

Addis Ababa University
School of Graduate Studies
Department of Biology



**AN INVESTIGATION INTO SOME ASPECTS OF THE EPIDEMIOLOGY OF
CUTANEOUS LEISHMANIASIS IN SARIS, ADDIS ABABA**

A thesis submitted to the School of Graduate Studies:

In partial fulfillment of

the requirements for the Degree of Master of Science in Biology

(Biomedical Streamscience)

Girum Erenso Degu

July 2006.

DEDICATION

To:

My beloved parents Yeshihareg Kebebew and Erenso Degu,
My sister Lidia Erenso, My brother Henock Erenso
and
My grandmother, Aregash Teklegiorgis.

ACKNOWLEDGEMENTS

This thesis is the culmination of my MSc study at the Faculty of Science, Department of Biology, and hence I would like to make use of this opportunity to extend my sincere gratitude to all the staff members who directly or indirectly got involved in my studies and my fellow friends at AAU, for making my study fruitful.

I earnestly owe my most sincere gratitude and appreciation to my supervisor, Professor Asrat Hailu, for his constant encouragement, guidance, and constructive criticisms in the preparation of this manuscript by sacrificing his precious time. Indeed, he has timelessly and tirelessly guided and encouraged me throughout the course of my studies and thesis completion. Despite his immense tasks and responsibilities, he has virtually made himself available at my disposal and offered me his limitless counseling, and constructive suggestion. Moreover, he has inspired and aroused my interests for further research in the area of medical parasitology and related fields. Really, Professor Asrat Hailu is genuine and has been very considerate, inspiring, supportive and brotherly during the course of this research.

Sincere appreciation also goes to the School of Graduate Studies for financing my research project. Indeed, the author is pleased to further express his deepest appreciation and thanks to the Department of Biology for providing me this study opportunity.

Thanks go to Anteneh Yihune, Solomon Aragie, Yohannes Negash, Chirotaw Ayele, Tamrat, Tesfaye Gelana and Wossenseged Lemma, my fellow friends in AAU, for all

the support and for being there always especially at times when it mattered most and necessary.

It would be a remiss if I do not thank some of the staff in the Department of Biology, Mrs. Mulualem and Mrs. Banchialem who directly or indirectly provided me their immeasurable assistance.

Last but not least, I am greatly indebted and wish to thank my beloved parents (Yeshihareg Kebebew and Dr. Erenso Degu) who gave me unreserved support both financially and morally. My sister, brother and Efrata gave me their support during my study. Their patience and continuous encouragement are much acknowledged. Sincere appreciation and thanks to my beloved uncle, Dr. Fassil Kebebew for his constant encouragement throughout the course of this study.

Table of contents	page
ACKNOWLEDGEMENTS	iii
Table of contents	v
List of Figures	viii
Abbreviations	ix
Abstract	x
1 INTRODUCTION	1
1.1 LEISHMANIASIS:.....	1
1.1.1 EPIDEMIOLOGY OF LEISHMANIASIS.....	3
1.1.2 The life cycle of <i>Leishmania</i>	6
1.1.3 THE VECTOR.....	9
1.1.4 RESERVOIR HOSTS OF LEISHMANIASIS	11
1.1.5 PATHOGENESIS AND CLINICAL SPECTRUM:	12
1.1.5.1 LOCALIZED CUTANEOUS LEISHMANIASIS (LCL):	14
1.1.5.2 DIFFUSE CUTANEOUS <i>LEISHMANIASIS</i> (DCL):.....	15
1.1.5.3 MUCOCUTANEOUS <i>LEISHMANIASIS</i> (MCL).....	15
1.1.5.4 RECIDIVANS CUTANEOUS LEISHMANIASIS (RCL)	16
1.1.5.5 VISCERAL LEISHMANIASIS (VL):	16
1.1.5.6 POST KALA-AZAR DERMAL LEISHMANIASIS (PKDL):.....	18
1.1.6 DIAGNOSIS OF LEISHMANIASIS	18
1.1.7 TREATMENT	22
1.2 LEISHMANIASIS IN ETHIOPIA	24
1.2.1 CUTANEOUS LEISHMANIASIS IN ETHIOPIA	26
1.3 PROBLEM OF STATEMENT	29
1.4 HYPOTHESIS	29
1.5 Objective	30

1.5.1	General.....	30
1.5.2	Specific	30
2.	MATERIALS AND METHODS.....	31
2.1	STUDY AREA	31
2.2	STUDY POPULATION	34
2.3	STUDY DESIGN.....	37
2.4	DIAGNOSIS	38
2.4.1	Smear	38
2.4.2	Culture.....	38
2.4.3	Leishmanin skin test (LST).....	38
2.5	DOCUMENTATION OF ANIMALS that CO-EXISTED WITH THE RESIDENTS	39
2.6	ETHICAL CONSIDERATIONS.....	39
2.7	STATSTICAL DATA ANALYSIS.....	39
3.	RESULTS	40
3.1	HUMAN CL IN SARIS, ADDIS ABABA	40
3.2	Present cases of cutaneous leishmaniasis	41
3.3	Distribution of lesions of CL on the body	42
3.4	Leishmanin Skin Test Results.....	44
3.5	RELATIONSHIP OF CL TO AGE AND SEX.....	44
3.6	RELATIONSHIP OF CL TO THE STUDY SITES.....	44
3.7	Animals Living Within the Households	45
4.	DISCUSSION	46
5.	CONCLUSION AND RECOMMENDATIONS	53
6.	REFERENCES:	55
	ANNEXES.....	65
	DECLARATION	69

List of Tables

Table 1. Sex and age distribution of the study population within each site.....	41
Table 2. Prevalence of CL along the Bulbula river basin by age and sex	42
Table 3. The distribution of lesions of cutaneous leishmaniasis on the body, their duration and stage development.....	43
Table 4. Prevalence of cutaneous leishmaniasis in the three study sites.	45

List of Figures

Figure 1 Morphological forms of <i>Leishmania</i> parasites.	6
Figure 2 Life cycles of Leishmania Parasites.	8
Figure 3 Adult sandfly.....	9
Figure 4 A map of Saris showing the three study sites.....	32
Figure 5 Pictures showing the construction of the living houses at the study sites.....	33
Figure 6 Pictures showing hyrax habitats and vegetation around the study sites.....	36
Figure 7 Surveying strategy for covering one study site.	37
Figure 8 Household size of the study population	40

Abbreviations

AAU	Addis Ababa University
ALERT	All African Leprosy Rehabilitation Training
CL	Cutaneous Leishmaniasis
DALYS	Disability Adjusted Life Years
DAT	Direct Agglutination Test
ELISA	Enzyme Linked Immunosorbent Assay
IFAT	Indirect Fluorescent Antibody Test
ITN	Insecticide Treated Net
LCL	Localized Cutaneous Leishmaniasis
LST	Leishmanin Skin Test
PCR	Polymerase Chain Reaction
PKDL	Post Kala-azar Dermal Leishmaniasis
RCD	Recidivan Cutaneous Leishmaniasis
VL	Visceral Leishmaniasis
WHO	World Health Organiztion

Abstract

Cutaneous leishmaniasis (CL) is one of the endemic diseases in Ethiopia. It's widespread over several parts of the highlands of the country. Currently, it is becoming a major challenge in the highlands near and/or surrounding Addis Ababa. However, there was little information on the prevalence of CL around Bulbula river basin in Saris adjoining Saris Abo, Yoseph and Worku Sefer study sites, the suburbs of Addis Ababa. A survey was carried out to describe some aspects of the epidemiology of cutaneous leishmaniasis in the surroundings of Bulbula river in Saris (Addis Ababa) especially Saris Abo, Yoseph and Worku Sefer. A house to house survey of the study population (1010 persons) was undertaken of Saris Abo, Yoseph and Worku Sefer. Clinical, smear, culture and Leishmanin skin test (LST) were used to detect *Leishmania* parasite. Based on this investigation; Saris Abo, Yoseph, and Worku Sefer study sites had infection rates of, 3.6%, 0.6% and 1.1%, respectively. The overall cutaneous leishmaniasis prevalence rate was 1.7% (17/1010). More active cases were found aggregated around hyrax colonies in Saris Abo site and most common in age group 10-19 years. Males were found to be the most affected by the disease as compared to females. Since the males' activities are outdoors in the field, infection could probably have occurred near hyrax colonies (extra-domiciliary transmission). Univariate multiple and pairwise comparisons showed significant differences in age groups 10-19 and below ($P= 0.03$), study sites ($P= 0.002$) and between sex ($P= 0.02$). This study suggests that CL has been found to be a public health problem surrounding the Bulbula river basin, especially in the study site of Saris Abo where the highest infection rate was recorded (3.6%).

Key words: Epidemiology, Cutaneous Leishmaniasis, Saris Abo, Yoseph, Worku Sefer.

1 INTRODUCTION

1.1 LEISHMANIASIS:

Leishmaniasis is a disease that has been the cause of great suffering and death for hundreds of years. Representations of skin lesions and facial deformities have been found on pre-Inca pottery from Peru and Ecuador dating back to the first century AD (www.emedicine.com). They are evidence that some forms of leishmaniasis prevailed as early as this period. The discovery of parasites in lesions of cutaneous or visceral leishmaniasis was reported in the late 1800s and early 1900s. In 1756 Russel first described the disease in English; Borovsky noted the protozoal nature of the organism in 1898; Leishman identified the parasite in 1903 (reviewed in Bryceson and Hay, 1998).

Leishmaniasis is a collective term given to a protozoal disease caused by a member of the genus *Leishmania* and transmitted by sandflies of the genera *Phlebotomus* and *Lutzomyia* (Panosian and Wyler, 1983). The members of the genus *Leishmania* are responsible for causing a wide spectrum of disease that ranges from a simple self healing cutaneous lesion (Localized Cutaneous Leishmaniasis, LCL) through extensive cutaneous and mucocutaneous involvement (Mucocutaneous Leishmaniasis, MCL and Diffuse cutaneous Leishmaniasis, DCL) to a more potentially fatal viscerising form, Visceral Leishmaniasis (VL) and the Post Kala-azar Dermal Leishmaniasis (PKDL).

Cutaneous leishmaniasis (CL) is unsightly and stigmatising but ephemeral, mucocutaneous causes deformity and is highly distressing, while VL is potentially life threatening. Each of the three forms of the disease is caused by a different species of

Leishmania. In the Old World four species of *Leishmania* are known to be pathogenic for Man. They are: *Leishmania major*, *Leishmania tropica*, *Leishmania aethiopica* and *Leishmania donovani*.

The global epidemiology of these diseases has changed, characterized by (re)emergence in many parts of the world, ascribed to population movement or man-made environmental changes, AIDS and other immunosuppressive conditions (Desjeux, 2001). After malaria and lymphatic filariasis, the leishmaniasis form the third most important group of vector-borne diseases, responsible for an estimated 1.81 million disability adjusted life-years (DALYs) and 57,000 deaths annually (WHO, 2002).

On the basis of development in the sandflies, the genus *Leishmania* has been divided into two subgenera. Development of organisms belonging to the subgenus *Leishmania* is restricted to the anterior portion of the alimentary tract of the *Phlebotomus* sandflies (suprapylarian development), whereas organisms belonging to the subgenus *Viannia* develop in the midgut and hindgut of the sandflies *Lutzomyia spp.* (peripylarian development). *Viannia* contains the complex of *L. brasiliensis* (*L. brasiliensis*, *L. peruviana*), *L. mexicana*, *L. amazonensis* and *L. guyanensis* complex (*L. guyanensis*, *L. panamensis*) (Desjeux, 2001). The subgenus *Leishmania* contains the complex *L. donovani* (*L. infantum*, *L. donovani*, and *L. chagasi*), *L. major*, *L. tropica* and *L. aethiopica* (Lainson and Shaw, 1987).

1.1.1 EPIDEMIOLOGY OF LEISHMANIASIS

The *Leishmaniasis* is a globally widespread group of diseases caused by obligatory, intracellular, haemoflagellate protozoan parasites of the genus *Leishmania* (Family, Trypanosomatidae). Leishmaniasis is not a single disease but a variety of syndromes that differ remarkably with one another. The WHO considers *Leishmaniasis* as one of the most important parasitic diseases (WHO, 1990). *Leishmaniasis* is endemic in the tropical and subtropical regions of 88 countries on the five continents (Africa, Europe, Asia, Central and Latin America). Sixteen are in developed countries, 72 are in developing countries, and 13 of them are among the least developed (Desjeux, 1996).

A total of 350 million people are at risk and 12 million are afflicted world-wide. There are 1-1.5 million new cases of CL and 500 000 of VL per year (Desjeux, 1996). Over 90% of cases are found in 3 regions: Sudan/Ethiopia/Kenya; India/Bangladesh/Nepal and Brazil (WHO, 1991; WHO, 1996), with as many as 100 000 deaths every year (Ashford *et al.*, 1992). Out of 1-1.5 million new cases, CL represents 50 to 75 percent and 500,000 cases of VL per year are likely (WHO, 1998 & 2000). These only represent the tip of the iceberg since not all infected individuals develop a disease (Hommel, 1999).

Leishmaniasis forced itself upon medical attention as an increasingly significant problem over the last decades. Increasing risk factors make leishmaniasis as growing public health concern around the globe. Because of its importance, *Leishmaniasis* is considered as one of the 6 diseases selected by WHO for its special programme for research and training in

tropical diseases (WHO, 1984). It ranks only second to malaria among human protozoan diseases (Chang *et al.*, 1985).

The epidemiology of leishmaniasis is diverse with 20 *Leishmania* species that are pathogenic for humans and 30 sandfly species that have been identified as vectors. Each *Leishmania* species has its own biotope with its own geographical distribution zone and complex of parasite, reservoir and vector and their particular intimate relationship within this setting (WHO, 1996). Furthermore, epidemiological patterns of leishmaniasis is in evolution because of an accumulation of risk factors, like immuno-suppression, human and environmental changes and drug resistance (Lane, 1993).

The epidemiology of leishmaniasis in a given area is directly dependent on the behavior of the human and/or animal population in relation to the cycle of transmission. There is a variety of factors that influence the transmission of the disease. Some are the following:

- Proximity of residence to sandfly breeding and resting sites (Werneck *et al.*, 2003).
- Type of housing (Kolasczinski *et al.*, 2004).
- Occupation (Weigle *et al.*, 1993).
- Extent of exposure to sandfly bites (Weigle *et al.*, 1993).
- Natural resistance (genetic or acquired) to infection (Ashford *et al.*, 1992).
- Virulence of the parasite species (Kettle, 1993).
- Zoonotic and/or anthroponotic reservoirs (Lane, 1993).
- The vectorial capacity, which is defined as the number of infective bites delivered per human per annum (Dye, 1992).

