Infection, Genetics and Evolution xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

Research paper

Molecular epidemiology of human cutaneous leishmaniasis in Jericho and its vicinity in Palestine from 1994 to 2015

A. Al-Jawabreh [a,b,c,d,*], K. Dumaidi [c], S. Ereqat [b,d,e], H. Al-Jawabreh [a,b], A. Nasereddin [b,d], K. Azmi [b,d,e], F. Barghuthy [f], S. Sawalha [g], I. Salah [a], Z. Abdeen [b,d]

[a] Leishmaniases Research Unit, Jericho, Palestine
[b] Al-Quds Public Health Society, Jerusalem, Palestine
[c] Department of Medical laboratory Sciences, Faculty of Allied Health Sciences, Arab American University in Jenin, Palestine
[d] Al-Quds Nutrition and Health Research Institute, Al-Quds University, East Jerusalem, Palestine
[e] Biochemistry and Molecular Biology Department, Faculty of Medicine, Al-Quds University, Abu Deis, East Jerusalem, Palestine
[f] Laboratory Department, Arab Health Center, Jerusalem, Palestine
[g] Ministry of Health, Ramallah, Palestine

ARTICLE INFO

Article history:
Received 26 March 2016
Received in revised form 1 June 2016
Accepted 2 June 2016
Available online xxxx

Keywords:
Cutaneous leishmaniasis
Jericho
Palestine
L. tropica
L. major

ABSTRACT

Cutaneous leishmaniases (CL) are vector-borne parasitic diseases endemic in many countries of the Middle East including Palestine. Between 1994 and 2015, 2160 clinically suspected human cases of CL from the Jericho District were examined. Stained skin tissue smears and aspirates were checked by microscopy and cultured for promastigotes, respectively. For leishmanial species identification, amplification products from a PCR-ITS1 followed by RFLP analysis using *Hae* III. Data were analyzed using Epi Info free-software. The overall infection rate was 41.4% (895/2160), 56.3% (504/895) of the cases were male, 43.7% (391/895) female, 60.5% (514/849) children under age 14, 41.3% (259/627) of the cases were caused by *Leishmania major* and 57.3% (359/627) by *Leishmania tropica*. The case numbers peaked in 1995, 2001, 2004, and 2012. Statistically-significant clusters of cases caused by *L. major* were restricted to the Jericho District; those caused by *L. tropica* were from the districts of Jericho, Bethlehem, Nablus and Tubas. CL is seasonal and trails the sand fly season. Distribution of cases was parabolic with fewest in July. The monthly total number of cases of CL and just those caused by *L. major* correlated significantly with temperature, rainfall, relative humidity, evaporation, wind speed and sunshine ($P < 0.05$, $r^2 = 0.7–0.9$ and $P < 0.05$, $r^2 = 0.5–0.8$, respectively). Cases caused by *L. tropica*, significantly, had a single lesion compared to cases caused by *L. major* ($P = 0.0001$), which, significantly, had multiple lesions ($P = 0.0001$). This and previous studies showed that CL is present in all Palestinian districts. The surveillance of CL has increased public awareness and molecular biological methodology for leishmanial species identification is an essential addition to classical diagnosis. The overall results are discussed, correlated to climatic and environmental changes and large-scale human activities.

1. Introduction

Within the last century starting from 1910, cutaneous leishmaniasis (CL) in Palestine has received attention by scientists including Palestinians, French and Germans with appearing and disappearing foci over time. Then Palestinian and Israeli scientists worked extensively on wider and deeper scales in the Jordan Valley. Within few years of discovering that the diseases now known as the leishmaniases were caused by protozoan parasites, amastigotes were seen by light microscopy in stained smears of skin tissue from dermal lesions of inhabitants living in the area specified (Canaan, 1916, 1929, 1945; Huntemüller, 1914). The means for characterizing and differentiating leishmanial parasites were not available then and all cases of human CL were described as being caused by a single species, i.e., *Leishmania tropica*. Separation of this clinically defined 'species' into the two definite species *L. tropica* and *Leishmania major* came much later and was based firstly on biological and epidemiological criteria, then also on serological, indicating antigenic, differences, and finally corroborated by biochemical and genetic differences (Bray et al., 1973). Classically, the species *L. tropica* is considered to be anthroponotic, being transmitted from person to person by female sand flies of the species *Phlebotomus sergenti* (Al-Jawabreh et al., 2004; Schnur et al., 2004), rather than zoonotic and being transmitted from infected animals to people. However, recent studies have indicated that in some cases, at least, the species *L. tropica* is also zoonotic with the rock hyraxes, *Procavia capensis*, serving as the animal reservoir

* Corresponding author at: Leishmaniases Research Unit, Jericho, Palestine.
E-mail address: islahjr@yahoo.com (A. Al-Jawabreh).

http://dx.doi.org/10.1016/j.meegid.2016.06.007
1567-1348/

(Jacobson et al., 2003; Svobodova et al., 2006; Talmi-Frank et al., 2010). In a focus north of the Sea of Galilee (Lake of Tiberias), female sand flies of the species *Phlebotomus arabicus* were found harbouring parasites of the species for *L. tropica* and are considered to be their specific local vectors in addition to the more ubiquitous sand flies of the species *Ph. sergenti* (Jacobson et al., 2003; Svobodova et al., 2006). Human infections caused by either species occur seasonally in parallel with sandfly vector abundance, which increases from mid-spring through to mid-autumn (Muller et al., 2011; Orshan et al., 2010; Sawalha et al., 2003; Schlein et al., 1982).

The geographical area encompassing the southern part of the Jordan Valley down to and including the northern shores of the Dead Sea with the city of Jericho at its center is a typical focus of zoonotic cutaneous leishmaniasis (CL) caused by the species *Leishmania major* with female sand flies of the species *Phlebotomus papatasi* and desert rodents, i.e., sand rats, of the species *Psamommys obesus* serving as the vectors and animal reservoir, respectively (Gunders et al., 1968a, 1968b; Schlein et al., 1982, 1984). Despite the Jordan Valley and Dead Sea area being known as a focus of zoonotic CL caused by the species *L. major*, the species *L. tropica* has also been implicated in causing human cases of CL in this area and those bordering it. Human cases of CL caused by the parasites of both species, *L. tropica* and *L. major*, have been reported in all the other West Bank Palestinian districts (Al-Jawabreh et al., 2003, 2004; Azmi et al., 2012; Jaffe et al., 2004). Those caused there by *L. major* are probably importations, following visits to the Jordan Valley and Dead Sea area. The situation concerning leishmaniases in general in the Gaza Strip is unknown with no autochthonous infection reported.

