Spread of Leishmania killicki to central and south-west Tunisa

A Bouratbine, K Aoun, J Ghrab, Z Harrat, Ms Ezzedini, S Etlijani

SPREAD OF *LEISHMANIA KILLICKI* TO CENTRAL AND SOUTH-WEST TUNISIA

BOURATBINE A.*,**, AOUN K.*,**, GHRAB J.**, HARRAT Z.***, EZZEDINI M.S.****
& ETLIJANI S.*****

Summary:

Twenty cutaneous leishmaniasis (CL) cases were notified from December 2001 to February 2002, in a small village in the district of Oueslatia (governorate of Kairouan, central Tunisia) which is an endemic focus of infantile visceral leishmaniasis due to *Leishmania (L.) infantum* and that had never been concerned previously by CL. The parasite typing of two isolates obtained from two children that have never left the region has identified *L. killicki*. This species had only been reported previously in a limited focus of Tunisian Southeast. In October 2002, an epidemiological survey with isoenzym characterization of the parasite led in a well-known focus of zoonotic cutaneous leishmaniasis of South-West Tunisia also revealed the presence of *L. killicki*. These results suggest the spread of this species and stress the need of further investigations for a better control of CL in Tunisia.

KEY WORDS : Cutaneous leishmaniasis, *Leishmania killicki*, epidemiology, Tunisia.

Résumé : EXTENSION GÉOGRAPHIQUE DE *LEISHMANIA KILLICKI* VERS LE CENTRE ET LE SUD-OUEST TUNISIEN

Entre décembre 2001 et février 2002, 20 cas de leishmaniose cutanée (LC) ont été répertoriés à Aïn Jloula, une petite localité de la délégation de Oueslatia (Tunisie centrale), foyer classique de leishmaniose viscérale à Leishmania infantum. *Deux isolats prélevés à partir des lésions de deux enfants ont mis en évidence* Leishmania (L.) killicki, *espèce rapportée jusque là uniquement dans des micro-foyers du sud-est tunisien. En octobre 2002, une étude menée à Métlaoui (sud-ouest tunisien), foyer historique de leishmaniose cutanée zoonotique à* Leishmania major, *révèle également la présence de* L. killicki. *Ces résultats suggèrent l'extension géographique de cette espèce au centre et au sud-ouest tunisien et appèlent des investigations complémentaires afin de mieux cerner l'épidémiologie de cette forme de leishmaniose cutanée en Tunisie.*

MOTS CLÉS : *leishmaniose cutanée,* Leishmania killicki, *épidémiologie, Tunisie.*

INTRODUCTION

Three noso-geographical forms of cutaneous leishmaniasis (CL) coexist in Tunisia: in northern Tunisia, the most humid part of the country, sporadic cutaneous leishmaniasis (SCL) is caused by *Leishmania (L.) infantum* (Aoun *et al.*, 2000). In central and southern Tunisia, semi-arid and arid respectively, zoonotic cutaneous leishmaniasis (ZCL) caused by *L. major* is the prevalent form with thousands of cases reported each year since the start of a large epidemic in 1982 (Ben Ismail & Ben rachid, 1989; Anonymous, 2003). In the arid area of the south-east, is located the small and only so far reported sporadic hypoendemic rural focus of *L. killicki* considered in 1986 as a new species within the *L. tropica* complex (*L. tropica* MON8) (Rioux *et al.*, 1986) (Fig. 1). More recently this species has gained the status of a complex (Rioux & Lanotte, 1993).

From December 2001 to February 2002, cases of CL emerged in central Tunisia, in an area located between the classic northern SCL foci and the expanded endemic area of ZCL, which justified an epidemiological investigation of the outbreak and parasite typing (Fig. 1). Unexpectedly, two children that have never quit the region bore a parasite that was identified as *L. killicki*. In October 2002, an epidemiological survey led in the historical ZCL focus of Metlaoui (South-Western Tunisia) revealed the presence of the parasite (Fig. 1). This suggests that areas endemic for *L. major* and *L. killicki* overlap to a certain extend. The purpose of this paper is to describe the epidemiological and clinical features of CL in these two new foci of *L. killicki*.