- Density, seasonality, longevity and flight range of sandfly populations (Al-Awsik and Abukhamsin, 2004).
- Anthrophilia or zoophilia of sandflies and degree of it (*Bucheton et al.*, 2002).

Distribution of leishmaniasis is limited by the distribution of the sandfly, its susceptibility to cold climates, its tendency to take blood from humans or animals only and its capacity to support the internal development of specific species of *Leishmania*. Since 1993, regions that are *Leishmania*-endemic have been expanded significantly, accompanied by a sharp increase in the number of recorded cases of the disease (www.emedicine.com). The geographic spread is due to factors related mostly to development. These include massive rural-urban migration and agro-industrial projects that bring non-immune urban dwellers into endemic rural areas. Man-made projects with environmental impact, like dams, irrigation systems and wells, as well as deforestation, also contribute to the spread of leishmaniasis (El- hassen *et al.*, 2001; Hommel, 1999).

Leishmania/HIV co-infections can lead to epidemiological changes which modify the traditional patterns of zoonotic transmission. Co-infected patients harbour a high number of *Leishmania* in their blood so there is also a risk of them becoming reservoirs of the disease as in anthroponotic foci in Bangladesh, India, Nepal and East Africa. Consequently, there is an increased risk of future epidemics. Cruz *et al.* (2002) experimentally found that sandflies can be infected through a blood meal containing a very small quantity of blood from co-infected patients. There is also a chance of being

transmitted through sharing of needles as it is shown in south-western Europe co-infected patients (Cruz *et al.*, 2002).

1.1.2 The life cycle of *Leishmania*

All *Leishmania* parasites are digenetic: they need to pass through two hosts (i) invertebrate (sandfly) and (ii) vertebrate (mammals, including humans). *Leishmania* parasites can take two morphological appearances, promastigote (long flagellated parasite) and amastigote (round, intracellular parasite, without free flagellum) (Fig. 1 A & B). These forms correspond to the changes in direct environment (sandfly gut and macrophage, respectively) and are accompanied by biochemical modifications and changes in gene expression (Molyneux & Killick-Kendrick, 1987; Hommel, 1999).

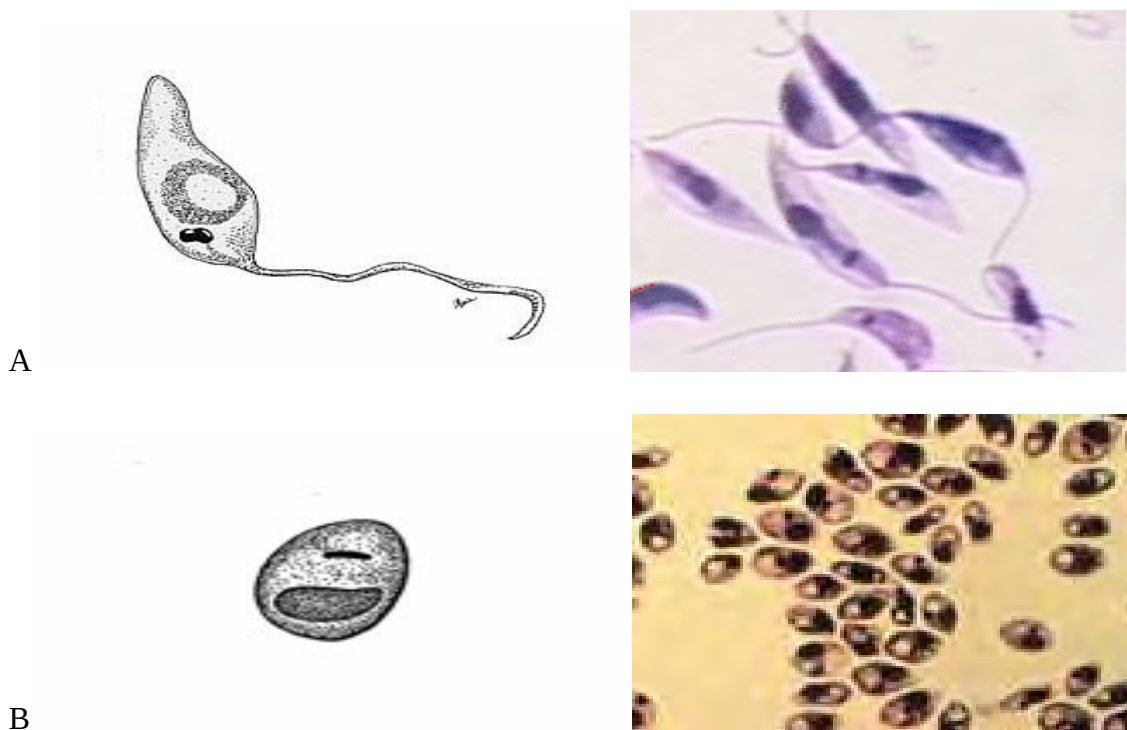


Figure 1. Morphological forms of leishmania parasites. A. Promastigotes-long flagellated parasites; B. Amastigotes- round, intracellular parasites, without free flagellum.

All *Leishmania* parasites alternate from a flagellated promastigote form in the gut of phlebotomine sandflies to a non-flagellated intracellular amastigote form in the mammalian host (Jaffe and Mchon, 1983). Although there are several different species of *Leishmania* that infect man, they all share this type of life cycle in which an infected sand fly bites a suitable mammal, transmitting the *Leishmania* promastigotes directly from the proboscis or by regurgitating them during feeding.

The female sandfly picks up infected cells from the skin with its blood meal. The amastigotes are released in the midgut of the insect, transform to the procyclic stage and start multiplying actively without penetrating the hemocoele. After few days, numerous procyclic conquer the gut of the insect. Then the elongated procyclic promastigote attach to the midgut epithelium by inserting their long flagella between the microvilli that line the midgut. They migrate to the cardiac valve, where they transform into short, spherical, non-dividing promastigotes. Then the parasites are released from the midgut and penetrate the pharynx (proboscis) as metacyclic promastigotes, also termed paramastigote. From proboscis the metacyclic promastigotes are ousted to the new mammalian host.

Metacyclic promastigotes enter the skin of the vertebrate host when the infected sandfly takes its next blood meal. It may inoculate 10-200 promastigotes into the dermis. Within the macrophages and related cell types, they rapidly transform into amastigotes, remain within the phagocytic vacuole, where they develop and multiply. At some stage, this infected cell, which may harbor up to 20 or more amastigotes, bursts and released free amastigotes, which infect other cells. Infected macrophages move from the skin to other

tissues, infecting the spleen, liver and bone marrow, while certain parasites exhibit a specific tropism for each given host, e.g., viscerotropism or dermatotropism. The features that control this tropism have not yet been elucidated but are thought to include host and parasite genetics as well as the status of immunity (Molyneux & Killick-Kendrick, 1987; Hommel, 1999).

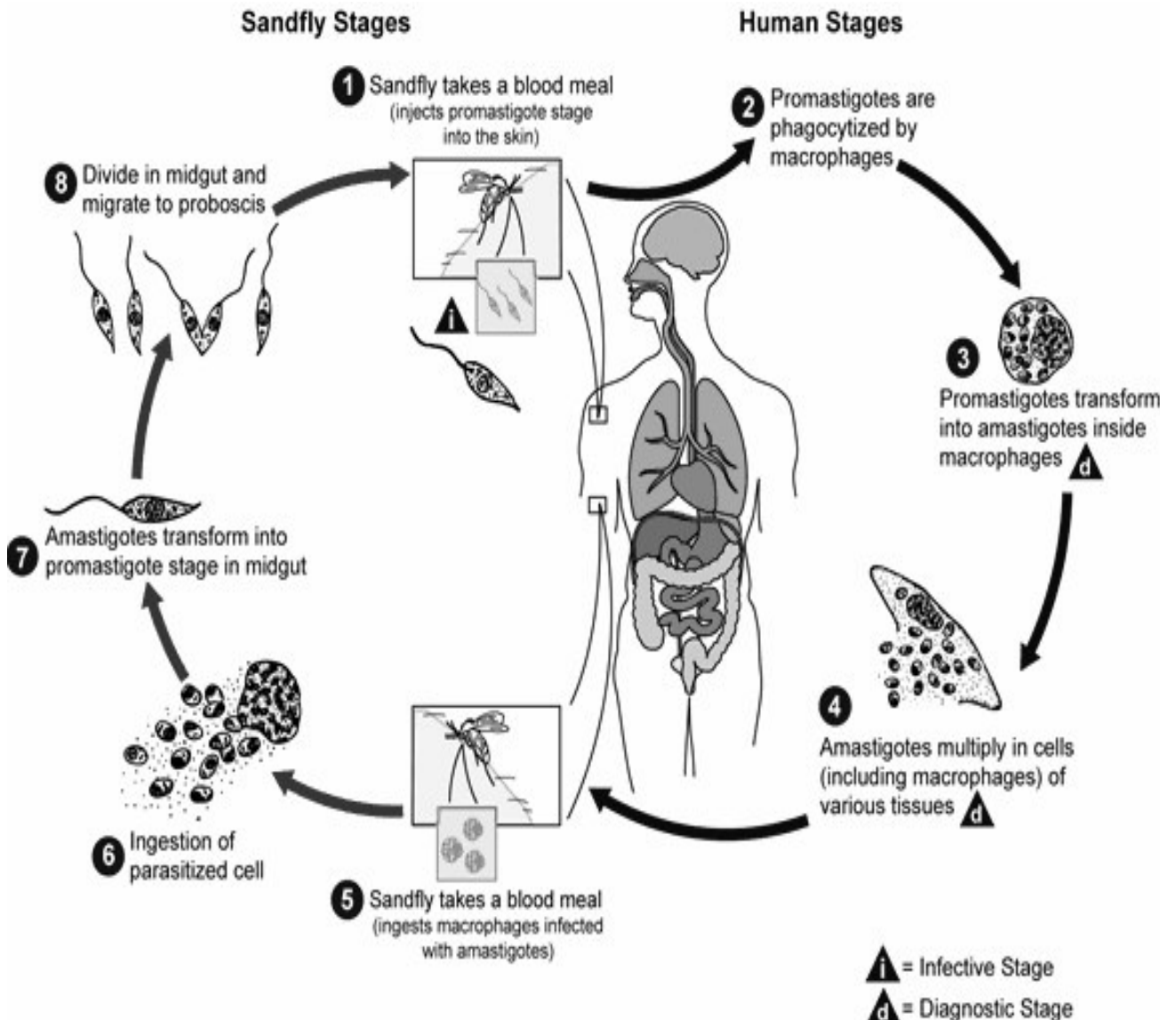


Figure 2 Life cycle of *Leishmania* parasites. There are two transmission cycles: anthroponotic cycle, when man is the host; and zoonotic cycle, when animals are the hosts (Source: CDC).

1.1.3 THE VECTOR

Sandflies that are vectors of *Leishmania* parasites are insects of the order Diptera, family Psychodidae, and subfamily Phlebotominae of the genus *Phlebotomus* in the Old World (divided in to 12 subgenera), in the New World *Lutzomyia* (divided into 25 subgenera and species groups) and *Psychodopygus* (Fig. 3). There are more sandfly species in the New World than the Old World and this is often quoted as the reason that there are more species of *Leishmania* in the New World (León *et al.*, 1996). The biology of many species of these vectors is unknown (Lewis *et al.*, 1987).



Figure 3 Adult sandfly.

Phlebotominae sandflies occupy most of the zoogeographical regions of the world (Palearctic, Oriental, Afro tropical, Nearctic, Neotropical and Australasian). Each of the zoogeographical regions has its own characteristic sandfly fauna. Sandfly numbers are related to natural factors such as rainfall and temperature and may increase with global warming. Among all factors, temperature is considered to limit the worldwide

distribution of sandflies. In extreme temperature, sandflies survive in diapause (WHO, 1984; Lewis, 1982).

Sandflies live in dark, damp places, and are relatively weak fliers, with a range of only 50 meters from the breeding site. Unlike mosquitoes, they fly silently and their small size (2-3mm long) allows them to penetrate mosquito nets (Tayeh *et al.*, 1997). Burrows of several reservoir hosts of leishmaniasis, damp portion of a cave and termite hills provide ideal habitats for the breeding of vector species. The immature stages develop in a variety of habitats with suitable temperature and high humidity. The developmental period of immature stages depends upon temperature, humidity and larval diet (Gemetchu, 1977). Gemetch (1977) also recorded 48 days from egg laying to adult emergence.

Most sandfly vectors are active from dusk to dawn, with a peak biting immediately after sunset and have diurnal resting sites: e.g., houses, cellars, stables, caves, fissures in walls, rocks or soil, dense vegetation, tree holes, burrows of rodents and other mammals, bird's nests, and termitaria (Killick-Kendrick, 2002). The choice of resting sites depend on host distribution, temperature, moisture soil and vegetation types. They may have endophilic and/or exophilic habits. Sandflies usually repose during the day in burrows, tree hollows, caves or buildings. After sundown, they leave the shelters to remain active throughout the night. Approximately 80 species of phlebotomine sandflies are the vectors for the *Leishmania* parasites: some *Leishmania* species can be transmitted by one sandfly species only, while others are more permissive (Sacks & Kamhawi, 2001).

1.1.4 RESERVOIR HOSTS OF LEISHMANIASIS

Leishmaniasis is primarily a zoonotic disease in which wild and domestic animals such as the fox, jackal, rodents, hyraxes and wolves serve as reservoir hosts. Other animals in the surrounding areas can become infected and these are referred to as secondary or incidental hosts. Of all the potential animal hosts, domestic dogs by far play the most important role in harboring and transmitting the disease to humans due to the close association between humans and dogs as pets (WHO, 1991; Arias *et al.*, 1996). In anthroponotic VL, due to *Leishmania donovani*, such as in India and Sudan, man is the principal reservoir host. Ashford (1996) recognized hosts as reservoir when they are abundant or gregarious, long lived or survive at least during non transmission season of the parasite, remain infected for long time with out acute disease and present the parasite in their skin or circulation for sandfly bite.

In Ethiopia, *Procavia capensis* and *Heterohyrax brucei* in the family Procaviidae are the main animal reservoir hosts of CL. *Procaviidae* is the only extant family in the order *Hyracoid* which is considered to have evolved in Africa before the Oligocene or 40 million years ago (Walker, 1975). Hyraxes are widely distributed in Africa, Arabia and the Mediterranean regions (Walker, 1975; Corbet, 1979). *Procavia*, *Heterohyrax* and *Dendrohyrax* are the three genera in the family Procaviidae.

Hyraxes live in a wide variety of habitats and are found in altitude ranging from sea level to the height of 4650m in Kenya (Kingdon, 1971; Walker, 1975). A narrow temperature range (from 3 to 10°C) within hyrax holes as opposed to the large temperature range

(41.8°C to 5°C) of external environment in which they are known to live, augmented their poor thermoregulatory system (Kingdon, 1971).

