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## Epidemiological and clinical features of cutaneous leishmaniasis in Jenin District, Palestine, including characterisation of the causative agents in clinical samples

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### ABSTRACT

During 2002–2009, 466 cases of cutaneous leishmaniasis (CL) were reported from Jenin District, Palestine, affecting both genders. The average annual incidence was 23 cases per 100 000 inhabitants, increasing with age in children. Most cases presented a single lesion, generally on the face. Diagnosis and species identification was done by applying internal transcribed spacer 1 (ITS1) RFLP analysis to 47 isolates, of which 44 (93.6%) were *Leishmania tropica* and 3 (6.4%) were *L. major*. RFLP analysis was also performed on 256 skin tissue scrapings spotted onto filter papers, showing that 138 (53.9%) were positive, of which 50.7% were infected with *L. tropica*, 17.4% with *L. major* and 2.9% with *L. donovani* s.l., and 29.0% could not be identified. This is the first report from Palestine on human CL caused by *L. infantum*. Nine of the strains of *L. tropica* were subjected to multilocus enzyme electrophoresis, six of which belonged to the zymodeme MON-137 and three to a new zymodeme (MON-307). This separation was corroborated by excreted factor serotyping. This observation modifies the classical epidemiological view of CL in Palestine. Jenin District is an active focus of CL caused by *L. tropica*, where *Phlebotomus sergenti*, the putative vector, is abundant. These data suggest that CL is a zoonotic infection, but an animal reservoir has not been found.

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### 1. Introduction

Cutaneous leishmaniasis (CL), caused by *Leishmania major* and *L. tropica*, is an important public health

problem in Palestine.<sup>1–4</sup> The epidemiology of CL, a zoonotic vectorborne disease caused by *L. major*, has been studied in depth,<sup>1,3,5,6</sup> whereas that of *L. tropica* has not. From 1990–1999, the highest rate of CL was in the vicinity of Jericho [Palestinian Ministry of Health (PMOH)], where *L. major* was the main cause, followed by Jenin District (JD), where *L. tropica* has been its main cause. Outbreaks were not investigated in depth in order to estimate the average annual incidence of CL and to identify the species of *Leishmania* involved. *Leishmania tropica* has, rarely, been the cause of visceral leishmaniasis (VL).<sup>7–9</sup> Classically, CL

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caused by *L. tropica* is considered to be anthroponotic, however in Palestine and Israel it appears to be zoonotic, with rock hyraxes (*Procapra capensis*) serving as the reservoir host in Israel.<sup>10,11</sup> The vector for *L. tropica* has not been unequivocally identified in JD. *Phlebotomus sergenti*, which is the main vector in Israel,<sup>12</sup> is also the putative vector in Palestine.<sup>13</sup>

Recently, new foci of CL caused by *L. tropica* appeared in different parts of Palestine. The northern region of the West Bank is considered to be a main focus of *L. tropica* and most cases were reported from JD. Endemicity is still increasing, but the distribution of cases and factors affecting transmission are unknown. Owing to the increasing number of CL cases occurring in JD in recent years, and to discern its risk to the local population, clinical and epidemiological information from 2002–2009 was gathered and collated and is presented in order to increase the awareness of physicians and clinics of CL, including what is currently known about its causes and transmission.

## 2. Materials and methods

### 2.1. Geographic distribution and topography

All cases of CL analysed in this study came from JD, located in the northern part of the West Bank, which is a hilly region of approximately 592 km<sup>2</sup> with elevations of 90–750 m a.s.l. The rainy season in JD is from mid October to the end of April [data from the Applied Research Institute in Jerusalem (ARIJ), 2008], with its peak in January and February, and the mean annual rainfall is 528 mm. The mean annual relative humidity ranges between a mean of 39% in summer and mean of 84% in winter. However, the onset and continuation of a sand fly season requires an optimal combination of humidity and temperature. Since JD is in a mountainous region and winter temperatures can be very low with snow in some years, while summer temperatures can be very high, the annual average maximum and minimum temperatures have little bearing on sand fly abundance and the average maximum and minimum temperatures are given for June and August and were 34.2 °C and 17.3 °C, respectively (ARIJ, 2008).

The total population of JD at the end of the period under consideration was 259 361 (10.9% of the Palestinian population), living in 96 localities (Palestinian Central Bureau of Statistics, 2009). The inhabitants are predominantly villagers working in and dependent on agriculture. Most houses have yards where sheep, goats, chickens and other domestic animals are kept. Houses are built of concrete and stone, with the more affluent residents frequently building in suburban areas, where most cases of CL and, in fact, also those of VL are reported.

### 2.2. Collection of epidemiological and clinical data

The Ethics Research Committee of the University of Al-Quds (Palestine) approved all activities involving human subjects. Epidemiological data were collected on 466 CL cases seen from 2002–2009. Clinical data were obtained for 256 patients admitted to the clinics of PMOH

for treatment in Jenin from whose lesions biopsy samples were taken for laboratory diagnosis of CL.

