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Original article

Phlebotomus sergenti (Parrot, 1917) identified as *Leishmania killicki* host in Ghardaïa, south Algeria

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Abstract

Since 2005, an outbreak of human cutaneous leishmaniasis (CL) in Ghardaïa, south Algeria, was studied and one output of these investigations was the identification of two *Leishmania* species, *Leishmania major* and *Leishmania killicki*, as the CL causative agents. In the present study, we were curious to focus on sand fly fauna present in this area and detection of *Leishmania*-positive sand fly females. Sand flies (3717) were collected during two seasons using sticky papers and CDC light traps in urban, rural and sylvatic sites. Twelve *Phlebotomus* species were identified. *Phlebotomus papatasi* was dominant in the urban site while *Phlebotomus sergenti* and *Phlebotomus riouxi/chabaudi* were dominant in the sylvatic site. Out of 74 *P. sergenti* females captured by CDC light traps in the sylvatic site populated by Ghardaïas' Gundi (*Massoutiera mzabi*), three ones were hosting *Leishmania* promastigotes. PCR-RFLP and sequencing of seven single-copy coding DNA sequences identified the promastigotes as *L. killicki*. Furthermore, laboratory experiments revealed that *L. killicki* isolate sampled from a CL patient inhabiting the studied region develop well in *P. sergenti* females. Our findings strongly suggest that the human cutaneous leishmaniases caused by *L. killicki* is a zoonotic disease with *P. sergenti* sand flies acting as hosts and vectors and gundi rodents as reservoirs.

Keywords: Cutaneous leishmaniasis; *Leishmania tropica killicki*; *Phlebotomus sergenti*; Ghardaïa; Algeria

1. Introduction

In Algeria, leishmaniases occur in two clinical forms: visceral leishmaniasis (VL) caused by *Leishmania infantum* and cutaneous leishmaniasis (CL) caused by three *Leishmania* species: *L. infantum, Leishmania killicki* and *Leishmania major* which is the most widespread one [1]. During 2005, almost thirty thousands (30,000) CL cases were reported in the country, the province of Ghardaïa being particularly affected with more than two thousands cases recorded. In this region, the disease is caused by either *L. killicki* or *L. major*, which coexist sympatrically [2].

Recently, *L. killicki* spread was reported from central and south-western areas of Tunisia, e.g. Metlaoui and Ain Jloula [3–5], and *L. killicki* parasites were isolated from patients also in Kenya, Yemen [6] and Libya [7]. Apparent increase of the geographic distribution of *L. killicki* may correspond with historical difficulties in distinguishing between *L. major* and *Leishmania tropica* complex which coupled with a low disease burden may have masked the presence of the parasite from past discovery [8].

CL caused by *L. major* is a zoonosis with rodents serving as reservoir hosts. In Algeria, *L. major* is transmitted by *Phlebotomus papatasi*, gerbils *Psammomys obesus* and *Meriones*

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shawi were proven as reservoirs [9,10]. On the other hand, transmission cycle of *L. killicki* is still unknown.

L. killicki (Rioux, Lanotte and Pratlong 1986) was first described in Tataouine, southeast Tunisia by Rioux et al. (1986) [12] and in the same year included in the *L. tropica* complex. Species denoted as *L. tropica* are highly heterogeneous [13], strains are readily distinguishable by antigenic, biochemical and molecular techniques [14], nevertheless, the position of *L. killicki* as a separate species is still questionable. Based on the similarity with *L. tropica*, Rioux et al. (1986c) [15] first mentioned *Phlebotomus sergenti* as a suspected vector of *L. killicki*. However, since its description in Algeria in 1917 by Parrot [16], *P. sergenti* was never found in this country infected by promastigotes and neither was any other sand fly species belonging to subgenus *Paraphlebotomus*.

CL caused by *L. tropica* complex is usually regarded as an anthroponosis occurring in hyperendemic situations where man-to-man transmission is well ascertained. *L. tropica* parasites were, however, isolated from black rats, dogs and rock hyraxes (*Procavia capensis*) [13,17–19] and recently new zoonotic foci of *L. tropica* are emerging in different parts of the Mediterranean countries [19], including Tunisia and Algeria [2]. The location of sporadic human cases in the province of Ghardaïa also suggests zoonotic transmission.

In the present study, we proved *P. sergenti* as a competent vector of *L. killicki*. We found natural infection in *P. sergenti* captured in the neighborhood of the Mzab Gundi's (*Massoutiera mzabi*) burrows with *L. killicki* and experimentally confirmed susceptibility of *P. sergenti* to *L. killicki*.

