EPIDEMIOLOGY OF CUTANEOUS LEISHMANIASIS DUE TO *LEISHMANIA TROPICA* AT UTUT, NAKURU DISTRICT, KENYA

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1995

DECLARATION

I Adam Kiplagat Toroitich hereby declare that this thesis is my original work and has not been presented for a degree in any other University.

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Signature

I Dr. L. W. Irungu hereby declare that this thesis has been submitted for examination with my approval as principal University supervisor.

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I Dr. D. K. Sang hereby declare that this thesis has been submitted for examination with my approval as second supervisor; Ministry of Health, Division of Vector Borne Diseases.

..... 23.2.95

Signature

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ABSTRACT

A cross-sectional immunological, clinical and parasitological investigation carried out on a sample population of 220 individuals in a new focus of cutaneous leishmaniasis at Utut area, Rift Valley Province in Kenya revealed a total positive leishmanin skin reactivity rate of 29.0%. This was compared with a reactivity rate of 23.4% in an adjacent area, Kongasis. The prevalence rates of active lesions and scars were 19.4% and 6.5% respectively for Utut and 3.7% and 6.9% respectively for Kongasis. The leishmanin test reactivity and clinical profile by age in the study area was typical of a recent endemic focus of cutaneous leishmaniasis, and depicted an age distribution disease pattern consistent with people's habitation in temporary shelters and often in caves in the Utut focus of cutaneous leishmaniasis . Males were more commonly infected than females: 31.3% males and 26.7% females in Utut, and 28.0% males and 18.9% females in Kongasis. Amastigotes were detected in 4 (30.8%) of 13 patients found with cutaneous lesions.

Four species of sandflies: *Phlebotomus guggisbergi*, *P. saevus*, *P. aculeatus* and *Sergentomyia schwetzi* were distributed sympatrically throughout the study area. Gut promastigotes which grew in Novyl, MacNeal, Nicolle (NNN) culture were isolated from *P. saevus*. This gave a parasite rate of 23.5%. A total parasite rate of 16.7% was obtained in a sample of sandflies which included *P. guggisbergi*

and *P. aculeatus* in which no parasites were detected. The distribution of previously proven vector *P. guggisbergi* and other suspected vectors of the genus *Phlebotomus* suggest that cases of cutaneous leishmaniasis may have contracted the disease from nearly all the sites as people moved frequently in the Utut focus.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

1.1.1 Definition

Human leishmaniasis is a complex of diseases caused by protozoan parasites of the genus *Leishmania*. Clinically the disease manifests as cutaneous, mucocutaneous and visceral leishmaniasis. Cutaneous leishmaniasis of the Old World is caused by three parasites, *Leishmania aethiopica*, *L. major* and *L. tropica*. *L. aethiopica* may cause diffuse cutaneous leishmaniasis. Occasionally *L. donovani* the aetiological agent of kala-azar, causes cutaneous leishmaniasis (Kirk, 1938; Manson-Bahr, 1955). *L. tropica* may cause visceral leishmaniasis (Groyl *et al.*, 1993). It causes leishmaniasis recidivans (Sang *et al.*, 1994), a chronic disease in which a cutaneous lesion enlarges over the years, healing at the centre while advancing at the periphery. The disease is usually destructive and disfiguring.

In the New World, *L. braziliensis* complex causes cutaneous and mucocutaneous leishmaniasis while the *L. mexicana* complex causes cutaneous and diffuse cutaneous leishmaniasis. Human leishmaniasis is transmitted by sandflies of the genus *Phlebotomus* in the Old

World and the genus *Lutzomyia* in the New World (WHO, 1984). There is at present no conclusive evidence incriminating sandfly species of the genus *Sergentomyia* as vectors of leishmaniasis to man.

1.1.2 Morphology and life cycle of *Leishmania* parasite

Two forms exist in the life cycle of *Leishmania*. The amastigote form is found in the mononuclear cells of reticulo-endothelial system of the liver, spleen, bone marrow or skin of man and of animal reservoirs. This form is aflagellate, usually oval shaped with conspicuous nucleus and rod shaped kinetoplast. Its mode of reproduction is by binary fission.

When a female sandfly vector bites an infected host the amastigotes ingested transform in the gut into promastigotes which are elongate motile flagellates. Reproduction of promastigotes in sandflies is by binary fission. The promastigotes migrate forward to the pharynx of the sandfly and transform into metacyclic promastigotes infective to man (Killick-Kendrick, 1990).

1.1.3 Global prevalence and incidence of leishmaniasis

Among the human protozoan diseases leishmaniasis ranks second to malaria in terms of medical and socio-economic importance (Lainson, 1982). Due to its importance, leishmaniasis has been considered as one of the six diseases in WHO/UNDP/World Bank Special Programme

for Research and Training in Tropical Diseases (WHO, 1984). The prevalence rate of the disease is 12 million people, with an annual incidence rate of 0.6 million cases and 350 million at risk of infection (WHO, 1990).

Leishmaniasis is more rapidly spreading and more devastating than was previously thought because of migration, change in socio-economic structure and large scale displacement (WHO, 1993). The challenge posed by the disease has resulted in many countries adopting appropriate control programmes as part of the primary health care system. These include identifying endemic foci, reducing morbidity, interrupting transmission and keeping the size of reservoir hosts to a minimum (IDRC, 1992).

These control measures have been limited by dearth information on the transmission cycle in many localities with either the reservoir hosts or the vector species still to be identified and by lack of good pharmacological support against the disease (IDRC, 1992; WHO, 1993).

1.1.4 Distribution of leishmaniasis in Kenya

Human leishmaniasis is an important disease in endemic localities of Kenya. Its occurrence and spread is determined by suitable microhabitat favouring the existence of sandfly vectors and reservoir hosts. Visceral leishmaniasis caused by *L. donovani*

(Manson-Bahr, 1955) and transmitted by *P. martini* (Minter, 1963) occurs in low-land arid regions. These include Machakos, Kitui, Meru, Baringo, West Pokot, Turkana and Kajiado Districts.

Cutaneous leishmaniasis occurs in the highland regions of Kenya: the disease caused by *L. aethiopica* occurs in southern slopes of Mount Elgon (Mutinga and Ngoka, 1970, Kungu *et al.*, 1972) at an altitude between 1750 to 1900 metres (Sang *et al.*, 1993a). Cutaneous leishmaniasis in Baringo District is caused by *L. major* (Muigai *et al.*, 1987). Other highland areas of cutaneous leishmaniasis include Nakuru, Laikipia, Nyandarua and Nyeri Districts where *L. tropica* occurs (Mebrahtu *et al.*, 1988; Sang *et al.*, 1992a, 1993b).

1.2 Literature review

1.2.1 Transmission of cutaneous leishmaniasis in Kenya

The vector of cutaneous leishmaniasis due to *L. aethiopica* on Mt. Elgon has been found to be *Phlebotomus pedifer* which inhabit caves (Mutinga, 1975; Sang and Chance, 1993). Disease transmission to man has been attributed to infected sandfly bites in caves or in houses close to caves (Sang *et al.*, 1993a). Rock hyrax has been proven as a reservoir host of *L. aethiopica*. *P. guggisbergi* has been proven as the vector in the foci of cutaneous leishmaniasis due to *L. tropica*, with rock hyrax as a reservoir (Lawyer *et al.*,

1991; Sang *et al.*, 1992a,b). Human infections with *L. tropica* possibly occurred in caves inhabited by *P. guggisbergi* (Sang *et al.*, 1994). A naturally infected *P. duboscqi* has been found to transmit cutaneous leishmaniasis due to *L. major* (Beach *et al.*, 1984).