The inclusion criterion was the presence of one or more cutaneous lesions with a signed physician's report of suspected CL. A questionnaire was completed for each case presenting cutaneous lesions, recording the patient's full name, age, gender, number of lesions, lesion sites and duration, and patient's address, work place and travel history.

### 2.3. Collection of samples

Tissue aspirates were collected and seeded into semi-solid normal rabbit blood agar medium and were spotted onto filter papers (GB002; Schleicher and Schuell, Dassel, Germany).<sup>14,15</sup> Mass culture of promastigotes was done at 26 °C.<sup>16</sup>

### 2.4. DNA extraction

DNA was extracted from cultured promastigotes according to van Eys et al.<sup>17</sup> and from infected tissue skin samples spotted onto filter papers using a high purification template PCR kit (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's instructions.

### 2.5. DNA amplification and analysis

Two PCR-based methods were employed: one described by Rodgers et al.<sup>18</sup> that amplifies a 120 bp sequence in the conserved region of the leishmanial minicircle kDNA; and another by Schonian<sup>19</sup> that amplifies the ribosomal internal transcribed spacer 1 (ITS1). All PCRs were carried out in a volume of 25 µl using PCR-Ready Mix Supreme (Syntezza Bioscience, Jerusalem, Israel). Five microlitres of eluted DNA from tissue on filter paper was used in the PCR. Amplicons were analysed electrophoretically in 2% agarose gels and were visualised by UV light. For species identification, 10 ng of DNA of *L. infantum* MHOM/TN/1980/IPT1, *L. tropica* MHOM/IL/1998/LRC-L747 and *L. major* MHOM/SU/1973/5ASKH was used as controls. Reaction buffer without leishmanial DNA was included as a PCR-negative control. The ITS1 PCR product (15–20 µl) was digested with the restriction endonuclease *HaeIII* or its prototype (*BsuRI*; MBI Fermentas, Amherst, NY, USA) according to the manufacturer's instructions, and the restriction fragments were separated electrophoretically in 3% agarose gels containing ethidium bromide (0.3 µg/ml) for visualisation by UV light.

### 2.6. Characterisation and identification by excreted factor (EF) serotyping and multilocus enzyme electrophoresis (MLEE)

EF serotyping was done according to Schnur and Zuckerman,<sup>20</sup> and MLEE was performed according to Pratlong et al.<sup>21</sup> and Rioux et al.<sup>22</sup>

## 2.7. Statistical analysis

$\chi^2$  and NPar tests were used to analyse the epidemiological data using SPSS V.17.0 (SPSS Inc., Chicago, IL, USA).

## 2.8. Collection and identification of sand flies

Sand flies were collected on 18 nights in 2008 and 20 nights in 2009 during May through to the end of September. In total, 1492 sand flies were collected from four foci in JD where the highest numbers of human CL cases had occurred using CDC miniature light traps (John W. Hock Company, Gainesville, FL, USA) and aspirator collection as described by Killick-Kendrick.<sup>23</sup> Collection was done inside and near the entrances of caves as well as in and around homes. After examining the sand flies for leishmanial infections, their heads and genitalia were mounted on microscope slides in Berlese fluid for taxonomic identification.<sup>24</sup> Females belonging to *Phlebotomus* spp. were identified by the structures at the base of the spermathecal ducts. Females that were seen to have imbibed blood or had swollen abdomens suggesting infections after the digestion of blood were dissected and examined for the presence of promastigotes.

## 3. Results

### 3.1. Geographic distribution and demographics

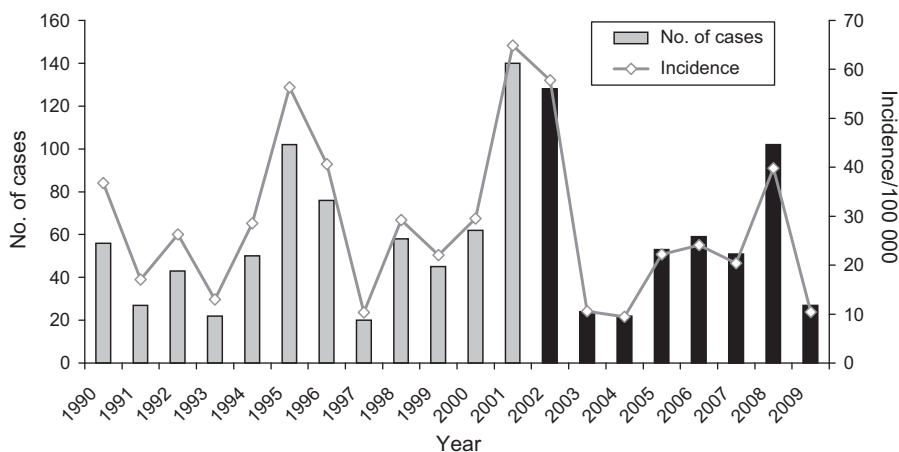
From January 2002 to July 2009, 466 individuals attending the clinics of the PMOH in Jenin City were registered as human cases of CL. This was done solely on the basis of the development and clinical appearance of lesions and place of habitation of the individual. Parasitological confirmation and identification of the leishmanial species were not done at that time. Figure 1 shows the trends in the occurrence of CL during 2002–2009 when the average annual incidence was 23.0 per 100 000 inhabitants. The prevalence of CL was

