

**ANNUAL REPORT**  
June 2003- June 2004

Submitted to the US Agency for International Development; Bureau for Global Programs; Field Support and Research; Center for Economic Growth

**VISCERAL LEISHMANIASIS IN THE WEST BANK AND  
ISRAEL – DISTRIBUTION AND RISK FACTORS,  
USING GIS**

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Project Number: C20-025  
Grant Number: TA-MOU-00-C20-025

Grant Project Officer: William H. Crane  
Project Duration: June 2002-March 2006

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## EXECUTIVE SUMMARY

Visceral leishmaniasis is a disease that infects people and dogs and can be fatal. Dogs and wild canine species are the major reservoir for this disease, which is transmitted to people by sand flies. An outbreak of visceral leishmaniasis took place in central Israel and the Palestinian Authority during the last decade. The project "Visceral leishmaniasis in the West Bank and Israel – distribution and risk factors, using GIS" is intended to reveal the ecological factors that determine the geographical spread of visceral leishmaniasis. We are employing a geographical information system (GIS) analysis to investigate how these factors affect the presence of visceral leishmaniasis, in order to be able to forecast where this disease might outbreak.

During the second year of the project, a database on sandfly species in Israel and the Palestinian Authority was created with more than 14,000 flies from 44 trapping locations from the north to the south. *Leishmania infantum* DNA was detected by a molecular method in some sandflies species that act as disease vectors. The sandflies distribution is a key element for the understanding of the epidemiology and spread of visceral leishmaniasis. Databases on human and canine visceral leishmaniasis have been created and the prevalence of infection in the domestic dog and wild canids populations were determined. A non-invasive molecular technique for the detection of infection in dogs was developed, and comparative genotyping of parasite isolates from the Palestinian Authority and central Israel. With this data, we are able to conclude that the recent disease outbreak was a result of local infection possibly due to incursion of humans into animal habitat, and not due to migration of infected animals from the northern disease focus. Risk factors for the disease will be assessed during the last year of the project when the distribution of infection will

be analyzed with the data on sandflies and geographical environmental maps. Collaboration between the Palestinian and Israeli scientists has been continuous and a Palestinian-Israeli spatial analysis workshop was held with a large number of Palestinian student participation.

## SECTION I

### A) Research Objectives

1. Determination of the impact of geographic and environmental factors on the prevalence of visceral leishmaniasis in Israel and the West Bank (Palestinian Authority).
2. Determination of the potential importance of the presence of certain reservoir hosts and vector sand flies for the spread of the disease.
3. Employment of geographic information system (GIS) and spatial statistics for the analysis of risk factors and devising a model that would predict where disease outbreaks may occur.

### B) Research Accomplishments

The project has accomplished these goals set for the second year:

- [A] Sandflies database - A survey leading to the creation of a large database on sandfly species in Israel and the West Bank has been carried out.

The purposes of this survey were:

- (1) To map where sandflies are found in Israel and the West Bank
- (2) To investigate what sandfly species are found in different geographical locations.
- (3) To map where the sandfly species that act as vectors for *Leishmania infantum* are located.

The distribution data on sandflies is a key element for the understanding of the epidemiology and spread of visceral leishmaniasis and risk factors for this disease.

The database that we have formed includes a total of 8,396 sandflies that were collected and analyzed during the project's duration. In addition, for the purpose of adding information on additional sandfly locations, we have added 6,332 sandflies that were caught during earlier times and were not analyzed, or analyzed in part and needed a revision on their speciation. In total, 14,728 sandflies from 44 locations were analyzed (see table no. 1 and figure 1).

Table 1 – Numbers and locations of sandflies caught and analyzed

Sandflies caught during the study	8,396
Sandflies caught prior to the study and re-analyzed	6,332
<b>Total number of sandflies trapped</b>	<b>14,728</b>
Number of trapping locations in the West Bank (Palestinian Authority)	16
Number of trapping locations in Israel	28
<b>Total number of trapping locations</b>	<b>44</b>

The vectors of *L. infantum* in our region and the eastern Mediterranean are *Phlebotomus (Laroussius) pertilewi*, *Phlebotomus (Lar.) neglectus*, *Phlebotomus (Lar.) syriacus* and *Phlebotomus (Lar.) tobbi*. The preliminary results of the study show that these 4 species are found in the West Bank and Northern and Central Israel, and their presence roughly parallels the spread of canine and human visceral leishmaniasis as established during year 1 of this project (figure 2). In addition, PCR performed on wild-caught *Phlebotomus perfiliewi* and *Phlebotomus syriacus* in the West Bank disease focus of Jedidah has detected *L. infantum* DNA in these two species, indicating these two vectors are probably of importance in transmitting visceral leishmaniasis in the West Bank (Appendix no. 1). In contrast, *Phlebotomus papatasi*, the vector of *Leishmania major*, a causative agent of cutaneous leishmaniasis, was found mostly in the Jordan valley, Jericho and Arava, where visceral leishmaniasis is not present. The originally planned sandfly ELISA for detection of *Leishmania* parasites in sandflies was replaced with a more advanced technique of direct PCR to detect *Leishmania* DNA in sandflies. Direct PCR has the advantage of enabling the speciation of the *Leishmania* parasites in the sandflies by DNA sequencing or PCR and restriction fragment length polymorphism (RFLP).

The findings on sandflies will be analyzed in more detail during the third year of the project, however, they provide important evidence for the role of sandfly species populations in the spread of visceral leishmaniasis in the Palestinian Authority and Israel.

