ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

PRESENT STATUS OF CUTANEOUS LEISHMANIASIS, HYRAXES AND *PHLEBOTOMUS LONGIPES* IN META ABO, SEBETA

WORKU NEGASH

JUNE, 1988

PRESENT STATUS OF CUTANEOUS LEISHMANIASIS, HYRAXES AND *PHLEBOTOMUS LONGIPES* IN META ABO, SEBETA

A Thesis
Presented to the
School of Graduate Studies
Addis Ababa University

In Partial Fulfillment
of the Requirements for the Degree of
Master of Science in Biology

---

by
Worku Negash
June, 1988

ACKNOWLEDGEMENTS

I wish to express my deep gratitude to Dr. Teferi Gemetchu, Associate Professor, Department of Biology, Faculty of Science, Addis Ababa University for the supervision of the work, guidance, support, encouragement and help in data interpretation.

I am also very grateful to Ato Asrat Hailu for material and intellectual assistance from the beginning to the end. My sincere thanks also go to the Institute of Pathobiology, A.A.U., for the assistance and cooperation.

Thanks is due to Dr. Dick Ashford, Liverpool School of Tropical Medicine, for his comments on the proposal during his brief stay in Ethiopia. Dr. Mesfin Tadese deserves special thanks for his identification of plant specimens.

Similarily, I would like to thank the National Meteorological Service Agency, Addis Ababa, for providing me with climatic data of Sebeta and the Swedish Agency for Research Cooperation with Developing Countries (SAREC) for funding my project.

Lastly, my acknowledgement goes to Wt. Fekerte Tulu who promptly and patiently typed this dissertation.

TABLE OF CONTENTS

Table of Contents (Cont'd.)

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Study on the present status of cutaneous leishmaniasis (CL), hyraxes and *Phlebotomus longipes* was made from March, 1987 to April, 1988.

A house to house survey on the population (1129 persons) of Meta Abo hills revealed 13 active cases (11.5/1000 and 43 (38/1000) individuals with scars of CL. Of all changes in human attributes during the past seventeen years, the recently started villagization seems to have a reducing tendency on prevalence. Active cases were found aggregated around hyrax habitats and most common in youth and middle ages. Males were more affected by the disease than females. It was, therefore, suggested that most infections probably occurred near hyrax habitats.

From a year round field observation on hyraxes, it was demonstrated that hyraxes produced youngs twice a year (i.e. in October and March) and that their low diel activity occurred mainly in the early mornings and late afternoons. Hyraxes of the area were seen in small colonies (2-8) and were not abundant (about 23 individuals in two habitats).

Ten hyraxes were trapped from two of the three selected hyrax habitats. Although all specimens displayed characteristic features of *Heterohyrax* spp., variation in form was observed

between the two populations seen in Meta Abo area. Five specimens were examined for leishmanial infection but all turned negative.

Population estimates of Phlebotomus longipes have shown a unimodal peak in August and September. Low population occurred during the dry months, namely, December to January.

Dissections of 834 females revealed a bimodal distribution of parous rates. One peak occurred at the end of the dry months (December and January) and the other in May and June. This may indicate two distinct generations in a year.

Only 1 out 514 (0.2%) females (fed, gravid and parous) collected from the three sites was found infected with leishmanial parasites.

From the population estimates and distribution of parous rates, it was concluded that the peak transmission season was in August and September.

1. INTRODUCTION

Italian authors were the first to describe cutaneous leishmaniasis (CL) in Ethiopia (Martoglio, 1912; Monti, 1937; Poggi, 1937 and Cupi and Cattapan, 1942); cited by Ayele et al.(1981). Reports of several researchers (Balzer et al., 1960; Poirier, 1964; Price and Fitzherbert, 1965; Bryceson and Leithead, 1966; Lemma et al., 1969; Ashford et al., 1973; 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 (Ayele et al.,1981). To date CL has been reported from ten administrative regions of Ethiopia (Ayele et al.,1981). However, it is suspected that there is no region free from CL including Addis Ababa (Sarojini et al., 1984).

On the basis of its distinct epidemiology, geographical distribution and clinical manifestations, the causitive agent of CL in Ethiopia was determined as *Leishmania aethiopica* by Bray et al.(1973).

Much of the information concerning the epidemiology of CL in Ethiopia has been obtained from studies in endemic foci such as Ochollo, Aleku, Kutaber and Sebeta (Lemma et al.,1969; Ashford et al.,1973). Cutaneous leishmaniasis due to *L.aethiopica* is a zoonosis as are most of the leishmaniasis 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 rocks hyraxes (Procavia capensis = P.habessinica 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 (microfocal) clusturing of cases (Wilkins, 1972) suggest possible man to man transmission. However, this may not maintain the parasite.

No rodents have been reported to be naturally infected with L. aethiopica except a single report by Mutinga (1975b) from the Giant rat, Cricetomys sp., the significance of which is not known. In Kenya, Heterohyrax johnstoni and Dendrohyrax arboreous are the reservoir hosts of L. aethiopica.

The natural invertebrate hosts (P.longipes and P.pedifer) have never been reported to be vectors elsewhere except for P.pedifer in Kenya (Mutinga, 1975a; Mutinga, 1971).

So far CL due to L.aethiopica is only found in Ethiopian and Kenyan highlands. CL in Ethiopia is restricted to areas between 1500 and 2700m (Ashford and Smith,1985).

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 of above 800 mm.

Clinical manifestations of CL due to L.aethiopica ranges from self-healing localized cutaneous leishmaniasis (LCL) to diffuse cutaneous leishmaniasis (DCL). The latter form of the disease is thought to be due to the immunological incompetence of the host (Bryceson and Leithead,1966; Bryceson and Nichol,1966; Bray and Bryceson, 1969). More recently, 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). Although heterogeniety is known among the various isolates of L. aethiopica (Schnur,1986), its identity as a single species has been confirmed by more objective means of characterization (Le Blancq et al.,1986a; Gardner et al., 1974; Schnur and Zuckerman, 1977; Chance, 1979).

Extensive entomological (Foster, 1972a,b; Foster et al.,1972) and quantitative epidemiological studies (Wilkins, 1972) were made at Sebeta after a preliminary survey by Lemma et al. (1969). Unsuccessful attempts were made to isolate leishmanial parasites from rodents (Lemma et al.,1969,1970) in Meta Abo focus.

Distribution of sandflies and leishmaniasis is not static (Lewis, 1971,1974) and changes as several factors change. Alekseeve et al.(1986) have recently reported that agricultural practice has suppressed the activity of leishmaniasis focus in five years. It is now seventeen to nineteen years since Lemma et al. (1969), Wilkins (1972) and Foster (1972a,b) made epidemiological investigations in Meta Abo, Sebeta. So far nothing has been done to control CL in Meta Abo area except for treatment of some cases who managed to visit the Institute of Pathobiology, Addis Ababa University and All African Leprosy and Rehabilitation Training Center (ALERT). During the past seventeen to nineteen years ecological and social changes are known to have taken place around Meta Abo as a result of nationalization of land.

The bionomics of *P.longipes*, seasonality, age structure, infection rate and vector status in the habitat of the natural host (i.e. hyraxes) have not been clearly elucidated in previous studies carried out in Sebeta.

The aims of the present study are to:

1. Make a year round observation on the availability and behaviour of hyraxes in selected sites in Meta Abo, Sebeta.
2. Try and isolate leishmanial parasites from hyraxes trapped in this area.

3. Survey the human population for scars or lesions.

4. Study the vector status of P.longipes in and around hyraxes habitats by determining the seasonality and age structure of the sandfly, degree of anthropophily, infection rates and possible peak transmission season.

Information obtained will provide a base line data on the present situation of leishmaniasis in Sebeta and how, when and where transmission occurs. In the present study emphasis had been given to number, activities, habits and infections of hyraxes and sandflies of the area.

1.1 Hyraxes: Ecological Note

Procavidae is the only extant family in the order Hyracoidea which is considered to have evolved in Africa before the Oligocene or 40 million years ago (Walker,1975). At present hyraxes are widely distributed in Africa, Arabia and the Mediteranean regions (Kingdon, 1971; Walker, 1975; Corbet, 1979). Procavia, Heterohyrax and Dendrohyrax are the three genera in the family Procavidae. The first two represented by P.capensis and H.brucei in Ethiopia. Both Procavia and Heterohyrax comprise allopatric forms which posed considerable taxonomic problems (Corbet, 1979). P. habessinica, for example, was considered to constitute more than one species (Ashford, 1977). However, Corbet (1979) named P.habessinica as P.capensis. This nomenclature is now accepted by Ashford and Smith (1985).

Hyraxes live in a wide variety of habitats and found in altitude ranging from sea level to a hight of 4650 in Kenya (Kingdon, 1971; Walker, 1975).

It is apparent that rock hyraxes select suitable habitats which provide protection from predators and shelter from extreme climatic and other conditions. They inhabit cavities and crevices in rock out crops, cliffs and boulder rock formations, mountains and escarpments. 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 (Sale, 1966; Kingdon, 1971) augmented their poor thermoregulatory system. Hyraxes living in rock habitats avoid isolated holes, those facing prevailing wind and those which are large enough for predators to enter (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 outcroping rocks in Zway and Aleku (Ashford,1977).

Heterohyrax brucei is known to feed in the early morning and late afternoon (07:00 to 10:00 and 16:00 to 18:00) hours and may be active at night in moon light (Kingdon, 1971). *Procavia capensis* of Kutaber area feeds at dusk and dawn; throughout the day in a dry month (Ashford *et al.*,1973). Hyraxes are known to be catholic feeders.

Hyraxes have long gestation period (upto 230 days) and a female usually produces two youngs (Delany and Happold, 1979), although upto six youngs have been recorded (Walker, 1975). No accurate estimation of colony size is possible because of limited diel activity of hyraxes outside their holes. Under no circumstances would one find all colony members outside. Authorities have estimated 5 to 60 members in a colony (Walker, 1975; Kingdon, 1971).

1.2 Cutaneous leishmaniasis of the Old Wórld

Distribution of leishmaniasis is changing because of several factors (Lewis, 1971, 1974; Maskovski and Duhanina, 1971; WHO, 1984; Ashford, 1986; de Raadt, 1986) so that monographes are revised from time to time. This may be due to actual change in disease prevalence and incidence or technological achievements in taxonomy and identification of parasites and vectors as well as successful efforts in case detection.

Leishmaniasis may increase or decrease depending on the type and intensity of human interference in the habitat of the vectors or reservoirs and/or change in the behavior of vector or reservoir host species. Lewis (1974) recognized human activity to have initial and ultimate effect. In general, urban development and activities such as deforestation, irrigation and other human activities initially bring people into contact with vectors, the ultimate effect of which is destruction of breeding and resting sites of vectors or the threatening of natural hosts. Irrigation schemes and military movements in the arid zones of *L.major* in the Middle East (Ashford,1986), agricultural activity (irrigation) in Senegal (Dedet *et al.*, 1979) and in USSR (Neronov and Guinin, 1971) resulted in several epidemics or created man-made foci. Provision of water and vegetation may attract or create an ideal condition for the reservoir hosts (eg. *Rhombomys*) and vector species (eg. *P.papatasi*). Conversely, destruction of burrows by ploughing or raising of water table through irrigation have negative effects (Lewis, 1974; Lewis, 1971). All of these situations depend on the response of vector or reservoir species to human encroachment (Bray,1983).

The Parasite

Leishmania major (=*L.tropica major*) and *Leishmania tropica* (=*L.tropica minor*) were earlier separated into distinct types by Bray *et al.*(1973) on the basis of classical means. This classification has been later supported by biochemical means (Chance, 1979;Greenblatt *et al.*,1983; Le Blancq *et al.*,1986b; Le Blancq and Peters, 1986). As far as is known Old World cutaneous leishmaniasis (OWCL) is caused by the above two species and *L.aethiopica* and rarely by *L.donovani*.

L.major is widely distributed in the Old World stretching over the arid tropical and subtropical regions.

L.donovani causes cutaneous diseases in Sudan but the majority of cases are suspected to be due to L.major (Ashford and Smith,1985). A parasite resembling L. major has been isolated from Arvicanthis niloticus in the Omo Valley in Ethiopia (Haile and Lemma, 1977), and from several rodents in Kenya (Mutinga and Nkogo, 1986; Githure, 1986). L.major is also found in North and West Africa (eg. Libya, Senegal) (Dedet et al., 1979; Ashford, 1986). It also stretches from Mongolia, Soviet Asia and Afghanistan through the Middle East (Le Blancq et al., Ashford, 1986b).

L.tropica is less widely distributed than L.major (Ashford, 1986). L. tropica causes CL in urban areas and is found in Europe, Asia (eg. Israel, Iran) and Africa (eg. Tunisia and Uganda) (Le Blancq and Peters, 1986).

Sandfly Vectors

The taxonomy of sandflies is still a controversial issue and whether to consider sandflies as an independent family or assign them a subfamily status is not yet settled. For practical reasons, Lewis et al.(1977) proposed a stable classification which recognized five genera in the subfamily Phlebotominae. They are Phlebotomus and Sergentomyia of the Old World and Warileya, Brumptomyia and Lutzomyia of the New World. This system of classification has been widely accepted although it is not "immune" to oppositions. For example, Ready et al. (1980) in Lane (1986) have strongly proposed genus level for the Psychodopygus although this had been assigned a subgenus status in the genus

Lutzomyia by Lewis et al.(1977) and WHO (1984). Lewis (1982) rejected the proposal of Ready et al.(1980) as he believed that this would unnecessarily lead to multiplication of genera. The major problem in identification of sandfly is the presence of cryptic species (WHO, 1984; Lane,1986). In Ethiopia P.longipes and P.pedifer females are morphologically indistinguishable. Laboratory colonization studies of P.longipes (Foster et al.,1970) and P.longipes and P.pedifer (Gemetchu, 1974; 1977) have revealed several biological and behavioural information.

Only Phlebotomus and Lutzomyia have proven or suspected vectors o[illegible] in the Old World and in New World, respectively. There is no clear indication about vector status of Sergentomyia. Several Sergentomyia species are known to bite man (Lewis, 1974). S.ingrami was shown to be attracted to a mammalian host (Mutinga et al.,1986a) and was found infected with untyped Leishmania sp. considered as L.major by the behaviour of the isolate in animal model (Mutinga et al., 1986b). The finding of these authors has not yet been fully accepted.

Seventy species or subspecies are reported to be proven or suspected vectors of leishmaniasis in the world (WHO,1984). Among the fifty two species or subspecies listed by Killick-Kendrick (1978) twenty eight species of Phlebotomus are proven or suspected of being vectors of leshmaniasis in the Old World. Six out of eleven subgenera of Phlebotomus have species that serve as vectors of leishmaniasis (Lewis, 1982; WHO, 1984). These are Phlebotomus, Paraphlebotomus, Synphlebotomus, Larroussius, Adlerius and Euphlebotomus. Larroussius, Phlebotomus and Paraphlebotomus are the most important subgenera.

