ADDIS ABABA UNIVERSITY
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Investigations on the parasite, vector and reservoir host of cutaneous leishmaniasis in Addis Ababa, Silti, Merabete and the Awash Region, Ethiopia

By

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ABBREVATIONS

CI = Body coefficient index
CL = Cutaneous Leishmaniasis
DCL = Diffused cutaneous leishmaniasis
DNA = Deoxyribonucleic acid
EDTA = Ethylen Diamine Tetra Acetic Acid
HCl = Hydrochloric acid
HIV = Human immuno deficiency virus
ITS = Intergenic transcribed spacer
kb = killo base
KDNA = Kinetoplast DNA
LCL = Localized cutaneous leishmaniasis
MCL = Muco cutaneous leishmaniasis
ML = Mandibular length;
NNN = Novy-MacNeal-Nicolle medium
PBS = Phosphate buffer saline
PCR = Polymerase chain reaction
PKDL = Post kalazar dermal leishmaniasis
RLFP = Restriction fragment length polymorphisms
RNA = Ribonucleic acid
SDS = Sodium Dodesylsulfate
TEA – buffer = Tris-Acetate- EDETA buffer
VL = Visceral leishmaniasis
WHO = World health organization
ZCL = zoonotic cutaneous leishmaniasis

ABSTRACT

From January 2005 to May 2006, investigations on the parasite, vector and reservoir host of cutaneous leishmaniasis in Addis Ababa, Silti, Merabete and Awash Region, were carried out in different areas of Addis Ababa, Silti, Merabete and Awash-7. Addis Ababa and Silti were discovered recently as *Leishmania* foci of cutaneous *leishmaniasis* in the highlands of Ethiopia. Awash valleys were areas where sympatric *Leishmania tropica* and *L. aethiopica* were discovered for the first time from sandflies. Merabete is not very well known for cutaneous leishmaniasis in humans, but visceral leishmaniasis was reported from children in the associated lowland. The current *Leishmania* epidemics in South Eastern Addis Ababa along the gorges of Bulbulla and Akaki rivers was related to settlement of people near the Bulbulla - Akaki river gorges where hyraxes and sandflies (*Phlebotomus longipes*) co-existed in crevices and cracks of the basalt rocks. It was the area where the zoonotic *Leishmania* cycle was maintained. In Silti, however, the epidemics was related to the recent increase in number of hyraxes and sandflies as a result of the ecological changes of the Kerate gorge that bisects the center of the town (Kibet) and synanthropic adaptations of the hyraxes. The possible reservoir hosts that were examined for natural infection of *Leishmania* were 79 hyraxes, 12 *Rattus rattus*, 14 *Praomys* spp., 41 bats, 3 mongooses and 3 genet cats. Samples from skin, blood, liver, spleen and bone marrow were cultured in NNN medium in addition to Giemsa stain. *Leishmania* parasites were isolated in NNN medium only from three bush hyraxes (*Heterohyrax brucei*) in Addis Ababa. The hyraxes from the other areas were negative for *Leishmania* in NNN medium. The total infection rate of *H. brucei* in Addis Ababa was 6.3% (3/48) and the highest infection rate was from the Saris area, 11.1% (2/18). The hyrax infection rates in Kality were 5.9 %(1/17). No infected hyraxes were found in Kilinto (Akaki). The giemsa stains of the above tissues were negative for amastigote stage of *Leishmania* in the macrophages. *Leishmania* infection was not found in the guts of sandflies dissected. Seasonality and age dependent infections in hyraxes were observed. Infections in hyraxes were found only in April, June and September after spring peak sandfly abundance and biting passed. In Addis Ababa, no adults (0/16) were found infected, but infections occurred only in juveniles (2/25) and subadults (1/10).

