

A Historical Overview of the Classification, Evolution, and Dispersion of *Leishmania* Parasites and Sandflies in Morocco

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Abstract

In Morocco, as in most countries around the Mediterranean, leishmaniasis is an important public health problem. The situation has become worrying since the 1970's and continues to be more complicated. The objective of this work was to realize a historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and Sandflies in Morocco. The status of the different leishmaniasis could be illustrated in three epidemiological entities: zoonotic cutaneous leishmaniasis (ZCL) due to *L. major* transmitted by *P. (Phlebotomus) papatasi*, Anthroponotic cutaneous leishmaniasis (ACL) due to *L. tropica* transmitted by *P. sergenti* and cutaneous and visceral leishmaniasis (VL) due to *L. infantum* transmitted by *P. ariasi*, *P. perniciosus*, and potentially by *P. longicuspis*. Actually, VL occurred mainly in Northern Morocco. The ACL is widely distributed in the central and western Morocco. The ZCL occurred mainly in the south and south-east of the Atlas Mountains.

Keywords: Leishmaniasis; Parasites; Sandflies; Morocco

Introduction

In Morocco, as in most countries around the Mediterranean, leishmaniasis is an important public health problem [1]. The situation has become worrying since the 1970's and continues to be more complicated. With the agro-sylvo-pastoral practices, hydraulic development, overpopulation, and migration, the disease becomes more extensive in recent years, and the emergence of new Leishmanian foci has been noted. In 2011, Moroccan Ministry of Health reported 4319 cases [2] of cutaneous leishmaniasis (CL) and 107 cases of visceral leishmaniasis (VL).

Cutaneous leishmaniasis has been known in Morocco for almost a century. It was considered a sporadic affection. Foley et al. [3] reported the existence of the endemic buds in the southeast Morocco. Cases of Oriental buds were reported in Figuig [4] and the Moroccan Atlas [5]. In 1977, with the halting of DDT spraying operations against malaria in the south of the country, it began the development of epidemic fashion. Several epidemic outbreaks erupted in the sub-Saharan zones, due to *L. (Leishmania) major* [6].

The first mention of human visceral leishmaniasis in Morocco was mentioned by Klippel and Monier [7] in Meknes in 1921. Observations recorded thereafter showed a wide dispersion of the disease in the various provinces of the country. If the regions of the North: Tangiers [4], Fez [8], Casablanca [9] and Azrou are frequently cited, other publications located further south: Irherm, Tata and Goulmime [10].

Since 1970s, the situation of leishmanioses become worrisome in Morocco, which has led to the development of a Franco-Moroccan study program, under the direction of Professor Rioux, to analyze the different Leishmanian foci. The trapping of sandflies and the isolation of *leishmania* were carried out. According to Rioux [11], *L. major*, *L. tropica* and *L. infantum* were identified as the parasite species responsible for the diseases.

Before 1993, the lack of epidemiological information system based on collected data on leishmaniasis means that the available data hardly reflect the actual epidemiological situation. Between 1994 and 1999, taking into account the case reports during this period, the situation of the different leishmaniasis could be illustrated in three epidemiological entities [12]: zoonotic cutaneous leishmaniasis (ZCL) due to *L. major* transmitted by *P. (Phlebotomus) papatasi*, Anthroponotic cutaneous leishmaniasis (ACL) due to *L. tropica* transmitted by *P. sergenti* and cutaneous and visceral leishmaniasis (VL) due to *L. infantum* transmitted by *P. ariasi*, *P. perniciosus*, and potentially by *P. longicuspis*. Actually, VL due to *L. infantum* occurred mainly in Northern Morocco. The ACL due to *L. tropica* is widely distributed in the central and western Morocco, in contrast ZCL due to *L. major* occurred mainly in the south and south-east of the Atlas mountains. The objective of this work was to realize a historical overview of the classification, evolution, and dispersion of *Leishmania* parasites and Sandflies in Morocco.

Zoonotic cutaneous leishmaniasis due to *L. major*

In southern and eastern Morocco, ZCL due to *L. major* was characterized by its endemo-epidemic character. The first outbreak was identified in the south of the Anti-Atlas, in the palm groves of Wadi Tata, where a major outbreak of cutaneous leishmaniasis occurred in the late 1970s. After the Tata epidemic two new outbreaks have been identified: one in Ouarzazate and the other in Oujda [6,13].

The enzymatic analysis of the isolates obtained at the beginning of the epidemic allowed identifying *L. major* MON-25 [14], the only zymodeme involved throughout the Maghreb. The intra-vector infestation was demonstrated in the Akka-Ighan douar (East of Tata) by the identification of a female *P. (Phlebotomus) papatasi* carrying *L. major's* promastigotes [13].