2. Materials and methods

2.1. Study area

Localization of Ghardaïa (32°30′/32°41′N; 03°37′/03°42′E) and description of the region was presented by Harrat et al. (2009) [2]. For the entomological investigations, three sites with different biotopes were prospected in respect to the incidence of human cases, population density and abundance of wild rodents. **Urban** site was located in Ksar (Castle) of Ghardaïa (32°29′N; 03°42′E), one of the five cities of Ghardaïa province, where high number of CL cases was reported during the outbreak in 2005 [20]. This site is characterized by a high concentration of houses and inhabitants, some of them breeding goats, chickens and donkeys. **Rural** site was located in the Oases of Ghardaïa named Ghaba (32°29′N; 03°39′E) where inhabitants of urban sites move during hot summer season to rest in their second homes dispersed in the palm plantations. **Sylvatic** site consists of natural caves in the rocky hill of Chaabat Telli (32°48′N; 03°63′E), located in the periphery of Ghardaïa's valley. These caves provide shelters for the Mzab Gundi (*M. mzabi*), a rodent frequently encountered in this area.

2.2. Sand fly collection, dissection and identification

Samplings were performed in 2008 and 2009, twice a month from April to November, using sticky traps and four CDC light traps (John W. Hock, Gainesville, FL). Sticky traps were placed in each site, near human dwellings, in front of rodents' burrows and inside the caves. Captured flies were stored in 70% alcohol and most of them subsequently treated by KOH 20% and embedded in Marc André medium for species identification using keys of Abonnenc (1972) [21], Dedet (1984) [22] and Depaquit (1998) [23].

Female sand flies caught by CDC light traps in sylvatic site in October 2009 were washed in 70% ethanol and dissected in sterile saline. For species identification, the head and the posterior part of the abdomen of each sand fly were mounted in a drop of sterile saline and the gut was examined for the presence of promastigotes under microscope. Upon microscopical detection of *Leishmania* infection, the material from the slide was divided into two parts. The first part of each gut containing promastigotes was aseptically inoculated into NNN medium (9‰ sterile saline; 50 μg/ml streptomycin; 100.000 UI penicillin; 1.000 μg/ml Nystatine). The second part of parasite suspensions was placed in duplicate wells containing a drop of Tris-EDTA buffer for DNA extraction and *Leishmania* identification as a backup in case of unsuccessful cultivation.

In some specimens belonging to *Phlebotomus riouxi/chabaudi*, the thorax was preserved in alcohol and used for DNA extraction and molecular identification based on PCR-RFLP and sequencing of the cytochrome *c* oxidase I (COI) gene (see below).

2.3. DNA extraction, Leishmania typing and P. riouxi/chabaudi identification

Extractions of total DNA from the material preserved in Tris-EDTA buffer (parasites from naturally infected sand flies) and in ethanol (sand fly thorax) were performed using a DNA extraction kit (Roche, France) according to the manufacturer's instruction.

Samples containing *Leishmania* parasites from naturally infected sand flies were analyzed by the sequencing of seven single-copy coding DNA sequences (Multi Locus Sequence Typing) located on six different chromosomes [C. Ravel, unpublished]. Obtained sequences (GenBank accession number Ph3:HM135420–HM135426; LEM4995: HM135427–HM135433; Ph13:HM135434–HM135440) were concatenated (4839 bp in all) and compared to 22 *L. tropica* complex strains and one *L. major* strain as an out-group (Table 1). Analysis was performed using PhyML 3.0 software under GTR substitution model and 1000 bootstraps.

The molecular identification of specimens belonging to sibling species *P. riouxi/chabaudi* was based on PCR-restriction fragment length polymorphism (PCR-RFLP) and on direct sequencing of a 689 bp variable region of COI gene as described by Boudabous et al. (2009) [24].

2.4. Development of L. killicki in P. sergenti

P. sergenti colony originating from females collected in Amnun, Northern Israel in 2001 was maintained in conditions described by Benkova and Volf (2007) [25]. Female sand flies

Table 1
Geographical origin and host of the 22 *Leishmania tropica* s.s. and one *L. major* (LEM0062) strains.