1. INTRODUCTION

Leishmaniasis is a manifestation of diseases caused by obligate intracellular protozoan in the genus *Leishmania* that belong to the order Kinetoplastida, Family Trypanosomatidae. It is transmitted by female sandflies of genus *Phlebotomus* (Old World) and *Lutzomia* (New World). The sandflies ingest the amastigote stage of the parasite during blood meal on vertebrate hosts. The parasite is transformed into metacyclic promastigote in the sandfly gut and transmitted to hosts during second meals (Sacks and Perkins, 1984; Lainson and Shaw, 1987). According to Shaw (1994), the genus *Leishmania* includes 30 species of *Leishmania* parasitizing mammals, including 21 species, which infect man. The genus *Leishmania* is divided into the subgenera *Leishmania*, *Viannia* and *Sauroleishmania* (Lainson and Shaw, 1987). The *Leishmania* like parasites of lizards (*Sauroleishmania*) are not known to cause natural infection in humans. Other *Leishmania* species, which are unknown as human parasites include *L. enriettii,* which is known from isolates of domestic guinea pigs and *L. hertigi* of South American porcupines and other parasites of Sloth's (Bray, 1974; Ashford, 1996). More recently Cupolillo *et al.* (2000) have proposed the separation of the genus *Leishmania* in to two divisions, *Euleishmania* and *Paraleishmania.* The *Euleishmania* is composed of the subgenera *Leishmania* and *Viannia* as described by Lainson and Shaw (1987) and the *Paraleishmania* consists of *L. heritigi, L. deanei, L. colombiensis* and *L. equatoriensis.*

The leishmaniases are endemic in 88 countries with a total of 350 million people at risk. It is believed that 12 million people are infected by leishmaniasis worldwide. About 1.5 million new cases of cutaneous leishmaniasis (CL) and 0.5 million visceral leishmaniasis (VL) occur every year (WHO, 1990; Desjeux, 2001). The epidemiology of leishmaniasis depends on massive migrations (El-Hassen and Zijilstra, 2001; Kolaczinski *et al.*, 2004), urbanizations (Tesh, 1995), and intrusions into *Leishmania* foci (Sang, 1992a; Weigle *et al.*,1993), building of irrigation schemes and dams (Ben Rachid *et al.*,1987) and individual risk factors such as malnutrition or famine and immunodeficiency (Dujardin, 2006).

1.1. CLINICAL FORMS OF *LEISHMANIASIS*

A. Cutaneous leishmaniasis (CL)

Cutaneous leishmaniasis can be divided into localized cutaneous leishmaniasis (LCL), diffused cutaneous leishmaniasis (DCL) and recidivate. Localized cutaneous leishmaniasis (LCL) is the type of manifestation with sores or ulcers on exposed parts of the body such as arms, legs and face, which remain localized and may heal spontaneously. Diffused cutaneous leishmaniasis (DCL), however, consists of painless nodular lesions over wide area of the body which is non self-healing. Recidivate is a type of CL with new eruptions evolved at the border of the past scaring lesion after many years. In Old World, LCL can be caused by four parasites: *L. aethiopica, L. major, L. tropica* and *L. infantum* (WHO, 1990). *L. aethiopica* is restricted in high lands of Ethiopia and Kenya (Lemma *et al.,* 1969; Mutinga, 1971; Ashford *et al.,* 1973; Hailu and Formmel, 1993). In Ethiopia, DCL is caused by *L. aethiopica* (Bryceson and Leithead, 1966; Bray and Bryceson, 1969 and Belehu, 1980) while in New World, DCL caused mainly by *L. amazonensis* (Grimaldi, 1989). DCL occurs also in immuno-deficient patients with no species-specific relation (Ramos-Santos, 2003). Recurrence of cutaneous leishmaniasis in HIV- patients with healed localized cutaneous leishmaniasis due to *L aethiopic*a was reported by Berhe *et al.* (1995). *L. major* is endemic in desert and Savannah regions of North Africa, the Middle East, and Central Asia (Bray, 1974; Le Blancq *et al.,* 1986). *L. tropica* has also a potential to recidivate. It is found in Middle East, South West Asia and North India (WHO, 1984; Le Blancq and Peters, 1986a; Lainson and Shaw, 1987). *L. tropica* is rarely found to be the causative agent of VL (Magil, *et al.,* 1993). CL can be caused by *L. infantum* in Mediterranean region (Alvar *et al.,* 1997), while in most patients it causes VL. In New World, CL is caused by *L. mexicana, L. amazonensis, L. panamensis, L. guyanensis, L. braziliensis* and other agents of the disease (Lainson and Shaw, 1987; Grimaldi, 1989).

