

# Outbreak of Cutaneous Leishmaniasis in Northern Israel

Raymond L. Jacobson,<sup>1</sup> Carol L. Eisenberger,<sup>1</sup> Milena Svobodova,<sup>5</sup> Gad Baneth,<sup>3</sup> Julia Sztern,<sup>1</sup> Jorge Carvalho,<sup>1</sup> Abdelmajeed Nasereddin,<sup>1</sup> Mustafa El Fari,<sup>4</sup> Uri Shalom,<sup>2</sup> Petr Volf,<sup>5</sup> Jan Votypka,<sup>5</sup> Jean-Pierre Dedet,<sup>6</sup> Francine Pratlong,<sup>6</sup> Gabriele Schonian,<sup>3</sup> Lionel F. Schnur,<sup>1</sup> Charles L. Jaffe,<sup>1</sup> and Alon Warburg<sup>1</sup>

<sup>1</sup>Department of Parasitology, The Kuvim Center for Study of Infectious and Tropical Diseases, The Hebrew University Hadassah Medical School, and <sup>2</sup>Division of Pest Surveillance and Control, Ministry for the Environment, Jerusalem, and <sup>3</sup>The Hebrew University of Jerusalem, School of Veterinary Medicine, Rehovot, Israel; <sup>4</sup>Institut für Mikrobiologie und Hygiene, Charité Campus Mitte, Berlin, Germany; <sup>5</sup>Department of Parasitology, Charles University, Prague, Czech Republic; <sup>6</sup>Laboratoire de Parasitologie, Montpellier, France

This study describes a new focus of cutaneous leishmaniasis (CL) due to *Leishmania tropica*, in the Galilee region of northern Israel. Thirty-three cases from 4 villages (northern part) and from the city of Tiberias (southern part) have been clinically diagnosed since 1996. Parasites from 13 patients and from 6 sand flies were characterized by isoenzyme electrophoresis, 2 immunological methods, and 3 polymerase chain reaction (PCR)-based methods. Isolates from the northern part were antigenically similar to *Leishmania major* and were different from other *L. tropica* isolates, including those from the southern part of the focus. They belonged to a newly reported zymodeme and were separable from all known Israeli *L. tropica* isolates, by use of 2 different PCR-based methods. Five (5.2%) of 97 *Phlebotomus (Adlerius) arabicus* and 2 (1.2%) of 162 *Phlebotomus (Paraphlebotomus) sergenti* females from the northern part of the focus were found to be infected with *L. tropica*. Three of 29 hyraxes (*Procavia capensis*) were positive for *Leishmania* ribosomal DNA. Thus, the northern part of this emerging focus of CL in Israel is distinct from all known *L. tropica* foci. *P. arabicus* is the main vector, and it transmits parasites that are different from other *L. tropica* isolates, with respect to antigenic, molecular, and biochemical parameters.

The leishmaniasis—cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL)—are parasitic diseases with a wide range of clinical symptoms. Leishmaniasis currently threatens 350 million people in 88 countries around the world, with 1–1.5 million new cases of CL reported annually [1].

In Israel, the epidemiology of CL due to *Leishmania*

*major* has been investigated and clearly defined as being zoonotic, with *Psammomys obesus* and *Meriones crassus* as the main reservoir hosts and *Phlebotomus papatasi* as the vector [2, 3, 4]. However, outbreaks of CL due to *L. tropica* were rarely investigated in-depth, and, to estimate annual CL incidence rates of 0.17–7 cases/100,000 persons, cases were usually grouped together with cases due to *L. major* [5]. In a clinical setting, cutaneous lesions due to *L. tropica* last much longer and are more difficult to treat than those due to *L. major* [6]. Moreover, *L. tropica* infections can result in life-threatening VL, making correct diagnosis and treatment of crucial importance [7, 8]. Although CL due to *L. tropica* was classically considered to be anthroponotic, zoonotic outbreaks have been reported sporadically in Greece [9], Saudi Arabia [10], Kenya [11, 12], and Jordan [13, 14]. Outbreaks occur frequently in newly inhabited areas, and the number of cases is relatively small, compared with those in urban outbreaks of anthroponotic CL. In Saudi Arabia and Morocco, the most important proven vector of *L. tropica*

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Reprints or correspondence: Dr. R. L. Jacobson, Dept. of Parasitology, The Kuvim Center for Study of Infectious and Tropical Diseases, The Hebrew University Hadassah Medical School, P.O. Box 12272, Jerusalem 91120, Israel (jacobsr@cc.huji.ac.il).

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is the sand fly *Phlebotomus (Paraphlebotomus) sergenti* [10, 15], whereas, in Kenya, *Phlebotomus (Larrousius) guggisbergi* appears to be the main vector [16].

In terms of zymodemes and other genetic markers, *L. tropica* isolates are very heterogeneous [17, 18]. The animal reservoir hosts of *L. tropica* are not easily identified, because infection rates are very low. Rock hyraxes (*Procavia capensis*) were found to be infected with a Namibian variant of *L. tropica* [19], and, in Kenya, *Procavia johnsoni* were found to be infected [20]. In Syria and Morocco, dogs have recently been found to be infected with *L. tropica* [21, 22] but are thought to be accidental hosts rather than true reservoirs. Although many rats (*Rattus rattus*) have been examined, only 2 isolates from rats from Iraq and Kenya have ever been characterized as *L. tropica* [23, 24].

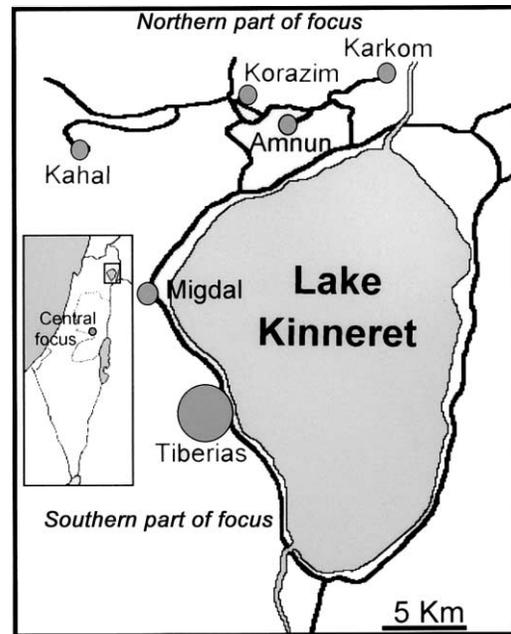
In the present study, we describe a new focus of CL in northern Israel. Molecular, biochemical, and immunological characterization of parasite isolates from patients, as well as from sand flies, prove the causative agent to be *L. tropica*. Parasites from the northern part of the focus are different from all other *L. tropica* isolates that have been characterized to date. Ecological studies implicated *Phlebotomus arabicus* as the main vector and rock hyraxes as probable reservoir hosts.

