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Leishmaniasis in the West Bank, Palestine: Epidemiology, Sandfly Vectors and Reservoir Hosts

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TO

MY DEAR FATHER, MOTHER, FIANCÉE, BROTHERS AND SISTERS FOR THEIR ENCOURAGEMENT, WITH LOVE AND RESPECT

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ABSTRACT

In the West Bank, Palestine, two forms of leishmaniasis occur. Visceral leishmaniasis (VL) due to *Leishmania infantum* affects human (mainly infants) and cutaneous leishmaniasis (CL) caused by *L. tropica* and *L. major* affects a broader age group.

During the last decade, there seems to have been an increase in both the incidence and geographical spread of the disease. This study was conducted to investigate regional differences in the occurrence of the disease and to provide data about sandfly vectors and reservoir hosts in different leishmaniasis foci in the West Bank.

Reported leishmaniasis cases in the West Bank from 1990 to 1999 were collected, also data was gathered by active case finding and visiting to special dermatology clinics in Jenin district. Light and sticky-paper traps were used to catch sandflies, furthermore samples of blood and tissues from incriminated animal hosts were collected from different localities through the West Bank.

A total of 832 cases of CL and 127 cases of VL were reported from the West Bank districts other than the Jordan Valley. According to the year of infection, the annual incident rates for both CL =24 % and VL = 30 % was highest in 1994. Northern districts were most affected; 37 % of CL cases and 39 % of VL were reported from Jenin district, indicating that an epidemic of CL and VL occurred in the middle of 1990s. The majority of patients lived in rural areas. Cases of CL were from all age groups and both sexes. The ages of VL patients ranged from 5 months to 46 years, 114 (90 %) were younger than 5 Years old.

A survey of sandflies was carried out in different leishmaniasis foci in the West Bank to determine the composition of the sandfly fauna. A total of 528 sandflies was caught from inside and outside houses mainly *P. papatasi, P. sergenti*, *P. major syriacus*, *P. tobbi*, and *P. perfiliewi* which were increminated vectors of CL or VL in other leishmaniasis foci in the neighboring countries, indicating the high risk of acquiring the disease in the area.

The possible contributory factors of the recent leishmaniasis outbreak in Jenin district were also studied and discussed.

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CHAPTER ONE

1. INTRODUCTION

Leishmaniases are a complex of diseases that present with variable clinical manifestation. They are caused by single-celled protozoan parasites of different species of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae). The promastigote stage inhabit the alimentary tract of the females of Phlebotomine sandflies; while the amastigote stage lives and multiply in macrophages and other phagocytic cells of the reticuloendothelial cells of the vertebrate hosts includes several mammalian species including man. Transmission among the mammalian hosts is predominantly by the bit of the infected sandfly.

Leishmaniasis is a public health problem in the world, as about 12 millions people are infected world wide and 350 millions are considered at risk. There are three clinical forms of leishmaniasis visceral (VL), cutaneous (CL) and mucocutaneous (WHO, 1990).

During the last two decades it had been noted that leishmaniasis was recognized as a public health problem of considerable importance in Palestinian territories and neighboring Israel (Arda, 1983; Klaus *et al*., 1994; Qubain *et al*., 1997; Benth *et al*., 1998). Two forms of leishmaniases, visceral and cutaneous, are found to be endemic in these areas (Greenblatt *et al*., 1985).

Since Huntermuller (1914) first reported CL in the Jordan Valley, many epidemiological studies had been carried out in this area, the causative agent, vector, and reservoir animal, were identified as: *Leishmania major*, *Phlebotomus papatasi,* and *Psammomys obesus,* respectively (Greenblatt *et al*., 1985). Cases reported between 1948 and 1967 were sporadic and few, as a result of extensive use of DDT for malaria eradication (Naggan *et al*., 1970). However, until 1978, reports on leishmaniasis in other parts of West Bank are lacking.

In 1978, Blum described a new endemic focus of CL in Salfit town. Arda (1983) and Arda and Kamal (1989) described some epidemiological aspects of CL in the West Bank during the period from 1972 to 1989.

However, little is known about the distribution of the disease in the West Bank during last decade. Although the number of leishmaniasis cases, CL and VL, in other parts of West Bank other than Jordan Valley have been on the increase (Preventive Medicine Department, Palestinian Ministry of Health, 1997), most of these cases were recorded in Jenin district. Thirteen species of sandflies were identified, nine species were *Phlebotomus* and four species were *Sergentomya* genera (Sawalha, 2000). Furthermore seven species of *Phlebotomus* genus reported in the Jenin district were found to be as suspected or proven vectors for CL or VL in other leishmaniasis foci in different countries.

The presence of so numbers of sandfly species, suspected vectors of CL and VL, in leishmaniasis foci in the Jenin district explained the presence of CL and VL foci within close proximity (Sawalha, 2000) and not as previously suggested (Lewis, 1982).

1.1. STATEMENT OF THE PROBLEM

Although leishmaniasis has been reported from all districts of West Bank and it is growing public health problem of considerable importance, Jenin and Jericho districts have deserved special attention.

During the last decade the numbers of CL and VL cases have increased, and most cases of both forms of the disease were reported from the Jenin district. The ecology of sandfly vector in the Jenin district were studied (Sawalha, 2000) but the distribution of leishmaniasis cases and the factors affecting disease transmission in the district are still unknown. On the other hand, another study has been carried out to investigate leishmaniasis epidemiology in the Jordan Valley, but studies on the epidemiology of the disease and the sandfly vectors in other West Bank districts are still lacking.

This study is intended to amplify our knowledge of the ecology and epidemiology of leishmaniasis in the Jenin district as well as the sandfly fauna in the West Bank in order to survey which sandfly species are present in different disease foci.

1.2. JUSTIFICATION AND SIGNIFICANCE

Although leishmaniasis, CL and VL, has been known in West Bank for a long time no efforts were made to control the disease in effective way; this is partly due to lack of information on the epidemiology, vector, and reservoir animal of the disease.

This study was designed to investigate epidemiology of leishmaniasis in West Bank districts excluding Jordan Valley. More emphasize was given to study the ecological factors affecting disease transmission in the area, where most cases of Leishmaniasis in West Bank had been reported. Studying these ecological factors is very important for control the disease. Also determination of the most susceptible areas for the disease should be useful for effective control and decreasing the cost of insecticides and their potential side effects.

This study is intended to: (1) serve as a pilot experiment that provide guidance to further investigation, to avail some suggestions which may be used in the control of the disease through its vector and reservoir hosts, (2) give information that might help in explaining outbreaks of the disease, and (3) study of sandfly species of the area might help in identifying sandfly fauna of the West Bank and the suspected species of vectors of leishmaniasis which has a great value to provide base line data for the control of sandflies.

1.3. PURPOSE OF THE STUDY

1.3.1. Long term Goal

The aim of this research is to assess the leishmaniasis problem in the West Bank and to provide information on the incidence of the disease, ecological factors affecting reservoir animals, sandfly vectors and leishmaniasis transmission. A major theme of this work was to provide data about leishmaniasis and sandflies in different districts of the West Bank. On the basis of this information suitable control and preventive measures would be implemented by health authorities.

1.3.2. Specific Objectives

In details the objectives of this study was designed to determine:

1. The distribution of leishmaniasis in the West Bank with emphasis on disease foci in the Jenin District.
2. Sandfly fauna of West Bank, excluding areas studied before (Jenin and Jericho districts).
3. Differences of disease incidence between central and peripheral areas bordering agricultural and natural areas.
4. Sex and age distribution of the disease and the location of ulcers on the exposed parts of human body.
5. The presence of suspected reservoir hosts for CL and VL in the Jenin district. And trapping some mammals for dissection; and
6. Possible ecological factors that may have influenced the epidemic of CL and VL during the last 10 years in the Jenin district.

1.4. REVIEW OF LITERATURE

1.4.1. Etiological Agents of Leishmaniasis

The different species of *Leishmania* are morphologically indistinguishable at their amastigote as well as their promastigote stages. They have been identified on the basis of isoenzyme profiles.

Since the discovery and identification of the genus *Leishmania* Ross in 1903, the number of species *Leishmania* has increased continuously. Since there are few morphological differences among the species of leishmania, features such as clinical forms, epidemiological cycles, host and vector types and geographical distribution were used for identification and classification of *Leishmania* parasites. Only after the 1970s intrinsic criteria, such as immunological, molecular and biochemical character began to be used for *Leishmania* classification, however the taxonomy is far from being established (Macedo *et al*., 1992).

Twenty one out of thirty *Leishmania* species that infect mammals infect humans, and are transmitted by about thirty species of sand flies (Herwaldt, 1999). Leishmania species can be classified into two main groups according to the clinical manifestations that they cause. The first group includes species that cause visceral leishmaniasis, include: *L. donovani* in the Indian subcontinent, East Africa, and southern Asia including Iran. *L. infantum* in Mediterranean, and Middle East regions, and *L. chagasi* in the New World.