Implementation of the Oslo accords signed in 1993 led to significant changes in all the Palestinian districts relinquished to the Palestinian authorities among them the refurbishing of government institutions like the Palestinian Ministry of Health and new development in urbanization and agriculture (Arabic-Islamic States, 2016). It also enabled the return of Palestinians domiciled in other countries, in some of which various leishmaniases are endemic, leading to the possible importation of non-indigenous types of leishmanial parasite. In addition to urbanization and agriculture; conflict is another factor highly correlated to the CL outbreak as in the case of civil war in Syria (Al-Salem et al., 2016). This article is a study of human CL that occurred in the Jericho District during the 21-year period between 1994 and 2015. It aimed to correlate fluctuations in the numbers of human cases with changes in climatic and environmental conditions, and human activities like immigration, urbanization and agriculture while also comparing the number cases caused by *L. major* with those caused by *L. tropica* where possible.

2. Materials and methods

2.1. Study design

A long-term cross-sectional study on the prevalence of human CL was conducted, covering the 21-year period between 1994 and 2015, which included a descriptive analysis of the clinical manifestations seen and demographic parameters of the cases encountered.

2.2. Study area

The study was conducted in the Jericho District, which includes the Palestinian part of the Jordan Valley, which is part of one of the classical foci of human CL. The city of Jericho (A'riha) (latitude of 31° 52′ N and longitude 35° 28′) is the only city in the Palestinian part of the Jordan Valley with a large population of 22,609 inhabitants, reaching 52,154 inhabitants when the outlying villages and refugee camps are included (Palestinian Central Bureau of Statistics, 2015a). In addition to part of the Jordan Valley, the Jericho District incorporates the eastern part of Jerusalem (Al-Quds) and the cities of Bethlehem, Ramallah, Nablus, and Tubas and the countryside surrounding them. The total area of the Jericho District is about 593 km^2 and it has the lowest population density of all the Palestinian districts at 71 persons per km^2 (Palestinian Central Bureau of Statistics, 2009, 2015a). The Jordan Valley in the vicinity of Jericho has a unique topography with an elevation of 300 m below sea level, which drops to 400 m below sea level at the southern end of the Dead Sea (Applied Research Institute Jerusalem, 2011c). The Jericho District is a hot, dry, semi-arid area with a rainy season, approximately, from October to March, an annual cumulative rainfall averaging 166 mm, a mean average temperature of 20.4 °C and absolute minimum and maximum temperatures of −0.4 °C and 46.4 °C, respectively (Palestinian Central Bureau of Statistics, 2015b; Palestinian Metrological Authority, 2015).

2.3. Human cases

Between February 1994 and June 2015, 2260 patients with skin lesions suggestive of CL attended or were referred to the Leishmaniases Research Unit (LRU) in Jericho for laboratory diagnosis and confirmation as cases of CL. The patients were Palestinians living in the Jericho District and its vicinity with the exception of a few patients who were temporary sojourners working on international projects in the aforementioned areas. A patient's data sheet was filled in for each patient prior to taking tissue samples. The data sheet included: demographic data such as name, address, age and sex; a clinical description; and epidemiological information such as travel history and number, positions and duration of lesions.

2.4. Diagnosis

Diagnosis included conventional and molecular diagnostic methods.

2.4.1. Microscopy

Five touch smears were prepared from lesion(s), stained with Giemsa stain, and examined microscopically for amastigotes (Al-Jawabreh et al., 2006).

2.4.2. In-vitro culture

Beginning in 1998, dermal tissue aspirates were cultured as described previously (Al-Jawabreh et al., 2003).

2.4.3. DNA extraction

Beginning in June 1997, dermal tissue scrapings from lesions were blotted onto autoclaved Whatman no. 4 filter papers (Whatman International Ltd., England) for leishmanial DNA extraction as described previously (Al-Jawabreh et al., 2004). However, samples before that date were extracted from Giemsa-stained smears. In this case, the stained dermal tissue was removed by spreading 50 μl of lysis buffer onto the surface of the slide and scratched off with a sterile surgical blade into a sterile 1.5 ml micro-centrifuge tube. The procedure was repeated several times until a total volume of 250 μl of lysis buffer accumulated in the tube. Then, the DNA was extracted as described above for filter papers.

2.4.4. PCR amplification

The ribosomal internal transcribed spacer 1 (ITS1) region separating the genes coding for ssu rRNA and L5.8S rRNA was amplified by a PCR, using the primers LITSR and L5.8S as described elsewhere (Al-Jawabreh et al., 2004, 2006; El Tai et al., 2000; Schonian et al., 2003). At a later stage, DNA was accurately quantified, using the Thermo scientific NanoDrop 2000 spectrophotometer. Also, commercial master mix kit PCR-Ready from Syntezza (Syntezza Bioscience Ltd., Jerusalem) was used for amplification of DNA.

2.4.5. Restriction fragment length polymorphism (RFLP)

PCR products derived from the ITS1 region were digested with the restriction enzyme *Hae* III according to the conditions recommended by the supplier (Promega, Promega Corporation, USA) and as described

Please cite this article as: Al-Jawabreh, A., et al., Molecular epidemiology of human cutaneous leishmaniasis in Jericho and its vicinity in Palestine from 1994 to 2015, Infection, Genetics and Evolution (2016), http://dx.doi.org/10.1016/j.meegid.2016.06.007

by others (Al-Jawabreh et al., 2004; El Tai et al., 2000; Schonian et al., 2003).

2.5. Statistical analysis

Epi Info™ statistical package (CDC free-software) was used to analyze data, which included distribution and frequency tables and graphs. Spot mapping of cases of CL was performed using Epi Info 7. Contingency 2 × 2 tables for infections of CL and risk factors were tested using the two-tailed Fisher's exact test and chi square. SaTScan™ v8.0 Freeware was used to detect statistical evidence for purely spatial and space-time clustering of cases caused by *L. major*, cases caused by *L. tropica* and the total number of cases. SaTScan™ v8.0 input files included number of cases per locality, year of infection, population size of location in the year of infection, and the exact latitude-longitude coordinates of each location. Data were analyzed based on discrete Poisson model with level of statistical significance considered at P-value ≤0.05 (Kulldorff, 1997).

