Acta Tropica 177 (2018) 89–93

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Natural infection of *Ctenodactylus gundi* by *Leishmania major* in Tunisia

Wissem Ghawar[a,b,*], Jihène Bettaieb[a,b,c], Sadok Salem[a,b], Mohammed-Ali Snoussi[a,b], Kaouther Jaouadi[a,b], Rihab Yazidi[a,b], Afif Ben-Salah[a,b,c,d]

[a] Department of Medical Epidemiology, Institut Pasteur de Tunis, 13, Place Pasteur, BP 74 1002, Tunis-Belvédère, Tunisia
[b] Laboratory of Transmission, Control and Immunobiology of Infections (LR11IPT02), Institut Pasteur de Tunis, 13, Place Pasteur, BP 74 1002, Tunis-Belvédère, Tunisia
[c] Faculty of Medicine of Tunis, University Tunis El Manar, Tunis, Tunisia
[d] Department of Family and Community Medicine, College of Medicine and Medical Sciences (CMMS), Arabian Gulf University (AGU), Manama, Bahrain

ARTICLE INFO

Keywords:
Ctenodactylus gundi
Leishmania major
Leishmania tropica
Rodent reservoir
Cutaneous leishmaniasis
Tunisia

ABSTRACT

Incriminating new rodent species, as reservoir hosts of *Leishmania* parasites is crucial for understanding the transmission cycle of cutaneous leishmaniasis in Tunisia. *Ctenodactylus* (*C.*) *gundi* was previously described as extremely abundant in all Tunisian *Leishmania* (*L.*) *tropica* foci in south Tunisia besides its presence in *L. major* endemic area. The aim of this study was to detect *Leishmania* species parasites among *C. gundi* in two endemic regions in Tunisia: Sidi Bouzid and Tataouine.

Total DNA was isolated from the spleens and the livers of 92*C. gundi*. Leishmaniasis clinical manifestations were detected among 11 rodents (12%). *Leishmania* parasites were detected in 30 (32.6%) rodents using direct exam method. *Leishmania* DNA was detected in 40 (43.5%) *C. gundi* by combining results among spleens and livers using ITS1-PCR. Positive samples were confirmed to be *L. major* except for only one specimen which was *L. tropica*.

These results demonstrated, for the first time, the high natural infection rate of *C. gundi* with *L. major* parasites in Tunisia. Hence, *C. gundi* should be considered as potential reservoir host of *Leishmania* parasites causing cutaneous leishmaniasis in Tunisia.

1. Introduction

Cutaneous leishmaniasis (CL), caused by three species of *Leishmania* parasites: *Leishmania* (*L.*) *major*, *L. tropica* and *L. infantum*, represent the most important and endemic disease in Tunisia (Ben-Ismail and Ben Rachid, 1989).

Leishmania major with the single zymodeme MON-25 is the principal agent causing zoonotic CL in Tunisia with more than 90% of the registered cases of leishmaniasis (Aoun and Bouratbine, 2014). Since 2002, zoonotic CL caused by *L. major* was endemic into the whole center and southern parts of the country, and occurs in 15 out of the 24 governorates (Salah et al., 2007). The cyclic epidemics of the disease depend on climatic factors (Toumi et al., 2012) with an annual incidence ranges from 2000 to 10000 cases (Bettaieb et al., 2014). This incidence presents a close spatial association with the abundance of *Phlebotomus papatasi*, the proven vector (Chelbi et al., 2009). However, *L. major* has been recently detected from other species of phlebotomine sand flies in Tunisia: *Sergentomyia minuta* (Jaouadi et al., 2015) and *Sergentomyia clydei* (Ayari et al., 2016). Wild rodents such as *Psammomys* (*P.*) *obesus*, *Meriones* (*M.*) *shawi* and *Meriones libycus* were incriminated as the reservoir hosts of *L. major* parasites (Ben-Ismail et al., 1987a; Ben-Ismail et al., 1987b; Ben-Ismail et al., 1989; Fichet-Calvet et al., 2003; Ghawar et al., 2011b). However, these parasites were recently detected in other small mammals such as *Mustela nivalis* and *Atelerix algirus*; but their role as reservoir hosts was not confirmed (Chemkhi et al., 2015; Ghawar et al., 2011a).