The second group includes *Leishmania* species that cause Cutaneous Leishmaniasis, include: *L. mixicana* complex (*L. mexicana mexicana*, *L. mexicana amazonensis,* and *L. mexicana venzuelensis*), and the *L. Braziliensis* complex (*L. braziliensis braziliensis*, *L. braziliensis panamensis*, and *L. braziliensis guyanensis*) in the new world, and by *L. tropica*, *L. major*, and *L. ethiopica* in old world (Berman, 1997; Sacks *et al.*, 1993).

1.4.2. Life Cycle

The infection cycle begins when female sandflies ingested amastigotes while feeding on infected mammalian host; engorgement is quickly followed by production of peritrophic membrane by the epithelial cells lining the midgut of the sandfly. Procyclic promastigotes develop from amastigotes within 12-24 hours undergo rapid division prior to their transformation to nectomonads 2-5 days post feeding, in the anterior abdominal midgut. As the peritrophic membrane break down many nectomonads become attached to the thoracic mid gut by their flagella. The forward migration is accompanied by transformation to haptomonad and promastigote forms, during the final stage of infection; the anterior movement progresses into the oesophagus and pharynx. The invasion of the fore gut is accompanied by appearance of the metacyclic form, which move forward into the pharynx, cibarium and proboscis. The metacyclic forms were found to be the only infective stage for experimental animals (Sacks *et al.,* 1984).

Infected sandfly during feeding injects the promastigotes in the vertebrate host which are taken up by phagocytosis and endocytosis. Promastigotes transform into amastigote forms, which multiply by binary fission. Infection may lead to destructive lesions of the skin or pathological changes in internal organs.

1.4.3. Clinical Forms

Leishmaniasis manifests itself in man in three forms namely cutaneous, mucocutaneous and visceral leishmaniasis.

1.4.3.1. Cutaneous Leishmaniasis

Depending on the different species of parasite or the type of zoonotic cycle concerned and also the immune responses developed by the host, clinical features of CL tend to differ between and within regions (WHO, 1984).

CL is a condition due to infection of reticuloendothelial cells of the skin by *Leishmania* parasites. *L. tropica, L. major* and *L. aethiopica* are mainly the causative agents of Old World CL, while those in New World include *L. mexicana* complex and *L. braziliensis* complex (WHO, 1990).

L. major, usually causes zoonotic or "rural" CL, occurs on an exposed part of the body, characterized by acute, rapidly ulcerating and commonly with multiple lesions. The initial papule develops to a nodule, which is elevated above the level of the surrounding skin in forms of volcanic nodules. The nodule ulcerates and the lesion some times appear as flat ulcers with slightly raised margins. Other characteristics of the lesions include satellite papule, skin crease orientation (Sacks *et al.,* 1993). The lesion caused by *L. major* heals in 2-6 months (Klaus *et al.,* 1994).

Leishmania tropica, cause anthroponotic or urban CL. It usually produces single dry lesion, ulceration of the skin often leading to disfiguring scars. The lesion usually heals in about a year. The lesions caused by *L. tropica* are larger, last longer and more difficult to treat than those caused by *L. major* (Klaus *et al.,* 1994).

Leishmania recidivans (lupoid or tuberculoid chronic form) may last for many years, usually affecting the face and is characterized by widely disseminated nodules and thickening of the skin does not ulcerate. This disease does not heal spontaneously and tends to replace after treatment. The parasite load is high and patients are leishmanin negative.

Leishmaniasis recidiva (lupoid) is another form of CL caused by *leishmania tropica,* but after healing of the primary lesion, new lesions start at the edge of the scar. It is very chronic lasting for 20-40 years, resistant to drugs and destructive if not treated.

Post Kala-azar dermal leishmaniasis is caused by *L. donovani* may occur in many endemic areas of Kala-azar. It commences 6 months to one or several years after apparent cure of VL. It is a multiple noduler infiltration of the skin especially the face (WHO, 1990).

1.4.3.2. Visceral Leishmaniasis

VL is a condition due to infection of reticuloendothelial cells through the body. VL is caused in the Old and New World by parasites of *L. donovani* complex and *L. chagasi* respectively (WHO, 1990). The parasite invades the reticuloendothelial system of nearly all internal organ particularly the liver, spleen bone marrow and lymph nodes. The incubation periods lies between 2 to 6 months; however, there are considerable variations, and it may range from 10 days to over one year, or even several years. Common symptoms are fever, pallor of mucocutaneous, malaise, weight loss and the usual clinical signs are non-trend splenomegaly, with or without hepatomegaly. Patients have anemia thrombocytopenia and leukopenia. Although the existence of an asymptomatic and subclinical form has been reported. The mortality rate for untreated cases varies from 75 % to 95 %. In North Africa and the Middle East, children at the age between 1 to 4 years are more affected. The disease also occurs predominantly in males (WHO, 1984).

1.4.3.3. Mucocutaneous Leishmaniasis

Mucocutaneous Leishmaniasis (espundia) is a condition due to infection of reticuloendothelial cells initially of the skin and subsequently of the mucosa of the mouth and nose. It is mainly prevalent in the New World (South America) where it is caused by *L. braziliensis* and *L. panamensis,* sporadic cases of the disease occurred in the Old World and the causative agents are *L. aethiopica* and *L. donovani* (WHO, 1984).

1.4.4. Epidemiology and Geographic Distribution

The epidemiology of leishmaniasis is varying greatly. Important determinants of epidemiology of leishmaniasis include the presence and characteristics of the vector, the existence of reservoir hosts that maintains the disease in nature and provides source for infection for human. For example *P. papatasi* was excluded from being a vector of VL following artificial infection studies carried out by Heyneman (1963).

In the Old World endemic foci of leishmaniasis are scattered over wide areas, from China across Asia, India, Iran, and Afghanistan, the Middle and Near East, the Mediterranean Basin, Portugal, Ethiopia, East and West Africa and the Sudan. In the New World the disease is found in area covering Mexico to the northern part of Argentina (WHO, 1990).

Visceral leishmaniasis occurs in four epidemiological forms depending on the geographical distribution. In the Mediterranean area the reservoir is the domestic dog and the vectors are *P. perniciosus* and *P. chinensis*. In Indian Kala-azar the main vector is *P. argentipes* and human is the reservoir host. The African form occurs in semi-arid country across Africa south of Sahara, small rodents are the reservoir hosts and the vectors are *P. orientalis* and *P. martini.* In the South and Central American Kala-azar vectors belong to the subgenus *Lutozomyia* and dogs are the reservoir host. CL due to infection with *L. tropica* complex occurs in the countries of the Mediterranean especially southern Europe and North Africa, in Africa south of Sahara, in the Middle

East, southern Russia, north-west India and north China. The suspected vectors are *P. papatasi, P. sergenti* and *P. pedifer* (WHO, 1990).

Many of the leishmania parasites are zoonotic and the intrusion of human into sylavatic cycle may result in greater exposure to sandflies and hence, high risk of infection. In certain areas of leishmaniasis in the Old and the New World, transmission can be domestic or peridomestic. Many vectors transmit leishmaniasis to people who made a contact with them through agriculture, road-building, military movements, herding in forests, charcoal burning and other activities (WHO, 1984). The human behavior like manmade ecological changes, large numbers of non-immune immigrants intruding into an established sylavatic zoonotic area of endemic leishmaniasis, increase the risk of transmission to human.

1.4.5. The Vector (Sandflies)

Sandflies represent a well-defined group of insects belonging to the subfamily Phlebotominae, family Psychodidae and order Diptera. There are about 700 known species of sandflies including in the subfamily. These species belong to 6 genera, namely *Phlebotomus, Sergentomyia* and *Chinius* in Old World and *Lutzomyia*, *Brymotomia* and *Warileya* in the New World. Only about 70 species of *Phlebotomus* and *Lutzomyia* were involved in transmission of disease to man (Killick-Kendrick, 1990; WHO, 1990, Lane, 1993).

Sandflies are delicate blood sucking insects and can be easily distinguished from other Diptera insects by their brownish color, small size (1.5-2.5 mm), hairy appearance, long legs, jerky flight pattern and characteristic manner in which they hold their pointed wings at an angle of 45° above their body (lane, 1993).

Sandflies are wide spread throughout the tropics and subtropics; their distribution is limited primarily by climatic conditions. Sandflies distribution, taxonomy, biology ecology and relation to disease is reviewed by Kirk & Lewis (1951), Quate (1964), Lewis (1974), Killick-Kendrick (1978), Lewis (1982), Ward (1985), Lewis and Ward (1987), Peter and Killick-Kendrick (1987), WHO (1984, 1990) and Lane (1993).