3. Results

3.1. Study population and demographic parameters

The study population consisted of 2160 suspect human cases of CL who were referred to the LRU in Jericho. Out of the 2160 suspected cases, 41.4% could be confirmed as CL (n = 895) (Fig. 1(a)). The genotyping of DNA extracted from infected skin tissues and leishmanial strains isolated from the human cases diagnosed as cases of CL showed that 57.3% (359/627) of the cases were caused by *L. tropica*, 41.3% (259/627) were caused by *L. major* and for nine (1.4%) of the cases of CL the species of *Leishmania* could not be determined (Fig. 1(b)). About 56.3% (504/895) of the cases were male and 43.7% (391/895) were female, and 60.5% (514/849) were children under 14 years old. Male and female cases were equally infected by the species *L. major* and *L. tropica* (P = 0.12). However, cases caused by *L. tropica* were significantly more frequent in adults (P = 0.02). Of the cases under 19 years old, 67.3% (572/849) were schoolchildren. Of the adult cases according to occupation, 13.7% (69/502) were soldiers, 9.8% (49/502) were housewives, 6.5% (33/502) were workers and 3% (15/502) were farmers.

Between 1994 and 2015, there were four peak periods in the numbers of cases of CL recorded: the first was in 1995 with cases caused only by *L. major*; the second was in 2001 with case caused equally by *L. major* and *L. tropica*; the third was in 2004 with cases caused mainly by *L. major* and some by *L. tropica*; the fourth was in 2012 with cases caused by *L. tropica* (Fig. 2).

3.2. Geographic distribution and CL clusters

The CL cases in the study came from 77 Palestinian localities, which included cities, villages, refugee camps and Bedouin encampments. Most of them (628/876 = 72%) were from the Jericho District. Cases diagnosed as caused by *L. tropica* came from 64 localities covering all 11 West Bank districts, and those diagnosed as caused by *L. major* came from 20 localities covering only nine of the West Bank districts (Fig. 3(a)). Implementing purely spatial analysis by SaTScan revealed 12 statistically significant clusters of cases caused by *L. tropica* and 6 of cases caused by *L. major* (Fig. 3(b)). However, implementing the space-time statistical analysis revealed 13 significant clusters of cases caused by *L. tropica* and six of cases caused by L. major in years between 2002 and 2012 (Fig. 3(c)). In both analyses, all the statistically significant clusters caused by *L. major* were restricted to the Jericho District, but the Jericho District villages, refugee camps, Bedouin encampments and Jericho City itself provided significant clusters caused by *L. major* and *L. tropica*. Although purely spatial analysis located disease clusters over the entire study period as a result of accumulation of cases, however, weaker or silent clusters may appear temporarily for a short period of time which can only be revealed by space-time analysis particularly when there is more than one genotype circulating. In Fig. 3, cluster 8 appeared by purely spatial analysis, while clusters 13 and 14 appeared only by space-time analysis.

3.3. Clinical picture

More than half (56%, 455/788) of the cases of CL had a single lesion, 21.5% (170/788) had two lesions and 22.5% (172/788) had three or more lesions. Cases caused by *L. tropica*, significantly, tended to have a single lesion compared to cases caused by *L. major* (P = 0.0001), which, significantly, caused more cases with multiple lesions (P = 0.0001). The head and face appear to be the main sites for the bites of the sand fly vectors (53%, 401/758), followed by the upper extremities (29%, 220/758) and then the lower extremities (18%, 137/758). More of the cases with lesions on the lower extremities were, significantly, caused by *L. major* compared with those caused by *L. tropica* (P = 0.0043). On the head, the cheek and forehead were the most predominant sites of infection with more lesions on the forehead, significantly, having been caused by *L. major* than *L. tropica* (P = 0.006) (Table 1). In half of the cases, the time between the appearance of a lesion and seeking healthcare was a month or more and only 9% sought help within the first week, with no significant difference between cases caused by *L. major* and cases caused by *L. tropica* (P = 0.16).

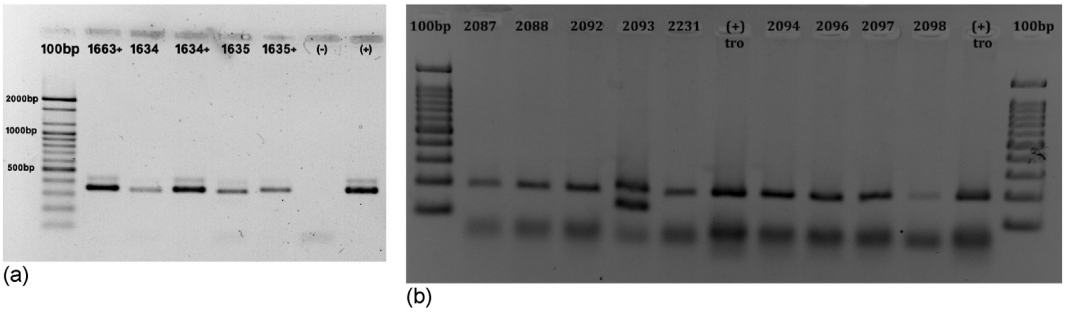

Fig. 1. The molecular biological differentiation of strains of *Leishmania major* and *L. tropica* by amplification of their ribosomal internal transcribed spacer 1 (ITS1), using a polymerase chain reaction (PCR), followed by restriction analysis of the PCR products after their digestion with *Hae* III: (a) the amplified ITS1 products of leishmanial DNA samples isolated from human cases of CL (1663, 1634, 1635) run in a 1.4% agarose gel with a negative control of nuclease-free distilled water (−) and a positive control of amplified ITS1 from a strain of *Leishmania turanica*, MRHO/MN/83/MNR-6, (+) as described by Al-Jawabreh et al. (2006) and where bands of 300–350 bp indicated the presence of leishmanial parasites and confirmed a diagnosis of leishmaniasis; (b) restriction fragment length polymorphism (RFLP) run in a 2% agarose gel where the sample 2093 displays two bands, one of 203 bp, the other of 132 bp, indicating that it came from a strain of *L. major* while the other samples, actually, displaying four bands of 185, 57, 53, and 24 bp came from strains of *L. tropica*, however, only the band of 185 bp is clearly visible here as the rest are either hidden in the primer dimers or unapparent owing to their small size.

