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Research paper

Increased prevalence of human cutaneous leishmaniasis in Israel and the Palestinian Authority caused by the recent emergence of a population of genetically similar strains of *Leishmania tropica*

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ABSTRACT

Twelve unlinked microsatellite markers were used to determine the microsatellite profiles of 50 newly and 46 previously typed strains of *L. tropica* from various Israeli and Palestinian foci. Their microsatellite profiles were compared to those of 99 previously typed strains of *L. tropica* from 15 countries. Israeli and Palestinian strains of *L. tropica* fell into three different groups, one of which contained 75 of the 96 Israeli and Palestinian strains. This population separated from all the others at the first hierarchical level by Bayesian statistics and formed a distinct monophyletic group on applying genetic distance and allele frequency analyses. The second cluster contained ten Israeli strains from a specific focus north of the Sea of Galilee, which were previously shown to differ from all other strains of *L. tropica* in their serological, biochemical and molecular biological parameters. This cluster was closely related to clusters comprising strains of *L. tropica* from Africa. Four Israeli and five Palestinian strains fell into different genetic entities mostly related to strains from Asian foci of CL.

Importation during numerous migrations of humans and, perhaps, infected reservoir animals in the past and, now, through modern travel is the most likely explanation for the existence of so many locally encountered genetic variants of *L. tropica* in the Israeli-Palestinian region. Geographical and ecological variation may play a role in expanding the genetic heterogeneity once given importations had become established in different foci. Currently, one population is expanding in the area comprising almost all of the Palestinian and Israeli strains of *L. tropica* isolated since 1996 and investigated in this study, which differ clearly from all other strains of whatsoever origin. This population seems to result from the re-emergence of a previously existing genotype owing to environmental changes and human activities.

1. Introduction

In Israel and the Palestinian Authority, two indigenous species of *Leishmania* (Kinetoplastida: Trypanosomatidae), *L. major* and *L. tropica*, cause most of the human cases of cutaneous leishmaniasis (CL). A third indigenous species, *L. infantum*, is the main cause of human cases of visceral leishmaniasis (VL) however, recently the first four cases of human CL caused by *L. infantum* were reported in the Israeli-Palestinian region (Azmi et al., 2012b).

Originally, the species *L. major* and *L. tropica* in the Israeli-Palestinian region differed in their distribution, depending on differences in their ecological habitats and modes of transmission. Most of the human cases of CL diagnosed in the study area were caused by *L. major* and these cases occurred mainly in the Jordan and Arava Valleys and in foci along the Western Negev. CL caused by *L. major* is a zoonotic disease and the distribution of the parasites was governed primarily by the need of soft soil for burrowing by the desert rodents, mainly *Psammomys obesus*, that serve as the animal reservoir. However, *L. tropica* was distributed more widely and mainly in the hilly and mountainous regions. Human cases of CL caused by *L. tropica* were sparse and it was considered to be anthroponotic (reviewed, Jaffe et al., 2004). More recent field studies carried out in Israel have suggested that some of the

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human cases of CL caused by *L. tropica* are zoonoses with rock hyraxes (*Procavia capensis*) serving as the animal reservoir (Jacobson et al., 2003; Svobodova et al., 2006).

Epidemiological surveys and field studies carried out from the late 1990s onward have documented a very significant increase in the number of human cases of CL caused by *L. tropica* and their wider geographical distribution throughout the central area of the Israeli-Palestinian region (Al-Jawabreh et al., 2004; Azmi et al., 2012a, 2012b; Schnur et al., 2004; Singer et al., 2008) and the emergence of new foci on the western side of the Sea of Galilee by the City of Tiberias and in the Lower Galilee north of this 'sea' (Fig. 1) (Svobodova et al., 2006; Vinitsky et al., 2010). Based on records for the years 2002 to 2009, many human cases of CL caused by *L. tropica* were recorded in the Jenin District, with an average annual incidence of 23.0 per 100,000 inhabitants (Azmi et al., 2012b). In some cases, the increase in the number of cases coincided with an increase in the numbers of rock hyraxes (*P. capensis*) close to human habitations, especially where the cases have occurred. In fact, a number of the hyraxes caught in the northern foci close to the Sea of Galilee were shown to be positive for DNA from *L. tropica* (Jacobson et al., 2003; Svobodova et al., 2006).

L. tropica is a very heterogeneous parasitic species associated with a broad and quite complex clinical spectrum of disease ranging from simple CL presenting as either single or multiple lesions to relatively rare cases of leishmaniasis recidivans (LR); rarer cases of visceral leishmaniasis (VL), including some that have also displayed post kala azar dermal leishmaniasis (PKDL) after treatment; and even an occasional case of oro-nasal and naso-pharyngeal leishmaniasis (Azmi et al., 2012a; Magill et al., 1993; Sacks et al., 1995; Schnur et al., 1981; Schnur and Greenblatt, 1995). Many previous studies have exposed the considerable serological (Azmi et al., 2012a; Jacobson et al., 2003; Jaffe et al., 1990; Schnur et al., 2004), biochemical (Azmi et al., 2012a; Le Blancq and Peters, 1986; Mebrahtu et al., 1992; Nimri et al., 2002; Pratlong et al., 1991; Rioux et al., 1990) and genetic heterogeneity (Azmi et al., 2012a, 2013; Krayter et al., 2014a; Schonian et al., 2001) of this species.