1.2.2 Cutaneous leishmaniasis caused by *L. tropica* in Kenya

Prior to 1985, *L. tropica* was not known to occur in Kenya. The first isolates of the parasite came from 3 patients who had visited various parts of Kenya (Mebrhatu *et al.*, 1987). Subsequently autochthonous *L. tropica* were isolated from Laikipia District (Mebrahtu *et al.*, 1988). Cases of cutaneous leishmaniasis due to *L. tropica* have since been identified in the highland regions of Nakuru and Samburu Districts in Rift Valley and Nyandarua, Nyeri, and Isiolo Districts in central Kenya where the disease occurred at endemic levels (Sang, 1991; Sang *et al.*, 1993b). Among these areas Utut in Nakuru District has been identified as an intense zoonotic focus of cutaneous leishmaniasis (Sang *et al.*, 1994).

1.2.3 The Utut focus

Utut is a fertile area with diverse species of plant biota that have attracted migrant charcoal burners since 1979 (Sang *et al.*, 1994). The area has numerous lava rocks forming caves and rock crevices which have been identified as sandfly habitats (Sang *et*

al., 1992a). Transmission of zoonotic cutaneous leishmaniasis in this area has been found to involve *P. guggisbergi*, based on recent characteristics described by Killick-Kendrick *et al.* (1991, 1993) and the rock hyrax, *Procavia johnstoni* (Sang *et al.*, 1992a,b). Recently *Phlebotomus aculeatus* has been suggested as a possible vector of cutaneous leishmaniasis in the focus (Johnson *et al.*, 1993).

Epidemiological studies in this focus showed that people of all ages were infected probably by bites of sandflies harbouring *Leishmania* in the caves or in the working places proximity to the caves and rock crevices (Sang *et al.*, 1994). The disease was found to have a short incubation period ranging from 2 weeks to 3 months and resulted in single or multiple cutaneous lesions which healed in 1 to 2 or more years, but some presented with a chronic state identified as leishmaniasis recidivans.

1.2.4 Assessment of the risk of contracting cutaneous leishmaniasis in Utut

The risk of contracting cutaneous leishmaniasis has been assessed by calculating the risk on the basis of prevalence of both past and present infections in each age group of the human population in a focus of disease transmission (Aston and Thorley, 1970; Maskovskis and Duhanina, 1971).

1.2.4.1 Active and passive case detection

The active and passive case detection has been recommended by the World Health Organisation as a method of elucidating distribution of disease in the human population to infer endemicity or risk of infection with leishmaniasis (WHO, 1984). Its application in Utut has indicated 83 (50%) active lesion rate relative to 30 (18%) scar rate in a population of 425 people in 1989, and 95 (54.9%) people with active lesions relative to 75 (43.4%) with scars in 173 cases of a changing population in 1991 (Sang *et al.*, 1994). The occurrence of active lesions has been used as a presumptive evidence of active transmission (Kadaro *et al.*, 1993). In an old focus of leishmaniasis, high scar rates have been observed among the older age groups (Moskovskis and Duhanina, 1971).

Detection of *Leishmania* parasites from cutaneous leishmaniasis lesions has been considered as an important diagnostic tool in the epidemiology of cutaneous leishmaniasis (WHO, 1984). In Utut focus *Leishmania* parasites were detectable in lesions of mostly up to 2 years duration but thereafter failed to be detected although the lesions continued to grow (Sang *et al.*, 1994). The delay in development of cutaneous leishmaniasis lesions resulting in non detection of cases clinically has been attributed to the variability in incubation period and the slow progress of morbidity (Sanchez *et al.*, 1992).

1.2.4.2 Leishmanin (Montenegro) skin test

Leishmanin skin test (Montenegro, 1926) has been recommended as a simple and an effective tool for investigating the epidemiology of leishmaniasis (WHO, 1984). The test has been investigated in various parts of the world and found to be quite specific for leishmaniasis except that tuberculosis and leprosy occasionally give positive results (Aston and Thorley, 1970). The test has been shown to be highly sensitive for *Leishmania tropica*, giving a positive result in early infections (Dostrovsky *et al.*, 1952).

Leishmanin skin test has been found to be positive in early cutaneous leishmaniasis caused by *L. tropica*, and in late East African kala-azar after spontaneous or therapeutic cure (Manson-Bahr, 1961) but negative or weakly positive in post kala-azar dermal leishmaniasis (Manson-Bahr *et al.*, 1959). A negative test has been detected in diffuse cutaneous leishmaniasis cases although the test could turn positive after treatment (Cahill, 1965). A positive test in an apparently normal individual has been used as presumptive evidence of subclinical or cryptic infection with leishmaniasis (Manson-Bahr, 1961; Southgate, 1964; Pampiglione *et al.*, 1974; Kadaro *et al.*, 1993), which may turn overt under certain circumstances for example poor nutrition (Ali and Ashford, 1993b) and infection with human immunodeficiency virus (HIV) (WHO, 1992).

Leishmanin skin test has been used to quantify the risk of contracting leishmaniasis in an endemic area when the population is exposed to potential bites by infected sandflies (Manson-Bahr, 1959, 1961; Southgate and Oriedo, 1962, 1967; Southgate, 1964; Southgate and Manson-Bahr, 1967; Leeuwenburg *et al.*, 1983; Ali and Ashford, 1993a,b).

1.2.5 Vectors of *Leishmania tropica*

Interrupting the transmission as one of the methods of controlling cutaneous leishmaniasis in an endemic focus has been hampered by paucity of information on transmission cycles, with either the reservoir hosts or the vector species or both still to be identified (Killick-Kendrick and Ward, 1981; IDRC, 1992). Killick-Kendrick (1990) has described the criteria for incriminating a sandfly species as a vector of leishmaniasis to man. The criteria include the abundance and distribution of sandfly species consistent with the distribution of disease in human population in a focus, the existence of an anthropophilic sandfly species where human infections occur and the isolation of indistinguishable *Leishmania* from a sandfly species, a reservoir host and man. The characteristics at the base of spermathecae of females and the aedeagus of males have been used to identify sandflies into species since these characters could be seen when examining the flies for parasites (Killick-Kendrick *et al.*, 1991, 1993). In Utut focus parasite isolates from *P. guggisbergi* and human patients have been

typed as *L. tropica* zymodeme (MON-119) (Sang *et al.*, 1992a).

1.2.5.1 Distribution of sandfly species

Sufficient abundance and wide distribution of sandfly species in accordance with the distribution of leishmaniasis in man has been considered important for the maintenance of parasite transmission in nature (Killick-Kendrick and Ward, 1981).

The use of standard sampling methods: light traps, sticky paper traps, direct search and capture by suction tubes and human bait have been recommended for reliable comparison of sandfly species density and distribution in a focus of human leishmaniasis (WHO, 1984). Some studies have shown that the area of maximum distribution of *Phlebotomus pedifer* coincided with the distribution of cases of cutaneous leishmaniasis in a focus (Sang and Chance, 1993; Sang *et al.*, 1993a).