190.1 per 100 000. Peaks of infection occurred in 2002 and 2008. A similar periodic peak of infection was seen in 1995 (Figure 1 based on data from previous public health records and from the current study), suggesting a cycle of increasing and decreasing infection rates. Cases initiated towards the end of the sand fly season, more or less from May to November in Palestine,<sup>24</sup> in a given year are often recorded in the following year and peaks of infection can span two consecutive years as in 2001 and 2002 (Figure 1).

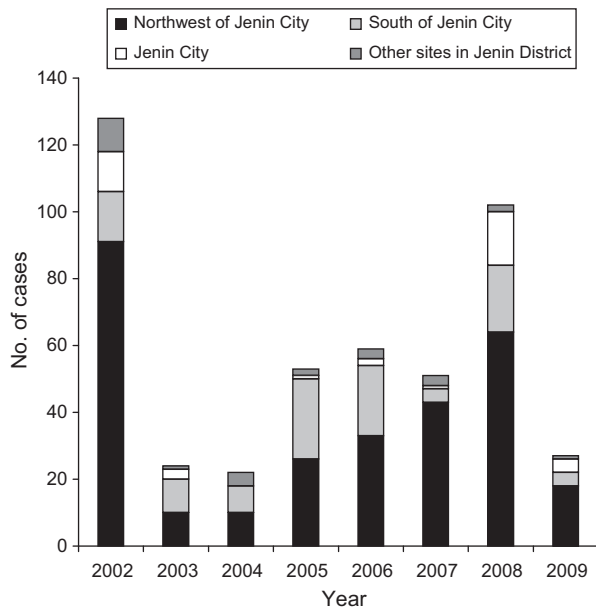
The annual number of reported cases of CL varied between 128 cases in 2002 and 22 cases in 2004, with rates varying between 8.9 to >50.5 per 100 000 inhabitants. The highest number of cases and incidence per 100 000 inhabitants were, respectively, 128 and 50.5 in 2002 and 102 and 39 in 2008. Most cases had dry lesions of long duration and, of the 212 patients seen between the years 2006–2008, 170 (80.2%) developed signs and symptoms of disease between January and May, indicating a long incubation period (data not shown), a situation classically accepted as associated with CL caused by *L. tropica*.

Although the patients were distributed throughout JD, most were from south of Jenin City, mainly Qabatya, and northwest of Jenin City at the villages of El Yamoon and Silat El Hartheyia (Figure 2 and Supplementary Figure 1). In fact, the highest infection rate during the period of this study occurred in the area northwest of Jenin City, where 63.3% of all the cases occurred with a total average annual incidence of 119.4 cases per 100 000 inhabitants; most of these cases (288; 61.8%) occurred in the vicinity of El Yamoon and Silat El Hartheyia at an altitude of 140–200 m a.s.l. Lower infection rates were found in the southern part of JD (106; 22.7%) and in urban Jenin (39; 8.4%). The lowest infection rates (2.8% and 1.1%, respectively) occurred in the eastern and western parts of JD, with a total average annual incidence of 5.8 and 2.0 per 100 000 inhabitants, respectively.

Cases were of all ages. Figure 3 shows the age and gender distribution. The mean age was 22 years (median



**Figure 1.** Cases of cutaneous leishmaniasis (CL) recorded for Jenin District, Palestine, during 1990–2001 (grey, taken from public health records) and 2002–2009 (black, from this study). The histogram and line graph show the number of new cases of CL per year and the yearly rate of cases per 100 000 inhabitants, respectively (population 259 361 in 2009).



**Figure 2.** Annual distribution of cases in Jenin District, Palestine, during 2002–2009 by main locality: Jenin City; south of Jenin City (Qabatya); northwest of Jenin City (El Yamoon and Silat El Hartheyia); and other sites in Jenin District (including Aba, Araqa, Arabeh, Beit Qad, Deir Abu Da'if, Hashemeya, Kafr Dan, Meselya, Raba, Sanur, Serees, Al Shuhada and Ya'bad, places where just one or two cases occurred).