It is apparent that rock hyraxes select suitable habitats which provide protection from predators. They inhabit cavities and crevices in rock out crops, cliffs and boulder rock formations, mountains and escarpments. Hyraxes living in rock habitats avoid isolated holes that are large enough for predators to enter and exposed to the wind (Sale, 1966). In the genus *Heterohyrax*, some members have secondarily adapted to arboreal life. *H. brucei* was found to live entirely in large trees in the absence of outcropping rocks (Ashford, 1977). Hyraxes are known to feed in the early morning and late afternoon; active from dusk to dawn. Hyraxes are known to be catholic feeders (Ashford *et al.*, 1973).

Hyraxes have long gestation period (up to 230 days) and a female usually produces two to six young's (Walker, 1975). Though accurate estimation of colony size is difficult because of limited diel activity outside their holes, Walker (1975) and Kingdon (1971) estimated 5 to 60 members in a colony. Not all colony members are found outside at once.

1.1.5 PATHOGENESIS AND CLINICAL SPECTRUM:

Leishmaniasis is a variable disease with a variety of syndromes that are manifested alone or in combinations (Garnham, 1987). The incubation period ranges from a few days to several months. The sandflies are biting humans and animals in the uncovered and hairless areas of the body. At the inoculation site an erythematous nodule appears. The

nodule grows to an ulcer with a raised edge. This sore remains often in that stage without further development and when it heals it leaves scar tissue. Scars can even disable if they are on the face or over a joint.

After inoculation of the parasite through the sandfly bite, *Leishmania* promastigotes are phagocytosed in the skin by activated macrophages. Patients with acute *Leishmaniasis* fail to produce T helper cell 1 (Th1) cytokines and the parasite interferes with the killing mechanism of the macrophages (Bogdan *et al.*, 1990; Russo *et al.*, 1992). The parasites transform into amastigotes and start to divide. Amastigotes have an affinity for macrophages and endothelial cells of arterioles and capillaries, leading to tissue lysis and necrolysis. Then one of the following events finds place:

- The immune system kills the parasites and the person becomes immune to reinfection by that species.
- A local infection develops until either the immune system of the host eradicates it or is defeated by it permitting dissemination.
- The infection disseminates to the viscera (*L. infantum*, *L. chagasi*), oronasal mucosa (*L. brasiliensis*) or skin (*L. aethiopica*, *L. mexicana*).

Parasites multiply in the cells of the mononuclear phagocyte system like blood monocytes, macrophages, histiocytes, epithelioid cells, Kupffer cells of the liver, reticuloendothelial cells in spleen and lymphoid tissue (Bogdan *et al.*, 1990).

The disease presents itself in humans in different forms with a broad range of clinical manifestations (CL, MCL, DCL, VL, PKDL and Recidivans Cutaneous Leishmaniasis (RCL). All forms can have a devastating consequence. Leishmaniasis is a disease in

which the clinical diversity reflects a complex interplay between the virulence of the infecting species and the host's immune response. At one extreme, LCL demonstrates a vigorous immune response, with most cases resolving with out intervention. This form exhibits a TH1 immune response, with interleukin 2, interferon gamma, and interleukin 12 as the prominent cytokines that induce disease resolution. At the other extreme, with VL or DCL, patients exhibit relative anergy to the *Leishmania* organism and have a prominent TH2 cytokine profile (Russo *et al.*, 2002).

1.1.5.1 LOCALIZED CUTANEOUS LEISHMANIASIS (LCL):

In the Old World LCL is known as oriental sore. It produces skin lesions, sometimes as many as 200 on the face, arms and legs, causing serious disability and permanent scars (WHO, 1998). In the Old World it is mainly caused by *Leishmania major*, *Leishmania tropica* and *Leishmania aethiopica* (WHO, 1990) and sporadically by *L. infantum* and *L. donovan* (Aliaga *et al.*, 2003; Belhadj *et al.*, 2003). In the New World CL is caused by *L. mexicana*, *L. braziliensis*, *L. peruviana*.

Ninety percent of all cases of CL occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria, with 1-1.5 million new cases reported annually world-wide (WHO, 1996). *L. major* usually produces self-healing lesions, on the other hand, *L. tropica* is usually more chronic, and its most severe form, RCL, is very difficult to treat. In the New World, *L. mexicana* usually produces relatively benign lesions but some locations such as the ear's pinna are very difficult to treat in general (Desjeux, 1996). LCL lesions are those, which evolve from small nodular lesions through the ulcerative stages to self curing lesions that

ultimately, leave behind a typical scar. Parasites are usually scanty during late stages of LCL.

1.1.5.2 DIFFUSE CUTANEOUS *LEISHMANIASIS* (DCL):

DCL is less common, chronic in evolution and principally difficult to treat. It produces lesions resembling leprosy, which do not heal spontaneously, due to deficiency of the immune response (Desjeux, 1996; WHO, 1998). Consequently, patients develop multiple widespread cutaneous papules and nodules and are anergic to LST. DCL is due to *L. aethiopica* in the old world and *L. amazonensis* in the new world (Desjeux, 1996). DCL never heals spontaneously and tends to relapse after treatment.

DCL lesions are characterized by multiple, diffuse, non-ulcerating nodular lesions (Bryceson, 1970; Belehu, 1981). In contrast to LCL and MCL, parasites are abundant in DCL lesions. A depressed cell mediated immunity, but high antibody titers are characteristic features of DCL (Akuffo *et al.*, 1987).

1.1.5.3 MUCOCUTANEOUS *LEISHMANIASIS* (MCL)

Also called 'espundia', MCL produces disfiguring lesions to the face, destroying the mucous membranes of the nose, mouth and throat (Desjeux, 1996; WHO, 1998). It is mostly related to *Leishmania* species of the New World such as *L. braziliensis*, *L. panamensis* and *L. guyanensis*, but mucosal lesions have been reported in the Old World due to *L. donovani*, *L. major* and *L. infantum* in immunosuppressed patients (Desjeux, 1996). In such lesions, ulcerations and swelling are characteristic. Parasites in MCL

lesions are scarce, presumably due to strong antibody and delayed hypersensitivity reactions (Belhu, 1981). Ninety percent of all cases of MCL occur in Bolivia, Brazil and Peru (WHO, 1996).

1.1.5.4 RECIDIVANS CUTANEOUS LEISHMANIASIS (RCL)

RCL is relatively uncommon clinical variant of leishmaniasis. It is a condition that can result in infection with *L.tropica*. It has the clinical picture of Lupus vulgaris and represents persistence of the infection in the face of a vigorous immune response (Pettit, 1962). It presents as recurrence of lesions at the site of apparently healed lesions years after the original infection. Typically, RCL lesions occur on the face and presents as an enlarging papule, plaque, or coalescence of papules that heals with central scarring. Relentless expansion at the periphery may cause significant facial destruction.

1.1.5.5 VISCERAL LEISHMANIASIS (VL):

Also known in Asia as 'black fever' or 'kala-azar' is the most severe form of the disease, the parasite invades internal organs (spleen, liver, bone marrow) and the consequences are usually with an almost 100% mortality rate if left untreated. It is characterized by irregular fever, loss of weight, splenomegaly, hepatomegaly and/or lymphadenopathy and anaemia. Of the 500,000 new cases of VL, which occur annually, 90% are in 5 countries Bangladesh, Brazil, India, Nepal and Sudan (Desjeux, 1996; WHO, 1996 & 1998). VL is caused by *L. donovani* on the Indian subcontinent and in East Africa, by *L. infantum* in the Mediterranean region and by *L. chagasi*, which is closely related to or not

distinguished from *L. infantum* (Mauricio *et al.*, 2000), in the New World mainly in Brazil, Peru and Paraguay (Berman, 1997).

The number of *Leishmania*/HIV co-infections will continue to rise in the coming years and there are indications that cases are no longer restricted to endemic areas. The overlapping geographical distribution of VL and AIDS is increasing due to two main factors: the spread of the AIDS pandemic in suburban and rural areas of the world, and the simultaneous spread of VL from rural to suburban areas. There are important clinical, diagnostic, chemotherapeutic, epidemiological and economic implications of this trend (Cruz *et al.*, 2002).

In eastern Africa, cases of *Leishmania*/HIV co-infection have been reported in Ethiopia, Kenya, Malawi and Sudan (WHO, 1996). The risk of overlap is increasing due to a number of factors: mass migration, civil unrest, resettlement programmes and promiscuity and prostitution in refugee camps. In North Africa, a few cases have been reported in Algeria, Morocco, and Tunisia and in western Africa 1 case in Cameroon and 1 case in Guinea Bissau (WHO, 1996). AIDS and VL are locked in a vicious circle of mutual reinforcement. VL accelerates the onset of full-blown AIDS, and shortens the life expectancy of HIV-infected people, while HIV spurs the spread of VL. The gridlock produce cumulative deficiency of the immunoresponse, as *Leishmania* parasites and HIV destroy the same cells (WHO, 1998).

1.1.5.6 POST KALA-AZAR DERMAL LEISHMANIASIS (PKDL):

Endemic to India and the Sudan, this form of leishmaniasis develops months to years after the patient's recovery from VL (WHO, 1990), but there are cases without any previous known history of VL (El-Hassan *et al.*, 1992). In PKDL the viscerotropic parasite becomes dermatrophic as a consequence of treatment (Kuba and Al-Gindan, 1989). As in Leprosy, the wide clinical spectrum of PKDL reflects the immune response of the individual to the *Leishmania* organism. Lesions may be numerous and persist for decades.

The disease is characterized by the development of macules, papules and nodules, which first appear around the mouth; those which do not heal spontaneously become denser and spread over the entire body (Berman, 1997). The interval between the end of treatment of VL and the onset of PKDL is variable: PKDL may appear during or directly after treatment (Zijlstra *et al.*, 1995) to up to 2 years post treatment (Zijlstra *et al.*, 1991). The etiology is unknown but possibilities include inadequate treatment or re-infection of patients previously cured of VL. These patients may play an important role as reservoir of infection in VL transmission (Addy & Nandy, 1992; WHO, 1990).

1.1.6 DIAGNOSIS OF LEISHMANIASIS

An epidemiological study of leishmaniasis relies on sensitive, specific, reproducible, and cheap diagnostic tests. Since Leishman identified the parasite in 1901, the definite diagnosis of leishmaniasis relies on detection of the parasite in aspirate material or by culturing of aspirates or tissue. The diagnosis is often made on the basis of a clinically typical lesion in conjunction with an appropriate history of exposure. However, there are a

number of mimics, and the treatment may be toxic, so pathological confirmation should be sought- preferably by demonstrating the organism in tissue and/or culture. Unfortunately this is not always possible in practice. The best approach is to use several methods (Hepburn, 2003). The various diagnostic methods employed in the diagnosis of leishmaniasis include: microscopy, culture, leishmanin skin test (LST), serodiagnosis, and molecular diagnosis: Polymerase chain reaction (PCR).

Parasitological diagnosis, the gold standard, depends on microscopic examination of skin lesions using smears and cultures of dermal scrapings or examination of sections obtained from skin biopsy (Cuba *et al.*, 1984). Biopsy smears may be used for culture, inoculation into hamsters, impression smears or immunohistology in tissue sections (Sells *et al.*, 1981). Lesions smears and culture are best in cases with cutaneous lesions and biopsy and hamster inoculation when mucosal lesions are predominant. This method is simple and cheap with limited sensitivity. Culture is more sensitive, easy and less invasive than microscopy. The draw back is its sensitivity to bacterial and fungal contamination. Parasitological diagnoses using smears, cultures or biopsy is unsatisfactory and requires well trained personnel (Chulay *et al.*, 1983; Del Mar Sanz *et al.*, 1991).

LST measures delayed type hypersensitivity reaction to *Leishmania* antigens. Leishmanin is a suspension of washed promastigotes in a solution of 0.5 % phenol in saline. (Leeuwenburg *et al.*, 1983; Cuba *et al.*, 1984). In active VL, it is negative but within several months to a year after recovery, individuals elicit a positive response. It is useful to detect active MCL and CL with about 80-100% sensitivity (Weigle *et al.*, 1991).

Overall, it is a good test for epidemiological surveys of a population to identify groups at risk of infection from CL (Weigle *et al.*, 1991).

Serological tests reveal the presence of antibody and are useful in both individual diagnosis and epidemiological surveys. A number of methods have been described (reviewed by Kar, 1995), including indirect immunofluorescent antibody test, IFAT, (Badaro *et al.*, 1983; Pappas *et al.*, 1985); enzyme- linked immunosorbent assay, ELISA, (Hommel *et al.*, 1978; Pappas *et al.*, 1985); direct agglutination tests, DAT, (Harith *et al.*, 1986 & 88; Meredith *et al.*, 1995; Hommel *et al.*, 1997), and a variety of immunoblotting methods test have been developed (Evans & Pearson, 1988; Rolland *et al.*, 1994). The use of these serologic tools in diagnosis of CL has been assessed by a number of studies (El Amin *et al.*, 1986; Hommel *et al.*, 1997). However, not any serologic tool is yet available for routine diagnosis.

There are various problems with serological assays, including the fact that persistence of antibodies may be a problem in endemic area; certain individuals may have a high level of reactive antibodies in the absence of the organism (false positive), conversely anti-parasite antibodies when induced may not be present until some time after the initiation of infection (false negative). The possible cross-reactivity with other pathogens, e.g. malaria, trypanosomiasis, schistosomiasis and leprosy (Abdallah, 1980) and the fact that most serological tests cannot readily distinguish between current, sub clinical or past infections (Hommel *et al.*, 1997) limited its performance. The performance of serological tests is particularly poor in patients co- infected with HIV (Hommel, 1999).

Nowadays, DAT is widely used in the diagnosis of VL and for use in field situation. DAT is a relatively economical, fast and simple technique, with high sensitivity and specificity (Hommel *et al.*, 1997; Meredith *et al.*, 1995), thus one of the most widely used immunological tests that have been applied in diagnostic and epidemiological studies. DAT showed its value in large-scale sero-epidemiological surveys in eastern Sudan (Zijlstra *et al.*, 1991), Ethiopia (Hailu, 2002) and in the Himalayas (Rab and Evans, 1995). The main disadvantages of it are cross-reactivity (Hommel *et al.*, 1997), the persistence of the antibodies after apparent cure (Hommel *et al.*, 1997; Zijlstra *et al.*, 1991), and the thermal instability of the aqueous antigen (Zijlstra *et al.*, 1997).