Phlebotomine sandflies occupy most of the zoogeographical regions of the world, namely, Palaearctic, Oriental, Afrotropical, Nearctic, Neotropical and Australasian region (Lane, 1986). Each of the zoogeographical regions has its own characteristic sandfly fauna. Most of all factors, temperature is considered to limit the worldwide distribution of sandflies (WHO, 1984; Lewis, 1982). Unlike their relatives (Nematoceran) sandflies breed in biotopes largely independent of surface water (Lewis, 1971). In extreme temperatures sandflies survive in diapause.

The large number of species in the genus Phlebotomus, in Ethiopia, is unusual to the features of Afrotropical sandfly fauna (Ashford,1974). Sergentomyia is the principal genus in this zoogeographical region. Species of the two genera overlap with distinct altitudinal strata of dominance. Species of the genus Phlebotomus dominates in higher altutides while the other outnumber in lower elevations (Ashford,1974). This gives an intermediate position to sandfly fauna in Ethiopia.

The subgenus Larroussius includes most of the vector species of the Old World, visceral leishmaniasis due to L. donovani and CL due to L. aethiopica in the highlands of Ethiopia and Kenya. *P. longipes* and *P.pedifer* occupy diverse biotopes in Ethiopian highlands (Ashford,1974). Their distribution is limited by altitude unlike hyraxes (Ashford, 1977). *P.pedifer* is found in Kenya (Minter,1964; Lewis *et al*,1972) and also reported from Sudan by several authorities (Peters *et al*.,1977; White, 1977; Lewis *et al*., 1972) (all cited by Lewis 1978).

The subgenera *Phlebotomus* and *Para Phlebotomus* consist of vector species of *L.major* and *L.tropica*, respectively (WHO,1979; Nadim *et al*., 1979). *Phlebotomus duboscqi* and *Phlebotomus papatasi* are the known vectors of *L.major*. *P. duboscqi* is an important vector of *L.major* in Kenya (Beach *et al*., 1984; Mutinga *et al*.,1986b; Kaddu, 1986), possibly in Sudan (Ashford and Smith, 1985) and Sahelian belt of West Africa (Dedet *et al*., 1979 WHO,1984). *P.Papatasi* is the prime vector of *L.major* in the other parts of the Old World (Le Blancq *et al*., 1986b; Schnur, 1986). *L.tropica* is mainly transmitted by *P. sergenti* in much of its range (WHO, 1984; Le Blancq and Peters, 1986). Although *P.papatasi* is common in the various endemic areas of *L.tropica*, the distribution of *P.sergenti* better correlates with the disease (WHO, 1984; Le Blancq and Peters, 1986).

Reservoir host

L. major is primarily a parasite of rodents. In East Africa, murine rodents are the major reservoir hosts. *Arvicanthis niloticus* in Ethiopia (Haile and Lemma, 1977) and several rodent species including Arvicanthis, Tatera, Taterillus and Aesthomys in Kenya are known to carry L.major (Githure, 1986; cited Githure et al., 1984; Mutinga and Ngoka,1983). In Senegal Arvicanthis, Tatera and Mastomys are the reservoir hosts (Bray,1983; Dedet et al., 1979). L. major is maintained by Meriones and Psammomys species in Israel, Saudi Arabia, Libya and Morrocco (Bray, 1983; Ashford,1986). The various species of Rhombomys and Meriones are the major reservoir hosts in Mongolia, Soviet Asia, Afghanistan and parts of the Middle East (Bray,1983; Le Blancq et al., 1986b).

L.tropica is widely known as causitive agent of anthroponotic cutaneous leishmaniasis in the Old World (WHO,1984; Ashford, 1986; Schnur,1986). However, there is no general agreement regarding the wild host of the parasite. L.tropica has been isolated from dogs and rodents (Bray,1983; Ashford, 1986, WHO, 1984).

1.3 Breeding, Resting and Feeding Habits of Sandflies

The immature stages develop in a variety of habitats with suitable temperature and high humidity. The larvae feed on decomposing organic matter.

Burrows of several reservoir hosts of cutaneous leishmaniasis provide ideal habitats for the breeding of vector species, especially in arid zones of L.major. It is well known that P.papatasi breeds well in Rhombomys burrows (Bray,1983). In Kenya breeding places of several vector species are identified (Mutinga and Odhiambo,1986b; Mutinga 1986; Mutinga et al.,1986b). According to the above studies, P.pedifer breeds on the moist portion of a cave. It is also demonstrated that sandflies breed in termite hills (eg. P.martini) and animal burrows (eg. P.martini, P.duboscqi) where adults rest. Some used wide range of breeding sites. Hyrax holes are beyond the reach of man and is difficult to fully investigate whether or not P. langipes actually breeds in the habitat of hyraxes eventhough larvae and pupae have been collected from nearby caves by Foster(1972a).

Knowledge of basic requirements on the development of immature stages is gained from laboratory studies. The developmental period of immature stages depends upon temperature, humidity and larval diet (Foster et al., 1970; Gemetchu, 1971,1976,1977). In general, 53 to 100 days were recorded from egg laying to adult emergence.

Most sandfly species of the Old World are active from dusk to dawn and rest during the day in appropriate resting sites. The choice of resting sites depend on host distribution, temperature, moisture soil and vegetation types. Sandfly species may have endophilic or exophilic habits. Some others (eg. P.langeroni) may be exophilic in peridomestic sites and may enter human dwellings to feed (Said et al., 1986). However, there is no demarcation in resting habits among several species. P.papatasi rest outdoor in Sudan (Lewis,1971) but in the majority of cases they rest inside houses. P.longipes is sylvatic in the various Ethiopian highlands but it rests where it has fed in Sebeta area (Foster,1972a) and in Addis Ababa it rests in houses P.pedifer is peridomestic in Ochollo (Gamo Gofa) (Ashford et al., 1973) unlike the situation in Kenya (Mutinga, 1975a).

Many adult sandflies of both sexes feed on sugar. The gut of sandflies is normally sterile and a chance contamination is fatal to the insects (Adler and Theodor, 1957; Killick-Kendrick,1979). Natural source of sugar meals and their role in transmission of leishmaniasis are not well known. Several postulates and evidences were proposed by authorites. Killick-Kendrick (1979) recruited aphids or coccids as the major source of sugar meals in nature. This is based on the availability of the above sources in sandfly habitats and its high nutritive value. It is observed that P.longipes, P.orientalis, S.africana magna and S.bedfordi probe on stems and leaves of different plants. Schlein et al. (1985b) have experimentally shown that P.papatasi fed, in the same manner as above, selectively on several plant species brought from their natural habitats.

It is demonstrated that the crop contains antibacterial factors (Schlein et al.,1985a). The various meals (sugar, blood) are routed selectively by the various modes of feeding in P.papatasi (Schlein et al.,1986 Schlein & Warburg, 1985). Sugar feeding mode directs the food to the crop. The presence of antibacterial factors in the crop and behavioural selective routing of meals are considered as systems of control and avoidance of bacterial contamination which are aimed to protect the sandflies (Schlein *et al*.,1986).

Only female sandflies are equiped with piercing-sucking type of mouth parts which enable them to feed on vertebrate blood. Except in some autogenous species (eg. *P.papatasi*) a female needs at least on bloodmeal to develop and lay her eggs.

Species of the genus *Phlebotomus* are generally mammal feeders and those of *Sergentomyia* feed on reptiles. However, species of *Sergentomyia* are known to feed on mammals including man (Lewis, 1971).

Mammalophilic sandflies are either zoophilic, anthropophilic or opportunistic. Some species which live in houses or peridomestic sites feed mainly on man by preference (eg. *P.argentipes* in India). However, nowadays several species are reported to be opportunistic (Mutinga *et al*.,1986a; Javadian *et al* 1977). A good example of this is the situation of *P. papatasi* in an Iranian village. It is observed that *P.papatasi* has a wide range of natural host.

1.4 Leishmanial in Sandfly

Behaviour of leishmania parasites in the gut of sandfly vary depending on the parasite - sandfly combination (Killick-Kendrick, 1979; Molyneux, 1977). Mammalian species of Leishmania display three morphological forms, namely,amastigote, promastigote and paramastigote. Amastigote engorged with bloodmeal of a sandfly divides in the midgut, more than one time and later transforms to promastigote forms. Promastigotes mainy multiply in the stomach region of the midgut as the peritrophic membrane is seen to break anteriorly (Gemetchu, 1974; Molyneux 1977 Killick-Kendrick, 1978,1979). Route of migration and developmental sites vary depending on species of Leishmania (Killick-Kendrick,1979). All Leishmania species of mammals other than the L.braziliensis complex migrate anteriorly to the thoracic midgut (anterior midgut)., oesophagus and cibarium.

Duration of the life cycle of Leishmania in sandflies ranges from 4 to 18 days (WHO,1984). In Ethiopia, P.longipes, fed on lesions of CL human cases, developed anterior infection (anterior midgut infection) 7 to 10 days later (Lemma et al.,1969) and 8 to 14 days (Foster,1972b). Similarily fed P.pedifer transmitted L.aethiopica to hamster after 9 to 13 days (Mutinga and Odhiambo,1986a).

The only study on the behaviour of L.aethiopica in naturally infected vectors are those of Ashford et al.(1973) in Ethiopia and Kaddu and Mutinga (1981) in Kenya. The former authors have noted the pattern of parasite distribtuion in relation to abdominal conditions of the female P.longipes. L.aethiopica promastigotes were found attached to the midgut of naturally infected P.pedifer (Kaddu and Mutinga, 1981).

Transmission is mainly by the bite of infected vector species. However, controversial evidences on the mechanism of transmission are still in progress. Multiple probing by infected sandflies as a result of difficulty in obtaining bloodmeal increased rate of transmission (Beach et al.,1984, 1985). There is also evidence that attached parasites reduced the pumping ability of cibarium and pharynx (Jefferies et al.,1986). In addition, the proboscis forms are considered to be the infective stages of Leishmania promastigotes (Killick-Kendrick and Molyneux,1981; Killick-Kendrick and Ward,1981).

2. MATERIALS AND METHODS

2.1 The Study Area

Sebeta is about 25 kms south-west of Addis Ababa along Addis Ababa-Jimma road. Meta Abo area is located about 5 kms west of Sebeta town. The road running to Jimma divides Meta Abo area into a flat plain farmland to the south and a stony farmland on the hills to the north and lies on the southern slopes of Mt. Wuchacha. A side road from Meta village runs to Meta Abo Brewery. Meta-Suba road (leading to Menagesha forest, a conservation area) branches northwest at about the midpoint of Meta village. The road serves as a boundary for a plain farmland to the west (now villagized) and the Western slopes of Meta Abo hills to the east (now deserted). The inhabitants of the hills were transferred to the new area one to two years ago. In spite of the transfer the farmers still farm on their old farms. On the other hand peasants on the eastern slopes of Meta Abo hills were re-organized on one of the hill tops and in a valley (not shown on the map) without much displacement.

The focus of the present study is the Meta Abo hills (i.e., the eastern, western and northern slopes) from where peasants were transferred or re-organized. Three hyrax habitats which harboured sandflies were selected. Two of the study sites are distributed in the lower Meta Abo valley (S_1) and the northern hills (S_3) corresponding to Zone C and Zone D of Wilkins (1972), respectively. The

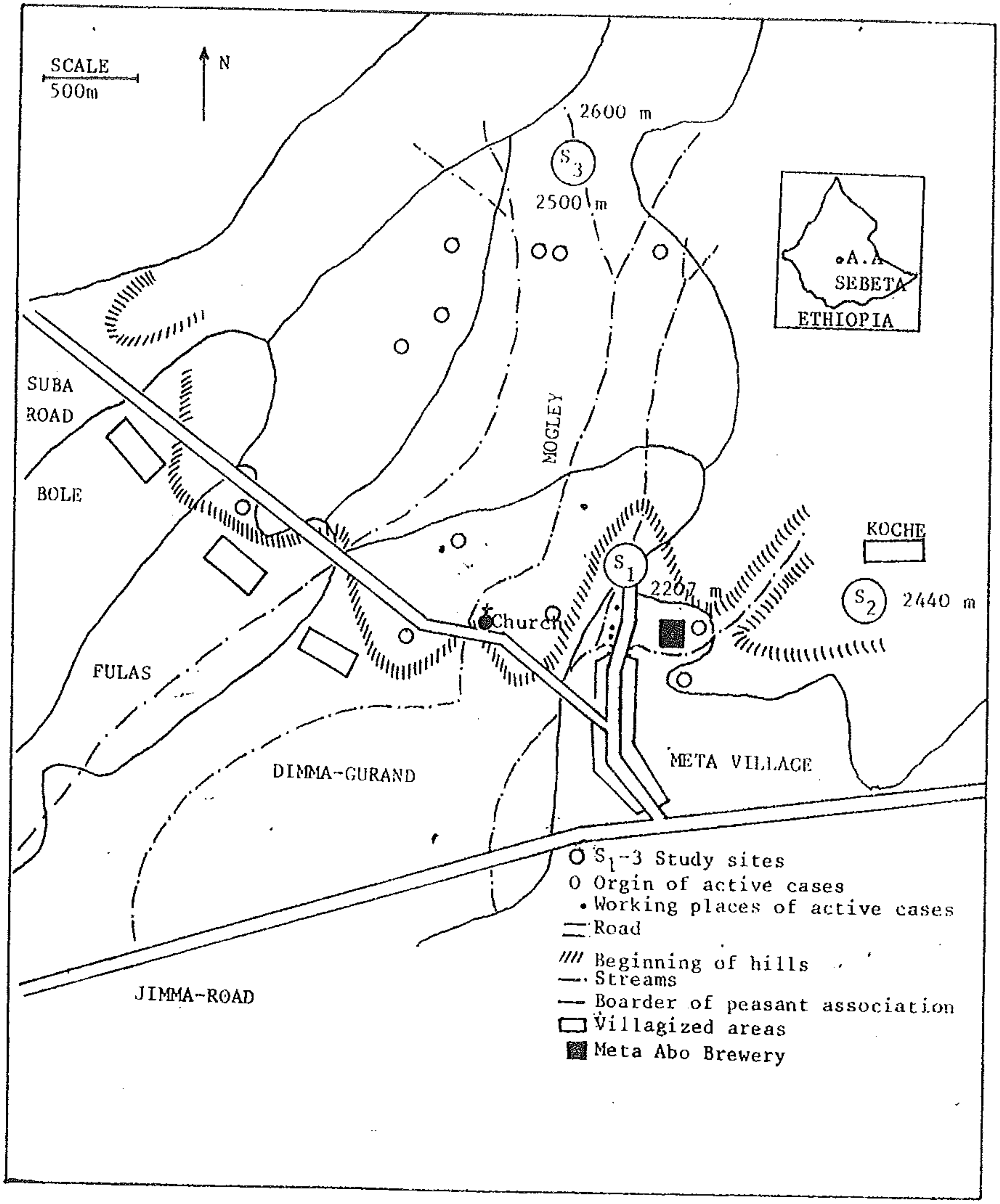

Figure 1. Sketch map showing the different study sites, Meta Abo area

third site (S_2) is situated on the eastern hills. Entomological and reservoir host studies were restricted within hundred meters of hyrax habitat. Most of the hills were covered to determine the prevalence of cutaneous leishmaniasis.