16.0 years; interquartile range 9–30 years), with the oldest case being an 86-year-old male and the youngest a 1-year-old female. Of the 466 cases diagnosed as CL, 257 (55.2%) were male and 209 (44.8%) were female, a ratio of 1.2:1 with no significant association with gender.

Approximately one-half (49.6%) of the infections were in patients under 15 years old, with the incidence appearing to increase with age; 14.7% in those up to and including 4 years old and 26% in those >5 years old, with significant differences ( $p < 0.05$ ) compared with the adult group. The lowest number (82; 17.6%) was in the group aged >40 years. Combining gender with age groups showed that adult males aged 20–39 years were at the highest risk ( $p < 0.05$ ) (Figure 3).

### 3.2. Number of lesions and body sites involved

The 466 cases presented a total of 686 lesions. The head was most commonly affected (235/466; 50.4%), especially the cheek (173; 37.1%), followed by the upper limb (168; 36.1%) and then the lower limb (33; 7.1%). Multiple lesions involved one or more parts of the body in 30 cases (6.4%). Single lesions occurred in 331 cases (71.0%); most of these were on the head (174/331; 52.6%), followed by the upper limb (139/331; 42.0%). Two lesions were seen in 83/466 cases (17.8%), and 52/466 cases (11.2%) showed from three to seven lesions. Males had more lesions on the upper limb than females (117/168; 69.6%), and females had more lesions on the head (128/235; 54.5%) ( $p < 0.05$ ), mainly the cheek (101/173; 58.4%) (Figure 3).

Figure 4 correlates the number of lesions with age. There were 225 children, who had 48.6% (161/331) of the single lesions.

### 3.3. Detection and identification of leishmanial parasites causing cutaneous leishmaniasis in Jenin District

Interviews with the dermatologists who referred suspected cases to PMOH clinics indicated that most of the skin lesions that were seen and treated by them as if they were CL were 0.5–5 cm in diameter, dry in appearance, and showed granulation and papules appearing at their periphery. Skin tissue from 256 suspected cases was smeared on glass slides and stained with Giemsa stain. This was done by local laboratory staff not well practiced in this technique. On microscopic examination for amastigotes, they proved to be poor in dermal tissue, more like blood films and not very useful. All were negative for amastigotes, but this result was not conclusive.

However, skin tissue from these patients was also smeared onto filter papers for leishmanial DNA analysis. The 120 bp fragment amplified by the kDNA PCR is present and diagnostic for all species of *Leishmania* and in this study was diagnostic for the cases of CL as it was found in 249 (97.3%) of the 256 skin samples tested; cases giving a positive kDNA PCR were considered confirmed cases of CL and included cases of CL caused by *L. major*, *L. tropica* and *L. infantum*. The ITS1 PCR-RFLP was used for species identification. The ITS1 PCR amplified the diagnostic 300 bp fragment in 138 (53.9%) of the 256 samples. The RFLP pattern of the ITS1 amplicon of 98 (71.0%) of the 138 strains clearly enabled identification of their leishmanial species. The other 40 samples (29.0%) could not be identified owing to weak PCR amplification. *Leishmania tropica* was identified as the causative agent in 70/138 samples (50.7%), *L. major* in 24/138 (17.4%) and, surprisingly, *L. donovani* s.l. in 4/138 (2.9%).

Partial sequencing of ITS1 revealed identical sequences for the latter four patients, which corresponded to that of the *L. infantum* reference strain MHOM/TN/1980/IPT1 (data not shown). These sequences have been submitted to GenBank under accession numbers **JN181861–JN181864**.