### **[B] Analysis of isolates, speciation and genotyping**

Thirty seven *L. infantum* strains isolated from dogs or humans in northern Israel, the West Bank and central Israel were studied using random amplified polymorphic DNA – polymerase chain reaction (RAPD-PCR) or DNA fingerprinting with the human multilocus probe 33.15. The origins of the outbreak of leishmaniasis in central Israel and the West Bank were investigated by examining genetic polymorphism in analysis of the patterns obtained by RAPD-PCR or DNA fingerprinting and compared to isolates from the older focus in Northern Israel, where this disease was known for decades. Analysis generated three clades, but the strains were distributed differently by each method. No correlation was seen between clades generated by RAPD-PCR and geographic origin of the strain. However DNA fingerprinting separated the strains geographically into northern (clade A) and central (clade B & C) genotypic groups. These results suggest that the emergence of visceral leishmaniasis in central and the Palestinian Authority is not due to parasite spread from the old northern to the new central focus. The re-emergence of the disease might be due to changing ecological and environmental conditions leading to the increased human incursions into and modifications of existing habitats where sylvatic transmission was taking place.

This part of the study has been submitted for publication in a leading scientific journal.

**(C) Human and canine leishmaniasis databases**

Databases on the cases of canine and human leishmaniasis in Israel and the Palestinian Authority from 1994 to the present year have been constructed and are continuously updated and analyzed on a monthly basis.

- (1) Domestic dogs database - 166 cases of canine leishmaniasis were recorded from 1994 to April of 2004. All cases were diagnosed by positive serology and most were also positive by culture and/or PCR. The average annual number of cases was 16 (range 1 in 1994 – 33 in 2000). These included infected dogs detected by passive surveillance, referrals from local veterinarians suspecting the disease, and by active surveillance in foci of the disease. During 1994-2003, canine leishmaniasis was detected in animals from 47 locations in central and northern Israel and the West Bank. Of these cases, 25% were from northern Israel and 75% from the central region. Ninety percent of the cases originated from rural settlements and 10% from urban settings. A clear overlap was found between the regions in which canine and human visceral leishmaniasis are present. Interestingly, no clinical human cases have been found in some of the most active canine foci of the disease in Israel such as the villages of Nataf and Klil, whereas in West Bank foci of active human visceral leishmaniasis, only a few infected dogs are found. These interesting findings warrant further research and may be related to different nutritional conditions of children (the main risk group for human visceral leishmaniasis) or to environmental factors.

A summary of our findings on canine leishmaniasis was presented recently in a veterinary scientific congress. The abstract of this talk will be published shortly in a veterinary journal (Abstract in Appendix no. 2).

A wide-based serosurvey of canine leishmaniasis has been completed. This survey was described in more detail in the first year's annual report. It included 936 dogs from the West Bank and Israel. Six out of 790 dogs (0.76%) randomly tested were seropositive for visceral leishmaniasis, and 22 of 146 (15%) from disease foci were positive. This study indicated that visceral leishmaniasis is mostly a focal disease, and dogs from disease foci are more likely to be infected than dogs randomly selected from the general dogs population. However, the number of disease foci as shown in the database of infected dogs, is high, and the disease is spread in multiple foci throughout central and northern Israel and the West Bank.

(2) Human database –

- (1) West Bank database – A general database of human visceral leishmaniasis cases from 1994 to the present year (2004) includes roughly 150 cases. In order to get more specific details on the location of each patient (by village, not just by district as is reported by the Palestinian Ministry of Health), we

have investigated each district separately and are in the initial stages of forming a detailed database. We have detailed databases for the districts where visceral leishmaniasis is the most prevalent and are expecting to finish the database from all the districts within one month. In the Hebron area, our database includes 51 cases of human visceral leishmaniasis from 13 different villages. In the Jenin region, it includes 50 cases from 16 villages. Patients were all children 12 years or younger (age range 1-12 years; median age = 2 years).

- (2) Israel – Fourteen cases are currently in the database. These are mostly young children as in the West Bank.

The number of human infantile cases of visceral leishmaniasis in the Palestinian Authority is higher than we initially expected. Further analysis on the locations of patients and risk factors for the disease will be carried out in the third year of the project.

- (3) Wild canine study - Sera from wild canids ( jackals, foxes, wolves) caught by the National Reserves Authorities were analyzed for seropositivity to *Leishmania infantum*. This component of the study has been completed. Of 158 jackal (*Canis aureus*) sera, 7.6% were seropositive. Of 93 fox (*Vulpes vulpes*) sera, 2% were seropositive, and of 16 wolf (*Canis lupus*) sera, 1 (6%) were seropositive.

**[D] Improved molecular diagnosis of canine visceral leishmaniasis –**

We have found that detection of *Leishmania infantum* infection in dogs by non-invasive PCR using samples taken by a cotton swab from the conjunctiva, is superior to invasive diagnosis by aspirates from the spleen or by PCR of blood. This new technique was developed during this project will be useful for prevalence studies and also for direct diagnosis of infection in dogs.

A paper on this study was recently published in the Journal of Infectious Diseases (Appendix no. 3).

**[E] Training: Palestinian-Israeli Workshop on Spatial Analysis of Infectious Diseases**

A three days Palestinian-Israeli workshop on spatial analysis and GIS (geographic information system) techniques in the study of infectious diseases was held during September 15-17<sup>th</sup>, 2000 in Hebron, West Bank. The aim of the workshop was to study and practice spatial analysis techniques as tools for epidemiological research with special emphasis on vector-borne diseases, in particular leishmaniasis. The workshop was attended by 16 participants of which 10 were Palestinian, 4 Israeli and 2 Americans. Prof. Mark L. Wilson from the Department of Epidemiology, School of Public Health, at the University of Michigan, Ann Arbor, who is the spatial analysis consultant for this project, tutored most of the lectures and practical sessions in the workshop. Several sessions were devoted to group discussions of the students' projects and the implications of spatial analysis.

Some more time was spent on direct counseling of individual students. The participants were encouraged to describe their projects and future plans to the whole group, and received comments and suggestions from their peers and seniors. A session was also devoted to the use of on-line internet resources for spatial analysis. A report on this workshop was added as an appendix to the July 2003- December 2003 semi-annual management report.