* Laboratoire de Parasitologie-Mycologie, Institut Pasteur de Tunis.
** Unité de recherche "Parasitoses émergentes", Faculté de Médecine de Tunis.
*** Laboratoire de Parasitologie, Institut Pasteur d'Alger.
**** Direction régionale de la Santé Publique, gouvernorat de Kairouan.
***** Direction régionale de la Santé Publique, gouvernorat de Gafsa.
Correspondence: Pr Aïda Bouratbine, Laboratoire de Parasitologie-Mycologie, Institut Pasteur de Tunis, 13, Place Pasteur, B.P. 74, 1002, Tunis Belvédère, Tunisie.
Tel : 00 216 98 343 479 – E-mail: aida.bouratbine@pasteur.rns.tn

MATERIALS AND METHODS

STUDY AREAS

- Focus of Aïn Jloula

Between December 2001 and February 2002, 20 active cases of CL, confirmed by examination of Giemsa-

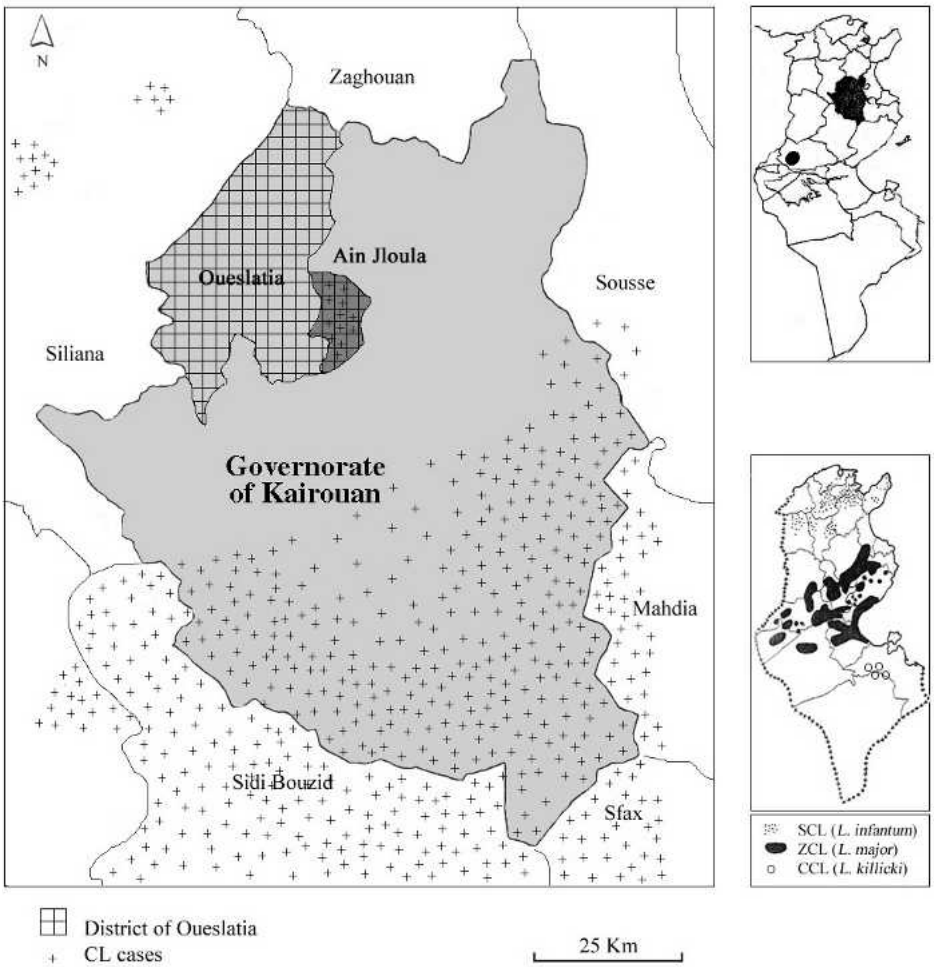

Fig. 1. – Geographical situations of the emerging focus of cutaneous leishmaniasis (CL) of Aïn Jloula (district of Oueslatia, Governorate of Kairouan, Central Tunisia) and the historic focus of zoonotic CL of Metlaoui.
Two maps of Tunisia are on the right: the first, in the top right, shows the governorate of Kairouan and the city of Metlaoui shaded black; the second, below, gives details on the geographical distribution of the three forms of CL encountered in Tunisia (Ben Ismail & Ben Rachid, 1989).
The main map reproduces the governorate of Kairouan. The district of Oueslatia is located northern the governorate. The locality of Aïn Jloula is situated in the southern west part of the district. Outside the limits of Kairouan are the neighbouring governorates: Zaghouan in the north, Siliana in the west, Sousse and Mahdia in the east, Sidi Bouzid and Sfax in the south. The main map shows the geographical distribution of CL cases reported by the Directory of primary health care during the season of transmission 2001-2002 (Anonymous, 2003).

stained smears, were recorded for the first time by the regional Primary Heath Care Unit of the district of Oueslatia, governorate of Kairouan, Central Tunisia in the setting of the National program of leishmaniasis control. The study of the disease distribution per locality revealed that cases emerged in Aïn Jloula, a small locality of about 5,000 inhabitants (Fig. 1). The area is composed of small hills lying along the southern versant of the Atlas Mountains, at a moderate altitude (150-200 m). Geographically, the area belongs to the semi-arid zone in which visceral leishmaniasis caused by *Leishmania infantum* is known to be endemic (Bouratbine *et al.*, 1998). Some few kilometres north are found some sporadic foci of CL due to *L. infantum* and some few kilometers south-east is the most septentrional border of the steppic extension area of LCZ due to *L. major* (Fig. 1).