## METHODS

**Study areas.** The focus is divided into a northern part and a southern part. The northern part lies 5 km north of Lake Kinneret (Sea of Galilee), in the eastern, lower Galilee region of northern Israel, at 32° 55' N, 35° 36' W (figure 1). The area investigated, ~75 km<sup>2</sup>, encompasses the villages of Amnun (at sea level), Kahal (100 m above sea level), Karkom (100 m above sea level), and Korazim (150 m above sea level), totaling ~2000 inhabitants. The villages studied comprise ~400 modern single-family houses surrounded by gardens and built on basalt or limestone rock formations. Many boulders from the cleared land have been piled into large mounds, separating individual plots and surrounding the villages. These boulder mounds are inhabited by numerous rock hyraxes (*P. capensis*).

The southern part includes the city of Tiberias (population, 38,952 [2001 census]), where human cases were diagnosed in the neighborhood of Moradot Tveria (150–200 m above sea level) on the elevated western slopes of the city. The village of Migdal (140 m below sea level and 4 km north of Tiberias) was another locality where human cases were found (figure 1).

**Case finding.** Health professionals in the area alerted the authors when new cases appeared in the village and suburban foci. Visits were made to the infected individuals, and biopsy specimens of lesions were seeded in blood agar medium containing 200 IU/mL penicillin, 200 µg/mL streptomycin (Teva), and 1500 µg/mL 5-fluorocytosine (Sigma). Lesion material was also smeared on microscope slides and was stained with Giemsa



**Figure 1.** Map depicting the focus of cutaneous leishmaniasis, in the Galilee region of northern Israel. Inset, Map of Israel showing location of the central Israel focus, near Jerusalem.

and blotted on filter paper, for polymerase chain reaction (PCR) analysis.

**Collection and identification of sand flies.** Sand flies were trapped by use of Centers for Disease Control and Prevention miniature light traps (John W. Hock) placed in the gardens of houses and among the boulders surrounding the villages. Collections were made from June to September during 3 consecutive years (2000–2002) by trapping at various intervals in all the villages composing the northern part of the focus. For identification, flies were dissected, and the head and genitalia were mounted in either Hoyer's or Berlese's medium and were identified by use of several keys [25, 26, 27]. Sand flies collected for parasite isolation were kept in a humid and cool environment and were rapidly transported to the laboratory. The female flies were dissected on sterilized microscope slides, in sterile physiological saline. The guts were examined for the presence of parasites by use of a phase-contrast microscope. Infected guts were seeded in blood agar medium containing 200 IU/mL penicillin, 200 µg/mL streptomycin, and 1500 µg/mL 5-fluorocytosine. Heads and genitalia were mounted in Hoyer's medium for identification.

**Collection of animals.** Hyraxes were trapped by use of raccoon traps (Tomahawk) baited with fresh leaves and vegetables. Animals were anesthetized by a qualified veterinarian using ketamine (10 mg/kg intramuscular; Parke-Davis). Samples of blood, lymphatic tissue, and skin were seeded in NNN medium containing 200 IU/mL penicillin, 200 µg/mL streptomycin, and 1500 µg/mL 5-fluorocytosine, were blotted on

**Table 1. DNA genotyping of *Leishmania* isolates analyzed in this study.**

Focus, <i>Leishmania</i> isolate's WHO code	Origin	Species	PPIP-PCR	ITS-SSCP
Present study				
IARA/IL/00/Amnunfly1	Northern	<i>L. tropica</i>	LtB2	b
MHOM/IL/00/Omri	Northern	<i>L. tropica</i>	LtB2	b
MHOM/IL/02/Ofri	Northern	<i>L. tropica</i>	ND	b
MHOM/IL/97/P963	Southern	<i>L. tropica</i>	LtB1	B
MHOM/IL/01/LRC-L836	Southern	<i>L. tropica</i>	ND	B
MHOM/IL/01/LRC-L837	Southern	<i>L. tropica</i>	ND	B
MHOM/IL/01/LRC-L838	Southern	<i>L. tropica</i>	ND	B
Other Israeli foci				
MHOM/IL/96/P837	Central Israel	<i>L. tropica</i>	LtB1	B
ISER/IL/98/LRC-L757	Central Israel	<i>L. tropica</i>	LtB1	B
ISER/IL/98/LRC-L758	Central Israel	<i>L. tropica</i>	LtB1	B
MHOM/IL/90/P283	Central Israel	<i>L. tropica</i>	LtA1	A
International reference strains				
MHOM/SU/74/SAF-K27	Soviet Union	<i>L. tropica</i>	LtA1	A
MHOM/IQ/66/L75	Iraq	<i>L. tropica</i>	LtA1	A
MHOM/IL/59/LRC-L22	Southern Israel	<i>L. tropica</i>	LtA1	a
MHOM/EG/90/LPN65	Mt. Sinai	<i>L. tropica</i>	LtB1	B
MHOM/TN/80/LEM 163	Tunisia	<i>L. killicki</i>	LtK	C
MPSA/IL/83/PSAM398	Israel	<i>L. major</i>	LmA1	D
MHOM/TN/80/IPT1	Tunisia	<i>L. infantum</i>	LiA1	E

**NOTE.** ITS-SSCP, internal transcribed spacer–single-strand conformation polymorphism; Lt, *L. tropica*; Lm, *L. major*; Li, *L. infantum*; ND, not done; Northern, northern part of the focus; PPIP-PCR, permissively primed intergenic polymorphic–polymerase chain reaction; Southern, southern part of the focus; WHO, World Health Organization.

