FINAL REPORT
April 2001- August 2005

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

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

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EXECUTIVE SUMMARY

Visceral leishmaniasis (VL) is a potentially fatal disease that infects people and dogs. Dogs and wild canine species are the major reservoir for this disease which is transmitted to people by sand flies. An outbreak of VL took place in central Israel and the Palestinian Authority during the last decade. This project was intended to reveal the ecological factors that determine the geographical spread of VL.

Databases on sand flies, infected humans, and infected dogs in Israel and the Palestinian Authority were constructed, merged, plotted on maps and analyzed using geographic information system (GIS) software. The databases on sand fly species in Israel and the Palestinian Authority included more than 14,000 flies from 44 trapping locations from the north to the south. Data included 190 human patients diagnosed during 1990-2003 and 189 infected dogs.

Infection in humans and dogs was found in the West Bank and the mountainous and hilly parts of central and northern Israel, but not in the southern and eastern arid and desert regions. Infection was present mainly in rural settings, however, urbanization of the disease was evident and poses a threat to the health of large populations. The distribution of sand fly species that are capable of transmitting visceral leishmaniasis emerged as a key element for the understanding of the epidemiology and spread of VL. A comparison of sand fly trapping sites where vector species were found to locations of non-vector species indicated that vectors favor regions with a high annual rainfall (mean 550 ml) and a rich vegetation of olives groves and natural forests. The incidence rates of human VL in children under the age of 10 years, which comprised 98% of the 176 patients in the West Bank, were calculated for every village, cluster of sites and district. Locations of low disease incidence were compared to locations of high incidence using multifactorial regression analysis. The geographic variables that emerged as having a significant effect on the incidence of human VL included: land uses with highly vegetated areas, a high annual rainfall (average 550 ml), low temperatures in October, relatively high altitude (300-550 meters above sea level) and high wind speed at ground level (average 5.2 meters/second).

DNA fingerprinting of *Leishmania infantum* isolates by several techniques showed that parasite isolates from northern Israel differed from isolates originating in central Israel and the West Bank (Palestinian Authority). The findings of different northern and central genotypes suggested that the emergence of infection in Israel and the West Bank in the recent decades was a result of re-appearance of the infection from a local source, perhaps due to increased human incursions into natural habitats where transmission was taking place, and probably not due to importation of infection to the central focus from the older northern focus. Additional products of this study that will be useful for future research are the non-invasive molecular diagnostic techniques for canine leishmaniasis developed during this study and the molecular detection of *Leishmania* in sand flies. A survey of leishmaniasis in wild canine species suggested that jackals are involved with the epidemiology of VL in the Middle East with a seroprevalence rate that greatly surpasses that of dogs in the general population..

This study is the first in the Middle East to address VL using GIS and one of the first in the world to find links between geographic risk factors and the presence of leishmaniasis. It's most important achievement was the definition of ecological risk factors and the statistically-significant linkage between geographic conditions and the presence of VL. The description of risk factors for VL in the PA and Israel will serve as a model for understanding the epidemiology of this disease in the Middle East and elsewhere. It can be used to forecast where outbreaks of infection may take place and also to plan prevention and intervention programs targeted on areas of high risk or involving risk factors that can be modified.

The project has considerably strengthened cooperation between Palestinian and Israeli scientists. The mutual training, workshops, writing of collaborative research papers and presentations in scientific congresses have encouraged investigators from the Arab and Israeli universities to carry out more mutual research. This collaboration has also served to encourage scientists from other Arabic countries such as Tunisia and Morocco to join research proposals with Israeli and Palestinian partners. In addition, Palestinian and Israeli students that trained under the project have formed good relationships and venues of communication and will continue to work together and maintain these ties.

RESEARCH OBJECTIVES

Visceral leishmaniasis (VL) caused by the parasite *Leishmania infantum* is a severe disease that is generally fatal in people if not diagnosed and treated in time. In the Middle East, the dog is the primary peri-domestic reservoir host and the disease is transmitted to humans by phlebotomine sand-flies. Visceral leishmaniasis has emerged during the last decade in central Israel and the Palestinian Authority (PA) where it had been reported mostly in children under the age of 3 years (Abdeen et al., 2002; Jaffe et al., 2004).

The overall aim of this project was to study the risk factors for VL in the West Bank and Israel using Geographic Information System (GIS). Our hypothesis was that risk factors for the spread of VL can be determined by examining geographic and environmental parameters. In this cooperative study, we constructed data bases on VL in the West Bank and Israel and compared areas with a high prevalence of the disease with areas of low prevalence. We analyzed this epidemiological data together with environmental, geographical and botanical information using GIS technology and found statistically-significant relationships between the presence of disease and ecological determinants.

The specific objectives of the project were:

1. Determination of the impact of geographic and environmental factors on the prevalence of visceral leishmaniasis in Israel and the West Bank (PA).
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 prediction where disease outbreaks may occur.

This project fits with other collaborative efforts between Israeli and Palestinian scientists and medical personnel that focus on the fight against infectious diseases and environmental health hazards that threaten both sides. Brucellosis and echinococcosis are additional diseases that have an important impact on public health in the Middle East as a whole and in Israel and PA in particular. This study is the first in the Middle East to address VL using GIS and one of the first in the world to find links between geographic risk factors and the presence of VL (El Naim et al., 2003; Bavia et al., 2005). In addition to defining risk factors for the disease in the Palestinian Authority and Israel, this project has also produced and put together comprehensive information on VL and it's sand fly vectors which comprise a thorough up-to-date report on the state of VL in this area. This information will be valuable in planning control programs against this disease in this region. The study was supported by the Geography Department at the Hebrew University who provided the geographic databases, the

Palestinian Health Ministry who supplied the information on the cases of human leishmaniasis in the West Bank and the Israeli Ministry of Environment who was helpful with collection of data and samples from wild canids.

METHODS AND RESULTS

[A] Construction of databases -

Visceral leishmaniasis is a zoonotic vector-borne disease which involves interaction between 3 major elements: the sand fly vector, the canine reservoir host, and humans who become infected by the disease agent *L. infantum*. In order to study the epidemiology and geographic spread of VL we constructed databases that contained details on each of these elements (**Table 1**). The coordinates of all locations were recorded and data were plotted on a computerized map of the region using the Arcview® software.

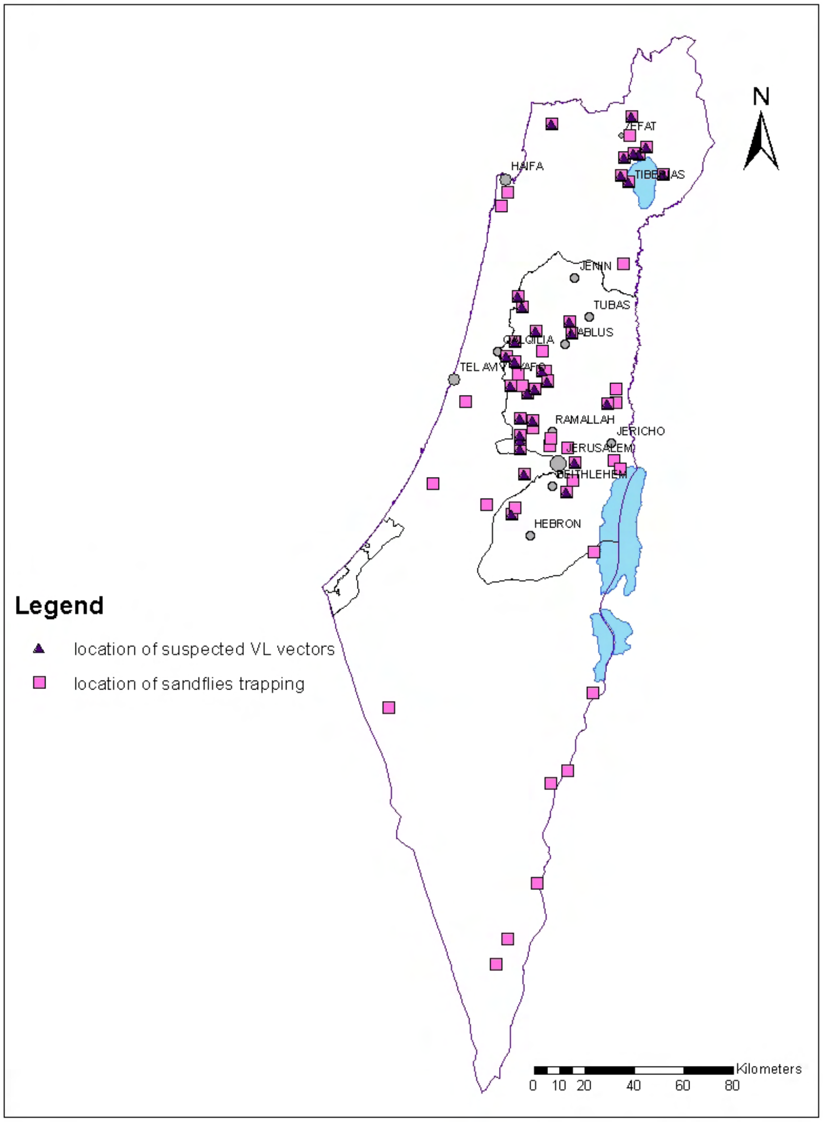
Table 1 – Details on databases constructed for the study 1994-2004

Database	Details
Sand flies Israel & Palestinian Authority	No. of sand flies caught - 14,728
	No. of trapping locations in Israel -28
	No. of trapping locations in the PA - 16
Human visceral leishmaniasis in Israel	No. of patients - 14
Human visceral leishmaniasis in the PA	No. of patients - 176
Canine visceral leishmaniasis in Israel	No. of infected dogs - 177
Canine visceral leishmaniasis in the PA	No. of infected dogs - 12

(A1) **Sand flies database** – Sand flies are the vectors of leishmaniasis. However, there are many species of sand flies present in the study area. Most of these species are not vectors of VL and are either non-vectors of any *Leishmania* parasite or vectors of cutaneous leishmaniasis spp. which include *Leishmania major* and *L. tropica* in this region. There are 4 sandfly spp. that have been described as vectors in the eastern Mediterranean (Killick-Kendrick, 1999; Jaffe et al., 2004). The vectors of *L. infantum* in the eastern Mediterranean are *Phlebotomus* (*Laroussius*) *perfiliewi*, *Phlebotomus* (*Lar.*) *neglectus*, *Phlebotomus* (*Lar.*) *syriacus* and *Phlebotomus* (*Lar.*) *tobbi*. Although we proved the presence of *L. infantum* in some of these eastern Mediterranean vector species during this study by PCR, we cautiously call them "suspected vectors" in Israel and the PA since a transmission study proving that each of these spp. can transmit *L. infantum* by feeding on an infected animal and infecting a naive animal has not been carried out.

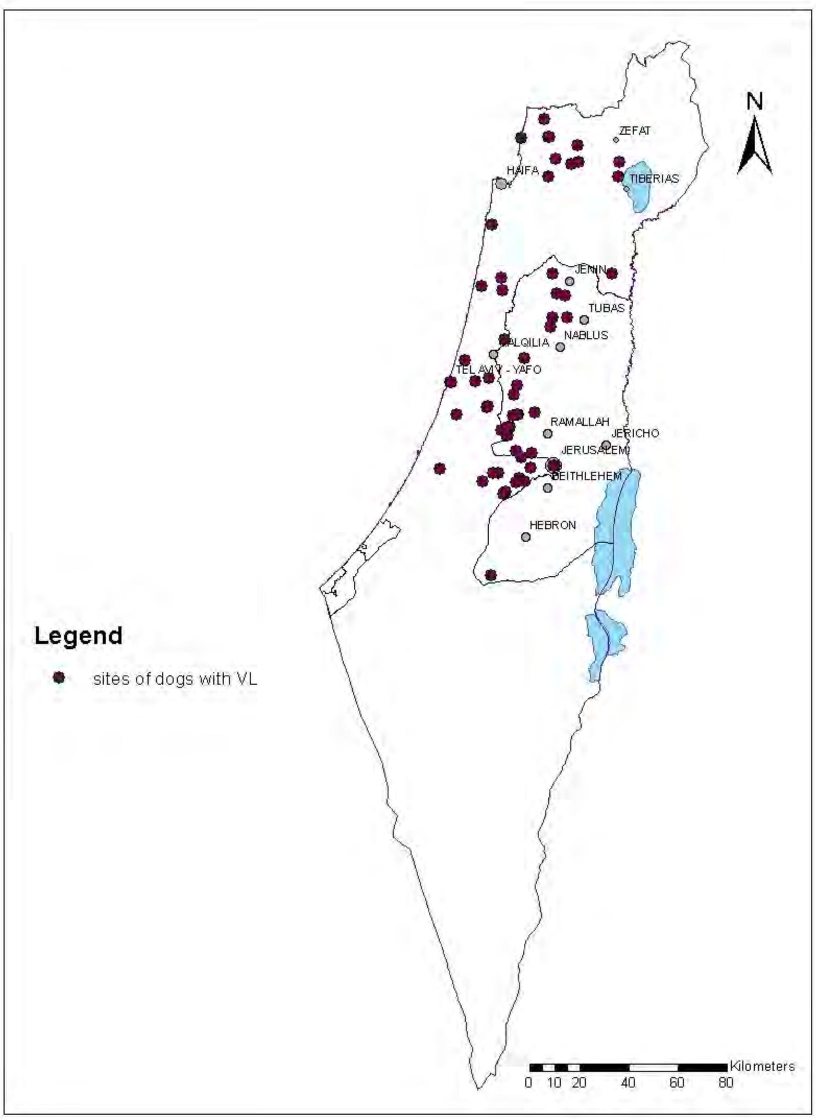
The database was constructed by putting together results from sand fly trapping in multiple locations in Israel and the PA carried out during the study period (**Figure 1**). The trapped sand flies (n = 8,400) were analyzed for species, sex, location and date of trapping. In addition, older collections of sand flies including an additional 6,300 flies were re-analyzed by the same entomologist for this study. In total there were 44 locations of trapping, 28 in Israel and 16 in the PA.. The locations were spread all over Israel and the PA from the southern Negev desert and Arava valley, to the central mountain region, the coastal plain and the Galil region in the north. The data on location of sand fly trapping was divided into locations where suspected vectors were found and locations where only other spp. of sand flies were caught. These data were plotted on a computerized map of the region using the Arcview® software (**Figure 1**).