Recently, several molecular biological techniques have been developed for the sensitive detection and identification of pathogens. The main approaches to nucleic-acid-based detection are (i) hybridization using DNA probes (ii) amplification techniques including the polymerase chain reaction (PCR) for the detection of DNA, nucleic acid sequence based amplification (NASBA) and reverse-transcriptase PCR (RT-PCR) for the detection of RNA.

PCR allows the sensitive, specific and fast detection of minute amounts of pathogen DNA. It has been used in a limited extent but provided rapid diagnosis with very impressive results (Saiki *et al.*, 1988). It is one of the newest techniques used to identify leishmaniasis and shows significant promise as a method applicable for both detection and speciation. Most research laboratories have reported higher sensitivity and specificity with PCR than with other currently available diagnostic methods. Attempts have been

made to utilize molecular technology to improve diagnostic speed, convenience and specificity. But, they remain research tools (Saiki *et al.*, 1988; Weiss, 1995).

1.1.7 TREATMENT

There is no single optimal treatment for all forms of leishmaniasis because of the natural diversity of the parasites together with the wide spectrum of clinical disease that they produce. Each endemic area would establish its own optimum treatment regimen based on efficacy and toxicity, without neglecting the practical difficulties of administration and cost. Since 1941, the pentavalent antimony (sodium stibogluconate or meglumine antimonite) has been the main stay of antileishmanial therapy (Herwaldt, 1999). Other measures include freezing, local heat, oral ketokonazole, rifampicin and topical paromomycin. Surgical excision usually is not recommended because of the risk of relapse and cosmetic disfigurement (Herwaldt and Berman, 1992).

The treatment of leishmaniasis depends on the clinical form of the disease. Lesions due to different species vary in both their severity and response to treatment- American CL tends to be more severe and longer lasting than old world CL. Some infections in the old world, especially simple cutaneous lesions due to *L. major*, are often self-healing and induce immunity to reinfection and treatment of these is generally not recommended, unless the lesions do not heal within 6-9 months. In case of chronic lesions due to *L. tropica* the treatment is based on pentavalent antimonials intramuscularly or intravenously at 10-20 mg/kg/day until cure (Herwaldt, 1999; Hepburn, 2003).

Treatment of other forms, such as VL and MCL infections, mainly relies on the pentavalent antimonials sodium stibogluconate (Pentostam) or meglumine antimoniate (Glucantime), the first -line drugs except when resistance exists, and the usual dose is 20 mg/kg/day for 30 days (Desjeux, 1996). They are expensive and need to be given by injection. The second-line drugs in case of resistance -amphotericin B and pentamidine, used in cases unresponsive to antimonials, need careful management to avoid serious side effects. They are used intravenously over several hours on alternate days from 0.1 mg/kg/day up to 1 mg/kg/day with a maximum total dose of 3g. For VL, aminosidine, alone or in association with pentavalent antimonials, has shown good efficacy but it is still under evaluation. Amphotericin B, included in liposomes, has proven to be very efficient but its use is still limited and expensive (Baily & Nandy, 1994; WHO, 1998). PKDL cases should be given high priority as they are considered to be residual reservoir, able to disseminate the disease. Patients should be treated by pentavalent antimonials at 20 mg/kg/day for 3 to 4 months (Desjeux, 1996).

Treatment for co-infected patients is aimed at clinical and parasitological cures and prevention of relapses. Unfortunately, in such patients' treatment failure, relapse due to drug resistance and drug toxicity are very common (Cruz *et al.*, 2002). However, WHO still suggests since the economical condition of the endemic areas is very poor, and for reason of meeting effectivity with low cost of treatment, pentavalent antimonials are the drugs of choice in those areas. The main alternative drugs include pentamidine, amphotericin A and amphotericin B encapsulated in liposomes. This encapsulation

reduces the occurrence of side-effects, but relapses still occur and the drug remains extremely expensive (Bryceson and Hay, 1998).

Most CL lesions are self-limiting and may heal spontaneously within one to five years. In spite of this, treatment is justified in a variety of cases, namely, early lesions, multiple lesions, lesions involving cosmetically sensitive sites, mucosal lesions, disseminated lesions, and patients with significant immunosuppression (Kubba and Al-Gindan, 1989). Unfortunately, to date there is no safe, simple and effective treatment and the PVA compounds, “the best drugs of a bad bunch,” still remain the mainstay of treatment in the majority of cases. Cure rates have varied from zero to 100% in different areas of the world (Stratigos, 1980).

1.2 LEISHMANIASIS IN ETHIOPIA

Leishmaniasis is one of the most important vector-borne diseases in Ethiopia and presents many examples in and challenges for disease ecology and epidemiology. It is caused by *Leishmania donovani* (VL, in the lowlands), *L. aethiopica* (CL, in the highlands), and occasionally *L. major* and *L. tropica* (reviewed in Hailu and Frommel, 1993).

The discovery of cases of DCL in Ethiopia by Balzer *et al.* (1960) has stimulated work on several aspects of leishmaniasis in Ethiopia. Initial interest was concentrated on cases of DCL seen in hospital (Poirier, 1964; Bryceson and Leithead, 1966). However, Bryceson and Nichol (1966) reported cases with active and healed oriental sore in

several areas and concluded that the infection was usually acquired in early life and that 10 to 20% of the population had had the disease at some time.

CL (oriental sore) was common in the eastern edge of the highland plateau in former Wollo, Dembidollo in Wollega and the Sebeta area of Shoa province. And DCL was a rare aberrant form (Lemma *et al.*, 1969). Detailed epidemiological surveys by Ashford *et al.* (1973) established the endemicity of CL and were able to identify the vectors and reservoir hosts for *Leishmania aethiopica*. Though all ages appeared susceptible to infection, most individuals appeared to contract the disease in childhood or youth. In Sebeta the risk of infection among males exceed that among females by over 50%, but elsewhere the prevalence was similar in both sexes (Lemma *et al.*, 1969).

The transmission cycles of the *Leishmania* and their geographic distribution in Ethiopia is gradually being elucidated and is partially understood. Schaller and Kuls (1972) reported the occurrence of CL in the highlands and of VL in the lowlands in western and southern Ethiopia. Both diseases are wide spread in Ethiopia.

Several phlebotomine species are known or implicated to transmit the different forms of the disease in Ethiopia. These include: *Phlebotomus martini* and *P. celiae* (Gebre-Michael and Lane, 1996), *P. orientalis* (Hailu *et al.*, 1995), *P. pedifer* and *P. longipes* (Ashford *et al.*, 1973) and *P. duboscqi* (Gebre-Micahel *et al.*, 1993). Other potential vector species (*P. alexandri*, *P. saevus*) are known to exist in the endemic area; some of these species have been found naturally infected with yet unidentified *Leishmania spp.* (Balkew *et al.*, 1999).

Leishmaniasis in Ethiopia reflects spatially varied disease ecologies, and each ecological situation has to be dealt with separately. The increased risk of leishmaniasis; increasing population movements in and out of endemic areas, discovery of new transmission sites, and widespread malnutrition and recurrent famine are major challenges in the control of leishmaniasis in Ethiopia. The wide ecological diversity in its physical, biotic, and human environments are also an important considerations in the control of leishmaniasis in Ethiopia. Highland CL is associated with rock hyraxes, the anthroponotic type VL with termite mounds, and zoonotic VL with deeply cracking clay soils of Acacia-Balanites woodland. This diversity is a major challenge, and its various problems will have to be addressed (Hailu and Frommel, 1993).

1.2.1 CUTANEOUS LEISHMANIASIS IN ETHIOPIA

Italian authors were the first to describe CL in Ethiopia (Martoglia, 1912; Monti, 1937; Poggi, 1937; Cupi and Cattapan, 1942 cited by Ayele *et al.* (1981). Reports of several researchers (Balzer *et al.*, 1960; Poirier, 1964; Lemma *et al.*, 1969; Ashford and Smith, 1985) have shown that the disease is prevalent in many highland areas of Ethiopia. The exact distribution of the disease is not yet defined as cases from previously undescribed areas are still being reported. It is also suspected that there is no region free from cutaneous *Leishmaniasis* including Addis Ababa (Sarojini *et al.*, 1984).

The main causative agent of CL, *Leishmania aethiopica* (Bray *et al.*, 1973), in Ethiopia is naturally maintained zoonotically by rock hyraxes and several species of *Phlebotomus*. CL due to *L. aethiopica* is widely spread in the highlands of Ethiopia and Kenya

(Ashford *et al.*, 1973; Mutinga, 1975)). Ashford (1977) described the distribution of the disease in terms of topographical types, namely gorges and escarpments of the central plateau and the western edge of the plateau with annual rainfall above 800mm.

Cutaneous leishmaniasis due to *L. aethiopica* is a zoonosis as are most of *Leishmaniases* of the old world. However, the composition of the parasite system in which the disease is maintained indefinitely is distinct. In Ethiopia, the parasite is maintained among two species of rock hyraxes (*Procavia capensis* and *Heterohyrax brucei* and two species of Phlebotomine sandflies namely *Phlebotomus longipes* and *Phlebotomus pedifer* (Ashford *et al.*, 1973; WHO, 1984; Ashford and Smith, 1985). Man acquires the disease when he intrudes into the system. The frequent and chronic infection in man (Lemma *et al.*, 1969), intense man-sandfly contact in Sebeta and Ochollo (Foster *et al.*, 1972; Ashford *et al.*, 1973) and spatial and temporal clustering of cases (Wilkins, 1972) suggest possible man to man transmission. However, this may not maintain the parasite.

The ecology of Ethiopian highlands CL is unique. No rodents have been reported to be naturally infected with *L. aethiopica* except a single report by Mutinga (1975) from the Giant rat, *Cricetomys spp.*, the significance of which is not known. In Kenya, *Procavia johnstoni* and *Dendrohyrax arboreous* are the reservoir hosts of *L. aethiopica* (Mutinga, 1975). The natural invertebrate hosts (*P. longipes* and *P. pedifer*) have never been reported to be vectors of *L. aethiopica* elsewhere except for *P. pedifer* in Kenya (Mutinga, 1975).

Three distinct clinical types of Cutaneous *Leishmaniasis* have been reported to occur in Ethiopia. The commonest form is LCL which is generally restricted to the face, arms or legs and is eventually self healing. This form of the disease may occur on, or spread to, mucous membranes and termed MCL (Humber *et al.*, 1986). In contrast, DCL is not self-healing, and the lesions are progressive and eventually spread over large areas of the body. DCL is associated with immunological unresponsiveness to *Leishmania* antigens (Belehu and Humber, 1981). However, Genene *et al.* (1986) have given an immunological evidence for an active role of parasites in clinical manifestations of DCL and LCL patients. This supports the epidemiological findings of Lemma *et al.* (1969).

1.3 PROBLEM OF STATEMENT

There are cases of CL in the catchment of Bulbula River in Addis Ababa (Asrat personal communication). So far nothing has been done to control CL in this area except for treatment of some cases at AAU medical faculty and ALERT. This region is important from the point of view that dense population living in this area; it is within Addis Ababa, the capital city of Ethiopia. There is the ongoing infrastructure development in this area. So, knowing the epidemiology of cutaneous leishmaniasis in this region is important for initiating appropriate clinical management and treatment of the disease.

Information obtained will provide a base line data on the present situation of leishmaniasis in Addis Ababa and how and where transmission occurs.

1.4 HYPOTHESIS

CL in the surroundings of Bulbula river is endemic with significant morbidity, which can be ascribed to the presence of suitable ecological settings for transmission i.e. sandfly and hyrax habitats in close proximity to human dwellings. Furthermore, ecological and social changes in Saris (surroundings of Bulbula River basin) due to intensive infrastructural development like construction of bridges, road and houses are key determinants.

1.5 Objective

1.5.1 General

- To get pertinent information regarding some aspects of the epidemiology of cutaneous leishmaniasis in the surroundings of Bulbula River (Addis Ababa).

1.5.2 Specific

- To establish the prevalence of cutaneous *leishmaniasis* among people living in the catchment of Bulbula river.
- To determine household and environmental risk factors associated with the transmission of Cutaneous *Leishmaniasis*.
- To recommend further action for cutaneous *leishmaniasis* control on the basis of the data obtained.

2. MATERIALS AND METHODS

2.1 STUDY AREA

This study was carried out in Saris under Nefassilk-Lafto sub city, one of the suburbs of Addis Ababa surrounding Bulbula River where hyrax colonies were found. A preliminary survey was conducted to select representative study sites in different ecological set-ups of Bulbula river, as designated by different infrastructural development like bridges, roads and houses. The criteria for study site selection was ecology suggestive of the transmission of CL (e.g. high altitude, temperature, favorable micro-habitats for the breeding of sandflies, presence of hyrax burrows, etc), settlement around hyrax burrows and new infrastructural developments. Based on this information, pertinent investigation sites (Saris Abo, Yoseph and Worku Sefer) were selected. Generally, the area lies around Bulbula River occupying the south eastern part of Addis Ababa, situated between 9.02'N latitude and 38.42'E longitude. The target sites are located at an altitude range of 2330 to 2600m a.s.l. The sites are found along the Addis- Debrezeit asphalted highway (ring road extends from Bole International Airport to Debrezeit). A map of Saris showing the three study sites appears in Figure 4. The average minimum/maximum temperature of the area is 11/27°C. The area like most of the country has six rainy months with two periods of rainfall; the “big” and “small” rains of June to September and March to April, respectively, with an average annual rainfall of 1079mm. October to January are considered to be the dry months.