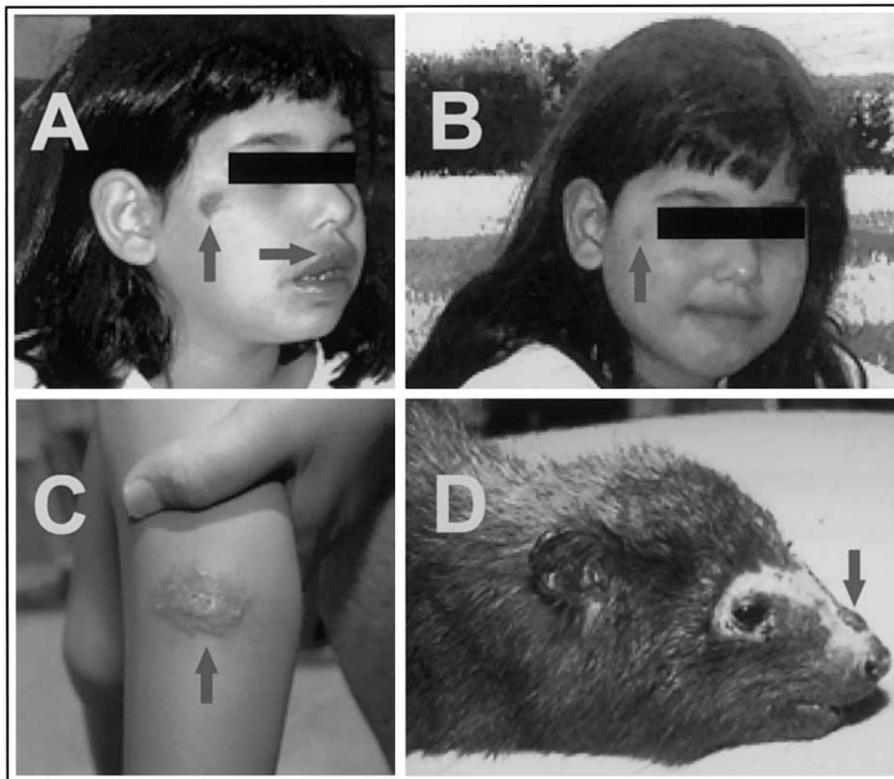
filter paper for DNA extraction, were smeared on microscope slides, and were stained with Giemsa. Hyraxes found to be negative for *Leishmania* species were released in their original place of capture, whereas those found to be positive were kept in holding pens for 2 months and were euthanized by a licensed veterinarian.

**Parasite culture.** For mass cultivation and characterization, promastigotes from blood agar cultures were seeded in Dulbecco's modified Eagle medium supplemented with L-glutamine (final concentration, 4 mmol/L), 4.5 mg/L glucose, 200 IU/mL penicillin, and 200 µg/mL streptomycin (Biological Industries) [28].

**Parasite identification.** Representative isolates obtained during the present study are designated as follows: sand fly *P. arabicus* (northern part of the focus): IARA/IL/2000/Amnunfly1; patients (northern part of the focus): MHOM/IL/2000/Omri and MHOM/IL/2002/Ofri; patients (southern part of the focus): MHOM/IL/1997/P963, MHOM/IL/2001/LRC-L836, MHOM/IL/2001/LRC-L837, and MHOM/IL/2001/LRC-L838; patients and sand flies (central Israel focus [included for comparison of local strains]): MHOM/IL/1996/P837, ISER/IL/1998/LRC-L757, ISER/IL/1998/LRC-L758, and MHOM/IL/1990/

P283. Isolates from patients and from sand flies were compared to the following international reference strains: *L. tropica*: MHOM/SU/1974/SAF-K27, MHOM/IQ/1966/L75, MHOM/IL/1959/LRC-L22, and MHOM/EG/1990/LPN65; *Leishmania killicki*: MHOM/TN/1980/LEM163; *L. major*: MPSA/IL/1983/PSAM398; and *Leishmania infantum*: MHOM/TN/1980/IPT1 (table 1). Five methods were used to identify the parasites obtained in this study.

**Serotyping.** Initial screening of the isolates was performed by gel diffusion of the glycoconjugates secreted into the culture media, by use of several antileishmanial serum samples (polyclonal antibody [pAb]) for 3 d at 4°C (excreted factor [EF] serotyping [29]). Specific *Leishmania* monoclonal antibodies (MAbs) were used in indirect immunofluorescence antibody tests to determine the surface antigenic epitopes of the parasites [30]. In brief, promastigotes from initial culture tubes of the new isolates and known controls of *L. major*, *L. tropica*, and *L. infantum* were placed in individual wells of fluorescent antibody slides (Bellco), were air-dried, and were fixed in cold acetone. The preparations were blocked with 5% fetal calf serum in PBS for 1 h at room temperature. Mouse MAbs specific for *L. major* (T1), *L. tropica* (T11, T14, and T15), *L. tropica/L. major* (T3),



**Figure 2.** Cutaneous leishmaniasis lesion and infiltration of the lips due to *Leishmania tropica*, in a girl from the northern part of the focus. *A*, Before treatment. *B*, Same patient 15 d after treatment with sodium stibogluconate. *C*, Leishmaniasis lesion on the arm of a young patient from a suburb of Tiberias (southern part of the focus). *D*, Alopecia and lesions on a rock hyrax (*Procavia capensis*). DNA extracted from these lesions were positive for *L. tropica* internal transcribed spacer region 1 DNA, by polymerase chain reaction (figure 5).

and *L. infantum/Leishmania donovani* (D2) were applied for 1 h at 37°C. Goat anti-mouse IgG fluorescein isothiocyanate antibody was applied for 40 min at 37°C. The preparations were washed, were mounted in 3% 1,4-diazabicyclo[2,2,2]octane (Sigma) in PBS/glycerol, and were viewed by use of a Zeiss Axiovert microscope.

**PCR-based typing.** Genomic DNA was amplified by permissively primed intergenic polymorphic-PCR (PIIP-PCR), as described elsewhere [31]. The PIIP-PCR were modified by use of 20 ng/ $\mu$ L of genomic DNA and were amplified with 5  $\mu$ mol/L of a single *Leishmania*-specific primer, 2B (5'-CAG GAG CGC GCA CAC GCA CAC ACG), and 2 U of recombinant *Taq* DNA polymerase (MBI Fermentas).

The ribosomal internal transcribed spacer region 1 (ITS1), lying between the *ssu* rRNA and 5.8S rRNA genes, was amplified with the following *Leishmania*-specific primers: LITSR (5'-CTGGATCATTTTCCGATG-3') and L5.8S (5'-TGATACCAC-TTATCGCACTT-3'), as described elsewhere [32]; 15–20  $\mu$ L of these amplicons, containing the amplified ITS1 region, were digested for 2 h with *Hae*III (Hybaid GmbH), as described elsewhere [33].

**Single-strand conformation polymorphism (SSCP) analy-**

**sis.** Analysis of ITS1 (SSCP-ITS1) was performed by denaturing the double-stranded PCR products and running 10–15  $\mu$ L/lane on agarose gels, as described elsewhere [33].

**Isoenzyme electrophoresis.** Two isolates from the northern part of the focus were characterized at the National *Leishmania* Centre, Montpellier, France, by starch gel electrophoresis using 15 enzymatic systems to generate isoenzyme profiles. *L. infantum* MHOM/FR/78/LEM (zymodeme MON-1) was used as the reference strain [17].