Strain	International code	Origin	Host
LEM0163	MHOM/TN/80/LEM163	Tunisia	*Homo sapiens*
LEM4018	MHOM/TN/2000/26LC	Tunisia	*Homo sapiens*
LEM3987	MHOM/TN/2000/000a	Tunisia	*Homo sapiens*
LEM4995	MHOM/DZ/2005/LIPA07	Algeria	*Homo sapiens*
LEM1015	MHOM/YE/86/LEM1015	Yemen	*Homo sapiens*
LEM0955	MHOM/YE/86/LEM955	Yemen	*Homo sapiens*
LEM3956	MHOM/IL/96/LRC-L691	Israel	*Homo sapiens*
LEM2001	MHOM/EG/90/LPN65	Egypt	*Homo sapiens*
LEM3322	MHOM/JO/96/JH-88	Jordan	*Homo sapiens*
LEM1824	MHOM/KE/86/EB103	Kenya	*Homo sapiens*
LEM2454	MHOM/KE/92/EB000	Kenya	*Homo sapiens*
LEM2313	IGUG/KE/91/000	Kenya	*Phlebotomus guggisbergi*
LEM1828	ISER/MA/89/LEM1828	Morocco	*Phlebotomus sergenti*
LEM1694	ISER/MA/89/LEM1694	Morocco	*Phlebotomus sergenti*
LEM1452	MHOM/MA/88/LEM1452	Morocco	*Homo sapiens*
LEM0588	MHOM/GR/82/SER-L60	Greece	*Homo sapiens*
LEM1904	MHOM/GR/88/LA615	Greece	*Homo sapiens*
LEM3919	MHOM/TR/99/LSL43	Turkey	*Homo sapiens*
LEM1451	MHOM/MA/88/LEM1451	Morocco	*Homo sapiens*
LEM1314	MHOM/MA/88/LEM1314	Morocco	*Homo sapiens*
LEM0617	MHOM/IL/80/SINGER	Israel	*Homo sapiens*
LEM2869	MHOM/JO/93/JH67	Jordan	*Homo sapiens*
LEM0062	MHOM/YE/76/LEM62	Yemen	*Homo sapiens*

were infected by feeding through a chick-skin membrane on heat-inactivated rabbit blood containing 10^6/ml 5-day-old promastigotes of *L. killicki* (MHOM/DZ/2004/LIPA11 isolated from patient). Blood-engorged females were separated and maintained at a constant temperature of 25 °C with a free access to 50% honey as a sugar source. On days 2, 7 and 10 after the blood meal, females were dissected and their guts were checked microscopically for the presence and location of *Leishmania* promastigotes. The parasite density was graded according to accepted criteria [26], i.e. <100, 100–1000 and >1000 parasites/gut were graded as weak, medium and heavy infection, respectively.

3. Results

3.1. Sand fly fauna

In Ghardaïa focus, 12 sand fly species were found, seven belonging to genus *Phlebotomus* and five to genus *Sergentomyia*. In total, 3.717 sand flies were collected during two seasons. List of species and their abundance is given in Table 2. *Phlebotomus* (*Phlebotomus*) *papatasi* (Scopoli) and *P.* (*Paraphlebotomus*) *sergenti* (Parrot) were prevalent; in the whole focus they represent 89.7% of catches of genus *Phlebotomus* (63.2% and 26.5%, respectively). Among genus *Sergentomyia*, *Sergentomyia minuta* and *Sergentomyia fallax* were the most numerous (63.8% and 33.5%, respectively).

Transect line through Ghardaïa's valley included three different catching sites: urban, rural and sylvatic ones. In urban sites, *P. papatasi* was the only *Phlebotomus* species caught (Fig. 1). On the other hand, the majority of *P. sergenti* (99.2%) was caught in the wild site in the vicinity of gundi's burrows and only 0.8% was collected indoor in the rural site. As expected, sand fly fauna in rural and sylvatic sites was more diverse than in the urban site. While in the rural site *P. papatasi* was highly predominant and other five *Phlebotomus* species occurred in densities less than 1%, in the sylvatic site three dominant species were found: *P. papatasi*, *P. sergenti* and *P. riouxi/chabaudi* (Fig. 1).

Morphological examination of *P. riouxi/chabaudi* did not result in unambiguous identification. Phylogenetic and PCR-RFLP analysis of COI gene revealed that all nine studied samples from Ghardaïa's valley belong to *P. riouxi* and according to sequence analysis they compose an Algerian clade (data not shown).

3.2. Leishmania-infected flies and parasite typing

In total, 325 sand fly females captured in the wild site in natural caves of Chaabat Telli hill were dissected. Three out of 74 dissected females (4%) of *P. sergenti* (Ph 3, Ph 13 and Ph 30) were found infected by *Leishmania* promastigotes (Table 2). Those three females positive for leishmania parasites

Table 2
Phlebotomine sand flies captured by sticky traps (ST) and CDC light traps in Ghardaïa valley, Algeria.