1.2.5.2 Anthropophily of the sandfly species

The vectorial status of a sandfly species has been assessed by its man biting activities in areas where human cases occur. In Utut focus, man-biting *P. guggisbergi* was found in Msalaba site where it was also reported as the site of most human infections (Sang *et al.*, 1994). Man-biting sandflies in other case sites of cutaneous leishmaniasis due to *L. tropica* have been identified as *Phlebotomus*

species: *P. saevus*, *P.aculeatus*, *P. pedifer*, *P. longipes*, and *Sergentomyia* species: *S. schwetzi*, *S. garnhami*, *S. graingeri* and *S. adleri* (Mebrahtu *et al.*, 1988; Lawyer *et al.*, 1989; Sang, 1991; Sang *et al.*, 1993b).

In the Old World except in Kenya *L. tropica* has been considered as an urban parasite, transmitted by both peridomestic and anthropophilic sandfly *P. sergenti* (Lewis, 1971; WHO, 1984). Both *P. sergenti* and *P. saevus*, belong to the subgenus *Paraphlebotomus*, thus suggesting *P. saevus* is a potential vector of *L. tropica* in Kenya (Mebrahtu *et al.*, 1988; Lawyer *et al.*, 1989; Sang, 1991; Sang *et al.*, 1993b).

1.3 Objectives of the study

The population in Utut is not stable since people move frequently into and out of the area for charcoal burning and poles for constructing huts in the adjacent area. These socio-economic activities may introduce people into the transmission sites of the disease. In other related foci cases of cutaneous leishmaniasis occurred by encroachment of new comers into previously unoccupied land (Sang *et al.*, 1993a). Part of the population of Utut has emigrated completely to the adjacent new settlement schemes or to the original homes while others still inhabit the area awaiting resettlement elsewhere. The presence of the proven vector and the reservoir host suggest a likelihood of frequent new human cases of

cutaneous leishmaniasis in immigrants and other non immune, susceptible, individuals. That other sandflies have been found in the case sites of cutaneous leishmaniasis due to *L. tropica* could be of epidemiological significance in Utut focus. Cutaneous leishmaniasis in Utut is important notwithstanding the working time lost, the psychological impact and the cost of treating the disease. The study is therefore important as a baseline for future control and treatment of cutaneous leishmaniasis in the Utut focus.

The general aim of the study was to determine the transmission dynamics of cutaneous leishmaniasis caused by *L. tropica* in the Utut focus, in view to understanding the disease distribution in population, and vectorial status of the sandfly fauna present. The specific objectives of the study were:

1.3.1 To elucidate possible vector species, their distribution and their parasite rates.

1.3.2 To determine the risk of contracting cutaneous leishmaniasis by examining the population inside and outside the Utut focus.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Study Area

Utut is situated near Lake Elmentaita (36°15'E,0°30'S) at an altitude of 2000 metres above sea level, at the floor of the Rift Valley, Kenya. It covers an area of about 20 square kilometres and is bordered by Mau escarpment to the south and Kinangop Plateau to the east. To the north it borders the southern shore of Lake Elmentaita. It is 5 km off the main Nairobi-Nakuru Road at Gilgil Figure 1 shows a sketch map of Utut.

The area has numerous caves and rock crevices formed by volcanic lava flow, inhabited by phlebotomine sandflies, rock hyraxes *Procavia johnstoni* and occasionally by people. The area is a government forest reserve, with diverse vegetation of trees, shrubs and herbs in sharp contrast to the surrounding savannah grassland. Wild animals include buffalos, *Syncerus caffas*, baboons, *Papio anubis*, vervet monkeys, *Cercopithecus aethiops*, leopards, *Panthera leo* and diverse antelope species. A brief account of the fauna and flora of Utut has been described by Sang *et al.* (1994).

Rainfall is unevenly distributed through out the year; but the mean total is 891.6 mm per year. Major occupation is charcoal burning although livestock keeping and growing of crops such as beans, maize and to a limited extent, wheat, are practiced in the surroundings of Utut.