Figure 1 - Locations of sand fly trapping in Israel and the PA showing locations where suspected vectors were found and locations where only non-vectors of *L. infantum* were detected.



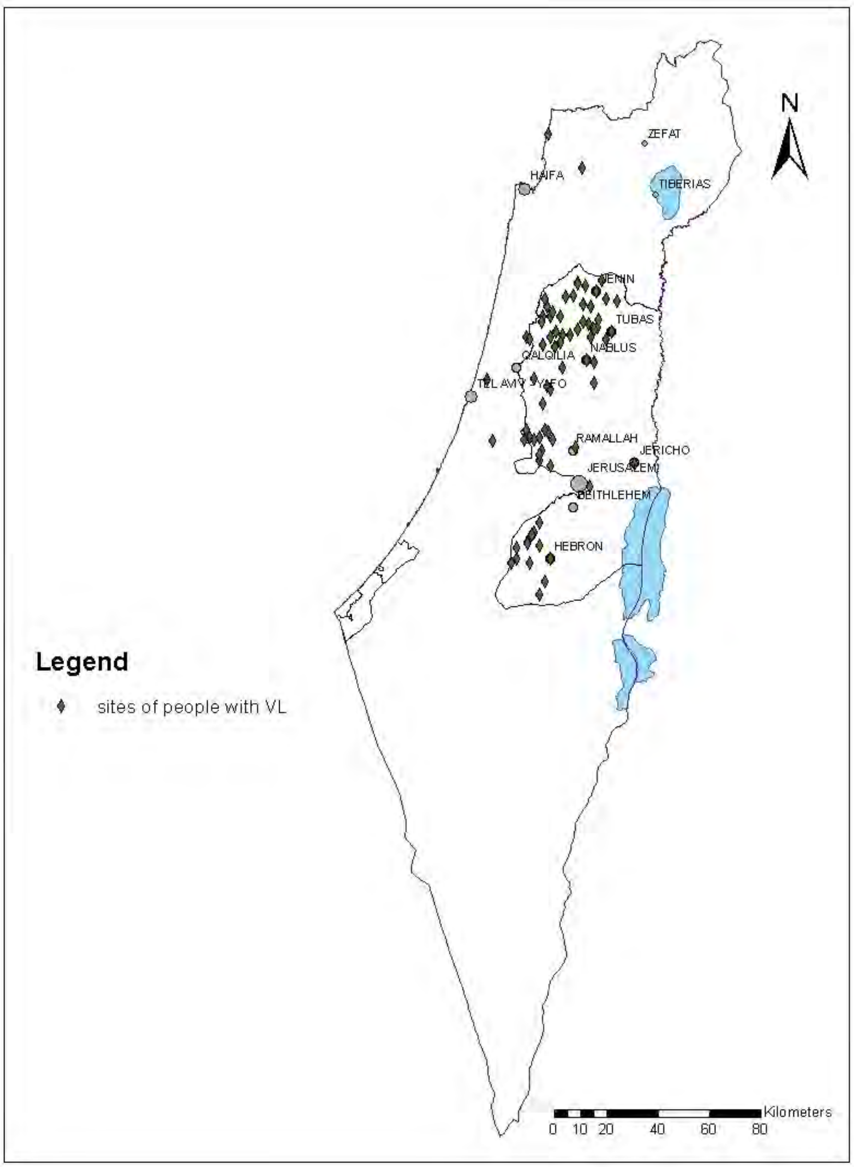
(A2) **Canine visceral leishmanasis databases and geographical spread** – Dogs are the peri-domestic reservoir of *L. infatnum*. Data was collected on the locations and number of dogs infected with VL in Israel and the PA during 1994-2004. Canine VL is a reportable disease in Israel and every new case of infection must be reported to the authorities. Our laboratories in the Hebrew and Al Quds University are the only labs. which carry out the diagnosis of this disease in Israel and the PA. Dogs were diagnosed by serology, culture of the parasite and/or PCR of lymph nodes, spleen or blood. The databases were derived from the records of the Israeli Ministry of Agriculture, Dr. Baneth and Prof. Abdeen's laboratories. The dogs were analyzed according to location, age, sex, method of diagnosis and whether they were detected by passive surveillance, e.g. referred by a local veterinarian suspecting the disease, or by active surveillance during a survey of this disease in a village. In all, 177 dogs from Israel and 12 from the PA were identified with infection. Of these, the majority, 128 (72%) dogs were detected by passive surveillance. The dogs originated from 49 different sites, of which 38 (78%) were rural settlements and 11 (22%) were urban locations. For statistical comparisons of prevalence, only dogs detected by passive surveillance were used. Infected dogs were found mostly in the central region of the Judean and Samarian hills and in the northern Gail region, with a few cases also along the coastal plain (**Figure 2**)

Figure 2 - Locations of canine visceral leishmaniasis sites in Israel and the PA.



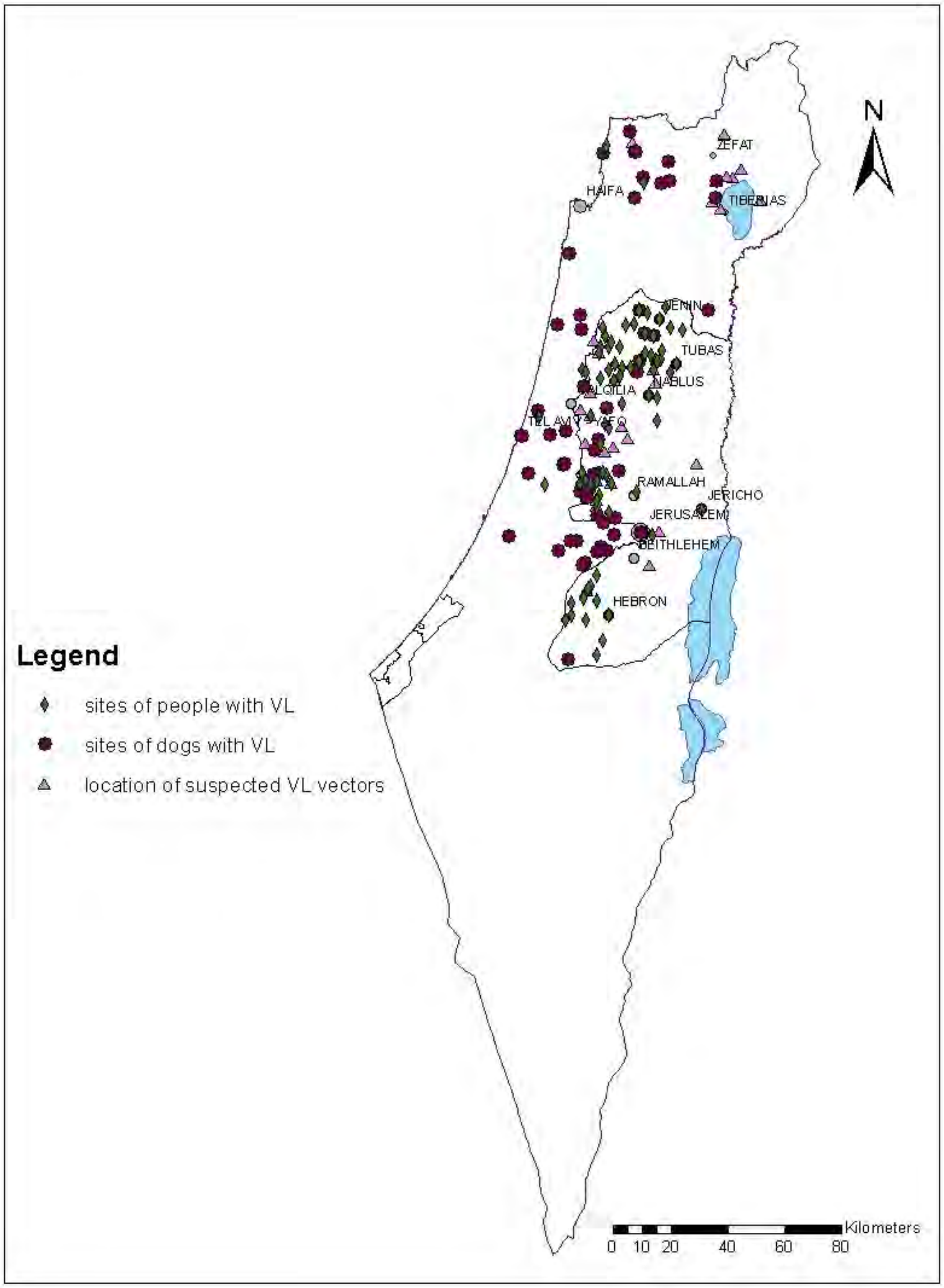
(A3) **Human visceral leishmanasis databases and geographical spread –** Human VL is a reportable disease in both Israel and the PA. The data on human infection was derived from the Palestinian and Israeli Ministries of Health and from the laboratories which perform the diagnosis in the Hebrew and Al Quds Universities. Diagnosis was achieved by bone marrow or spleen biopsies, culture, PCR, or serology. The datasets included information on age, sex, location, district and date of diagnosis. In the PA, there were 176 cases of human VL during the study period, or which 173 (98%) were children younger than 10 years. The mean age of the patients was 2.3 years and 75% of the children were between 0-3 years of age. In Israel there were only 14 cases of VL between 1995 and 2004 including 5 cases in children, 5 cases of Ethiopian immigrants (3 HIV+) who probably acquired the disease outside of Israel, 2 cases in adults and 2 cases with insufficient data. The geographic distribution of human VL included cases mostly from the Jenin, Ramallah and Hebron districts of the West Bank (**Figure 3**).

Figure 3 – Sites of human visceral leishmaniasis in Israel and the PA.



(A4) **Overlapping of human and canine VL with the presence of sand fly vectors** – Maps with the locations of human VL, canine VL and locations of suspected vectors were overlaid, merged and compared (**Figure 4**). A clear overlap in the locations of VL and suspected vectors can be seen. No cases of human or canine VL were reported in southern Israel where suitable sand fly vectors of VL were not found, although vectors of cutaneous leishmaniasis were present.

Figure 4 – Sites of human and canine visceral leishmaniasis in Israel and the PA, overlaid on the locations where suspected sand fly vectors of the disease were trapped.



[B] Studies of VL foci

Studies of specific foci of visceral leishmanaisis were conducted in several foci with high or low prevalence of infection using a finer resolution with examination of the micro-environment. The studies were conducted in the West Bank villages of Al Jedidah, Siris, Alyamoon and Quabatiyah near Jenin in the northern West Bank, and Kharas near Hebron in the southern West Bank. In Israel, studies were carried out in Nataf and Nili in the Jerusalem region, and in Klil and Kahal in the northern Galil region.

The studies included testing of dog populations by serology, isolation of parasites from positive dogs, trapping of sand flies, speciation of sand flies and performing PCR for detection of parasites in the vector sand flies.

The seroprevalence of dogs for *L. infantum* ranged between 5% in Alyamoon to 25% in Klil. Aerial photographs and maps of the villages were obtained and each house was identified. The houses where owners of infected dogs lived were signaled but no clear association could be found between the location of an owners house within the village the infection status of the dog. This is probably due to the fact that most of the dogs in these villages roam freely and may become infected also away from their owner's house.

(B1) **Sand fly fauna of Al-Jedidah (West Bank)**: Sand flies were trapped in houses, caves, domestic animal shelters, and olive groves in Al-Jedidah, a village near Jenin that is a focus of VL. The density of sand flies was found to be unusually high in the village and its vicinity. Identification of samples of males revealed *Ph. perfiliewi, Ph. syriacus* and *Ph. tobbi,* potential vectors of VL as well as *Ph. sergenti,* a vector of *L. tropica.* Population densities were highest in caves and close to animal sheds (**Fig. 5**) PCR performed on wild-caught *Ph. perfiliewi* and *Ph. syriacus* in Al-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.

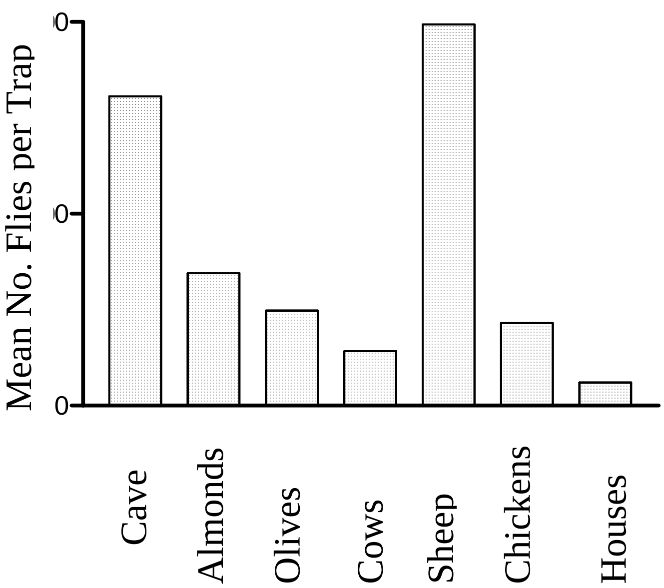


Fig. 5: Comparative densities of sand flies in different habitats in Al-Jedidah

(B2) **Sand fly fauna of Nataf (Israel):** Sand fly rapping carried out in the village of Nataf where a large percentage of the dogs were found infected with *L. infantum.* Both potential VL vectors, *P. syriacus* and *P. tobbi* constitute the major species throughout the transmission season (**Fig. 6**).