**[F] Disease foci studies**

A study of micro-environmental data collection and analyses by GIS in positive and negative village foci for VL which are disease foci was carried out in Nataf in Israel and Jdidah in the northern West Bank. In addition, epidemiological information was collected in more "positive" villages including Klil, Nili and Sirs and in "negative" dog village populations. The data collected in the foci is under analysis for risk factors of infection. Preliminary results of these analyses suggest that ownership of farm animals and poultry may be risk factors for canine and human visceral leishmaniasis on a focal scale.

**[E] GIS analysis of macro-environmental data –**

This final component of the project involves the overlaying of the disease distribution map over environmental database maps (topography, land use, temperatures, soil types). An important part of this component is the overlay of the sandfly species distribution map formed by the data collected in the first two years of this project (section [A]) over the disease distribution map and the environmental maps. Analyses of the distribution pattern of the disease facilitate the determination of risk factors. Initial results from the disease distribution comparisons indicate that visceral leishmaniasis in dogs and humans is mostly present in the central and Northern Israel and in the central hills of the West Bank (Figs. 1 & 2). The foci are mostly in hilly or mountainous regions with a cooler climate, and generally away from deserts and from the coastal plain close to the Mediterranean. Also, most infected dogs and humans are from rural villages and not from urban settings.

**B) Scientific Impact of Collaboration**

The connection between the Palestinian and Israeli principal investigators remained intensive during the project's second year. Prof. Abdeen and Dr. Baneth communicate by phone or e-mail on a near to daily basis. In addition to that, meetings to discuss the research progress are held roughly every month. Al Quds University and the Hebrew University are within a one-hour drive of each other. The meetings often include students and other research collaborators. The closeness of the partners' research institutes is an advantage that allows a strong cooperative capacity with exchange of students and scientific techniques know-how. Testing for the visceral leishmaniasis such as serological analyzes, PCR for *Leishmania* DNA and culturing of parasites are often performed by one side for the other, and the Palestinian laboratories have attained the capacity to perform these tests.

Prof. Mark Wilson from the Department of Epidemiology, University of Michigan in Ann Arbor, the GIS advisor to the project, has visited the Hebrew and Al Quds Universities for a second time in September of 2003. During the September visit, an Israeli-Palestinian GIS workshop was held. Both visits included meetings with Palestinian and Israeli students and visits to field sites. These activities further strengthened the collaboration between the Israeli and Palestinian scientists and their research teams.

### **C) Description of Project Impact**

The project's results will be used for understanding where in Israel and the West Bank does visceral leishmaniasis occur, to identify the ecological risk factors and for devising a model that would help predict where disease outbreaks may occur in the future.

The data that has been collected during the first two years of the project is extensive and includes a large number of sandflies collected in multiple locations, a thorough investigation on the prevalence and distribution of human leishmaniasis in the West Bank and Israel during the past decade, a wide-based database on clinical canine leishmaniasis and a serosurvey of dogs and wild canids. This data reveals that the disease is even more prevalent and widespread than expected when the project was initiated. The main risk of human disease is clearly in the West Bank with over 150 cases during 10 years of the potentially-fatal infantile disease. Spatial analysis of the data collected in the project's first two years will be processed during the last year of the project and will facilitate a summary of the epidemiology of visceral leishmaniasis, identification of risk factors, and formulation of precautions to be taken by the local health authorities. International dissemination of the knowledge gained on visceral leishmaniasis in the West Bank and Israel in this project is made by scientific publications (Appendix no. 1) and by presentations in local and international scientific congresses (Appendix no. 2). More mutual Palestinian-Israeli presentations and publication will follow.

### **D) Strengthening of Developing Country Institutions**

Research in the Palestinian Authority is conducted by Prof. Abdeen in the field and laboratory in close cooperation with the principal investigator (Dr. Baneth) from the Hebrew University. The research program involves Palestinian students and technicians at Al Quds. These students and technicians have been trained at the Hebrew University, and are directly involved with research at the Hebrew University.

A Palestinian student involved in this project, Ms. Suheir Zoloum, is participating in a Masters of Public Health (MPH) degree program at the Hebrew University School of Public Health. Before entering the MPH course, Suheir trained for 3 months in molecular techniques for the detection of leishmaniasis at the Hebrew University. Ms. Kifaya Azmi, a Palestinian technician at the Al Quds University medical lab has trained in the Hebrew University and is currently performing ELISA assays and isolation of *Leishmania* strains in Al Quds. Ahmad Amro, another Palestinian student, has carried out the epidemiological data collection on human leishmaniasis in the West Bank and keeps in close contact with Dr. Baneth.

The Israeli-Palestinian GIS workshop held in September 2003, in which 10 Palestinian students affiliated with Al Quds University participated, was another important aspect in the strengthening of the Palestinian research capacity. The direct tutoring of students, group discussions and demonstration of use of internet resources for research were helpful for students in improving their research projects.

#### **E) Future Work**

The project has achieved the goals set for year 1 and 2. Large databases have been created, data has been mapped and molecular analysis of *Leishmania* isolates has been carried out. The major goal ahead is the analysis of the large amount of data collected in the first and second years and the interpretation of the results. Some of the initial data collected and the molecular techniques that were developed, such as the non-invasive PCR have already been published by us. However, there is more information to be presented at future international scientific congress, and in the form of scientific manuscripts authored by the Israeli and Palestinian principal and co-principal investigators, together with research students.

## **SECTION II**

#### **A) Managerial Issues**

Despite the difficult political situation in Israel and the West Bank, the project has progressed and has reached it's final year with fulfillment of the research plan made. Entrance permits into Israel for students from the West Bank wishing to reach Jerusalem are difficult to get at times. These permits are also restrictive and often allow the Palestinian students to stay at Al Quds or the Hebrew University during certain hours and not overnight. Movement within the West Bank is also complicated at times due to the road blocks and inspection points. However, it is still possible to collect samples such as dog sera and sand flies, in the West Bank. When direct communication between the researchers and students is difficult, transfer of data is done via telephone and e-mail.

#### **B) Budget changes**

No major budget changes are currently needed.

#### **C) Special Concerns**

No protocols addressing the special concerns analysis made when the project proposal was written have been changed.