Sandflies are mainly crepuscular or nocturnal, usually encountered immediately after sunset. During the daytime they rest in dark humid places. The main requirements for sandfly resting sites are optimal physical conditions such as still air, optimum conditions of temperature (28° C) and relative humidity. The resting sites vary with different species. The domestic species are frequently found resting in humid dwellings between cloths, behind cupboards and hanging pictures as well as in the wall crevices. The wild species are often found resting in damp microhabitats such as rock crevices, caves, cracks and fissures in soil, bank of steams, animal burrows and leaf litter in forests (Kirk and Lewis, 1951).

The two sexes need to feed on sugar for general activities and they may obtain this from aphids and coccids secretions and / or by direct piercing from plants (Schlein and Warburg, 1986); in addition female sandflies need vertebrate blood to mature and lay eggs during their gonotrophic cycle (WHO, 1984).

Killick-Kendrick (1978) suggested that sugar meals play important role in sandflies ecology and epidemiology of leishmaniasis because possible preference by sandfly for particular plants may restrict its distribution, consequently the distribution of the parasites it transmits. In addition the type of sugars and frequencies with which they are taken by a particular species of sandflies may be a factor in the insects ability to transmit *Leishmania* parasites.

1.4.6. The Reservoir Hosts of Leishmaniasis

A reservoir animal is defined by WHO (1984) as the ecological system in which the parasite population is maintained indefinitely. Reservoir hosts play an important role in the epidemiology of leishmaniasis. There is one primary reservoir host for given leishmania species in a particular focus, but other mammals in the same area may become infected, and serve as secondary and incidental hosts.

Wild animals keep most *Leishmania* species in natural foci of infection. In zoonotic CL, the primary reservoir hosts of *L. major* are wild rodents such as *Psammomys obesus* in north and West Africa and *Rhomboys opimus* in central Asia. Hyraxes are reservoir host of *L. aethiopica* in East Africa (WHO, 1990).

The animal reservoirs of *L. tropica* are not well defined. Parasites of this species isolated from dogs in Kabul, Iran and Morocco (Dereure *et al*., 1991) and from rats in Iraq (El-Adhami, 1976). In all of these cases infection has been considered accidental rather than primary reservoir host.

In general, the principal reservoir hosts of VL in the Old World were canines, such as fox, jackal and wolf that had been found infected with *L. infantum* in both the New and Old World. Among domestic animals the dog is the principal reservoir of *L. infantum* in most endemic areas, especially in the Mediterranean region (WHO, 1990).

1.4.7. Control of Leishmaniasis and Sandflies

The most successful integrated control programs of leishmaniasis combine the control of the vector and the reservoir animal when the later is known. This method was applied successfully in USSR, rodenticides and mechanical methods were used to destroy the net work burrows of the great gerbil (*Rhombomys opimus*) which is the main reservoir host of zoonotic CL (Ashford and Bettini, 1987).

In general, reservoir control must be considered as an important part of the integrated control of leishmaniasis, however, different animals can act as reservoirs of the same parasite and even if one species is completely eliminated another can take its place. Also sandflies are generally opportunistic in their feeding habits and for this reason they can turn to another host if any particular species of animal should be eradicated. For these reasons reservoir control should be considered as only one method in complex programs to control the leishmaniasis (WHO, 1990).

Vector control is considered to be the main method for prevention of leishmaniasis. For the proper organization of sandfly control, precise knowledge of their biology, ecology is important. The principle methods used in control of sandflies and leishmaniasis are application of chemical insecticides sometimes in conjunction with environmental management. DDT is still one of the recommended insecticides (Rozendaal, 1997). Other control methods, such as biological predators and genetical and ecological measures, are difficult to apply and sometimes not effective. An integrated program of control, including all other measurements was recommended, but applied in very rare cases (Viokov, 1987).

Alternative control methods for sandflies are currently tested in different parts of world include the use of the insecticide-impregnated bed nets and curtains (Rozendaal, 1997). Organic remnants and piles of bricks and stones in construction sites constitute potential breeding and resting sites for sandflies; elimination of these factors from human habitats decrease the risk of leishmaniasis (WHO, 1990). The habitat of rural population to keep their animals in residence areas attracts both anthropophilic and zoophilic vectors and decrease the possibility of acquiring the disease (Rozendaal, 1997).

1.5. EPIDEMIOLOGY OF LEISHMANIASIS IN PALESTINE AND VICINITY

In the West Bank, Palestine, both cutaneous and visceral leishmaniasis occur and are a growing public health problem (Arda, 1983; Greenblatt *et al*., 1985; Klaus *et al*., 1994; Qubain *et al*., 1997; Benth *et al*., 1998).

1.5.1. Cutaneous Leishmaniasis

Cutaneous leishmaniasis has been reported in Palestine since the beginning of the last century. The Jordan Valley, and specially Jericho town, is recognized as a hyperendemic area of CL in the country; where it is called “Jericho Boil”. Huntimular was the first to detect *Leishmania* parasites from cases of CL in the Jordan Valley in 1914; and since that time many cases of CL still to occure every year as well as several outbreaks were discribed from different parts of Palestine, and so that much attention had been given to the diseas (Greenblatt, *et al*., 1985). The rate of human cases depend on the number of people enter the zoonotic focus in the nature (Naggan *et al*., 1970).

In Palestine, two forms of CL have been established i.e. *L. major* and *L. tropica*. The epidemiology of the first form has been studied in great detail. It is a zoonotic disease described from different parts of Palestine and especially from the Jordan Valley; the parasite is harbored by *P. obesus* and *Meriones crassus* and the vector is *P. papatasi* (Schlein *et al.*, 1984). Sporadic cases of CL caused by *L. tropica* were reported from mountainous area in the northern parts of the West Bank, reservoir host and sandfly vector were not identified (Klaus *et al.*, 1994).

Blum (1978) found that the incidence of CL in the peripheral areas of Salfit town was twice that found in the centre of town because of the natural increasing of the researvoir animals (*Rattus rattus*) in peripheral areas. 14 % of the sampled population were leishmanin positive and its incidence was found to increase with age; and no active lesion was observed above the age of 25 years.

Most CL cases were reported between June and November, but cases have been reported in every month of the year (Y. Schlein, personal communication). The main infectious period is during the summer months. Based on the time of acquiring the infection of CL from different Leishmaniasis foci the highest exposure months were June and July (Klaus *et al.*, 1994).

Dispite leishmaniasis is a reportable disease by law in West Bank and Israel, many cases were not reported and the estimated cases are several handreds per year (Greenblatt *et al*. 1985; Y. Schlein, unpublished work). Table 1 summarizes reported cases and outbreaks of CL in the West Bank and Israel since early of the last century.

1.5.2. Visceral Leishmaniasis

Reports of VL cases were scattered; cases were reported from different parts including Petah Tiqwa, Jerusalem and the Jezreel Valley (Greenblatt *et al.*, 1985). In addition, imported cases between Jewish immigrants from neighboring countries to Palestine were also reported.

During 1960s, 45 sporadic cases of VL were reported from different Arab villages in Gallilee; about 42 % of cases reported from three closed villages. All the affected were children under 8 years old. The intensive uses of DDT in Israeli's settlements, during 1950s and thereafter, prevents the occurrence of VL in these communities, also the decrease of cases in Arab villages was contributed to the same reason. During 1971 to 1979 only one case of VL was reported in Israel (Greenblatt *et al*., 1985).

Table 1. Reported cases and outbreaks of CL in West Bank and neighboring Israel documented from 1914 to 1994.

Year	**Number of cases**	**Infected locality(s)**	**Comments**	**Reference**
1914	Few	The Jordan Valley	The first Parasitology proven cases	Huntimular (1914)
1914-1948	Unknown	Palestine	Scattered reports of the presence of CL	Greenblatt *et al*. (1985)
1967	125	The Jordan Valley	An epidemic resulted from entering non-immune soldiers into hyper-endemic area	Naggan *et al*. (1970)
1967-1974	377	Different localities in Israel	Reported by Ministry of Health, Israel	Greenblatt *et al*. (1985)
1972-1980	131	Salfit District	CL was unknown (or very rare) before 1972; its prevalence peaked in 1973 and then dropping. In 1978, 14.5 % of residents were leishmanin positive.	Arda (1983); Blum (1978)
	57	Northern districts of West Bank	33, 12 and 12 cases were reported from Jenin, Tulkarm and Nablus Districts respectively.	Arda (1983)
	24	Jordan Valley	From different parts of the Jordan Valley	
	11	Southern districts of West Bank	8 cases from Rallah and 3 cases from Hebron.	
	Total = 223	West Bank	In addition to 14 imported cases	
1980-1982	780	Different localities in Israel	Israeli Ministry of Health reported cases. 60 were soldiers whom based in an endemic area in Negev desert during 1982.	Greenblatt *et al*. (1985)
1983-1987	173	Northern districts of West Bank	Most of cases were diagnosed during 1986-87; 62 of these cases were found in Qabatyah where the disease wasn't reported before.	Arda and Kamal (1989)

1984-1985	50	Negev desert	50 cases were occurred in a village in the desert.	Y. Schlein, unbublished work
1988-1989	69	West Bank	36 cases from Jenin district and 22 from Jericho district.	Arda and Kamal (1989)
1988-1992	371	Different localities in Israel	Diagnozed in clinic from different parts of Israel; most cases from Jordan Valley.	Klaus *et al.* (1994)
1992-1994	200	Different localities in Israel	Reported by Ministry of Health, Israel	Y. Schlein, unbublished work

al. 1985). During 1980 to 1991, about 12 cases of VL were reported from the northern parts of Israel (Greenblatt *et al.* 1985; Ephros *et al*., 1994). In 1994, a child from central Israel was found infected with VL (Baneth *et al*. 1998). Arda and Kamal (1989) have shown out that 15 cases of VL were reported at AL Watany Hospital in Nablus; cases were reported from different parts of the West Bank, 13 cases of these occurred before 1978 and only 2 cases were reported during 1978-1989.