B. Muco-cutaneous leishmaniasis (MCL)

Muco-cutaneous leishmaniasis (MCL) or espundia produces lesions, which can lead to disfiguring destruction of mucous membranes of the nose, mouth and throat cavity. MCL is mainly caused by *L. braziliensis* but is also occasionally caused by *L. aethiopica* (Bryceson, 1969) *and L. guyanensis* (Grimaldi, 1989).

C. Visceral Leishmaniasis (VL) or kala azar

Visceral leishmaniasis (Kala azar) is the most severe form and if untreated it usually is fatal. The classical VL is characterized by fever, malaise, weight loss and hepatomegaly. Patients in India often develop hyperpigmentation, which led to the name kala-azar, meaning black fever in Hindi (Pearson and de Queiroz Sousa, 1996). It is caused by *L. donovani* in India and Africa (Le Blancq and Peters, 1986b) and by *L. infantum* in Mediterranean regions, which can also causes CL, MCL, DCL and PKDL. (Alvar *et al.*, 1997). In Latin America, VL is caused by *L. chagasi* (Lainson and Shaw, 1987; Gramaldi, 1989). Post kala azar dermal leishmaniasis (PKDL) is caused due to complication of VL which is characterized by occurrence of skin lesions, or nodules, mainly on the face, after 2-7 years of unsuccessful treatment of VL (Zijlstra and El-Hassen, 2001). In India, patients with PKDL are considered to be important reservoirs of the parasites (Thakur and Kumar, 1992).

1.2. VECTORS OF *LEISHMANIA*

Sandflies are small, fragile, nocturnally active insects with weak, direct flight capability. Both sexes require sugar for energy, obtained from variety of sources, including the vascular tissues of plants (Alexander and Usma, 1994). The normal ovarian development in sandflies requires blood meal. Following ingestion of blood meal is the secretion of proteolytic enzymes from the midgut (Gemetchu, 1974; Alexander, 2000; Jacobson, 2003).

According to Kellick-kendrick (1990), there are four criteria to consider sandflies as vectors (1) the sandflies support development of the parasite after infecting blood meal has been digested and defecated (2) sand flies should feed on man and/or reservoir host (3) isoenzyme analysis of the parasite in man and reservoir hosts should indicate similarity with naturally infected sandflies (4) the vectors should readily be attracted and feed on the animal host in nature.

The two proven vectors of *L. major* are *P. duboscqui* (Mutinga *et al.,* 1989) and *P. papatasi* (Dhiman *et al.,* 1983) and the only known vectors of *L. aethiopica* are *P. longipes* and *P. pedifer* (Mutinga, 1971; Foster 1972; Ashford *et al.,* 1973). The most probable vector of *L. tropica* in all foci seems *P. sergenti* (Ashford *et al.,* 1992; Jacobson *et al.*, 2003). *P. arabicus* and *P. saevus* were found infected with *L. tropica* in Ethiopia in addition to *P. sergenti* and considered as implicated vecters of *L. tropica* (Gebre-Michael *et al.,* 2004). The vectors of *L. donovani* are *P. argentipes* in India (Dhiman *et al.,* 1983) *P. celiae* and *P. martini* in East Africa (Mutinga *et al.,* 1989; Gebre-Michael and Lane, 1996). The *vectors* of *L. infantum* in the Mediterranean regions are *P. ariasi* (Killick-Kendrick, 1987), *P. perfliwi* (Bettini *et al.,* 1986) and *P. perniciousus* (Bettini *et al.,*1986). The vectors of American visceral leishmaniasis due to *L. chagasi* are *Lu. longipalpis* and *Lu. evansi* (Travi *et al.*, 1990).