#### **D) Collaboration, Travel, Training and Publications**

During the second year of the project, collaboration between the Palestinian and Israeli researchers and students commenced and intensified.

##### **1. Mutual researchers contacts and meetings -**

The principal Israeli and Palestinian investigators keep in touch on a regular basis and meet frequently to discuss issues related to the project and its progress. The principal investigators have also collaborated in writing scientific manuscripts (Appendix no. 1) and congress presentations (Appendix no. 2).

## 2. Training –

Much of the technical training of Palestinian technicians and students in animal handling and laboratory techniques was carried out during the first year. The second year laboratory training included training in performance of serology by ELISA and PCR on tissues and sandflies.

In addition, a three days workshop on Spatial Analysis and GIS was carried out in September of 2003 and tutored by Prof. Mark Wilson from the University of Michigan (please see the July- December 2003 semi-annual report).

## 3. Travel -

Travel for the purpose of this project was restricted to local travel to field sites for the purpose of data and sample collections. This took place by both Israeli and Palestinian research teams. The international travel included in this project is Prof. Wilson's visit from Michigan to Israel.

## 4. Publications:

Two papers were published during the past year with partial support from this project. An additional research manuscript on the genotyping of *Leishmania* isolates has been submitted for publication. A research abstract from a scientific congress has been presented with full support from the project and will be published. Next year, we expect to submit one or two manuscripts summarizing the project's findings and conclusions with full support from the US-AID CDR program.

- (1) Jaffe CL, Baneth G, Abdeen ZA, Schlein Y, Warburg A. Leishmaniasis in Israel and the Palestinian Authority. *Trends in Parasitology*, 2004, 20: 328-332.

This paper is a review on leishmaniasis in Israel and the Palestinian Authority. It was published in the most highly ranked parasitology journal. This review deals with both cutaneous and visceral leishmaniasis and describes some of the findings made during the project's earlier stages, in addition to findings made by other researchers (Appendix no. 1).

- (2) Baneth G, Abdeen AZ, Strauss Avoli D, Maccoradino A, Jaffe CL. The epidemiology of canine visceral leishmaniasis in Israel and the West Bank – an update. To be published in the *Israel Journal of Veterinary Medicine*.

This abstract was presented at the 28<sup>th</sup> Veterinary Symposium in April 2004 at Beit Dagan, Israel. It describes our findings on canine visceral leishmaniasis during the first and some of the second year of the project (Appendix no. 2).

- (3) Strauss-Ayali D, Jaffe CL, Burshtain O, Gonen L, Baneth G. Polymerase Chain reaction using noninvasively obtained samples, for the detection of *Leishmania infantum* DNA in dogs. Journal of Infectious Diseases. 2004, 189: 1729-1733.

This manuscript was published by Dr. Baneth's research group on conjunctival PCR. This non-invasive technique was developed with partial support from the USAID- CDR program and is also being applied now by the Palestinian research team (Appendix no. 3).

Requests for American Embassy Tel Aviv or A.I.D. actions:

We have no special requests at this stage. Thank you.



Figure 1 – Locations of sandfly trapping in Israel and the West Bank (Palestinian Authority). Trapping at close sites were marked with a single signs.

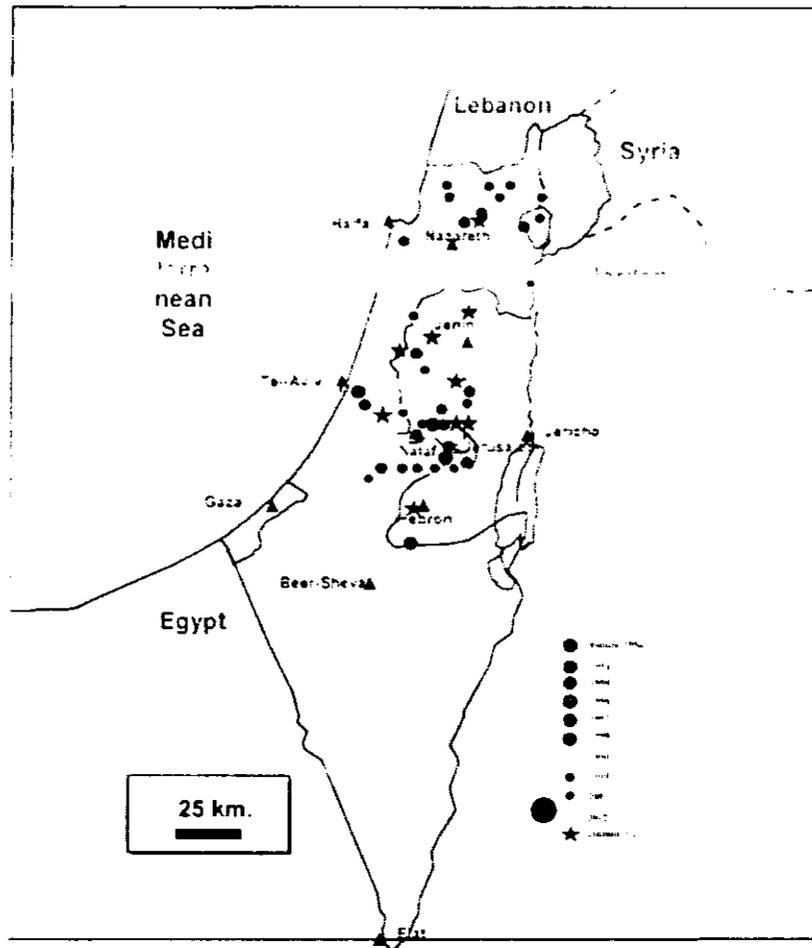


Figure 2 – A schematic presentation of the distribution of visceral leishmaniasis in dogs (circles) and humans (stars) in Israel and the West Bank. The distribution of infection in dogs is arranged according to the year of initial detection in the different disease foci.