The Meta Abo area ascends from 2200m at the feet of the hills (eg. Meta Village) to 2430m at the top of eastern hills and 2600m at the upper limit of hyrax colony in the northern hills. Waterfalls at several points (including hyrax cliffs) feed streams which flow south-ward along the Meta Abo valley. The main stream is diverted to irrigate plantation of citrus fruits during the dry months. Because of this a waterfall near hyrax habitat (S_1), in the lower Meta Abo valley (a recreation site), usually dries out.

The primary vegetation has been removed by intense human activity. Nowadays, spiny shrubs such as Rosa abyssinicus and Carissa edulis predominate much of the hills. Various species of trees are either concentrated within a few meters radius or scantly distributed over several meters, or restricted to the banks of streams. Juniperus procera, Croton macrostachym, Podocarpus gracilior, and Ficus sp., which probably constitute some of the primary vegetation, are very scantly represented among the riverine vegetation. Though few in number, Juniperus procera and Erica arobrea still predominate in higher altitude (2600m) in the northern hill. . The former species is also found on the hills sides north of the brewery interspersed with Eucalyptus plantation.

Meta Abo area lies within the Ethiopian central plateau. There are nine rainy months with an annual rainfall greater than 800mm. The big rains occur from June to September. October to January are the dry months. March and April are periods of the small rains.

Over 155 families or more than 1000 persons depend on cultivation of cereal crops on the hill tops, sides and in the valleys. Severe water erosion has removed much of the soil to the extent that several abandoned farmlands are nowadays rocky. Meta Abo brewery is the most important industry in the area. The employees reside in Addis Ababa, Sebeta town, Meta village and in Meta Abo valley. Citrus fruit plantation and small scale animal fattenning are being practiced .

2.2 Study for Cutaneous Leishmaniasis in Meta Abo

House to house survey was conducted in April, 1988. Cutaneous leishmaniasis clinical survey form as recommended by Ayele *et al* (1981) was modified to meet the need for changed social condition (i.e villagization). This form was prepared to obtain information on sex distribution and household of the population. Exposed body surfaces of each available individual were physically examined for scar and/or lesion in cooperation with staff of the Institute of Pathobiology, Addis Ababa University. Oriental sore (OS) has a vernacular name, "Shahign", in various endemic areas of Ethiopia including Sebeta (Lemma *et al*.,1986).

Information obtained from family heads (eg. age of scar or date of onset of lesion) were mainly used for analysis.

2.3 Selection of Study Sites

The study area was visited in December 1986 January and February 1987, inorder to identify hyrax and sandfly habitats. Out of several likely hyrax habitats (Ashford *et al.*,1973) three habitats were positively identified. Potential resting sites of adult sandflies within a hundred meter radius of these hyrax habitats were searched taking into account the works of Foster (1972a) and Ashford *et al.* (1973). Some negative sites but with considerable potential as resting sites were reserved for concurrent observation since seasonal variation in resting sites of *P.longipes* has been reported by Foster (1972a) in Meta Abo area.

2.4 Vegetation Types of the Study Sites

A total of forty plant specimens (shrubs, trees, etc.) were collected from the three study sites (within hundred meters radius) in September, 1987. Prior to collection observations were made inorder to be familliar with the vegetation of the area. Value from one plus (+) to four (++++) were given to each plant specimen according to its relative abundance.

2.5 Observation on Hyraxes

2.5.1 Field Observation

The number of hyraxes seen in the early morning and late afternoon hours using a binocular was recorded for a year.

Ten observations were made for the whole day. Site 1 was selected for a regular count of population because of its suitability to observe and count hyraxes in the early mornings and late afternoons. A less regular observation was done on hyraxes in Site 3. Sentinel posts and feeding grounds of hyraxes in S_2 were the least conspicuous. The site was recognized as hyrax habitat based on the regular presence of fresh pellets at the entrance of a hole.

2.5.2 Search for Leishamanial Infection in hyraxes

Locally made snare traps (Hailu Mscthesis,1987) were set at the entrances of caves and rock holes and on feeding grounds.

Hyraxes trapped by this method were maintained and examined at the Institute of Pathobiology, Addis Ababa University, where the necessary conditions were already established. Tip of nose, lips and bare sides of ears were sterilized with Savlone and 70% alcohol and shaved. Samples for smears and culture were taken from the normal skin. Smears were fixed with absolute methanol & stained in Giemsa as recommended by Ayele *et al*. (1981). Slides were later examined for leishmanial parasites using a compound microscope and oil-immersi . lens. Samples were inoculated into NNN culture (Novy, MacNeal and Nicholle's) medium. One hundred units of Penicillin and a hundred μg of streptomycin were added to hundred ml of Lock's solution (the overlay) inorder to control bacterial contamination. Culture materials were incubated at 24^{o}C and examined every seven days for 2 month before discarding. Survived hyraxes were similarily treated again.

The animals were tentatively identified according to Kingdon (1971) and Ashford et al.(1973). Dead specimen were preserved as skin and skull for a later confirmation.

2.6 Collection of Sandflies

Sandflies, particularily *Phlebotomus longipes*, were collected with aspirator from their resting sites. *P.longipes* was the dominant or the only species available in hyrax habitat to deserve attention. Collection was done every fortnight from March 1987 to April 1988. Collections were consistent with respect to time of the day for a given site.

2.7 Estimation of Sandfly Population

No standard method was developed to quantify sandflies because of the diversity of biotopes they occupy. In the present study, catch per unit effort type of estimation was chosen. Resting adult sandflies were thoroughly searched and caught with aspirator within a hundred meter radius of the three hyrax habitats every fortnight, by myself. The first fifteen minutes catch was recorded separately inorder to follow the consistency of efforts. Resting sandflies in an abandoned house (made of stone, allover) were simply counted (i.e. without removing them with aspirators) concurrently to see the effect of sandfly collection on the population from the various collection sites in the study area. The walls and parts of the roof were marked into narrow blocks. This method has been used by Foster (1972a) to determine the seasonal abundance of *P.longipes* in Addis Ababa. Population index was calculated for each month applying the relation used by Ashford *et al.* (1973) for the sandflies of Kutaber, Wollo administrative region, Ethiopia.

$$\text{Population Index} = \frac{\;\frac{100n\bar{a}}{n_a}\;}{\frac{1}{N}}$$

where a = the number of flies per search

$\bar{a}$ = the monthly average per site

n = the number of months

N = the number of sites.

2.8 Mounting and Identification

Male sandflies were preserved in 70% ethyl alcohol. Specimens were later washed in distilled water for 24 hours cleared in Nesbitt's solution (Minter, 1963) for 48 hours and mounted in gum chloral mountant. Specimens were identified according to Lewis *et al.*(1974).

2.9 Dissection for Age Structure and Infection Rate

Female sandflies, collected by searching, were held mainly in KM-vials used by Gemetchu (1977) for colonization experiments. Sandflies could be kept alive in vials stoppered with cotton for longer periods. Live specimens were transported to the laboratory and dissected as soon as possible.

Females were killed and washed in 2% Savlone and dissected in physiological saline on a slide. Dissections were carried out by detaching the head and drawing out the entire gut posteriorly. The gut (especially the midgut) was examined under 10X and 40X objectives for leishmanial promastigotes. At the same time the conditions of the accessory glands and the gut were noted and females were classified as parous, nulliparous, gravid and fed. Head and terminalia of the dissected female were mounted in gumchloral mountant for a later identification, as mentioned above.

2.10 Human Exposure

Two individuals with exposed heads and arms, were seated adjacent to hyrax cliff (S_1) usually from 19:00 hours to 21:00 hours. The same two individuals were similarily exposed in a hotel 25 meters away from this cliff from 21:00 to 23:00 hours on the same day. Biting sandflies were removed with aspirator or test tubes as soon as the sandfly started probing. Wind flow and temperature reading were taken concurrently.

2.11 Isolation of Leishmanial parasites from Sandflies

Density, location and motility of promastigotes in the gut of an infected sandfly was recorded. Promastigotes were suspended in sterile physiological saline solution, on a slide, and asceptically inoculated into NNN-medium with sterile disposable syringe. Culture were incubated at 24°C and regularily subcultured.

Sample of positive culture from 4^{th} sub-passage was harvested by centrifuging at 4000 rpm for 10 minutes in a refrigerated centrifuge. One ml of mixed phase promastigotes ($5x10^5$ promastigotes) was inoculated into the nose of a gelada baboon and ears of grivet monkey by sub-cutaneous injection. Positive cultures samples from 5^{th} sub-passage were sent to England in Sloppy Evans medium (Evans et al.,1984) and also in bottles containing NNN-medium for isoenzyme characterization.

RESULTS

3.1 Human Cutaneous leishmaniasis in Meta Abo, Sebeta

Data were obtained from the population of Meta Abo hills during April 19-23,1988 .

1129 persons lived in 193 families.79.8% of the population were peasant farmers. The rest (20.19%) worked at the Meta Abo Brewery. Household size was large; 68.39% of the families had five or above household (Table 1).

Table 2 shows sex structure of the study population. In general, males were slightly more than females (51.2:48.8). Obvious sex differences is seen among persons working at the brewery.

3.1.1 Present and Past CL Cases

Thirteen relatively recent cases,11.51/1000, were found in Meta Abo hills (Table 3). Parasitological examinations were made on two active cases. One was smear and culture positive whereas the other was negative. 43 persons, 38/1000 of the population, were with characteristic scar of CL.

3.1.2 Relationship of Sites of Scar/lesion of CL to Preferred *P.longipes* Biting Sites as Observed in Meta Abo

Table 4 shows the relationship between sites of scar/ lesion to the preferred biting sites of *P.longipes*. Sites of *P.longipes* bite were recorded during collection from their resting places. Most bites were on part of the head (68.8%) and most commonly (17%) on the nose. A similar distribution of scar/lesion on the human body was observed.

Table 1. Distribution of Household in Families Living in Meta Abo Hills

Number of persons in household	1	2	3	4	5	6	7	8	9	10	11	12	13
Number of families	3	6	26	26	31	28	31	13	15	6	15	11	2

Table 2. Sex-Distribution in Meta Abo Study Population

Village	No. of Males (%)	No. of Females (%)	Total
Dimma-Guranda	130(50.58)	127(49.42)	257
Meta Abo Brewery Workers	123(53.95)	105(46.05)	228
Koche	65(50)	65(50)	130
Fulas	89(51.45)	84(48.55)	173
Bole	171(50.15)	170(49.85)	341
Total	578(51.2)	551(48.8)	1129

Table 3. Active Cases of Cutaneous leishmaniasis seen in Meta Abo, Sebeta, During April 19-23,1988

Age	Sex	Site(s)	Duration of lesion (months)	Clinical Features	Time Since Transfer to villagized areas (Birth Place)
7	Male	Cheek	12	Ulcerative	Not transferred
9	Male	Cheek	14	Just Healing	12 months
15	Male	Nose	?	Ulcerative	Not trans.
21	Female	Nose	2	Progressive*	13 months (Borena)
22	Male	Forehead	2	Progressive°	Not trans.
27	Female	Nose	7	?	Not trans.
30	Female	Nose	8	Healing	Not trans.
30	Female	Nose	2	ulcerative	12 months
30	Female	Nose	36	Tiny ulcer	13 months
30	Female	Lips/Nose	?	Mucocutaneous ?	13 months
41	Male	Nose	18	Progressive	Not trans. (Debre Berhan)
45	Female	Nose	18	Healing	Not trans. (Fiche)
43	Male	Nose	24	Healing	Not trans.

*Culture and Smear negative

°Culture and smear positive.

Table 4 Relationship Between Preferred *P.longipes* Biting Sites and Sites of scar/lesion of Cutaneous leishmaniases Seen in Meta Abo, Sebeta.

Site	Preferred Sites of *P.longipes* Bite	Sites of Scar/lesion
Nose	24 (17.02)	29(51.79)
Forehead	19 (13.48)	5(8.93)
Cheek	19 (13.48)	16(28.57)
Eyelid	12 (8.51)	0(00.00)
Ear	16 (11.35)	1(1.79)
Lips	2 (1.42)	2(4.35)
Chin	5 (3.55)	1(1.79)
Neck	10 (7.09)	1(1.79)
Arms	19 (13.48)	0 (0.00)
Others	15 (10.64)	1 (1.79)
	141	56

Table 5. Number of Cutaneous leishmaniasis Cases in the Various age Groups in Meta Abo, Sebeta

Age group	Village (Mean Age)					
	Dimma-Guranda	Brawery Workers	Koche	Fulas	Bole	Total
0-9	2	1	0	0	0	3
10-19	3	3	1	3	3	13
20-29	4	3	0	1	7	15
30-39	2	3	1	3	3	12
40-49	3	3	1	2	2	11
Over 50	1	1	0	0	0	2
Total	15	14	3	9	14	56

Most were on the head (96.43%) and 51.79% of the cases had them on the nose.

3.1.3 Relationship of CL to Age and Sex

Active cases were found in most age groups (Table 3). The disease was common in all age groups but very rare in early childhood (no case below 7) and old age (over 50 years old) (Table 3 and Table 5).

Lesion were equally distributed among both sexes. In spite of almost 50:50 sex ratio of the population (Table 2), more males were found with scar/lesion than females (Table 6). This suggested that more males were affected than females (X^2= 5.19, df 5).

3.1.4 Relationship of CL to Locality

Based on areas of origin (i.e. before villagization) the human population was divided into four localities. The three localities corresponded to the three selected hyrax habitats (sites 1-3). The fourth locality comprised the western hills of Meta Abo.

Table 7 shows the population size and percent with scar or lesion. The disease was most common in site 1 (6.31%) and site 3 (6.8%).

Age of scar/lesion was recorded during the survey. 62.5% of cases occurred during the past 10 years (Table 8). Recent cases were more numerous in S_1 and S_3 than the other sites. Cases therefore, clustered in recent years, especially in Site 1 and Site 3 (S_1+3). This by itself is not strictly comparable. So, the relative incidence was calculated in order to correct for population size (Wilkins, 1972). This was done as follows:

Table 6. Number of Female and Male Cases of Cutaneous leishmaniasis with Scar/lesion Found in the Different Villages, Meta Abo, Sebeta

Village	Male Cases		Female Cases		X^2
	Observed	Expected	Observed	Expected	
Dimma-Guranda	11	7.59	4	7.41	3.10
Meta Abo Brewery Workers	8	7.55	6	6.45	0.06
Koche	1	1.5	2	1.5	0.33
Fulas	5	4.63	4	4.37	0.06
Bole	10	7.52	5	7.48	1.64
Total	35		21		5.19

Table 7. Distribution of Cutaneous leishmaniasis Cases by Localities in Meta Abo, Sebeta

	Site 1	Site 2	Site 3	Western Hills
Number of families	47	21	43	82
Population	317	130	262	420
Number affected	20	3	18	15
% Affected	6.31	2.36	6.87	3.57

Table 8. Age of Scar/lesion of Cutaneous leishmaniasis Cases in the Various Sites, Meta Abo, Sebeta.