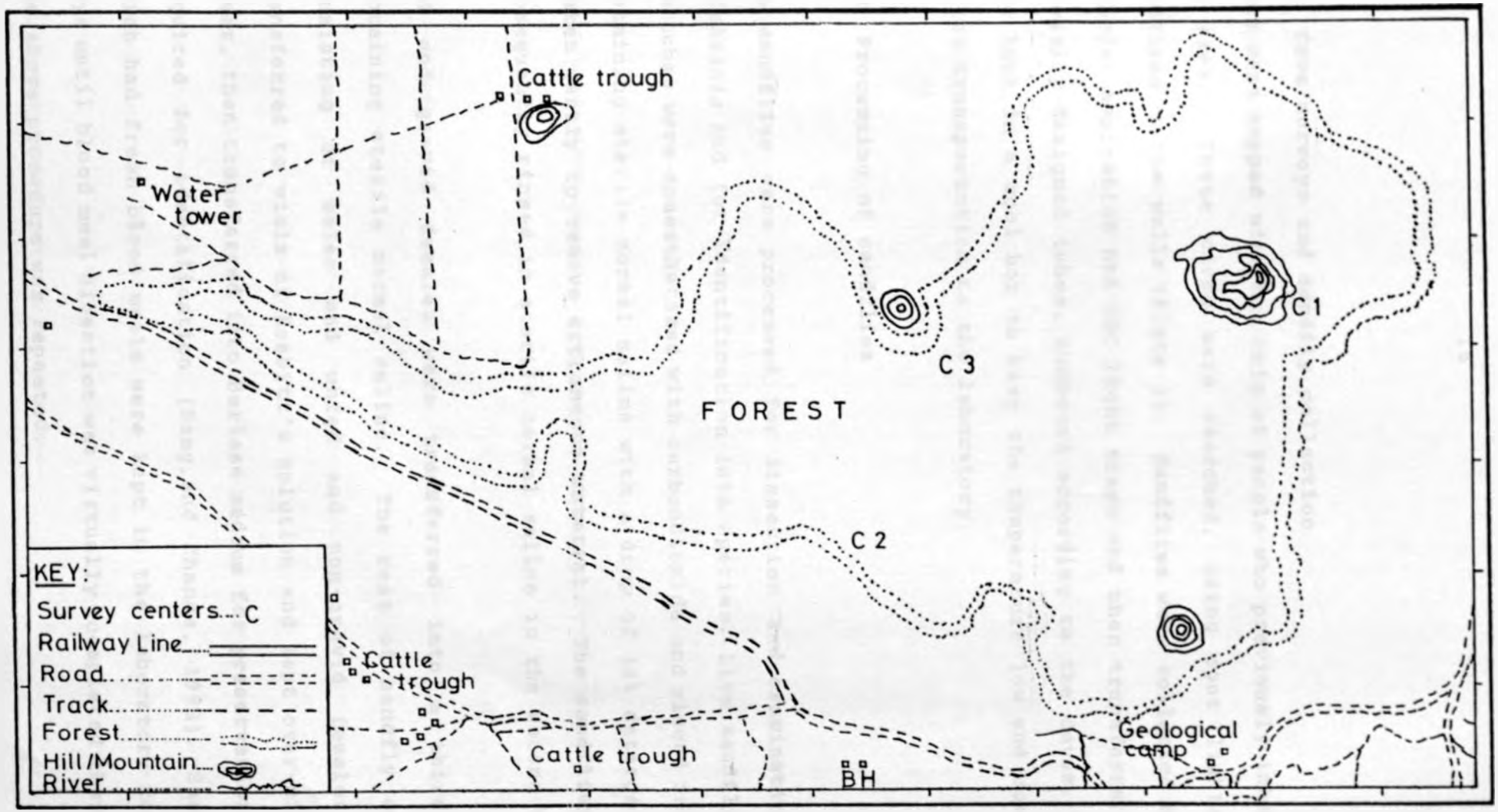

FIGURE 1. SKETCH MAP OF UTUT

2.2 Cave surveys and sandfly collection

Caves were mapped with the help of people who previously inhabited the area. These caves were searched, using spot lights for sandflies on the walls (Plate 1). Sandflies were collected by two methods: aspiration and CDC light traps and then transferred into specially designed tubes, numbered according to the caves. These were kept in a cool box to keep the temperature low and constant during transportation to the laboratory.

2.3 Processing of sandflies

The sandflies were processed for dissection and examination for *Leishmania* and for identification into species. Live sandflies in the tubes were anaesthetized with carbondioxide and placed in vials containing sterile normal saline with a drop of 10% detergent and shaken gently to remove extraneous material. The sandflies were subsequently rinsed in sterile normal saline in the second vial.

Fed and gravid females were transferred into a third vial containing sterile normal saline. The rest of sandfly catches consisting of males and unfed and non-gravid females were transferred to vials of Nesbitt's solution and kept over-night to clear, then transferred into Berlese medium for preservation until required for identification (Sang,and Chance, 1993). Sandflies which had fresh blood meals were kept in the laboratory for 4-10 days until blood meal digestion was virtually completed thereafter the above procedure was repeated.

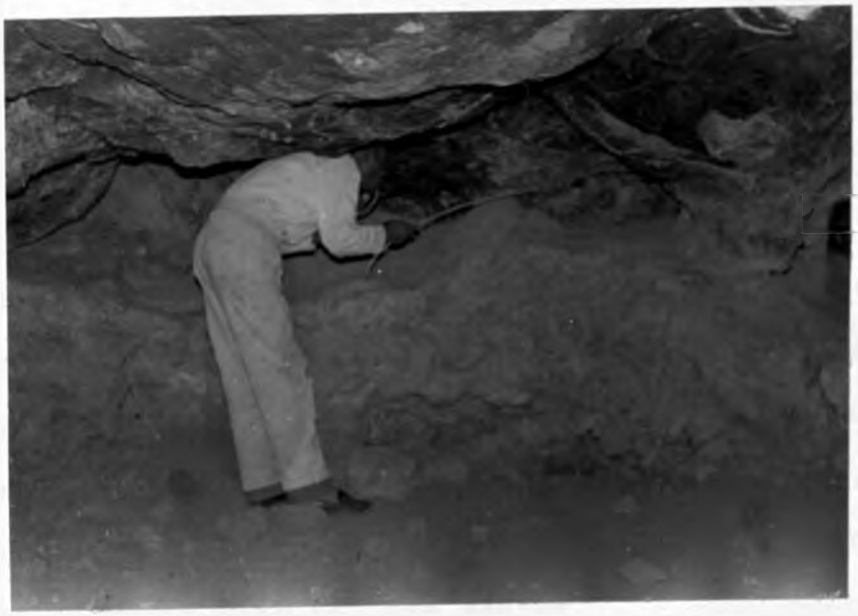

Plate 1. Active search for sandflies in a cave at Utut

2.4 Dissection of gravid sandflies and isolation of promastigotes

Each fed and gravid female sandfly was picked from the vial by means of alcohol sterilized mounting entomological needles, and placed on a drop of normal saline supplemented with penicillin on a dry clean alcohol sterilized microscope slide. Dissection was carried out by chopping off the head and transferring it to another slide with a drop of gum chloral mountant. While holding the thorax with one needle the gut was gently drawn out by pulling the terminal abdominal segment with the second needle.

The terminal segment was separated from the gut and transferred onto the same slide containing the head and further dissected in a drop of saline to expose the spermathecae for identification into species (Leger *et al.*, 1983; Killick-Kendrick *et al.*, 1991, 1993). A coverslip was then placed and permanent preparation was obtained by irrigating it with Berlese medium.

The gut was lacerated to release contents into suspension and an alcohol sterilized coverslip was placed. This was microscopically examined, under X 40 objective, for promastigotes. When positive, the promastigotes were withdrawn into a sterile 2 ml. disposable syringe and needle and inoculated into blood agar slants of Novyl, MacNeal, Nicolle (NNN) media as described by Sang and Chance (1993). A solution of 1,000,000 units of penicillin was made in 10 ml. of sterile normal saline. 1 ml. of this was inoculated into NNN media under sterile conditions. In positive sandflies small quantities of NNN were injected beneath the cover slip on the slide

and the contents withdrawn were inoculated into NNN media. Cultures were kept at room temperature and examined weekly for any growth of promastigotes.

2.5 Dissection of males, and unfed and non-gravid sandflies for identification

Sandflies were removed from Berlese medium and each male was placed separately in a drop of gum chloral mountant on a microscope slide. The head was placed upside down to expose cibarial and pharyngeal regions as described by Sang and Chance (1993). In females a slit was made between the 5th and 6th segments then pulled apart to expose the base of spermathecal ducts attached to distal segments for speciation (Leger *et al.*, 1983; Killick- Kendrick *et al.*, 1991, 1993).

2.6 Sandfly behaviour: Human biting and nocturnal activities

Sandflies in caves attracted to and landed on man during the day were aspirated before they could bite. Sticky paper traps were set at night to catch sandflies inside and outside one of the large caves. All the sandflies were processed and dissected as described by Sang and Chance (1993) and identified into species (Leger *et al.*, 1983; Killick-Kendrick *et al.*, 1991,1993).

2.7 Study Population

Total population of Utut was estimated as 425 in the 1989 National census. Although new comers continued to arrive more people have

been emigrating to new settlement schemes. The study population was chosen from Utut, Sait but extended to an adjacent area of Kongasis. The inhabitants were contacted through the chief, the primary school headmaster and other prominent people. The purpose of the exercise was explained and the people were asked to assemble at their respective centres.

A cross-sectional study was done to elucidate immunological, clinical and parasitological status of the population. Each subject was interviewed by means of a standard questionnaire which comprised of demographic variables such as name, age, sex, original place of residence, and the initial settlement site at Utut. The nature of cutaneous lesions and leishmanin skin test were included. Excitable infants were excluded from the study.

The school teachers or the parents verified the ages of the children. The investigators occasionally estimated the ages when the individuals could not recall by physical appearance and by memory of events of particular significance.

A case was defined as an individual in the area who had characteristic ulcerated or nodular lesions (Sanchez, *et al.*, 1992; Sang *et al.*, 1994). The controls were normal individuals from non endemic areas and not known to have been exposed to leishmaniasis. All the parasitological and skin test studies were performed by informed consent of each individual as an extension of an on-going study.