# Leishmaniasis in Israel and the Palestinian Authority

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Cutaneous and visceral leishmaniasis in the Middle East has been known since the early 1900s. Recent epidemiological studies show that they are re-emerging as important public health problems in areas long believed to be disease free. Cutaneous leishmaniasis, caused by *Leishmania tropica*, has become a significant problem in northern Israel and parts of the West Bank, whereas zoonotic foci of *Leishmania major* in the Jericho area and Negev desert present a threat to increasing populations. Canine leishmaniasis, caused by *Leishmania infantum*, is now highly prevalent in central Israel and encroaching on urban areas. Recent studies on the vectors and reservoir hosts, in addition to the molecular characterization of *Leishmania*, are helping us understand the dynamics of these diseases.

*Leishmania* spp. are the causative agents of leishmaniasis, parasitic diseases with a wide range of clinical symptoms: cutaneous (CL); mucocutaneous; and visceral (VL). Leishmaniasis currently threatens 350 million people in 88 countries worldwide, with 1–1.5 million CL and 500 000 VL cases reported annually [1,2]. In Israel and the Palestinian Authority, similar to other parts of the eastern Mediterranean basin, *Leishmania major* and *Leishmania tropica* cause CL, and *Leishmania infantum* causes VL. Notification of the Ministry of Health (MOH) of these diseases is required by law in both jurisdictions, but the number of CL cases remains consistently under-reported [3,4]. This can occur for several reasons including the fact that CL frequently heals without treatment, people fail to seek medical care, and lack of reporting by physicians and diagnostic laboratories [4]. Until recently, these diseases were thought to be restricted to geographically defined areas [5,6] with CL occurring primarily in the Negev Desert, Jordan and Arava Rift Valleys, and VL limited to the Galilee region of northern Israel. However, recent studies [4,7–9] suggest that the areas affected by these diseases are expanding, and probably overlap in many parts of Israel and the Palestinian West Bank (Figure 1).

Much of this new information on leishmaniasis is a result of cooperative efforts between Palestinian and

Israeli researchers that were initiated more than 10 years ago, and have stood the test of turbulent times, funding difficulties and geopolitical obstacles [10].

## Cutaneous leishmaniasis

Ecological, clinical, epidemiological and entomological studies on leishmaniasis conducted in Israel and the Palestinian Authority in the past decades have contributed considerably to our understanding of these diseases. The presence of CL in Jericho was documented scientifically at the beginning of the 20th century [11,12]. Saul

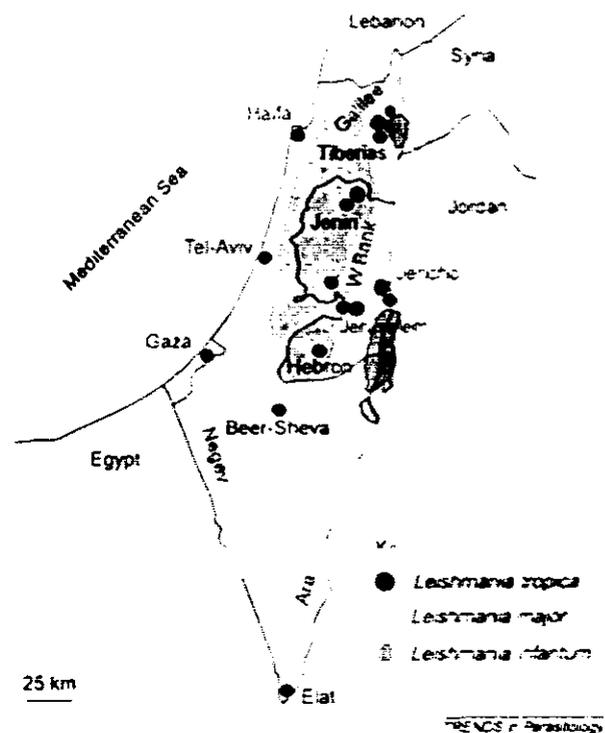


Figure 1. Cutaneous and visceral leishmaniasis in Israel and the Palestinian Authority. *Leishmania major* and *Leishmania tropica* are responsible for cutaneous disease, and *Leishmania infantum* for visceral disease. The regions where *L. major*, *L. tropica* and *L. infantum* have been found are shown. No cases of autochthonous disease have been reported from Gaza. Source of data: L. Schnur, WHO Jerusalem Leishmania Reference Center and from Refs [4,5,7–9,16,29,34,44–46,52].

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Available online 19 May 2004

Adler and Oskar Theodor were the first to study Phlebotomine sandflies and the role they played in transmission of CL in the region [13]. The vector of *L. major* (referred to in early papers as *L. tropica* or *L. major tropica*, synonymized by *Phlebotomus papatasi* [13] and the ability of *Ph. papatasi* to transmit promastigotes by bite was proven experimentally [14]. Following the 1967 Israeli administration of the West Bank, CL became a serious problem with the number of civilian cases more than quadrupling and peaking at ~125 that same year [3]. A second, larger peak of ~250 cases per year was observed in Israel between 1982 and 1984 following the Peace Agreement with Egypt and redeployment of the Israeli Defense Forces (IDF) in non-developed areas of the Western Negev [15]. Since the 1990s, the incidence of CL according to the Israeli MOH has remained more or less constant at between 0.5 and 2.5 cases per 100 000 with a small peak occurring in 1995–1996 [3]. Information collected by the IDF on CL shows a trend similar to that found by the MOH (I. Grotto *et al.*, unpublished, see [http://parasitology-soc.md.huji.ac.il/new\\_page\\_5.htm](http://parasitology-soc.md.huji.ac.il/new_page_5.htm)), except that the incidence in soldiers is 25–60 times higher than that for civilians [3,16]. A peak in CL cases in the Palestinian Authority was also observed in 1995, when the incidence reached almost 16 cases per 100 000 (MOH-Palestine, Annual Report 2001, <http://www.moh.gov.ps/annual%20report/graphs/graph68.html>). Since 1995, the number of cases in the West Bank has steadily declined according to the Palestinian MOH, reaching 1.9 per 100 000 in 2000 and 2001 (<http://www.moh.gov.ps/annual%20report/graphs/graph68.html>). These statistics published by the MOHs or IDF do not differentiate between CL caused by *L. major* or *L. tropica*.