Age of Scar/lesion (Years)	S_1+3	S_2	Western Hills	Total
0-9	29	1	5	35
10-19	4	1	5	10
20-29	2	0	2	4
30-39	1	0	3	4
40+	2	1	0	3
Total	38	3	15	56

Relative incidence in S_1+3 = $\frac{O_{S_{1+3}}}{e_{S_{1+3}}} \div \frac{O_{S_{2+W}}}{e_{S_{2+W}}}$

Where,

$O_{S_{1+3}}$ = Observed number of cases in Site 1 and Site 3

$e_{S_{1+3}}$ = Expected number of cases in Site 1 and Site 3

$O_{S_{2+W}}$ = Observed number of cases in Site 2 and Western hills

$e_{S_{2+W}}$ = Expected number of cases in Site 2 and Western hills.

The relative incidence for S_{2+W} was similarily calculated.

The relative incidence for S_{1+3} over several years is shown in Table 9. All current and past cases in the last ten years were numerous in S_{1+3}. For periods over ten years ago, however, cases were slightly in excess in the Western hills. Moreover, the incidence rate in S_{1+3} was significantly higher than the other sites in all cases (X^2 = 6.15), in current cases (X^2 = 5.77) and in past cases in the last ten years (X^2 = 8.2) at 1 degree of freedom. However, insignificantly higher (X^2 = 0.314, 1df) incidence rate was observed in S_{2+W} for a period over ten years ago. It is possible, therefore, that S_{1+3} is an active and persistent focus and S_{2+W} was once important.

3.2 Observation on Hyraxes in Meta Abo, Sebeta

Several authorities have noted the presence of hyraxes in Sebeta area (Ashford, 1970; Foster *et al.*,1972) without

Table 9. The Relative Incidence of Cutaneous leishmaniasis in Site 1 and Site 3 and S_{2+W}.

Cutaneous leishmaniasis Cases		S_1+3	S_2+W	Relative Incidence S_1+3	S_2+W
Current Cases (lesion)	Observed	11	2		
	Expected	6.67	6.33	5.16	0.19
	Observed/Expected	1.65	0.32		
Past cases in the last 10 years	Observed	18	4		
	Expected	11.29	10.72	4.32	0.23
	Observed/Expected	1.6	0.37		
Cases over 10 years ago (with scar)	Observed	9	11		
	Expected	10.25	9.74	0.78	1.28
	Observed/Expected	0.88	1.13		
All cases (lesion, scar)	Observed	38	18		
	Expected	28.72	27.28	2	0.5
	Observed/Expected	1.32	0.66		

% of population in S_{1+3} = 51.28

S_{2+W} = 48.71

considering their exact identity, number, seasonality, infection rates and habitats. Infection in hyraxes from Sebeta was mentioned in Ashford *et al.* (1973). However, no source (documents or publication) was available to support their statement. In the present study, a year round counts of hyraxes were made in premarked sites. Hyraxes were also examined for leishmanial infection.

3.2.1 Identity of the Hyraxes

A total of ten hyraxes were trapped. Three adult females from Site 1 (S_1) and four adults (two males and two females) and three baby hyraxes from Site 3(S_3). Morphological variations have been observed in the two populations from the two sites, but both were tentatively identified as *Heterohyrax* Sp. Specimens from S_1 displayed distinctive *H.brucei* features. All were small (1570-1600 gms) and had narrow head, short muzzle and weak incisor teeth. They also displayed the yellow dorsal spot. Specimens from S_3 were darker and larger (above 1700 gms) and had rounded head, short muzzle, projecting weak incisor teeth and lacked the yellow dorsal spot. Unlike *Procavia*, a black dorsal streak was absent in all the specimens. This feature was considered to be the only distinctive feature of *Procavia capensis* in the Central Ethiopian plateau (Ashford, 1977). These characteristics, therefore, suggest that the specimens from S_3 could be a different form of *H.brucei*.

3.2.2 Habitat of the Hyraxes

Hyraxes in Meta Abo live in caves and narrow vertical cracks on bare cliffs eight to twelve meters high. Two colonies in S_3 and some hyraxes of unknown colony in S_2 occupy rock walls with dense vegetation cover (herbs and spiny shrubs). They were seen in small colonies (Table 10) and had conspicuous "Sentinel post" except in S_2. Hyrax habitats are found surrounded by human dwellings and farmlands. Here, hyraxes are closely associated with man.

3.2.3 Population Data on Hyraxes

It is difficult to count all the hyraxes at any one time. Some members of the colony remain inside their holes. A year round regular count from March 1987 to April 1988 for S_1 and S_3 is shown in Table 10.

Numbers observed varied little during the year except after trapping operation in November, 1987. Monthly average and the maximum count in a month for periods before November were least from July to September and highest from March to June. Assessment of outside activity during the dry months (i.e November to February) was obscured by the trapping.

Hyrax colonies in Meta Abo area were most active in the early mornings and late afternoons (Table 11). Out of 36 observations during the year, hyraxes were seen 33 times in the early morning hours (06:00-09:00) and 31 times in the late afternoon hours (16:00-18:00). They were never seen at noon and very rarely in the remaining hours of the day (Table 11). Exception to this generalization

Table 10. Monthly Mean and Maximum Number of Hyraxes Observed in Meta Abo, Sebeta, Between March 1987 and April 1988

Month (1987-1988)	Number of Hyraxes in Site 1		Number of Hyraxes in Site 3	
	Average (range)	Maximum	Average (range)	Maximum number (No. of colony)
March	6(5-6)	6	2(2)	2(1)
April	4(3-6)	6	3(3)	3(2)
May	5(2-7)	7	3(3)	3(2)
June	6(4-7)	7	4(2-6)	6(2)
July	3(2-4)	4	2(1-2)	2(2)
August	3(0-4)	4	1(1)	1(1)
September	3(2-4)	4	-	-
October	4(3-6)	6	8(2-9)	12(3)
November	1(0-3)	3	3(3)	3(2)
December	2(2)	2	4(4)	4(2)
January	2(2)	2	4(3-5)	5(2)
February	3(2-3)	3	1(1-2)	2(2)
March	3(3-4)	4	8(7-9)	9(2)
April	4(4)	4	4(4)	4(2)

was the condition of some colonies in S_3. At least a single hyrax was seen frequently during the afternoon hours of the day (Table 12). In this site two colonies live in isolated rockwalls facing westwards. So, they have to come out late in the morning to bask in the sun. The major cliffs in Meta Abo with the exception of parts of S_3 are oriented South-North and facing eastwards.

The number of hyraxes and colony size are difficult parameters to determine because of low outside activity. Only estimates can be given as an index of abundance and colony size. Observations have shown that hyraxes are rare in Meta Abo area. A good accuracy is expected from a year round observation. Inaccuracies due to low outside activity would be minimized by taking the maximum number of hyraxes observed in each colony. Based on this, more than seven individual hyraxes comprised a colony are found in S_1. Sixteen hyraxes in four colonies of 2 to 8 members live in S_3. The carrying capacity (rock walls) of the habitat and the various indicators of hyraxes (Pellet heaps, paths, feeding grounds) in S_2 suggested the presence of a smaller number of hyraxes and colony. In this site only a single hyrax was seen in August, 1987.

New-born young hyraxes were seen only in October, 1987 and March, 1988. Close observation on the youngs have shown that they fed frequently on leaves of climbers on the cliff (Site 1). So, it is much easier to catch sight of youngs than adults, especially in S_1 where they occupy the bare rock. Two to three youngs were usually seen together tended by possible mothers.

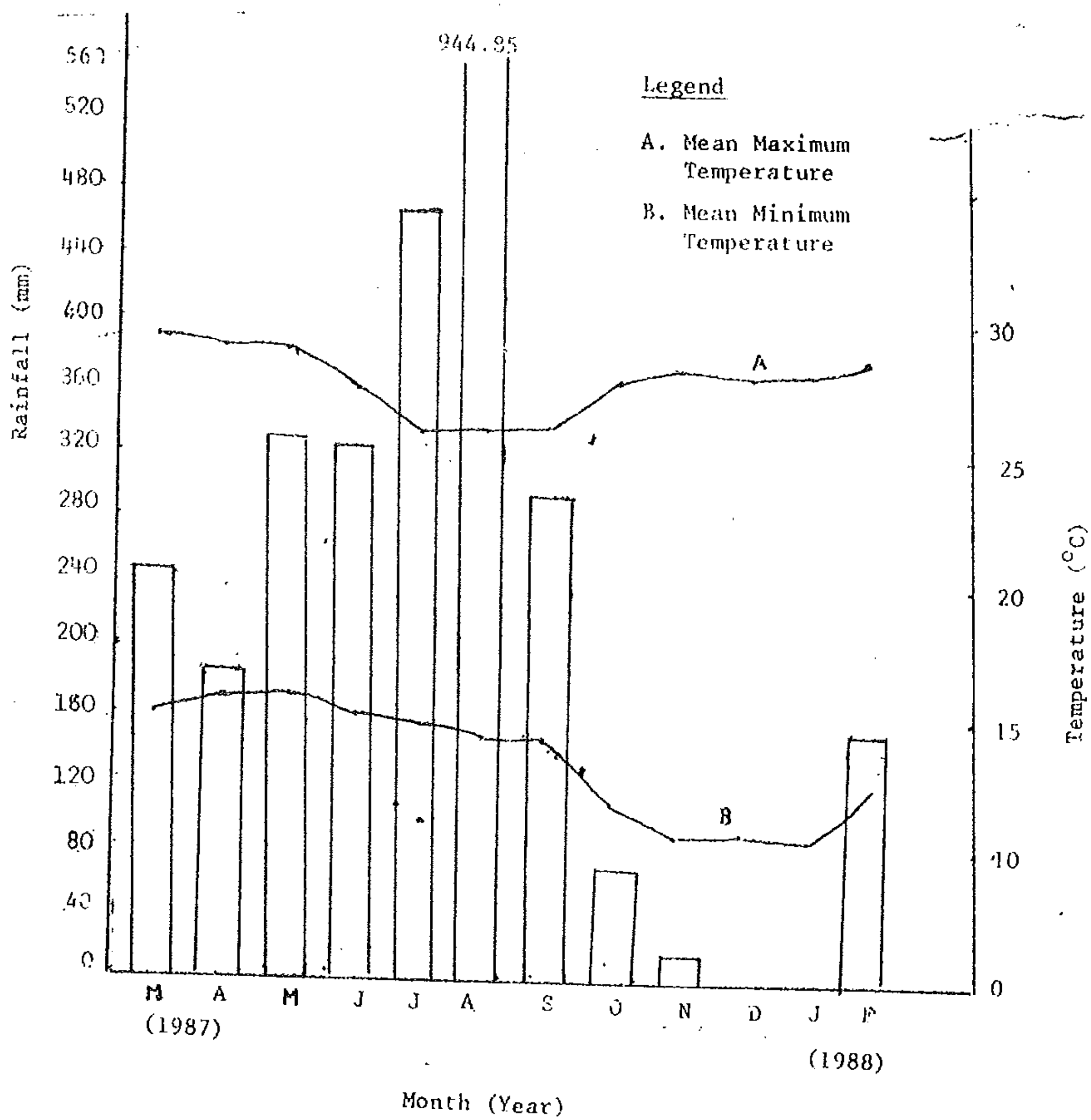

Figure 2. Monthly mean rainfall and maximum-minimum temperature of Sebeta area (data obtained from National Meteorological Service Agency)

3.2.4 Leishmanial Infection in Hyraxes

None of the five examined hyraxes were smear or culture positive. Few cultures were contaminated with bacteria and the rest were negative although none of them were contaminated. Two of the hyraxes were reexamined unsuccessfully.

3.3 Observations on Phlebotomus longipes in Meta Abo, Sebeta

3.3.1 Resting Habits of P.longipes

Adults of *P.longipes* rest in a variety of biotopes. They occupy both natural and man-made habitats. The major resting places were caves, occupied and abandoned houses, tree holes, cracks, cavities under tree root systems, under bridges and rock fissures. Excessively large caves adjacent to waterfalls were usually avoided.

P.longipes was mainly cave dwelling in S_2. Among trees, *Podocarpus gracilior*, *Juniperus procera*, *Croton macrostachym* and *Ficus* spp. were important resting habitats of *P.longipes* in S_1 and to a lesser extent in S_3. Peridomestic resting sites (e.g man-made habitats) were restricted to S_1 . The other resting places mentioned above were common to all sites.

The inside of hyrax holes are beyond the reach of man. Deep vertical cracks and caves where fresh pelletes were regularily seen at the entrances were considered homes of hyrax colonies. In general, *P. longipes* was found around hyrax habitats or holes.

Most of the negative micro habitats (those which harboured none or few sandflies) at the start of the study remained negative. This was especially true in excessively large caves. A small semicircular cave in S_1 became important resting site after a waterfall adjacent to it was dried out. None of the major resting sites were entirely abandoned. However, cavities under root systems along the banks of a stream and seepage holes under a bridge were abandoned during periods of flooding.

3.3.2 Biting Habits of P.longipes

Sandflies bite collectors inside its resting places. From July, 1987 to April, 1988, two collectors recorded 69 bites in a cave (S_2) and 101 bites in an uninhabited stone house (S_1). In June 1987 flies were observed biting 10 meters away from their resting sites in an overcast air, by day in large number. Fifteen females were caught biting by day (even at noon) in the hotel from August, 1987 to April,1988.

P.longipes man biting rate is shown in Table 11. 38 females were caught in 96 man hr. of exposure inside the hotel at the recreation garden in Meta Abo (biting rate was 0.396 bite/ man/hour). Only 9 females were caught biting outdoor in the same man-hr. exposure (biting rate of 0.094 bite/man/hour). Meteorological records have shown that most of the nights were cold (15.28 to 18.06^{o}C). It was also observed that most nights were windy varying from breeze to strong wind. The relationship of biting

Table 13. Phlebotomus longipes Man Biting Rate in Meta Abo, Sebeta

Month (1987-1988)	Indoor No. of Bites	Indoor Bites/man/hr.	Outdoor No. of Bites	Outdoor Bites/man/hr.
July	0	0.00	2	0.50
August	9	0.56	0	0.00
September	7	0.88	4	0.50
October	4	0.50	0	0.00
November	12	0.45	2	0.10
December	0	0.00	0	0.00
January	0	0.00	0	0.00
February	0	0.00	0	0.00
March	0	0.00	0	0.00
April	6	0.75	1	0.25
Total	38	0.39[illegible]	9	0.094

activity with meteorological conditions is difficult to assess from 9 females. Although bites occured in a relatively wide range of temperature (16.39°C - 18.06°C), three of the bites were in a calm and warm (18°C) night.