2.8. The leishmanin (Montenegro) skin test

The leshmanin skin test was performed using an Israeli strain of *Leishmania major* containing 10^5 promastigotes reconstituted in 0.5% phenol in sterile physiological saline (Sang, pers comm). The antigen was dispensed in aliquot into sterile rubber stoppered normal saline bottles. The skin over the flexor surface of the left forearm was cleaned with methylated spirits or 70% alcohol soaked in cotton, air dried and 0.1 ml of the antigen was inoculated intradermally, using sterile disposable 1 ml tuberculin or 2 ml syringes and disposable needles. All the tests and induration readings were done as described in other related studies (WHO, 1984; Ali and Ashford, 1993a,b; Sang, per comm).

2.9. Diagnosis of cutaneous leishmaniasis

Subjects were examined for external lesions and evidence of scars due to cutaneous leishmaniasis on the exposed parts of the body. Suspected lesions were thoroughly cleaned with alcohol and examined as described by WHO (1984), Kadaro *et al.* (1993) and Sang *et al.* (1993a, 1994). Parasites were obtained from the nodular edge of the lesion by slit skin technique and smear. The lesion was held between the finger and thumb to cause blanching and using a sterile surgical blade an incision, a few mm. long, was made through the intact epidermis into the dermis. The exposed surface was scraped to obtain tissue juice and cells then smeared onto clean microscope slides. The slides were kept in slide boxes for transportation to the laboratory, where the specimens were fixed in methanol and stained with 10% Giemsa and examined for amastigotes.

CHAPTER THREE

3.0 RESULTS

3.1 Sandfly fauna

190 male and 232 female sandflies were caught using aspirators and CDC light traps. This excluded 20 damaged specimens which could not be identified. Plate 1 shows phlebotomine sandflies resting on the wall of a cave. Four species of sandflies were detected. This included two of subgenus *Larroussius*: *Phlebotomus guggisbergi* and *P. aculeatus*; one of *Paraphlebotomus*: *P. saevus* and one of *Sergentomyia*: *S. schwetzi* (Table 1).

Table 1 Relative composition of sandfly species caught at Utut

Species	Number of: Male	Female	Total Number	% Composition
Phlebotomus guggisbergi	70	52	122	28.91
P. saevus	80	102	182	43.13
P. aculeatus	38	78	116	27.49
Sergentomyia schwetzi	2	0	2	0.47
Total	190	232	422	100.00

In terms of relative abundance *P. saevus* was the most predominant species and *S. schwetzi* was apparently a very rare species. *P. guggisbergi* and *P. aculeatus* ranked second and third respectively.

3.2 Parasite rates in sandflies

Among 232 female phlebotomine sandflies captured 24 were either freshly fed or gravid. Promastigotes were isolated from 4 out of 17 *P. saevus* dissected giving a parasite rate of 23.5%. The positive sandflies were among 66 *P.saevus* females captured at Msalaba and Stech Kubwa. A total parasite rate of 16.7% was detected which included 4 *P. guggisbergi* and 3 *P. aculeatus* in which no parasites were detected (Table 2).

Table 2 Parasite rate in fed and gravid females

Species	Total no. of females	Fed & gravid females: Total no.	No. positive	Parasite rate
Phlebotomus guggisbergi	52	4	0	0.00
P. saevus	102	17	4	23.53
P. aculeatus	78	3	0	0.00
Total	232	24	4	16.67

Two preparations of gut promastigotes isolated from sandflies at Msalaba site grew in NNN culture but diminished after sub culturing for six weeks. Also two similar preparations from sandfly catches at Stech Kubwa did not grow in culture when examined seven days after inoculation. Plate 4 shows some promastigotes, with conspicuous flagella, isolated from one of the infected phlebotomine sandflies.

Plate 2. Phlebotomine sandflies resting on the wall of a cave.

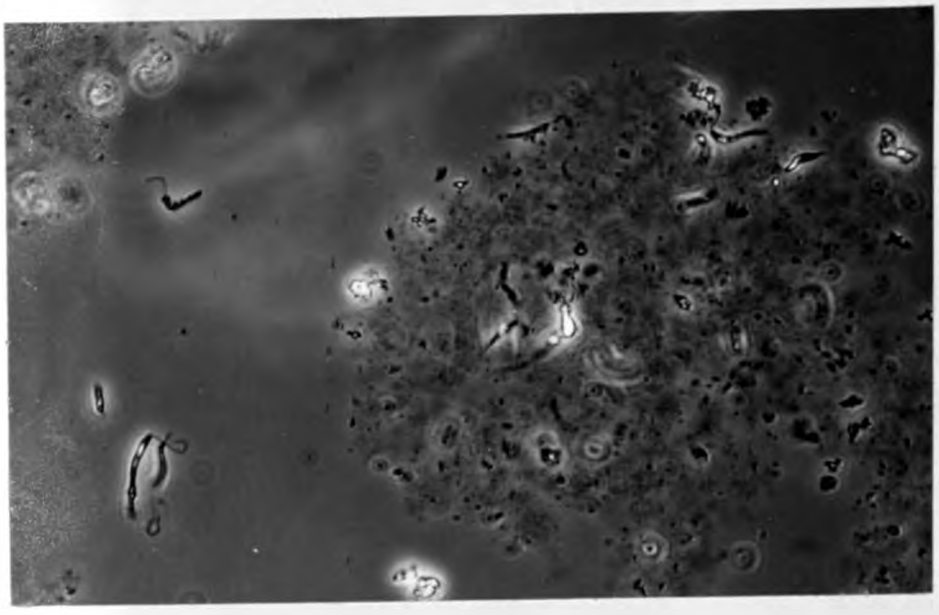

Plate 3. Gut promastigotes isolated from a sandfly

3.3 Distribution of sandfly fauna

Table 3 shows relative proportion of sandfly species in each site. Data from adjacent caves were grouped together to give a total species proportion. Most of the caves in all other sites were relatively inconspicuous. Two very large caves: one at Kahuruko and the other at Stech-Kubwa produced 55 (96%) and 19 (40%) specimens of *P. aculeatus* respectively, a species which was virtually absent elsewhere. Nearly all the 29 caves sampled had a history of human habitation.

Table 3 Relative population of sandfly species in each site, Utut

Site	% Species composition							
	P. guggisbergi		*P. saevus*		*P. aculeatus*		*S. schwetzi*	
	No	%	No	%	No	%	No	%
Sait/Muthuri	5	33.33	9	60.00	1	6.67	0	
Sait	16	39.02	17	41.46	8	19.51	0	
Muthuri	2	33.33	4	66.67	0			
Soko-Mjinga	34	54.84	17	27.42	11	17.74	0	
Kapturo	1	7.14	6	42.86	7	50.00	0	
Msalaba	41	34.75	67	56.78	9	7.63	1	0.85
Mathare	6	46.15	6	46.15	1	7.69	0	
Stech-Kubwa	14	17.07	46	56.10	22	26.83	0	0.00
Kahuruko	3	4.22	10	14.08	57	80.28	1	1.41
Total	122	28.91	182	43.13	116	27.49	2	0.47

3.4 Sandfly behaviour

3.4.1 Sandfly human biting activity

Females of *P. saevus, P aculeatus* and *S. schwetzi* were attracted to and bit man in an exercise carried out in two very large caves at Kahuruko and Stech-Kubwa respectively (Table 4).