The primary vegetation has been removed by intense human activity. Nowadays, evergreen spiny shrubs such as *Carissa edulis*, *Dodinio angostifolia* and *Opuntia-Ficus-Indica*; *Pennisitum* and *Asparagus spp.* interspersed with the non indigenous eucalyptus

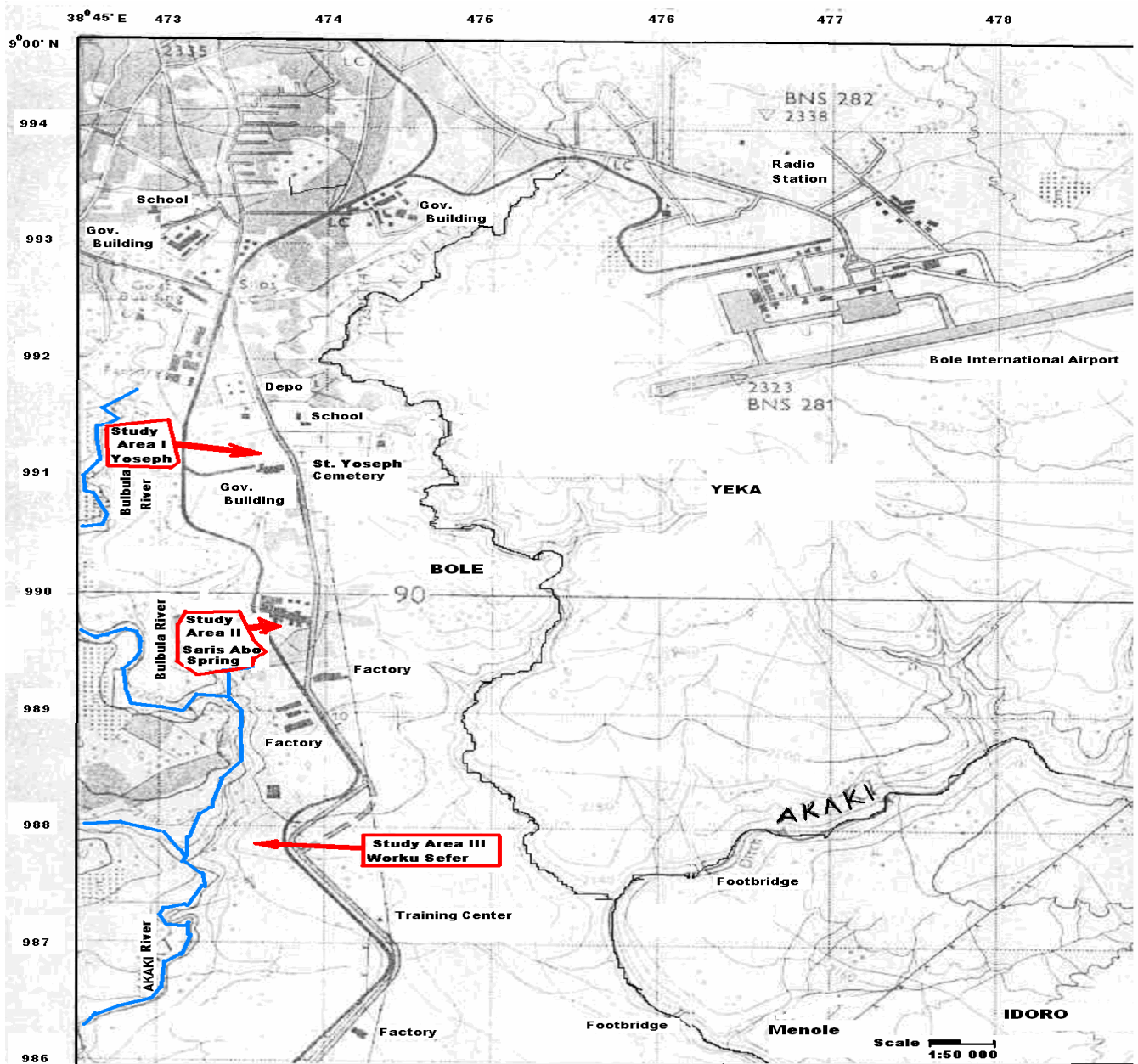


Figure 4. A map of Saris showing the three study sites.

trees predominate much of the hill. Various species of trees are either concentrated within a few meters radius or scantily distributed over several meters, or restricted to the banks of Bulbula River. *Acacia abyssinica*, *Croton macrostachym* and *Ficus sp.*, which probably

constitute some of the primary vegetation, are scantily represented among the riverine vegetation. The area is represented by rock outcrops where hyrax colonies may exist (Figure 5).



Figure 5. Pictures showing hyrax habitats and Vegetation around the study sites.

2.2 STUDY POPULATION

There is a high population density in the three study sites which is estimated to be about 7500 persons. The population came from different part of the country (e.g. Minjar, Becho, Butajira, Gojjam, Wellega and predominately from Tigray and Wello to escape a drought in their home region 10-20 years ago. People usually live in houses constructed of wood and mud (Figure 6). The areas are located in the catchment of Bulbula River. The disease is known among people in these areas as “Shahign” or “Kunchir”. Out of the 7500 people, samples of 1010 were selected for CL prevalence study.

The study population were people living 10-500m from hyrax colonies and consisted of all age groups (4 months to 85 years). Before commencing, preliminary surveys were undertaken at the areas having CL cases and details of the questionnaire and proposed research were discussed. The population was kept informed about all stages of the study and individuals participated only after obtaining their informed consent or, in the case of minors, with the consents of their parents or guardians. The head of each family was interviewed by means of a questionnaire. Two types of questionnaires were utilized (annex 1 and 2). The first form recorded all information about each household (number of inhabitants, their age, sex, occupation, socio-economic status, history suggestive of CL in any member) and data about possible CL risk factors [the type and number of both wild and domestic animals in the proximity of each household, the type of building materials used for roof and walls and the use of insecticide treated nets (ITNs)]. We assessed awareness about leishmaniasis by direct questions. The second form was used to take the individual data including all the suspected cases. It included detailed information about the clinical presentation, demographic data, duration of lesion, and size of lesion and

presence of scar. The houses were visited to collect information on socio-economic conditions and the period of residence of each member of the population. The personal information collected included the following: name, date of birth, sex, profession, time of residence, previous travel history and medical history with respect to lesions and treatment of CL. Clinical suspects of the disease were subjected for further diagnosis. Then, samples were taken from the lesion(s) of patients. Details of all family members, both in families with and with out cases, were recorded on the questionnaire. All patients who agreed to provide samples were those who signed consent forms (annex 3).



Figure 6. Pictures showing the construction of the living houses at the study sites.

2.3 STUDY DESIGN

A cross-sectional study (Kolasczinski *et al.*, 2004) was undertaken to assess the prevalence of CL. Within each study sites, households were sampled along perpendicular transects (i.e. north- south and east- west), with the survey teams starting from the central point and surveying 26 houses along each transects, i.e. a total of 52 households were surveyed in each study sites (i.e. Yoseph, Saris Abo and Worku Sefer) and a total of 156 households for the entire study.

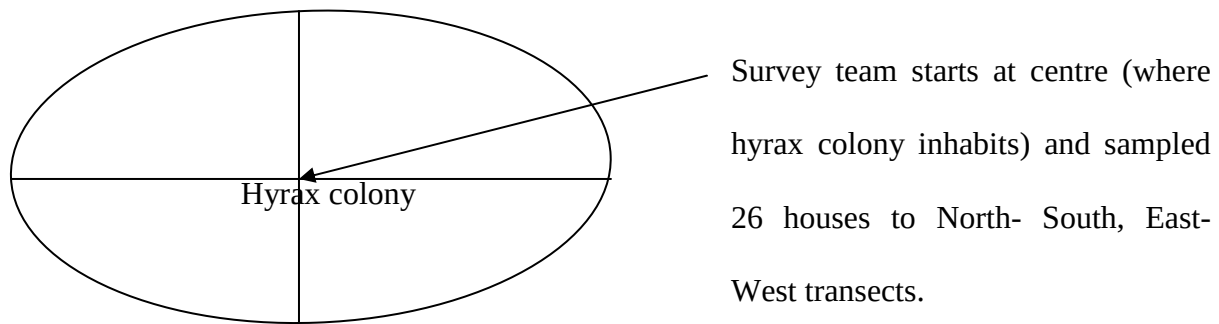


Figure 7 Surveying strategy for covering one study site.

Working along transects, every 5th household on both sides of the path were surveyed until the 65th house was reached and 13 households were surveyed. If the boundary of site was reached beforehand, the survey team turned around and surveyed every 3rd household, walking their way back towards the central point (where hyrax colony was found). If the 5th household was not willing, the immediate neighbor was included.

2.4 DIAGNOSIS

2.4.1 Smear

Smears were prepared by skin scrapping method. A small amount of lesion material was taken from patients for spreading on a glass slides. Smears were allowed to be air-dried, fixed with methanol and stained with a Giemsa solution. Then slides were examined with microscopy.

2.4.2 Culture

Skin snip taken from a patient was inoculated in to a biphasic NNN (Novy-macNeal-Nicolle) medium overlaid with Locke's solution that has previously been autoclaved at 15 lbs and 121°C for 20 minutes. The medium was incubated at room temperature and examined weekly over a five week period.

2.4.3 Leishmanin skin test (LST)

Leishmanin skin testing was carried out by medical laboratory technicians. It was carried out by intradermal inoculation of 0.1ml of a Leishmanin antigen containing 5×10^6 killed promastigotes per ml of 0.5 percent phenol-saline as recommended by Lemma *et al.* (1969). Results were read between 48-72 hours and areas of indurations 5mm in diameter or over were regarded as positive. Leishmanin was obtained from a WHO reference laboratory (ISS, Rome, Italy).

2.5 DOCUMENTATION OF ANIMALS that CO-EXISTED WITH THE RESIDENTS

- Both wild and domestic animals that co-existed with the community in the study sites were documented.

2.6 ETHICAL CONSIDERATIONS

Ethical clearance was obtained from the ethical clearance committee of Department of Biology, Addis Ababa University. Only volunteer patients were included in the study. Diagnosis was done using sterile and disposable materials and all activities in clinical examination as well as diagnosis were supervised by health personnel. Individuals found positive were treated with sodium stibogluconate 20mg/kg/day for 30 days free of charge.

2.7 STATSTICAL DATA ANALYSIS

The sample size to establish the prevalence of CL in Saris was 1010. Analysis was carried out using SPSS Version 13 Software and EPINFO. The data were analyzed using simple frequency distribution, Chi-square tests, Univariate multiple comparison and pair wise comparison tests. The differences were considered statistically significant when P-value less than 0.05. The factors tested were gender, age group and locality.

3. RESULTS

3.1 HUMAN CL IN SARIS, ADDIS ABABA

Demographics were obtained from the population of those who reside in the suburb of Addis Ababa surrounding Bulbula River in Saris from April to June, 2005 (Table 1).

A total of 1010 human persons who lived in the 156 households of the three study sites were recorded for the study. The mean age was 23.3 years. The minimum age was 4 months and the maximum was 85 years. Household size was large; 76.28% of the study households had five or above family members. The remaining 23.72% had four or below family members (Figure 8). The median size is 5 and the mode is 6. Most members of the population (71.78%) were less than 30 years old (Table 1). New houses were distributed along the sides of the river, the majority of them being constructed of mud. The socio-economic survey revealed that the majority of the population lived under precarious condition. The heads of the families were predominantly daily laborers who received meagre salary.

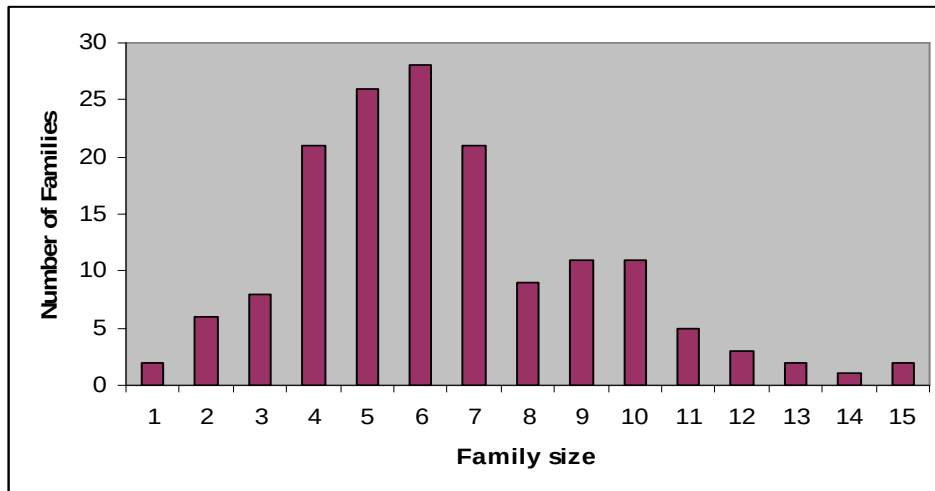


Figure 8. Household size of the study population.

The sex structure of the study population shows an almost one to one ratio (1.1:1). These were 534 Females (52.87%) and 476 males (47.13% (Tables 1or 2).

Table 1. Sex and Age distribution of the study population in each site.

Age group/years	Study sites									Total
	Yoseph			Saris Abo			Worku Sefer			
	F	M	Total	F	M	Total	F	M	Total	
0-9	33	27	60	17	25	42	29	25	54	156
10-19	61	45	106	55	53	108	58	60	118	332
20-29	50	29	79	40	28	68	54	36	90	237
30-39	22	23	45	14	16	30	22	18	40	115
>40	25	34	59	27	29	56	27	28	55	170
Total	191	158	349	153	151	304	190	167	357	1010

3.2 Present cases of cutaneous leishmaniasis

Persons with and with out CL infection, in all three study sites, are shown in Tables 2. Seventeen relatively recent cases were found in the three study sites. Out of the seventeen CL infected individuals, five were females (29.41%) and 12 were males (70.59)(Tables 2 or 3). Parasitological examinations (smear and culture) were made on 10 active cases. 10 of them were both smear and culture positive. Two were skin test positive and the remaining five cases were clinically diagnosed.

Table 2. Prevalence of CL along the Bulbula river basin by age and sex.

Infection	Age groups/years										Total
	0-9		10-19		20-29		30-39		>40		
	M	F	M	F	M	F	M	F	M	F	
With CL	4	0	6	4	2	1	0	0	0	0	17
With out CL	73	79	152	170	91	143	57	58	91	79	993
Total	77	79	158	174	93	144	57	58	91	79	1010

3.3 Distribution of lesions of CL on the body

The distribution of lesions of cutaneous leishmaniasis on the body, the duration and stage of development is shown (Table 3). Approximately, 88.2% (15/17) of the lesions were on the head and 11.8% (2/17%) were on the forearms.

Table 3. The distribution of lesions of cutaneous leishmaniasis on the body, their duration and stage of development.

Age/year	Sex	Sites	Duration of lesion (months)	Clinical features
4	M	Nose	36	Ulcerative
11	M	Nose	2	Ulcerative
10	F	Lips/nose	36	Healing
19	M	Cheek	2	Ulcerative
16	M	Nose	24	Healing
19	M	Eyelid	1	Healing
19	M	Forearm	2	Ulcerative
12	F	Forearm	1	Healing
27	M	Forehead	1	Healing
17	M	Cheek	48	Healing
28	M	Lips/nose	12	Healing
21	F	Nose	12	Tiny ulcer
6	M	Lips	6	Healing
18	F	Lips	0.5	Healing
15	F	Nose	4	Ulcerative
5	M	Nose	1	Ulcerative
5	M	Cheek	1	Ulcerative

3.4 *Leishmanin Skin Test Results*

Out of the 93 skin tested individuals only 3 were LST positive. Out of the three LST positives, two were diagnosed as CL cases.

LST Positives	Age/years	Duration of stay/ years	Clinical status	CL case in their family	Locality
1	27	20	healing	Two	Saris Abo
2	5	5	ulcerative	-	Yoseph
3	40	11	asymptomatic	Two	Saris Abo

N.B.: induration size was 5 mm in diameter.