1.5.3. Causative agents, reservoir animals and vectors

Both *leishmania major* and *L. tropica* cause cutaneous disease in Palestine. In the central Jordan valley, along the Dead Sea, *L. major* is transmitted from the fat sand rat, *Psammomys obesus,* to human by *P. papatasi*, (Schlein *et al*., 1982; Schlein *et al*., 1984). In contrast, *L. tropica* is mainly found in the northern region of the West Bank (Oren *et al*., 1991); neither the reservoir host nor the vector have been identified.

The major reservoir animal of VL in the area is dog whereby 4 % of domestic dogs in Wadi Hamam, northern Israel, were found to be seropositive, and parasites isolated were typed as *L. donovani sensu lato*

(Jaffe, *et al.*, 1988). Parasites were also isolated from seropositive dogs in central Israel were typed as *L. infantum;* the rate of seropositive examined animals from the area were 1-11.5 % in domestic dogs, 7.6 % in jackals and 5 % in foxes (Baneth, *et al.*, 1998).

Three species of rodents were found infected with *L. major* in the southern parts of Israel, namely *Psammomys obesus*, *Meriones crassus* and *Nasokia indica*. Other animals examined but found not to be infected included two foxes, three porcupines and three hyraxes (Schlein, *et al*. 1984). It had been suggested that rocky hyrax may have a role in the transmission of CL, because many cases of CL have been reported near their colonies in Kfar Adumim (Klaus *et al.,* 1994).

P.papatasi sandflies collected from three localities in the southern parts were found infected with *Leishmania* parasites (7-23 %). One female of *P. sergenti* caught from Arava area was found infected (Schlein, *et al.*, 1984).

Sawalha (2000) presumed on epidemiological and entomological surveys, that *P. papatasi* might be the principal vector of CL in the northern parts of West Bank. Since *P. papatasi* was the predominantly man biting species. Table 2 summarizes causative agents, sandfly vectors and reservoir animals reported from Palestine and neighboring Israel.

Table 2. Causative agents of leishmaniasis and their distribution, vectors and reservoir hosts, documented from Palestine and neighboring Israel.

Causative agent (clinical condition)	Geographical Distribution	Sandfly Vector(s)	Reservoir Animal(s)	References
L. major (Cutaneous leishmaniasis)	Central Jordan Valley	*P. papatasi*	*P. obesus*	Schlein *et al.* (1984)
	Dead Sea Region	-	*P. obesus*	Schlein *et al.* (1984)
	Kfar Adumim	-	-	Klaus *et al.*, (1994)
	Rift Valley (Arava)	*P. sergenti*	*P. obesus* *M. crassus*	Schlein *et al.* (1984)
	Keziot in the Negev desert (South western Israel)	*P. papatasi*	*P. obesus* *M. crassus*	Giladi, *et al.* (1984)
L. tropica (CL, VL* and leishmania recidivans)	Salfit	*P. papatasi* (suspected)	*Rattus rattus* (not confirmed)	Blum (1978)
	Northern Jerusalem	-	-	Y. Schlein (personal communication)
	Kfar Adumim	-	-	Klaus *et al.*, (1994)
	Mountains between Nablus and Jenin	-	Unknown	Klaus *et al.* (1994)
L. infantum (Visceral Leishmaniasis)	Northern Israel	-	Domestic dogs	Jaffe, *et al.*, (1988) Ephros *et al.*, (1994).
	Central Israel and West Bank	-	Dogs Jackals Foxes	Baneth, *et al.*, (1998)

1.5.4. Ecology of leishmaniasis

The zoonotic nature of CL in the Jordan Valley had been well established, so that infection is more likely to occur at the neighborhood of Jericho City, close to the sand rat *Psammomys obesus* (Rodentia: Gerbillidae) colonies (Naggan *et al*., 1970; Schlein *et al*., 1982). In this arid climate the sand rat burrows seem to provide sandflies with moist and relatively cool microhabitat essential for larva and adults. This closeness between the vector and reservoir animal lead to high rate of infection in both, which may reached to 56 % in *P. papatasi* (Schlein *et al*., 1982). On the other hand the rate of infection found in the same species of sandfly collected from Jericho city was less than

0.1 % (Adler and Theodor, 1926). Saliba *et al.* (1985) described an outbreak of CL, which occurred in the outskirts of Amman in the Jordanian desert; peak transmission of the outbreak took its place in the late summer of 1982. About two third of the patients were less than 15 years old.

1.5.5. Sandflies fauna

Phlebotomine sandflies (Diptera: Psychodidae) of Palestine are of great important in relation to CL, VL and sandfly fever (Greenblatt *et al*., 1985). Adler and Theodor (1926 a) were the first to study sandflies of Palestine and they isolated *Leishmania* parasites from *P. papatasi* caught from Jericho (Adler &Theodor, 1926 b).

Investigation of the sandfly fauna of Palestine resulted in identification twelve species of *Phlebotomus,* namely *P.* (*Paraphlebotomus*) *alexandri* Sinton, *P.* (*Adlerius*) *halepensis* Theodor, *P.* (*Paraphlebotomus*) *jacusieli* Theodor, *P.* (*Laroussius*) *major* Annandale, *P.* (*Paraphlebotomus*) *marismortui* Theodor, *P.* (*Laroussius*) *mascittii* Grassi, *P.* (*Phlebotomus*) *papatasi* (Scopoli), *P.* (*Laroussius*) *perfiliewi* Parrot, *P.* (*Paraphlebotomus*) *sergenti* Parrot, *P. (Adlerius) simici* Nitzulescu, *P. (Synphlebotomus)* Theodor and *P.* (*Laroussius*) *tobbi* Adler & Theodor; Table 3 summarize sandfly species of the genus *Phlebotomus* reported from Palestine and neighboring countries.

Table 3. Sandfly species of *Phlebotomus* genus reported from Palestine and neighboring countries.

Sandfly species	Locality	Comments	References
P. alexandri	Jordan Valley and southern Israel	Rare species	Y. Schlein (unpublished work), Schlein *et al.* (1982)
P. halepensis	Northern West Bank	Rare species	Sawalha (2000)
	Palestine	-	Lane *et al.* (1988)
P. jacusieli	Northern West Bank	-	Sawalha, 2000
	Northern and central Israel	Rare species	Y. Schlein (unpublished work)
P. major	Northern West Bank	Three subspecies were reported *P. m. syriacus, P. m. neglectus* and *P. m. major*	Sawalha, 2000

	Northern and central Israel	*P. m. syriacus.* Prevalent subspecies	Y. Schlein (unpublished work)
P. marismortui	Israel	Undefined location	Lewis (1982)
P. mascittii	Northern West Bank	*P. mascitti canaaniticus* and *P. mascitti mascitti*	Sawalha, 2000
	Northern and central Israel	Rare species	Y. Schlein (unpublished work)
	Palestine	*P. mascitti canaaniticus*	Lane *et al.* (1988)
P. papatasi	Northern West Bank	-	Sawalha, 2000
	Jordan Valley and southern parts of Israel	The dominant species and a proven vector of CL	Adler &Theodor (1926 a) Schlein *et al.* (1982) Schlein *et al.* (1984)
P. perfiliewi	Northern West Bank	-	Sawalha, 2000
	Palestine	*P. perfiliewi galilaeus*	Lane *et al.* (1988)
P. sergenti	Northern West Bank	-	Sawalha, 2000
	Different regions of Israel	More prevalent in northern and central regions	Y. Schlein (unpublished work)
P. simici	Palestine	-	Lane *et al.* (1988)
P. (Syn.) sp.	Northern West Bank	Reported from the area for the first time	Sawalha, 2000
P. tobbi	Northern West Bank	-	Sawalha, 2000
	Different regions of Israel	More prevalent in northern and central parts	Y. Schlein (unpublished work)
	Jordan Valley	Rare species	Schlein *et al.* (1982)

Also eleven species of *Sergentomyia* were identified from the area, including *S.* (*Parrotomyia*) *africana asiatica* Adler & Theodor, *S.* (*Sergentomyia*) *antennata* (Newstead), *S. christophersi* (Sinton), *S. clydei* (Sinton), *S.* (*Sergentomyia*) *fallax* Parrot, *S.* (*Parrotomyia*) *palestinensis* Adler & Theodor, and *S. squamipleuris* (Newstead), *S.* (*Sergentomyia*) *sinotoni* Pringle, *S.* sp. a, *S. theodori* (Parrot), and *S. tiberiadis* (Adler, Theodor & Lourie). Table 4 summarizes sandfly species of the genus *Sergentomya* reported from Palestine and neighboring countries.