Table 4 Composition of female sandfly species attracted to and bit man in two sites

Site	Activity	Species composition			
		P. saevus	*P. aculeatus*	*S. schwetzi*	*P. guggisbergi*
Kahuruko	Biting	2	53	1	0
	Resting	0	0	0	0
Stech-Kubwa	Biting	1	0	0	0
	Resting	1	0	0	1
	Total	4	53	1	1

During the human bait catches 1 female *P. guggisbergi* and 2 male and 1 female *P.saevus* were caught resting on the walls of the cave in Stech-Kubwa. At Kahuruko cave no sandfly was found resting during this exercise but all female sandflies were caught biting.

3.4.2 Sandfly night activity

Table 5 shows a sticky paper sandfly night catches by sex inside and outside the cave at Kahuruko. 2 male *P. saevus*, 12 male and 3 female *P. aculeatus* and 1 female *S. schwetzi* were caught inside the trap in the large cave at Kahuruko. A sandfly larva was also caught. In addition, 3 female *P. aculeatus* were caught outside the trap at the entrance of the cave.

Table 5 Sandfly night catches by sex inside and outside the cave at Kahuruko

Position	Species/sex					
	P. saevus		*P. aculeatus*		*S. schwetzi*	
	M	F	M	F	M	F
Inside cave	2	-	12	3	-	1
Outside cave	-	-	-	3	-	-
Total	2	-	12	6	-	1

3.5 The leishmanin (Montenegro) skin test

A total of 444 individuals were cross-sectionally screened by the leishmanin skin test. 224 of this did not turn up for reading, leaving a net study population of 220. This consisted of 109 (49.5%) males and 111 (50.5%) females of all age groups ranging from 2 years to 62 years. 172 (78.5%) of the population sample was less than 20 years. Indurations of 5 mm or more in diameter were recorded as positive.

In the study population 54 (24.5%) individuals were leishmanin skin test positive and 162 (73.6%) tested negative. The positive subjects included 44 (23.4%) from Kongasis centre and 9 (29.0%) from Utut centre (Table 8). The population in Kongasis engaged in some agriculture although some could frequent Utut for charcoal

burning or poles for construction of huts. Occupation in Utut was exclusively charcoal burning.

3.6 Clinical examination

In the total population 192 (87.3%) individuals had normal skin condition, 13 (5.9%) had active lesions and 15 (6.8%) had scars only. Most of the lesions were observed to be of lupoid type, healing at the centre while advancing at the periphery (Plate 4).

The population of Utut comprised of 16 (51.6%) males and 15(48.4%) females of ages 2 to 60 years out of which 23 (74.2%) had normal skin, 6 (19.4%) had active leishmaniasis lesions and 2 (6.5.%) had scars. Males were more commonly infected than females, the male: female ratio in infected individuals was 3:1.

In Kongasis centre the study population sample consisted of 93 (49.2%) males and 96 (50.8%) females of all age groups ranging from 3 to 62 years. 169 (98.4%) individuals had normal skin, 7 (3.7%) had active lesions and 13 (6.9%) had scars or healed lesions. Table 6 and Table 7 show cutaneous lesion and scar rates by age in population samples at Utut and Kongasis respectively.

Table 6 Cutaneous lesion and scar rates by age in a population sample at Utut

Age group (years)	No. Normal	No. Active lesion	No scars	% Normal	% Active lesion	% scars
0-9	10	4	1	66.7	26.7	6.7
10-19	1			100.0		
20-29	2	1		66.7	33.3	
30-39	2		1	66.7		33.3
40-49	4	1		80.0	20.0	
50-59	2			100.0		
60+	2			100.0		
Total	23	6	2	74.2	19.4	6.5

Table 7 Cutaneous lesion and scar rates by age in a population sample at Kongasis

Age group (years)	No. Normal	No. Active lesion	No. Scar	% Normal	% Active	% Scars
0-9	54		2	96.4		3.6
10-19	99	1		99.0	1.0	
20-29	6		5	54.5		45.5
30-39	7	3	1	63.6	27.3	9.1
40-49	1	3	4	12.5	37.5	50.0
50-59	1			100.0		
60+			1	100.0		
Total	168	7	13	89.4	3.7	6.9

Total prevalence rate of active lesions was higher for Utut than for Kongasis but there was virtually little difference in scar rates between the two centres. Active lesion rates were higher in lower age groups 0-19 and 20-29 years for Utut than in the same age groups for Kongasis. Generally, the scar rates increased with age in the two centres, but active lesion rate clearly increased with age in Kongasis compared with Utut. The youngest active lesion subject was a male aged 3 years from Utut.

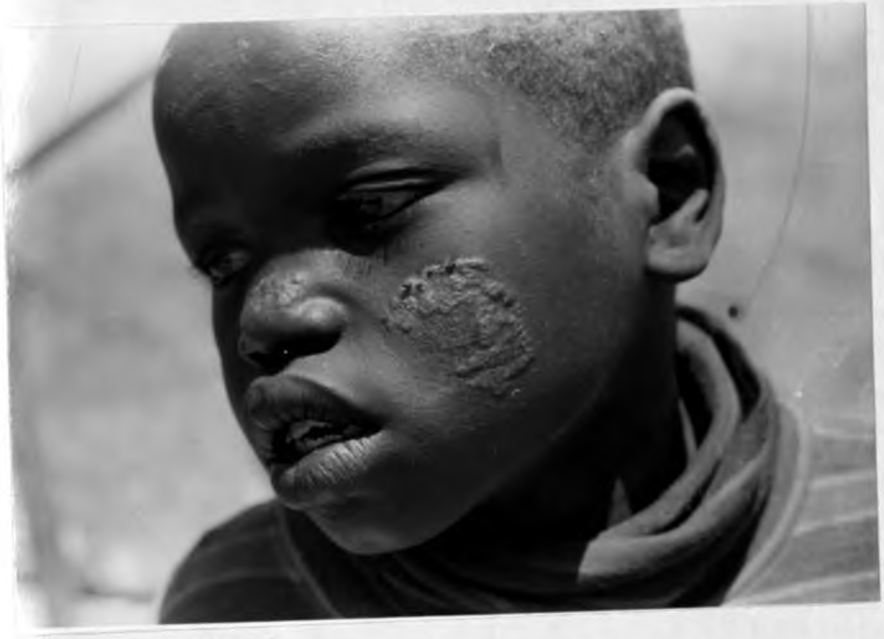

Plate 4. Cutaneous lesion on the face of a patient at Utut

Table 8 Leishmanin skin reactivity rate in population samples by centre and age group

	KONGASIS (*N=188)		UTUT (*N=31)	
Age group (years)	(Lst.-)	(Lst.+)	(Lst.-)	(Lst.+)
0-9	92.9	3.6	73.3	20.0
10-19	76.0	24.0	100.0	
20-29	63.6	27.3	66.7	33.3
30-39	27.3	72.7	33.3	66.7
40-49	25.0	75.0	100.0	
50-59		100.0	50.0	50.0
60+	100.0			100.0
Total	75.0	23.4	67.7	29.0

*N= Total sample size.