Please cite this article as: Al-Jawabreh, A., et al., Molecular epidemiology of human cutaneous leishmaniasis in Jericho and its vicinity in Palestine from 1994 to 2015, Infection, Genetics and Evolution (2016), http://dx.doi.org/10.1016/j.meegid.2016.06.007

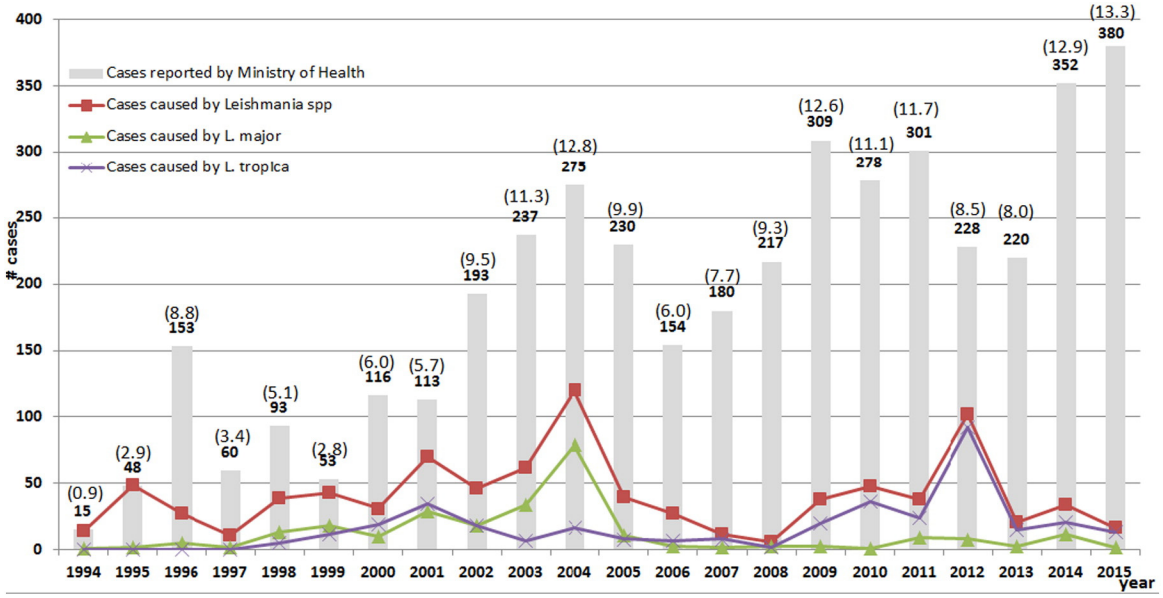

Fig. 2. Cases of CL recorded between 1994 and 2015. Genotyping revealed that cases from Jericho and its vicinity were caused by either *L. major* or *L. tropica* with the number of cases caused by each species changing over time. The bar graph in gray represents the official figures for cases of CL registered by Palestinian Ministry of Health (MoH). The numbers in brackets are the incidence rates per annum in Palestine (West Bank) and the numbers below them represent the total number of cases reported per annum. The line graphs represent the numbers of cases of CL from Jericho and its vicinity studied here and the corresponding numbers of the cases caused by either *L. major* or *L. tropica*, according to the parasites' genotypes.

3.4. Seasonality and climatic trends

The curve displayed in Fig. 4 approximates to a parabola with its apex of symmetry at July. The minimum number of cases occurred in summer, began to increase in September, i.e., the autumn, and continued to do so till the end of spring, i.e., May in the following year. The maximum number of cases was seen in the winter and peak occurred in December, January and February of the following year. The monthly distribution of cases of human CL caused by *L. major* more or less abided by the parabola and showed peaks in January and February of the following year while that caused by *L. tropica* deviated somewhat with peaks in March and April of the following year.

Regarding seasonality, Pearson's correlation was applied to the monthly occurrence of cases in relation to the climatic parameters shown in Fig. 4. The total number of cases of CL showed a significant correlation with all parameters ($P < 0.05$, $r^2 = 0.7–0.9$) for all the cases of

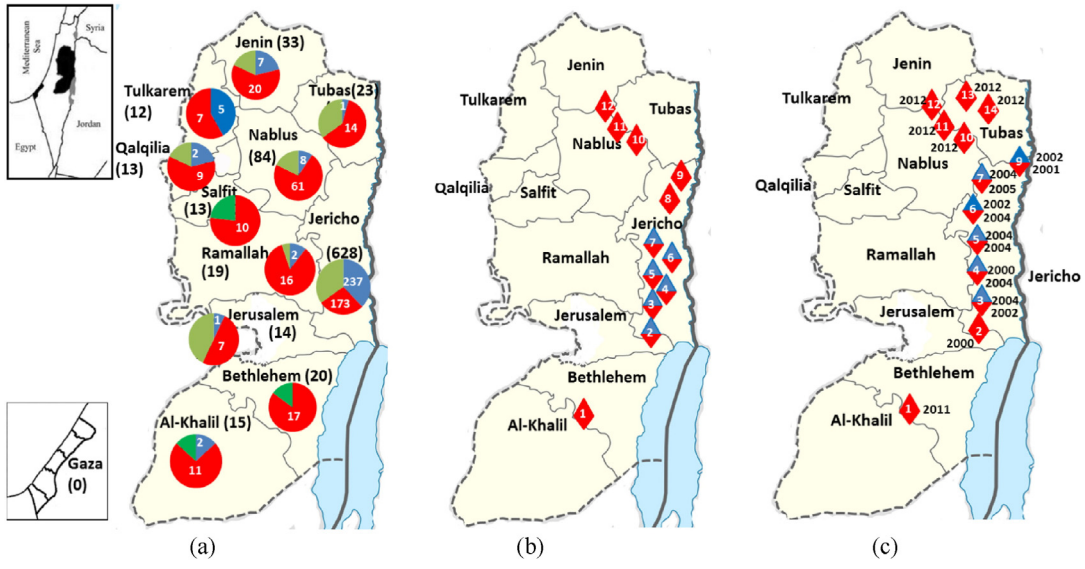

Fig. 3. Distribution of human CL cases. (a) Geographical distribution of human CL in the Palestinian West Bank region between 1994 and 2015: numbers of cases caused by *L. major* are in blue; numbers of cases caused by *L. tropica* are in red; numbers of cases where the species of *Leishmania* remained undetermined are in green. (b) Geographical distribution of human CL on implementing purely spatial analysis by SaTScan: blue triangles show the statistically significant clusters of *L. major*; red triangles show those of *L. tropica*. The numbers within the triangles indicate the location and its type, according to the following list: 1 = Rashydeh, Bedouin encampment; 2 = Nabi Musa, Bedouin encampment; 3 = Aqbat Jabr, refugee camp; 4 = Jericho, city; 5 = Ein-as-sultan, refugee camp; 6 = Al-A'uja, village; 7 = Fasayil, village; 8 = Al-Jiftlik, village; 9 = Zubaidat, village; 10 = Al-Nassarieh, village; 11 = Al-Badhan, village; 12 = Talluza, village; 13 = Tayaseer, village; 14 = Tammon, village. The last two localities are shown only in (c). (c) Geographical distribution of human CL on implementing the space-time analysis by SaTScan: blue triangles show the statistically significant clusters of *L. major*; red triangles show those of *L. tropica*. The year that each cluster occurred is given beside each triangle. The locations and their types are as listed in the legend of (b). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Please cite this article as: Al-Jawabreh, A., et al., Molecular epidemiology of human cutaneous leishmaniasis in Jericho and its vicinity in Palestine from 1994 to 2015, Infection, Genetics and Evolution (2016), http://dx.doi.org/10.1016/j.meegid.2016.06.007

Table 1
Bodily locations of the cutaneous lesions caused by *L. major* compared to those caused by *L. tropica*.