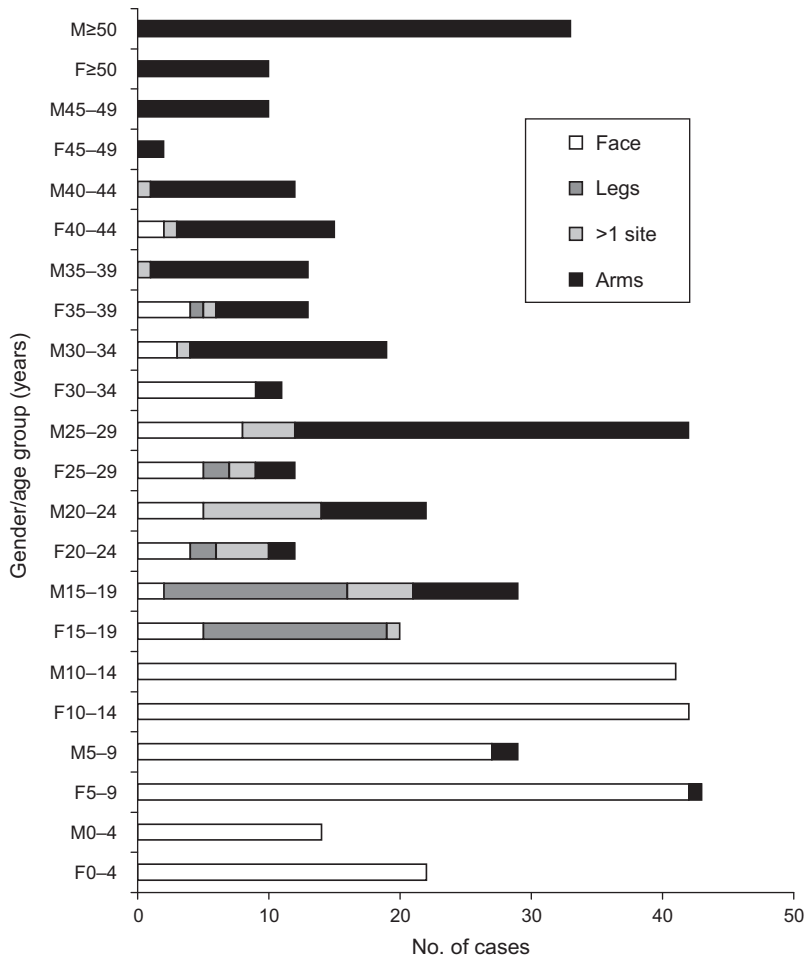
Sixty strains were isolated from dermal tissue aspirates by culture, 13 (22%) of which were lost through concomitant bacterial and fungal contamination. Of the 47 cultures, 44 (93.6%) were *L. tropica* by ITS1 PCR-RFLP and 3 (6.4%) were *L. major* (Figure 5).

### 3.4. Characterisation of leishmanial strains from culture

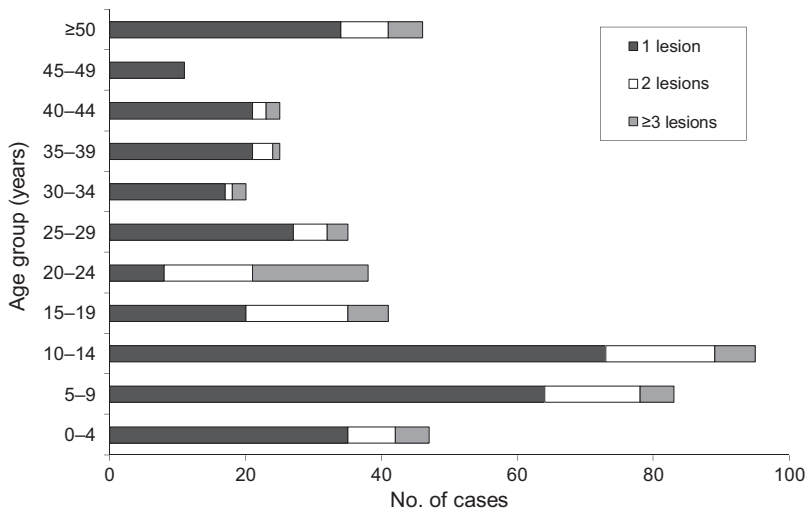
Unfortunately, cultures obtained from the cases shown to be caused by *L. infantum* by DNA analysis did not survive. However, EF serotyping of used medium from one of them showed the strain was EF subserotype B<sub>2</sub>, which was compatible with the DNA results.

EF serotyping of the 41 other cultures showed that 38 were either EF subserotype A<sub>2</sub> or A<sub>9</sub>B<sub>4</sub> and therefore *L. tropica*, and 3 were EF subserotype A<sub>1</sub> and therefore *L. major*. This was also compatible with the DNA results.

Nine strains of *L. tropica* were typed by MLEE and fell into two different zymodemes: six belonged to the zymodeme



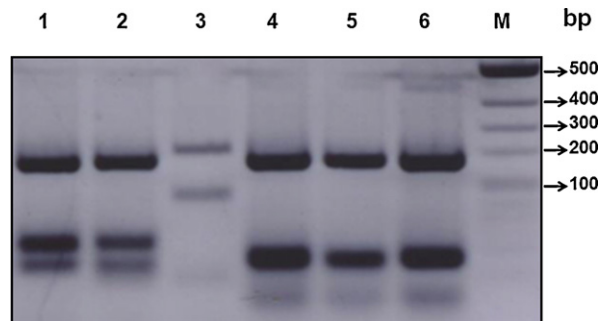
**Figure 3.** Anatomical distribution of cutaneous lesions by age group of cases from Jenin District, Palestine, during 2002–2009. F: females; M: males.  $p = 0.017$ , Mann–Whitney test.



**Figure 4.** Number of cutaneous lesions per patient and their anatomical distribution by age group in cases from Jenin District, Palestine, during 2002–2009.  $p = 0.032$ , Kruskal–Wallis test.