The increase in skin test positivity with age was very clear for Utut and Kongasis with almost 100% leishmanin skin positivity in the individuals of 50 or more years. The leishmanin positivity was higher in the age group 0-9 years for Utut than in the same age group for Kongasis. In each centre, the males showed a higher leishmanin reactivity rate in each age group than the females (Table 9).

Table 9 Age- and- sex-specific leishmanin positivity rate at Kongasis and Utut

	KONGASIS		UTUT	
Age group (years)	Male	Female	Male	Female
	(*n=26)	(*n=18)	(*n=5)	(*n=4)
0-9		6.3	22.2	16.7
10-19	18.6	18.1		
20-29	37.5			33.3
30-39	80.0		66.7	
40-49	85.7			
50-59	100.0		100.0	
60+				100.0
Total	28.0	18.9	31.3	26.7

*n=Total number of each sex leishmanin positive.

The leishmanin skin positivity in each sex in both centres increased with an increase in age. The total leishmanin skin positivity rate in males and in females from Utut was more than that in males and in females from Kongasis respectively. Some of the individuals from Utut and Kongasis who had cutaneous leishmaniasis lesions or scars were either leishmanin positive or negative. In Kongasis centre, 31 individuals had normal skin condition but were leishmanin positive and 3 individuals were leishmanin negative despite having scars due to cutaneous leishmaniasis. In Utut centre, 4 individuals had normal skin condition but were leishmanin positive and 1 individual was leishmanin negative despite having a scar due to cutaneous leishmaniasis, while 1 patient with an active lesion was leishmanin negative.

3.7 Parasitological diagnosis

Amastigotes were detectable in smears of 4 (30.8%) of 13 patients with active cutaneous leishmaniasis lesions. The patients included 3 males and 1 female from the two centres: Utut and Kongasis. Plate 5 shows the amastigotes with a characteristic rod-shaped kinetoplast and oval nuclei.

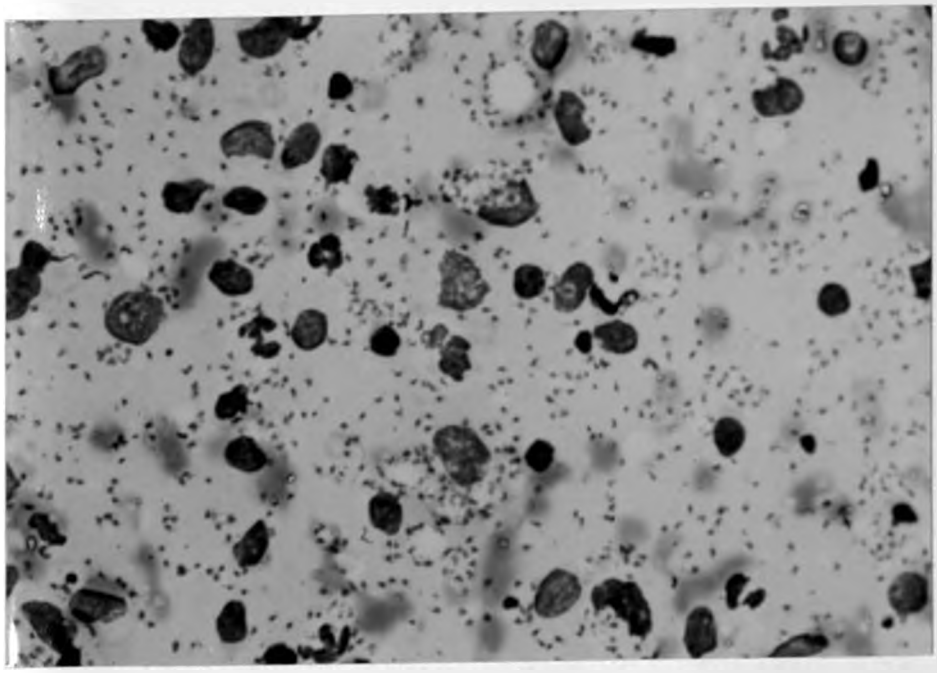

Plate 5. Amastigote smear from a cutaneous lesion of a patient

CHAPTER FOUR

DISCUSSION

Recent techniques described by Leger *et al.* (1983) and Killick-Kendrick *et al.* (1991, 1993) were used extensively in this study and enabled the identification of female sandflies of the subgenus *Larroussius*. A remarkable compliance to leishmanin skin test and to clinical and parasitological examination by the study population enabled an undertaking of a cross-sectional study.

Previous reports by Sang (1991) and Lawyer *et al.* (1991) that the vector of *L. tropica* was predominantly a cave inhabiter implied that the search for the sandflies had to be concentrated in the caves. The observed variation in abundance of each species could be explained either by limited sampling methods which were restricted to active search by aspiration and occasionally CDC light traps or by differences between species longevity and resting habitats. In similar studies sampling methods and species longevity have been found to influence species abundance or catches (Killick-Kendrick, 1990). Sandfly species favoured by their suitable microhabitat and have higher longevity than other species, may predominate in the samples of sandflies collected during a season.

In the present study sandflies were observed resting closer to the periphery of cave openings during the rains while they retreated

deeper into the caves or were completely absent in shallow caves during dry seasons. This may not only explain the fluctuations of sandfly populations but also the transmission of cutaneous leishmaniasis in the study area. In other related studies more sandfly vectors of *L. aethiopica* were caught in the wet than in the dry season (Sang and Chance, 1993). Seasonal sandfly population dynamics in the Utut focus is at present not properly understood.

The gut promastigotes which grew in culture could not be identified because they died out after six weeks of sub-culture. The cause of this was not immediately established but factors such as the efficiency of NNN culture media could be a possible explanation as cultures revealed no contamination on examination. Some studies have shown that, East African strains of *Leishmania* grow best in Schneider's *Drosophila* medium supplemented with either 20% (v/v) fetal bovine serum (FBS) or 15% (v/v) defibrinated rabbit blood (DRB), and that the cutaneous strains grow best in a mixture of Cunningham's SM medium and RPMI medium 1640 supplemented with 20% (v/v) FBS (Lightner and Githure, 1983). These culture media were not used owing to their unavailability at the time of this study. However, the *in vitro* cultivation of the strains was carried out before *Leishmania tropica* was discovered in Kenya and hence the suitability of these culture media in supporting the growth of *L. tropica* is not known at present. Previous typed isolates from the Utut focus confirmed the parasite as *Leishmania tropica* (MON-119) although a very rare dermotropic form of *L.donovani s.l.* (MON-82)

from one patient was detected (Sang, 1991; Sang *et al.*, 1992a). In the same focus Johnson *et al.* (1993) isolated *L. tropica s.l.* The parasite rate was computed on the assumption that sandflies which had had a blood meal were more likely to be infected than those which had never had a blood meal according to Killick-Kendrick (1990). In other studies sandfly infection levels have been explained in terms of infection levels in reservoir hosts and the periodic fluctuations in climate (Hoogstraal and Heyneman, 1969).

