Acta Tropica 119 (2011) 131–137

Contents lists available at ScienceDirect

Acta Tropica

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

Habitats of the sandfly vectors of *Leishmania tropica* and *L. major* in a mixed focus of cutaneous leishmaniasis in southeast Tunisia

Ahmed Tabbabi[a,b], Jamila Ghrab[a], Karim Aoun[a], Paul Donald Ready[b], Aïda Bouratbine[a,*]

[a] *Laboratoire de Parasitologie, LR 05SP03, Institut Pasteur de Tunis, 13 place Pasteur, 1002 Tunis, Tunisia*
[b] *Molecular Biology Laboratory, Department of Entomology, Natural History Museum, London, UK*

ARTICLE INFO

Article history:
Received 11 January 2011
Received in revised form 2 May 2011
Accepted 4 May 2011
Available online 13 May 2011

Keywords:
Phlebotomus sergenti
Phlebotomus riouxi
Cutaneous leishmaniasis
Leishmania tropica
Leishmania major
Tunisian habitats

ABSTRACT

From 2009 to 2010, 3129 sandflies were caught in CDC light traps placed in various habitats in Ghomrassen, Tataouine governorate, southeast Tunisia, a mixed focus of human cutaneous leishmaniasis caused by *Leishmania tropica* and *Leishmania major*. Species diversity was quantified in anthropogenic, semi-anthropogenic and semi-natural locations. Sandflies were identified according to morphological characters and also by the comparative sequence analysis of a fragment of the mitochondrial cytochrome b gene to distinguish between two putative local vectors of *L. tropica*, namely *Phlebotomus chabaudi* and *Phlebotomus riouxi*. The lowest sandfly diversities were found in *L. major* sites, where the incriminated vector *P. papatasi* predominated in the burrows of the rodent reservoir hosts (*Meriones*) as well as inside and outside houses of human cases. In *L. tropica* sites, the incriminated peri-domestic vector *Phlebotomus sergenti* was the most abundant species inside houses, whereas *P. riouxi* or *P. chabaudi* was the dominant species in the semi-natural rocky habitats favoured by the putative rodent reservoir, *Ctenodactylus gundi*. All specimens of *P. chabaudi* identified molecularly had the diagnostic cytochrome b characters of *P. riouxi*, indicating either that the latter represents only a geographical variant of *P. chabaudi* or that these two species may sometimes hybridize.

1. Introduction

In the Tataouine governorate, southeast Tunisia, *Leishmania tropica* and *Leishmania major* are the causative agents of cutaneous leishmaniasis (CL) (Bousslimi et al., 2010). CL caused by *L. tropica* is hypo-endemic and prevails in communities living in villages built on the flanks of the arid mountains of Tataouine (Bousslimi et al., 2010). Cases of CL caused by *L. major* probably emerged recently in this area as a result of susceptible populations settling in new neighborhoods at the margin of some villages (Bousslimi et al., 2010).

Among zymodemes attributable to the polymorphic *L. tropica* complex, *L. tropica* MON8 syn *Leishmania killicki* was found to be endemic in Tataouine region (Bousslimi et al., 2010). Recently, all 35 *L. tropica* zymodemes, the *L. killicki* zymodeme, and a new zymodeme related to *L. killicki* (MON-301 isolated in Algeria) have been considered as belonging to a single broad *L. tropica* complex. However, in this complex of four clusters, cluster A (including *L. killicki* parasites) is the most distant from the others (Pratlong et al., 2009). This fact may explain some regional features of the epidemiology of CL in North Africa.