Leishmania major occurs exclusively in the arid and semi-arid bioclimatic stages. The vector species is *P. papatasi* and its density

increases with increasing aridity. The main actors in the cycle were identified by the isolation of the parasite (*L. major* MON-25) in humans, the vector and the *Meriones shawi* reservoir [15,16].

Currently, foci of ZCL are linked to rural areas with degraded environmental and socio-economic conditions [17]. It is widespread from the Atlantic coast, south of the anti-atlas mountains to the north-east, crossing south saharan area of the Anti-Atlas and High Atlas mountains, and east of the middle Atlas mountains [18].

Anthroponotic cutaneous leishmaniasis due to *L. Tropica*

The ALC is an anthroponose whose proven vector is *P. sergenti* and the reservoir is the man [19]. In 1989, Marty et al. [20] located the first outbreak of ALC in Tanant in the region of Azilal after the diagnosis of this parasitosis in a Moroccan girl on the move in Nice. Other outbreaks, similar to Tanant, were identified in Smimou [21], in Taza [22], Zouagha My Yacoub [23], and Chichoua [24-26].

Phlebotomus sergenti, a proven vector of *L. tropica* [27], was abundant in Moroccan foci. The proof of its role as vector in Morocco was done in the Tanant home. *Leishmania tropica* is still regarded in Morocco as an anthroponose. The dog found repeatedly infected with the MON-102 and MON-112 zymodemes sporadically, however, is not considered a "real reservoir" [28]. This form of leishmaniasis rages in the semi-arid zones of the country ranging from the plateau of Tadla to the region of Agadir through Beni Mellal, Azilal, Marrakech, Kelaa and Essaouira. In these areas, the disease is found in rural douars between 400 and 1000 m above sea level in the vicinity of vast cedar forests. In this biotope common to several regions of Morocco, the disease evolves a hypoendemic form with a reduced number of cases. The enzymatic polymorphism of the parasite is considerable, *L. tropica* MON-102, MON-107, MON-112, MON-113, MON-122, MON-123, MON-279 [21]. Recently in the province of El Houcima, *L. tropica* MON-279 was identified in a dog with symptoms of canine visceral leishmaniasis [29]. Actually, the ACL is widespread in the semi-arid regions of centre and south-western Morocco [30].

Leishmaniasis due to *L. infantum*

Leishmania infantum present throughout the Moroccan territory, expressed in 2 clinical forms; Visceral and cutaneous. The MON-1 zymodem was isolated from a case of infantile visceral leishmaniasis in Taounate province [31]. The dog, the habitual reservoir of the MON-1 zymodem, is an excellent nosogeographic indicator: better than any other method, screening for canine visceral leishmaniasis makes it possible to locate foci of infection and to estimate their level of activity. Thus, in the Rif, the enzootic reached the frequency of 32.5% whereas it was only 3.1% in the High Atlas [32] and becomes sporadic in the Anti-Atlas [33]. *Leishmania infantum* MON-24 is an agent of human cutaneous leishmaniasis in the south [34] and in northern Morocco [34]. Its transmission cycle remains unknown. The VL form is widespread throughout the country. It is more frequent in the northern part with sporadic cases observed in other regions, particularly in the south [35].

Canine leishmaniasis is widespread in the northern part of Morocco and strains of *L. infantum* are very virulent. Changes of *L. infantum* antibody in a cohort of naturally infected dogs were studied in the provinces of Sefrou and Zouagha Moulay Yacoub [36]. The dogs were divided into 3 groups: a group of symptomatic dogs that produced high antibody titers (titers \geq 800) and developed lethal canine leishmaniasis; a group of dogs that showed low antibody titers and

remained asymptomatic throughout the follow-up and a group of dogs that became negative at the end of the study. The authors noted that adult dogs are more susceptible to the disease than young dogs [34].

Like *L. major* and *L. tropica*, the chorology of *L. infantum* is modeled on that of the vector, but there is at least three species, belonging to the subgenus *Larroussius*, share responsibility for transmission: *P. ariasi*, *P. perniciosus* and *P. longicuspis* [37,38]. The role of *P. perniciosus* and *P. ariasi* has been demonstrated in Spain [6], northern Morocco (Taounate) where a female of *P. ariasi* was found infested [39]. According to Rioux et al. [32], the three species: *P. ariasi*, *P. perniciosus* and *P. longicuspis* are responsible for transmission in humid and subhumid regions in northern Morocco, whereas in the south of the country, only *P. longicuspis* is known as a potential vector of *L. infantum* [33]. The association of morphometric, isoenzymatic and molecular tools in northern Morocco [40-43] has shown the existence of atypical morphs of *P. Perniciosus*, combined with *P. longicuspis*, and an introgression between *P. perniciosus* and *P. longicuspis*. The morphological analysis of the sub-genus *Larroussius* in central and south-west Morocco [44] showed that the atypical form of *P. perniciosus* appeared to be widespread in these regions.