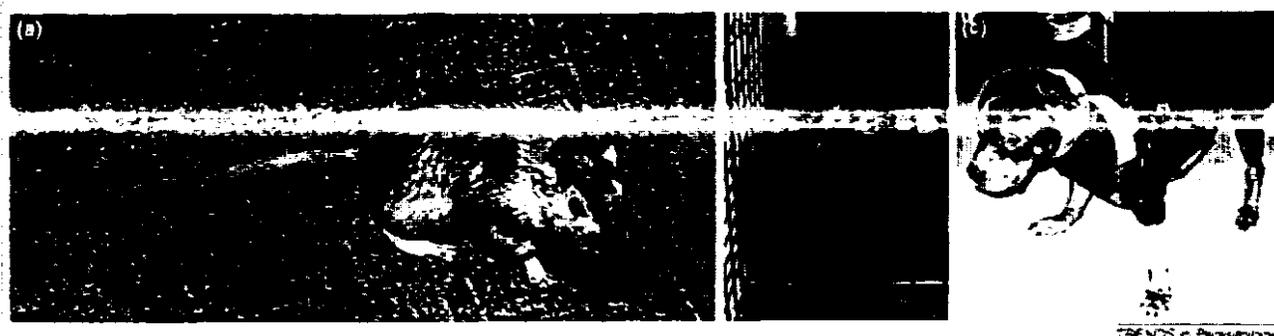
#### Leishmania major

Several early studies dealt with the ecology and prevention of transmission in areas near Jericho [17,18]. Later, Schlein *et al.* [19] confirmed the role of *Ph. papatasi* in transmission of *L. major* in foci of zoonotic CL in the central Jordan Valley (near Jericho), in addition to the Negev and the Arava deserts of southern Israel. These authors showed that the reservoir hosts

in the Jordan Valley and Arava were fat sandrats (*Psammomys obesus*, Figure 2), whereas the jird *Meriones crassus* was an important host in the Western Negev region close to the Egyptian border [19,20]. In recent years, *L. major* has been reported as a zoonotic agent in several zoonotic foci in southern Israel. Sporadic infections in *Gerbillus dasyurus* and *M. crassus* were also recorded [16].

Ecological studies on foci of *L. major* have indicated that the vector-competence of *Ph. papatasi* in arid desert habitats is linked to their ability to tolerate hunger, whereas sandflies from oases were refractory to infection [21,22]. Conversely, agriculture and other anthropogenic disturbances of natural habitats resulting in higher soil humidity apparently enhance zoonotic transmission in desert foci [23]. This could explain the yearly increase in incidence of CL caused by *L. major* in the Negev region of southern Israel [3,16]. However, habitat modification might also be useful in controlling sandflies and leishmaniasis near human dwellings [24]. Certain exotic plants (e.g. *Ricinus communis*) fed upon by *Ph. papatasi* females cause parasite death, whereas other plants, such as *Bougainvillea glabra*, frequently used in gardens were found to cause high mortality of the sandflies [25,26].

Isoenzyme profiles of *L. major* strains isolated from rodents, sandflies and CL patients in the Middle East and Africa are mostly zymodeme LON-1 or MGN-26 [27], although additional variants have been identified [28]. In Israel and the Palestinian Authority, three *L. major* variants (LON-1, LON-2 and LON-3) of the enzyme 6-phosphogluconate dehydrogenase (6-PGD) were found in different geographical areas (central Jordan Valley, Arava and Negev deserts) [27,29]. More recently, the permissively primed intergenic polymorphic-polymerase chain reaction (PPIP-PCR) was used to discriminate between *L. major* strains from these geographic regions [30]. Because *Ph. papatasi* is the vector for *L. major* in all regions of Israel and the West Bank, it was suggested that the microheterogeneity observed among the *L. major* strains from the Jordan Valley, Arava and Negev deserts could be a result of the differences in reservoir hosts [20].



**Figure 2.** Reservoir hosts of *Leishmania* spp. in Israel and the Palestinian Authority. (a) *Psammomys obesus* is the major reservoir host for *Leishmania major*. *Phlebotomus papatasi* sandflies breed inside their burrows and feed on the animals. Photograph by A. Warburg. (b) Rock hyraxes (*Procapra capensis*) are becoming common in and around rural, in addition to urban areas, in Israel and the West Bank. Photograph depicts hyraxes that were trapped live and checked for infection with *Leishmania* species. (Photograph by J. Votýpka, Charles University, Prague). (c) Photograph of a dog with canine visceral leishmaniasis. Dogs, foxes and jackals are infected and can act as reservoir hosts for *Leishmania infantum*. Note the dermal lesions and exfoliative dermatitis over the ears and face. Photograph by G. Baroni.

### *Leishmania tropica*

Until recently, relatively little information was available on the epidemiology of CL caused by *L. tropica* in Israel and the Palestinian Authority. Outbreaks were not investigated in depth, parasites of genetic diversity not characterized, and cases were usually grouped together with *L. major* [3]. Clinically, cutaneous lesions caused by *L. tropica* tend to last longer (6–12 months or longer for *L. tropica* versus 2–6 months for *L. major*) and are more difficult to treat than those caused by *L. major* [4,6]. Moreover, infections with *L. tropica* occasionally produce VL or viscerotropic leishmaniasis [31–33]. In recent years, new foci of CL are emerging in the Galilee region of northern Israel, the Judean desert east of Jerusalem, and the Jenin district of the Northern West Bank (Figure 1). Both urban and rural environments are affected, but most human cases are residents on the periphery of their settlements – their houses and gardens bordering open areas.

Rock hyraxes (*Procapra capensis*, Figure 2) are very numerous in the *L. tropica* foci studied to date [9,34]. Three out of 29 hyraxes examined in the Galilee focus were positive by PCR for *L. tropica* DNA [9], and a *L. tropica* isolate was obtained from one infected hyrax captured in the same focus (M. Svobodova and A. Warburg, pers. commun.).