**Table 1**  
Abundance of different sand fly species in four different foci of Jenin District, Palestine

Species	Foci								Total (%)
	El Yamoon		Silat El Hartheyia		Qabatya		Jenin		
	F	M	F	M	F	M	F	M	
<i>Phlebotomus sergenti</i>	108	131	33	16	3	2	1	1	295 (32.0)
<i>Phlebotomus papatasi</i>	40	23	84	68	7	3	2	5	232 (25.2)
<i>Phlebotomus tobbi</i>	44	42	21	6	39	52	1	1	206 (22.3)
<i>Phlebotomus syriacus</i>	17	51	2	1	3	11	1	1	87 (9.4)
<i>Phlebotomus perfiliewi</i>	4	1	15	18	2	2	0	0	42 (4.6)
<i>Phlebotomus neglectus</i>	13	15	0	3	3	5	0	0	39 (4.2)
Other species	14		2		5		0		21 (2.3)
Total	503		269		137		13		922



**Figure 5.** RFLP of the internal transcribed spacer 1 (ITS1) of representative isolates from Jenin District, Palestine, following digestion with *Hae*III. Lanes 1 and 2: *Leishmania infantum* MCAN/IL/1996/LRC-L692 and MHOM/TN/1980/IPT1, respectively; lane 3: *L. major* MHOM/SU/1973/5ASKH; lane 4: *L. tropica* MHOM/IL/1998/LRC-747; and lanes 5 and 6: *L. tropica* MHOM/PS/2002/LRC-L893 and MHOM/PS/2002/LRC-L890, respectively, from Jenin. M: DNA molecular marker phiX174 DNA/Hinf.

MON-137 and three belonged to a new zymodeme (MON-307) (K. Azmi et al., unpublished data).

### 3.5. Identification of sand flies

Of the 1462 sand flies trapped, 922 (63.1%) were species of *Phlebotomus* (Table 1) and 540 (36.9%) were species of *Sergentomyia*. The percentages of the different species of *Phlebotomus* were: *P. (Paraphlebotomus) sergenti*, 32.0%; *P. (Phlebotomus) papatasi*, 25.2%; *P. (Larroussius) tobbi*, 22.3%; *P. major syriacus*, 9.4%; *P. (Larroussius) perfiliewi*, 4.6%; and *P. major neglectus*, 4.2%. The remaining 2.3% belonged to the species *P. (Paraphlebotomus) alexandri*, *P. (Synphlebotomus) sp.*, *P. (Larroussius) mascitti*, *P. (Adlerius) kazeruni*, *P. (Adlerius) arabicus* and *P. (Adlerius) halepensis* (Table 1). None of the female sand flies that were dissected showed infections of promastigotes.

## 4. Discussion

In 1978, Blum<sup>25</sup> described a focus of CL at Salfit in the Salfit District of the West Bank, Palestine (32° 7'5.5" N, 35° 5'25" E) (Supplementary Figure 1). Arda and Kamal<sup>1</sup> described some epidemiological aspects of CL in the West Bank from 1972–1980 where the average annual incidence of CL was 5.04, 2.35, 0.34, 0.14 and 0.1 per 100 000 in the

districts of Salfit, Jericho, Jenin, Tulkarem and Nablus, respectively. Since then, JD has been an active focus of leishmaniasis since the 1970s. Cases of CL and VL occur, however published information on both is sparse.<sup>1,26</sup> The incidence rate of CL in JD has increased since then. At a local level, the overall annual incidence rate differs between urban localities, ranging from 2.0 per 100 000 in the west of Jenin City to 119.4 per 100 000 in the northwest, possibly owing to different distributions of reservoir animals and sand flies. These are affected by annual rainfall,<sup>6</sup> which ranges between 600–800 mm/year in the western parts and 350–550 mm/year in the eastern parts.

In the 1960s, cases of CL were low in the eastern part of JD. From 1990–2000, Sawalha<sup>13</sup> noted an abundance of potential vectors of CL in the eastern and central parts of JD when, according to the PMOH, there was an increase in cases. The latter could be explained by lifestyle changes, e.g. ceasing burning animal manure for cooking, which produced heavy smoke that kept sand flies away.

Infection rates appear unrelated to altitude but related to specific topographical and climatic characteristics of locations. For example, from 1990–2000 and from 2002–2009, 135 and 288 cases, respectively, were recorded from the vicinity of El Yamoon and Silat El Hartheyia, two neighbouring towns at an altitude of 140–160 m a.s.l. in the northwestern part of JD. At the same time, *P. sergenti*, the putative vector of *L. tropica*, was the most abundant sand fly species. Most cases were from the mountain of Abu Zreek, an undeveloped area between El Yamoon and Silat El Hartheyia. Only three cases were recorded from Deir Abu Da'if in the eastern part of JD at an altitude of 200 m a.s.l., from where 32 cases had been recorded between 1990 and 2000.<sup>13</sup> During the intervening period, rainfall dropped considerably causing drought conditions, which were more severe in the eastern part.