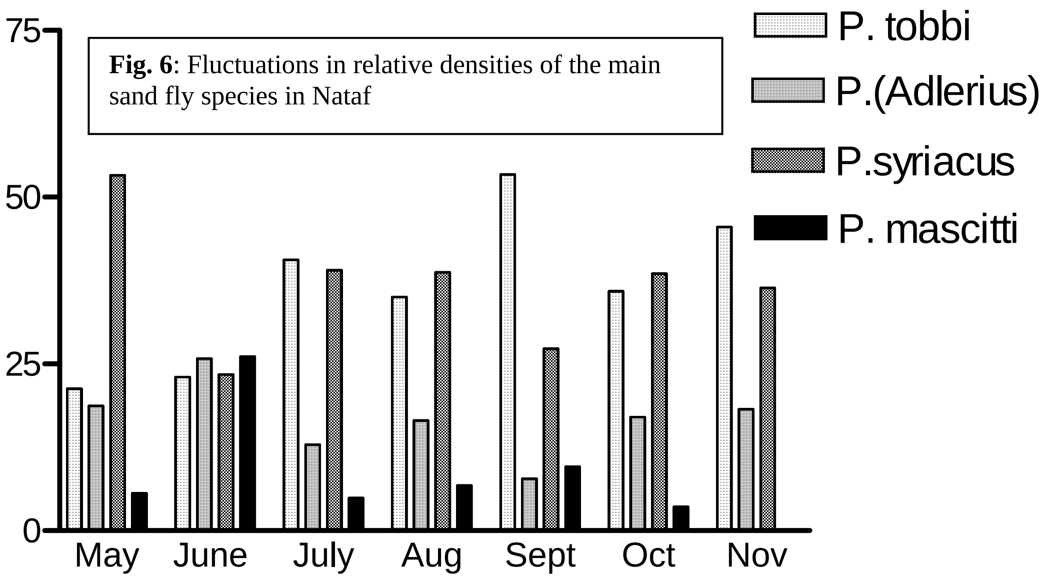


Fig. 6: Fluctuations in relative densities of the main sand fly species in Nataf

[C] <u>Analysis of *Leishmania* isolates, speciation and genotyping</u>

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. DNA fingerprinting separated the strains geographically into northern (clade A) and central (clades B & C) genotypic groups (**Table 2**; **Fig. 7**).

We concluded that the genotyping results suggest that the emergence of visceral leishmaniasis in Israel and the Palestinian Authority is not due to parasite spread from the old northern focus 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. These findings were published as a paper in the Journal of Clinical Microbiology (Nasereddin et al., 2005).

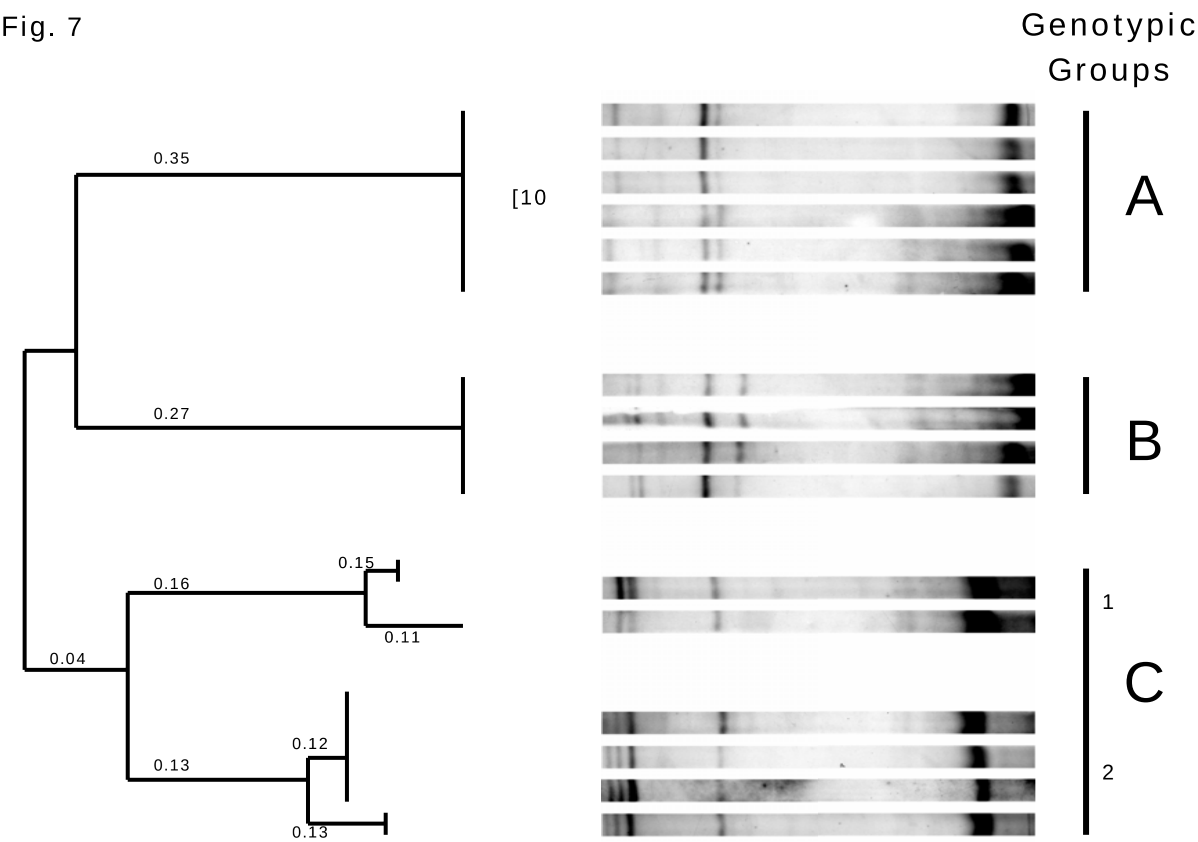
Table 2 - Sources of *L. infantum* strains examined and techniques used for characterization

Isolate LRC No.	***Source***[a]	***Origin***	**RAPD**[b]	***33.15***
L741	d	Alei Zahav	b	C
L742	h	Barkan	b	C
L921	d	Beit Arif (BArief)	-	A
L772	d	Har Adar (Hadar)	a	A
L808	d	Jenin (Jen1)	-	C
L807	d	Jenin (Jen2)	-	C
L773	h	Jenin (Jen3)	b	C
L745	h	Kiryat Sefer	b	C
L709	d	Klil (Kl1)	c	B
L705	d	Klil (Kl2)	b	B
L706	d	Klil (Kl3)	a	B
L708	d	Klil (Kl4)	b	B
L699	d	Klil (Kl5)	-	B
L911	d	Modi'in (ModS)	-	C
L894	d	Maskeret Bataya (Mbat)	-	C
L639	d	Nataf (Nat1)	a	A
L718	d	Nataf (Nat2)	b	A
L700	d	Nataf (Nat3)	-	A
L716	d	Nataf (Nat4)	c	A
L717	d	Nataf (Nat5)	c	C
L792	d	Nataf (Nat6)	c	A
L966	d	Nataf (Nat7)	-	A
L880	d	Neve Ellan (NevE)	-	C
L695	d	Nili (Nil1)	b	C
L697	d	Nili (Nil2)	c	C
L690	d	Nili (Nil3)	c	C
L692	d	Nili (Nil4)	c	C
L693	d	Nili (Nil5)	c	C
L696	d	Nili (Nil6)	-	A
L783	d	Reshafim	b	B
L842	d	Reut	-	C
L760	d, s	Rosh Ha'ayin	b	A
L689	d	Sataf1	-	C
L847	d	Sataf2	-	C
L869	d	Shilat	-	C
L905	d	Shoham	-	C
L720	d	Tzur Natan	b	C

[a] Source, d - dog, h - human, s – sand fly.
[b] Technique used to analyze the *L. infantum* strains is indicated as follows: RAPD - RAPD-PCR using the primer F10, clades a-c; 33.15, DNA fingerprinting with the human multilocus probe 33.15, clades A-C.

Figure 7 - DNA fingerprinting patterns and genotypes of Israeli and Palestinian *L. infantum* obtained with the human multilocus probe 33.15. The dendogram was calculated using the RAPDistance Package (version 1.04) based on pairwise comparison of the Southern Blot hybridization patterns. The number strains belonging to each genotypic group and subgroup (Clades A, B, C1 and C2) is given in square brackets.

Fig. 7



[D] Serosurvey of domestic and wild canines

The prevalence of *L. infantum* infection in dogs and in populations of indigenous wild canid species such as jackals and foxes was not previously determined in Israel and the PA. Although it has been shown by us that infection rates in some specific foci of the disease are high (Baneth et al., 1998; Abdeen et al., 2002), it was not known what the rates of seropositivity are in a randomly-collected sample of dogs. For this purpose sera of dogs (n=790) were collected from municipal dog pounds all over Israel, society for prevention of cruelty to animals (SPCA) facilities, private veterinary clinics and from dogs admitted to the Hebrew University Veterinary Teaching Hospital. A second group of dogs included dogs (n=146) from foci of endemic VL in Israel and the West Bank (PA). In addition, sera from wild canids (jackals, foxes, wolves) caught by the National Reserves Authorities were analyzed for seropositivity to *Leishmania infantum.* Serology was performed by ELISA as previously described for dogs and wild canids (Baneth et al., 1998).

Of 790 dog sera collected randomly, only 6 (0.76%) were positive, whereas 15% of the 146 sera from dogs living in the endemic foci were positive. This suggests that VL is highly focal with a relatively low incidence of disease in the general dog population, and a much higher prevalence in endemic foci. This finding further strengthens the notion that VL is present in areas where suitable conditions for transmission are present, hence justifying the need for a GIS study to determine the ecological risk factors for this infection.

The seroprevalence rates among wild canids were of special interest, since wild canids could constitute a natural reservoir for this infection and be responsible for infection of dogs or humans who live in close vicinity. 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 (**Table 3**). This suggests that mainly jackals are possible reservoir hosts for VL with a seroprevalence rate that greatly surpasses that of the randomly tested dogs.

Table 3 –Seropositivity to *Leishmania infantum* among domestic dogs and wild canids in Israel and the West Bank (PA).

Species	**Number tested**	**Number of seropositive**	**Percent of seropositive**
Dogs random collection	790	6	0.76
Dogs endemic foci	146	22	15
Dogs total	936	28	3
Jackal	158	12	7.6
Fox	93	2	2
Wolf	16	1	6

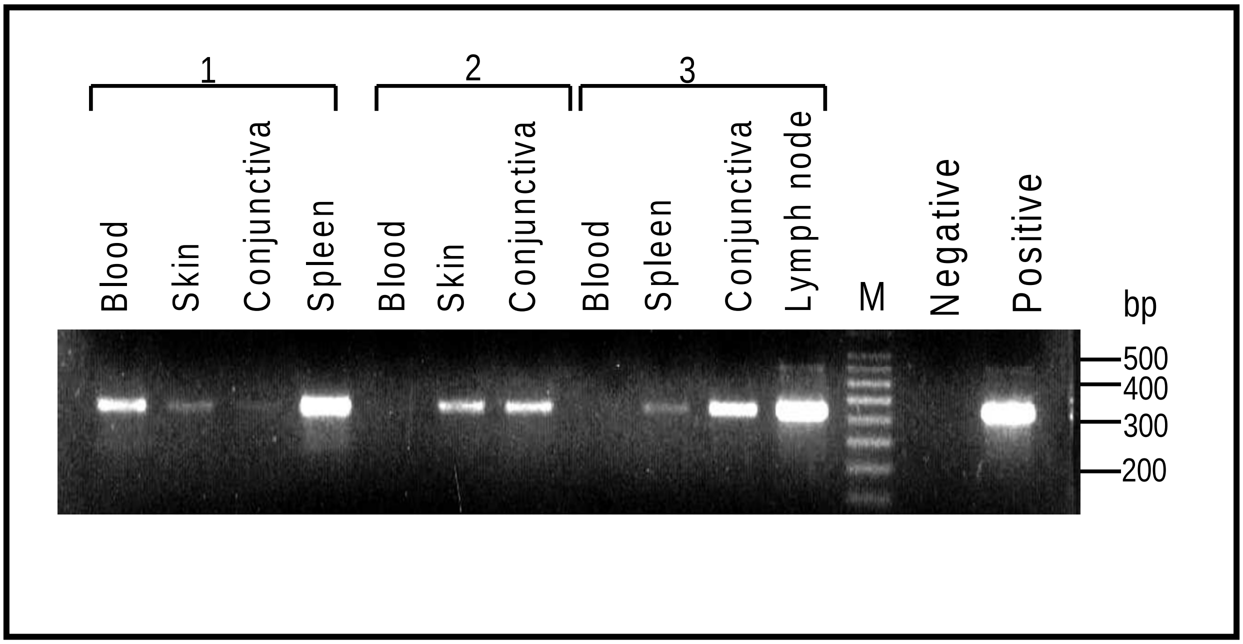
[E] Improved molecular diagnosis of canine visceral leishmaniasis and sand fly infection

(E1) **Non-invasive molecular diagnosis**

Confirmation of visceral leishmanaisis infection often requires a complimentary technique in addition to serology. Culture of the parasite from lymph node, spleen or bone marrow aspirates is often impractical, requires a long time for the result, and is not available in diagnostic laboratories that do not specialize in leishmaniasis. Detection of the parasite by microscopy in cytological specimens is often not possible even in dogs with severe disease symptoms, because very few parasites can be seen in the tissues in about 50% of the cases. Molecular diagnosis by PCR which detects specific DNA belonging to the *Leishmania* parasite is a very sensitive diagnostic method which has become common in diagnostic and research laboratories. During this study, we developed diagnostic PCR assays and evaluated their sensitivity in comparison to serology and culture. We placed special emphasis on non-invasive diagnostic techniques which could detect infection without having to perform highly invasive procedures such as bone marrow and spleen biopsies. Non-invasive sampling of dogs by collecting cells from the conjunctiva using a cotton swab, from which DNA was extracted was found to be superior to invasive diagnosis by aspirates from the spleen or by PCR of blood. This new technique is useful for direct diagnosis of infection and also for prevalence studies in dog populations.