Leishmania tropica is commonly stated to be anthroponotic (WHO, 1984). However, the relative paucity of CL cases in Tataouine region and their spatial distribution suggests that it might be a zoonosis (Ben Ismail and Ben Rachid, 1989). The putative reservoir host is the North African gundi (*Ctenodactylus gundi*) which is extremely abundant in the mountainous area of Tataouine (Ben Ismail and Ben Rachid, 1989). This wild rodent is also found in all emerging Tunisian foci of CL caused by *L. tropica*, which underlines its potential reservoir role (Bouratbine et al., 2005). *Phlebotomus (Paraphlebotomus) sergenti* is the confirmed vector of *L. tropica* in Morocco and throughout the Middle East and Central Asia (Al-Zahrani et al., 1988; Guilvard et al., 1991; Killick-Kendrick, 1990), and this phlebotomine sandfly (Diptera, Psychodidae) has been incriminated as a vector in southeast Tunisia (Tabbabi et al., personal communication). However, *P. (Paraphlebotomus) chabaudi* and the closely related species *Phlebotomus (Pa.) riouxi* were also suspected as vectors, based on their abundance in Tunisian and Algerian foci of CL caused by *L. tropica* (Bounamous et al., 1998).

Phlebotomus (Phlebotomus) papatasi is the confirmed vector of *L. major* in southwest Asia and North Africa (Killick-Kendrick, 1990), including central and southern Tunisia where the gerbil reservoir hosts are widespread (Ben Ismail et al., 1987; Ben Ismail and Ben Rachid, 1989).

The aim of the current study is to contrast the habitats of vectors and putative vectors in the mixed CL focus in southeast Tunisia,

* Corresponding author. Tel.: +216 71 890 827.
E-mail address: aida.bouratbine@pasteur.rns.tn (A. Bouratbine).

0001-706X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2011.05.002

in order to help assess the epidemiological roles of each sandfly species. The approach involves quantifying sandfly diversity.

2. Materials and methods

2.1. Study area

The study was carried out in the village of Ghomrassen and its surroundings, located in the Governorate of Tataouine, southeast Tunisia (Fig. 1). This well-known focus of CL caused by *L. tropica* is situated in a mountainous area at moderate altitude (c. 300 m above sea level) with an arid bioclimate (Gounot and Le Houerou, 1985). The average temperature is 22 °C and annual rainfall varies between 88 and 157 mm (data available from Tunisian Institute of Meteorology). A basalt rock escarpment determines the regional landscape. Sparse vegetation is dominated by steppe species. The area is pastoral with olive groves and cereals cultivated around water points. *C. gundi* is the most prevalent wild rodent living in the stony mountains. However, gerbils (*Meriones* species) are also found at the periphery of the village. During October 2008–September 2009, 5 *L. tropica* and 24 *L. major* CL cases were identified in specific households inside the village and in the marginal new neighborhoods, respectively (Bousslimi et al., 2010).

2.2. Sample collection

Captures of adult sandflies were made in the two summer seasons (July–September) of 2009 (147 trap-nights in July and 97 trap-nights in September) and 2010 (28, 32 and 102 trap-nights per month), by setting CDC miniature light traps (John W. Hock, USA) in anthropogenic, semi-anthropogenic and semi-natural sites situated at c. 300 m a.s.l (Fig. 1). Light traps were set before sunset and collected the following morning.

Captures were made in *L. major* sites around *L. major* CL cases (Fig. 1). Houses of *L. major* CL cases were located in marginal new neighborhoods at c. 305 m a.s.l. surrounded by cultivated vegetation. Sandflies were collected indoors and outdoors in human resting places and in animal sheds. In semi-anthropogenic environments, light traps were placed inside burrows inhabited by *Meriones* (Fig. 1).

Captures were also made in *L. tropica* sites (Fig. 1). Sites characterized as anthropogenic were houses of *L. tropica* CL cases. Sandflies were collected indoors and outdoors in human resting places and in animal sheds. Houses of *L. tropica* CL cases were built in the rocky mountainside surrounding the village at c. 288 m a.s.l. and the immediate habitat also contained flood-protection barriers ("Tabia") and some date palm trees. In this semi-anthropogenic environment, light traps were placed inside and near the entrances of caves and crevices inhabited by *C. gundi* at c. 324 m a.s.l. The semi-natural site was 6 km from the village, in the rocky mountain inhabited by *C. gundi* at c. 310 m a.s.l. (Fig. 1), where traps were placed inside and near the rocky habitats of this wild rodent. It was more abundant in the semi-natural site than in the semi-anthropogenic sites around houses.