**Laboratory infection of sand flies.** Laboratory-reared *P. papatasi* were from a colony originating from Kfar Adumim (~15 km east of Jerusalem). These flies were selected because *L. tropica* isolates from 2 patients and sand flies were antigenically similar to *L. major* (see Results section) and because *P. papatasi* is the natural vector of *L. major*. Five-day-old *P. papatasi* females were artificially infected through chick-skin membranes, with meals consisting of 10<sup>6</sup> promastigotes/mL of either *L. major* (IPAP/IL/84/Uvda [LRC-L465]) or the *L. tropica* isolate from *P. arabicus* (IARA/IL/00/Amnunfly1), in washed, inactivated rabbit blood [28]. Blood-fed flies were maintained on water and 50% solution of aqueous honey, at 26  $\pm$  1°C and 80% relative humidity. Flies were dissected 7 d after feeding,

**Table 2. Identification of sand flies from the northern part of the *Leishmania tropica* focus, in northern Israel.**

Sand fly species	Village								Total
	Korazim		Karkom		Kahal		Amnun		
	F	M	F	M	F	M	F	M	
<i>P. sergenti</i>	19	53	20	108	3	0	120	237	560
<i>P. arabicus</i>	11	35	1	3	1	24	84	271	430
<i>P. simici</i>	6	32	4	13	13	22	45	132	267
<i>P. tobbi</i>	46	251	11	40	100	114	72	171	805
<i>P. syriacus</i>	0	5	0	3	0	2	1	14	25
<i>P. papatasi</i>	0	0	1	0	11	29	0	1	42
<i>P. perfiliewi</i>	2	15	0	3	2	12	6	11	51
Total	84	391	37	170	130	203	328	837	2180

in physiological saline solution, and were examined by use of a phase-contrast microscope.

In an attempt to obtain live parasites from hyraxes, *P. papatasi* and *Lutzomyia longipalpis* were allowed to feed on anesthetized hyraxes that were positive for *Leishmania* species by PCR. *L. longipalpis* were used because this species has been shown to be susceptible to local strains of *L. tropica* (M.S., unpublished data). The natural vector in the region, *P. arabicus*, is not available for laboratory experiments.

## RESULTS

**Case findings.** On the basis of information received from local health professionals, visits for case findings took place in the suburbs of Tiberias, and patients from Amnun, Karkom, and Migdal were seen at the Department of Dermatology, Hadassah Hospital, Jerusalem. In 2001, 13 cases were parasitologically confirmed by smear and culture, and parasites were identified as *L. tropica* by ITS1-PCR. The clinical picture was typical

for *L. tropica*: ulcerating dry lesions of long duration, often on the face or upper extremities (figure 2A and 2C).

Twenty-one additional cases of CL (as diagnosed in hospital clinics by qualified physicians) were found retroactively from information provided by householders, after a postal survey and follow-up interviews. All cases were from the northern part of the focus: 10 were from Karkom, 6 were from Amnun, 4 were from Kahal, and 1 was from Korazim, and most lived on the fringes of their respective villages.

**Identification of sand flies.** Collection of sand flies was performed only in the northern part of the focus. Of the 2544 sand flies collected, 2180 were identified as *Phlebotomus* species (table 2), and 358 were identified as *Sergentomyia* species. The percentages of the *Phlebotomus* species identified were the following: *P. (Larrousius) tobbi*, 36.9%; *P. (Paraphlebotomus) sergenti*, 23.7%; *P. (Adlerius) arabicus*, 19.7%; *P. (Adlerius) simici*, 12.3%; *P. (Larrousius) perfiliewi*, 2.3%; *P. (Phlebotomus) papatasi*, 1.9%; *P. (Larrousius) syriacus*, 1.2%; and *P. (Paraphlebotomus) alexandri*, *P. (Larrousius) jacusieli*, and *P. (Larrousius) kazeruni*, <0.1%. In the village of Amnun, only 5 *P. sergenti* were caught during 10 nights in June, whereas 267 were caught during 4 nights in September. The numbers of *P. tobbi* and *P. arabicus* were significantly more evenly distributed throughout the season than were those of *P. sergenti* ( $P < .01$ , Fisher's exact test). Many sand flies were caught among the boulders where the hyraxes dwelled, but some were trapped in orchards and on external walls of houses.

**Parasite characterization.** Of the 502 gravid female *Phlebotomus* from the 4 villages, 5 (5.2%) of 97 *P. arabicus* and 2 (1.2%) of 162 *P. sergenti* were found to be infected with promastigotes. All the infections in the *P. arabicus* were deemed to be mature, post blood meal, whereas 1 *P. sergenti* infection was still encased in the blood meal, and the other was relatively light.

**Table 3. Characterization of *Leishmania* isolates from the northern part of the focus, by indirect immunofluorescent assay using species-specific monoclonal antibodies (MAbs).**

Variable	MAb				
	<i>L. major</i> T1	<i>L. major</i> and <i>L. tropica</i> T3	<i>L. tropica</i> T11	<i>L. tropica</i> T14	<i>L. infantum</i> D2
<i>P. arabicus</i> <sup>a</sup>	+++++	+++++	0	+	0
Man <sup>b</sup>	++++	++++	0	++	0
Girl <sup>c</sup>	+++++	+++++	0	0	0
<i>L. major</i> <sup>d</sup>	+++++	+++++	0	0	0
<i>L. tropica</i> <sup>d</sup>	±	+++	+++	+++++	0
<i>L. infantum</i> <sup>d</sup>	0	0	0	0	+

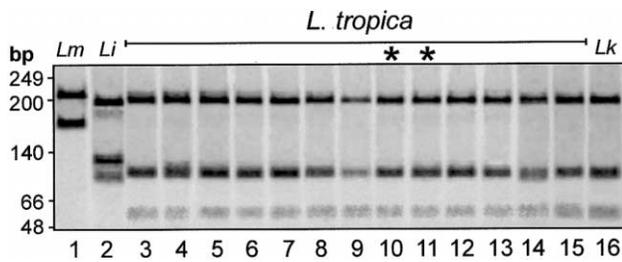
**NOTE.** +, Relative binding strength of MAbs to the surface of the promastigotes: +, very weak; +++++, very strong.

<sup>a</sup> IARA/IL/2000/Amnunfly1 (sand fly from Amnun); *P. arabicus*, *Phlebotomus arabicus*.

<sup>b</sup> MHOM/IL/2000/Omri (patient from northern part of the focus).

<sup>c</sup> MHOM/IL/2002/Ofri (patient from Karkom).

<sup>d</sup> Reference strains.



**Figure 3.** Restriction analysis of amplified ribosomal internal transcribed spacer region 1 of different *Leishmania* isolates. Lane 1, *L. major* (Lm) MPSA/IL/83/PSAM398. Lane 2, *L. infantum* (Li) MHOM/TN/80/IPT1. Lanes 3–15, different strains of *L. tropica*: 3, MHOM/SU/74/SAF-K27; 4, MHOM/IQ/66/L75; 5, MHOM/IL/90/P283; 6, MHOM/IL/96/P837; 7, ISER/IL/98/LRC-L757; 8, ISER/IL/98/LRC-L758; 9, MHOM/IL/59/LRC-L22; 10, IARA/IL/00/Amnunfly; 11, MHOM/IL/00/Omri; 12, MHOM/IL/01/LRC-L836; 13, MHOM/IL/01/LRC-L837; 14, MHOM/IL/01/LRC-L838; and 15, MHOM/IL/97/P963. Lane 16, *L. killicki* (Lk) MHOM/TN80/LEM163. \*Representative isolates from the northern part of the focus.