PCR for the internal transcribed spacer1 (ITS1; Schonian et al., 2003) was used in this study and a total of 92% of the symptomatic seropositive dogs were found to be positive by use of DNA from conjunctival swabs. Aspirates from lymph node and spleen were found to be positive in 86% and by culture in 74% of these dogs (**Fig. 8**). The sensitivity and specificity of conjunctival PCR were 92% and 100%, respectively. These findings were published in the Journal of Infectious Diseases (Strauss-Ayali et al., 2004).

Figure 8 – ITS-1 PCR from the conjunctiva, blood, skin scraping, spleen and lymph node from 3 dogs seropositive for canine leishmaniasis.



(E2) **Comparison of several blood PCR techniques and ELISA serology for the detection of canine leishmaniasis**

Domestic dogs from the northern West Bank (n=148) were screened for anti-leishmanial antibodies by enzyme-linked immuonosorbent assay (ELISA). Ten dogs (6.8%) were seropositive. Promastigotes were isolated from one seropositive dog and identified as *L. infantum* by Excreted Factor (EF) serotyping, isozyme electrophoresis, and Polymerase Chain Reaction (PCR). In addition to the ELISA, the internal transcribed spacer 1 (ITS1) -, modified ITS1 (mITS1) -, and kinetoplast DNA (kDNA) - PCRs, were used to validate this technique as a diagnostic tool for canine VL using blood. Each assay was performed on 60 blood samples. kDNA-PCR (13/60 positives, 21.7%) was the most sensitive of the assays examined followed by mITS1-PCR (9/60, 15.0%), ELISA (5/60, 8.3%) and ITS1-PCR (3/60, 5%). However, ITS1-PCR and mITS1-PCR were also capable of identifying the parasite species and indicated they belong to *L. infantum.* In view of its higher sensitivity, kDNA-PCR is recommended for the routine diagnosis of CVL. This study was performed by the Al Quds laboratory and has been accepted for publication in the Journal of Parasitology (Nasereddin et al, in press).

(E3) **Sand flies PCR for detection of *L. infatnum* infection**

The originally planned sand fly ELISA for detection of *Leishmania* parasites in sandflies was replaced with a more advanced technique of direct PCR to detect *Leishmania* DNA in sandflies using the ITS1 PCR. Direct PCR has the advantage of enabling the speciation of the *Leishmania* parasites in the sand flies by DNA sequencing or PCR and restriction fragment length polymorphism (RFLP). PCR was performed on wild-caught sand flies in the West Bank (PA). *Ph. perfiliewi* and *Ph. syriacus*, two sand fly spp. known as *L. infatnum* vectors in other countries in the Eastern Mediterranean were found infected with *L. infantum* in sand flies from the West Bank focus of Al Jedidah. This indicates that these two vectors are probably of importance in transmitting visceral leishmaniasis in the West Bank. The rate of infection in sand flies was < 1% similar to rates of *L. infatum* infection in sand flies elsewhere. The identity of *L. infantum* was verified by both sequencing and RFLP.

[F] <u>Ecologic and Geographic Analyses of Study Findings</u>

Overlaying of the data obtained with the construction of the databases over geographical and different ecologic conditions maps allowed us to make statistical analyses and look for different factors that effect the spatial distribution of human and canine VL, and also the presence of sand fly vectors. Mutlivariate statistical analysis was performed using the SPSS software.

(F1) <u>Sand Flies</u>

The locations where sand fly species who are potential vectors of VL were trapped were compared to the locations where non-vector spp. were found. A significant statistical difference in the annual rainfall was found between locations where suspected sand fly vectors were trapped vs. locations where non-vector spp. were trapped (P= 0.0226) (**Figs. 9 & 11**). The mean annual rainfall in sites of suspected vectors was 550 mm whereas the mean in sites of non-vectors was just above 350 mm. In addition, a significant statistical difference in the landuse patterns was found between locations where suspected sand fly vectors were trapped vs. locations where non-vector spp. were trapped (P= 0.015) with regions of suspected vectors being richer in vegetation. Vegetation in these sites included mostly olive groves and natural forests (**Fig. 10**). No statistical difference was found between locations where suspected sandfly vectors were trapped vs. locations where non-vector spp. were trapped when the following parameters were compared: altitude, mean temperatures in October or August, and wind speed at the surface level.

Figure 9 – Comparison in the annual rainfall between locations where suspected vector sand flies were trapped vs. locations of non-vector spp.

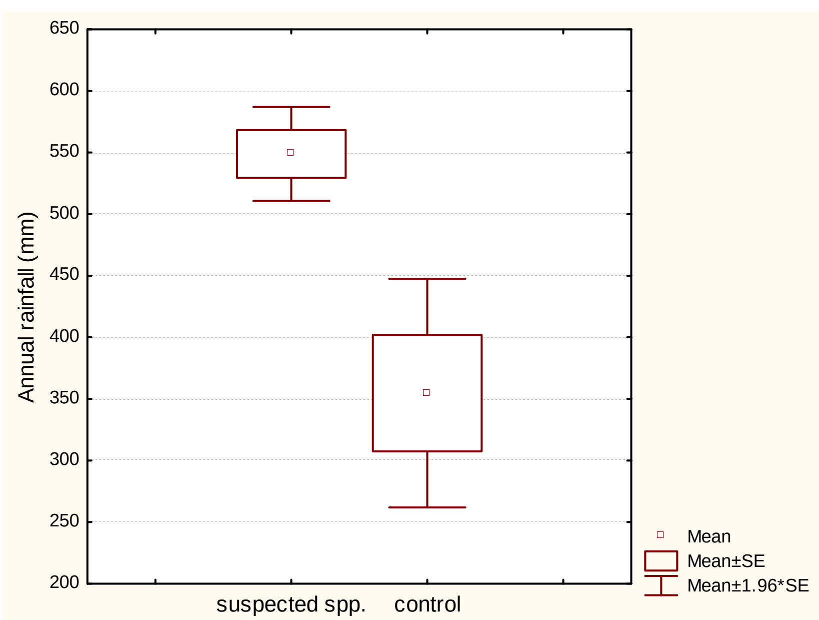


Figure 10 – Landuse in sites where suspected vector sand flies spp. were trapped.

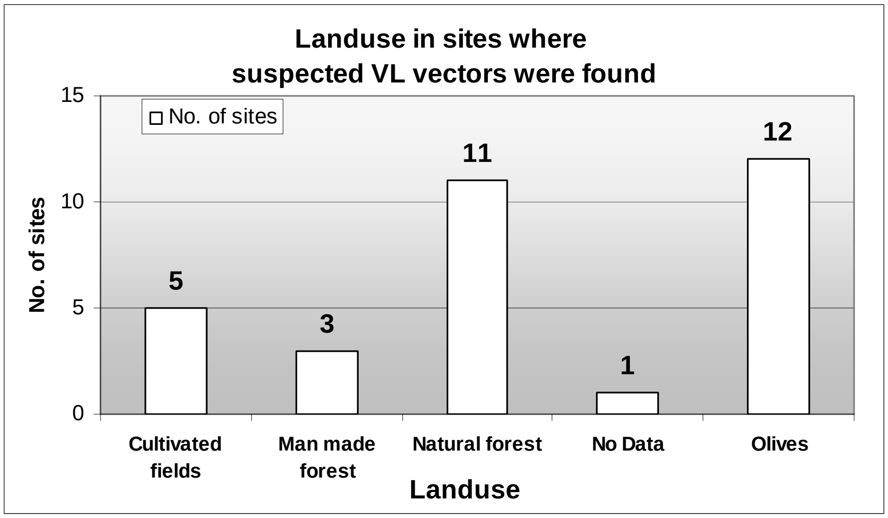
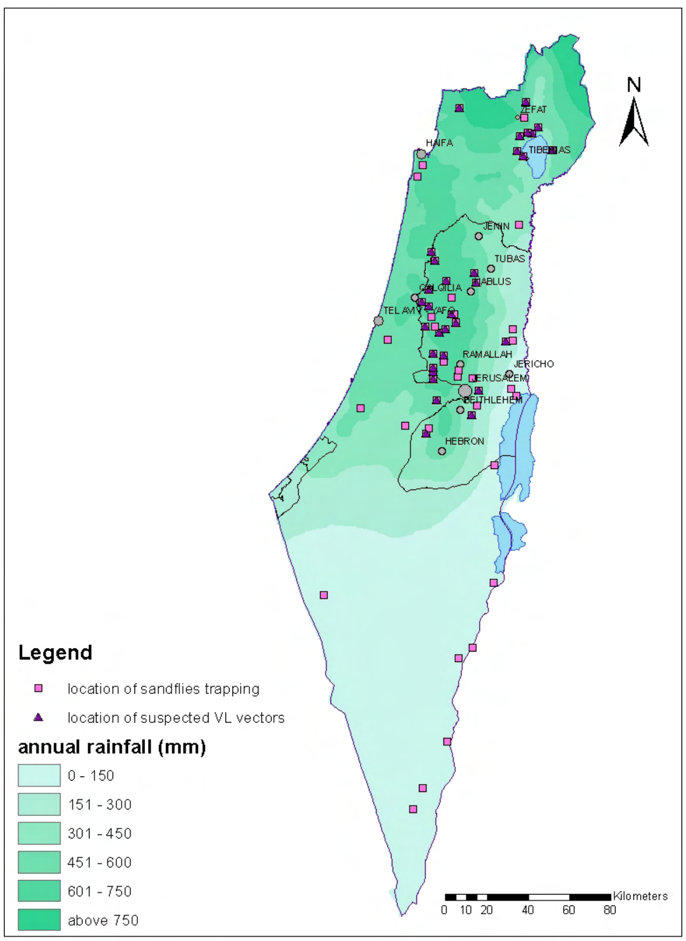


Figure 11 – A map of annual rainfall zones overlaid on the locations where suspected sand fly vectors of the disease were trapped and sites of non-vector trapping.

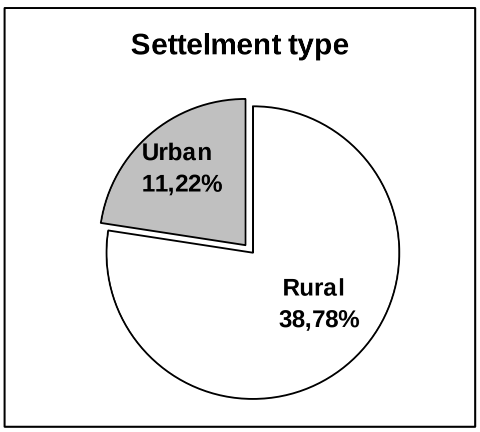


(F2) Canine leishmaniasis

One hundred and 77 dogs from Israel and 12 from the PA were identified with infection. Of these, the majority, 128 (72%) dogs were detected by passive surveillance. For the purposes of statistical analyses and tracking of epidemiologic trends, only dogs detected by passive surveillance were included in the study. All of the dogs detected by passive surveillance were from Israeli owners, however, a small minority of these dogs came from West Bank settlements. The 12 dogs with VL from the PA were all detected by the Al Quds University team by active surveillance. Seventy five percent of the dogs originated from central Israel, whereas 25% were from northern Israel. The analyzes of canine leishmaniasis were limited because there are no figures for the precise size of canine populations in most of the locations, hence no incidence rates could be calculated. This differs from our study on human VL where the size of the human populations of foci and districts was known and enabled the calculation of incidence and comparisons between locations and districts with calculations of risk factors.

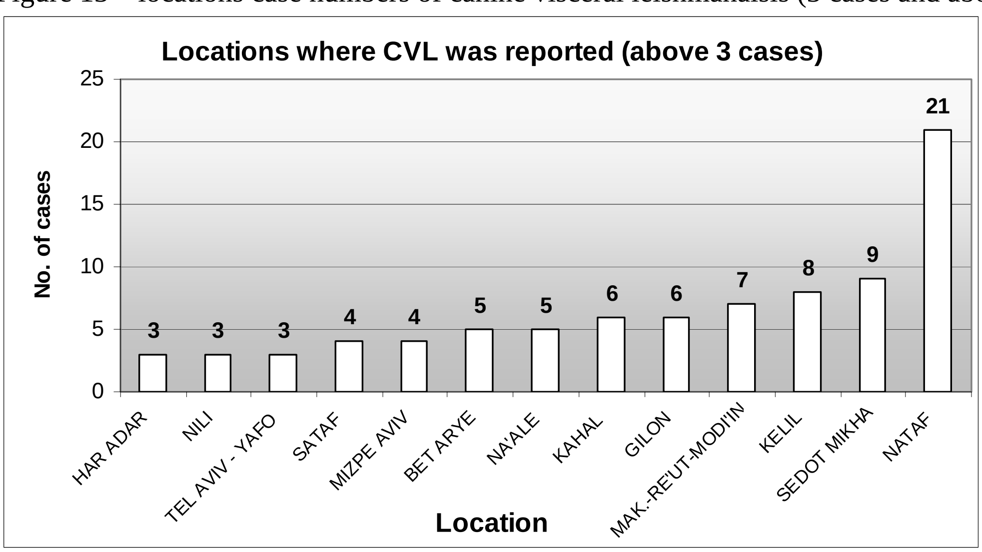
The mean annual number of canine cases during the previous 5 years was 18 and no seasonal trend for infection could be detected when analyzing the month of diagnosis. The dogs originated from 49 different sites, of which 38 (78%) were rural settlements and 11 (22%) were urban locations (**Fig. 12**).

Figure 12 – Division of canine leishmaniasis locations into urban and rural foci.



The geographic distribution of canine leishmanais can be seen in **Fig. 2** and the major locations of multiple cases are detailed in **Fig. 13**.

Figure 13 – locations case numbers of canine visceral leishmanaisis (3 cases and above).