Species (subgenus)	ST ♂/♀	Sub total	CDC ♂/♀[a]	Sub total	Total (%)	
P. (*Phlebotomus*) *papatasi*	469/113	582	238/329 (0/140)	567	1149	(30.9)
P. (*Paraphlebotomus*) *sergenti*	87/36	123	270/88 (3/74)	358	481	(12.9)
P. (*Paraph.*) *riouxi/chabaudi*	20/1	21	132/13 (0/11)	145	166	(4.5)
P. (*Paraph.*) *alexandri*			1/3	4	4	(0.1)
P. (*Larroussius*) *longicuspis*	4/0	4	6/4 (0/1)	10	14	(0.4)
P. (*Larroussius*) *perniciosus*	1/0	1	2/0	2	3	(0.1)
P. (*Larroussius*) *langeroni*	1/0	1			1	(0.02)
S. (*Sergentomyia*) *minuta*	632/329	961	146/104 (0/30)	250	1211	(32.6)
S. (*Sergentomyia*) *fallax*	276/146	422	104/110 (0/69)	214	636	(17.1)
S. (*Sergentomyia*) *antennata*	25/5	30	5/4	9	39	(1.1)
S. (*Grassomyia*) *dreyfussi*	0/9	9	0/2	2	11	(0.3)
S. (*Sintonius*) *christophersi*	0/2	2			2	(0.1)
Total	1515/641	2156	904/657 (3/325)	1561	3717	

[a] number of positive females/number of dissected females.

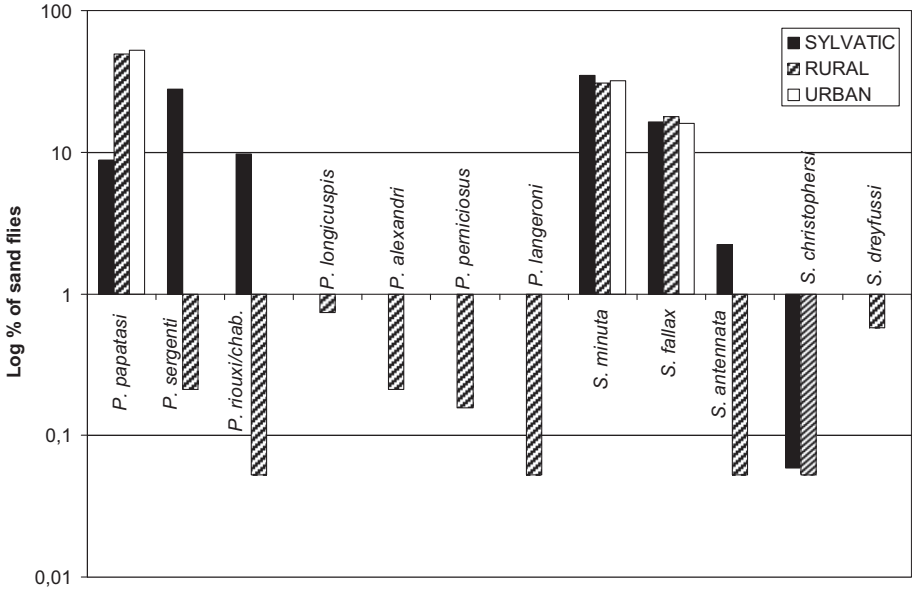

Fig. 1. Relative proportion of all sand fly species (sum of males and females) captured by sticky papers and CDC traps in sylvatic, rural and urban sites during 2008–2009 in Ghardaia, Algeria. (Please note that position of mosquito species labels will be changed – will be set above and below the line.)

were caught in the sylvatic area populated by the gundi. In all three cases, parasites were numerous and the midgut of females did not contain any blood or blood meal remains. In one female (Ph 30), colonization of the stomodeal valve was observed.

Two sequences of *Leishmania* originated from naturally infected *P. sergenti* females (Ph3 and Ph13) are identical and grouped with three *L. killicki* strains sequences (LEM4018, LEM0163 and L3987) (Fig. 2), the characterization of the third isolate (Ph30) was not successful. No leishmania isolate from sand flies was constituted in culture.

3.3. Development of L. killicki *in* P. sergenti

In laboratory experiments, *L. killicki* parasites from Algerian patient (MHOM/DZ/2005/LIPA11) developed well in laboratory-reared *P. sergenti*. After defecation, on days 7 and 10 post blood meal, promastigotes were present in 55%–48% of females, respectively (Fig. 3). Colonization of the stomodeal valve was observed in the 45% of infected females on day 7 and in the 80% of infected females on day 10.

4. Discussion

Twelve sand fly species (seven of genus *Phlebotomus* and five of *Sergentomyia*) collected in Ghardaïa focus represent more than one half of 23 species reported for Algeria [22,23,27]. Even though we report sibling species *P. riouxi/chabaudi*, according to PCR-RFLP and sequencing of nine representatives and based on previous studies focused on the study region of Ghardaïa's valley [24,28], apparently all captured specimens shall belong to *P. riouxi*. Since there was a hypothesis that the vector of *L. killicki* could belong to the subgenus *Paraphlebotomus* [15,29,30], we concentrated our efforts to the collection of infected sand flies in the sylvatic site.