Results showed that *L. tropica* isolates from the northern part were distinct from *L. tropica* isolates from the southern part—2 subfoci ~15 km apart. The parasites from the northern part produced EF serotype A<sub>1</sub>, which is typical of *L. major*, and reacted with anti-*L. major* serum (pAb), which does not react with most *L. tropica* isolates. This finding was in sharp contrast to findings regarding isolates from 5 patients from the southern part, isolates that produced serotypes of either A<sub>2</sub> or A<sub>3</sub>B<sub>2</sub>, which are typical of *L. tropica* strains found in central Israel (table 1).

The *L. major*-specific MAbs T1 and T3 reacted strongly with the surface of parasites from the northern part of the focus, whereas only 1 of the specific MAbs for *L. tropica*, T14, reacted very weakly. The other *L. tropica*-specific MAbs, T11 and T15, did not bind to the surface of these parasites at all (table 3).

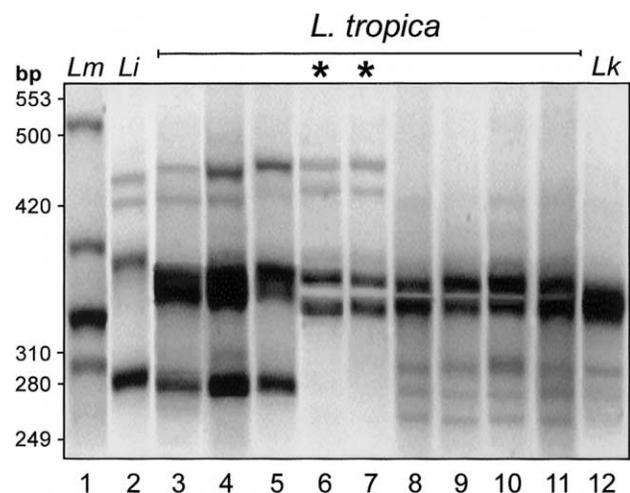
Restriction fragment-length polymorphism (RFLP) analysis of PCR-amplified ITS1 region showed that all isolates were identical to reference *L. tropica* isolates and were different from *L. major* and *L. infantum* isolates (figure 3). PPIP-PCR analyses showed that the sand fly isolate, IARA/IL/00/Amnun fly1, and the patient isolate, MHOM/IL/2000/Omri, (both from the northern part of the focus) were identical to each other but were different from other Israeli *L. tropica* isolates, including isolates obtained from patients in the southern part of focus (table 1). PPIP heterogeneity was evidenced by the absence of certain bands ranging from 260 to 300 bp. There was little similarity to *L. major* or *L. infantum* strains (figure 4). These results were confirmed by ITS1-SSCP analysis, by which the 3 banding regions for these 2 isolates from the northern part of the focus all appeared as distinct doublets, designated ITS-SSCP genotype b. Like most *L. tropica* strains from Israel, the isolates from patients from Tiberias had the ITS-SSCP genotype B (figure 5 and table 1).

In summary, isolates from the northern part of the focus

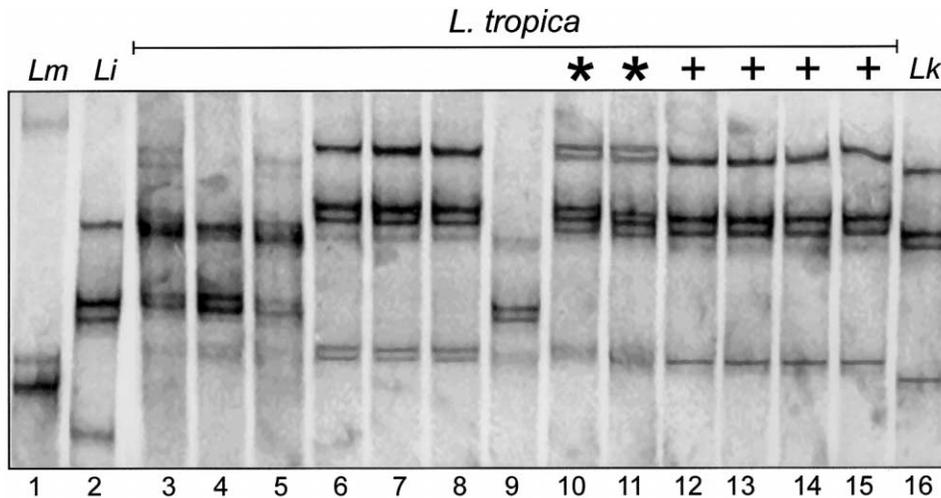
were antigenically similar to *L. major*, and their *L. tropica* DNA genotype profile differed from the isolates from the southern part of the focus. The parasites collected from patients from Tiberias were antigenically and genotypically indistinguishable from *L. tropica* isolates from central Israel. Isoenzyme profiles showed that parasites from the northern part of the focus (*P. arabicus* and a human case) were typical of the recently reported *L. tropica* zymodeme MON-265.

**Animal results.** Twenty-nine hyraxes were caught around the villages of Korazim and Amnun. Biopsy specimens were obtained from the nose, ear pinna, and lymph glands. ITS1-PCR products positive for *Leishmania* species were obtained in nasal and ear tissue and blood from 3 hyraxes. ITS1-PCR RFLP profiles were identical to *L. tropica* marker strains and were distinct from *L. major* or *L. infantum* (figure 6). Despite the presence of *L. tropica* DNA, microscopic examination and cultures from all the hyraxes were negative for *Leishmania* species. Furthermore, hamsters that were injected in the foot-pads with blood from the hyraxes that were positive for *Leishmania* species by PCR remained negative for >1 year. In the absence of a colony of the putative vectors, sand flies from colonies of *L. longipalpis* and *P. papatasi* were allowed to feed directly on a hyrax that was positive for *Leishmania* species by PCR, but, after 5 d, no infections were detected in 100 flies of each species.