In general, months of highest P.longipes biting activity was related to the number of resting P.longipes. In September, 1987 seven and four females bit indoor and outdoor, respectively, over a period of 8-man-hour. No females were caught biting from December to March.

3.3.3 Seasonal Abundance of P. longipes

3129 adults were collected from all sites in 28 searches in 14 months by one man. Monthly distribution of population indices is shown in Fig. 3. P.longipes was most numerous in S_2 (54.68%) followed by S_1 (35.70%) and S_3 (9.62%). Time of collection and nature of resting sites were not the same for all sites. In S_2 collections were made usually from 0900 hours onwards and in S_1 and S_3 from 1500 hours and 1000 hours onwards, respectively. Resting sites were more dispersed in S_3 and were diverse in S_1. In spite of the above variations the general trend in the seasonal fluctuation of the population was similar for all sites. Nevertheless, seasonality was relatively more marked in S_2 than the others. Ignoring results at the start of the study (periods of low efficiency) the population ranged from 12.98% in January to 59.96% in September.

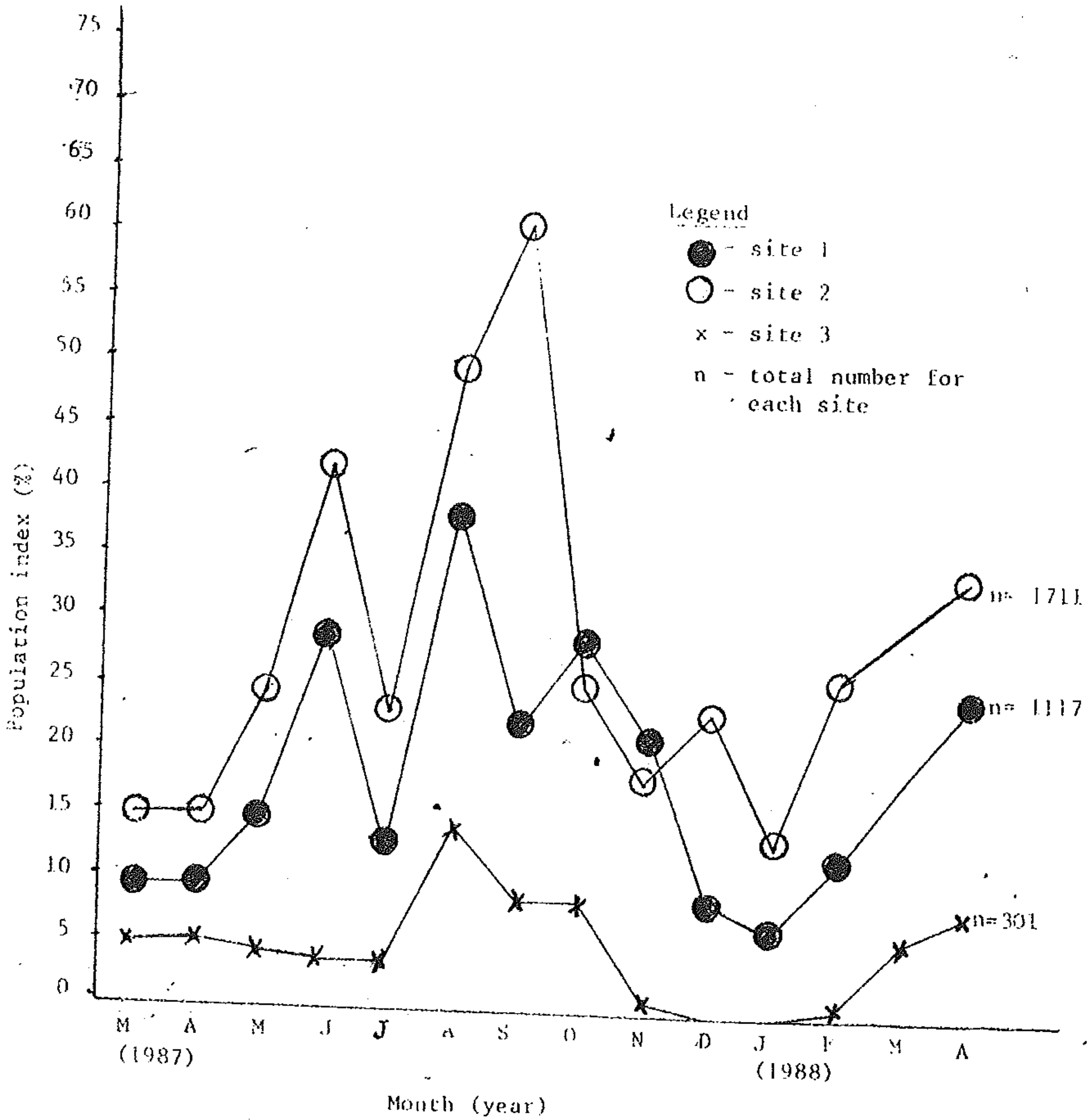

Figure 3 Monthly distribution of population indices of *Phlebotomus longipes* in each of the three study sites at Meta Abo, Sebeta

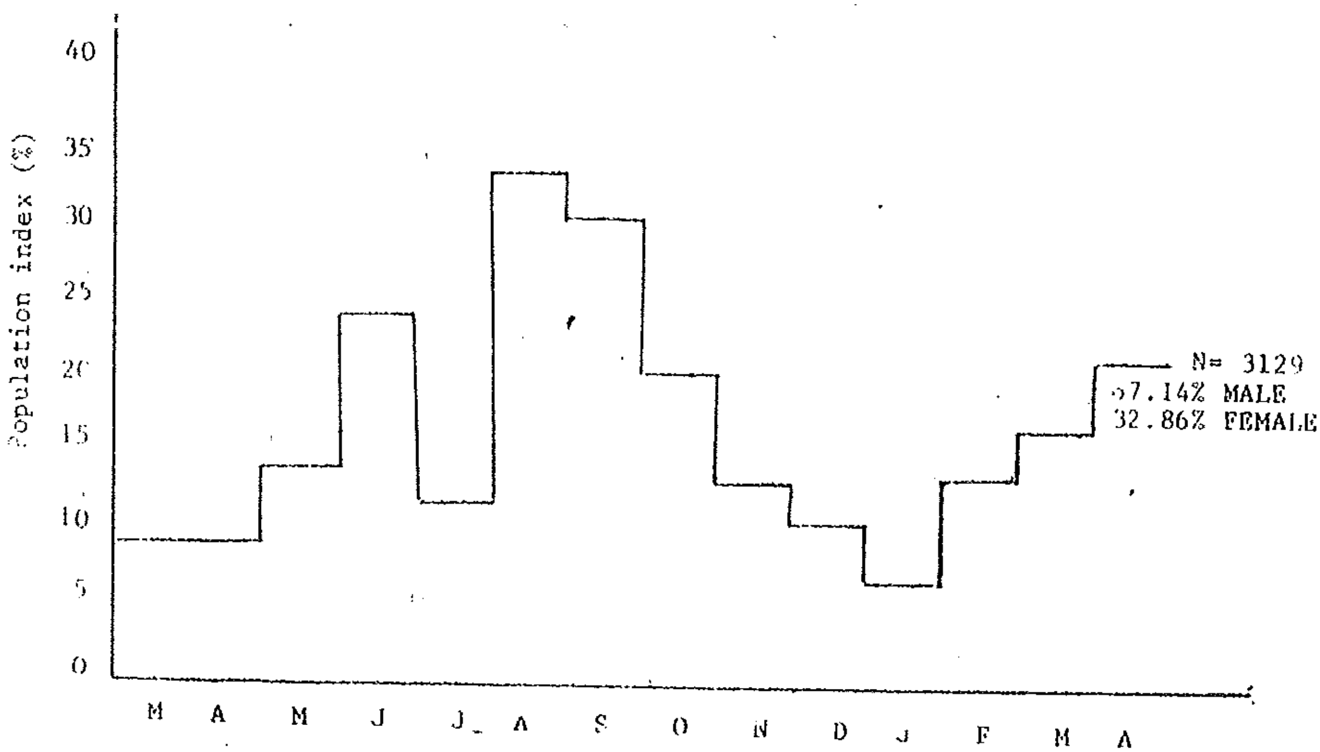

Figure 4. Monthly distribution of population indices of <u>Phlebotomus longipes</u> collected from the three study sites (S_1-3) in Meta Abo, Sebeta

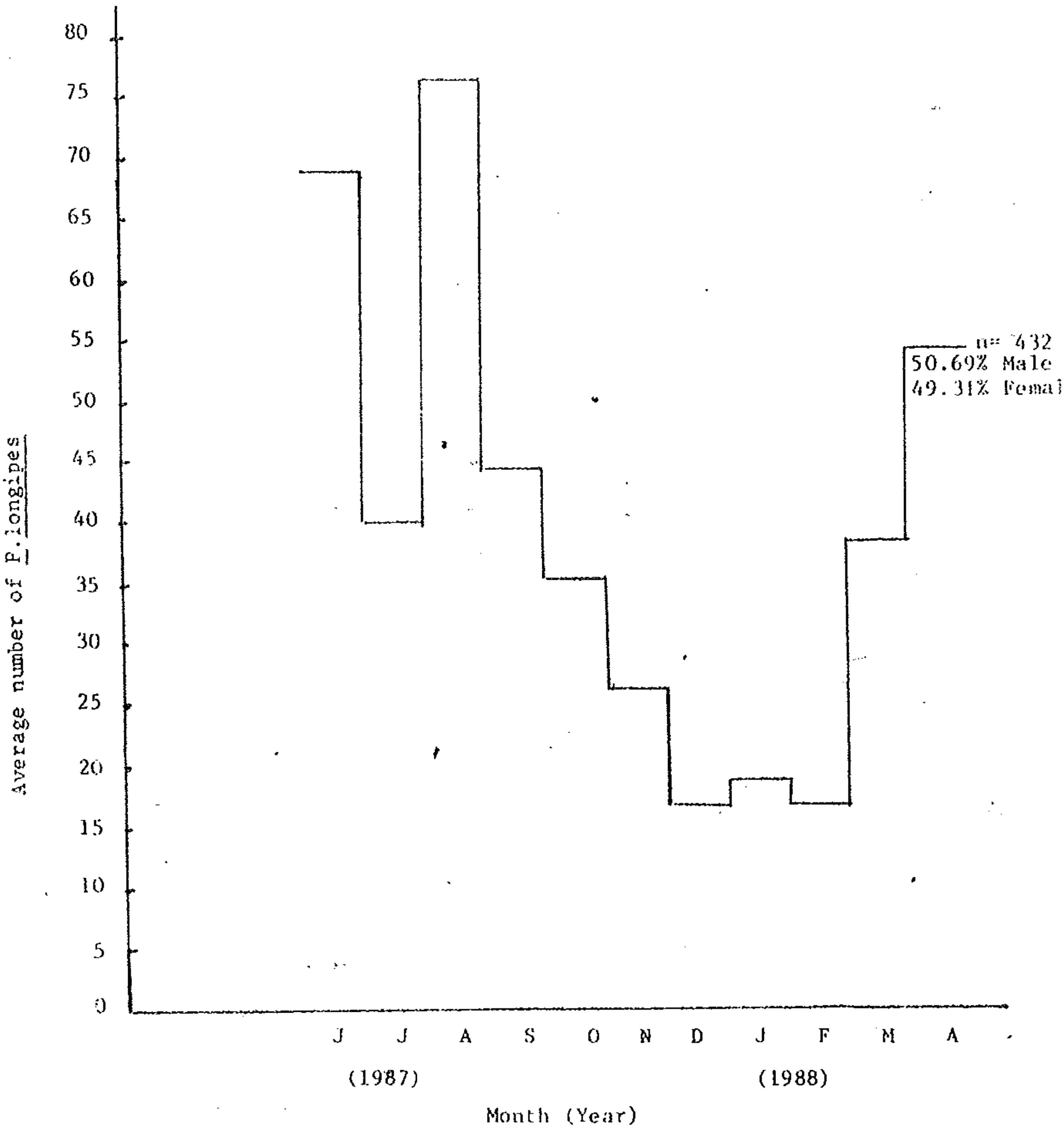

Figure 5. Monthly average of Phlebotomus longipes caught from site 2 by searching for 15 minutes.

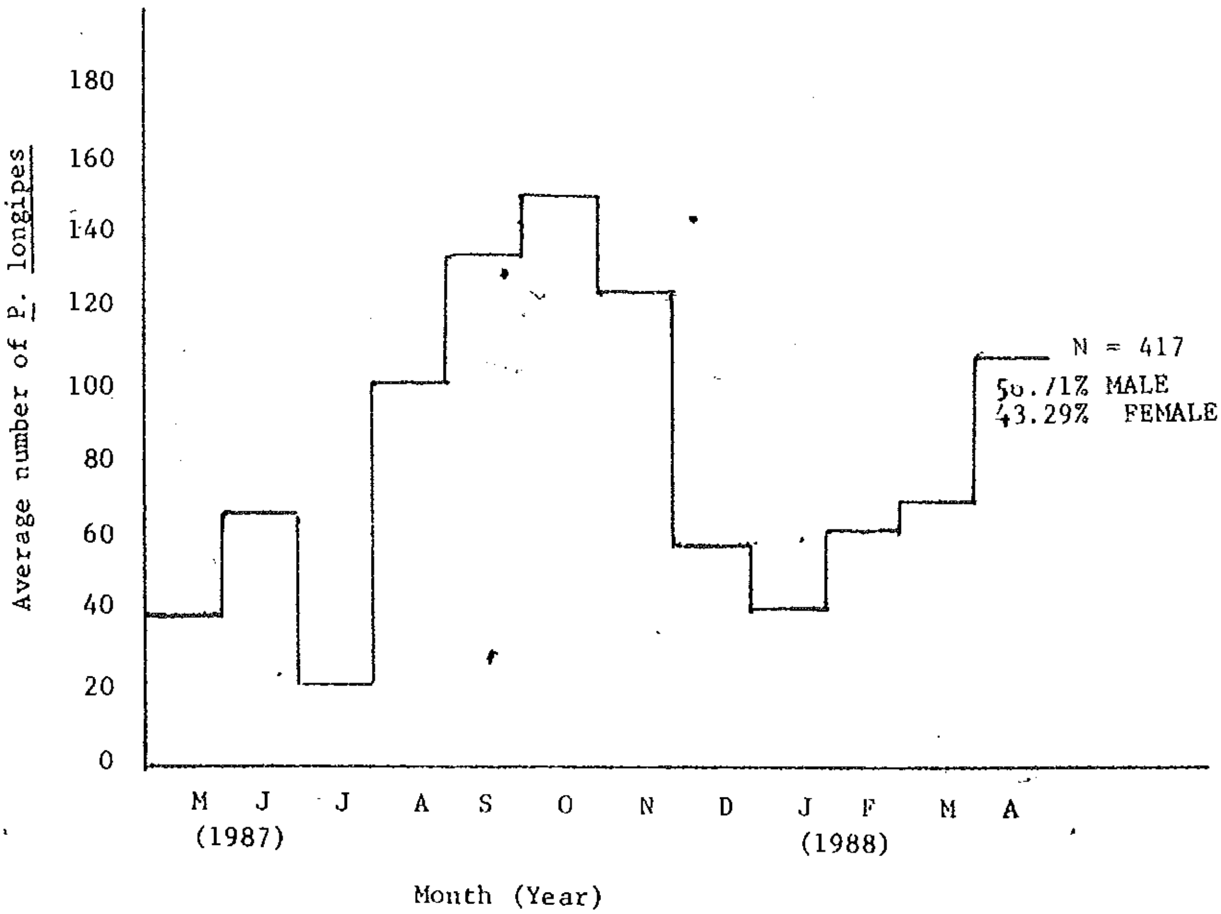

Figure 6. Monthly average *Phlebotomus longipes* by direct count in abandoned stone house, in site 1, Meta Abo, Sebeta

Figure 4 shows monthly population indices for all sites. Monthly average of P.longipes caught in 15 minutes (Fig. 5) agrees well with the monthly population indices (Fig.4). Results by direct counts (Fig.6) were basically similar to the monthly population index (Fig.4), although in the former (Fig.6) a shift in peak population period has been observed. The population index ranged from 6.4% in January to 33.2% in August. One peak abundance during August and September was observed. The population was conspicuously low during November to February. The relative abundance of P.longipes in different seasons shows some relationship to rainfall patterns (Fig.2). In the peridomestic site (an abandoned stone house), however, peak abundance occured during September to November.