The dominance of *P. papatasi* and *P. sergenti* in our sampling is in agreement with the fact that in the CL foci of Ghardaïa *L. major* and *L. killicki* occur sympatrically [2]. Both species belong to specific vectors by definition of Volf and Myskova (2007) [31]. *Phlebotomus papatasi* supports the development of only *L. major* [32,33], similarly, *P. sergenti* was demonstrated as susceptible to *L. tropica* [19,34,35].

Molecular analysis showed that the DNA of *Leishmania* promatigotes extracted from the two naturally infected *P. sergenti* females (Ph3, Ph 13) presented the same lineage of *L. killicki* (Fig. 2). Isolates from four patients obtained in 2004 belonged to *L. killicki* MON 301 by MLEE (Multi Locus Enzyme Electrophoresis) [2], whereas leishmania DNAs from two *P. sergenti* sand flies (Ph3, Ph 13) captured in 2009 are closely related to *L. killicki* MON 8 by MLST. Unfortunately, no *Leishmania* isolate from sand flies was available in culture for MLEE analysis and used genetic markers (MLST) did not allow differentiating between MON 8 and MON 301.

Although we demonstrated *P. sergenti* naturally hosting *L. killicki*, we cannot exclude the possibility that other species of subgenus *Paraphlebotomus* are involved in transmission of this parasite. In the sylvatic site, *P. (Paraphlebotomus) riouxi* was the second most abundant *Phlebotomus* species present. Susceptibility of this species to *L. killicki* is unknown as, despite significant effort of our group (JV and VD), no laboratory colony is available at the moment.

Around the Mediterranean basin, various populations of *P. sergenti* occur in both types of *L. tropica* foci, sylvatic (e.g. Kenya or North Israel [6,13,19];) as well as urban ones (e.g. South-East Turkey [36];). In Ghardaïa region, *P. sergenti* highly dominated in the sylvatic site. More than 99% *P. sergenti*, including three females infected with *L. killicki*, were caught in rocky places close to the gundis' burrows. This finding suggests that the Algerian CL due to *L. killicki* is a zoonotic disease.

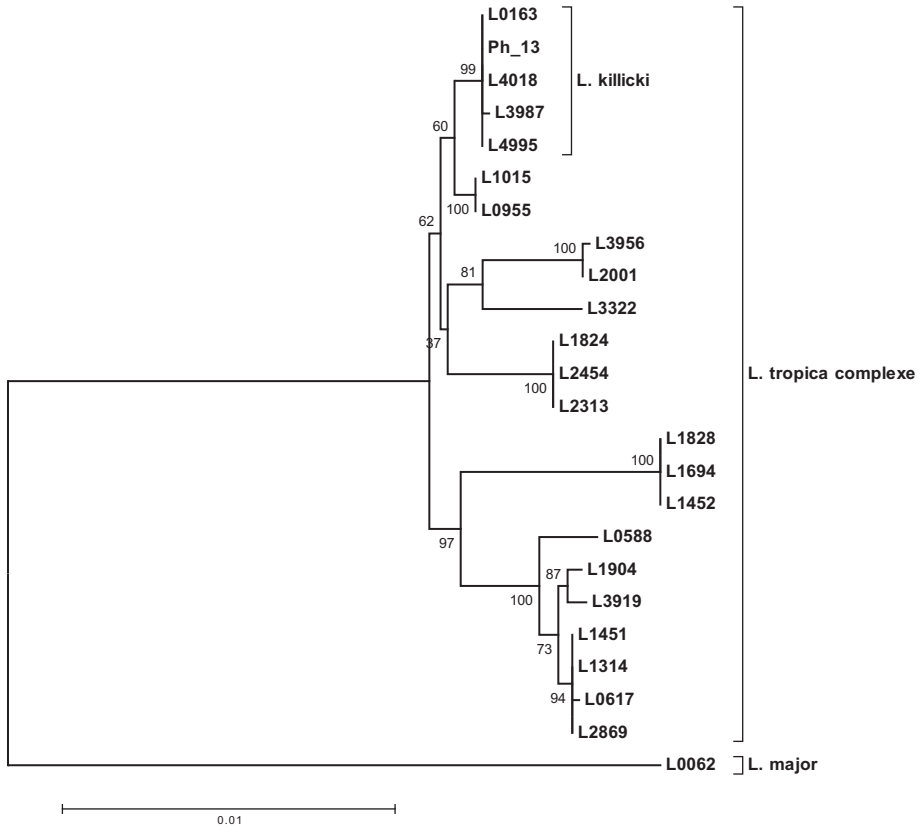

Fig. 2. Phylogenetic analysis (the maximum likelihood tree; GTR model) of 22 strains belonging to the *Leishmania tropica* complex based on sequences of seven single-copy coding DNA sequences. *L. major* LEM62 strain was used as an out-group. Sequences of Ph3 and Ph13 are identical.