• Focus of Metlaoui

The city of Metlaoui is known as a historic focus of ZCL (Chatton *et al.*, 1918), where cases are regularly recorded each year by the Primary Health Care Unit (Anonymous, 2003). It is located in an arid mountainous area at 200 m above the sea level, 60 km south of Gafsa (South-West Tunisia) and has a population of 42,000 inhabitants. Patients are listed mostly among different neighbourhoods.

Case survey

The medical records of cases diagnosed in the local Primary Health Care Unit of Aïn Jloula and Metlaoui and confirmed by Giemsa-stained smear examination were thoroughly reviewed. Patients' dwellings were identified and visited. Patients presenting active lesions were interviewed using a standardized questionnaire, including age, travel history and date of appearance of the lesions. The size, location and clinical description were noted. Family contacts and neighbours of index cases were also investigated.

Parasite typing

Samples were obtained from active lesions for culture on NNN medium. The growing parasites obtained from culture were then adapted to RPMI medium supplemented with fœtal calf serum and stored at – 80° C. The isoenzymatic profile was characterised, in Pasteur

Institute of Algiers, by electrophoretic analysis of 15 enzymes according to Rioux *et al.* (Rioux *et al.*, 1990). The following enzyme systems were studied : malate deshydrogenase (MDH, EC1.1.1.37), malic enzyme (ME, EC1.1.1.40), isocitrate desydrogenase (IDH, EC1.1.1.42), 6-phosphogluconate desydrogenase (6 PGD, EC1.1.1.44), glucose-6-phosphate desydrogenase (G6PD, EC1.1.1.49), glutamate deshydrogenase (GLUD, EC1.4.1.3), NADH diaphorase (DIA, EC1.6.2.2), purine nucleoside phosphorylase (NP1, EC2.4.2.1. et NP2, EC2.4.2.), glutamate oxaloacetate transaminase (GOT1 et GOT2, EC2.6.1.1), phosphoglucomutase (PGM, EC5.4.2.2), fumarate hydratase (FH, EC4.2.1.2), mannose phosphate isomerase (MPI, EC5.3.1.8), glucose phosphate isomerase (GPI, EC5.3.1.9). The reference strains used were MHOM/TN/80/LEM163 for the *L. killicki* complex, MHOM/FR/78/LEM75 (zymodeme MON-1), MHOM/DZ/82/LIPA59 (zymodeme MON-24) and MHOM/DZ/83/LEM425 (zymodeme MON-80) for the *L. infantum* complex, MHOM/MA/81/LEM265 (zymodeme MON-25) for *L. major* complex and and MHOM/SY/86/LIPA/154 (zymodeme MON-76) for the *L. tropica* complex.

RESULTS

Focus of Ain Jloula

Twenty patients living in the locality of Aïn Jloula were diagnosed as CL cases at the local Primary Health Care Unit during the transmission season 2001-02. They were geographically scattered, living in rural communities, on the flanks of the rocky hills that surround the village. All age groups were concerned by CL. Clustering of two to five cases among close contacts was observed in five communities. Five additional cases were discovered at the time of the investigation among family contacts and neighbours of index cases. The clinical investigation of 15 patients revealed a wide clinical polymorphism from small, weakly infiltrated lesions of the face to multiple, large, crusted ulcerated lesions of the limbs (Figs 2, 3). The classical crusted ulcerated forms were predominant. The face was involved in four cases and the limbs in 12. There were multiple lesions in nine patients (60 %) and single ones in six (40 %). Single lesions on the face were observed only in three children aged respectively of two, six and seven years. The diameter of the lesions varied from 0.5 to 8 cm (mean $\pm$ DS = 2 cm $\pm$ 2.4 cm).

Two isolates obtained from active lesions evolving in two children have been typed. The first patient aged of six years presented a small, weakly infiltrated, lesion of the face (Fig. 2) and the second one aged of seven years presented two small dry lesions, one on the face and the other on upper extremity. The two children belonged to two different communities. CL cases have been noted among their family and neighbours. The two isolates were characterized as *L. killicki*.