Body site	*L. major*	*L. tropica*	Total	P-value (significance)[a]
Arm	102	118	220	>0.05 (NS)
Leg	74	63	137	=0.0043 (S)
Head:	178	223	401	>0.05 (NS)
Cheek	68	81	149	>0.05 (NS)
Forehead	40	24	64	=0.006 (S)
Nose	17	30	47	>0.05 (NS)
Chin	14	33	47	>0.05 (NS)
Eye	18	14	32	>0.05 (NS)
Neck	9	19	28	>0.05 (NS)
Lip	4	13	17	>0.05 (NS)
Ear	8	9	17	>0.05 (NS)
Total	354	404	758	

[a] Fisher's exact test. S = significant, NS = not significant.

Table 2
Environmental and behavioral factors that appear to increase the risk of contracting CL.

Variable		CL	Non-CL	Odds ratio (95% confidence interval)	P-value[a] (significance)[b]
CL acquired in residential area	Yes	581	722	1.3 (1–1.7)	=0.037 (S)
	No	131	211		
Travelled within the last 3 months	Yes	143	216	0.8 (0.7–1.1)	=0.13 (NS)
	No	618	779		
Other family member(s) infected	Yes	286	287	1.5 (1.2–1.8)	P = 0.0001 (S)
	No	464	693		
Received treatment prior to diagnosis	Yes	197	223	1.3 (1–1.6)	P = 0.045 (S)
	No	506	721		
Agricultural farm within 200 m	Yes	314	384	1.3 (1.0–1.6)	P = 0.027 (S)
	No	317	489		
Pond or spring near residential area	Yes	244	301	1.2 (0.97–1.5)	P = 0.10 (NS)
	No	388	572		
Domestic animals/pet	Yes	198	220	1.5 (1.1–2.1)	P = 0.02 (S)
	No	83	137		

[a] Fisher's exact test.
[b] S = significant, NS = not significant.

CL. The number of cases of CL caused by *L. tropica* correlated significantly with the mean average temperature (°C), mean average rainfall (mm), mean average evaporation (mm), and mean average sunshine (hour/day) ($P < 0.05$, $r^2 = 0.3–0.4$). While, no correlation was found between the number of cases of CL caused by *L. tropica* and mean average relative humidity (%), mean average atmospheric pressure and mean average wind speed (km/h) ($P > 0.05$, $r^2 = 0.1–0.2$). On considering the number of cases of CL caused by *L. major*, there was significant correlation between the number of cases and all the climatic parameters considered ($P < 0.05$, $r^2 = 0.5–0.8$).

3.5. Risk factors

The risk factors affecting the rate of infection of humans with CL can be behavioral, owing to, both, people's life style and activities, and to environmental conditions such as climate and topography. Table 2 shows the statistical significance of factors affecting the contraction of infection of CL.

4. Discussion

Within mainly the district of Jericho during the 1994–2015, the overall infection rate was 41.5%, with an annual average incidence of 7.9 per 100,000 (0.9 to 12.9) within the whole area of the Palestinian West Bank (Fig. 2). During the surveyed period, peaks in the number of human cases of CL occurred. The first was in 1995–6, probably, owing to the introduction of unimmunized individuals such as returnees from abroad, following implementation of the Oslo agreement in 1993, and members of units of the Palestinian Police Force brought in from Palestinian districts free of leishmaniases. They were settled around the city of Jericho near farms and agricultural areas in close proximity to the habitats of sand rats (*Psammomys obesus*), which are the animal reservoir of human CL caused by *L. major* (Al-Jawabreh et al., 2003).

Genotyping the DNA from the leishmanial strains isolated from the 10 cases examined between 1994 and 1997 showed that they were all caused by *L. major*. It was only in 1998 that human cases caused by *L. tropica* started to appear in the study area, leading to the peak in 2001, which was shared with the cases caused by *L. major* (Al-Jawabreh et al., 2004; Schnur et al., 2004). The last peak during the study period occurred in 2004 and was caused mainly by *L. major*. After that, human cases caused by *L. major* were sporadic with those caused by *L. tropica* dominating as shown by the peak of 2012.

Kuldorff's SaTScan was applied to statistically expose the actual spatial and space-time clustering for both species. The six spatial and space-time clusters confirmed the localization of *L. major* to the district of