A particularly worrying aspect of the spread of canine leishmaniasis is the incursion of disease into urban areas. Although the majority of cases occurred in rural settlements (104/128; 82%), 24 cases (18%) were from cities (**Table 4**) and most dogs have never left their city according to the owners and thus were most probably infected within these cities. This suggests that active transmission of VL occurs within major cities in Israel including Modiin, Tel Aviv and Jerusalem. Infection in dogs could be associated with human VL.

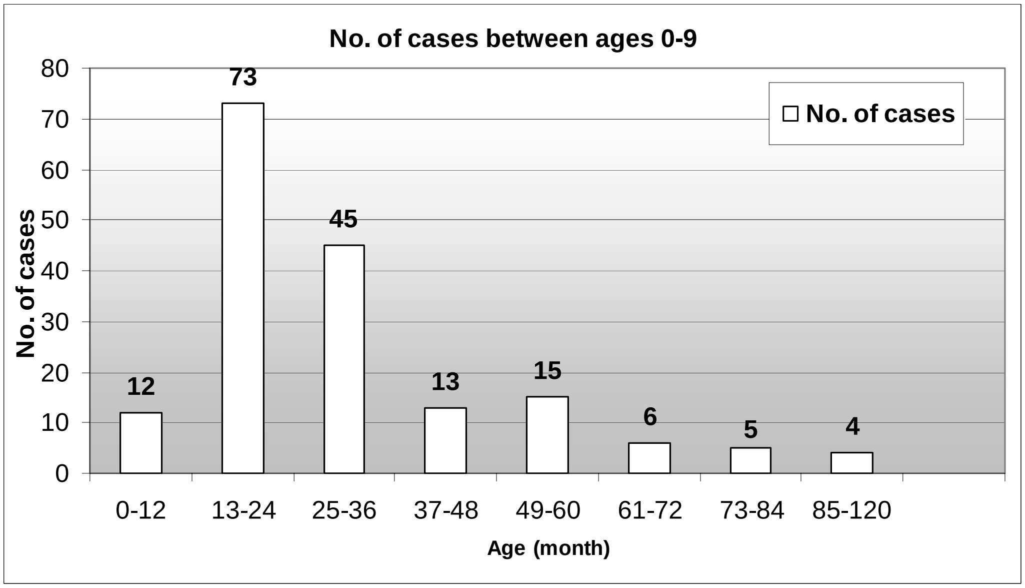
Table 4 – locations of urban canine leishmaniasis

Geographical Location	**Location**	**No. of cases**	**Years**
Judean Foothills	Beit Shemesh	1	1996
Center	Rishon Lezion	1	1997
Ramla	Maccabim-Reut Modiin	7	1998-2001, 2003 2002-2003
Petah Tiqwa	Rosh Ha'ayin	1	1999
Haifa	Hadera	1	2000
Judean Mountains	Jerusalem	2	2000, 2004
Ramla	Shoham	2	2001, 2003
Tel Aviv	Herzeliya	2	2002, 2004
Tel Aviv	Tel Aviv	3	2002-2004
Petah Tiqwa	Petach Tikva	1	2004
Total	11 urban locations	**24 cases**	

(F3) Human visceral leishmanasis

The database of human VL from the West Bank (PA) included a large number of patients which together with the data on the population of the locations from which these patients originated, enabled analyses of incidence and also comparisons of areas with low or high infection rates. Of 176 cases of human VL in the PA during the study period, 173 (98%) were children younger than 10 years. The mean age of the patients was 2.3 years and 75% of the children were between 0-3 years of age (**Fig. 14**).

Figure 14 – Age distribution of 173 human VL patients aged 0-9 years in the West Bank (PA)



In Israel there were only 14 cases of VL between 1995 and 2004 including 5 cases in children, 5 cases of Ethiopian immigrants (3 HIV+) who probably acquired the disease outside of Israel, 2 cases in adults and 2 cases with insufficient data. Due to the small number of autochthonous VL cases in Israel, only the cases from the PA were analyzed statistically for the impact of ecological risk factors. Figures on the population of each district, village and city in the West Bank for each year were available from the Palestinian Ministry of Interior, from the Palestinian census data and from institutional websites containing these data.

Ninety of the 173 (52%) VL patients who were under the age of 10 years were males and there was no statistically-significant difference between females and males (**Fig. 15**). There was also no seasonality pattern for the month of diagnosis of VL among children in the study **(Fig. 16**). The annual numbers of children patients under the age of 10 years fluctuated from 32 in 1995 to 1 in 2002 with the highest incidence of 2.3 children/100,000 in 1999 and a mean annual incidence of 1.15 per 100,000 children (**Fig. 17**). Incidence rates were calculated from 1997 to 2003. In comparison, the mean annual incidence of VL in children 0-10 y old in Israel calculated over 11 years was roughly 0.048 per 100,000.

Figure 15 – Sex of children with visceral leishmaniasis in the West Bank (PA)

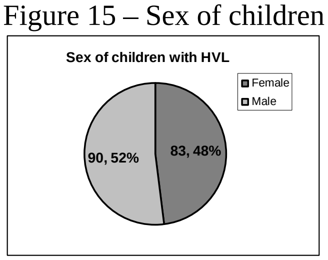


Figure 16 – Month of diagnosis of children under the age of 10 years with VL in the West Bank (PA).

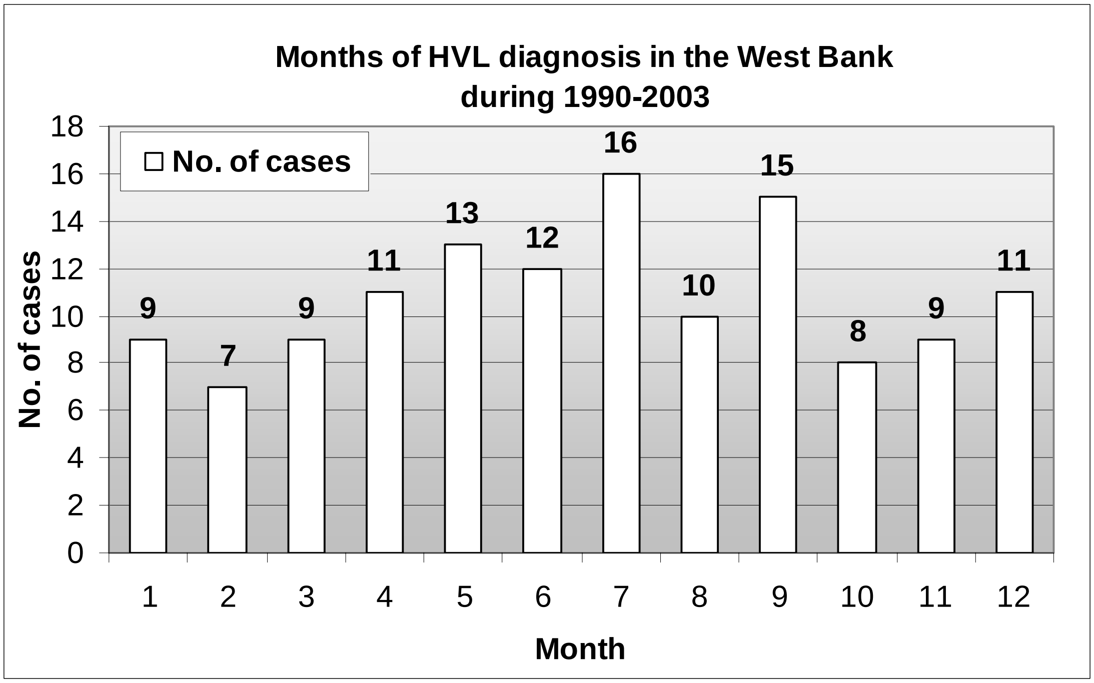
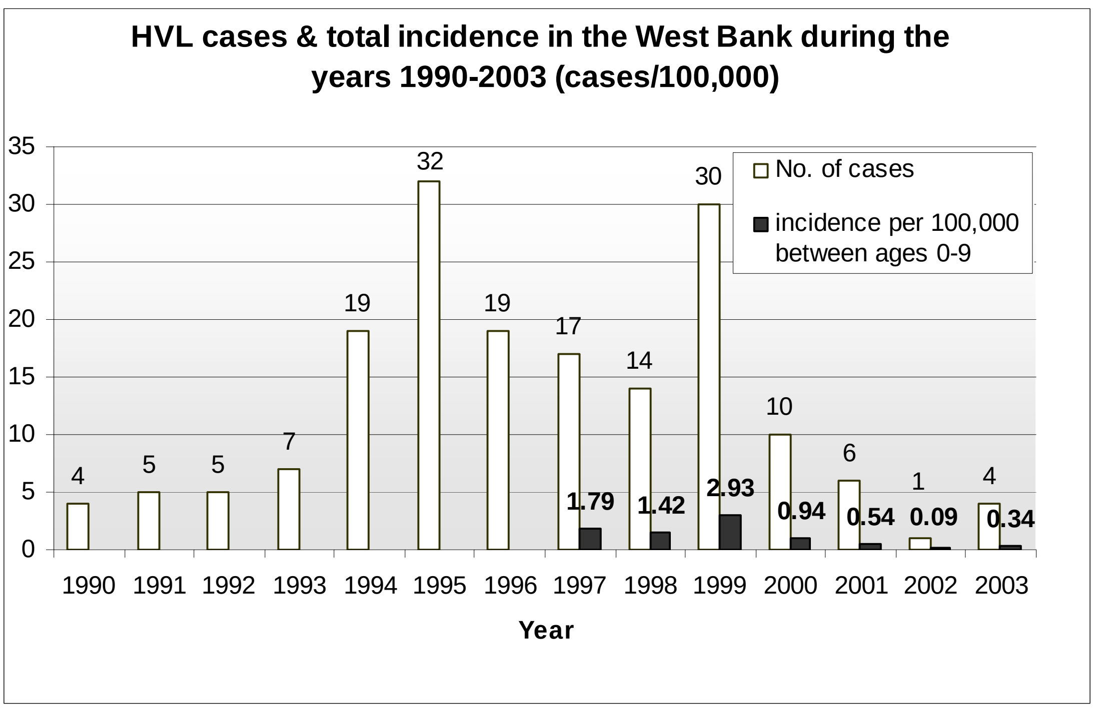


Figure 17 – Human VL and incidence of disease in the West Bank (PA)



The distribution of human VL patients under the age of 10 years in the different West Bank districts is shown in **Table 5**. The districts of the West Bank (PA) are shown if **figure 18**. The highest number of patients was in the southern district of Hebron from which 38% of the patients originated. This was followed by the northern districts of Jenin+ Tubas with 32%, and Tulkarem+Qualkilia+Salfit in the northwestern region with 25%. Very few patients were recorded in the central Bethlehem and Jericho districts and in the Nablus district.

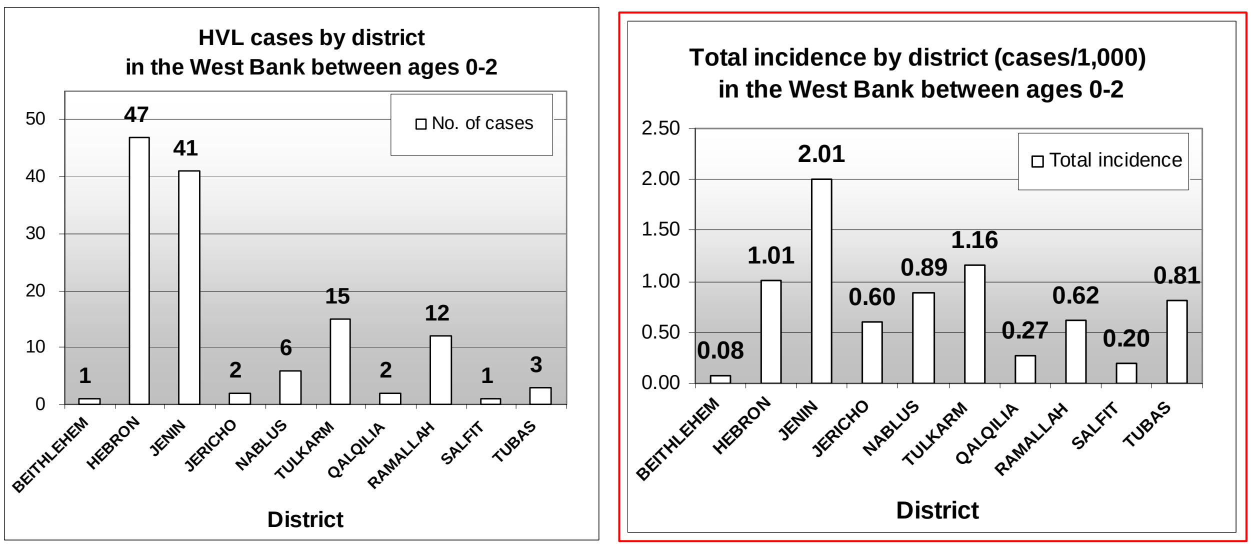
Table 5 – Distribution of VL patients under 10 years of age in the West Bank (1990-2003) by district and year.

Year District	**90**	**91**	**92**	**93**	**94**	**95**	**96**	**97**	**98**	**99**	**00**	**01**	**02**	**03**	**Total number (%)**
Hebron				1	1	4	13	6	7	18	9	4		2	65 (37%)
Jenin + Tubas	3	2 1	2	2	9	14 1	3 1	7	4	5					54 (32%)
Tulkarem + Qualkilia + Salfit	1	1 1	1	1	4 4	8		1 1		1 1				2	25 (15%)
Ramallah			2	2	3	3	1	1	2	3	1		1		19 (11%)
Nablus						2	1		1	1		2			7 (4%)
Jericho								1		1					2 (1.5%)
Bethlehem				1											1 (0.5%)
Total	4	5	5	7	19	32	19	17	14	30	10	6	1	4	173 (100%)

Figure 18 – The districts of the West Bank (PA).