The proven vector *Phlebotomus guggisbergi* was collected in the present study. This concurred with previous studies by Sang *et al.* (1992a) in which the specimen was positively identified according to the technique of Killick-Kendrick *et al.* (1991, 1993). The finding of positive *P. saevus* and its anthropophilic activity in the present study suggests that this sandfly is most probably a vector of cutaneous leishmaniasis in Kenya. Nevertheless, further studies are needed to establish the form of promastigotes in this sandfly and their transmission in various foci of cutaneous leishmaniasis. The subgeneric relationship between *P. saevus* and *P. sergenti*, the proven vector of *L. tropica* in the Old World, and possibility of vectorial role of the former, have been described (Mebrahtu *et al.*, 1988; Lawyer *et al.*, 1989; Sang., 1991; Sang *et al.*, 1993b). *P. aculeatus* from Utut has been reported to harbour *L. tropica s. l.* (Johnson *et al.*, 1993). These findings suggest that the epidemiology of cutaneous leishmaniasis in Utut and probably

other foci of similar characteristics in Kenya could be much more complex than was previously thought, with a possibility of an overlap in the transmission of *Leishmania* parasite species (Sang, pers comm).

The 29 caves sampled were distributed throughout the study area, although other sites with caves were inaccessible due to complex landscape. Nearly all caves had history of human habitation and harboured sandfly species distributed sympatrically but with variable proportions. This has a significant implication in the epidemiology and transmission of cutaneous leishmaniasis in that infections had been inferred to occur in caves where man and sandflies co-existed or in human working places close to caves or rock crevices (Sang *et al.*, 1994). The distribution of sandflies in nearly all endemic foci is poorly understood because of dearth information on adult sandfly breeding habitats and the larval requirements (WHO, 1984). The trapping of a sandfly larva from one of the caves may imply that sandflies live and probably breed in these caves.

Infected sandflies were among the catches from two out of the 9 sites: Stech-Kubwa and Msalaba. In other related studies Msalaba was reported to be the site of most cases of cutaneous leishmaniasis due to *L. tropica* (Sang *et al.*, 1992a, 1994). The distribution of the proven vector and of suspected vector sandflies throughout Utut indicates that disease transmission

possibly occurs in nearly all the 9 sites.

Sang *et al.* (1992b) have established the reservoir role of rock hyrax, *Procavia johnstoni* at Utut. Casual observations indicated that rock hyraxes occurred in caves inhabited by sandflies. This implied that infections occurred when man intruded into the zoonotic cycle of transmission between the sandfly and the animal reservoir host. Accidental human infections when man intrudes the cycle of transmission has been observed in other endemic areas of cutaneous leishmaniasis (Ashford *et al.*, 1973; Sang *et al.*, 1993a).

Cutaneous leishmaniasis in this focus is popularly known as "Utut disease" by the inhabitants. The lupoid, chronic cutaneous leishmaniasis lesion which healed at the centre while advancing at the periphery was similar to one described by Sang *et al.* (1994) as "leishmaniasis recidivans". The higher rates of active cutaneous lesions in Utut than in the adjacent area, Kongasis, implied that Utut residents were more frequently exposed to infection than the Kongasis population which occasionally visited Utut. The occurrence of active lesions, detection of amastigotes and isolation of promastigotes in sandflies indicated an active transmission at Utut. In other related studies detectable *Leishmania* from active lesions has been used as a presumptive evidence of active transmission (Kadaro *et al.*, 1993).

There was no attempt to determine the duration of lesions as described by Sang *et al.* (1994) but parasites were detectable in this study up to 3 months following appearance of a lesion in a male aged 3 years in the Utut focus. In similar studies Sang *et al.* (1994) reported detectable *Leishmania* parasites from lesions of up to 2 years duration although the lesions continued to grow in size.

The steady increase of active cutaneous lesion and scar rates to a higher level in adulthood in the population sample, thus depicting an age-specific disease pattern was consistent with other endemic areas (Southgate, 1964; Southgate and Manson-Bahr, 1967; Leeuwenburg *et al.*, 1983; Ali and Ashford, 1993a) and with human habitation in caves or in "daki". In other related studies high cutaneous leishmaniasis scar rates suggestive of past infection have been found among people of older age groups in a focus of an active transmission (Moskovskis and Duhanina, 1971; Sang *et al.*, 1993a, 1994). That there was more infection in males than in females implied a change in occupational sex-related exposure to disease. Males cut trees for poles or charcoal burning but females usually fetch firewood from Utut. Males may be thus more at risk of infection than the females probably because of duration of exposure to infected sandfly bites.

The introduction of the parasite into a non-immune population, migrants who have not been previously exposed to cutaneous leishmaniasis, and a high rate of disease transmission may explain

the high rate of prevalence at Utut. In similar studies, a high rate of leishmanin skin positivity and of cutaneous lesions could be explained by presence of a non-immune population and a high rate of disease transmission (Kadaro *et al.*, 1993; Sang *et al.*, 1994). The increase in leishmanin positivity has been found to reflect a delayed-type hypersensitivity reaction against *Leishmania* antigen expected to persist for life and possibly be protective to reinfection (Southgate and Manson-Bahr, 1967; Southgate and Oriedo, 1967; Leeuwenburg *et al.*, 1983; Kadaro *et al.*, 1993).

Occurrence of leishmanin skin negativity in a patient with an active cutaneous leishmaniasis lesion at Utut implies that the individual may not have yet been sensitized. The presence of leishmanin positivity, without evidence of cutaneous leishmaniasis active or healed lesions, suggested possibly the occurrence of subclinical infections with leishmaniasis which has been reported frequently in other related studies (Manson-Bahr, 1961; Kadaro *et al.*, 1993; Ali and Ashford, 1993a; Sang , pers com). In some studies the delay in the development of cutaneous leishmaniasis lesions resulting in non detection of cases clinically has been explained by variability in incubation period and a slow progress of morbidity (Sanchez *et al.*, 1992).

Normal controls from non endemic areas and not known to have been exposed to *Leishmania* showed a negative leishmanin skin test. World Health Organisation (1984) has recommended the use of cases and

control population to define leishmanin positivity under local conditions. The fact that some leishmanin skin positive individuals from Kongasis had not visited or lived in Utut suggested possibility of disease having been contracted elsewhere by the settlers. Longitudinal studies could be required to substantiate this hypothesis.

Conclusion

The distribution of proven vector *P. guggisbergi* and other possible vectors of phlebotomine species in Utut suggests that cases of cutaneous leishmaniasis may have contracted the disease throughout the focus as they moved frequently or settled in different sites. Isolation of promastigotes from *P. saevus* suggests its vectorial status although parasites were not typed.

The progressive increase of leishmanin skin positivity and cutaneous leishmaniasis lesions to adulthood implied that Utut was typical of an endemic focus of cutaneous leishmaniasis and that people of all ages were at risk of contracting the disease as they continue to immigrate into the area.

People in the age group 20 to 29 years were relatively at highest risk of contracting cutaneous leishmaniasis, consistent with their frequent movement in the focus when burning charcoal. Leishmanin skin positivity in people from Kongasis who had not visited Utut

suggested that leishmaniasis could have been contracted from other foci of leishmaniasis transmission in Kenya. It is recommended that longitudinal studies be conducted to justify this hypothesis. Further studies are required to establish the *Leishmania* from *P. saevus* in Utut focus.

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