Sidi Bouzid governorate, in central Tunisia, is the most important foci of this disease with 25–30% of the Zoonotic CL reported cases annually (Salah et al., 2007).

Chronic CL caused by *L. killicki* (belonging to the *L. tropica* complex) (Chaara et al., 2015; Pratlong et al., 2009) and transmitted by *Phlebotomus sergenti* (Tabbabi et al., 2011a) was usually recognized as an anthroponotic infection. This disease was firstly described in Tataouine governorate, in south Tunisia and form mixed zones with the zoonotic CL (Aoun et al., 2015). Recently, *L. tropica* parasites were detected, using molecular tools among small samples of wild animals such as: *Ctenodactylus* (*C.*) *gundi*, Rock Hyraxes (*Procavia capensis*), Golden Jackals (*Canis aureus*) and Red Foxes (*Vulpes vulpes*) around the Old

* Corresponding author at: Department of Medical Epidemiology, Pasteur Institute of Tunis, 13, Place Pasteur, BP 74 1002, Tunis – Belvédère, Tunisia.
E-mail addresses: ghawarwissemmed@yahoo.fr (W. Ghawar), bettaiebjihene@yahoo.fr (J. Bettaieb), sadok-salem@live.fr (S. Salem), snoussidali@yahoo.fr (M.-A. Snoussi), kaouther.jaouadi@pasteur.tn (K. Jaouadi), rihabyazidi@yahoo.fr (R. Yazidi), afif.bensalah@pasteur.rns.tn (A. Ben-Salah).

http://dx.doi.org/10.1016/j.actatropica.2017.09.022
Received 6 January 2017; Received in revised form 6 May 2017; Accepted 25 September 2017
Available online 28 September 2017
0001-706X/

World (Bousslimi et al., 2012; Jaouadi et al., 2011; Talmi-Frank et al., 2010a; Talmi-Frank et al., 2010b). The implication of these animals in the sylvatic cycle of *L. tropica* may support the presence of a zoonotic transmission cycle and could influence disease emergence. In Tunisia, the North African rock-dwelling rodent: *C. gundi* habits in the mountainous area of Tataouine (the original focus of *L. tropica*) as well as in all emerging Tunisian foci of CL caused by *L. tropica* (Ben-Ismail and Ben Rachid, 1989; Bouratbine et al., 2005; Haouas et al., 2005). Thus supports the implication of this rodent species as possible reservoir in the *L. tropica* life cycle in Tunisia.

The main purpose of the present study was to investigate the different species involved in the natural *Leishmania* infection of *C. gundi* collected from two CL endemic regions in Tunisia: Sidi Bouzid and Tataouine.

2. Materials and methods

2.1. Study site and rodent trapping

Rodents were trapped from three study sites in Sidi Bouzid and Tataouine regions, in central and south Tunisia, respectively. *Ctenodactylus gundi* were trapped in Khbina (average altitude 231 m; N35 1215.96 E9 42.3824) in Sidi Bouzid Governorate; Guermessa (average altitude 297 m; N32 5931.56 E10 15.138) and Mdhila (average altitude 374 m; N33 0238.46 E10 22.1422) in Tataouine Governorate. *Ctenodactylus gundi* were abundant in the rocky-mountains of the three study sites in addition to the presence of *P. obesus* and *M. shawi* as previously described for Khbina (Sidi Bouzid) (Ghawar et al., 2011b).

Trappings were done by placing pincer traps recovered by sands, in areas where rodents have been spotted by vision or by the presence of their wastes. Three expeditions in which we have realized eight field trips (one week for each one) were realized: two in March 2010 in which rodent from Sidi Bouzid were collected; two between May and July 2011, and four between April and May 2013 in which rodents from Tataouine were captured with an equal alternation between the two study sites.

2.2. Clinical manifestations of Leishmania

Each captured rodent was identified, sexed, and searched for cutaneous lesions in the different parts of the body. Clinical signs of *Leishmania* infection were assessed through skin examination. Signs included depilation, hyper-pigmentation of the higher edge of the ear, the presence of small nodules and/or partial destruction of organs.

