VL in terms of sex, age and seasons before 2000 are not available; only the reported cases from 2000 to 2014 have been analyzed in this study (Fig. 1).

### 2.3. Sampling and diagnosis

For the identification of the causative agent of the disease, medullary samples were taken from patients with VL, who had consulted UH in different Moroccan provinces. Individual forms were completed for each patient: date of admission/notification, residence, sex and age. These slides were examined microscopically for the detection of *Leishmania* amastigotes by the leishmaniasis provincial laboratory and sent to the national laboratory of leishmaniasis in INH for control and confirmation. Molecular exam was done for all slides received by the National Institute of Hygiene (INH).

### 2.4. DNA extraction and PCR-Lei70 analysis

DNA was extracted and purified from positive smears using a kit "high pure template PCR" (Roche Molecular Biochemicals, Indianapolis, IN) according to the instructions of the manufacturer. The conserved region of the ssu-rRNA *L. infantum* gene was amplified using the primer pair Lei70R and Lei70L by PCR-Lei70 approach for identification of the *Leishmania* parasites. The product was loaded and analyzed on 1.5% agarose gels by electrophoresis and visualized by UV lights (Spanakos et al., 2002; Chargui et al., 2009). Positive controls contain DNA of *L. infantum* (MHOM/MA/1998/LVTA), *L. tropica* (MHOM/MA/2010/LCTIOK- 4), and *L. major* (MHOM/MA/2009/LCER19-09). Negative controls (distilled water) were included during PCR to ensure reliability and validity and to check for possible contaminations of the amplification reactions. PCR product was followed with the Lei70 analysis using RsaI enzyme (Gibco- BRL).

## 3. Statistical analysis

Statistical analysis was performed with the SPSS software package 22 and analyzed by the software RChi2 of Pearson for comparison of percentage. For all test, the significance level was 0.05.

## 4. Ethics statement

According to ethical approval of this retrospective study, all samples were anonymized. For the hospital survey the data was extracted from the patient records by the clinician in charge onto an individual questionnaire format, without copying any personal identifiers that would allow identification of individual patients. Study design and procedures were revised and approved by the Ethical Committee of Charitè Faculty of Medicine and Pharmacy, Rabat, Morocco.

## 5. Results

### 5.1. Temporal distribution of VL in Morocco

During the period 1990–2014, the Moroccan Ministry of Health noted 2421 human VL cases in Morocco. Over 1990–2014 the temporal evolution of the number of new VL cases was unstable. There are two annual epidemiological phases: a hypoendemic phase from 1984 to 1994 (less than 50 cases/year) and a highly endemic phase from 1999 to 2014 with a significant increase and an average of 124 cases per year. The maximum number of cases was recorded in 2006 with 170 cases (Fig. 2).

### 5.2. Distribution of VL according to provinces from 1990 to 2014

Two thousand four hundred and twenty one (2421) VL cases were reported between 1990 and 2014 from the 57 of Morocco to

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