Two sandfly species were implicated as the vectors of *L. tropica* in the region. In the Galilee focus, both *Phlebotomus (Adlerius) arabicus* and *Phlebotomus (Paraphlebotomus) sergenti* were found naturally infected with *L. tropica*, but heavy infections suggesting ability to transmit were found only in *Ph. arabicus* [9]. To date, *Ph. arabicus* has not been found in any other *L. tropica* focus including the Judean desert focus where *Ph. sergenti* was implicated as the vector with 4 out of 105 females harboring *L. tropica* promastigotes [34]. The relatively low prevalence of *L. tropica* in humans in the Judean desert was attributed to the reluctance of *Ph. sergenti* to enter homes and bite humans [34]. *Phlebotomus sergenti* was also found in an endemic area near the Palestinian town of Jenin, northern West Bank [35].

Recent studies using molecular tools to characterize the parasite isolates from patients with CL [30,36] have helped to clarify the distribution of *L. tropica* and *L. major*, but have raised additional points of interest. *Leishmania major* alone was long considered to be responsible for all cases of CL in the city of Jericho and surrounding Jordan Valley. Molecular characterization of patient isolates from this region showed that 22.5% of the cases from the city

were actually caused by *L. tropica* [37]. However, the question remains whether active *L. tropica* transmission also occurs in the Jordan Valley and the city of Jericho (similar to urban foci of *L. tropica* in neighboring countries), or are these cases imported from other areas of the West Bank.

*Leishmania tropica* is genetically a very heterogeneous species and strains are readily distinguishable using antigenic, biochemical and molecular markers [38–40]. In Israel and the West Bank, heterogeneity in parasite characteristics was demonstrated within CL foci and between them. For example, parasite antigens from the

northern part of the Galilee focus are more similar to *L. major* than to *L. tropica* when examined using monoclonal antibodies and excreted factor serotyping [9]. However, DNA analysis by several techniques identifies *L. tropica* in the Galilee focus. Interestingly, parasites from Tiberias (only 15 km away at the southern end of the focus) are antigenically more similar to *L. tropica* isolated in the Judean desert, 110 km to the south, than to parasites from the northern part of the Galilee focus [9]. This difference in parasite antigens correlates with the presence of different putative vectors in the two parts of the Galilee focus. *Phlebotomus arabicus*, the proven vector in the northern part of this focus, was not found in the southern part of the focus where *Ph. sergenti* is the suspected vector (A. Warburg, unpublished). Furthermore, preliminary results of experimental infections indicate that *Ph. sergenti* is refractory to *L. tropica* from the northern part of the Galilee focus, but highly susceptible to *L. tropica* from the Judean Desert (M. Svobodova, pers. commun.).

Genomic fingerprinting using a mini-satellite probe (33.15) separated the *L. tropica* strains into two clusters that were not entirely congruent with geographical distribution [34]. These groupings were also supported by other techniques such as isoenzyme analysis, excreted factor serotyping, PPIP-PCR and single-strand conformation polymorphism (SSCP) analysis [34].

Several strains from CL cases in the Jenin district have been typed and were all identified as *L. tropica* (L. Schnur, pers. commun.). This is in agreement with the ecology and the presence of *Ph. sergenti* in this region [35].

### Visceral leishmaniasis

Human and canine VL were first identified in the Galilee region during the British Mandate in Palestine in the 1930s [41,42]. A review on VL in the 1940s suggested that this disease was widespread throughout the region with cases occurring primarily among Arab communities in the Jaffa, Jerusalem, Haifa and Nablus districts [43]. During the period 1950–1994, human VL essentially disappeared from central Israel and was confined to a single focus in the Galilee region of northern Israel [44–46]. H. Palty, MSc Thesis, Hebrew University, 1961. Approximately 113 cases, almost all in young children, were reported from many villages in this region between 1950 and 1993. However, during the past few decades, clinical human VL in the Galilee has declined significantly. Only seven cases were reported from northern Israel in the 1990s, although asymptomatic human VL is common and clinical canine

VL is common. A hypothesis that a culling campaign of jackals and foxes in the late 1950s has been frequently postulated to be responsible for the disappearance of VL from central Israel [8,47], but there is no concrete evidence to support this hypothesis.

In 1994, the first case of canine VL was diagnosed in Nataf, central Israel, located 12 km west of Jerusalem (Figure 1) [8]. In the same year, the first human VL case in >30 years from this region was also diagnosed. Since then, canine VL has been diagnosed in 37 localities in central Israel and >90% of the human VL cases in Israel have originated in this region [47]. A retrospective study in 1989–1993 of 50 human VL cases from the Jenin District,

West Bank showed a peak in 1994 (21 cases), just when the first cases of human and canine VL appeared in Israel [7]. Human VL cases in the Hebron, Tulkarm and Ramallah districts of Palestine (32, 17 and 15 cases, respectively) were reported during this same period [47]. In the past, most of the cases of canine VL were found in rural settings but, recently, a few cases have appeared in urban areas such as Tel Aviv, Rishon Le Zion, Modi'in and Jerusalem, suggesting that this disease could be making inroads into densely populated areas.

As in other endemic regions around the Mediterranean basin, human VL in Israel and Palestine caused by *L. infantum* is primarily a disease of young children, with dogs (Figure 2) and other wild canids acting as the natural reservoir hosts. This epidemiological pattern is liable to change because of the AIDS epidemic, as has happened in southern Europe, where two-thirds of the VL cases now occur in young adults 20–40 years old, co-infected with HIV [48]. In Israel, several *Leishmania*–HIV co-infections have been documented in the past decade [47]; however, this is not the case in the Palestinian Authority where infantile VL remains an important cause of morbidity [7].