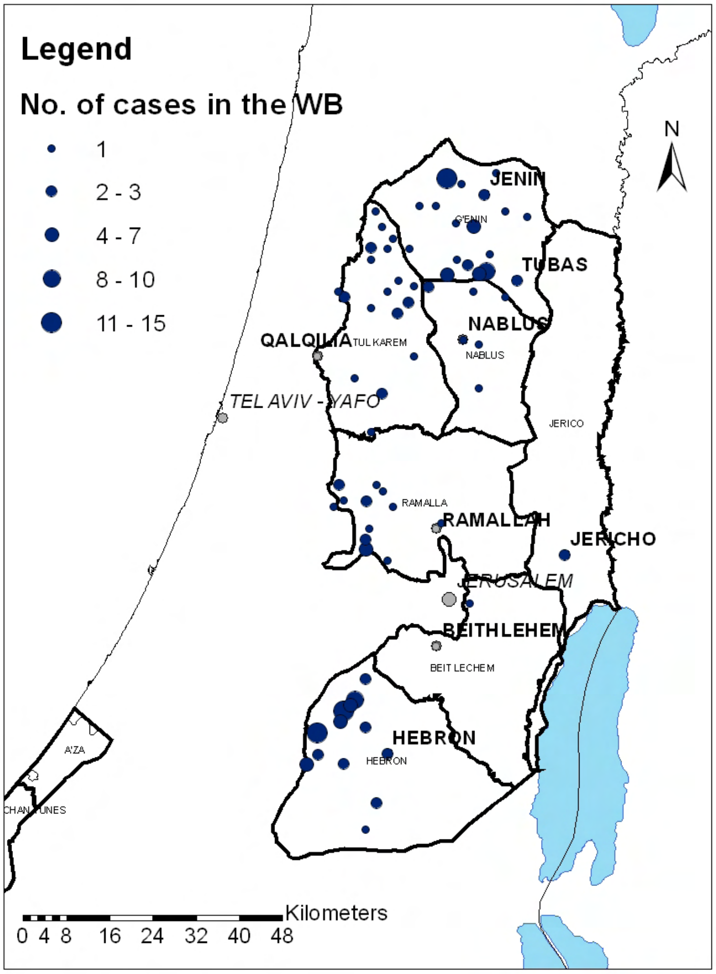
The age group which included the majority of the patients was the 0-2 years (0-36 months) group (**Fig. 14**). When analyzing the numbers of patients and incidence of disease in that age group by district, it was found that although the highest number of patients was in the Hebron district (n=41), the incidence of disease per 1,000 children aged 0-2 years was highest in the Jenin district (n=41) with 2.01 patients per 1,000 population of 0-2 years. This was two times higher then the incidence of disease in the Hebron district (Fig. 19) and indicated that the Jenin region is indeed a district where children are at high risk for this potentially fatal infection (Abdeen et al., 2002).

Figure 19 – The number of human VL patients and the incidence of infection by district among children aged 0-2 years in the West Bank (PA)



The distribution and clustering of human VL patients in the West Bank are shown in figure 20. Cases were found in several clusters : (1) Hebron west; (2) Ramallah west; (3) Tulkarem northeast; (4) Tulkarem north; (5) Jenin south; (6) Jenin northwest. These clusters are also in line with the geographical barriers and habitats present in those regions.

Figure 20 – Distribution and clustering of VL patients in the West Bank (PA).



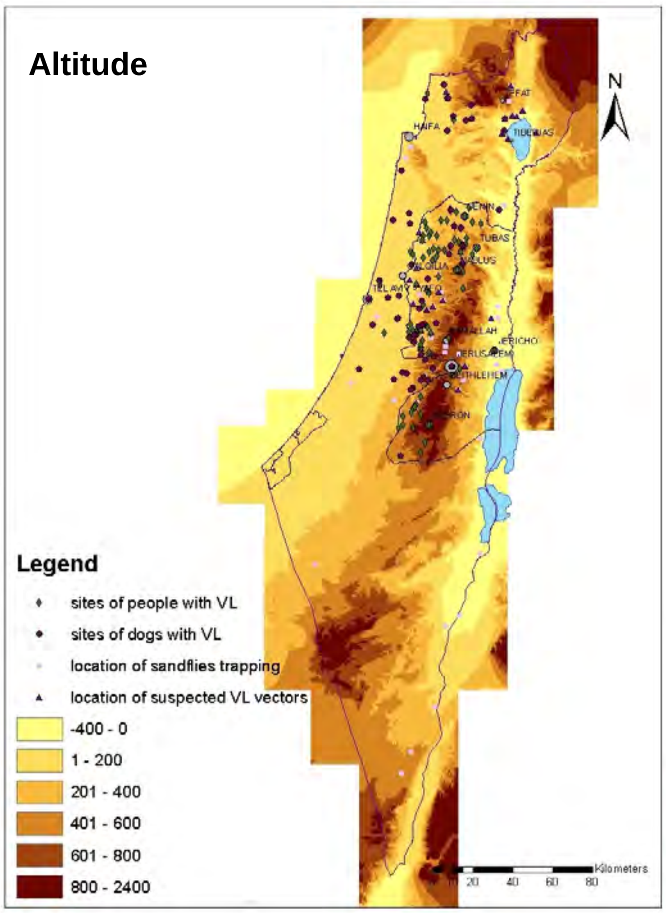
[G] <u>Ecological risk factors for visceral leishmaniasis</u>

The databases created during this study were merged and chartered on a GIS map using locations marked by coordinates. These databases included human VL in the PA and Israel; canine VL and locations of suspected vector sand flies. In order to analyze the data, this map was overlaid on the following maps:

(1) Geographic altitude (**Fig. 21**)
(2) Annual rainfall (**Fig. 21**)
(3) Mean temperatures in January (the coldest month) and August (the warmest month) (**Fig. 22**)
(4) Soil types
(5) Land use (**Fig. 23**)
(6) Wind directions (**Fig. 23**)

A comparison of the locations of sand fly vectors, infected dogs and infected human patients was able to provide a qualitative picture of where disease transmission is present and how the geographical risk factors effect it's distribution. However, the incidence data for the human disease was information which allowed statistical analyses for the effect of ecologic determinants and definition of risk factors for the presence of the disease. The ecological risk factor analyses for VL in the West Bank (PA) are detailed in the next chapter.

Figure 21 – A map of Israel and the Palestinian Authority showing altitudes, annual rainfall, and the location of human and canine visceral leishmaniasis, sites where sand fly species that are potential vectors of visceral leishmaniasis were trapped and all other sand fly trapping sites.



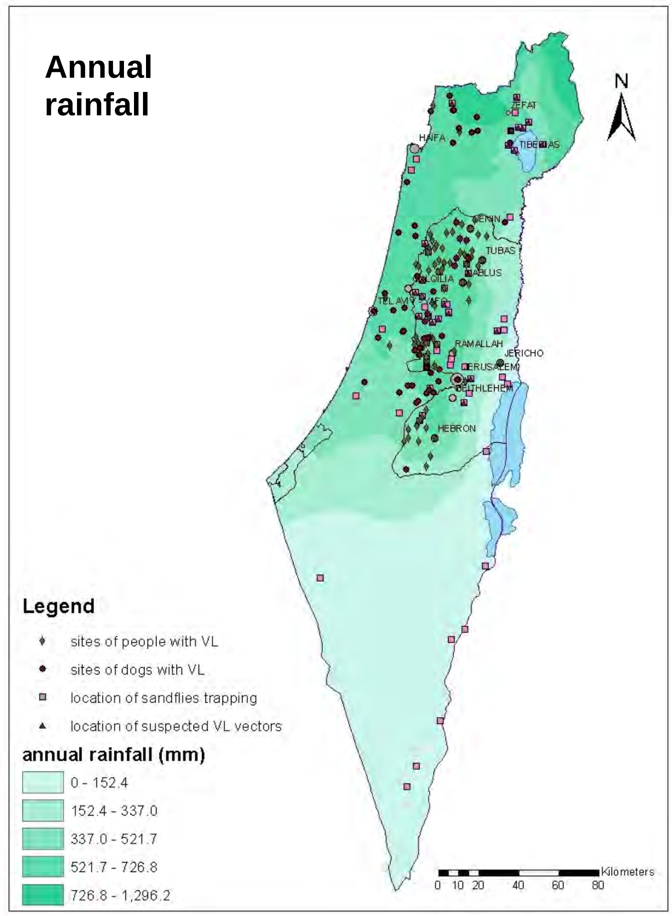


Figure 22 – A map of Israel and the Palestinian Authority showing the average temperatures in January (winter) and August (summer) and the location of human and canine visceral leishmaniasis.

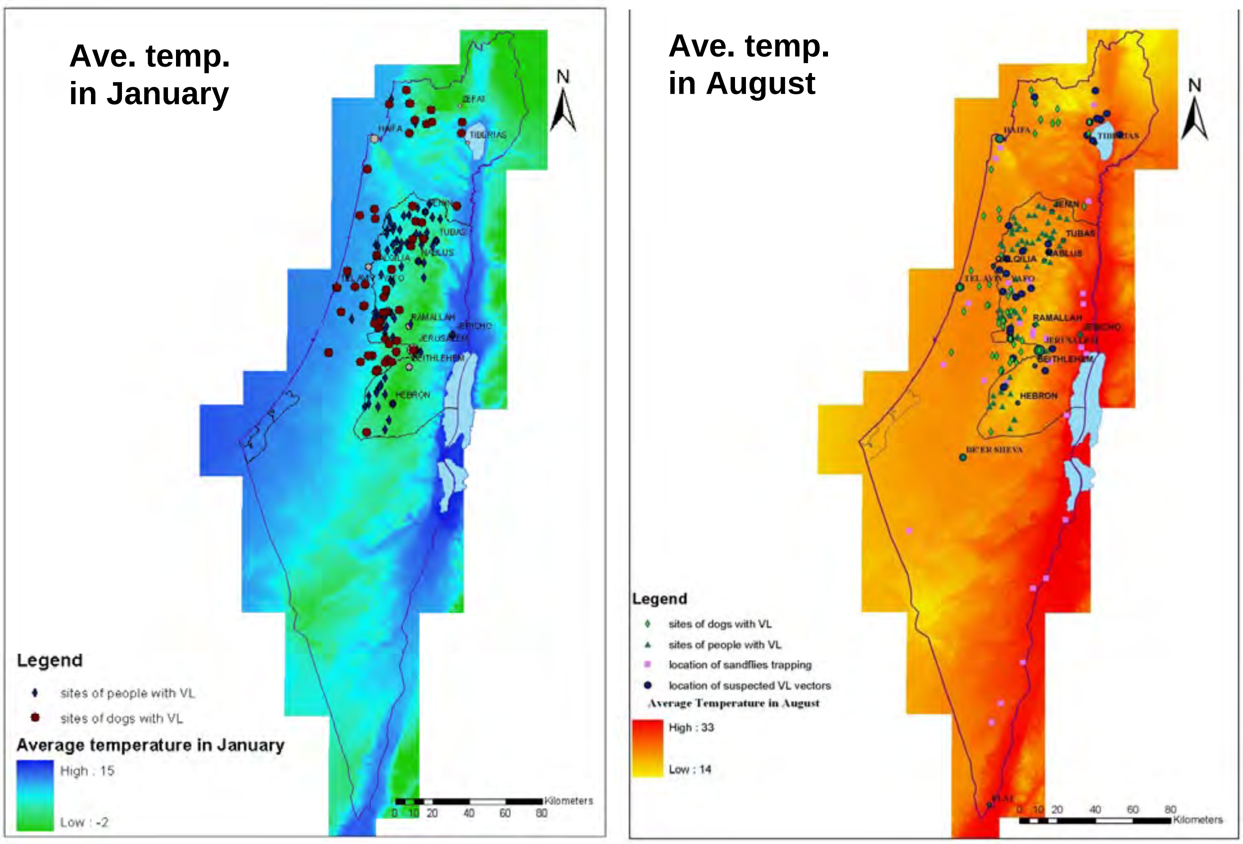
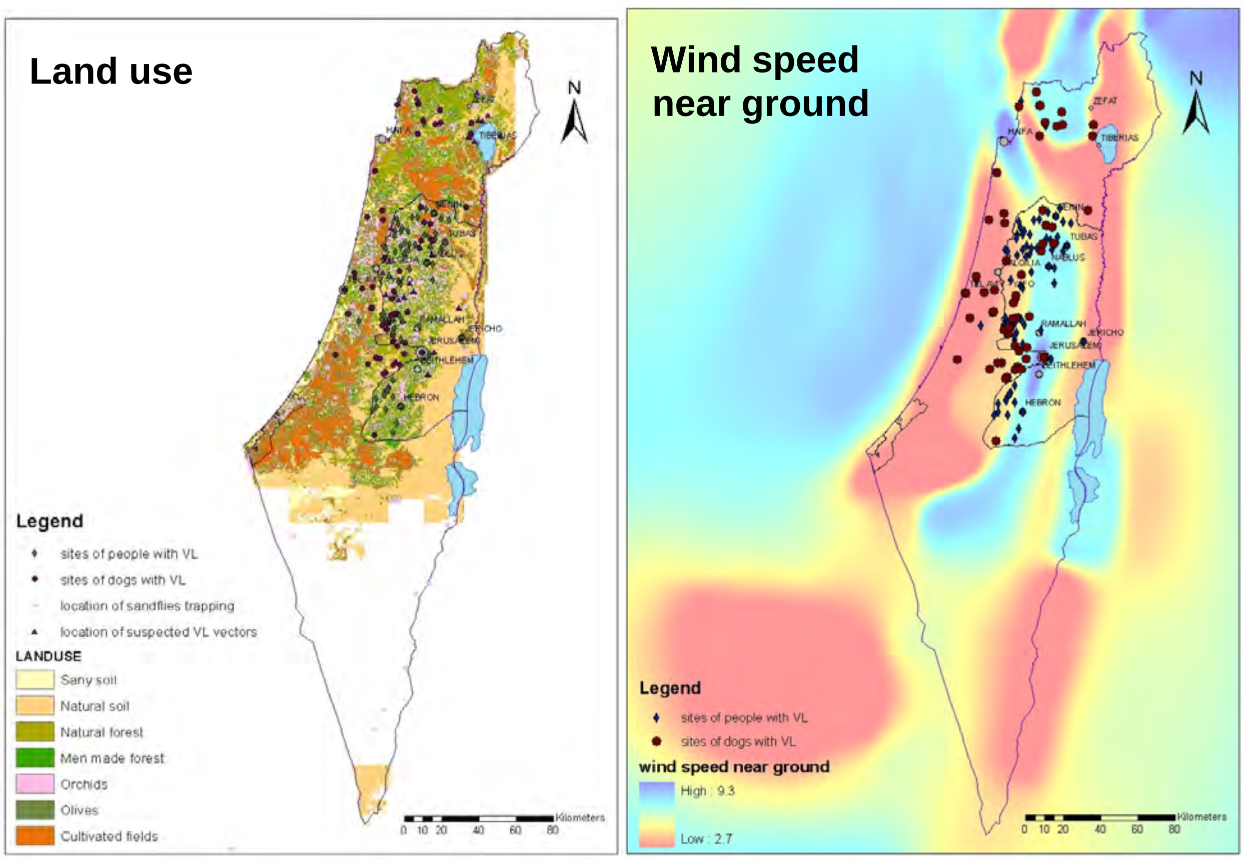


Figure 23 – A map of Israel and the Palestinian Authority showing land use, wind speed near ground and the location of human and canine visceral leishmaniasis, sites where sand fly species that are potential vectors of visceral leishmaniasis were trapped and all other sand fly trapping sites.