Adult sandflies shelter during the day in dark, humid places with wind protection such as tree holes, animal burrows, and crevices or under rocks (Ashford *et al.,* 1973; Yaghoobi-Ershadi and Javadian, 1996; Alexander, 2000). In the classical rural zoonotic foci in North Africa, South Asia and part of Middle East, *P. papatasi* rest in the great gerbil (*Rhombomys opimus*) burrows (Bray, 1974; Ashford, 1996; Yaghoobi-Ershadi and Javadian, 1996). Termite hills in East Africa are favorite resting places for *P. martini* and *P. celiae* and many other sandflies in the area (Mutinga and Kanau, 1986; Gebe-Michael *et al.,* 1996). *P.* orientalis in Sudan and Ethiopia, *is* found only in or near *Acacia-Balanites* forests (Fuller *et al.,* 1976; El-naiem, 1997; Zijlstra and El-Hassen, 2001). In Ethiopia and Kenya, *P. longipes* and *P. pedifer* rest in caves, hollow tube inside trees and cracks where hyraxes live (Ashford *et al.,* 1973; Mutinga and Odhiambo, 1986). Searching for developmental stages of sandflies in their natural habitats is difficult, tedious and has proved to be remarkably unproductive (Killick–Kendrick, 1999). The eggs are laid in terrestrial micro-habitats rich in organic matter that provides food for the larvae (Alexander, 2000; Feliciangeli, 2004).

1.3. RESERVOIR HOSTS OF *LEISHMANIA*

Most leishmaniases are zoonotic diseases with one or more reservoir hosts maintaining the disease. A host is by definition a habitat for a parasite at least temporarily. A mammalian host responsible for the long-term maintenance of a population of infectious agent is a reservoir host. A *Leishmania* disease is called zoonosis if the host range includes man and other mammals and anthroponotic if only humans are involved in transmission cycle. According to Ashford (1996), reservoir hosts fulfill the following criteria: (1) abundant or gregarious (2) long lived or survive at least during non transmission season of the parasite (3) remain infected for long time without acute disease and (4) present the parasite in their skin or circulation for sandfly bite.

In Ethiopia and Kenya, the reservoir hosts of zoonotic cutaneous leishmaniasis (ZCL) due to *L. aethiopica* are hyraxes. The behavioral and ecological requirements of hyraxes in *L. aethiopica* endemic areas make them good reservoir hosts of leishmaniasis. They are long-lived, gregarious, and share a habitat with *Phelobotomus* spp. and enhance the environment by accumulation of organic matter in their latrine for sandfly breeding (Ashford *et al.*, 1973). Both the mammals and vectors are infected at least seasonally (Ashford, 1977). The intense outbreaks *L. aethiopica* infections of man are usually due to exposure to an area where a parasite is maintained by hyraxes (Ashford *et al.*, 1973; Ashford, 1997). This is typical example of zoonotic transmission. Reports of ZCL due to *L. tropica*, however, are scarce and search for reservoir hosts is intensive. The dogs and the rats so far described are not true reservoir hosts, but incidental hosts. Dogs in some areas were victims as humans (Ashford, 1996). The only suspected animal, where more than one specimen has shown some suggestion of being a reservoir host, is the hyrax. Both in Kenya and Israel, hyraxes have been found infected (Sang *et al.*, 1992b; Ashford and Sang 2001; Jacobson *et al.*, 2003). The search for more infected hyrax in the zoonotic foci would confirm whether this animal is the only sole reservoir host of this parasite.