**Artificial infection of sand flies.** The isolate from the *P. arabicus* failed to infect any of the *P. papatasi* that fed on the



**Figure 4.** Amplification of *Leishmania* isolates by permissively primed intergenic polymorphic-polymerase chain reaction. Lane 1, *L. major* (Lm) MPSA/IL/83/PSAM398. Lane 2, *L. infantum* (Li) MHOM/TN/80/IPT1. Lane 3, *L. tropica* MHOM/SU/74/SAF-K27. Lane 4, MHOM/IQ/66/L75. Lane 5, MHOM/IL/90/P283. Northern part of the focus: Lane 6, IARA/IL/00/Amnunfly1; lane 7, MHOM/IL/00/Omri. Southern part of the focus: Lane 8, MHOM/IL/97/P963. Central Israel focus: Lane 9, MHOM/IL/96/P837; lane 10, ISER/IL/98/LRC-L757; lane 11, ISER/IL/98/LRC-L758; and lane 12, *L. killicki* (Lk) MHOM/TN/80/LEM163. Note extra band on isolates from the northern part of the focus (\*).



**Figure 5.** Single-strand conformation polymorphisms of the internal transcribed spacer region 1–polymerase chain reaction product of *Leishmania* isolates. Lane 1, *L. major* (*Lm*) MPSA/IL/83/PSAM398. Lane 2, *L. infantum* (*Li*) MHOM/TN/80/IPT1. Lanes 3–15, different strains of *L. tropica*: 3, MHOM/SU/74/SAF-K27; 4, MHOM/IQ/66/L75; 5, MHOM/IL/90/P283; 6, MHOM/IL/96/P837; 7, ISER/IL/98/LRC-L757; 8, ISER/IL/98/LRC-L758; 9, MHOM/IL/59/LRC-L22; 10, IARA/IL/00/Amnunfly; 11, MHOM/IL/00/Omri; 12, MHOM/IL/01/LRC-L836; 13, MHOM/IL/01/LRC-L837; 14, MHOM/IL/01/LRC-L838; and 15, MHOM/IL/97/P963. Lane 16, *L. killicki* (*Lk*) MHOM/TN80/LEM163. Isolates from the northern part of the focus (\*) different from isolates from the southern part of the focus (+).

artificial blood meal, whereas 68.8% of the *P. papatasi* that fed on *L. major* were infected, most with heavy and mature infections.

## DISCUSSION

The present study has described a new focus of zoonotic CL due to *L. tropica*, in the Galilee region of northern Israel, where the annual incidence rate was calculated to be 10.8 cases/100,000 persons, a relatively high figure for zoonotic CL. Despite its relatively small size, this focus appears to comprise 2 epidemiologically distinct parts, a northern, rural (village) part and a southern, more urban part (Tiberias). Most of our work concentrated on the northern part, whence all the entomological and animal-reservoir data were derived. Data on parasite isolates indicate that the southern part is both similar to other known foci in our region and different from the northern part.

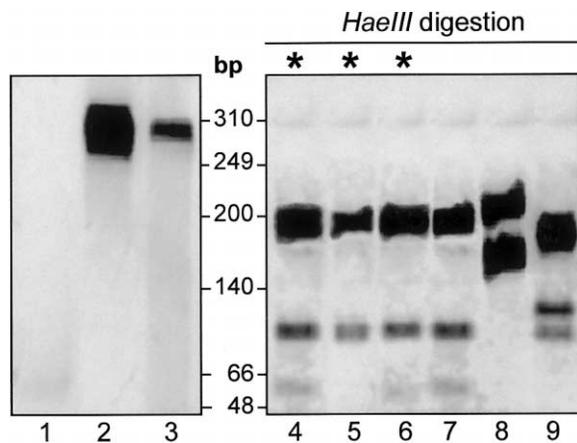
*L. tropica* lesions are notoriously resistant to topical medication, such as paromomycin [6]. During the present study, 2 case patients from the northern part of the focus with particularly long-lasting lesions required systemic treatment with sodium stibogluconate. The first patient had 2 lesions, 1 on the upper lip and 1 on the face (figure 2A); the second patient had 1 lesion, on the eyelid. Both patients were treated to achieve radical cure and to reduce tissue damage (Department of Dermatology, Hadassah Hospital, Jerusalem).

Sand flies were seldom seen within houses, and, therefore, all trapping was performed in the surrounding gardens and artificial boulder mounds. Three species of sand flies, *P. (Larrousius) tobbi*, *P. (Paraphlebotomus) sergenti*, and *P. (Adlerius)*

*arabicus* were found in large numbers (table 2). *P. tobbi* was found in relatively high numbers (36.9% of the *Phlebotomus* species caught in our study area), but none were found to be infected. At present, *P. sergenti* is the only proven or suspected sand fly vector of *L. tropica* throughout its range [10, 15, 34–36]. In recent reports from Jordan, *P. sergenti* was found in large numbers in 2 *L. tropica* foci [13, 14], 1 of which was only 60 km from the focus we have described here. Furthermore, in a focus of CL in Kfar Adumim (15 km east of Jerusalem), *P. sergenti* was the only species found to be infected with *L. tropica* (figure 1, inset, and table 1).

In the present study, *P. sergenti* constituted 25.7% of the total catch but was abundant only in September, which is toward the end of the transmission season, a phenomenon also seen in Morocco [15]. Although 2 females were found to be infected, 1 in Karkom and 1 in Amnun, infections were either very slight or were too immature for us to determine whether these infected flies would serve as competent vectors. On the other hand, *P. arabicus* was found in high numbers during the entire transmission season, and 5% of the females harbored heavy and mature *L. tropica* infections.

Several species of sand flies belonging to the subgenus *Adlerius* have been incriminated in the transmission of VL. *P. simici* is considered to be a probable vector of VL in the eastern Mediterranean region [37], and *Leishmania* DNA has been amplified from several different species in a focus of VL near Athens, Greece [38]. Although *P. arabicus* has never been positively incriminated as a vector of leishmaniasis, it is a suspected vector of *L. infantum* (VL) in Yemen and Saudi Arabia [39].



**Figure 6.** Left, *Leishmania* internal transcribed spacer region 1–polymerase chain reaction (ITS1-PCR) product from hyrax tissue. Lane 1, Negative control; Lane 2, positive control; Lane 3, hyrax tissue. Right, Restriction fragment–length polymorphism products from *HaeIII* digestion of ITS1-PCR. Isolates of *L. tropica* from the northern part of the focus (\*): Lane 4, Hyrax; lane 5, human; lane 6, *P. arabicus*. Marker strains: Lane 7, *L. tropica* MHOM/IQ/66/L75; lane 8, *L. major* MHOM/SU/73/5ASKH; and lane 9, *L. infantum* MHOM/TN/80/IPT1.

Here we report, for the first time, naturally infected *Phlebotomus (Adlerius)* species and provide proof that *P. (Adlerius) arabicus* is an important vector of *L. tropica* in this emerging focus of CL.

Although the isolates from *P. arabicus* were phenotypically similar to *L. major*, as evidenced by serotyping of secreted phosphoglycans and promastigote surface epitopes (table 3), they were not infectious to *P. papatasi*, which is the natural vector of *L. major*. Interestingly, they also failed to infect colonized *P. sergenti* originating from Amnun (northern part of the focus) that had fed on an infected mouse. However, they were infectious to *Phlebotomus (Adlerius) halepensis* (originating in Jordan), which is more closely related to *P. arabicus* (M.S. and P.V., unpublished data).