The vectors of *L. infantum* in Israel and Palestine have not been determined, but several putative vectors, *Phlebotomus (Laroussius) perfiliewi*, *Phlebotomus (Lar.) neglectus*, *Phlebotomus (Lar.) syriacus* and *Phlebotomus (Lar.) tobbi* have been found in the northern West Bank in foci of canine or human disease [35], and *L. infantum* DNA was detected by internal-transcribed spacer region 1 (ITS1)-PCR in wild-caught *Ph. perfiliewi* and *Ph. syriacus* (K. Bader and A. Nasereddin, pers. commun.). *Phlebotomus (Laroussius)* spp. have been implicated as vectors of *L. infantum* in other foci around the Mediterranean sea [49].

*Leishmania infantum* generally shows less heterogeneity than other species of *Leishmania* [50,51]. In Israel, >60 strains of *L. infantum* have been isolated from dogs and humans since 1994. All the strains were identical by ITS1-PCR [36] and had B2 excreted factor serotypes typical of *L. donovani* complex. A total of 14 dog and human strains were also characterized by zymodeme analysis, of these thirteen belong to the MON-1 zymodeme [50]. DNA fingerprinting and microsatellite analysis of the strains from the new focus (central Israel and/or West Bank) and the old focus (Galilee) suggests that *L. infantum* did not spread from the Galilee into central Israel and the West Bank [50]; C. Jaffe and G. Baneth, unpublished).

### Concluding comments

The epidemiology of CL in Israel and the Palestinian Authority appears to be undergoing a transition. Foci of *L. major* in the central Jordan Valley that were very active in the 1960s and 1980s are subsiding, perhaps as a result of intensive agriculture that has fragmented the natural habitats of *P. obesus*, the reservoir host. However, CL caused by *L. tropica* is spreading into new areas and threatening urban populations in cities such as Tiberias, Ma'ale Adumim, Jericho and Kabatiya. This could be a result of housing construction, irrigation and other changes that produce favorable habitats near human

settlements for hyraxes, a reservoir host for *L. tropica*. Finally, canine VL is increasing throughout the entire central region. Although clinical disease remains rare in Israel, human VL is more common among the Palestinian population [47]. The epidemiology of CL and VL in Israel are not known and further study is needed. The areas affected by CL and VL in the Palestinian Authority and Israel are expanding, and could eventually overlap. These findings require heightened awareness among physicians, veterinarians and public health authorities, and will require good co-operation between the responsible Israeli and Palestinian authorities in order to stem the continuing spread of these diseases.

### Acknowledgements

We thank all those people who helped facilitate Israeli–Palestinian scientific collaboration. Research performed at the authors' laboratories was supported by grant number SO 2203-1 from the Deutsche Forschungsgemeinschaft (DFG), The Palestinian-Israeli-German Co-operative project on Leishmaniasis in Israel and The West Bank, the Center for the Study of Emerging Diseases, the USAID-CER Program, grant number TA-MOU-00-C26-025PH and the Israeli Ministry for the Environment, grant number 802-2.

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### Soldiers in Iraq face things that bring bumps in the night

US troops in Iraq have discovered an unexpected adversary in cutaneous leishmaniasis. The parasite, *Leishmania* sp., transmitted through sandfly bites, is so widespread that the bumps and sores associated with the disease have been nicknamed the Baghdad boil. During the first Gulf war, only 20 US soldiers contracted leishmaniasis, but this number has now rocketed. For example, in one unit of 750 men, 200 had contracted the disease. This dramatic increase is thought to be the result of troops arriving in Iraq during the peak sandfly season. Many are remaining for longer periods and sleeping outside at night, exposing themselves to the sandfly at its peak feeding time.

The military is taking a serious approach to treating the disease, even pulling troops with confirmed cases out of Iraq and flying them back to Washington for treatment. Doctors have instructed the soldiers on the symptoms of leishmaniasis and urged them to wear spray, however, with the threat of enemy bullets flying overhead, the application of insect repellent has not yet become a top priority for many soldiers in the field.

For more information please go to: <http://www.cnn.com/2004/HEALTH/04/16/baghdad.boil.ap/index.htm>

## **THE EPIDEMIOLOGY OF CANINE VISCERAL LEISHMANIASIS IN ISRAEL AND THE WEST BANK – AN UPDATE**

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Presented at the 28<sup>th</sup> Veterinary Symposium. April 2004. Beit Dagan, Israel

To be published in the Israel Journal of Veterinary Medicine

Canine visceral leishmaniasis was reported in central Israel in 1994. A database of the canine cases of visceral leishmaniasis in Israel and the West Bank was constructed and analyzed for the year of diagnosis, geographic location and type of settlement (rural vs. urban). In all, 166 cases of canine leishmaniasis were recorded from 1994 to April of 2004. All cases were diagnosed by positive serology and most were also positive by culture and/or PCR. The average annual number of cases was 16 (range 1 in 1994 – 33 in 2000). These included infected dogs detected by passive surveillance, referrals from local veterinarians suspecting the disease, and by active surveillance in foci of the disease. During 1994-2003, canine leishmaniasis was detected in animals from 47 locations in central and northern Israel and the West Bank. Of these cases, 25% were from northern Israel and 75% from the central region. Ninety percent of the cases originated from rural settlements and 10% from urban settings. Nataf in the Judean hills was the location with the highest number of cases (n=45), followed by Klil (n=21), Nili (n=11) and Modiin-Maccabim-Reut (n=10). The annual number of urban cases and the % of urban cases is gradually rising and reached 4 urban cases (19% of the annual cases) in 2003. The number infected dogs is probably underestimated since not all dogs with clinical signs compatible with leishmaniasis are brought for diagnosis and because dogs can carry infection asymptotically. The ratio between the number of detected human and canine visceral leishmaniasis cases in Israel is about 1:10. A clear overlap can be found between the regions in which canine and human visceral leishmaniasis are found, however, no clinical human cases have been found in some of the most active rural canine foci of the disease such as Nataf and Klil.

Financial support: US-Israel Cooperative Development Research Program, Economic Growth. US Agency for International Development (Grant TA-MOU-00-C20-025)