2.3. Species identification

Sandflies were identified according to morphological characters described by Croset et al. (1978), Léger et al. (1983) and Boussaa et al. (2008). For distinguishing between *P. riouxi* and *P. chabaudi*, heads and genitalia of individual sandflies were removed, mounted on microscope slides and identified using the morphological characters of Depaquit et al. (1998) and Bounamous et al. (2008). The form of the male aedeagus is sharply pointed in *P. chabaudi* but bevelled in *P. riouxi*, and the basal process of the male coxite is smaller and less tufted in *P. chabaudi* than in *P. riouxi* (Depaquit et al., 1998). It was not possible to distinguish the females of *P. chabaudi* from those of *P. riouxi* on the basis of their spermathecae. The presence of antero-lateral teeth of the pharyngeal armature in *P. chabaudi*, never observed in *P. riouxi*, was used as a differential criterion (Bounamous et al., 2008).

The dissected thorax and attached anterior abdomen was used for the molecular typing of 6 *P. chabaudi* and 15 *P. riouxi* specimens. DNA extraction was performed as described by Esseghir et al. (1997). Amplifications targeted a mitochondrial cytochrome b gene fragment using CB3-PDR/N1N-PDR primers as previously described (Esseghir et al., 1997). PCR products were purified using Geneclean II, Bio 101 Inc., and cycle-sequenced using an ABI Prism® Big Dye™ Terminator, Cycle Sequencing Ready Reaction Kit and AB1 3130 sequencing system (ABI, PE Applied Biosystems), with the same primers used for PCR. DNA sequences from both strands were aligned and edited by eye using Sequencher™ 3.1.1 software (Gene Codes Corporation). Multiple sequence alignments were then assembled using CLUSTAL W Multiple Sequence Alignment Program using default parameters. Phylogenetic analyses were performed using PAUP software (Swofford, 2002). Relationships were inferred based on genetic distances using the Neighbor Joining (NJ) option with default settings, and with a homologous sequence from a Moroccan specimen of *P. sergenti* as the least ambiguous outgroup.

2.4. Biological diversity analysis

To assess the diversity of sandfly populations in different sites the following parameters and indices were used (Spellerberg and Fedor, 2003):

- Specific richness (S), which is the number of species present in the habitat;
- Relative abundance, which is the proportion of individuals among the species (p_i);
- Simpson's diversity index (D), which is based on both the number of species and the proportional abundance of species.

D is calculated as follows: the proportion of species i relative to the total number of species (p_i) is calculated and squared. The squared proportions for all the species are summed, and the reciprocal is taken:

$$D = \frac{1}{\sum_{i=1}^{S} pi^2}$$

The lowest possible value of this index is 1, which represents a community containing only one species. The higher the value, the greater is the diversity. The maximum value is the number of species in the sample.

Equitability (E_D) was calculated by taking D and expressing it as a proportion of the maximum value D could assume if individuals in the community were completely evenly distributed (D_{max}, which equals S when there is one individual per species). Equitability takes a value between 0 and 1, with 1 being complete evenness.

$$E_D = \frac{D}{D_{max}} = \frac{1}{\sum_{i=1}^{S} pi^2} \times \frac{1}{S}$$

3. Results

A total of 406 traps-nights produced a total of 3129 sandflies (2010 males and 1119 females). According to morphological identification, 11 species were recorded in the area: *P. (P.) papatasi*, *P. (Pa.) sergenti*, *P. (Pa.) riouxi*, *P. (Pa.) chabaudi*, *P. (Pa.) alexandri*, *P. (Larroussius) perniciosus*, *P. (La.) longicuspis*, *Sergentomyia (Sergentomyia) fallax*, *S. (S.) minuta*, *S. (S.) antennata* and *S. (S.) christophersi*.