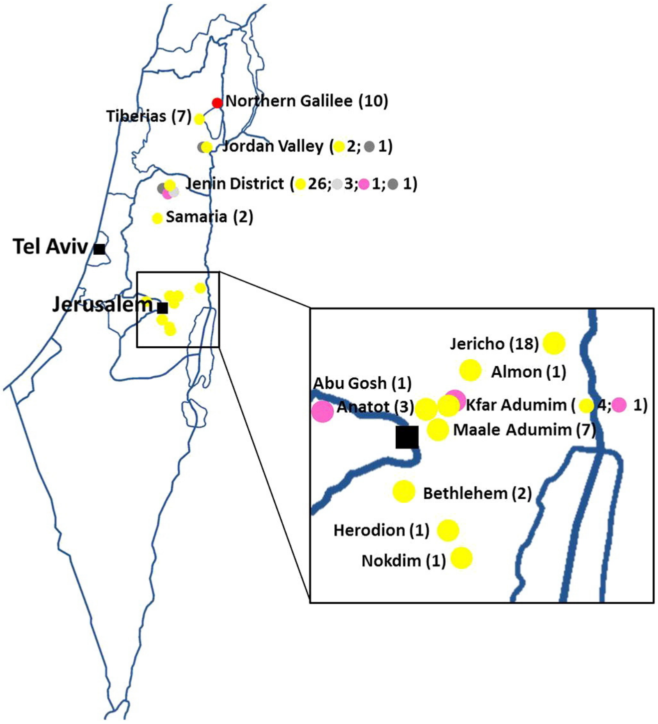

Fig. 1. Distribution of strains of *L. tropica* in the Israeli-Palestinian region. Map showing the geographical distribution of the Israeli and Palestinian strains, indicating their assignment to the different populations inferred by Bayesian statistics. The colours in the circles represent the different populations. The numbers in parentheses are the number of strains of the given populations. Two strains from Jenin had shared membership and have been excluded from the figure as have two other strains owing to their unspecified geographical origins. This map is a modification based on the one shown at https://commons.wikimedia.org/w/index.php?curid=491722 under a CC BY 2.5 license by Golbez.

In an earlier application of multilocus microsatellite typing (MLMT) (Schwenkenbecher et al., 2006), 21 independent microsatellite markers were used on 48 strains of *L. tropica* from different Israeli and Palestinian foci that fell into three distinct clusters of strains, named I, II and IV. Their 'cluster I' comprised most of the strains of *L. tropica* from the different Israeli foci and those in the Palestinian foci in Jenin and Jericho Districts. Their 'cluster IV' comprised strains of *L. tropica* from the focus of human CL north of the Sea of Galilee, where, in contrast to other foci, human CL caused by *L. tropica* is transmitted mainly by female sand flies of the species *Phlebotomus arabicus* and less so by *P. sergenti* known to be the usual vector of *L. tropica*. Their 'cluster II' comprised many other strains of *L. tropica* of various sources and geographical origins, and, interestingly, two older Israeli strains isolated in 1949 and 1990, and four more recently isolated Palestinian strains of *L. tropica* from the Jenin District. The existence of, at least, three different genetic categories of Israeli and Palestinian strains of *L. tropica* based on microsatellite profiles was paralleled by the different strains' distinctive antigenic, isoenzyme profile and kDNA attributes (Azmi et al., 2012a, 2013; Jacobson et al., 2003; Schnur et al., 2004).

So far, MLMT has proved to be the most discriminating means for identifying and distinguishing strains of *L. tropica*. However, the use of 21 markers is expensive, labour-intensive and time-consuming. To minimize these limitations, the set of microsatellite markers has been reduced from 21 to 12 and proven to have the same discriminatory power in population genetic studies (Krayter et al., 2014b). Here, the reduced set of microsatellite markers was applied to the genetic characterization of more recently isolated Israeli and Palestinian strains of *L. tropica*, mainly from human cases of CL but also from sand fly vectors and animal hosts, whose microsatellite profiles were compared to those of the previously isolated strains of *L. tropica* from the Israeli-Palestinian region and, also, to strains of *L. tropica* from various sources and of many different geographical origins.

2. Materials and methods

2.1. Ethical clearance

The strains of *L. tropica* from human cases were isolated during routine diagnosis with no unnecessary invasive procedures and with written and/or verbal consent recorded at the time of clinical examination. Case data were encoded upon sample collection to confer anonymity. The Ethical Committees of Al-Quds University and the Hebrew University-Hadassah Medical School, Jerusalem, approved all activities involving human subjects.

2.2. Parasite strains

Fifty strains of *L. tropica*, 31 Palestinian and 19 Israeli, were studied, all of which were isolated from human cases of CL between 2000 and 2011, except for two of the Israeli ones, one of which came from a hyrax of the species *Procavia capensis*; the other from a female sand fly of the species *P. sergenti*. The provenances of these test strains are listed in Table S1 in bold print.

Their microsatellite profiles were compared to those of 145 strains of *L. tropica*, most of which had been typed previously (Krayter et al., 2014a, 2014b; Schwenkenbecher et al., 2006). The strains used for comparison were isolated mainly from human cases of CL and a few from human cases of VL, female sand flies and hyraxes, 21 of which came from Israeli foci and 25 from Palestinian foci, and the rest from other various geographical locations (Table S1, Fig. 1). Microsatellite profiles of eight strains of *L. aethiopica* (Table S1) were also included for comparison (Krayter et al., 2015).

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