Sandflies in Morocco

Sandflies were subject of several studies in Morocco before leishmaniasis was considered a major public health problem. After the observations and notes of Ristorcelli [45-49] on sandflies harvested in the south and in eastern Morocco, Gaud [48] made a first study on sandflies in Morocco. He gave a general overview of the regional distribution and seasonal frequency of sandflies across the country [49-51] and in the Rabat region [52]. More detailed studies on the biology of sandflies in Figuig region were done by Parrot and Durand-Delacre [53]. The work of Bailly-Choumara et al. [54] presented a geographical and bioclimatic synthesis of the existed data on sandflies from Morocco and on their own research between 1965 and 1970.

Beginning 1970s, the work of Rioux, in collaboration with the Moroccan authorities, was aimed to analyze different households [11]. The Rioux team's missions in northern and southern Morocco allowed studying the inventory of sandflies fauna [55-57], to study the correlations between vector/bioclimat [58] and to consider predictions of the distribution of leishmaniasis with climate change [32]. Rioux et al. [59] reported *P. (Larroussius) mariae* in Meknes region at Ouarzazate. This species was found by Lambert et al. [60] in the High Atlas of the semi-arid and cold sub-humid stages. Leger et al. [61] conducted an ecological and systematic study of Complex *S. (Sergentomyia) antennata* of southern Morocco. *P. (Paraphlebotomus) riouxi* was reported in Morocco by Depaquit et al. [62]. The work of Guernaoui et al. [63-65] in southwestern Morocco updated Rioux data in this region and revised the distribution of *Larroussius* according to the observations of Benabdenni et al. [42] in northern Morocco. Preliminary studies [66,67] in Marrakech revealed a high-risk of leishmaniasis status by demonstrating three leishmania vector species: *P. papatasi*, *P. sergenti* and *P. longicuspis*. In addition to ecological and epidemiological studies, biochemical analyzes have also been used to characterize populations of sandflies in Morocco. Thus, in the Moroccan Rif, isoenzymatic analysis revealed a diagnostic allele at the hexokinase locus between *P. perniciosus* and *P. longicuspis* and showed that the identification of males could also be done on the Number of midline bristles of coxites, lower in *P. perniciosus* than in *P. longicuspis* [40].

Currently, the list of sandflies species in Morocco contains 22 species, divided into 13 species of the genus *Phlebotomus* and 09 species of the genus *Sergentomyia* [68]: *P. (Phlebotomus) bergeroti* Parrot, 1943; *P. (P.) papatasi Scopoli*, 1786; *P. (Paraphlebotomus) alexandri Sinton*, 1928; *P. (Para.) chabaudi Croset*, Abonnenc et Rioux, 1970; *P. (Para.) kazeruni* Theodor et Mesghali, 1964; *P. (Para.) sergenti* Parrot, 1917; *P. (Larroussius) ariasi* Tonnoir, 1921; *P. (L.) chadlii* Rioux, Juminer et Gibily 1966; *P. (L.) langeroni* Nitzulescu, 1930; *P. (L.) longicuspis* Nitzulescu, 1930; *P. (L.) mariae* Rioux, Croset, Léger et Bailly-Choumara 1974; *P. (L.) perfiliewi* Parrot, 1930; *P. (L.) perniciosus* Newstead, 191; *S. (Parrotomyia) africana* Newstead, 1912; *S. (P.) lewisi* Parrot, 1948; *S. (Sergentomyia) antennata* Newstead, 1912; *S. (S.) fallax* Parrot, 1921; *S. (S.) minuta* Rondani, 1843; *S. (S.) schwetzi* Adler, Theodor and Parrot, 1929; *S. (Sintonius) christophersi* Sinton, 1927; *S. (S.) clydei* Sinton, 1928; *S. (Grassomyia) dreyfussi* Parrot, 1933.

Conclusion

In Morocco, leishmaniasis are diverse and complex diseases for several reasons: Multiplicity and complexity of parasitic cycles: anthroponotic for *L. tropica* and zoonotic for *L. major* and *L. infantum*; Diversification of reservoirs of parasites: rodents for *L. major* and canids for *L. infantum*; Different geographic distributions: *L. major* in the Saharan regions, *L. tropica* in semi-arid zones and *L. infantum* in the north and central and sub-humid regions.

Leishmaniasis is a reportable disease in Morocco, and is still a real public health problem. The two clinical entities observed visceral and cutaneous are widely distributed throughout the territory.

Leishmaniasis in Morocco constitutes three well differentiated nosogeographic entities requiring codified control measures adapted to each one. The implementations of the control program and its generalization made it possible to implement various control measures. An information system allows, in particular, monitoring of the epidemiological situation and the degree of implementation of the different control measures. While screening and treatment of human cases is operational in the various endemic provinces, vector and animal reservoir control (rodent and dog) remains limited and insufficient.

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