3.3.4 Seasonal Distribution in P.longipes Parous Rates

Parous rates ranged from 25% to 68.42% and fluctuate between 50% and 35% for much of the months (considering unfed females). This is shown in Figure 7. Parcus rate was high (above 50%) in May and June (beginning of the big rains) and December and January (during the dry season). It was least at the end of the dry season in February. It was also observed that the proportion of parous females was low during peak abundance and highest during least population size (Figs. 4 and 7). Parous rate expressed as the percentage of females in all physiological states was shown in Figure 8. Parous rate was obscured by the high proportion of gravid and fed females in December and January.

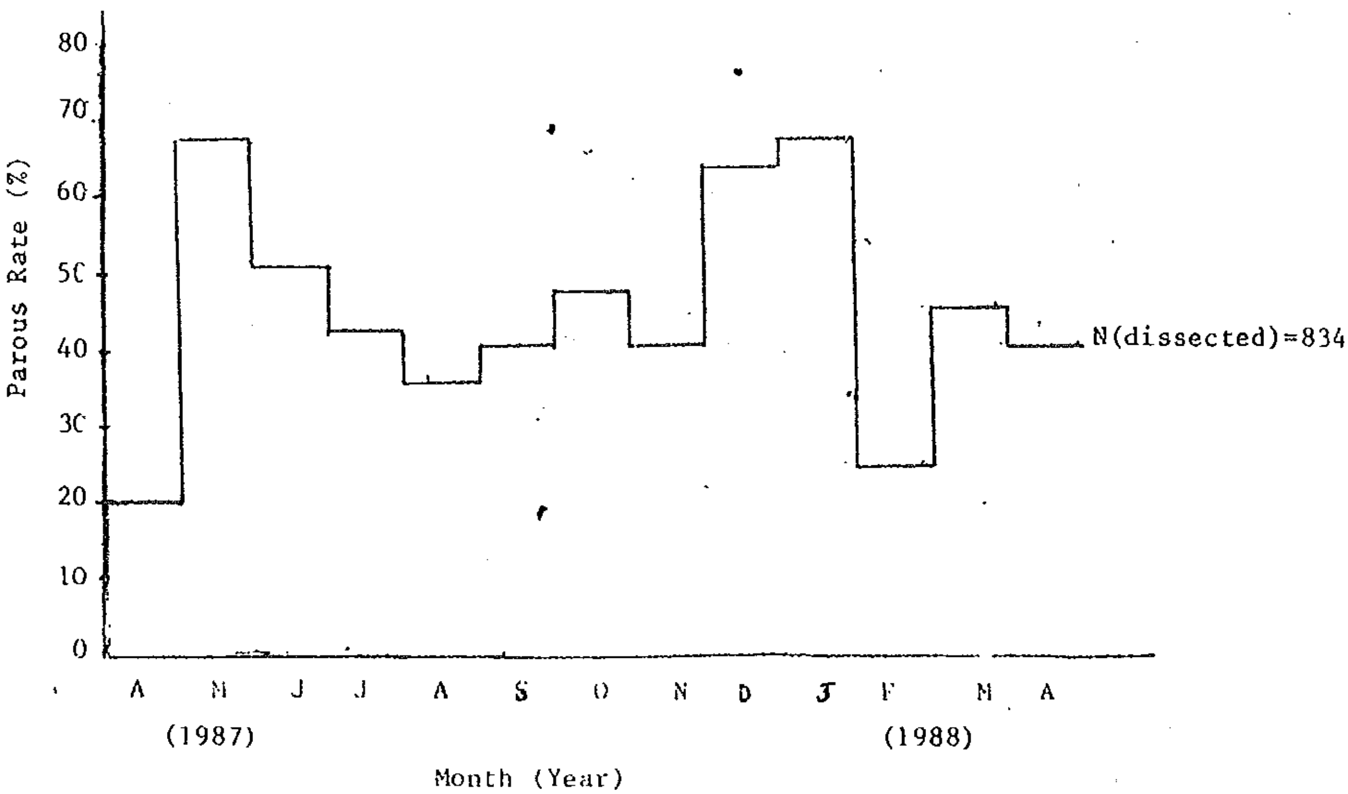

Figure 7. Monthly parous rate of unfed female P. longipes.

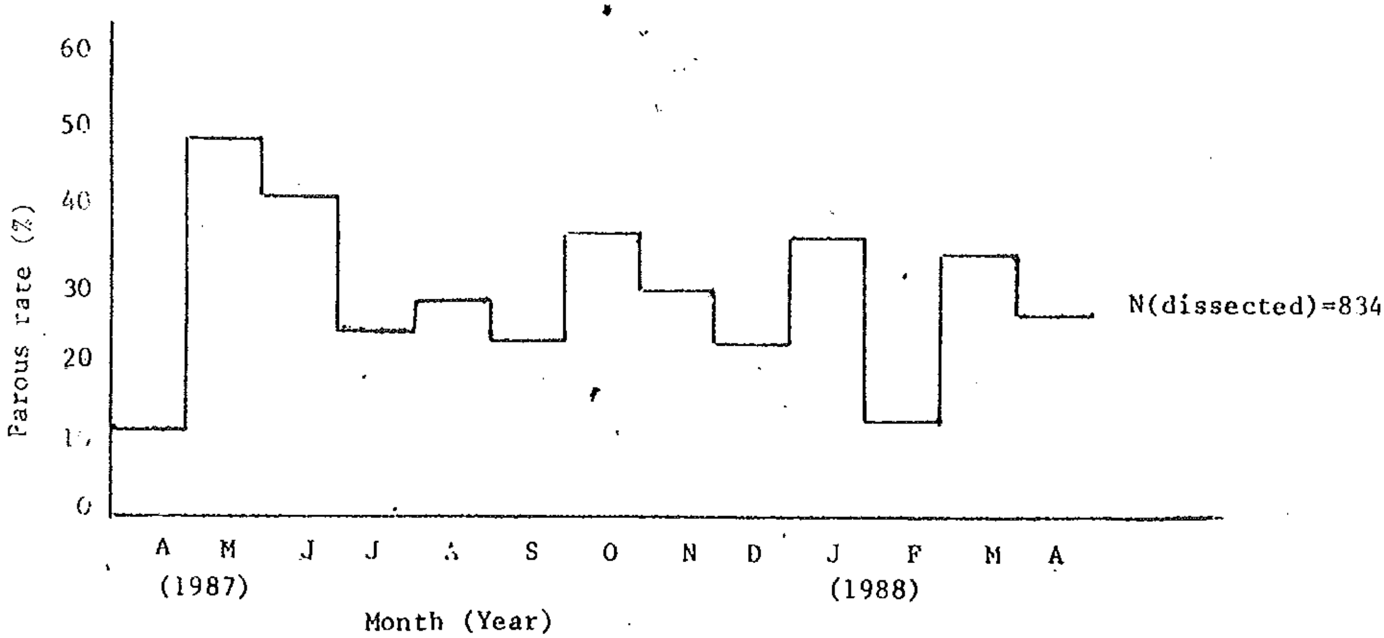

Figure 8. Monthly parous rate of all female P. longipes dissected.

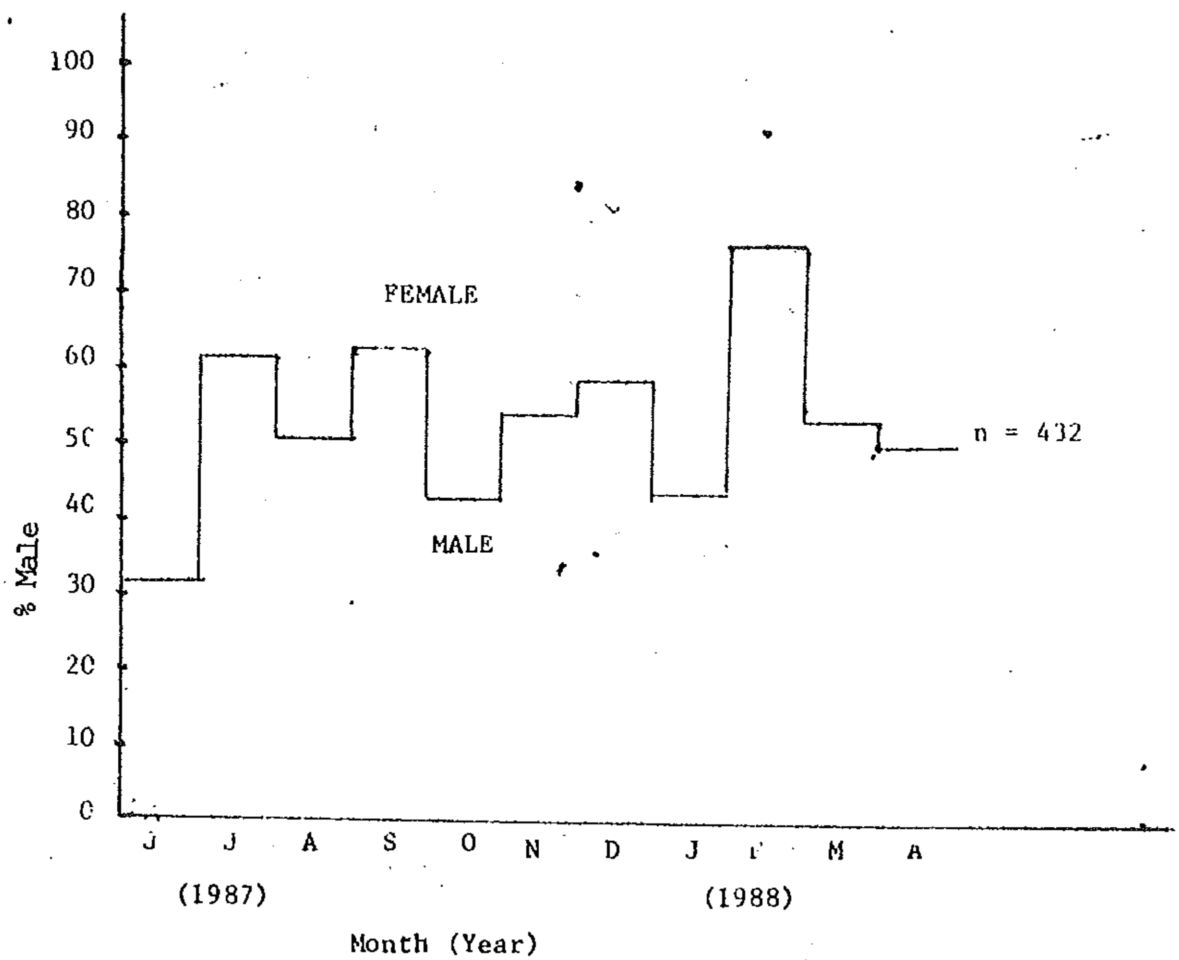

Figure 9 : Monthly sex-ratio of a smaple of *P. longipes* population

Female population fluctuated between 40% and 60% females for much of months (Fig.9). However, it was 68.84% in June and only 23.53% in February.

3.3.5 Leishmanial infection in *P.longipes*

834 females were dissected. One out of 514 (0.20%) parous , fed and gravid female *P. longipes* was found infected with flagellates. The infected female was fed; about 3/4 of the bloodmeal was already digested. Flagellates were dense in the stomach (abdominal midgut). Promastigotes were passed through five series of subcultures in NNN-media from 7/6/87 to 27/8/87. Parasites failed to infect the inoculated animals.

4. DISCUSSION AND CONCLUSION

4.1 Cutaneous leishmaniasis in Man

One parasitologically proven and twelve likely oriental sore (OS) active cases (Table 3) were found from survey of the population living in Meta Abo hills (Table 2). However, it is borne in mind that all the likely sores observed in the present study may not actually be due to leishmanial infection (WHO, 1984). The fact that OS is well known by the local inhabitants and has a vernacular name 'Shahign' has led Wilkins (1972), and now myself to assume the undiagnosed "Shahign' cases (both scars and lesions) as OS cases. The use of vernacular names (eg. Kuncher, etc.) in the epidemiological studies of OS in different parts of Ethiopia has been also emphasized by Lemma et al. (1969) and Ashford et al.(1973). It is thus taken that the information obtained on the number of possible active cases of CL and those with characteristic scars would be adequate to relate the present situation of CL in Meta Abo with entomological and reservoir host findings.

Household (Table 1) has slightly increased in the last seventeen years since Wilkins (1972) reported his findings (51% of the population lived in households of 5 or more). Reduced infant mortality and population growth might have contributed to the increase. It is assumed that this might have change the age structure of the population.

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. However, they have observed lesions of two to five years duration. History of active cases in the present study is within this range, 2-36 months (Table 3).