The present study brought a crucial knowledge in the comprehension of the epidemiological cycle of the disease and might be also useful for adequate control measures. Next, the effort should be focused to prove the reservoir host of *L. killicki*. In Algeria, highly suspected candidate is Mzab Gundi (*M. mzabi*), the rodent particularly abundant in Ghardaïa area. Similarly, in Tataouine and other *L. killicki* foci in Tunisia, the most abundant mammal is Common Gundi (*Ctenodactylus gundi*) [37]. The search for animals infected by *L. killicki* would then elucidate whether the gundis are only one of potential sources or the sole reservoir of this parasite.

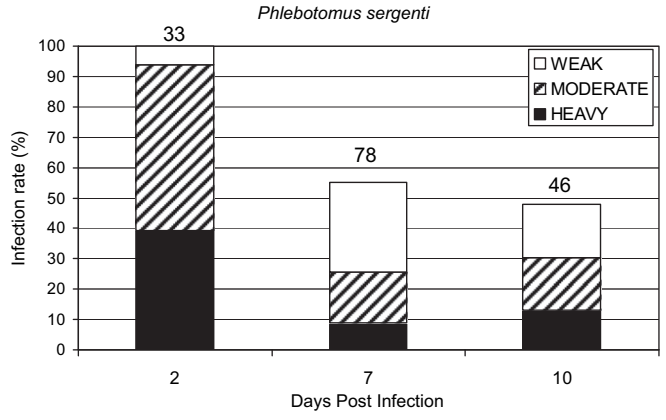

Fig. 3. Development of *Leishmania killicki* (strain LKA1 isolated from patient in 2005; MON 301) in *Phlebotomus sergenti*. Infection rates and density of *Leishmania* parasites in sand fly midguts on days 2, 7 and 10 p.i. were classified into three categories: heavy (more than 1000 promastigotes per gut; black bars), moderate (100–1000; shaded bars), and light (1–100; white bars). Numbers above the bars indicate the total number of dissected females.

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References

[1] Z. Harrat, B. Hamrioui, M. Belkaid, O. Tabet-Derraz, Current point of leishmaniasis epidemiology in Algeria, Bull. Soc. Pathol. Exot. Mar. 88 (1995) 180–184.

[2] Z. Harrat, S.C. Boubidi, F. Pratlong, R. Benikhlef, B. Selt, J.P. Dedet, C. Ravel, M. Belkaid, Description of a dermotropic *Leishmania* close to *L. killicki* (Rioux, Lanotte and Pratlong, 1986) in Algeria, Trans. R. Soc. Trop. Med. Hyg. 103 (2009) 716–720.

[3] A. Bouratbine, K. Aoun, J. Ghrab, Z. Harrat, M.S. Ezzedini, S. Etlijani, Spread of *Leishmania killicki* to central and south-West Tunisia, Parasite 12 (2005) 59–63.

[4] N. Haouas, N. Chergui, E. Chaker, M. Ben Said, H. Babba, S. Belhadj, K. Kallel, F. Pratlong, J.P. Dedet, H. Mezhoud, R. Azziez, Anthroponotic cutaneous leishmaniasis in Tunisia: presence of *Leishmania killicki* outside its original focus of Tataouine, Trans. R. Soc. Trop. Med. Hyg. 99 (2005) 499–501.

[5] K. Aoun, F. Amri, E. Chouihi, N. Haouas, K. Bedoui, R. Benikhlef, J. Ghrab, H. Babba, M.K. Chahed, Z. Harrat, A. Bouratbine, Epidemiology of *Leishmania* (*L.*) *infantum, L. major* and *L. killicki* in Tunisia: results and analysis of the identification of 226 human and canine isolates, Bull. Soc. Pathol. Exot. 101 (2008) 323−328.

[6] D.K. Sang, F. Pratlong, R.W. Ashford, Identity of *Leishmania tropica* in Kenya, Trans. Roy. Soc. Trop. Med. Hyg. 86 (1992) 621−622.

[7] K. Aoun, N. Bousslimi, N. Haouas, H. Babba, A. El-Buni, A. Bouratbine, First report of *Leishmania killicki* Rioux, Lanotte et Pratlong, 1986 in Libya, Parasite 13 (2006) 87−88.