(G1) Mutlifactorial regression analyses for the effects of geographic & climate factors on the spatial distribution of HVL in the West Bank (PA)

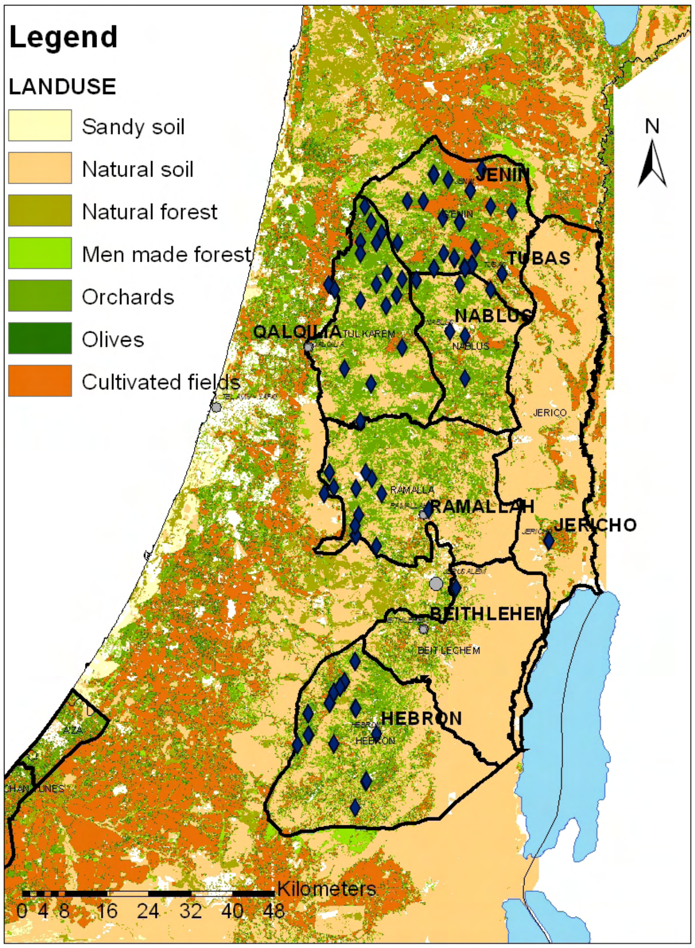
The longitudinal incidence data on the human VL which was available from the database created during this study was useful in combination with the geographical databases available from the Geography Department at the Hebrew University. The incidence rates of human VL in children under the age of 10 years, which comprised 98% of the patients in the West Bank, were calculated for every village, cluster of sites and district. Locations (villages) of low disease incidence were compared to locations of high incidence using multifactorial regression analysis. The variables that emerged as having a significant effect on the incidence of disease are outlined in **table 6**. These included: land uses, average annual rainfall, average temperatures in October (but not in August or January), altitude and wind speed at ground level.

Table 6 - Mutlifactorial regression analyses for the effects of geographic & climate factors on the spatial distribution of HVL in the West Bank (PA). The statistically-significant variable ($P < 0.05$) are bolded.

	Variable	***Beta***	***T***	***Sig T***
1	**Land use**	**0.455771**	**6.160**	**<0.0001**
2	**Average annual rainfall**	**-0.596024**	**-4.005**	**0.0001**
3	**Ave. temp. in October**	**-3.208345**	**-2.473**	**0.0145**
4	**Altitude**	**-1.941081**	**-2.015**	**0.0456**
5	**Wind speed at ground level**	**0.409259**	**2.002**	**0.0471**
6	**Year**	**-0.159147**	**-1.919**	0.0568
7	**Ave. temp. in August**	**1.067431**	**1.827**	0.0696
8	**Rainfall during a specific year**	**0.110749**	**1.149**	0.2522
9	**Ave. temp. in January**	**0.383812**	**0.552**	0.5820
	(Constant)	**357.629415**		

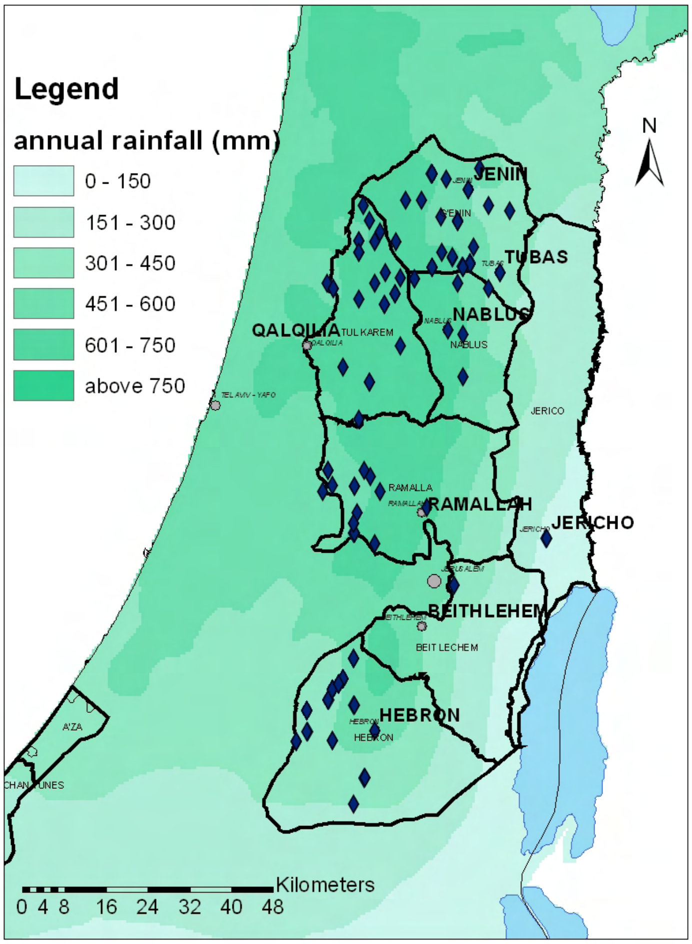
Landuse – A high incidence of VL was associated with a high degree of vegetation, mostly orchards, men-made forests and olives (**Fig. 24**). This is in agreement with our finding on the suspected sand fly vectors of VL in this region, which favored locations of rich vegetation.

Figure 24 – Map showing the locations of human VL cases and the different land uses in the West Bank (PA).



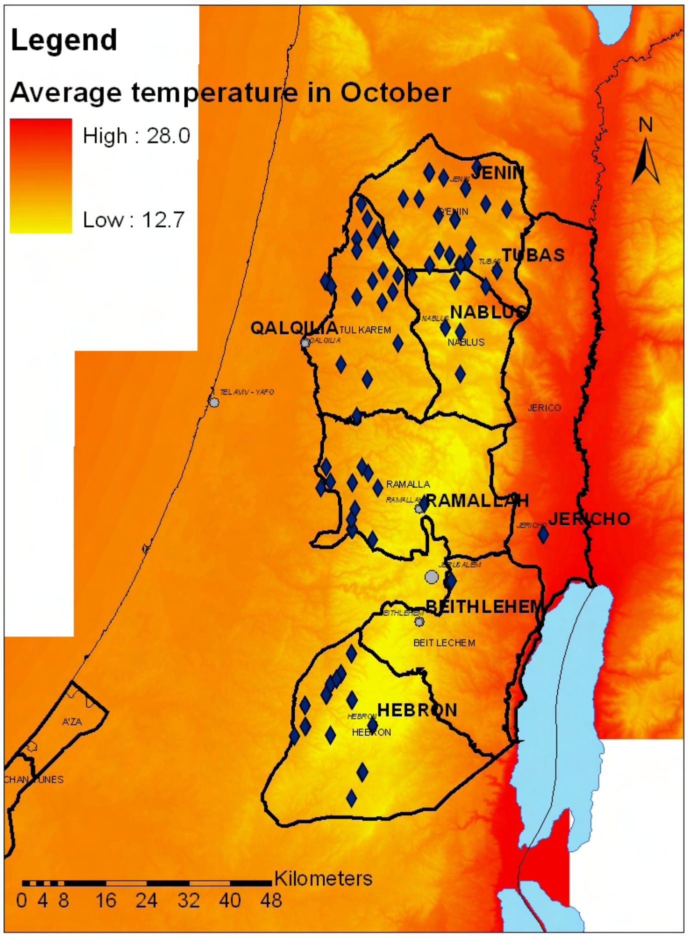
Average annual rainfall – A high incidence of human VL was associated with a high annual rainfall (average 550 ml.) relative to this area of the Mediterranean. Figure 25 shows that VL patients were found mostly in the western aspects of the West Bank and away from the dry regions of the Judean desert and the eastern Samarian hill slopes in the eastern West Bank. The findings of a relationship between patient locations and a high annual rainfall are in agreement with the relationship found in this study between the locations of suspected sand fly vector spp. and a high annual rainfall.

Figure 25 – Map showing the locations of human VL cases superimposed on the zones of average annual rainfall in the West Bank (PA).



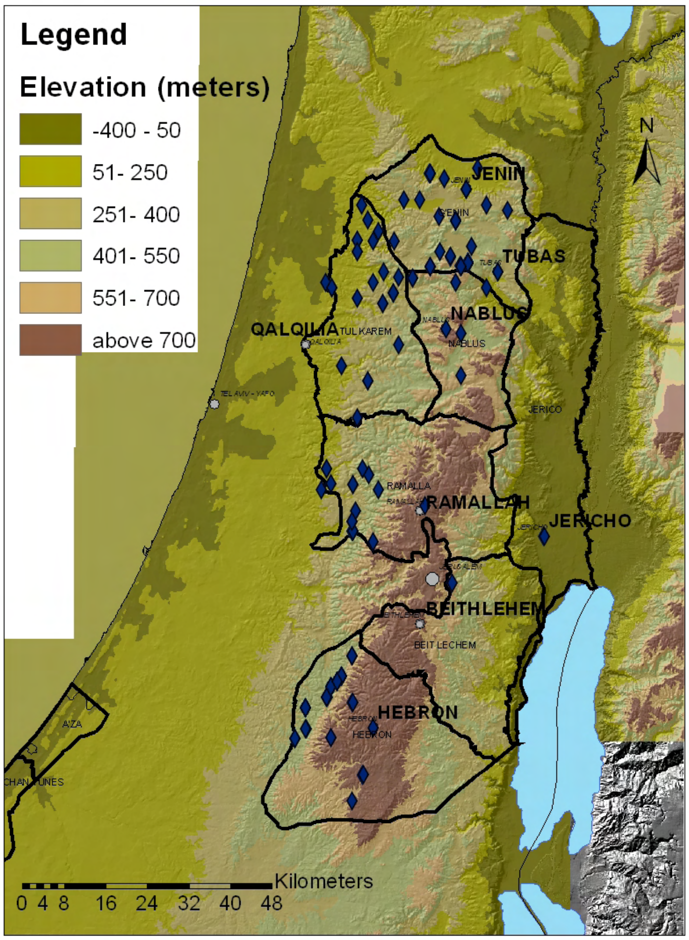
Average temperature in October – Human VL was found in areas of relatively low temperatures during October (**Fig. 26**). The statistically-significant preference of low temperatures is probably also related to the specific requirements that vector sand fly spp. have. Although the suspected vectors tended to be trapped in cooler locations in the sand fly component of this study, the difference in temperatures between trapping locations of vector and non-vector spp. was not statistically significant.

Figure 26 – Map showing the locations of human VL cases superimposed on the zones of average temperatures during October in the West Bank (PA).