The prevalence rate in Meta Abo hills was found to be 11.5/1000. This is almost twice as high as that of Wilkins (1972) which was 5.5/1000. Lemma *et al.* (1969) encountered 5 active cases in 185persons and their studies concentrated to the present S_1 and S_3 (Fig. 1) where the disease was considered to be more prevalent while wilkins (1972) worked over a large area which included the plains of Meta Abo. Prevalence rate in Ethiopia varies between 2/1000 in kutaber to 100/1000 in Ochollo (Ashford, 1977). Meta Abo falls between the two extremes. While the climatic situation in Meta Abo (Fig.2) is similar to Kutaber, hyraxes and *Phlebotomus longipes*, however, live close to human habitation somewhat similar to Ochollo. Furthermore, while in Ochollo, human dwellings are actually built on hyrax and sandfly habitats in Meta Abo we see that hyrax and sandflies are slightly physically separated from human dwellings. Still sandflies could freely move around in the Meta Abo area to sustain effective transmission of the disease. Proportion of persons with scar was 38/1000 close to Wilkins (1972) findings (32 [illegible]) and indicating an unchanged potential risk to the disease.

In spite of the increase household which has an influence on the density of the population and age structure, the pattern and degree of the disease have not changed much. Also this demographic chang s hasn't had any influence on the intensity of transmission.

It was observed that P. longipes preferred the face, especially the nose which corresponded well with the site of scar/lesion on man (Table 4). It is known that sites of scar/lesion represent area of inoculation of leishmanial parasites by the vector sandflies (Lewis, 1971; Lemma et al., 1969; Bray, 1983). However, influencing factors of this preference is not yet well established. It is assumed that CO_2 expired from the nose and mouth might contribute tremendously (Bray,1983).

Previous reports indicate that CL is more prevalent in children and youth (Lemma et al., 1969; Ashford, 1977) in Ethiopia. A slightly different result was obtained from the previous study (Table 3). Active cases and even those with scars (Table 5) were most common in age group 20 to 40 years. Lesions were absent in subjects below seven and above fifty years of age. Previous observations have shown that the disease was common in all age groups (Wilkins, 1972; Lemma et al.,1969). Although the present finding is not supported by age structure or leishmanian skin test, restriction of lesions to the middle age group reflects that the disease is affecting the most active group and that transmission may be taking place away from human dwellings.

The changed household does not correspond with the age distribution of lesion. We should have expected more children with lesions. It is possible that small children and old people who might possibly be infected in houses near hyrax habitats would be safe now where they are living far away (due to villagization) from sandflies and hyraxes. This is why Wilkins (1972) also observed low infection rate in people living in the plains of Meta Abo.

Scar/lesion was more common in males than females (Table 6). This varies from the previous observations in Meta Abo area (Wilkins, 1972) where more infected females were observed. Risk of infection is higher in those age groups and sexes who frequently visit hyrax habitats (Ashford et al., 1973; Ashford, 1977).

Active cases were found aggregated near hyrax habitats (Fig. 1-sites 1-3). In addition, the disease was most prevalent in S_1 and S_3 (Table 7 and 9). These are areas previously determined to be endemic (Lemma et al., 1969). These areas were later confirmed to be the most important microfocus in Meta Abo area (Wilkins, 1972). The western hills, which corresponded to Wilkins (1972) Zone B, is now considered more important than when it was reported.

From Table 8, it can be seen that the number of scar/ lesion tend to be more numerous in recent years. The same observation prompted Wilkins (1972) to suggest that the disease originated recently. My observation of a similar increasing tendency after seventeen years contradicts Wilkins (1972) conclusion. I believe the disease has been there throughout but changes have taken place in the degree of exposure by the different age groups and sexes.

Observation on the prevalence of the disease suggests that it is still important. People have moved away from sandfly/hyrax habitat. The degree of association between man and the wild habitat, in the future, may determine the fate of CL in the human population of Meta Abo.

4.2 Hyraxes in Meta Abo Area

Hyraxes of the area are *Heterohyrax* spp., although different forms were seen among specimens from S_1 and S_3 which are only two kilometers apart. *Heterohyrax* spp. is known in a wide range of altitude (Kingdon, 1971; Ashford *et al*., 1973). Rocks and cliffs were considered as the most important requirements of hyraxes in Ethiopia (Ashford,1977). Therefore, existence of the two different forms of the same species in Meta Abo indicates either they are territorial (Kingdon, 1971) or they tend to live permanently in a habitat they occupy. The local people neither complain of hyraxes as pests nor use them as resources. Hyraxes are affected little by direct human encroachment, thus making them a good reservoir host (Bray, 1983).

Seasonal variation in hyrax outside activity was not conspicuous (Table 10). However, low outside activity in August and September occured during the peak season of *P.longipes* (Fig. 3). Ashford *et al*. (1973) have reported that outside activity is highest during dry months. Although more adequate data is required to comment on this, in response to climatic factors the hyraxes are most available for bite during the period of peak abundance.

Diel activity of hyraxes in the Meta Abo area was very low similar to the majority of hyrax species (Kingdon, 1971) and hyraxes in Ethiopia (Ashford et al., 1973). They were most active in the early mornings and late afternoons (Table 11 and 12). So, hyraxes are available for sandfly bite for most of the hours of the day.

Hyraxes were observed to be rare in Meta Abo area. Over 23 hyraxes in 5 colonies were estimated for S_1 and S_3 together. Observations have shown that breedin is seasonal in Meta Abo. The young were produced twice a year. A female produced mostly two and rarely three youngs. Thus, their reproductive potential is low. It is possible, therefore, that hyraxes are rare in Meta Abo partly because of their low reproductive potential.

Leishmanial parasites were not recovered from five hyraxes both in cultures and smear. Previous observations have shown high infection rates in Kutaber (1 out of 5), Ochollo (4 out of 19) and Aleku (6 out of 22)(Ashford et al., 1973). Flemmings observation (Ashford et al.,1973) in Meta Abo is not complete enough to warrant any discussion now. Observation on five hyraxes, in the present study, should not be considered small in relation to the population size of hyraxes in Meta Abo area. L. aethiopica inoculated hyraxes failed to turn positive in culture (Hailu. M.Sc. thesis, 1987). This may lead to suggest that isolation of lesihamnail parasites from naturally infected hyraxes may not be easy. Bray (1983), however, suggested that a good reservoir easily presented the parasites to the vector.

Although I still believe that the major source of infection would be hyraxes the possibility of anthroponotic transmission should not be underestimated as has been suggested by Wilkins (1972) and Ashford et al. (1973).

Hyraxes are very restricted in their movements away from their holes. Sandflies may also rarely fly away from suitable microhabitat except in clam weathers. Under such conditions man would probably get infected during activity very near to hyrax /sandfly habitats.

4.3 Phlebotomus longipes of Meta Abo and its Importance

The present study showed that P.longipes inhabit a variety of habitat which included caves, rock holes, uninhabited stone house, inhabited hotel and buttresses of trees. Previous studies (Lemma et al., 1969; Foster, 1972a; Foster et al.,1972; Ashford et al., 1973; Gemetchu, personal communication) have also indicated that such habitats had served as resting places for P.longipes. It is believed that extradomestic resting sites (eg. cave, rockholes, etc) might serve as both resting and breeding sites. It is here that hyraxes live. On the other hand sandflies could travel freely during calm weather to enter human dwellings. Here unfed; fed or gravid females could be found. Normally a female feeds and rests in a dark corner to digest its bloodmeal. It is presumed, however, that gravid females have to leave human habitation in search of suitable area for oviposition and larval development.

The resting of adults in human habitation must be taken as a temporary event. However, the very fact that they can enter human habitation and feed on man could be instrumental in the transmission of CL from hyraxes to man or even from man to man. On the other hand it is believed that sandflies mainly rest in their wild habitats where the hyrax host are found. Here, both food and suitable breeding grounds are readily available. There is no need for sandflies to leave these microhabitats unless they are in need of plant sugar or unless the hyraxes had abondoned the habitat for unknown reasons. Therefore, under suitable conditions sandflies will remain in the microhabitat and maintain enzootic transmission of CL between hyraxes. In this case man gets infected while intruding in these habitats.

Caves adjacent to waterfalls were not used by P.longipes as resting sites during the wet season. Water stagnates at the waterfall stream junction. Obviously, the water table is excessively high in the vicinity of these sites due to seepage. It is known that sandflies prefer humidity but not excessively wet biotopes (WHO, 1984). It was also observed that an avoided cave was re-infested after the adjacent waterfall dried out. Thus, choice of extradomestic sites were partly influenced by the moisture content of the soil.

Although P.longipes man biting rate was relatively high in warmer and calmer weather conditions,biting has also occurred in cold and moderate wind condition depending on the abundance of P. longipes. Indoor biting rate was observed to be higher than outdoor (Table 13). Outdoor collections were made near hyrax colonies where a larger number of sandflies presumably rested. In addition to hyraxes, small rodents and monkeys are available in the vicinity. Rodents were reported to be unattractive hosts (Foster et al., 1972). In Meta Abo non-domestic sites, bovids were found to be important sources of bloodmeal (Foster et al.,1972). However, the engorged females were collected from Eucalyptus plantation, at 200 meters away from the hyrax colonies in S_1. The low outdoor manbiting rate may possibly be associated with a readily available source of food (the hyraxes) for P. longipes in outdoor situations.

Seasonal distribution of P.longipes as determined by the direct count (Fig. 6) and catch per unit time (Fig. 5) closely agreed with the population index (Fig. 4). Although inaccuracies due to weather conditions and behaviour of sandflies are unavoidable, the population index may be regarded as a reliable estimate of the population of P. longipes in the three hyrax habitats. Simple methods such as the present one, offer adequate information on abundance and estimate of population density where skill of the collector, efforts and time of collections are constant (Southwood, 1978; WHO, 1984).

P.longipes was observed to prevail throughout the year (Figs. 3, 4). This is a characteristics of tropical species where the rainfall pattern shows a short dry season (Minter, 1964). Observations of Foster (1972a) in Addis Ababa, and Ashford et al. (1973) in Kutaber, have also shown that P.longipes is a perennial species. Emergence is, therefore, possible throughout the year. However, one peak in the population was observed in the big rains (August and September) indicating a unimodal patterns of abundance very similar to the observations of Foster (1972a) in Meta Abo and Ashford et al. (1973) in Kutaber. The lowest population was observed at the end of the dry season in January. Although excessive rains and draught are reported to cause diapause in the tropics (WHO, 1984), the effect of excessive rain in Meta Abo hills, except possibly for high humidity by the waterfall, can be ruled out.

A peak in population in August and September, as shown by the population index (Fig. 4) indicates a period of mass emergence. In nature, therefore, mass emergence could have been stimulated by high humidity during August and September while draught could have suppressed emergence during November to January.

In spite of a unimodal peak abundance, the distribution of parous rate was observed to be bimodal (Fig. 7). One occurred in the dry season (December and January) and the other just before the big rains (May and June). So, two conspicuously old generations were indicated. Observations of Foster (1972a) have shown no conspicuous peak in parous rates. The present study concentrated to three hyrax habitats (i.e the area was demarcated). The other months were with mixed population other than February. Large proportion of young population in February indicated the first mass emergence after four months of draught. So, two distinct generations were found in Meta Abo area, one after the draught and the other in August after the July runoff.

It was observed that proportion of engorged and gravid females was remarkably high in December and January. This indicates high rate of feeding and ovposition during the dry season. This can be explained by the combined effect of high temperature and low competition for bloodmeal source.

Seasonal distribution of sex ratio (Fig. 9) has indicated that male population was dominant in the young population (February). This is possible as it has been known that males always emerged before females in sandflies. Conversely, females outnumbered the old population in June. This suggested that the overall male to female ratio was 1:1.

P.longipes is a perennial species in Meta Abo area. In addition, parous females are available throughout the year. It is believed that this would guarantee transmission throughout the year. Peak transmission season is a result of high density and high parous rate (WHO, 1984). Thus, in Meta Abo transmission is most intense during August and September.

The infection rate in P. longipes, as observed by Foster (1972b) in Meta Abo, was 0.16% (2 out of 1216 females dissected). In the present study 1 out the total

834 (0.12%) or 1 out of 514 (0.20%) parous, fed and gravid females dissected were found infected with leishmanial promastigotes. My observation compares very well with that of Foster (1972b). However, the infection rate observed in P.longipes in Meta Abo, is very low compared to Ashford et al.(1973) work in Kutaber.

5. REFERENCES

Adler, S. and Theodor, O. (1957). Transmission of disease agents by pblebotamine sandflies. Ann. Rev. Ent., 2, 203-226.

Alekssev, A.N., Krasnonos, L.N. and Zhakhangirov, SH.M. (1986). The impact of agricultural cultivation of virgin lands in the Golodnaya steppe on the natural focus of zoonotic cutaneous leishmaniasis. Tropical Disease Bulletin, 84, abstract no.1557(1987).

Ashford, R.W. (1970). A possible reservoir for Leishmania tropica in Ethiopia. Trans. Roy. Trop. Med. Hyg., 64,936-937.

Ashford, R.W. (1974). Sandflies (Diptera: Phlebotominae) from Ethiopia: Taxonomic and biological notes. J. Med. Ent., 11,605-616

Ashford, R.W. (1977). The comparative ecology of Leishmania aethiopica. Colleoques internationaux du CNRS, No. 239- Ecologie des leishmaniases., 233-240.

Ashford, R.W. (1986). Leishmaniasis in the Middle East. Recent advances in epidemiology. Insect. Sci. Applic., 7(2), 157-160.

Ashford, R.W., Bray, M.A., Hutchinson, M.P. and Bray, R.S. (1973). The epidemiology of cutaneous leishmaniasis in Ethiopia. Trans. Roy. Soc. Trop. Med. Hyg., 67, 568-601.

Ashford, R.W. and Smith, D.H. (1985). Leishmaniasis in Sudan, Ethiopia and Kenya. In, Leishmaniasis, Chang/Bray eds., Elsevier Publishers B.V., London., enter, pp. 377-391.

Ayele, T., Habte Gaber, E. and Belehu, A. (1981). Leishmaniasis in Ethiopia: A handbook. Institute of Pathobiology, Addis Ababa University, pp. 86.

Balzer, R.J., Destombes, P., Schaller, K.F. and Serie, C. (1960). Leishmanioise cutanee peudo-lepromateuse on Ethiopie. Bull. Soc. Path. Exot., 53, 293-298.

Beach, R., Kiilu, G., Hendricks, L., Oster, C and Leeuwenburg, J. (1984). Cutaneous leishmaniasis in Kenya. Transmission of Leishmania major to man by the bite of naturally infected Phlebotomus duboscqi, Trans. Roy. Soc. Trop. Med. Hyg., 78, 747-751.

Beach, R., Kiilu G. and Leeuwenburg, J. (1985). Modification of sandfly biting behaviour by Leishmania leads to increased parasite transmission. Am. J. Trop. Med. Hyg., 34,278-292.

Bray, R.S. (1983). The zoonotic potential of reservoirs of leishmaniases 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. Roy. Soc. Trop. Med. Hyg., 67, 345-348.

Bray , R.S. and Bryceson, A.D.M. (1969). The identity of strains of Leishmania from Ethiopian diffuse cutaneous leishmaniasis. Trans. Roy. Soc. Trop. Med. Hyg., 63, 524.