Table 4. Sandfly species of the genus *Sergentomya* reported from Palestine and neighboring countries.

Sandfly species	**locality**	**Comments**	**References**

S. africana asiatica	Jordan Valley	-	Schlein *et al.* (1982)
	Southern Israel	-	Schlein, *et al.*, (1984)
S. antennata	Jordan Valley	Dominant species	Schlein *et al.* (1982)
	Southern Israel	Dominant species	Schlein, *et al.*, (1984)
S. christophersi	Northern West Bank	-	Sawalha, 2000
S. clydei	Southern Israel	Rare species	Schlein, *et al.*, (1984)
S. fallax	Northern West Bank	-	Sawalha, 2000
	Jordan Valley	Rare species	Schlein *et al.* (1982)
	Southern Israel	Rare species	Schlein, *et al.*, (1984)
S. palestinensis	Jordan Valley	Rare species	Schlein *et al.* (1982)
	Palestine	-	Lane *et al.* (1988)
S. squamipleuris	Southern Israel	Rare species	Schlein, *et al.*, (1984)
S. sinotoni	Jordan Valley	Rare species	Schlein *et al.* (1982)
S. sp. a	Palestine	-	Lane *et al.* (1988)
S. theodori	Northern West Bank	Dominant species	Sawalha, 2000
S. tiberiadis	Northern West Bank	-	Sawalha, 2000
	Palestine	-	Lane *et al.* (1988)
	Southern Israel	-	Schlein, *et al.* (1984)

1.5.6. Incrimination of Vectors

Janini *et al.* (1995) collected 1446 females of *P. papatasi*, and about 50 of *P. alexandri* and *P. alexandri*. None of these flies were infected, but 14 of 686 of *P. papatasi* collected from *Ps. obesus* burrows were infected.

Four females of 3624 *P. papatasi*, caught from Jericho during 1925, were found infected with *Leishmania* parasites (Adler & Theodor, 1926 b). *P. papatasi* was the only species of sandflies found in a focus of CL in Salfit area (Blum, 1978). Schlein *et al.*, (1982) recorded 29 infected out of 70 *P. papatasi* females collected from *Psammomys* burrows or near them in the Jordan Valley. Yuval (1991) found that 7.4% of *P. papatasi* trapped from *P. obesus* burrows in southern Jordan Valley were infected with *L. major*, with highest peak during July and August, and along the sandfly season.

Adler and Theodor (1957) have suggested that three species of sandflies *P. major, P. perniciosus* and *P. longicuspis* are probable vectors of VL in the Mediterranean region, and the first one is the only proven vector so far, in the region.

In Israel, it has been found that *P. perfiliewi* is a zoophilic species and rarely feeds on man, and this explain the very low incidence of VL in the area although 20% of dogs were infected during 1930s-1950s (Adler & Theodor, 1957).

1.5.7. Control of Leishmaniasis

The widespread spraying of insecticides such as residue Pirethroids and Organo Phosphorous compounds, once a year in all infected areas, were used during sandflies season. In addition, anticoagulant compounds and Lenet were used for control of rodents and stray dogs respectively. These procedures were employed since 1996, which before the only control measure used was spraying patients' houses, who refer to governmental health clinics, with insecticides one to two weeks after their referring.

3.1.2 Visceral leishmaniasis in the West Bank

From February 1990 through March 1999, 127 cases of VL were recorded among the Palestinian population, with a mean of 14.1 cases / year (annual range =3-32). Table (13) shows the distribution of reported cases of VL by year of notification in the West Bank; 25 % of cases were reported in 1995. Table (14) shows distribution of cases according the year of infection; about 30 % of cases occurred during 1994. 50 of 127 patients (39 %) occurred in Jenin district; followed by Hebron, Tulkarm and Ramallah districts with 25.2 %, 13.4 % and 11.8 %, respectively. Figure (5) shows distribution of VL cases in 63 localities in West Bank.

Table 13. Reported cases of VL and rate per 100,000 inhabitants in West Bank districts from March 1990 till end of February 1999.

District	Reporting Year										Total (%)	Rate per 100,000 inhabitants
	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999		
Jenin	3	2	2	2	11	14	4	6	4	2	50 (39.3)	27.8
Hebron	-	-	-	1	1	4	11	8	7	-	32 (25.2)	8.9
Tulkarm	-	1	1	1	4	8	-	1		1	17 (13.4)	14.4
Ramallah	-	-	1	2	3	3	1	3	2	-	15 (11.8)	7.9
Nablus	-	-	-	-	-	2	1	-	-	-	3 (2.4)	1.3
Salfeet	-	1	-	-	2	-	-	-	-	-	3 (2.4)	7
Tubas	-	1	-	-	-	1	1	-	-	-	3 (2.4)	9.3
Qalqilia	1	-	-	-	-	-	-	1	1	-	3 (2.4)	4.7
Beithlehem	-	-	-	1	-	-	-	-	-	-	1 (0.8)	0.8
Jericho	-	-	-	-	-	-	-	-	-	-	0 (0.0)	0.0
Total	4	5	4	7	21	32	18	19	14	3	127 (100)	7.7

Table 14. Reported cases of VL and rate per 100,000 inhabitants in West Bank districts from March 1990 till end of February 1999.

District	Year of infection										Total
	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	
Jenin	1 (2.0)	2 (4.0)	4 (8.0)	1 (2.0)	3 (6.0)	21 (42.0)	4 (8.0)	4 (8.0)	7 (14.0)	3 (6.0)	50
Hebron	-	-	-	1 (3.6)	1 (3.6)	-	11 (39.3)	7 (25.0)	6 (21.4)	2 (7.1)	28
Tulkarm	-	-	2 (15.4)	-	2 (15.4)	8 (61.5)	-	-	-	1 (7.7)	13

Ramallah	-	-	-	1 (8.3)	2 (16.7)	2 (16.7)	2 (16.7)	1 (8.3)	2 (16.7)	2 (16.7)	12
Tubas	-	-	-	-	-	1 (33.3)	2 (66.7)	-	-	-	3
Salfit	-	-	1 (33.3)	-	-	-	-	-	-	-	3
Qalqilia	-	1 (33.3)	-	-	-	-	-	1 (33.3)	1 (33.3)	-	3
Nablus	-	-	-	-	-	1 (50.0)	1 (50.0)	-	-	-	2
Beithlehem	-	-	-	-	-	-	1 (100)	-	-	-	1
Total	1 (0.9)	3 (2.6)	7 (6.1)	3 (2.6)	9 (7.8)	34 (29.6)	21 (18.3)	13 (11.3)	16 (13.9)	8 (7.0)	115