Four-host parasite-ecology systems have been described for *L. major* based on the principal hosts namely *Psammomys*, *Meriones, Rhombomys* and *Arvicanthis* and *Phlebotomus* sand fly vectors (Bray, 1974; Ashford, 1987; Ashford, 1996). The animals, which tend to satisfy the criteria for reservoir hosts are the great gerbils (*Rhombomys opimus*). Sometimes, it would be difficult to say whether *R. opimus* is the primary host of *L. major* because only few infected *R. opimus* survive after the winter breeding season to insure the parasite maintenance (Rioux *et al.*, 1992). This indicates rodents are not the original hosts. *L. major* is also found in the absence of *R. opimus* in some part of Central Asia where the parasite was believed to be maintained by the *Meriones species*, or by *Nesokia indica* in Iran and in some foci in Palestine (Ashford, 1996). In sub-Saharan Africa, *L. major* has been isolated from different species of *Arvicanthis* spp., but the population of these rodents fluctuates to greater extent and the parasite may depend on other reservoirs such as *Mastomys* spp. for its maintenance. Of these, the *Arvicanthis/Phlebotomus* has been assumed to be the most primitive system and an

evolutionary processes originating with *Arvicanthis* transferring to *Meriones* and then to the other hosts has been postulated (Ashford, 1987). In sub-Saharan Africa *L. major* has more than one reservoir hosts (Arvicanthis, *Mastomys* and *Tatera* species) including man that are required to maintain the parasite (Ashford *et al.,* 1997). The epidemics of *L. major* in Khartoum (Sudan) between 1985 and 1987 where the disease had never been in Khartoum and no reservoir hosts reported before was assumed to be due to anthroponotic transmission of the disease to local non-immune population by influx of migrants from endemic areas. (EL- Hassen and Zijlstra, 2001).

Apart from anthroponotic transmission in India, *L. donovani* is zoonotic through out its ranges (Le-Blancq and Peters, 1986b). In East Africa, the reservoir hosts of visceral leishmaniasis are unknown. Dogs are the principal domestic reservoirs while wild canids (foxes, jackals, and wolves) serve as the major sylvatic reservoirs of *L. infantum* and *L. chagasi* (Mauricio *et al.,* 1999). According to Ashford *et al.*, 1997, dog is secondary host for *L. infantum* as it is the only reservoir host that suffers serious disease. The frequent presence of sandflies in caves and crevices commonly inhabited by bats suggests that they may provide a blood source for these sandflies (Mutinga, 1975).

1.3.1. Hyraxes

Hyraxes are members of Hyracoidea, family Procavidea and have short legs, a rudimentary tail, and round ears. The relatives of hyraxes are elephants, sea cows, elephant-shrews, aardvark and golden moles, which collectively are called the Afrotheria (Springer *et al.*, 1997). Rock, bush and tree hyraxes are the three groups of hyraxes in the genera *Procavia, Heterohyrax* and *Dendrohyrax,* which are superficially similar in size and appearance (Hoeck, H. N., 1982; Barry and Shoshani, 2000). Hyraxes are endemic to Africa and Middle East. The distributions of rock hyraxes extend into the Arabian Peninsula from Lebanon to Saudi Arabia (Corbers, 1979; Barry and Shoshani 2000). *H. brucei* and *P. capensis*, some time occupying the same habitat and their separation in field could be difficult (Corbet, 1979; Barry and Mundy, 1998). Sympatric *Procavia* and *Heterohyrax* share common holes and latrine (Hoeck, 1975; Corbet, 1979; Barry and

Mundy, 1998). Synchronous annual birth, gestation period, reproductive seasons of the two genera were also reported to be the same (Barry, 1994). Due to different reproductive anatomy and sexual behaviors they do not interbreed (Hoeck *et al.*, 1982). Interspecific competition is avoided by differential feeding habits. Rock hyraxes feed mainly on grass while bush and tree hyraxes feed on vegetables, (Hoeck, 1975; Deniro and Epistein, 1978).

Hyraxes have a poor ability to regulate their body temperature and a low metabolic rate for their body size. Body temperature is maintained mainly by gregarious huddling, long periods of inactivity, and basking. They are dependent on shelters (boulders and tree cavities) that provide relatively constant temperature and humidity (Rübsamen *et al.* 1982; Barry and Shoshani, 2000).