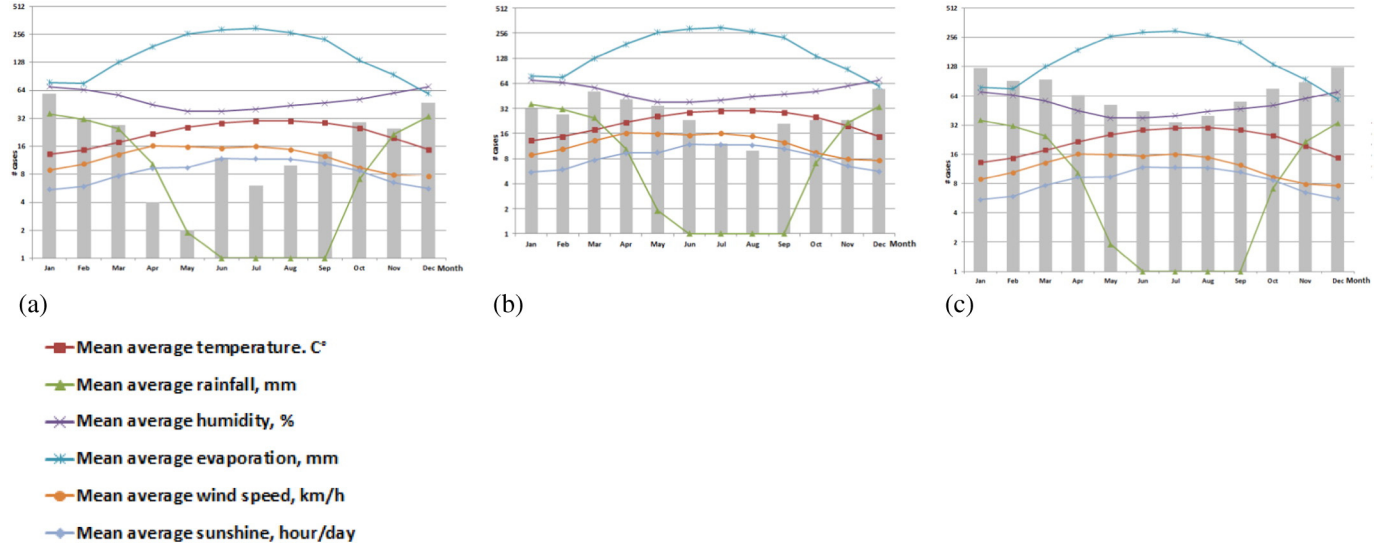

Fig. 4. Cumulative diagrams covering the years 1994 to and including 2014, showing line graphs representing various climatic parameters compared to bar graphs representing number of human cases of CL caused individually by *L. major* (a) and *L. tropica* (b), and for both added together (c). Note each figure is drawn as a calendar year, which allows for the parabola to be seen clearly but the sand fly season begins, approximately, in mid to late spring and cases of CL are caused from then on with some becoming evident only in the following calendar year.

Please cite this article as: Al-Jawabreh, A., et al., Molecular epidemiology of human cutaneous leishmaniasis in Jericho and its vicinity in Palestine from 1994 to 2015, Infection, Genetics and Evolution (2016), http://dx.doi.org/10.1016/j.meegid.2016.06.007

Jericho with *L. tropica* also being present during some years of the study period, while *L. tropica* encompassed four out of 12 spatial clusters and six out of 13 space-time clusters outside the district of Jericho (Fig. 3). It appears that the focus of *L. major* in the district of Jericho is the source of human CL caused by *L. major* seen in the rest of the Palestinian West Bank with cases encountered there having acquired there CL when visiting the vicinity of Jericho. Conversely, the cases of CL caused by *L. tropica* occurring within the human population in and around Jericho are probably acquired while visiting the neighboring hilly and mountainous districts that encompass the foci of *L. tropica* (Al-Jawabreh et al., 2004).

If an animal reservoir is involved in the transmission and spread of human CL caused by *L. tropica* in the Palestinian West Bank region, the most likely candidate would be rock hyraxes (*P. capensis*), which have been found infected with *L. tropica* in neighboring areas (Talmi-Frank et al., 2010). Regarding this, from about the year 2000 the local population started complaining of large numbers wild animals, presumably hyraxes, invading their farms and homes and even coming close to the city of Jericho itself on its western side by Wadi Qelt where many Bedouin inhabitants have acquired CL caused by *L. tropica.* In comparison, rodents of the species *P. obesus* are known to be the animal reservoir of *L. major* in parts of North Africa and the Middle East and were plentiful in the wider environs of the city of Jericho some years ago but the numbers have declined in recent years and they are hardly seen now.

The change in agriculture, leading to decreased humidity in the soil and the atmosphere just above it along with other factors such as less rainfall, dryer climate and drop of the Dead Sea level might have contributed to the change seen regarding the local leishmaniases. Furthermore, the population of Jericho has doubled in the last two decades, causing rapid urbanization with the building of new housing and industrial zones and their concomitant infrastructures. This has reduced the amount of surrounding arable land, decreased the amount of water for irrigation and, subsequently, reduced agricultural activity. It has also invaded and destroyed the foci of zoonotic CL that are closer to the city.

Approximately, from June to September is the dry season with the number of human cases of CL caused by *L. major* and *L. tropica* is at its minimal as shown by the histogram in Fig. 4, which correlates with several climatic conditions that seem to act together, leading to the seasonality of CL. At that time of year, rain fall is 0 mm and daylight sunshine and its concomitant heat become longer and more intensive, causing extremely high temperatures, high rates of evaporation and decreasing humidity, all of which are unfavorable for the reservoir animals, vectors and human hosts by reducing the more lush winter and spring vegetation cover and agricultural activity.

The parabolic distribution of human cases (Fig. 4) might indicate the presence of sand flies all the year round. However, an interpretation as such gives a false impression. In fact, there is a sand fly season (Schlein et al., 1982, 1984). It begins around late spring with the transformation and emergence of adult sand flies that were wingless, non-flying larval stages in the ground during the colder part of the year. Throughout the whole of the sand fly season, the adult sand flies breed, increasing the sand fly population. The females of the species *Ph. papatasi* and *Ph. sergenti* feeding on infected reservoir animals and, later when available, human cases pick up, respectively, infections of *L. major* and *L. tropica*. With this, infections begin to occur in humans and more human cases occur as the sand fly population increases. By late summer and autumn the numbers of sand flies are at their maximum and most human cases are initiated at this time. The sand fly season ends with the disappearance of the sand flies at the end of autumn. Human cases are not initiated after this. However, the human cases initiated during the summer and autumn become patent and are exposed as such in the late autumn and winter. The delay in their appearance is owing to the incubation period in humans, which is longer for *L. tropica* (four to six or more months) than for *L. major* (one to two months).

CL caused by *L. major* and *L. tropica* occurs as ulcers, nodules or papules (Olliaro et al., 2013) but it is difficult to determine whether lesions are cause by either *L. major* or *L. tropica* solely on the clinical picture. However, statistical analysis based on comparing the types of lesions with the genotypes of the leishmanial parasites causing them showed that a single large ulcer is, significantly, more likely to be caused by *L. tropica* while multiple lesions are more likely to be caused by *L. major*. Many cases in the study group had a single severe lesion, which persisted for more than six months and were either resistant to Pentostam™ or showed delayed resolution. On identifying the parasites, these cases proved to have been caused by *L. tropica* (Al-Jawabreh and Nasereddin, 2007; Al-Jawabreh et al., 2004, 2006).