Parasites isolated from *P. arabicus* (IARA/IL/00/Amnunfly1) and from human cases, from both the northern and the southern parts of the focus, were characterized by biochemical, serological, and molecular techniques. Isoenzyme profiles showed that the parasites belonged to *L. tropica* zymodeme MON-265, a zymodeme previously described to be only from Jordan (unpublished data, J-P.D. and F.P.) but different from those prevalent elsewhere in Israel [17]. All isolates had identical ITS1-RFLP profiles that, in general, were indistinguishable from those of *L. tropica* isolates (figure 3). However, the secreted phosphoglycans and surface epitopes of the isolates from the northern part serologically resembled those of *L. major* and were very different from those of other known *L. tropica* isolates. PPIP-PCR, a highly sensitive technique, consistently differen-

tiates *L. tropica* isolates from the northern part of the focus from other Israeli *L. tropica* strains (figure 4). SSCP analysis that can detect single base-pair differences confirmed that isolates from the northern part of the focus were distinct from those from the southern part (figure 5).

Anthroponotic transmission in the focus is very unlikely, since the number of CL cases is too small and their distribution in time and space is too sporadic to constitute an adequate reservoir. Rock hyraxes were seen frequently around the villages throughout the year, but they ventured much closer to human habitation during summer, when food in their natural habitats became scarce. Hyraxes live in large areas of the Middle East, Ethiopia, Kenya, and Namibia, where they constitute proven or suspected reservoirs of CL due to *Leishmania aethiopica* and *L. tropica* [20, 40, 41]. So far, we have found that 3 (10.3%) of 29 hyraxes from the northern part of the focus in Israel were positive for *Leishmania* DNA by PCR, DNA that was identical to DNA extracted from both sand fly and human isolates of *L. tropica* (figure 6). Intensive study of the focus is continuing through active case finding, with a search for additional reservoir hosts and attempts to obtain positively infected hyraxes or other putative reservoir hosts.

Recent reports suggest that *L. tropica* is rapidly spreading, with a 3-fold increase in the number of cases in the West Bank from 2000 to 2001 [5]. The West Bank and Israel are undergoing constant changes due to population growth, urbanization, introduction of new agricultural practices, and hostilities. Large-scale environmental modifications tend to promote outbreaks of infectious disease. This is particularly true of zoonotic diseases with animal reservoir hosts and vectorborne diseases transmitted by arthropods. Human activity can enhance the likelihood of disease emergence, by a variety of interacting environmental pathways. Denser human populations come in closer contact with potential reservoir hosts, and environmental deterioration can lead to increased numbers of sand flies that feed on animals and humans. Therefore, epidemiological and ecological studies that both assess risk factors for spread of the disease and formulate preventive measures are of utmost importance.

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## References

- Desjeux P. The increase in risk factors for leishmaniasis worldwide. *Trans R Soc Trop Med Hyg* 2001;95:239–43.