3.5 *RELATIONSHIP OF CL TO AGE AND SEX*

Active cases were found in the age groups 0-9, 10-19 and 20-29 (Tables 2 or 3). With regard to the age group evaluation, there was a significant difference between age groups 10-19 and 20-29 ($P= 0.03$), 30-39 ($P= 0.005$). As indicated in Table 2, no individual was infected by the disease after age 29. This study indicated that the younger age groups were the most affected, especially age group 10-19.

3.6 *RELATIONSHIP OF CL TO THE STUDY SITES*

Saris Abo study site had a significant higher infection as compared to the two Yoseph and Worku Sefer ($X^2= 10.16$, $DF=2$, $P= 0.006$). Among the study population at the three study sites only 17/1010 (1.7%) persons were found infected by the disease (CL) (Tables 2 and 4).

Table 4. Prevalence of cutaneous leishmaniasis in the three study sites.

Study population	Study sites			Total
	Yoseph	Saris Abo	Worku	
With out CL	347	293	353	993
With CL	2 (0.6%)	11 (3.6%)	4 (1.1%)	17 (1.7%)
Total	349	304	357	1010

There were significant mean infection differences between Yoseph and Saris Abo ($P=0.002$) and Sari Abo and Worku Sefer ($P=0.012$). Prevalence of CL between study sites showed marked differences. An increase in CL prevalence was observed on human population living in Saris Abo which has a high proximity to hyrax habitat.

3.7 Animals Living Within the Households

Out of the 156 studied households, 5.77% kept cats only, 14.1% kept dog and cat, 8.33% kept dog and cow and 14.74% kept only dogs.

4. DISCUSSION

We have investigated the epidemiology of CL in Saris along the Bulbula river basin. Though no parasite species identification was carried out in the present study; the causative parasite and the reservoir hosts have been identified by Lemma (2005, unpublished data) as *Leishmania aethiopica* and *Heterohyrax brucei*, respectively. The sandfly vector, *Phlebotomus longipes*, was found during an entomological survey of Saris in 2005 (Lemma, unpublished data) and this species is widely distributed in many CL endemic areas in Ethiopia and had been found infected with *Leishmania* (Lemma *et al.*, 1969). The present study shows significant cases of CL among the settlements along the Bulbula river basin.

Though LST was not undertaken on the whole study populations, the skin test profile shown in suggests that there is a very low rate of skin test positivity in all the age groups. This indicates that the population have either a very low rate of exposure to the circulating leishmanial parasites (Ali *et al.*, 2002) and/or the host characteristics (immune status, genetic factors and etc) inhibits the sensitivity and potency of a given LST antigen (Torelba, 1997). This requires further investigation.

Duration of lesion is known to vary (Lemma *et al.*, 1969; Ashford, 1977). According to Lemma *et al.* (1969), the duration of lesions was about 12 months. Ashford (1977) observed lesions of two to four years duration. History of active cases in the present study is two weeks to four years duration of lesions (Table 3). It is known that sites of lesions represent area of inoculation of leishmanial parasites by the sandflies (Lewis, 1987; Bray,

1983). It was observed that sandfly prefer to bite on the more exposed parts of the face especially the nose (88.2%) (Table 3). Influencing factors of this preference is not yet well established. It is assumed that CO₂ expired from the nose and mouth might contribute tremendously (Bray, 1983).

The present study revealed that cutaneous leishmaniasis is prevalent along the Bulbula river basin. Of the surveyed population (1010), an overall 1.7% was found to be infected with CL. The prevalence of CL was significantly higher among those living in Saris Abo (3.6%) than Yoseph (0.6%) and Worku Sefer (1.1%). This study reflected spatially varied disease ecology. The Saris Abo ecological situation seemed to be important for the potential spread of CL. In Ethiopia, infection rates varied significantly from place to place depending primarily on the proximity of hyrax colonies and vector habitats to human dwellings, human activities (such as land use, firewood collection, herding domestic animals) and environmental factors, including vegetation type, topography, wind direction and intensity (Ashford *et al.*, 1973; Bray, 1983; WHO, 1990). Saris Abo is located along the Bulbula river basin with different vegetation types, topography and newly developed area, with new social settings, changing the environmental conditions, may be favoring the presence of *Heterohyrax brucei* colonies, the main animal reservoir hosts, and allowing for the vector sandflies (*Phlebotomus longipes*) to inhabit the peridomestic area.

Considering the age group, age groups 10-19 and below were found to be the most infected by CL (Table 2), implying, younger individuals are at risk. The greatest rates of

infection were in those 10-19 years of age (10/17 cases) followed by 0-9 (4/17 cases) and 20-29 years of age (3/17 cases).

Generally, the low infection rates suggest that the parasite might have been seen somewhat recently in the study sites with new settlement forming in peri-urban settings and creating new human foci of the disease. Also, it is to be expected that increased prevalence of the disease (CL) in Saris Abo could be due to the suitable ecological habitat of the vector (*Phlebotomus longipes*) and hyrax colonies (*Heterohyrax brucei*) which serve as reservoir hosts. As observed in the current investigation, more CL cases were recorded in Saris Abo where 3.6% of the human subjects were infected. Moreover, 0.6% and 1.1% of the human subjects were infected in Yoseph and Worku Safer, respectively. The difference in infection rates in the three study sites might be due to the difference in rate of exposure to sandflies and the proximity of hyrax foci to human dwellings.

The Pearson chi-square for the localities ($X^2 = 10.16$, $DF = 2$, $P = 0.006$) clearly revealed that there was significant difference between the three sites with likelihood ratio 9.44, $DF = 2$, $P = 0.009$). The relative infection rates in the three sites were 2/17 (11.8%) for Yoseph, 11/17 (64.7%) for Saris Abo and 4/17 (23.5%) for Worku Sefer whereas percent infection in each site was 0.6% for Yosef, 3.6% for Saris Abo and 1.1% for Worku Sefer. Saris Abo being the most affected site by CL.

Our chi-square tests indicated that risk of active cases was significantly associated with site, age and gender ($P = 0.02$). The results also indicated a significant clustering of cases in Saris Abo where humans settled in close proximity to the hyrax colonies. Clustering of

CL cases is not uncommon, as also observed in Afghan by Kolaczinnsiky *et al.* (2004) where humans were the reservoir.

During our preliminary survey, one individual who has come from CL endemic area was encountered with CL infection. Though this seemed the disease might have been introduced from CL endemic area, our evidence points to local transmission as being the main cause of CL among the settlers. This idea is also supported by the works of Lemma (2005) who has isolated *Leishmania aethiopica* from *Hetrohyrax bruce*. Moreover, all of the seventeen individuals with CL infection were born in these site support the above opinion. None of them reported traveling to other areas within the period that transmission of the disease would have been possible. If the disease has recently been introduced to this area, one would expect the risk of CL to be unrelated to age, since every one would be non-immune to infection. By contrast, in endemic conditions, immunity will develop in most individual and only new comers by birth or immigration will be susceptible. Most cases would then be observed among the very young or immigrants (Ashford *et al.*, 1992). So, CL in Saris seems to be a hypoendemic condition since the age groups 10-19 and below were the most infected.

The results indicated a significant clustering within the age groups 10-19, the site Saris Abo, human dwellings in high proximity to hyrax colonies and relative high focal transmission. This conclusion is supported by the significant clustering of infection found in Kabul (Reithinger *et al.*, 2003) and the small scale (300m) spatial clustering in areas endemic for VL in Teresenia, Brazil (Werneck *et al.*, 2003). Clustering within the age

groups 10-19 may arise due to a variety of factors including heterogeneity to exposure (due to entomological, environmental or behavioral factors) and/or differences in susceptibility to infection (due to genetic or immunological factors). Werneck *et al.* (2003) suggested that transmission in a Brazilian population was limited to areas corresponding to the short flight range of sandflies and the reservoir distributions. Further investigation is needed to examine the relative contributions of entomological, environmental, immunological, and genetic factors for susceptibility of infection.

The CL infection rate varied in different age and sex groups, and particularly high in the young (10-19) and below as compared to the early age groups. By pair wise comparison, males were found to be significantly infected in the current investigation ($P= 0.023$). From the multiple comparison analysis in this study, the relative influence of the different covariates on the probability of developing CL was evaluated. Age, the presence of hyrax colonies, and male sex were associated with the risk of disease in the study area, particularly in Saris Abo area. The data indicate that CL could be transmitted within the locality due to the following situations:

- I. Hyrax colonies harboring the sandflies
- II. Age, teenagers due to higher level of exposure to the parasite occurring near working fields or places surrounding the Bulbula river basin where hyrax colonies exist on the bank of the river basin and some vegetation types growing in the study locality. Similar points were reported by Bucheton *et al.* (2002) in their study of VL in eastern Sudan.

Regarding sex, there has been a report that male hamsters had significantly more severe *Leishmania (Viannia) spp.* infection (Travi *et al.*, 2002) than female hamsters when lesion size, lesion severity (degree of tissue necrosis), parasite burden in the draining lymph node, and rate of parasite dissemination were evaluated. Likewise, though unclear in this study, CL infection in male individuals could be high as compared to the female, due to the sex related differences. They further emphasized the notion that the gender differences in disease evolution were the result of the sex hormone milieu of the animal. Firstly, prepubertal male animals, which would have significantly lower androgen levels than adult males, had smaller and/or less severe lesions than the adults until late in the course of infection, when the androgen levels would be equivalent. Secondly, administration of testosterone to female hamsters resulted in a dramatic increase in lesion size. These investigations underline an inherent increase in disease susceptibility in male hamsters and suggest that an androgen-related permissive immune response may contribute to the increase in disease prevalence among men in endemic areas.

Even though geographical systems and remotely sensed satellite data were not used, we investigated the spatial distribution of CL and identified defined risk areas of transmission by locating hyrax burrows. In common with many vector-borne diseases, the distribution of CL is influenced by environmental factors, which affect the distribution of vectors and the reservoir host. Specifically, sandflies reproduce optimally between 23-28°C and a relative humidity of 70-100%, whereas temperatures below 10°C and above 40°C are generally considered unfavorable for their survival (Kellick-Kendrick, 1999). Such specific environmental conditions required for vector propagation

is obtained in this area and makes Saris (along the Bulbula River) an ideal place for CL transmission. Study in the Sudan showed that occurrence of VL and its vector *Phlebotomus orientalis* were associated with temperature, rainfall and soil type (Elnaiem *et al.*, 2003). Taking in to consideration the weather and geographical data obtained from National Meteorological Service in Addis Ababa for 2005, for the study sites, that is a temperature of 11/27°C and altitude of 2330-2600m a.s.l, and the remoteness from hyrax colony restricted the occurrence of CL. Moreover, it appears reasonable to assume that an animal reservoir must be an important factor in the distribution of the disease. This finding is in agreement with that of Wilkins (1971).

Protective efficacy of insecticide treated nets for prevention of CL could not be discussed in the present study since none of the study population used ITNs. However, study conducted in Kabul by Reyburn *et al.* (2000) indicated that ITNs provide significant protection against CL in Afghan refugee camps. Regarding the association of keeping cows or dogs within the compound and the presence of CL infection, Bucheton *et al.* (2002) observed a significant association between VL infection and keeping cows within a compound as cows probably have a positive effect on the density of sandflies around houses, cow dung provides a rich environment for the development of early stages of *Phlebotomus* and also has a bait factor, attracting flies (zoophilic behavior of sandflies). In this study, there was no significant association observed except obtaining CL cases with three households who keep cows within their compound. This requires further investigation.

5. CONCLUSION AND RECOMMENDATIONS

CL is one of the threatening health problems in most parts of Ethiopia in general and around Bulbula River in particular. A current investigation showed that the disease has been expanding from time to time with the relative contributions of entomological, environmental, immunological and genetic factors in susceptibility to infection. The high risk groups were those among the age groups 10-19 and below, males and individuals settled high proximity to hyrax colony. Its control measures remain a big challenge in the country with different geographical positions- highlands and lowlands areas where CL is an endemic disease. Therefore, there is an urgent need for a more effective sandfly control through use of ITN or biological means directed against hyrax colonies. Generally, the present investigation highlights the importance of CL among the community living around Bulbula river basin and forms the basis for targeting control efforts.

In the present study the relative contribution of age, sex, vectors, reservoir host, and environmental risk factors are not clearly elucidated. Therefore, there is a need for more population-based studies that carefully figure out the CL status of the population, identification of the types of *Leishmania* parasites and investigation of the all possible contributions of other risk factors (e.g. vectors, reservoir, etc).

Understanding the ecological structure of a zoonotic disease is a crucial prerequisite for applying efficacious control measures. The biology of reservoir hosts and vectors as well as their respective spatial distribution with the underlying ecological determinants are key epidemiological parameters to be brought in to consideration when planning preventive

measures. Ecological understanding of the spatial distribution of the pathogen within the reservoir host populations could help to resolve epidemiological morbidity patterns. The settlers, in the study sites, got infected in high proximity to hyrax colony. This study identified both hyrax and sandfly habitats as an important risk factors for contracting CL. Taking these basic facts into consideration when planning settlements in CL endemic regions may help to reduce subsequent CL morbidity.

Among the various types of leishmaniasis, CL is undoubtedly the least understood and, until recently, the least studied of the major parasitic diseases of man. Although it is known that CL is a serious and growing problem in many parts of the world, accurate data concerning the extent of the problem has not been available, and basic epidemiological information, such as the identity of the *Leishmania* parasite, the vector, non-human reservoirs and conditions for transmission is still unknown in many foci. In the past few years, due to its increasing public health importance, there has been a marked increase in investigation of the epidemiology of CL, which has resulted in significant advances in knowledge. Nowadays, CL cases were reported from all suburbs of Addis Ababa; therefore, an intensive work must be done in Addis Ababa as a whole to clearly elucidate the distribution of CL and a delineation of the boundaries of the major foci, the essential characteristics of each major focus, including a etiological agents, known or suspected reservoir hosts and known or suspected phlebotomine vectors.