The head was the body site most affected, which was so in other foci in the local and geographically wider regions (Khan and Zakai, 2014; Saroufim et al., 2014). This is, possibly, owing to female vectors being attracted by human hosts' exhaled CO_2 and the sand flies biting and feeding on their faces. Dress codes might also play a role. As the hands tend to be mobile most of the time, this leaves just the face as a feeding site. Lesions appearing on the cheek, the forehead and the lower extremities were, significantly, caused more by *L. major* than by *L. tropica*, which was in congruence with other studies (Bousslimi et al., 2010). Another reason for the difference in the bodily location of lesions caused by the two species could be attributed to the biting and feeding behavior of their respective female sand fly vectors. Female sand flies of the species *Ph. papatasi* are the regional vectors for parasites of the species *L. major* and female sand flies of the species *Ph. sergenti* are for the parasites of the species *L. tropica*; although in a focus just north of the Sea of Galilee, female sand flies of the species *Ph. arabicus* are also vectors of the species *L. tropica* (Jacobson et al., 2003; Svobodova et al., 2006). Furthermore, in some cases, clinical differences can be attributed to hosts' genetic variation (Al-Jawabreh and Nasereddin, 2007; Kobets et al., 2012).

Despite health awareness, more than 50% of the cases tended to seek medical care only one month after the appearance of the lesion, mainly owing to either negligence or underestimation of the situation and thinking it will resolve automatically; especially as CL starts as a small papule and remains so for two to three weeks before ulcerating (Table 1).

This study showed that most of the human cases of CL encountered in the vicinity of the city of Jericho got their infections locally and 19% had travelled away for about three months or more before the appearance of their lesions. A family member with a history of CL is a significant indication that other family member will probably contract CL sooner or later; especially knowing that most of the cases in the study group got infected in their residential area. Other risk factors associated with a significantly increased risk of acquiring CL are the raising of domestic animals, especially sheep and goats, and living close to worked agricultural land with regular irrigation increases (Table 2). Both of these factors ensure an optimal habitat for the reservoir animals and vectors, providing humidity, breeding sites, vegetation and, possibly, alternatives for vectors to feed on other than humans, which, unintentionally, might also protect humans.

5. Conclusion

Following the creation of the Palestinian Ministry of Health in 1994, the Palestinian health system has been actively involved in determining the magnitude of local leishmaniases by implementing a surveillance system that incorporates active and passive components, carried out by official and unofficial health personnel. This depended on increasing public awareness and diligently actively finding and following up human cases of CL and reporting them. As more than one leishmanial species circulate in the overall focus, the stringent diagnostic protocols included a PCR-based genotyping method that identified the leishmanial species involved in each diagnosed case. This indicated prognosis and treatment when necessary, and assisted in ascertaining the epidemiology. Human immigration from other local regions and from abroad into the focus, climatic and environmental factors, e.g., lowered precipitation

Please cite this article as: Al-Jawabreh, A., et al., Molecular epidemiology of human cutaneous leishmaniasis in Jericho and its vicinity in Palestine from 1994 to 2015, Infection, Genetics and Evolution (2016), http://dx.doi.org/10.1016/j.meegid.2016.06.007

and reduced artificial irrigation, and large-scale human activity, especially urbanization and agriculture have had an impact, most probably, through their effect on the sand fly vector and animal host populations. During the study period, a shift was seen to have occurred from most human cases having been caused by *L. major* to most human cases being caused by *L. tropica*.

Acknowledgments

This work received financial support from grants of the Deutsche Forschungsgemeinschaft (DFG), Scho 448/6-1-3, Scho 448/8-1, Scho 448/8-2 that extended from 1998 until 2015. It also received support from EurNegVeg COST Action TD1303 (Cost 037/13). At one time during the study WHO Eastern Mediterranean Region (EMRO), Division of Communicable Diseases (DCD) and the WHO Special Programme for Research and Training in Tropical Diseases (TDR): the EMRO/DCD/TDR Small Grants Scheme for Operational Research in Tropical and Communicable Diseases financially supported this work.

We thank Dr. L. F. Schnur for going over our manuscript.

References

Al-Jawabreh, A., Nasereddin, A., 2007. Leishmaniasis recidivans in a Palestinian Bedouin child. Skinmed 6, 250–252.

Al-Jawabreh, A., Barghuthy, F., Schnur, L.F., Jacobson, R.L., Schoenian, G., Abdeen, Z., 2003. Risk assessment of cutaneous leishmaniasis in the endemic area of Jericho in Palestine. East Mediterr. Health J. 9, 805–815.

Al-Jawabreh, A., Schnur, L.F., Nasereddin, A., Schwenkenbecher, J.M., Abdeen, Z., Barghuthy, F., Khanfar, H., Presber, W., Schonian, G., 2004. The recent emergence of *Leishmania tropica* in Jericho (A'riha) and its environs, a classical focus of *L. major*. Tropical Med. Int. Health 9, 812–816.

Al-Jawabreh, A., Schoenian, G., Hamarsheh, O., Presber, W., 2006. Clinical diagnosis of cutaneous leishmaniasis: a comparison study between standardized graded direct microscopy and ITS1-PCR of Giemsa-stained smears. Acta Trop. 99, 55–61.

Al-Salem, W., Pigott, D.M., Subramaniam, K., Haines, L.R., Kelly-Hope, L., Molyneux, D.H., Hay, S.I., Acosta-Serrano, A., 2016. Cutaneous leishmaniasis and conflict in Syria. Emerg. Infect. Dis. 22, 931–933.

Applied Research Institute Jerusalem, 2011c. Jericho, Palestine; 2008–2010. In: Unit, G. (Ed.), Applied Research Institute Jerusalem, Bethlehem, Palestine.

Arabic-Islamic States, 2016. Peace Agreements & Related, Declaration of Principles on Interim Self-Government Arrangements ("Oslo Agreement"), 13 September 1993.

Azmi, K., Schonian, G., Nasereddin, A., Schnur, L.F., Sawalha, S., Hamarsheh, O., Ereqat, S., Amro, A., Qaddomi, S.E., Abdeen, Z., 2012. Epidemiological and clinical features of cutaneous leishmaniases in Jenin District, Palestine, including characterisation of the causative agents in clinical samples. Trans. R. Soc. Trop. Med. Hyg. 106, 554–562.

Bousslimi, N., Aoun, K., Ben-Abda, I., Ben-Alaya-Bouafif, N., Raouane, M., Bouratbine, A., 2010. Epidemiologic and clinical features of cutaneous leishmaniasis in southeastern Tunisia. Am.J.Trop. Med. Hyg. 83.

Bray, R.S., Ashford, R.W., Bray, M.A., 1973. The parasite causing cutaneous leishmaniasis in Ethiopia. Trans. R. Soc. Trop. Med. Hyg. 67, 345–348.

Canaan, T., 1916. The Jericho boil (in German). Arch. Schiffund Tropenhygiene 20, 109–119.