At first, Arda and Kamal<sup>1</sup> reported that there had been no cases from Qabatya, but later mentioned 62 cases that were diagnosed clinically. Here, 66 cases from Qabatya were diagnosed parasitologically and/or by molecular biological methods. The occurrence of cases from new foci in Meselya, Jalbun, Hashemeya, Araqa and Arabeh could be related to either introduction of the vector and/or the arrival of reservoir animals from existing foci. Caves and cervices in the rocks, piles of wood and stones, and stone walls around houses on the periphery of towns and villages supposedly offer good conditions for sand flies to

rest and possibly breed. Rock hyraxes are considered the natural animal reservoir of CL caused by *L. tropica*.<sup>10,11</sup> The piles of boulders cleared from arable land provide a perfect habitat, although there are fewer in the central parts of the residential areas where hyraxes have also been seen. Towns expanded massively during the 1990s. New houses were constructed at town peripheries where sand flies are more abundant.<sup>13</sup> Peripheral areas receive poorer services, e.g. less refuse collection, exposed wastewater and solid waste dumps that attract stray dogs and wild animals, including wild canines possibly serving as reservoir hosts. In fact, during a survey on VL caused by *L. infantum* in JD, a dog was caught, sampled and proved to be infected with *L. tropica*.<sup>27</sup> Dogs carrying *L. tropica* have been caught in Morocco<sup>28,29</sup> and Syria.<sup>29</sup>

Cases of CL caused by *L. tropica* occur sporadically at the periphery of human habitation and are unlikely to be anthroponotic in JD as elsewhere.<sup>30,31</sup> This suggests zoonotic transmission, as in Israel.<sup>10,32</sup> Blum<sup>25</sup> checked rats from Salfit and saw amastigotes in stained smears, but cultures were not made for species identification. El-Adhami<sup>33</sup> isolated a strain (MRAT/IQ/1973/MRCB-IBF) from *Rattus rattus* (black rat) caught in Baghdad that was *L. tropica*.<sup>34,35</sup> We caught and examined eight black rats that were uninfected (unpublished data). Svobodova<sup>36</sup> infected rats with *L. tropica* by feeding infected sand flies on them, demonstrating the potential of rats as a reservoir of CL caused by *L. tropica*.

Most patients visited clinics from January to May. Klaus et al.<sup>37</sup> suggested that exposure to CL normally occurs from May to November with a peak from June to July. Sawalha<sup>13</sup> found sand flies to be most abundant between June and August. It is reasonable to assume that infections occurred mainly from June to November in JD. This is supported by low numbers of cases from July to November compared with those at the end of the same year and in the first half of the next year.

Here, the increase in the annual incidence between the peaks of infection in the years 2002 and 2008 might indicate a 6–7-year cycle (Figure 1). Prior to this and taken from older public health records, there was a peak in the number of cases in 1995 where the incidence reached almost 16 per 100 000, declining to 1.9 per 100 000 by 2000 and 2001, possibly modulated by more efficient spraying against sand flies after a year of more cases.

All age groups were involved (Figure 3), with more cases among children, which increased with age. More males aged 20–39 years were affected. Most lesions were on the head and upper limbs owing to human behaviour during summer months when people spend the early evening outdoors. This is also the sand fly season. People do not use bed nets. Females were often more infected on their faces than males, who are more often infected on their hands and upper limbs. This gender difference is statistically significant and is probably associated with dress codes and type of employment. Blum<sup>25</sup> also noted this and attributed it to differences in dress and behaviour between age groups and between males and females. Killick-Kendrick<sup>23</sup> suggested that sand flies are more often attracted to the faces of people because of the carbon dioxide-enriched atmosphere on exhalation.

Clinics of the PMOH did not differentiate between CL caused by *L. major* and *L. tropica*. *Leishmania major* was accepted as the cause of all cases of CL as it is called Jericho boil in Palestine and the area around Jericho is a focus of CL caused by *L. major*.<sup>3</sup> *Leishmania tropica* also exists close to there.<sup>4</sup> Taking account of the need to differentiate leishmanial species, this study attempted to combine detection of leishmanial parasites in biopsy aspirates by a kDNA PCR with species identification by ITS1 PCR-RFLP.