2. Greenblatt CL, Schlein Y, Schnur LF. Leishmaniasis in Israel and Vicinity. In: Chang K-P, Bray RS, eds. *Leishmaniasis*. Amsterdam: Elsevier, **1985**: 415–26.
3. Schlein Y, Warburg A, Schnur LF, Le Blanq SM, Gunders AE. Leishmaniasis in Israel: reservoir hosts, sandfly vectors and leishmanial strains in the Negev, Central Arava and along the Dead Sea. *Trans R Soc Trop Med Hyg* **1984**; *78*:480–4.
4. Wasserberg G, Abramsky Z, Anders G, et al. The ecology of cutaneous leishmaniasis in Nizzana, Israel: infection patterns in the reservoir host, and epidemiological implications. *Int J Parasitol* **2002**; *32*:133–43.
5. Anis E, Leventhal A, Elkana Y, Wilamowski A, Pender H. Cutaneous leishmaniasis in Israel in the era of changing environment. *Public Health Rev* **2001**; *29*:37–47.
6. Klaus S, Frankenburg S. Cutaneous leishmaniasis in the Middle East. *Clin Dermatol* **1999**; *17*:137–41.
7. Magill AJ, Grogg M, Gasser RA, Sun W, Oster CN. Visceral infection caused by *Leishmania tropica* in veterans of operation desert storm. *New Engl J Med* **1993**; *328*:1383–7.
8. Oren R, Schnur LF, Ben Yehuda D, Mayner V, Okon E, Rachmilewitz EA. Visceral leishmaniasis: a difficult diagnosis and unusual causative agent. *J Infect Dis* **1991**; *164*:746–9.
9. Garifallou A, Schnur LF, Stratigos JD, et al. Leishmaniasis in Greece II. Isolation and identification of the parasite causing cutaneous leishmaniasis in man. *Ann Trop Med Parasitol* **1984**; *78*:369–75.
10. Al-Zahrani MA, Peters W, Evans DA, Chin C, Smith V, Lane RP. *Phlebotomus sergenti*, a vector of *Leishmania tropica* in Saudi Arabia. *Trans R Soc Trop Med Hyg* **1988**; *82*:416.
11. Mebrahtu YB, Lawyer PG, Ngumbi PM, et al. A new rural focus of cutaneous leishmaniasis caused by *Leishmania tropica* in Kenya. *Trans R Soc Trop Med Hyg* **1992**; *86*:381–7.
12. Sang DK, Njeru WK, Ashford RW. A zoonotic focus of cutaneous leishmaniasis due to *Leishmania tropica* at Utut, Rift Valley Province, Kenya. *Trans R Soc Trop Med Hyg* **1994**; *88*:35–7.
13. Kamhawi S, Abdel HS, Arbaji A. A new focus of cutaneous leishmaniasis caused by *Leishmania tropica* in northern Jordan. *Trans R Soc Trop Med Hyg* **1995**; *89*:255–7.
14. Saliba EK, Saleh N, Oumeish OY, Khoury S, Bisharat Z, al-Ouran R. The endemicity of *Leishmania tropica* (zymodeme MON-137) in the Eira-Yarqa area of Salt District, Jordan. *Ann Trop Med Parasitol* **1997**; *91*:453–9.
15. Guilvard E, Rioux JA, Gallego M, et al. *Leishmania tropica* au Maroc. III. Role vecteur de *Phlebotomus sergenti*: a propos de 89 isolates. *Ann Parasitol Hum Comp* **1991**; *66*:96–9.
16. Lawyer PG, Mebrahtu YB, Ngumbi PM, et al. *Phlebotomus guggisbergi* (Diptera: Psychodidae), a vector of *Leishmania tropica* in Kenya. *Am J Trop Med Hyg* **1991**; *44*:290–8.
17. Rioux JA, Lanotte G, Serres E, Pralong F, Bastien P, Perieres J. Taxonomy of *Leishmania*. Use of isoenzymes. Suggestions for a new classification. *Ann Parasitol Hum Comp* **1990**; *65*:111–25.
18. Schonian G, Schnur LF, El-Fari M, et al. Genetic heterogeneity in the species *Leishmania tropica* revealed by different PCR-based methods. *Trans R Soc Trop Med Hyg* **2001**; *95*:217–24.
19. Grove SS, Ledger JA. *Leishmania* from a hyrax in South West Africa [letter]. *Trans R Soc Trop Med Hyg* **1975**; *69*:523–4.
20. Ashford R, Sang DK. Review of *Leishmania tropica* infection in East Africa [abstract 55]. In: Program and abstracts of the Second International Congress on *Leishmania* and Leishmaniasis (Hersonissos, Crete, Greece). Athens: Hellenic Pasteur Institute, **2001**:14.
21. Dereure J, Rioux JA, Khiami A, Pralong F, Perieres J, Martini A. Ecoepidemiology of leishmaniasis in Syria. II. Presence, in dogs, of *Leishmania infantum* Nicolle and *Leishmania tropica* (Wright) (Kinetoplastida-Trypanomatidae) [in French]. *Ann Parasitol Hum Comp* **1991**; *66*:252–5.
22. Guessous-Idrissi N, Berrag B, Riyad M, Sahibi H, Bichichi M, Rhallem A. Short report: *Leishmania tropica*: etiologic agent of a case of canine visceral leishmaniasis in northern Morocco. *Am J Trop Med Hyg* **1997**; *57*:172–3.
23. El-Adhami B. Isolation of *Leishmania* from a black rat in the Baghdad area, Iraq. *Am J Trop Med Hyg* **1976**; *25*:759–61.
24. Massamba NN, Mutinga MJ, Kamau CC. Characterisation of *Leishmania* isolates from Laikipia District, Kenya. *Acta Tropica* **1998**; *71*:293–303.
25. Lewis DJ. A taxonomic review of the genus *Phlebotomus* (Diptera: Psychodidae). *Bull Br Mus Nat Hist (Ent)* **1982**; *45*:121–209.
26. Artemiev MM. Revision of sandflies of the subgenus *Adlerius* (Diptera, Phlebotominae, Phlebotomus) [in Russian]. *Zool Zhurnal* **1980**; *59*:1177–93.
27. Lewis DJ, Buttiker W. Insects of Saudi Arabia: the taxonomy and distribution of Saudi Arabian Phlebotomus sandflies (Diptera: Psychodidae). In: Witmer W, Buttiker W, eds. *Fauna of Saudi Arabia. Basle: Meteorology and Environmental Protection Administration, 1982*: 353–83.
28. Schlein Y, Jacobson RL. Linkage between susceptibility of *Phlebotomus papatasi* to *Leishmania major* and hunger tolerance. *Parasitology* **2002**; *125*:343–8.
29. Schnur LF, Jacobson RL. Surface reaction of *Leishmania*. IV. Variation in the surface membrane carbohydrates of different strains of *Leishmania major*. *Ann Trop Med Parasitol* **1989**; *83*:455–63.
30. Jaffe CL, Sarfstein R. Species-specific antibodies to *Leishmania tropica* (minor) recognize somatic antigens and exometabolites. *J Immunol* **1987**; *139*:1310–9.
31. Eisenberger CL, Jaffe CL. *Leishmania*: identification of Old World species using a permissively primed intergeneric polymorphic–polymerase chain reaction. *Exp Parasitol* **1999**; *91*:70–7 (erratum: *Exp Parasitol* 1999; *92*:159).
32. Schonian G, Akuffo H, Lewin S, et al. Genetic variability within the species *Leishmania aethiopia* does not correlate with clinical variations of cutaneous leishmaniasis. *Mol Biochem Parasitol* **2000**; *106*:239–48.
33. El Tai NO, Osman OF, El-Fari M, Presber W, Schonian G. Genetic heterogeneity of ribosomal internal transcribed spacer (its) in clinical samples of *Leishmania donovani* spotted on filter paper as revealed by single-strand conformation polymorphisms (sscp) and sequencing. *Trans R Soc Trop Med Hyg* **2000**; *94*:1–5.
34. Rowland M, Munir A, Durrani N, Noyes H, Reyburn H. An outbreak of cutaneous leishmaniasis in an Afghan refugee settlement in north-west Pakistan. *Trans R Soc Trop Med Hyg* **1999**; *93*:133–6.
35. Tayeh A, Jalouk L, Cairncross S. Twenty years of cutaneous leishmaniasis in Aleppo, Syria. *Trans R Soc Trop Med Hyg* **1997**; *91*:657–9.
36. Volf P, Ozbel Y, Akkafa F, Svobodova M, Votycka J, Chang K-P. Sand flies (Diptera: Phlebotominae) in Sanliurfa, Turkey: relationship of *Phlebotomus sergenti* with the epidemic of anthroponotic cutaneous leishmaniasis. *J Med Entomol* **2002**; *39*:12–5.
37. Killick-Kendrick R. Phlebotomine vectors of the leishmaniasis: a review. *Med Vet Entomol* **1990**; *4*:1–24.
38. Aransay AM, Scoulica E, Tselentis Y. Detection and identification of *Leishmania* DNA within naturally infected sand flies by seminested PCR on minicircle kinetoplastic DNA. *Appl Environ Microbiol* **2000**; *66*:1933–8.
39. Lewis DJ. The phlebotomid sandflies of Yemen Arab Republic. *Tropenmed Parasitol* **1974**; *25*:187–97.
40. Ashford RW, Bray MA, Hutchinson MP, Bray RS. The epidemiology of cutaneous leishmaniasis in Ethiopia. *Trans R Soc Trop Med Hyg* **1973**; *67*:568–601.
41. Sang DK, Njeru WK, Ashford RW. A possible animal reservoir for *Leishmania tropica* s.l. in Kenya. *Ann Trop Med Parasitol* **1992**; *86*:311–2.

## ERRATUM

In an article in the 1 October 2003 issue of the *Journal* (Jacobson RL, Eisenberger CL, Svobodova M, et al. Outbreak of Cutaneous Leishmaniasis in Northern Israel. *J Infect Dis* 2003; 188:1065–73), there is an error in lines 10 and 11 of the “Identification of sand flies” subsection on page 1069: the subgenus

*Larrousius* (shown in parentheses) is incorrect; the correct designations are *Phlebotomus* (*Paraphlebotomus*) *jacusieli* and *Phlebotomus* (*Paraphlebotomus*) *kazeruni*. The authors regret this error.