Bryceson, A. and Leithead, C.S. (1966). Diffuse cutaneous leishmaniasis in Ethiopia. Ethiop. Med. J., 5, 31-33.

Bryceson, A. and Nichol, T.W.(1966). Cutaneous leishmaniasis in Wollege Province. Ethiop. Med. J., 5, 35-42.

Chance, M.L.(1979). The identification of Leishmania. In, problems in the identification of parasites and their vectors, Tylor A.E.R. and Muller L. (eds). Blackwell Scientific Publication Oxford, pp.55-74.

Corbet, G.B.(1979). The taxonomy of Procavia capensis in Ethiopia, with especial reference to the aberant tusks of Procavia capensis capillosa Brauer (Mammalia, Hyracoidea). Bull. Br. Mus. Nat. Hist. (Zool.), 36, 251-259.

Dedet, J.P. Derown, F., Hubert, B., Schnur, L.F. and Chance, M.L (1979). Isolation of Leishmania major from Mastomys erythroleucus and Tatera gambiana in Senegal (West Africa). Ann. Trop. Med. Parasit., 73, 433.

Delany, M.J. and Happold, D.C.D. (1979). Ecology of African Mammals. Longman, London. New York. pp. 391.

De Raadt, P. (1986). Leishmaniasis. Global impact on health. Insect. Sci. Applic., 7, 155-156.

Evans D.A.(1987). Leishmania. In, In vitro methods for parasite cultivation, A.E.R. Taylor and J.R. Baker (eds), Academic press, London, New York, pp. 52-75. De Raadt, P. (1986).

Foster, W.A.,(1972a). Studies on leishmaniasis in Ethiopia. III- Resting and breeding sites, flight behaviour, and seasonal abundance of Phlebotomus longipes (Diptera: Psychodidae). Ann. Trop. Med. Parasit., 66,, 313-328.

Foster, W.A. (1972b). Studies on leishmaniasis in Ethiopia V - Distribution of vector potential of Phlebotomus longipes (Diptera-Psychodidae). Ann. Trop. Med. Parasit. 66, 445-455.

Foster, W.A., Boreham, P.F.L. and Tempelis, C.H. (1972). Studies on leishmaniasis in Ethiopia. IV- Feeding behaviour of Phlebotomus longipes (Diptera: Psychadidae). Ann. Trop. Med. Parasit., 66 433-443.

Foster, W.A., Tesfa-Yohannes, T.M. and Tecle, T. (1970). Studies on leishmaniasis in Ethiopia. II- Laboratory culture and biology of Phlebotomus longipes (Diptera: Psychodidae). Ann. Trop. Med. Parasit., 64, 317-322.

Gardner, P.J., Chance, M.L. and Peters, W. (1974). Biochemical taxonomy of Leishmania. II-Electrophoretic variation of malate dehydrogenase. Ann. Trop. Med. Parasit. 68, 317-325.

Gemetchu, T. (1971). Liver and yeast as larval diets in colonization of sandfly (Phlebotomus longipes). Trans. Roy. Soc. Trop. Med. Hyg. 65, 682-683.

Gemetchu, T. (1974). The morphology and fine structure of the midgut and peritrophic membrane of the adult female, Phlebotomus longipes Parrot and Martin (Diptera: Psychodidae). Ann. Trop. Med. Parasit., 68, 11-124.

Gemetchu, T. (1976). The biology of a laboratory colony of Phlebotomus longipes Parrot and Martin (Diptera: Phlebotomidae). J. Med. Ent. 12, 661-671.

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 diffuse cutaneous leishmaniasis express different antigens from those in parasites derived from patients with localized cutaneous leishmaniasis. Ethiop. Med. J.,24,199.

Githure, J.I. (1986). Characterization of Kenyan Leishmania species and identification of Mastomys natalensis, Taterillus emini and Aethomys kaiseri as new hosts of Leishmania major. Ann. Trop. Med. Parasit, 80, 501-5)7.

Greenblatt, C.L., Slutzky, G.M., de Ibarra, A. and Snaly, D. (1983). Monoclonal antibodies for the serotyping of Leishmania strains. J. Clin. Microbiol., 18, 191-193.

Haile, T. and Lemma, A. (1977). Isolation of Leishmania parasites from Arvicanthis in Ethiopia. Trans. Roy. Soc. Trop. Med. Hyg., 71, 180.

Hailu, A. (1987). Rock hyraxes and cutaneous leishmaniasis in Ethiopia. M.Sc. thesis, Addis Ababa University.

Javadian, E., Tesh, R., Saidi, S. and Nadim (1977). Studies on the epidemiology of sandfly fever in Iran. III. Host feeding patterns of Phlebotomus papatasi in an endemic area of disease. Ann. Trop. Med. Hyg., 26, 294-298.

Jefferies, D., Livesey, J.L. and Molyneux, D.H.(1986). Fluid mechanics of blood-meal uptake by Leishmania - infected sandflies. Acta Tropica. 43, 43-53.

Kaddu, J.B. and Mutinga, M.J. (1981). Leishmania in Kenyan Phlebotomine sandflies. I-Leishmania aethiopica in the midgut of naturally infected Phlebotomus pedifer. Insect. Sci. Applic. 2,245-250.

Kaddu, J.B. (1986). Leishmania in Kenyan Phlebotomine sandflies. III-Advances in the investigations of vector capacity and vector parasite relationships of various species of sandflies in Kenya. Insect. Sci. Applic., 7, 207-712.

Killick-Kendrick, R. (1978). Recent advances and outstanding problems in the biology of phlobotomine sandflies. A review. Acta. Tropica., 35, 297-313.

Killick-Kendrick, R. (1979). The biology of Leishmania in phlebotomine sandflies. In, Biology of Kinetoplastida, Vol. 2 W.H.R. Lumsden and D.A. Evans (eds.),Academic Press, Inc., London. pp.396-449

Killick-Kendrick, R. and Molyneux, D.H. (1981). Transmission of leishmaniasis by the bite of phlebotomine sandflies: A possible mechanics. Trans. Roy. Soc. Trop. Med. Hyg., 75, 152-154.

Killick-Kendrick, R. and Ward, R.D.(Reporters) (1981). Ecology of Leishmania. Parasitology., 82, 143-152.

Kingdon, J. (1971). East African mammals (an atlas of evoluation in Africa). Vol. 1, Academic press, Baltimore, pp. 1326-1331.

Lane, R.P. (1986). Recent advances in the systematics of phlebotomine sandflies. Insect. Sci. Applic., 7, 225-230.

Le Blancq, S.M., Belehu, A. and Peters, W. (1986a). Leishmania in the Old World 3- The distribution of Leishmania aethiopica zymodemes. Trans. Roy. Soc. Trop. Med. Hyg., 80, 360-366.

Le Blancq, S.M. and Peters, W. (1986). Leishmania in the Old World. 2 Heterogeniety among Leishmania tropica zymodemes. Trans. Roy. Soc. Trop. Med. Hyg. 80, 113-119

Le Blancq, S.M., Schnur, L.F. and Peters, W. (1986b). Leishmania in the Old World. 1- The geographical and hostal distribution of Leishmania major zymodemes. Trans. Roy. Soc, trop. Med. Hyg. 80, 199-112

Lemma, A., Foster, W.A., Gemetchu, T., Preston, P.M., Bryceson, A. and Minter, D.M. (1969). Studies on leishmaniasis in Ethiopia. I. Preliminary survey into the epidemiology of cutaneous leishmaniasis in the highlands. Ann. Trop. Med. Parasit., 63, 455-472.

Lemma, A., Haile, T. and Foster, W.A. (1970). Epidemiological and experimental investigations on diffuse cutaneous leishmaniasis and localized cutaneous leishmaniasis. J. Parasitol., 56, 439-440.

Lewis, D.J. (1971). Phlebotomine sandflies. Bull. Wld. Hlth. Org., 44, 535-551.

Lewis, D.J.(1974). The biology of Phlebotomidae in relation to leishmaniasis. Ann. Rev. Ent., 19, 363-384.

Lewis, D.J. (1978). The phlebotomine sandflies (Diptera: Psychodidae). Bull. Br. Mus. Nat. Hist. Ent., 45, 121-209.

Lewis, D.J. (1982). A taxonomic review of the genus Phlebotomus (Diptera: Psychodidae). Bull. Br. Mus. Nat. Hist. Ent., 45, 121-207.

Lewis, D.J., Minter, D.M. and Ashford, R.W.(1974). The subgenus Larroussius of Phlebotomus (Diptera: Psychodidae). in the Ethiopian region. Bull. Ent. Res., 64, 435-442.

Lewis, D.J., Young, D.G., Fairchild, G.B. and Minter, D.M. (1977). Proposal for a stable classification of Phlebotomine sandflies (Diptera_ Psychodidae). Sys. Ent., 2, 319-332.

Maskovskij, D.D. and Duhanina, N.N. (1971). Epidemiology of leshmaniasis: general considerations. Bull. Wld. Hlth. Org., 44, 529-534.

Minter, D.M. (1963). Three new sandflies (Diptera: Psychodidae) from East Africa, with notes on other species. Bull. Ent. Res., 54, 483-495.

Minter, D.M. (1964). Seasonal changes in populations of phlebotomine sandflies (Diptera: Psychodidae) in Kenya. Bull. Ent. Res., 55, 421-435.

Molyneux, D.H. (1977). Vector relationship in the Trypanosomatidae. In, Advances in parasitology. Vol. 15 Ben Dawes (ed.)., Academic press, London. pp.43-53

Mutinga, M.J. (1971). Phlebotomus longipes, a vector of cutaneous leishmaniasis in Kenya [correspondance]. Trans. Roy. Soc. Trop. Med., Hyg., 65, 106

Mutinga, M.J. (1975a). Phlebotomus in the cutaneous leishmaniasis focus of Mt. Elgon, Kenya. E. Afr. Med. J., 52, 340-347.

Mutinga, M.J. (1975b). The animal reservoir of cutaneous leishmaniasis on Mt. Elgon, Kenya. E. Afr. Med. J., 52, 142-151.

Mutinga, M.J. (1986). Epidemiology of leishmaniasis in Kenya: Advances in research on vectors and animal reservoirs, and possible control measures. Insect. Sci. Applic., 7, 199-206.

Mutinga, M.J., Kyai, F.M., Kamau, C. and Omago, D.M. (1986a). Epidemiology of leishmaniasis in Kenya.- III: Host preference studies using various types of animal bait at animal burrows in Marigat, Baringo district. Insect. Sci. Applic.,7, 191-197.

Mutinga, M.J., Kyai, F.M. and Omago, D.M. (1986b). Investigations on the epidemiology of leishmaniases in Kenya. I-Studies on vectors of Leishmania major in Marigat, Baringo district, Kenya. Insect. Sci. Applic., 7, 181-189.

Mutinga, M.J. and Odhiambo, T.R. (1986a). Cutaneous leishmaniasis in Kenya. II-Studies on vector potential of Phlebotomus pedifer (Diptera: Phlebotomidae) in Kenya. Insect. Sci. Applic., 7, 171-174.

Mutinga, M.J. and Odhiambo, T.R. (1986b). Cutaneous leishmaniasis in Kenya, III- The breeding and resting sites of Phlebotomus pedifer (Diptera: Phlebotomidae) in Mt. Elgon Focus, Kenya. Insect. Sci. Applic., 7, 175-180.

Nadim, A., Javadian, E., Nousia, M.K. and Nayil, A.K. (1979). Epidemiology of cutaneous leishmaniasis in Afganistan, Part 2; Anthroponotic cutaneous leishmaniasis. Bull. Soc. Path. Exot., 72, 461-466.

Neronov, V.M. and Guinin, P.D. (1971). Structure of natural foci of zoonotic cutaneous leishmaniasis and its relationship to regional morphology. Bull. Wld. Hlth. Org., 44, 577-584.

Poirier, A. (1964). Notes sur les leishmaniases on Ethiopie. Annls. Inst. Pasteur Ethiopie.,5, 89.

Price, E.W. and Fitzherbert, M. (1965). Cutaneous leishmaniasis in Ethiopia: a clinical study and review of literature. Ethiop. Med. J., 3, 57-83.

Said, S.E., Beier, J.C., Elsawaf, B.M., Doha, S. and Kordy, E. (1986). Sandflies (Diptera: Psychodidae) associated with visceral leishmaniasis in EL Agamy, Alexandria Governate, Egypt, II-Field behaviour. J. Med. Ent., 23, 609-615.

Sale, J.B. (1966). The habitat of rock hyrax. J.E. Afr. Nat. Hist. Soc., 25 , 205-214.

Sarojini, P.A.; Humber, D.P., Yemane-Berhan, T.,Elizabeth, F., Ayele, B., Mock, B. and Wardorff, J.A. (1984). Cutaneous leishmaniasis cases seen in two years at the All African leprosy and Rehabilitation Training Centre Hospital. Ethiop. Med. J., 22, 7-11.

Schlien, Y., Palacheck, T. and Yuval, B. (1985). Mycoses, bacterial infection and antibacterial activity in sandflies (Psychodidae) and their possible role in the transmission of leishmaniasis. Parasitology., 90, 57-66.

Schlien, Y. and Warburg, A. (1985b). Feeding behaviour, midgut distention and ovarian development in Phlebotomus papatasi (Diptera: Psychodidae). J. Insect. Physiol., 31, 47-51.

Schlien, Y. and Warburg, A. (1986a).Phytophagy and the feeding cycle of Phlebotomus papatasi (Diptera: Psychodidae) under experimental conditions. J. Med. Ent., 23, 11-15.

Schlien, Y., Warburg, A., Schnur, L.F. and Gunders,A.E. (1982). Leishmaniasis in Jordan valley. II- sandflies and transmission in the central endemic area. Trans. Roy. Soc. Trop. Med. Hyg., 76, 582-586.

Schnur, L.F. (1986). Identity and taxonomy within the genus Leishmania. Insect. Sci., Applic., 7, 213-223.

Schnur, L.F. and Zuckerman, A. (1977). Leishmanial excreted factor (EF) serotypes in Sudan, Kenya and Ethiopia. Ann. Trop. Med. Parasit., 71, 273-294.

Southwood, T.R.E. (1978). Ecological Methods: with particular reference to the study of insect populations. 2nd ed., chapman and Hall., London, pp. 524.

Walker, E.P. (1975). Mammals of the world. 3rd ed. vol. 2. John Hopkin's University Press, Baltimore, pp. 1326-1331.

Wilkins, H.A. (1972). Studies on leishmaniasis in Ethiopia. VI: Incidence rates of cutaneous leishmaniasis at Meta Abo. Ann. Trop. Med. Parasit., 66, 457-466.

World Health Organization (1984). The leishmaniasis. Tech. Rep. Ser. No. 701. Report of a WHO Expert Committee, Geneva. pp. 140.