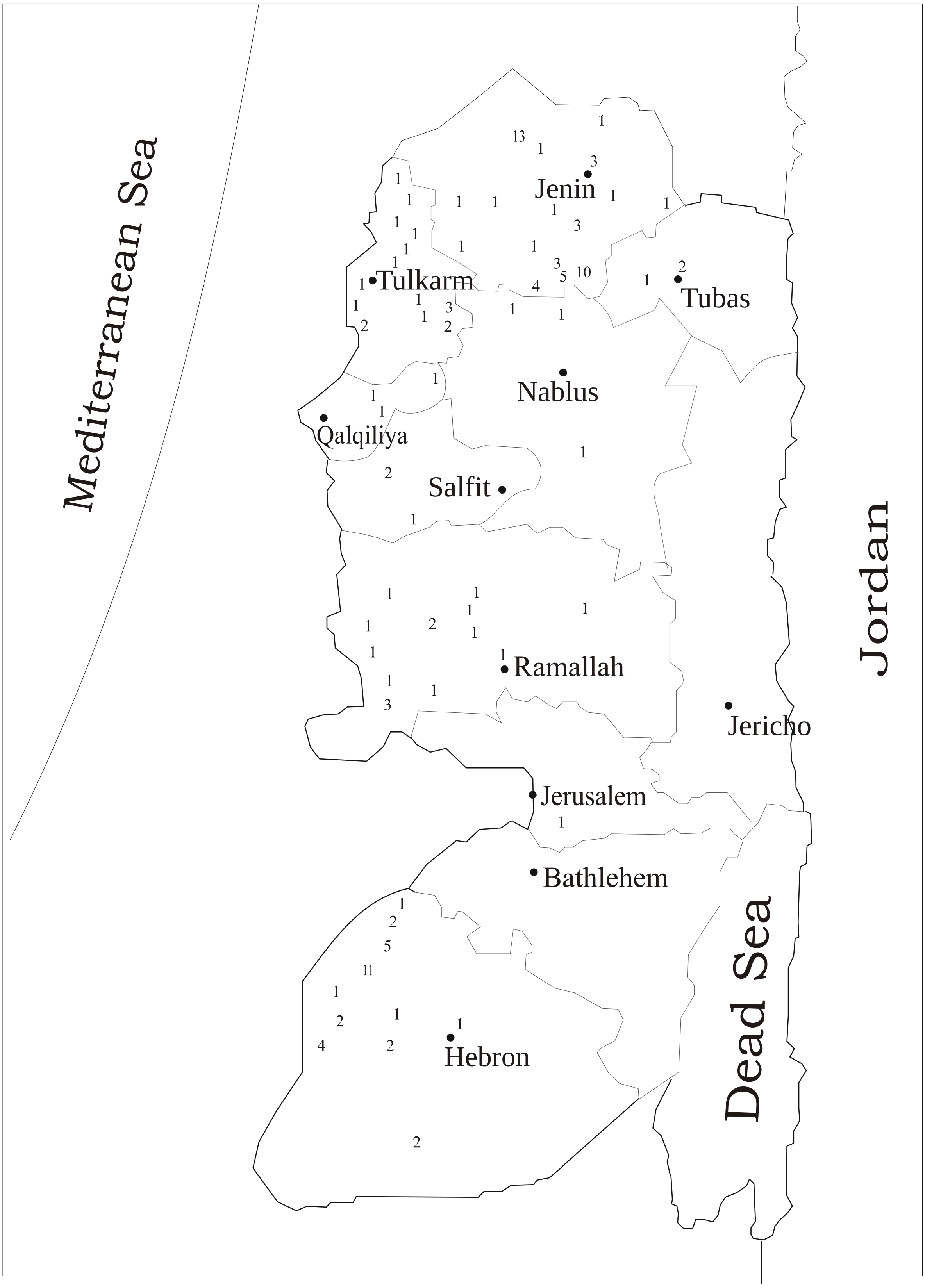

Figure 5. Geographic distribution of VL (number of cases / locality) in nine districts in the West Bank, 1990-1999.

Monthly distribution of VL cases showed that most cases were notified during June. Disease symptoms were noticed during the same period Figure (6).

Table (15) shows distribution of VL patients by age group and sex. The age of patients' ranged from 5 months to 46 years; 114 (90 %) were younger than 5 years old, and distribution by sex showed that there were no differences between both sexes.

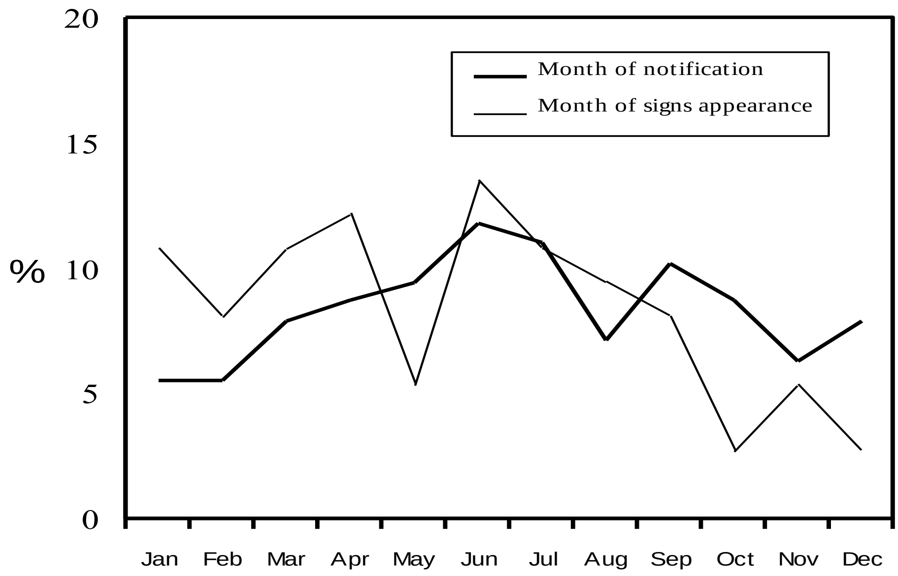

Figure 6. Percentage of monthly distribution according to the date of infection (thick line) and date of appearance of signs and symptoms (thin line) of VL cases reported from West Bank. 1990-1999.

Visceral disease mainly affects infants and young children throughout the northern region of the West Bank. From 1990 through 1999, many cases (127) were recorded throughout the

West Bank, giving a mean of 12.7 cases / year; Jenin district constituted 50 (39%) of the total cases 127 (61%).

Table 15. Distribution the numbers of VL patients by group and sex reported from the West Bank, 1990-1999.

District	Age (Years)								Sub-total		Total
	0-4		5-9		15-19		40+				
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	
Jenin	19	26	2	1	-	1	-	1	21	29	**50**
Hebron	14	13	4	1	-	-	-	-	18	14	**32**
Tulkarm	6	11	-	-	-	-	-	-	6	11	**17**
Ramallah	9	5	1	-	-	-	-	-	10	5	**15**
Tubas	2	1	-	-	-	-	-	-	2	1	**3**
Nablus	2	-	-	1	-	-	-	-	2	1	**3**
Salfit	2	-	1	-	-	-	-	-	3	-	**3**
Qalqilia	3	-	-	-	-	-	-	-	3	-	**3**
Beithlehem	1	-	-	-	-	-	-	-	1	-	**1**
Total	58	56	8	3	-	1	-	1	66	61	127

CHAPTER TWO

2. MATERIALS AND METHODS

Leishmaniases are zoonotic diseases that transmit from reservoir animals by sandflies vectors to human. The ecological factors have large effects on disease transmission. Thus, in a study about leishmaniasis epidemiology, it is important to study the ecological factors which affect the prevalence and geographical distribution of the disease in active foci. Also the sandfly species and suspected reservoir animals found in a certain endemic area may be used as an indicator to the epidemiology of the disease.

Therefore, such a study can provide base line data about different leishmaniasis foci in West Bank necessary to control the disease through its vector(s) and reservoir host(s) as well as provide information which help in explaining and predicting outbreaks of the disease.

All districts of West Bank were included in this study, except Jericho district, which was excluded because the epidemiology of leishmaniasis had been well studied in details (Schlein *et al.*, 1984). Epidemiological data on CL and VL were collected from the reports of other nine districts in West Bank, while ecological factors were studied in Jenin district that lies in the northern parts of the West Bank. Sandflies were collected from different localities in West Bank, except districts studied before, namely Jenin (Sawalha, 2000) and Jericho (Schlein *et al.*, 1982).

2.1. Design

The research design of this study was descriptive retrospective design. This design was chosen to investigate the factors that lead to the increase of leishmaniasis cases.

Although the study is descriptive, it attempts to put the problem in prospective by showing the relative degree to which different districts are affected, how the patterns of the disease occurrence vary between different areas and how the reservoir animal and vectors of the disease are associated with the different patterns of occurrence.

2.2. The Study Area

2.2.1. West Bank

The West Bank is bordered by Israel on the north, west and south. On the east the Jordan River and the Dead Sea from a natural border with Jordan. It is a hilly region located between the coastal plain in the west and the Jordan valley in the east with an average altitude of 2400 feet.

The total population size of the West Bank according to 1997 census was about 1,600,100 persons. Population density in the West Bank is high compared with neighboring countries. It is around 320 / km^2 because the considerable part of the land is still under Israel control.

The West Bank is divided into 10 districts: Jenin, Tubas, Tulkarm, Qalqiliya, Nablus, Ramallah, Salfit, Bethlehem, Jericho, and Hebron. It has 10 large towns, 430 villages and 18 refugees' camps. In general, 27 % of the population lived in urban communities.

The West Bank has a Mediterranean type climate. The mean monthly temperature over the last ten years ranged from 17.4° C in August. The mean annual relative humidity ranged from 84 % to 39 % while the mean annual rainfall was 528 mm (source: Palestine Meteorological Department). The year may be divided into a hot dry Summer, a warm Autumn, cold wet Winter and Spring.

Rainfall is limited to the winter and spring months from October to May. During the period of April to June, hot Khamsin winds from the south may occur and subsequently the atmosphere becomes hazy with dust from the desert. Rainfall in the West Bank varies greatly from east to west and from south to north according to topography. The annual rainfall ranges from 150 mm in the east to about 800 mm in the northwest. In the summer months there is no precipitation at all.