Focus of Metlaoui

Clinical investigations of 34 patients, who consulted the Primary Health Care Unit in October 2002, revealed that lesions were mostly large, wet, ulcerated, and located on limbs (88 %). 57 % of these lesions were single and 43 % were multiple. Five isolates have been characterized as *L. major* MON-25.

Visit to neighbourhoods permitted to detect other suspect CL cases. Two ten-year-old girls had particular clinical presentations. The first one presented recurrent reactivation of her face lesion with formation of satellite lesions at the periphery of the scar, a feature which is described with lesions caused by *L. tropica* in Kenya, a species identified as *L. killicki* (Sang *et al.*, 1992; Sang *et al.*, 1994) (Fig. 4). The other had a dry face lesion old of four years that resisted to treatment by Glucantime® (Fig. 5). Parasite from this latter has been characterized as *L. killicki*.

DISCUSSION

Three CL forms, with particular epidemiological and clinical features, can be distinguished in Tunisia. These forms affect separate areas of the country and are caused by three different species of *Leishmania*. CL which is caused by *L. infantum* is sporadic and occurs in the northern area, a classic focus of human and canine visceral leishmaniasis. Typically, the lesions consist of single small crusty ulcers of the face surrounded by a notable erythematous reaction (Chaffai *et al.*, 1988; Aoun *et al.*, 2000). Zoonotic cutaneous leishmaniasis caused by *L. major* is epidemic in the centre and the south where rodents *Psammomys obesus* and *Meriones shawi* are proven reservoirs (Ben Ismail & Ben rachid, 1989). *L. major* produces multiple lesions, localised most commonly on the limbs. Self healing occurs in less than eight months. Clinically, the lesions are polymorph, mostly large, wet, ulcerated and superinfected (Chaffai *et al.*, 1988) (Fig. 4). Finally CL caused by *L. killicki* is hypoendemic and occurs as scarce cases in micro foci located in the mountains of the south-east (Rioux, 1986). The reservoir seems zoonotic mainly because of the sporadic occurrence of cases and of the rural distribution of the disease (Ben Ismail & Ben rachid, 1989). Most patients with CL caused by *killicki* present a single lesion of the face. The clinical picture is polymorph. The lesions are readily dry, essentially characterised by a long evolu-

Fig. 2. – Small lesion, weakly infiltrated due to *L. killicki*.

Fig. 3. – Inflammatory lesion of cutaneous leishmaniasis (Focus of Aïn Jloula).

Fig. 4. – Satellite lesions at the periphery of the scar (Focus of Metlaoui).

Fig. 5 – Dry face lesion due to *L. killicki* (Focus of Metlaoui).

tion, lasting frequently several years (Rioux *et al.*, 1986).

The present report has described two new foci of *L. killicki* in Tunisia: a rural focus in Aïn Jloula in the Centre and a more urban one in Metlaoui in South-West. The two were far from the only known small foci of Southeast. The clinical picture of confirmed cases was similar to those described for lesions caused by *L. tropica* in Middle East: ulcerating dry lesion often on the face or on upper extremities that generally last a long time before healing and are difficult to treat (Jacobson *et al.*, 2003). Data on parasite isolates indicate a similarity with *L. killicki* known in the Tunisian foci and no heterogeneity was found. As described in south-eastern foci, anthroponotic transmission is very unlikely since the number of CL cases is too small and their distribution in space too sporadic to constitute an adequate reservoir. The rock hyraxes

which were suggested as reservoir hosts in Kenya (Sang *et al.*, 1994) aren't present in Tunisia. Whether the wild rodents *Ctenodactylus gondii* found in the mountainous areas of Aïn Jloula and Metlaoui play a role in the cycle of CL remains to be determined.

The investigation also revealed the concomitant presence of *L. killicki* in some ZCL Tunisian foci. In the focus of Metlaoui, cases of *L. killicki* seem rare compared to those caused by *L. major*, that is probably due to the force of transmission of each parasite species and to the cross immunity. In the focus of Aïn Jloula, although single dry lesions were present and characterised as *L. killicki*, they were few in number. Most patients developed rather rapidly multiple, large and inflammatory lesions of the limb, somewhat resembling those caused by *L. major* and the coexistence of these species can't be excluded especially since the latter strain is endemic in regions adjacent to the studied area.

CONCLUSION

Environmental modifications that caused the increase of the number of cases and the spread of *L. major* could also have favoured a concomitant spreading of *L. killicki* which haven't been noticed due to its rare cases comparing to the *L. major* ones. It is actually important to determine the real distribution and prevalence of this species for a better adequacy of control measures.

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