6. REFERENCES:

- Abdallah, R. E. 1980. Serodiagnosis of visceral leishmaniasis in an endemic area of the Sudan. *Ann. Trop. Med. Parasitol.* **74**: 415-419.
- Addy, M.& Nandy, A. 1992. Ten years of kala-azar in West Bengal, part 1. Did post kala-azar dermal leishmaniasis initiate the outbreak in 24-Parganas? *Bull. WHO* **70** 431-346.
- Ali, A., Berhe, N., Mengistu, G. and Gebre-Michael, T. 2002. Leishmaniasis survey in the Awash Valley: The magnitude of positive leishmanin reaction and its pattern in the middle Awash. *Ethiop. J. Health Dev.* **16** (2): 157-163.
- Aliaga, L., Cobo, F., Mediavilla, J. D., Bravo, J., Osuna, A.J, Amador, .M., Martin-Sanchez, J., Cordero, E. and Navarro, J. M. 2003. Localized mucosal leishmaniasis due to *Leishmania (Leishmania) infantum*: clinical and microbiologic findings in 31 patients. *Medicine (Baltimore)* **82**:147-158.
- Al-tawfik, J.A. and Abukhamsin, A. 2004. Cutaneous leishmaniasis: a 46-year study of the epidemiology and clinical features in Saudi Arabia (1956-2002). *Interna. J. Infect. Dis.* **8**: 244-250.
- Ashford R.W., Bray, M. A., Hutchnson, M., Bray, R.S. 1973. The epidemiology of cutaneous leishmaniasis in Ethiopia. *Trans R. Soc. Trop. Med. Hyg.* **67**: 568-601.
- Ashford, R.W. 1977. The comparative ecology of *Leishmania aethiopica*. Colloques internatiounex du CNRS., No.239- Ecologie des leishmanias. **pp.** 233-240.
- Ashford, R.W; Desjeux, P. & De Raadt, P. 1992. Estimation of population at risk of infection and number of cases of leishmaniasis. *Parasitol. Today* **8**: 104-105.
- Ashford, R.W. and Smith, D.H. 1985. *Leishmaniasis in Sudan, Ethiopia and Kenya*. In: Leishmaniasis. (Eds. Chang and Bray). Elsevier Publishers., London. **pp.** 377-391.
- Ashford, R.W. 1996. Leishmaniasis Reservoirs and their significance in control. *Clin. Dermatol.* **14**: 423-532.
- Ayele, T., Habte Gaber, E. and Belhu, A. 1981. *Leishmaniasis in Ethiopia: A handbook*. Institute of Pathobiology, Addis Ababa University, pp. 86.

- Badaro, R, Reed, S. G. & Carvalho, E. M. 1983. Immunofluorescent antibody test in American visceral leishmaniasis: sensitivity and specificity of different morphological forms of two *Leishmania* species. *Am. J. Trop. Med. Hyg.* **32**(3):480-484.
- Baily, G. G. & Nandy, A. 1994. Visceral leishmaniasis: more prevalent and more problematic. *J. Infect. Dis.* **29**: 241-247.
- Balkew, M., Hailu, A., Berhe, N. and Gemetchu, T. 1999. Leishmaniasis in the lower Omo plains, southwestern Ethiopia; the sandfly fauna. *Ethiop. Med. J.* **37**: 31-40.
- Balzer, R.J., Destombes, P., Schaller, K.F. and Serie, C. 1960. Leishmaniose Cutanee pseudolepromateuse en ethiopie. *Bull. Soc. Path. Exot.* **53**: 293-298.
- Belehu, A. and Humber, D.P. 1981. *Cutaneous leishmaniasis- Immunological features*. **In**: Leishmaniasis in Ethiopia. (Eds. Ayele, T., Habte-Gabriel, E. and Belehu, A.), Institute of Pathobiology, pp. 50- 54.
- Belehu, A. 1981. *Cutaneous leishmaniasis- Clinical features*. **In**: Leishmaniasis in Ethiopia. (Eds. Ayele, T., Habte-Gabr, E. and Belhu, A.), Institute of Pathobiology, pp. 45-49.
- Belhadj, S., Pratlong, F., Hammami, M., Kallel, K., Dedet, J. P. and Chaker, E. 2003. Human cutaneous leishmaniasis due to *Leishmania infantum* in the Sidi Bourouis focus (Northern Tunisia): epidemiological study and isoenzymatic characterization of the parasites. *Acta Trop.* **85**: 83-86.
- Berman, J. D. 1997. Human leishmaniasis: clinical, diagnostic, and chemotherapeutic development in the last 10 years. *Clin. Infect. Dis.* **24**(4): 684-703.
- Bogdan, C., Röhlhoff, M., Solbach, W. 1990. Evasion strategies of *Leishmania* parasites. *Parasitol Today* **6**: 183-187.
- Bray, R.S. 1983. The zoonotic potential of reservoirs of leishmaniasis in the old world. *Ecology of Diseases* **1**: 257-267.
- Bray, R.S., Ashford, R.W. and Bray, M.A. 1973. The parasite causing cutaneous leishmaniasis in Ethiopia. *Trans. R. Soc. Trop. Med. Hyg.* **67**: 345-348.
- Bryceson, A.D.M. 1970. Diffuse cutaneous leishmaniasis in Ethiopia. III. Immunological studies. *Trans. R. Soc. Trop. Med. Hyg.* **64**: 380-393.
- Bryceson, A.D.M and Hay, R.J. 1998. *Parasitic worms and Protozoa*. **In**: Rook/Wilkinson/Ebling Text book of dermatology. 6th edition. **pp.** 104-127.

- Bryceson, A. and Leithead, C.S. 1966. Diffuse cutaneous leishmaniasis in Ethiopia. *Ethiop. Med. J.* 5: 31-36.
- Bryceson, A. Nichol, T.W. 1966. Cutaneous leishmaniasis in Wollega province. *Ethiop. Med. J.* 5: 35-42.
- Bucheton, B., Kheir, M.M., El-Safi, S.H., Hammed, A., Mergani, A., Mary, C., Abel, L. and Dessien, A. 2002. The interplay between environmental and host factors during an outbreak of visceral leishmaniasis in eastern Sudan. *Micro. Infec.* 4: 1449-1457.
- Chang, K. P., Fong, D. & Bray, R. S. 1985. *Biology of Leishmania and leishmaniasis*. In: Leishmaniasis. (Eds. Chang, K.P. & Bray, R. S.). Vol.1. Elsev. Biomed. Press, Amsterdam, pp. 1-30.
- Chulay, J.D. and Bryceson, A. D. M. 1983. Quantitation of amastigotes of *Leishmania donovani* in smears of splenic aspirates from patients with visceral leishmaniasis. *Am. J. Trop. Med. Hyg.* 32: 475-479.
- Corbet, G.B. 1979. The taxonomy of *Procyon capensis* in Ethiopia. *Bull. Br. Mus. Nat. Hist.* 36: 251-259.
- Cruz, I., Morales, M.A., Noguer, I., Rodriguez, A. and Alvar, J. 2002. *Leishmania* in discarded syringes from intravenous drug users. *Lancet* 359 : 1124-1125.
- Cuba, C.C., Llanos-Cuentas, E.A., Barreto, A.C. 1984. Human mucocutaneous leishmaniasis in Tres Bracos Bahia, Brazil. An area of *Leishmania brasiliensis brasiliensis* transmission. I. Laboratory diagnosis. *Rev. Soc. Bras. Med. Trop.* 17: 161-167.
- Del Mar Sanz, M., Rubio, R., Casillas, A., Guijarro, C., Costa, JR., Martinez, R., De Dios, Garcia, J. 1991. Visceral leishmaniasis in HIV-infected patients. *AIDS* 5: 1275-1273.
- Desjeux, P. 2001. The increase in risk factors for leishmaniasis worldwide. *Trans. R. Soc. Trop. Med. and Hyg.* 54: 239- 243.
- Desjeux, P. 1996. Leishmaniasis: public health aspects and control. *Clin. Dermatol.* 14: 417-423.
- Dye, C. 1992. Leishmaniasis epidemiology: the theory catches up. *Parasitol. Today* 104: S7-S18.
- El Amin, E.R., Wright, E.P., Abdel Rahman, A.M., Kolk, A., Laarman, J.J., Pondman, K.W. 1986. Serodiagnosis of Sudanese visceral and mucosal leishmaniasis:

- comparison of ELISA-immunofluorescence and indirect haemagglutination. *Trans. R. Soc. Trop. Med. Hyg.* **80**: 271-274.
- Elbihari, S. & El-Hassan, A. M. 1987. The histopathology of naturally-occurring cutaneous leishmaniasis in the reservoir host *Psammomys obesus*. *Bull. Soc. Pathol. Exot. Filiales* **80** (4): 615-623.
- El-Hassan, A.M.; Ghalib, W.H.; Zijlstra, E.E.; Eltoum, I. A., Satti, M.; Ali, M. S. & Ali, H. M. A. 1992. Post kala-azar dermal leishmaniasis in the Sudan: clinical features, pathology and treatment. *Trans. R. Soc. Trop. Med. Hyg.* **86**: 245-248.
- El-Hassan, A. M.; Zijlstra, E. E.; Ismael, A. & Ghalib, W.H. 1995. Recent observations on the epidemiology of kala-azar in the eastern and central states of the Sudan. *Trop. Geograph. Med.* **47**:151-156.
- El-Hassan, A. M. and Zijlstra, E.E. 2001. Leishmaniasis in Sudan. 1. Cutaneous leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.* **95**: S1/1-S1/17.
- Elnaiem, D.E., Schorscher, J., Bendall, A., Obsomer, V., Osman, M.E., Mekkawi, A.M., Connor, S.J., Ashford, R.W. and Thomson, M.C. 2003. Risk mapping of visceral leishmaniasis: the role of local variation in rainfall and altitude on the presence and incidence of Kala-azar in eastern Sudan. *Am. J. Trop. Med. Hyg.* **68**: 10-17.
- El –Sibae, M. M.; Esa, N. M. & Morsy, T. A. 1993. Rodents and cutaneous leishmaniasis in Qasim, Saudi Arabia. *J. Egypt Soc. Parasitol.* **23** (3):667-673.
- Evans, T. G. & Pearson, R. D. 1988. Identification of leishmanial antigens in the sera of patients with American visceral leishmaniasis. *Infect. Immune.* **56**(12): 3139-3144.
- Foster, W.A., Boreham, P.F.L. and Tempelis, C.H. 1972. Studies on leishmaniasis in Ethiopia. IV- feeding behavior of *Phlebotomus longipes* (Diptera: Psychadidae). *Ann. Trop. Med. Parasitol.* **66**: 433-443.
- Garnham, P.C. 1987. *Introduction*. In: The leishmaniasis in Biology and medicine. (Eds. Peters, W. & Killick-Kendrick, R. vol 1. Orlando: Academic Press, xii-xxv.
- Gebre-Michael, T. and Lane, R.P. 1996. The role of *Phlebotomus martini* and *P. celiæ* (Diptera: Phlebotominae) as a vector of leishmaniasis in the Aba Roba focus, southern Ethiopia. *Med. Vet. Entomol.* **10**: 53-62.

- Gebre-Michael, T., Pratlong, F. and Lane, R.P. 1993. *Phlebotomus (Phlebotomus) dumboscqi* (Diptera: Phlebotominae), naturally infected with *Leishmania major* in southern Ethiopia. *Trans. R. Soc. Trop. Hyg.* **87**: 10-11.
- Gemetchu, T. 1977. Laboratory culture and biology of *Phlebotomus pedifer* (Diptera: Phlebotomidae). *Ethiop. Med. J.* **15**: 1-4.
- Genene, M., Akuffo-Adu, H. and Fehniger, T. 1986. Parasites derived from patients with DCL express different antigens from those in parasites derived from patients with localized cutaneous leishmaniasis. *Ethiop. Med. J.* **24**: 19-23.
- Hailu, A. 2002. The use of direct agglutination test (DAT) in serological diagnosis of Ethiopian cutaneous leishmaniasis. *Diagnostic Microbiology and Infectious Diseases* **42**: 251-256.
- Hailu, A. and Frommel, D. 1993. *Leishmaniasis in Ethiopia*. In: The Ecology of Health and Disease in Ethiopia (Eds., Kloos, H. and Zein, Z.A.). West view press, Boulder, Colorado, USA, **pp.** 275-388.
- Hailu, A., Balkew, M., Berhe, N., Meridith, S.E. and Gemetchu, T. 1995. Is *Phlebotomus (Larroussius) orientalis* a vector of visceral leishmaniasis in southwest Ethiopia? *Acta Trop.* **60**: 15-20.
- Harith, A. E., Kolk, A. H., Kager, P.A., Leeuwenburg, J., Muigai, R., Kiugn, S. & Laarman, J.J. 1986. A simple and economical direct agglutination test for serodiagnosis and seroepidemiological studies of visceral leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.* **80**: 583-586.
- Harith, A.E., Kolk, A.H.J., Leeuwenburg, J., Muigai, R., Huigen, E., Jelsma, T. & Kager, P.A. 1988. Improvement of a direct agglutination test for field studies of visceral leishmaniasis. *J. Clin. Microbiol.* **26**: 1321-1325.
- Hepburn, N.C. 2003. Cutaneous leishmaniasis: an overview. *J. Postgrad. Med* **49**: 50-54.
- Herwaldt, B.L. 1999. Leishmaniasis. *Lancet* **354**: 1191-1199.
- Herwaldt, B.L. and Berman, J.D. 1992. Recommendations for treating leishmaniasis with sodium stibogluconate (Pentostam) and review of pertinent clinical studies. *Am. J. Trop. Med. Hyg.* **46**: 296-306.
- Hommel, M. 1999. Visceral leishmaniasis: biology of the parasite. *J. Infect.* **39**: 101-111.

- Hommel, M.; Attar, Z.; Fargeas, C.; Dourado, C.; Monsigny, M.; Mayer, R. and Chance, M. L. 1997. The direct agglutination test: a non-specific test specific for the diagnosis of visceral leishmaniasis. *Ann. Trop. Med. Parasitol.* **91**(7):795-802.
- Hommel, M., Peters, W., Ranque, J., Quilici, M. and Lanotte, G. 1978. The micro-ELISA technique in the serodiagnosis of visceral leishmaniasis. *Ann. Trop. Med. Parasitol.* **72**(3):213-218.
- Humber, D.P., Yemene-Berhane, T. and Teklemariam, S. 1986. *Leishmaniasis in Ethiopia*. In: Ecology of Health and Disease in Ethiopia. (Eds. Ahmed, A. and Kloos, H.), Institute of Pathobiology, 12pp.
- Jaffe, C.L. and McMahon-Pratt, D. 1983. Monoclonal antibodies specific for *Leishmania tropica* 1. Characterization of antigens associated with stage and species-specific determinants. *J. Immunol.* **131**(4): 1987- 1993.
- Kar, K.1995. Serodiagnosis of Leishmaniasis. *Curr. Rev. Microbiol.*, **21**:123-152.
- Kettle, D .S. 1995. *Medical Veterinary Entomology*, 2nd edition. CAB International, 33pp.
- Killick-Kendrick, R. and Rioux, J. A. 2002. Mark-release-recapture of sand flies fed on leishmanial dogs: the natural life-cycle of *Leishmania infantum* in *Phlebotomus ariasi*. *Parassitologia* **44**:67-71.
- Kingdon, J. 1971. *East African mammals* (an atlas of evolution in Africa). Vol. 1, Academic press, Baltimore, **pp.** 1326-1331.
- Klaus, S.N., Frankenburg, S. and Ingber, A. 1999. Epidemiology of Cutaneous Leishmaniasis. *Clin. Dermatol.* **17**: 257-260.
- Kolasczinski, J., Brooker, S., Reyburn, H. and Rowland, M. 2004. Epidemiology of anthroponotic cutaneous leishmaniasis in Afghan refugee camps in northwest Pakistan. *Trans. R. Soc .Trop. Med. Hyg.* **98**: 373.378.
- Kubba, R. and Al-Gindan, Y. 1989. Leishmaniasis. *Dermatol. Clin.* **7**: 331- 351.
- Lainson, R. & Shaw, J. J .1987. *Evolution, classification and geographical distribution*. In: The Leishmaniasis in Biology and Medicine. London. (Eds. Peters, W. & Killick-Kendrick, R.). Academic press, **pp.**1-120.
- Lane, R. P. 1993. *Sandflies (Phlebotominae)*. In: medical Insects and Arachnids. (Eds. Lane, R. P. and Crosskey, R. W.). Chapman & hall, London, **pp.** 78-109.