[8] M.G. Shehata, A.M. Samy, S.A. Doha, A.R. Fahmy, R.M. Kaldas, B.D. Furman, J.T. Villinski, First report of *Leishmania tropica* from a classical focus of *L. major* in North-Sinai, Egypt, Am. J. Trop. Med. Hyg. 81 (2009) 213−218.

[9] S. Belazzoug, Isolation of *Leishmania major* Yakimoff, Schokhor, 1914 from *Psammomys obesus* Gretzschmar, 1828 (Rodentia: Gerbillidae) in Algeria, Trans. R. Soc. Trop. Med. Hyg. 77 (1983) 876.

[10] M.A. Izri, S. Belazzoug, F. Pratlong, J.A. Rioux, Isolation of *Leishmania major* in *Phlebotomus papatasi* in Biskra (Algeria). The end of an ecoepidemiological saga, Ann. Parasitol. Hum. Comp. 67 (1992) 31−32.

[12] J.A. Rioux, G. Lanotte, F. Pratlong, *Leishmania killicki* n.sp (*Kinetoplastida: Trypaosomatidae*). *Leishmania.* Taxonomie et phylogenèse. Applications écoépidémiologiques. (Coll. Int. CNRS/ INSERM. IMEEE, Montpellier, 1984, J. A. Rioux, G. Lanotte, F. Pratlong, P. Bastien, J. Perieres, 1990. Taxonomy of Leishmania. Use of isoenzymes, Suggestions for a new classification, Ann. Parasitol. Hum. Comp. 65 (1986) 111−115139−142.

[13] R.L. Jacobson, Leishmania tropica (Kinetoplastida: Trypanosomatidae) a perplexing parasite, Folia Parasitol. 50 (2003) 241−250.

[14] G. Schonian, L. Schnur, M. . el Fari, L. Oskam, A.A. Kolesnikov, W. Sokolowska-Kohler, W. Presber, Genetic heterogeneity in the species *Leishmania tropica* revealed by different PCR-based methods, Trans. R. Soc. Trop. Med. Hyg. 95 (2001) 217−224.

[15] J.A. Rioux, G. Lanotte, F. Petter, J. Dereure, O. Akalay, F. Pratlong, I.D. Velez, N.B. Fikri, R. Maazoun, M. Denial, D.M. Jarry, A. Zahaf, R.W. Ashford, M. Cadi-Soussi, R. Killick-Kendrick, N. Ben Mansour, G. Moreno, J. Périères, E. Guilvard, M. Zribi, M.F. Kennou, P. Rispail, R. Knechtli, E. Serres, Les leishmanioses cutanées du bassin méditerranéen occidental. De l'identification enzymatique a' l'analyse éco-épidémiologique. L'exemple de trois foyers, tunisien, marocain et français. in: J.A. Rioux (Ed.), Leishmania, Taxonomie et phylogenèse, Applications éco-épidémiologiques. IMEEE, Montpellier, France, 1986c, pp. 365−395.

[16] L. Parrot, Sur un nouveau phlébotome algérien *Phlebotomus sergenti*, sp. nov, Bull. Soc. Path. Ex 10 (1917) 564−567.

[17] B. El-Adhami, Isolation of *Leishmania* from a black rat in the Baghdad area, Iraq, Am. J. Trop. Med. Hyg. 25 (1976) 759−761.

[18] J. Dereure, J.A. Rioux, M. Gallego, J. Perieres, F. Pratlong, J. Mahjour, A. Saddiki, *Leishmania tropica* in Morocco: infection in dogs, Trans. R. Soc. Trop. Med. Hyg. 85 (1991) 595.

[19] M. Svobodova, J. Votypka, J. Peckova, V. Dvorak, A. Nasereddin, G. Baneth, J. Sztern, V. Kravchenko, A. Orr, D. Meir, L.F. Schnur, P. Volf, A. Warburg, Distinct transmission cycles of *Leishmania tropica* in 2 adjacent foci, Northern Israel, Emerg. Infect. Dis. 12 (2006) 1860−1868.

[20] Anonymous, Relevé, annuel des maladies à déclaration obligatoire, Année 2005. Ministère de la Santé de la Population et de la Réforme Hospitalière Direction de la Prévention, Algérie, 2005.

[21] E. Abonnenc, Les phlébotomes de la région éthiopienne (Diptcra, Psychodidae), Mem. ORSTOM 55 (1972) Paris.

[22] J.P. Dedet, K. Addadi, S. Belazzoug, Les phlébotomes (Diptera, Psychodidae) d'Algérie, Cah. O.R.S.T.O.M., sér. Ent. méd. et Parasitol 22 (1984) 99−127.