2.3. Detection of Leishmania parasites by parasitological methods

Each captured rodent was anesthetized by Ether, weighted to the nearest 0.1 g on a pan balance, and then sacrificed by cardiac exsanguination without producing any stress to the animal. External measurements (the head, the body, the tail, the hind feet, and the ear) were recorded. Samples of heart, liver, kidney, spleen, lung and brain were taken, then frozen, and stored in liquid nitrogen for molecular analysis. Both ears of each rodent caught were removed and macerated together in physiological saline. A subsample of the produced solution was smeared in a slide, stained by May-Grunwald-Giemsa, and observed at 1000X for direct examination of *Leishmania* amastigotes. The rest of the solution was placed in Novy-MacNeal-Nicolle (NNN) medium for parasites culture (Ghawar et al., 2011b).

2.4. DNA extraction and parasite detection by PCR-Restriction fragment length polymorphism (RFLP)

DNA from each rodent spleen and liver was extracted using the QIAamp® DNA Mini Kit (QIAgen, Germany).

The presence of the *Leishmania* DNA was tested by targeting the ribosomal internal transcribed spacer 1 (ITS1) using the primers LITSR and L5.8S followed by a Restriction Fragment Length Polymorphism (RFLP) analysis. ITS1-PCR products were digested by *Hae*III restriction enzyme using previously described protocol (Schonian et al., 2003). The following references strains were used as positive controls: *L. major* MON-25 MHOM/TN2009/S600, *L. killicki* MON-8 MHOM/TN/2011/MX and *L. infantum* MON-1 MHOM/TN/94/LV50.

2.5. Data analysis

Chi2 as well as Fisher exact tests permitted to compare association of categorical variables. STATA software version11 was used to carry out all statistical analysis (Stata Corporation).

2.6. Ethical statement

All animal experimentations comply with institutional, national and international guidelines.

The study and rodents handling protocols were approved by the ethics committee at Pasteur Institute of Tunis (5P50AI074178-04).

3. Results

A total of 92*C. gundi* were collected. Twenty of them (21.7%) were captured from Khbina in Sidi Bouzid governorate in 2010 and 72 (78.3%) were captured from Tataouine governorate in 2011 and 2013 subdivided into two sites: 21 (22.8%) from Guermessa and 51 (55.4%) from Mdhila.

Among the captured rodents, we found 45 (48.9%) males and 47 (51.1%) females.

3.1. Clinical manifestations

The clinical examination of all captured rodents showed various aspects of skin lesions. When we consider any clinical sign among the whole study sample (n = 92), disease was detected among 11 rodents (12%). Depilation, small nodules and hyper-pigmentation were the clinical signs observed in both ears (72.72%; n = 8) and the back (36.36%; n = 4) (Fig. 1) with a specimen showing a combined infection in these parts of body.

3.2. Infection prevalence

Leishmania infection prevalence among *C. gundi* was 32.6% (n = 30) using the direct examination of ears homogenates (Fig. 2) and 43.5% (n = 40) by combining result for both tested organs using the ITS1-PCR method. Unfortunately, we were unable to isolate *Leishmania* strains from these rodents by culture method due to contamination.

The overall infection rate was 57.6% (n = 53), by combining the results of Giemsa stained smears and ITS1-PCR.

Table 1 details the distribution of the infection rate and the identified positive samples among *C. gundi* according to the used diagnostic method by sampling site location.

The proportion of *C. gundi* with leishmaniasis lesions among those who showed a positive sample by any diagnostic method (direct exam and/or ITS1-PCR) was 13.2% (7/53). Thus, asymptomatic infection among positive samples was 86.8% (46/53). Moreover, five specimens were positives by the two used diagnostic methods and showing a clinical manifestation. Relationship between positivity rates determined by each diagnostic method and the clinical exam is presented in Fig. 3.

Additionally, *Leishmania* infection, determined by the molecular method, varied among *C. gundi* according to the tested organs. In fact, *Leishmania* DNA were detected in 25 versus 17 out of 92*C. gundi*, yielding a positivity rate of 27.2% versus 18.5% (p = NS) among