The inhabitants mostly work in agriculture, especially in the rural areas that have 60% of the total population. Although raising animals has decreased in the last years, domestic animals like sheep, goats, chickens, and others are still found, nearly, in all rural localities with different numbers, but the number of poultry houses has increased considerably and especially in certain localities. Most residents live in houses built of concrete and / or stone. Many of these houses were constructed in the peripheral areas.

In general human behavior in the rural areas of the West Bank is not different from district to another. During summer months (sandfly season) people spend the early evening in different activities at out doors sites of their houses. While the majority of people sleep indoors, young people may sleep at outdoors sites during the hot weather. People do not have the habit of sleeping under bed nets.

A preliminary survey for reported cases of leishmaniasis in different districts of West Bank was carried out at the beginning of the study. Based on the results of this preliminary survey, Jenin district was chosen to study the ecological factors and epidemiology of leishmaniasis with more emphasis for the following reasons: (1) It was one of the most infected district during the study period as shown by the preliminary survey. (2) Both forms of leishmaniasis were reported from many localities in the district and some of these localities had both forms of the disease. (3) The large number of leishmaniasis cases reported in the West Bank and the difficulties to reach to different areas. And (4) Jenin district was pointed out to be a focus of leishmaniasis since 1970s because of the many cases were reported from different district. The area was thus considered as an endemic and active focus of the disease up to now.

2.2.2. Jenin District

Jenin district is located in the northern part of the West Bank. It has 96 localities and covers an area of 592 km ^{2} with altitudes ranging between 90 and 750 meters above sea level (Applied Research Institute (ARIJ), 1996) and had a population of 195,299 in 1997 (Palestinian Central Bureau of Statistics, 1997).

Rainfall varies significantly in Jenin district from 778 mm in the west to 286 mm in the east and with mean annual rainfall of 528 mm. The district receives rainfall between middle October and end of the April, with peaks in January and February. Precipitation decreases to 12 % of the annual rainfall in March (ARIJ, 1996).

Wind blows from southwest and northwest, and being more northerly during the summer. The average wind speed ranged between 9.2 and 7.7 km / hour. There is also a little fluctuation in relative humidity in the district especially during summer months. It ranges from 63 to 66% during June to November, from 67 to 84 % during December to April, and it falls to 39% during May as a result of the dry winds that blow from the Arabian Desert (ARIJ, 1996).

Table 5 shows the dominant sandfly species, trees, topography, altitude and soil type in the most affected localities of Jenin district where 92 % of CL and 86 % of VL has been reported.

Table 5. Description of habitats of leishmaniasis foci in Jenin district where more than 90 % of cases were reported.

Locality	Topography	Soil type*	Altitude*	Plants of the area (trees)			Sandfly species**
				Olive	Fruit	Stone Fruit	
Aba	Hilly	Grumusols	150-200	+++	+	+	*P. papatasi*
Al Shuhada	Hilly	Grumusols, and Terra Rossas, Brown Rendzinas.	270-366	+++	+	+	*P. papatasi* *P. major syriacus*
Beit Qad	Hilly	Grumusols	190-290	+++	+	+	*P. papatasi* *P. perfiliewi* *P. tobbi*
Bir El Basha	Hilly	Brown Rendzinas and Pale Rendzinas	260-336	+++	+	+	*P. papatasi* *P. tobbi*
Deir Abu Da'if	Mountainous	Grumusols,and Terra Rossas, Brown Rendzinas.	180-302	+++	+	+	*P. papatasi* *P. perfiliewi* *P. tobbi*
El Yamun	Mountainous	Grumusols, and Terra Rossas, Brown Rendzinas.	130-251	+++	+	++	*P. papatasi* *P. perfiliewi* *P. tobbi* *P. major neglectus*
Jaba	Mountainous	Grumusols, And Terra Rossas, Brown Rendzinas.	350-610	+++	+	++	*P. papatasii* *P.perfiliewi* *P.tobbi*
Jadeida	Mountainous	Grumusols,And Terra Rossas, Brown Rendzinas.	360-539	+++	+	++	*P. papatasii* *P.perfiliewi* *P.tobbi* *P.major syriacus* *P.major neglectus*
Jenein	Mountainous	Grumusols	134-248	++	+	++	*P. papatasi* *P. perfiliewi* *P. tobbi* *P. major syriacus* *S. theodori*
Maythalun	Hilly	Grumusols	350-423	++	+	++	*P. papatasii* *P.perfiliewi*
Qabatiya	Mountainous	Grumusols,and Terra Rossas, Brown Rendzinas.	284-380	+++	+	++	*P. papatasi* *P. perfiliewi* *P. tobbi* *P. major syriacus*
Sanur	Mountainous	Grumusols	375-548	+++	+	+	*P. papatasi* *P. tobbi*
Silat El Harithiya	Hilly	Grumusols	150-195	+++	+	+	*P. papatasi* *P. perfiliewi* *P. sergenti*
Siris	Mountainous	Grumusols,And Terra Rossas, Brown Rendzinas.	375-500	+++	+	++	*P. papatasii* *P.perfiliewi* *P.tobbi* *P.major syriacus* *P.major neglectus*

* Applied Research Institute, (1996)
** Sawalha (2000).

2.3. Collection of the Epidemiological Data

2.3.1. Medical records

Data on leishmaniasis cases, reported in the West Bank, over nine years (March 1990 to the end of February 1999) were collected from the Ministry of Health, records of notifiable diseases.

The collected data included demographic (name, age, sex, and residence), type of leishmaniasis disease, date of onset of illness, date of diagnosis, occupation, origin of infection or travel history to other endemic foci, and the number and site of lesion or scars.

2.3.2. Private dermatology clinics

Visits had been carried out to all special dermatology clinics that patients from the Jenin district might refer to cure. These clinics included 3 clinics in Jenin City and 4 clinics in Nablus City. Dermatologists working in these clinics were interviewed to check whether leishmaniasis cases were reported to health authority, and to record available data about cases not notified to health authority, and to obtain information on the treatment used and its effectiveness and clinical picture.

Governmental hospitals, in the Nablus and Jenin districts to which VL patients are referred have also been visited to check on medical records. Interview with specialist at Al Watany hospital, where 76% of cases diagnosed and treated, were done in order to get information about the common signs and symptoms of the disease, treatment and its effectiveness, and diagnosis method used. The other cases (22 %) were diagnosis and treated at Jenin hospital and one case was diagnosed and treated at Al Motala’a hospital, in Jerusalem.

2.3.3. Active case finding

The last source of data collection was active investigation about leishmaniasis cases in the infected areas. During visiting to houses of leishmaniasis cases in Jenin district interviewees were asked whether they knew of other persons that have been infected with leishmaniasis. These names were compared those in medical records, and unreported cases were visited and epidemiological data were collected.

2.3.4. Diagnosis methods used

Collected data on diagnosis CL from dermatologist clinic and health centers in Jenin and Nablus districts included clinical picture and microscopic examination (Table 6). Demonstration of *Leishmania* parasite by microscopic method was used to confirm diagnosis especially for cases from a new foci and in case of infected wounds with long duration, recent scars not typical to CL but with no history of traumatic injury, etc.

Table 6. Number and percentage of CL cases in Jenin district diagnosed by different methods, 1990-1999.

Diagnosis Method used	Number	Percent
Clinical Picture	299	64.9
Microscopic Examination and Clinical Picture	72	15.6
Unknown	90	19.5
Total	461	100.0

A visceral leishmaniasis case was only considered if diagnosis was proven parasitologically, either by microscopic examination or culture of material for bone marrow accompanied by the following clinical manifestations: (1) high fever; (2) hepatosplenomegaly; (3) anemia and (4) leukopenia.

2.3.5. Determination of the year of infection

Determination of the year in which infection occurred is important to study factors affecting disease transmission. Year of infection was determined based on: (1) Incubation period of CL 7-60 days (Sacks *et al.*, 1993; Herwaldt, 1999) and incubation period of VL 1-3 months mean (Sacks *et al.*, 1993, Herwaldt, 1999). (2) The period consumed before seeking medical care for CL 1-4 months (Arda and Kamal, 1989) and nearly the same period is needed for seeking medical care and diagnosis of VL (Dr Loai Shahen personal communication; Qubain, 1997). (3) Multiplication of *Leishmania* parasites in the sandfly during 3-6 days (Sacks *et al.*, 1984). (4) The beginning of the sandfly season in May (Sawalha, 2000). (5) Both the date of referring to medical care and the date of the onset of signs and symptoms appearance. So that all cases referred to medical care or onsets of the disease appeared before July and August for CL and VL, respectively, were considered to have acquired infection of the previous year.

2.3.6. Mapping and interviews of leishmaniasis cases in Jenin district

Patients from the most affected localities in Jenin district (14 localities described in Table 5) were traced by their last known addresses. The location of patients' house where infection was suspected to have taken place were defined on a topographic maps from the Survey of Israel, 1996.