The discrepancy between the high percentage (97.3%) of positive samples revealed by kDNA PCR compared with the low percentage (53.9%) revealed by ITS1 PCR is due to the smaller size (120 bp) of the amplified kDNA product from the minicircles compared with the larger size (300 bp) of the amplified nDNA product from the ITS1 region.<sup>38</sup>

Most biopsies were positive for CL by the kDNA PCR, but species identification was achieved in only 53.9% of the PCR-positive samples. Most of the CL cases from JD were caused by *L. tropica*, confirmed by PCR-RFLP and EF serotyping of all strains, and MLEE of nine strains. MLEE revealed genetic heterogeneity among the strains of *L. tropica* and six strains identified previously were of the zymodeme MON-137; the other three were of the new zymodeme MON-307. Of the 24 cases of CL diagnosed in local clinics as having been caused by *L. major*, 11 said that they had visited known foci of *L. major* in the Jordan Valley and the Negev area. One was unsure of the geographical origin and 12 maintained that they had not travelled to areas where *L. major* circulates. This is unlikely in the latter 12 cases as they could only have contracted their infections where *L. major* circulates, unless they were infected through the bites of sand flies that had fed on other human cases caused by *L. major*, who had contracted their infections where *L. major* circulates. Alternatively, people passing through endemic regions and only stopping briefly often do not think that this may be sufficient to acquire an infection. Four cases of CL were caused by parasites of the '*L. donovani* complex', supposedly *L. infantum*. These patients said that they had not travelled outside of JD. CL caused by *L. infantum* has been described in other Mediterranean countries.<sup>39–41</sup> However, until this survey *L. infantum* had caused only human infantile and canine VL in JD.<sup>26,42</sup> This was the first record of CL caused by *L. infantum* in a Palestinian focus. The species composition of sand flies collected in JD during 2008–2009 showed that *P. sergenti*, the proven vector of *L. tropica* in several countries, including neighbouring Israel,<sup>11,12</sup> and the putative vector in Palestine, was the most abundant species. This correlated well with the occurrence of human cases of CL. *Phlebotomus tobbi* and *P. perfiliewi*, the proven vectors of *L. infantum* in other countries, were also caught in JD (Table 1). Domestic dogs with canine leishmaniasis caused by *L. infantum* have been reported.<sup>26</sup> *Phlebotomus papatasi*, the proven vector of *L. major* throughout North Africa and the Middle East, was also caught. However, the known animal reservoir of *L. major* does not exist locally in JD.

The following limitations of this study are worthy of attention. This study was based on people with cutaneous lesions visiting physicians and clinics and being defined clinically as CL cases. However, CL is usually chronic, self-limiting and self-curing and many cases do not bother to visit physicians or attend clinics so the number of cases



referred to here is probably lower than actually occurred during 2002–2009. An active home-by-home survey and referral of suspected cases to clinics for diagnosis and parasite identification would rectify this. Publication of information like that presented here should help to create greater awareness and thus improve future studies and assessments. Some patients with CL caused by *L. major* denied visiting any areas where *L. major* circulates. Possibly, as stated above, they do not understand that even a brief visit, i.e. driving through and just stopping at a viewing point or roadside cafe, is sufficient to get bitten by an infected sand fly. Physicians must be sure to point this out to their patients. This study was based wholly on human cases. No leishmanial strains have been isolated from sand flies or animals that might have proven to be vectors and reservoir hosts. Nor was DNA extracted from sand flies that might have implicated species of sand flies as vectors of any of the species of *Leishmania* causing human CL. The molecular biological diagnostic tests applied here to human clinical samples might have proved useful in this as it did in the diagnosis of human cases. The sand fly collections referred to here could be used for this in a further study.

In conclusion, cases of CL have been increasing in the northern part of the West Bank region and some areas have a higher density of infections than others. Human cases tend to present single 'dry' facial skin lesions. Most cases were caused by *L. tropica*. Cases caused by *L. major* occurred occasionally and were probably acquired in foci outside of JD. Four cases of CL without concomitant signs of VL were found to be caused by *L. infantum*. In the past, Palestinian physicians and clinics viewed CL as if it was one disease entity. The existence of three species of *Leishmania* (*L. tropica*, *L. major* and *L. infantum*) in close proximity makes species identification essential in the diagnosis of CL. The introduction of new and fast methods of diagnosis and consecutive identification of their causative agents enables a different, more precise approach to prognosis, treatment and response to the latter. It will also play an important role in deciding control strategies.

**Authors' contributions:** KA and ZA conceived the study; KA, SE, LFS and ZA designed the study; KA and AA were involved in the collection of samples; KA, SE and SEQ were involved in DNA extraction; KA was involved in PCR-RFLP; AN cultured the parasites and provided reference samples; LFS performed the excreted factor analysis; SS collected and dissected the sand flies; OH collected the patient samples; ZA provided the laboratory facilities and supervised the work; KA, GS AN and SEQ analysed the data; KA, GS, AA, OH, SS and ZA interpreted the data; KA and AN drafted the paper; KA, GS, ZA, SEQ, SE, SS, AA, OH and LFS critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. KA is guarantor of the paper.

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**Competing interests:** None declared.

**Ethical approval:** The Ethics Research Committee of the University of Al-Quds (Palestine) approved all the activities involving human subjects.

#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.trstmh.2012.06.005>.

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