[23] J. Depaquit, N. Léger, R. Killick Kendrick, Description de *Phlebotomus riouxi* n. sp. (Diptera: Psychodidae) d'Afrique du Nord, Parasite 5 (1998) 151−158.

[24] R. Boudabous, A. Bounamous, D. Jouet, J. Depaquit, D. Augot, H. Ferte, S. Berchi, A. Couloux, M. Veuille, H. Babba, Mitochondrial DNA differentiation between two closely related species, *Phlebotomus (Paraphlebotomus) chabaudi* and *Phlebotomus (Paraphlebotomus) riouxi* (Diptera: Psychodidae), based on direct sequencing and polymerase chain reaction − restriction fragment length, Ann. Entomol. Soc. Am. 102 (2009) 347−353.

[25] I. Benkova, P. Volf, Effect of temperature on metabolism of *Phlebotomus papatasi* (Diptera: Psychodidae), J. Med. Entomol. 44 (2007) 150−154.

[26] J. Cihákova, P. Volf, Development of different *Leishmania major* strains in the vector sand flies *Phlebotomus papatasi* and *P. duboscqi*, Ann. Trop. Med. Parasitol. 91 (1997) 267−279.

[27] S. Berchi, J.A. Rioux, A. Belmonte, J. Russo, A new *Phlebotomus* from Algeria, *Phlebotomus (Paraphlebotomus) kazeruni*, Ann. Parasitol. Hum. Comp. 61 (1986) 507−508.

[28] A. Bounamous, R. Boudabous, D. Jouet, D. Augot, H. Ferté, H. Babba, S. Berchi, J. Depaquit, Molecular and morphological characterisation of two closely related species belonging to the subgenus *Phlebotomus chabaudi* Croset, Abonnenc & Rioux, 1970 et *P. riouxi* Depaquit, Killick-Kendrick & Léger, 1998 (Diptera: Psychodidae), Parasite 15 (2008) 565−571.

[29] E. Serres, M. Roux, Pratique de la classification automatique. in: J.A. Rioux (Ed.), leishmania taxonomie et phylogénèse. Application éco-épidémiologique. IMEEE, Montpellier, 1986, pp. 27−39.

[30] R. Killick-Kendrick, Phlebotomine vectors of the leishmaniasis: a review, Med. Vet. Entomol. 4 (1990) 124.

[31] P. Volf, J. Myskova, 2007, Sand flies and *Leishmania*: specific *versus* permissive vectors, Trends Parasitol. 23 (1990) 91−92.

[32] R. Killick-Kendrick, M. Killick-Kendrick, Y. Tang, Anthroponotic cutaneous leishmaniasis in Kabul, Afghanistan: the low susceptibility of *Phlebotomus papatasi* to *Leishmania tropica*, Trans. R. Soc. Trop. Med. Hyg. 88 (1994) 252−253.

[33] P.F. Pimenta, E.M.B. Saraiva, E. Rowton, G.B. Modi, L.A. Garraway, S. M. Beverley, S.J. Turco, D.L. Sacks, Evidence that the vectorial competence of phlebotomine sandflies for different species of *Leishmania* is controlled by structural polymorphisms in the surface lipophosphoglycan, Proc. Natl. Acad. Sci. USA 91 (1994) 9155−9159.

[34] R. Killick-Kendrick, M. Killick-Kendrick, Y. Tang, Anthroponotic cutaneous leishmaniasis in Kabul, Afghanistan: the high susceptibility of *Phlebotomus sergenti* to *Leishmania tropica*, Trans. R. Soc. Trop. Med. Hyg. 89 (1995) 477.

[35] S. Kamhawi, G.B. Modi, P.F. Pimenta, E. Rowton, D.L. Sacks, The vectorial competence of *Phlebotomus sergenti* is specific for *Leishmania tropica* and is controlled by speciesspecific, lipophosphoglycan-mediated midgut attachment, Parasitology 121 (2000) 25−33.

[36] P. Volf, Y. Ozbel, F. Akkafa, M. Svobodová, J. Votýpka, K.P. Chang, Sand flies (Diptera: Phlebotominae) in Sanliurfa, Turkey: relationship of *Phlebotomus sergenti* with the epidemic of anthroponotic cutaneous leishmaniasis, J. Med. Entomol. 39 (2002) 12−15 (Erratum in: J. Med. Entomol. 39(3) 2002; iii).

[37] R. Ben Ismail, M.S. Ben Rachid, Epidémiologie des leishmanioses en Tunisie. in: AUPELF-UREF (Ed.), Maladies Tropicales Transmissibles (1989), pp. 73−80 Paris.