Location of patients' houses were plotted on a separate topographic map (Scale 1: 10,000) for each locality (Appendix B). Then altitude of the house and location in the central (built-up area on the map) or peripheral (far from built-up area on the map) of the locality were detected from the map. Every cluster of houses less than 300 m a part was given a symbol and plotted on the district map (figures 7 and 13).

Interviews were conducted with patients or their parents whose addresses were known. Information was collected about (1) the history of the disease in the area, (2) type of domestic and wild animals around the patients' houses, and (3) distribution of caves and crevices around the houses.

2.4. Sandflies Vectors

2.4.1. Collection methods used

Sandflies collections were carried throughout the sandfly season. Sampling techniques used were CDC light trap and knock down collection as described by Lewis (1973), Killick-Kendrick (1987) and WHO (1984).

Light traps: Centers for Disease Control (CDC) miniature light traps (model 512; John W. Hock Co., Gainesville, Florida, USA) were hanged at a height of 0.5 m above the ground level in collection sites. Traps were placed overnight, one to half an hour before sunset and collected within two hours after sunrise.

Sticky-paper traps: made of ordinary white paper sheets (21X 29 cm), coated on both sides with caster oil and fixed on sticks held vertically at a height of 0.25-0.3 m above the ground level. These traps were fixed overnight. Sandflies and other insects were trapped on the sticky oiled surface when they land. Sandflies were removed from oiled paper using a small fine brush, washed in 10 % domestic detergent and processed for identification.

2.4.2. Mounting and identification of sandflies

Sandfly specimens preserved in alcohol were carefully mounted in Berlese's medium on glass slide as described by Lewis (1967) and Killick-Kendrick (1987). Using a pair of fine dissecting needles, under binocular dissecting microscope, the head of each fly was severed and placed near the rest of the body. In case of the female samples, the last two abdominal segments were dissected out and mounted separately to examine the spermatheca. Care was taken to place the cover slip gently on each specimen, which was then left for at least 12 hours to clear. Sandfly identification was done under binocular microscope at 40X. The main features used for sandfly identification were the spermatheca of the female, the terminalia of the male and the pharyngeal and the cibarial toothed structures of both sexes. The taxonomic keys of Theodor (1958), Lewis (1967; 1978; 1982), Lane (1986), and Lane *et al.* (1988), were used for the identification of sandflies species.

2.5. Reservoir Hosts

In this part of the study of reservoir animals more attention was paid to rocky hyraxes, rodents, wild canines and dogs as possible reservoir hosts for *leishmania* parasites, because most of these animals were proved or suspected to act as such in many countries of Africa and Mediterranean region. Data about abundance and samples of blood and tissues for parasite infection of these animals were collected.

2.5.1. Abundance of suspected reservoir animals

In order to provide base line data about suspected reservoir animals and their role in disease transmission in different leishmaniasis foci abundance of different suspected reservoir animals, was approximately assessed by different ways. Data were collected from the fourteen localities in Jenin district where CL and VL were reported (Table 5). Collection of the data was limited to the areas around the affected houses. These areas were in general located in the peripheral of the infected localities. Interviewed patients and their parents were questioned about the presence of rocky hyraxes, rodents, wild canines and dogs in their neighborhoods.

Parameters used in this study for comparing the abundance of reservoir animals at neighborhoods of the patients of leishmaniasis in Jenin district are presented in Table 7. These parameters may not valid to describe density of reservoir animals in other leishmaniasis foci. Other factors that may influence or may be considered as an indicator for reservoir animals' abundance are also illustrated.

Table 7. Parameters employed in this study for comparing the abundance of reservoir animals in different leishmaniasis foci in Jenin district.

Reservoir animal and other factors	**Abundance**		
	High (+++)	**Moderate (++)**	**Normal or rare (+)**
Dogs and stray dogs	Dogs used as pets and for guard and hunting, always seen in the vicinity. Stray dogs are always seen in the area, residents complain of their risk.	Dog manly used for guard, always present in some houses. Stray dogs are some times seen, residents did not complain of their risk.	Domestic and stray dogs are very rare some times seen, residents did not complain of their risk.
Rocky hyraxes	Presence of at least two colonies near affected areas.	Presence of a small colony or few hyraxes near affected areas.	Present away from affected areas residence house. Only known to few people.
Foxes	Well known to residents and cases of predation were occurred in domestic areas.	Found away from residence areas and seen by hunters and sheepherders.	Known to sheep keeper and hunters.
Rats	Always seen at daytime and night and found their burrows around houses.	Seen around many houses and their burrows are found in areas of poor sanitation.	Seen around few houses and their burrows are rarely found.
Sheep and Goats	Presents in most houses and breading as a source of income is common.	Presents in some houses and breading as a source of income in some cases.	Presents in very few houses and breading as a source of income is rare.
Poultry houses	Many permanent and temporary poultry houses have been found in the affected area.	One or two of permanent and few temporary poultry houses have been found in the affected area.	Only the temporary poultry houses have been found in the affected area.
Caves	Mountainous area with rocky habitats, many caves of different sizes are found near affected areas.	Mountainous area with agricultural and rocky habitats, caves of different sizes are found near some houses in the affected areas.	Mountainous area with agricultural habitats and sporadic caves are found away from domestic areas.

To ensure validity and reliability of the presence of suspected reservoir animals and their abundance, visits to infected locality and observations were recorded. Other appropriate persons including sheepherders, hunters and health workers were interviewed. In addition colored photographs for wild animals were used during interviews.

2.5.2. Trapping and examining of the suspected reservoir animals

Different kinds of traps were used to capture wild animals (rats, rocky hyraxes and wild canine). Traps were set in four leishmaniasis foci in Jenin district namely Al Shuhada, Deir Abu daif and Qabatyah.

2.5.2.1. Rats

Sticky traps made from plastic sheets, covered with glue were used to catch rats. The trap consisted of a rectangle sheet 30 cm. in length and 17 cm. in width. About 400 traps were set for two nights, at different places outside houses of leishmaniasis cases on the outskirts of Qabatia. They were set early in the afternoon and collected early in the next morning. Traps baited with groundnuts, raw meat and cheese were set under small bushes, in the daily routes of rats and near rodents' barrows.

2.5.2.2. Rocky hyraxes

Deir Abu daif village was chosen for trapping of the hyraxes after the data about the distribution of hyraxes in leishmaniasis foci were known. Many attempts to collect the rocky hyraxes were done by using different methods cage traps, hunting dogs, and smoking by burning wood in their caves.

Cage traps with spring operated door were used to capture rocky hyraxes. These traps consisted of metallic frame 50 cm in length 30 cm in width and 40 cm high surrounded by wire gauze having a mesh of 1 cm, the cage provided with a door on its side which tied with spring. The door shuts immediately when the animal enters the cage for eating. Cages were baited with green leaves of almond, figs and different types of grasses and they were placed near burrows and along the routes, which the hyraxes used. Four traps were placed for ten hours during daytime for five days.

Two well-trained hunting dogs were used to catch hyraxes away from their colonies upon leaving burrows for grazing during daytime. Blocked hyraxes entered the metallic cage. Hunting was carried out during daytime for three times.

Wood were flamed inside burrows of hyraxes, in order to generate smoking which might obligate hyraxes to leave their burrows. Cages traps were placed on the openings burrows to catch escaped hyraxes. Three different colonies were flamed during daytime.

2.5.2.3. Wild Canine

Areas surrounding Al Shuhada village were selected to catch wild canine. Cage traps (similar to traps mentioned above) were used to capture wild canine. These traps were 80 cm in length, 40 cm in width and 60 cm. Traps were baited with meat and placed away from domestic areas overnight. Two traps were used for five night traps.

2.5.2.4. Domestic dogs

As part of interviews and visiting the affected houses in the Jenin district domestic dogs were examined for clinical symptoms of VL including skin lesion, lymphadenomegaly, weight loss and general weakness. A total of 23 dogs from Al Shuhda, Jaba'a, Kafr Dan and Siris were inspected visually. In other occasions, visits were made to houses of VL patients in Bet Anan Ramallah district and Kafr Thulth in Qalqiliya districts.

2.5.3. Detection of parasite in captured animals

Eight hyraxes and one wild canine (Marbled polecat, *Vormela peregusna*) were sent to the laboratory of College of Science and Technology, Al-Quds University for dissection. From each animal eight smears were made. Two blood films, two impression smears from the liver, two from bone marrow and two from the spleen. In addition biopsies of spleen, bone marrow and liver were cultured on NNN culture medium (WHO, 1996).

One hyrax with lesions suspected to be CL and two blood samples from dogs with signs of VL were sent to the WHO Jerusalem Reference Center for the Leishmaniasis-Kuvin Center for Enzyme-Linked Immunosorbent Assay (ELISA).

2.6. Meteorological Data

Meteorological data were obtained from the Meteorological Office, Ministry of Transport, Palestinian National Authority. The meteorological data included the annual and monthly rainfall in Jenin District from 1989 to 1998.