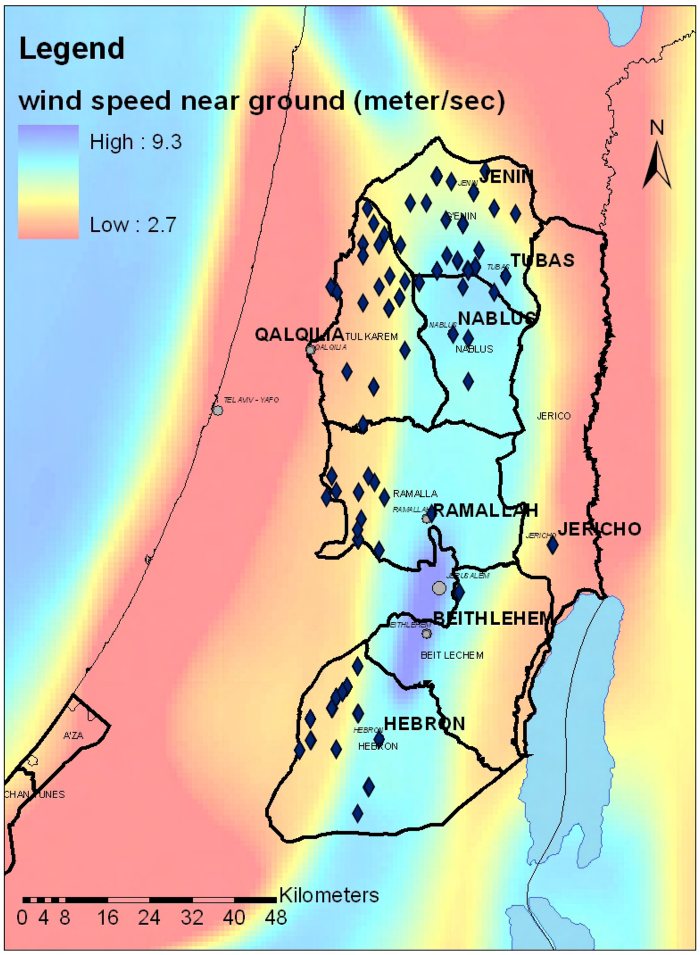
Altitude – Human VL was found mostly in areas of moderate to high altitudes of about 300-650 meters above sea level (**Fig. 27**). Most of the patients lived on the western slopes of the Judean and Samarian hills, an area with a high rainfall and extensive exposure to winds coming from the Mediterranean.

Figure 27 – Map showing the locations of human VL cases superimposed on the zones of geologic altitudes in the West Bank (PA).



Wind speed at ground level – Human VL was found in the WB mostly in areas of relatively moderate to high ground wind speed with an average of 5.2 meters/second.

Figure 28 – Map showing the locations of human VL cases superimposed on the zones of ground wind speed in the West Bank (PA).



[H] Discussion and Conclusions

This study included an in-depth evaluation of VL in the Palestinian Authority and Israel. It dealt with the epidemiology of this disease in several different layers of research and encompassed a multidisciplinary approach to this research topic. VL was studied in the first "layer" by the creation of databases and maps on infection in humans and dogs and by conducting a large scale survey on the sand fly spp. present in different geographic regions. The second "layer" of research included an active serosurvey of dogs and wild canids with the intent of learning about the role of wild canids and dogs in the epidemiology of the disease. The third "layer" was the molecular study with detection of infection in dogs and sand flies, and the genotypic comparison of isolates from different geographic regions leading to the understanding that there are several geographic-related genotypic groups of isolates. The fourth "layer" was the GIS and statistical analyses of the human VL incidence data with the geographic databases which enabled the definition of ecological risk factors for the presence of infection.

The results of this study indicate that geographical and climate factors influence infection with VL in Israel and the West Bank (PA). An overlap was found the habitats of the suspected sand fly vector spp. and the presence of infected reservoir dogs and people. Sand fly vectors that apparently transmit VL are common in the mountainous areas of central and northern Israel but not in the southern Negev desert, Arava valley and adjoining Jordan Valley where cutaneous leishmaniasis transmitted by other spp. of sand fly vectors is present, but there is no VL transmission. Therefore, the presence of VL is affected firstly by the capacity of the environment to support the existence of vector sand fly species. Our comparisons of sand fly trapping locations have shown that VL vector spp. in Israel and the PA favor regions with a high annual rainfall (mean 550 ml) and a rich vegetation of olives groves and natural forests. The geographic variables that emerged as having a significant effect on the incidence of human VL in the West Bank (PA) included: land use of highly vegetated areas, a high annual rainfall (average 550 ml), low temperatures in October, and relatively high altitude (300-550 meters above sea level) and high wind speed at ground level (average 5.2 meters/second). Interestingly, a study on VL caused by *L. donovani* in Sudan revealed that the annual rainfall and altitude were the best predictors of VL incidence (Elnaiem et al., 2003). Vegetation was shown to be an important risk factor for VL in another study employing GIS from Brazil (Bavia et al., 2005). These two factors were also identified as having a significant effect on the incidence of VL in the present study.

The presence of vector canids reservoirs in the vicinity of people are essential for the transmission of the disease. The findings of a high seroprevalence among jackals suggested that these wild canids could be associated with the transmission of infection. This is especially valid when realizing that infection often occurs in new settlements that incur into wild life habitat. The findings of different *L. infantum* genotypes separating them into northern and central genotypes suggested that the emergence of infection in Israel and the West Bank (PA) in the recent decades was a result of re-appearance of the infection from a local source perhaps due to increased human incursions into habitats where sylvatic transmission was taking place, and probably not due to importation of infection to the central focus from the older northern focus.

The improved non-invasive molecular detection methods for *L. infantum* in dogs and PCR of sand flies carried out in this study allowed the definite diagnosis of infection in dogs with a rapid molecular tool which is useful also for epidemiologic surveillance. In addition, we were able to detect *L. infantum* infection in *Ph. perfiliewi* and *Ph. syriacus* sand flies strengthening the previous assumption that these sand fly species are indeed vectors of infection in the West Bank and Israel.

The findings on human visceral leishmaniasis in the West Bank (PA) confirmed that the disease is mostly an infantile illness with young children being the majority of patients. The number of HIV+ patients in the West Bank is small. Unlike other regions of the Mediterranean where immunosupressed HIV+ patients have become the major risk group for VL, the disease in the West Bank and Israel is still primarily an infantile disease. The presence of frequent infection in dogs in Israel, where there are very few VL patients, indicates that VL does circulate in areas where there are few human patients. This is in line with publications showing large scale asymptomatic exposure to VL in some regions in Israel (Ephros et al., 1994; Adini et al., 2003). Although the human populations in Israel and the West Bank are exposed to the infection, the disease is only frequent among children in the West Bank. Differences in socioeconomic and nutritional status may be responsible for this striking difference in disease incidence among children in Israel and the West Bank. However, this should be tested in future studies. Another worrying aspect of the epidemiology of the disease is the increased urbanization of canine infection found in this study. Canine infection may be followed by human infection and spread of VL into highly densely populated areas.

This study elucidated the status of VL in the Palestinian Authority and Israel. It included a comprehensive survey detailing the locations and clusters of disease cases. In addition, it uncovered the geo-ecological risk factors for disease. These findings are imperative for forecasting where disease might occur and for planning prevention measures against this dangerous infection.

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IMPACT RELEVANCE AND TECHNOLOGY TRANSFER

This study created and brought together extensive data sets on visceral leishmaniasis. It combined data from various sources and analyzed it. It's most important achievement was the definition of ecological risk factors and the statistically-significant linkage between geographic conditions and the presence of visceral leishmaniasis. The definition of risk factors for VL in the PA and Israel will serve as a model for understanding the epidemiology of this disease in the Middle East and elsewhere. It can be used to forecast where outbreaks of infection may take place and also to plan prevention and intervention programs targeted areas of high risk or involving risk factors that can be modified. Additional products of this study that will be useful for future research are the non-invasive diagnostic techniques developed during this study, the sand fly *Leishmania* PCR and the genotyping technique used to compare *Leishmania* isolates from different geographic regions.

The outcomes of this study and the techniques developed during it could be useful to medical organization such as the World Health Organization (WHO), health ministries in the Middle East, ministries of agriculture and environment, nature protection agencies and non-governmental organizations that deal with health related issues and the fight against infectious diseases. The project has contributed to the education of Palestinian and Israeli students who have participated in it, studied molecular laboratory techniques and the use of GIS as a tool for epidemiologic research. The knowledge gained by students in this study will enable them to use GIS to study and understand similar problems such as other infectious diseases, health-related, environmental and agricultural problems.

PROJECT ACTIVITIES/OUTPUT

<u>Training and workshops</u>

Israeli-Palestinian workshops - The use of GIS for epidemiological studies such as the one which this project focused on was not a common practice in Israel or the PA. Therefore, during the planning of the project, we established contact with a U.S. GIS expert who took an active part in the construction of work plans and steering of the project. The GIS advisor to the project, Prof. Mark Wilson from the University of Michigan in Ann Arbor, visited the Hebrew and Al Quds Universities three times during the duration of the study, once in August of 2002, again in September of 2003, and lastly in July of 2005. Joint Israeli-Palestinian GIS workshops took place during the two last visits. These workshops included frontal lectures by Prof. Wilson on GIS, reports by the Israeli and Palestinian principal investigators, presentations by students and hands-on practical lessons. The workshops were usually two days long and carried out in the Kibbutz Tzora guest house near Jerusalem in Israel. All visits by Prof. Wilson included individual counseling of Palestinian and Israeli student's projects and visits to field sites. These activities further strengthened the collaboration between the Israeli and Palestinian scientists and their research teams.

The majority of the technical training was carried out during the earlier phases of the project. Student training in GIS and spatial analysis was carried out during the GIS workshop in September 2003 which included 16 participants (10 Palestinians, 4 Israelis, 2 American) (**Fig. 29**). The workshop in 2005 included 10 participants (4 Palestinian, 5 Israelis, 1 American) and included a summary of the findings, advanced analyses of the data and discussions on future research related to the study's topics.

Figure 29- Participants of the first Israeli-Palestinian GIS workshop, Kibbutz Tzora, September 2003.

Training - Training in laboratory methods took place during all years of the project. These training sessions were held between and within the research teams with techniques and methods transferred from more experienced staff to newer students. For instance, in Prof. Abdeen's laboratory in Al Quds University, techniques of performing serological tests and PCR that were learned by the technician Kifaya Azmi during training at the Hebrew University were transferred to the students Ahmad Amro and Suheir Erqkat. New techniques in genetic analysis of isolates were practiced by the Palestinians Abdelmajid Nassereddin and Kifaya Azmi initially in the Hebrew University and subsequently in Al Quds University.

Investigators meeting - Meetings between the principal investigators of the project, Prof. Abdeen and Dr. Baneth were held with a frequency of about every two months, usually in East Jerusalem and sometimes in the Hadassah Medical Faculty in West Jerusalem. These meetings strengthened the cooperation between the investigators and have led to more proposals for future mutual research and training activities.

Publications arising from the project:

Three papers have so far been published as a result of this project. An additional paper has been accepted for publication and another paper is in preparation currently. Three presentations in scientific congresses have been made with full support from the project and the research abstracts have been published (please see list below). An oral presentation summarizing the project's findings and conclusions was made at the WorldLeishIII congress in Sicily in April of 2005. The WorldLeish is a major international congress attended by most of the leading world scientists involved in the research of leishmanaisis.

Papers

(1) Jaffe CL, Baneth G, Abdeen ZA, Schlein Y, Warburg A. Leishmaniasis in Israel and the Palestinian Authority. *Trends in Parasitolgy,* 2004, 20: 328-332.
This paper is a joint Israeli-Palestinian 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.

(2) 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 paper describes the non-invasive PCR developed for VL diagnosis in dogs.

(3) Nasereddin A, Baneth G, Schonian G, Kanaan M, Jaffe CL.(2005). Molecular fingerprinting of *Leishmania infantum* strains following an outbreak of visceral leishmaniasis in central Israel. J. Clin. Microbiol. 43: 6054-6059.
The first author of this paper, Abed Nassereddin is a Palestinian who was trained at the Hebrew Univesity during this project and now works in Al Quds University and also at the Hebrew University. The paper describes the genotyping of *Leishmania* isolates and the relationship between genotype and geographic origin.

(4) Nasereddin A, Ereqat S, Azmi K, Baneth G, Jaffe CL, Abdeen Z. Serological survey with PCR validation for canine visceral leishmaniasis in Northern Palestine. J. Parasitol. In press (will be published in early 2006).
This paper describes the molecular and serologic detection of *L. infantum* in the blood of dogs from the Palestinian Authority. The first author, Abed Nassereddin is a Palestinian who was trained during this project. Most of the other authors are the Al Quds University team members.

(5) In preparation – A joint Palestinian-Israeli manuscript on the distribution of human and canine VL in Israel and the Palestinian Authority with description of geographical and ecological risk factors for infection.

Abstracts from presentations in scientific congresses

(1) Baneth G, Abdeen AZ, Strauss-Ayali D, Nassereddin A, Jaffe CL. The epidemiology of canine visceral leishmaniasis in Israel and the West Bank – an update. Israel Journal of Veterinary Medicine. 2004, 59: 53.
This joint Palestinian-Israeli abstract was presented at the 28th 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.

(2) Baneth G, Amro A, Strauss-Ayali D, Jaffe CL, Warburg A, Sawalha SS, Yossef K, Hopp M, Sztern J and Abdeen ZA. Visceral leishmaniasis in Israel and the Palestinian Authority 1994-2004. WorldLeishIII congress proceedings, Sicily, Italy. April, 2005.
This joint Palestinian-Israeli abstract summarizes the projects main findings.

(3) Baneth G, Amro A, Strauss-Ayali D, Jaffe CL, Warburg A, Sawalha SS, Yossef K, Hopp M, Sztern J, Abdeen ZA. A study of visceral leishmaniasis in Israel and the Palestinian Authority using geographical

information system (GIS). Presented at the annual meeting of the Israel Society for Parasiotology, Protozoology and Tropical Diseases. Kfar Hamakabiya, Israel. December 1st, 2005.Proceedings p. 13.

PROJECT PRODUCTIVITY

The project essentially accomplished all of the proposed goals. In some instances, there was a slight change of technique, for instance, sand fly ELISA for the detection of parasites was replaced sand fly PCR, but this was not a major deviation from the original plan and provided the same expected results using a more sensitive technique. No changes of significance were made from the original work plan.

FUTURE WORK

The project has considerably strengthened cooperation between Palestinian and Israeli scientists from Al Quds and the Hebrew University. The mutual training, workshops, writing of collaborative research papers and presentations in scientific congresses have encouraged the principal investigators to submit more mutual research proposals in partnership on leishmaniasis and on other infectious diseases. Palestinian and Israeli students that have trained under the project have also formed good relationships and venues of communication and will continue to work together and maintain these ties.