Canaan, T., 1929. The oriental boil: an epidemiological study in Palestine. Trans. R. Soc. Trop. Med. Hyg. 23, 89–94.

Canaan, T., 1945. Topographical studies in leishmaniasis in Palestine. J. Palest. Arab. Med. Assoc. 1, 4–13.

El Tai, N.O., Osman, O.F., El Fari, M., Presber, W., Schönian, G., 2000. 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. 94, 575–579.

Gunders, A.E., Foner, A., Montilio, B., 1968a. Identification of *Leishmania* species isolated from rodents in Israel. Nature 219, 85–86.

Gunders, A.E., Lidror, R., Montilo, B., Amitai, P., 1968b. Isolation of *Leishmania* sp. from *Psammomys obesus* in Judea. Trans. R. Soc. Trop. Med. Hyg. 62, 465.

Huntemüller, 1914. Neuartige Parasitenbefunde bei der Jerichobeule. Zentralbl. Bakteriol. Abt. I. Orig. Bd. 73, 137–141.

Jacobson, R.L., Eisenberger, C.L., Svobodova, M., Baneth, G., Sztern, J., Carvalho, J., Nasereddin, A., El Fari, M., Shalom, U., Volf, P., Votypka, J., Dedet, J.P., Pratlong, F., Schonian, G., Schnur, L.F., Jaffe, C.L., Warburg, A., 2003. Outbreak of cutaneous leishmaniasis in Northern Israel. J. Infect. Dis. 188, 1065–1073.

Jaffe, C.L., Baneth, G., Abdeen, Z.A., Schlein, Y., Warburg, A., 2004. Leishmaniasis in Israel and the Palestinian Authority. Trends Parasitol. 20, 328–332.

Khan, W., Zakai, H.A., 2014. Epidemiology, pathology and treatment of cutaneous leishmaniasis in Taif region of Saudi Arabia. Iran. J. Parasitol. 9, 365–373.

Kobets, T., Havelkova, H., Grekov, I., Volkova, V., Vojtiskova, J., Slapnickova, M., Kurey, I., Sohrabi, Y., Svobodova, M., Demant, P., Lipoldova, M., 2012. Genetics of host response to *Leishmania tropica* in mice — different control of skin pathology, chemokine reaction, and invasion into spleen and liver. PLoS Negl. Trop. Dis. 6, e1667.

Kulldorff, M., 1997. A spatial scan statistic. Commun. Stat. Theory Methods 36, 1481–1496.

Muller, G.C., Kravchenko, V.D., Rybalov, L., Beier, J.C., Schlein, Y., 2011. Characteristics of resting habitats of adult *Phlebotomus papatasi* in Neot Hakikar, an oasis south of the Dead Sea. J. Vector Ecol. 36 (Suppl. 1), S179–S186.

Olliaro, P., Vaillant, M., Arana, B., Grogl, M., Modabber, F., Magill, A., Lapujade, O., Buffet, P., Alvar, J., 2013. Methodology of clinical trials aimed at assessing interventions for cutaneous leishmaniasis. PLoS Negl. Trop. Dis. 7.

Orshan, L., Szekely, D., Khalfa, Z., Bitton, S., 2010. Distribution and seasonality of *Phlebotomus* sand flies in cutaneous leishmaniasis foci, Judean Desert, Israel. J. Med. Entomol. 47, 319–328.

Palestinian Central Bureau of Statistics, 2009. Census Final Results — Population Report West Bank. Second Part ed. Palestinian Central Bureau of Statistics, Ramallah-Palestine.

Palestinian Central Bureau of Statistics, 2015a. Localities in Jericho & Al Aghwar Governorate by Type of Locality and Population Estimates, 2007–2016, Ramallah-Palestine.

Palestinian Central Bureau of Statistics, 2015b. Meteorological conditions in the Palestine, annual reports 2003–2014. Summary of Main Findings Ramallah, Palestine.

Palestinian Metrological Authority, M.o.T., 2015. Climatic Bulletins, 2005–2014, Ramallah-Palestine.

Saroufim, M., Charafeddine, K., Issa, G., Khalifeh, H., Habib, R.H., Berry, A., Ghosn, N., Rady, A., Khalifeh, I., 2014. Ongoing epidemic of cutaneous leishmaniasis among Syrian refugees, Lebanon. Emerg. Infect. Dis. 20, 1712–1715.

Sawalha, S.S., Shtayeh, M.S., Khanfar, H.M., Warburg, A., Abdeen, Z.A., 2003. Phlebotomine sand flies (Diptera: Psychodidae) of the Palestinian West Bank: potential vectors of leishmaniasis. J. Med. Entomol. 40, 321–328.

Schlein, Y., Warburg, A., Schnur, L.F., Gunders, A.E., 1982. Leishmaniasis in the Jordan Valley II. Sandflies and transmission in the central endemic area. Trans. R. Soc. Trop. Med. Hyg. 76, 582–586.

Schlein, Y., Warburg, A., Schnur, L.F., Le Blancq, S.M., Gunders, A.E., 1984. 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. 78, 480–484.

Schnur, L.F., Nasereddin, A., Eisenberger, C.L., Jaffe, C.L., El Fari, M., Azmi, K., Anders, G., Killick-Kendrick, M., Killick-Kendrick, R., Dedet, J.-P., Pratlong, F., Kanaan, M., Grossman, T., Jacobson, R.L., Schonian, G., Warburg, A., 2004. Multifarious characterization of *Leishmania tropica* from a Judean desert focus, exposing intraspecific diversity and incriminating *Phlebotomus sergenti* as its vector. Am.J.Trop. Med. Hyg. 70.

Schonian, G., Nasereddin, A., Dinse, N., Schweynoch, C., Schallig, H.D., Presber, W., Jaffe, C.L., 2003. PCR diagnosis and characterization of *Leishmania* in local and imported clinical samples. Diagn. Microbiol. Infect. Dis. 47, 349–358.

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

Talmi-Frank, D., Jaffe, C.L., Nasereddin, A., Warburg, A., King, R., Svobodova, M., Peleg, O., Baneth, G., 2010. *Leishmania tropica* in rock hyraxes (*Procavia capensis*) in a focus of human cutaneous leishmaniasis. Am.J.Trop. Med. Hyg. 82, 814–818.

Please cite this article as: Al-Jawabreh, A., et al., Molecular epidemiology of human cutaneous leishmaniasis in Jericho and its vicinity in Palestine from 1994 to 2015, Infection, Genetics and Evolution (2016), http://dx.doi.org/10.1016/j.meegid.2016.06.007