- Leeuwenburg, J., Bryceson, A. D. M., Mbugua, G.G. and Siongok, T. K. 1983. The use of leishmanin skin test to define transmission of leishmaniasis in Baringo District, Kenya. *East. Afr. Med. J.* **60**: 81-84.
- Lemma, A., Foster, W.A., Gemechu, T., Preston, P.M., Bryceson, A. and Minter, D.M. 1969. Studies on leishmaniasis in Ethiopia: I Preliminary studies on cutaneous leishmaniasis in the highlands. *Ann. Trop. Med. Parasitol.* **63**: 455- 462.
- Lemma, W. 2006. Investigations on the parasite, vector and reservoir host of cutaneous leishmaniasis in Addis Ababa, Ethiopia. *MSc thesis*, AAU.
- León, A. M., Shaw, J. J. & Tapia, F. J. 1996. *A guide for the cutaneous leishmaniasis connoisseur*. In: Molecular and Immune Mechanisms in the Pathogenesis of Cutaneous leishmaniasis. (Eds. Tapia, F.J.; Ditar, G. C. & Sánchez, M. A.). Landes comp., pp.1-23.
- Lewis, D. J. and Ward, R. D. 1987. *Transmission and vectors*. In: Leishmaniasis in Biology and Medicine. (Peters, W. & Killick-Kendrick, R.). vol.1, Orlando: Academic Press, pp 235-262.
- Mauricio, I. L., Stothard, J. R. & Miles, M. A. 2000. The strange case of Leishmania chagasi. *Parasitol.Today* **16** (5): 188-189.
- Meredith, S.E.O., Kroon, N.C., Sondorp, E., Seaman, J., Goris, M.G., Van Ingen, C.W., Oosting, H., Schoone, G.J., Terpstra, W.J., Oskam, L. 1995. Leish-KIT, a stable direct agglutination test based on freeze-dried antigen for serodiagnosis of visceral leishmaniasis. *J. Clin. Microbiol.* **33**: 1742-1745.
- Molyneux D.H. & Killick-Kendrick, R. 1987. *Morphology, ultrastructure and life cycles*. In: The Leishmaniasis in Biology and Medicine. (Eds. Peters, W. & Killick-Kendrick, R.). London: Academic Press, pp. 121-176.
- Morsy, T. A.; Sabry, A. H.; Riffat, M. M. & Wahba, M. M. 1996. Psammomys obesus Cretzschmar, 1828 and zoonotic cutaneous leishmaniasis in Sinai Peninsula, Egypt. *J. Egypt. Soc. Parasitol.* **26** (2): 375-381.
- Mutinga, M.J. 1975. Phlebotomus longipes, a vector of cutaneous leishmaniasis in Kenya. *Trans. R. Soc. Trop. Med. Hyg.* **65**: 106-110.
- Panosonia, C.B. and Wyler, D.J. 1983. Acquired macrophage resistance to in vitro infection with leishmania. *J. Infect. Dis.* **148**: 1049-1054.

- Pappas, M.G., Hajkowski, R., Diggs, C.L. and Hockmeyer, W.T. 1985. Disposable nitrocellulose filtration plates simplify the Dot-ELISA for serodiagnosis of visceral leishmaniasis. *Trans. R. Soc. Trop. Med. Hyg.* **79**: 13-16.
- Pettit, J.H.S. 1962. Chronic (lupoid) leishmaniasis. *Br. J. Dermatol.* **74**: 127-131.
- Poirier, A. 1964. Notes sur les leishmanias on Ethiopie. *Annls. Inst. Pasteur Ethiopie* **5**: 89-94.
- Rab, M.A. and Evans, D.A. 1995. *Leishmania infantum* in the Himalayas. *Trans. R. Soc. Trop. Med. Hyg.* **89**: 27-32.
- Reithinger, R. & Davies, C. R. 1999. Is the domestic dog (*Canis familiaris*) a reservoir host of American cutaneous leishmaniasis? A critical review of the current evidence. *Am. J. Trop. Med. Hyg.* **61**(4): 530-541.
- Reithinger, R., Mohsen, M., Aadil, K., Sidiki, M., Erasmus, P. and Coloman, P.G. 2003. Anthroponotic cutaneous Leishmaniasis, Kabul, Afghanistan. *Emerg. Infec. Dis.* **9**: 727-729.
- Reyburn, H., Ashford, R., Mohsen, M., Hewitt, S. and Rowland, M. 2000. A randomized controlled trial of insecticide-treated bed nets chadders and or top sheets, and residual spraying of interior rooms for the prevention of cutaneous leishmaniasis in Kabul, Afghanistan. *Trans. R. Soc. Trop. Med. Hyg.* **94**: 361-366.
- Rioux, J. A.; Dereure, J.; Kiami, A.; Pratlong, F.; Sirdar, K. and Lambert, M. 1990. Ecoepidemiology of leishmaniasis in Syria: 1. *Leishmania major*. Yakimoff and Schokhar (Kinetoplastida- Trypanosomatidae) infestation of *Psammomys obesus* Cretzschmar (Rodentia-Gerbillidae). *Ann. Parasitol. Hum. Comp.* **65**(6):203-207.
- Rolland, L., Zilberfarb, V., Furtado, A. & Gentilini, M. 1994. Identification of a 94-Kilodalton antigen on *Leishmania* promastigote forms and its specific recognition in human and canine visceral leishmaniasis. *Parasit. Immunol.* **16**(11):599-608.
- Russo, D.M., Turco, S.J., Burns, J.M. and Reed, S. G. 1992. Stimulation of human T-lymphocytes by *Leishmania* lipophosphoglycan-associated proteins. *J. Immunol.* **148**: 202-207.
- Sacks, D. and Kamhawi, S. 2001. Molecular aspects of parasite-vector and vector-host interactions in leishmaniasis. *Ann. Rev. Microbiol.* **55**: 453-483.

- Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**: 487-491.
- Sarojini, P.A., Humber, D.P., Yemane-Berhan, T., Elizabeth, F., Ayele, B., Mock, B. and Wardoff, J.A. 1984. Cutaneous leishmaniasis cases seen in two years at ALERT. *Ethiop. Med. J.* **22**: 7-11.
- Sells, P.G., Burton, M. 1981. Identification of *Leishmania* amastigotes and their antigens in formalin fixed tissue by immunoperoxidase staining. *Trans. R. Soc. Trop. Med. Hyg.* **75**: 461-468.
- Stratigos, J.D. 1980. New aspects on cutaneous leishmaniasis. *Dermatosen* **5**: 139- 148.
- Tayeh, A., Jalouk, L. and Cairncross, S. 1997. Twenty years of cutaneous leishmaniasis in Aleppo, Syria. *Trans. R. Soc. Trop. Med. Hyg.* **91**: 657-659.
- Travi, B.L., Osorio, Y., Melby, P.C., Chanderasaker, B., Arteaga, L. and Saravia, N. 2002. Gender is a major determinant of clinical evolution and immune response in hamsters infected with *Leishmania* spp. *Infect. Immu.* **70** (5): 2288-2296.
- Walker, E.P. 1975. *Mammals of the world*. 3rd edition. Vol.2. Johnhpkin's University press, Baltimore, **pp.** 1326-1331.
- Weigle, K.A., Santrich, C., Martinez, F., Valderrama, L. and Saravia, N.G. 1993. Epidemiology of cutaneous leishmaniasis in Colombia: A longitudinal study of the natural history, prevalence, and oncidence of infections. *J. Infec. Dis.* **168**: 699-708.
- Weigle, K.A., Valderrama, L., Arias, A.L., Santrich, C. and Savaria, N.G. 1991. Leishmanin skin test standardization and evaluation of safety, dose storage, longevity of reaction and sensitization. *Am. J. Trop. Med. Hyg.* **44**: 260-271.
- Weiss, J. B. 1995. DNA probes and PCR for diagnosis of parasitic infections. *Clin. Microbiol. Rev.* **8**(1):113-130.
- Werneck, G.L., Costa, C.H.N., Walker, A.M., David, J.R., Wand, M. and Maguire, J.H. 2003. The urban spread of visceral leishmaniasis: clues from spatial analysis. *Epidemiology* **13**: 364-367.
- WHO. 2002. International Notes: Cutaneous leishmaniasis, Afghanistan. *WHO Weekly Epidemiological Record* **77**: 138- 139.

- WHO. 2000. Division of control of tropical diseases. Leishmaniasis control home page
WWW.who.int/health-topics/leishmaniasis.
- WHO. 1998. Leishmania and HIV in gridlock. Geneva: World Health Organization.
WHO/UNAIDS report. 15-25.
- WHO. 1996. The leishmaniasis and leishmania/HIV co-infections. *Inf. Circ.-WHO Mediterr. Zoon. Control Cent.* **40**: 11-13.
- WHO. 1991. Information on the epidemiological and control of the leishmaniasis by country on territory. *WHO/leish/91.30*. Geneva: World Health Organization.
- WHO.1990. Expert committee: Control of the leishmaniasis. Geneva: World Health Organization. *WHO Tech. Ser.* **793**.
- WHO. 1984. The leishmaniasis : WHO, Geneva. *World Health Organization Technical Report Series* 701.
- Wilkins, H.A. 1972. Studies on leishmaniasis in Ethiopia. VI: Incidence of cutaneous leishmaniasis at Meta Abo. *Ann. Trop. Med. Parasitol.* **66**: 466-475.
- Zijlstra, E. E.; Ali, M. S.; El-Hassan, A. M.; El-Toum, I. A.; Satti, M.; Ghalib, H. W.; Sandrop, E. & Winkler, A. 1991. Kala-azar in displaced people from southern Sudan: epidemiology, clinical and therapeutic findings. *Trans. R. Soc. Trop. Med. Hyg.* **85**: 365-369.
- Zijlstra, E. E., El-Hassan, A. M. & Ismael, A. 1995. Endemic kala-azar in eastern Sudan: post kala-azar dermal leishmaniasis. *Am. J. Trop. Med. Hyg.* **52**: 299-305.
- Zijlstra, E.E., Osman, O.F., Hofland, H.W., Oskam, L., Ghalib, H.W., El-Hassan, A.M., Kager, P.A., Meredith, S.E. 1997. The direct agglutination test for diagnosis of visceral leishmaniasis under field conditions in Sudan: comparison of aqueous and freeze-dried antigens. *Trans. R. Soc. Trop. Med. Hyg.* **91**: 671-673.

ANNEXES

Annex 1

Leishmaniasis Prevalence Survey in Saris, Addis Ababa.

Name _____ Age _____ Sex _____

Duration of stay at residence _____

Household number _____

Place of birth _____

If migrant; Place of origin _____ Date of arrival _____

Occupation _____ Religion _____

Q/N	Question	Coding	Answer
1	What is the type of wall surface?	1. mud 2. bricks. 3. stone. 4. wood 5. others	
2	What is the type of ceilings?	1. cloth 2. concrete 3. wood 4. others	
3	Do you keep any animals in your compound?	1. Yes. 2. No	
4	Total number of family numbers.	Exact number	
5	How long have you lived in this area?	Exact number of years	
6	Have you or some of your family number visited other areas?	1. Yes 2. No.	
7	Are any of your family numbers affected by leishmaniasis?	1. Yes 2. No.	

Annex 2

Complete household information for families with and without CL cases.

[illegible]

Annex 3

የስምምነት ቅጽ

ተራ ቁጥር _____

የካርድ ቁጥር _____

ስም _____

እኔ ከላይ በስም ተጠቃሹ የሻህኝ በሽታ (*Cutaneous leishmaniasis*) ሳይኖርብኝ እንደማይቀር ተነግሮኝ በሽታየን ለመመርመር ከቁስሌ ላይ ናሙና (specimen) እንደሚያስፈልገኝ ተነገርኛል። ይህ ናሙና የበሽታዬን ምንነት ለማወቅ የሚያስችል ምርመራ የሚካሄድበት ነው። እኔም ስለጥናቱ በሚገባ ከተረዳሁ በኋላ አስፈላጊ ናቸው ብዬ ያሰብሀቸውን ጥያቄዎች ጠይቄ አስፈላጊውን ማብራሪያ አግኝቻለሁ። በጥናቱ መረጃ መሰብሰቢያ መጠይቅ ላይ የተዘረዘሩት መረጃዎች ሁሉ በሚስጥር የሚያዙ መሆናቸውንና የስምምነት ቅጹም በሚስጥር ተይዞ ጥናቱ እንደሚካሄድ ተገንዝቤአለሁ።

ስለራሴ የምጠየቀውን መረጃ ያለመስጠት ለጥናቱ ያለመተባበርና በየትኛውም ወቅት ከጥናቱ እራሴን የማግለል መብቴ የተጠበቀ መሆኑ የተገለጸልኝ ሲሆን ይህንንም በማድረጌ ምክንያት በአጠቃላይ በሽታዬን ከመታከም የሚያግደኝ ነገር የሌለ መሆኑን በሚገባ ተረድቻለሁ። ስለሆነም ለተማሪው መስማማቴን በፊርማዬ ያረጋገጥኩት ሁኔታውን በመረዳቴ ነው። እነዲሁም ከጥናቱ የተረፈው ናሙና ወደ ፊት ለሌላ የዚሁ ተመሳሳይ የጥናት ተግባር ላይ ቢወልል ፈቃደኛ ነኝ።

ፊርማ _____

ቀን _____

DECLARATION

I, the undersigned, declare that this thesis is my original work. It has not been presented for a degree in this or any university and all the source materials used for the thesis have been duly acknowledged.

Name of the candidate Girum Erenso Degu

Signature _____

Place Addis Ababa

Date July, 2006.