Rock and bush hyraxes live in cohesive and stable family groups consisting of 3-7 related adult females, 1 adult territorial male, dispersing males, and the juveniles of both sexes. The territorial male repels all intruding males (Hoeck *et al.*, 1982). Females become receptive about once a year, and a peak in births seems to coincide with the rainy season (Barry and Mundy, 1998). Gestation is 7.5 months and the number of young per female bush and tree hyraxes ranges from 1-3 and in rock hyraxes from 1-4 and weaning up to 5 months. Reproductive maturity is generally attained at an age of 16-24 months age (Hoeck, 1982). Hyraxes are capable of living for longer than 10 years (Hoeck, 1982).

1.3.1.1. Taxonomy of hyraxes

Restriction fragment length polymorphisms of mitochondrial DNA show *Heterohyrax* and *Procavia* are closely related and *Dendrohyrax* is basal with in the family Procavidae (Barry and Shoshani, 2000). But *Heterohyrax* is less heavily built than *Procavia* (Barry and Mundy, 1998), with narrower muzzle (Skinner and Smithers, 1990) and smaller in size and rarely greater than 48 cm body length (Corbet, 1979). *Heterohyrax* has reddish or greyish brown hairs and *Procavia,* yellowish to reddish or greyish brown hairs (Barry and Shoshani, 2000) while *Dendrohyraxes* have long, wooly, grey or brown hairs (Jones,

levels. DNA (kDNA or genomic DNA) sequences, which are conserved with in the genus, are used for identification of the genus while Polymorphic DNA sequences in the genus used for identification of the species with in the genus (Rodriguez *et al.*, 1994; Margarita *et al.*, 1997; Schonian *et al.* 2000; Schonian *et al.*, 2001). The amplification of the internal transcribed spacer (ITS) in the ribosomal operon with the LISTR/L5.8S primers produces about 350 pb sized PCR-product for all *Leishmania* species. PCR-restriction fragment length polymorphism analysis or schizodeme analysis of ITS1-amplicon is used for identifications of all *Leishmania* species, except members of the *L. donovani* and *L. brasiliensis* complexes (Schonian *et al.* 2001).

1.5. LEISHMANIASIS IN ETHIOPIA

For the first time, CL in Ethiopia was described by Martoglio (as cited in Ashford *et al.*, 1973) before the wide spread of the disease was shown later by different investigators. The discovery of DCL in Ethiopia by Balzer *et al.*, (1960) interested many investigators. CL and DCL are two forms of diseases caused by single species, *L. aethiopica* (Bray and Bryceson, 1969; Bray, 1974; Chance *et al.*, 1978). Isoenzyme study of *Leishmania* parasites from CL and DCL patients from Ethiopia was indistinguishable from one another (Le Blancq *et al.*, 1986; Schonian *et al.*, 2000).

Both CL and VL are endemic in Ethiopia. The former is wide spread in highlands (Lemma *et al.*, 1969; Ashford *et al.*, 1973; Hailu and Frommel, 1993) while the latter mainly occurs in lowlands (below 1300m a.s.l) (Fuller *et al.*, 1979; Lindtjorn and Olafsson, 1983; WHO, 1990; Hailu and Frommel, 1993). The agents of CL reported in Ethiopia are *L. aethiopica* (Lemma *et al.*, 1969; Ashford *et al.*, 1973; WHO, 1990; Gebre-Michael *et al.*, 2004), *L. tropica* (Gebre-Michael *et al.*, 2003; Hailu *et al.*, 2006) and *L. major* (Haile and Lemma, 1977; Gebre-Michael *et al.*, 1993). The CL in Ethiopia is almost always due to *L. aethiopica*. Cases of CL due to *L. aethiopica* among a population of 30 million were reported to be between 50,000-70,000 (Belehu, 1980). *L. major* is very rare and so far limited to the lower Omo valley of Southern Ethiopia from where it was identified from *Arvicanthis* spp. (Haile and Lemma, 1977; Chance *et al.*, 1978) and Segen valley (Aba Roba) from where it was isolated from *P. duboscqi* (Gebre-

Michael *et al.*, 1993). In the highlands the two hyrax species (*Procavia capensis* and *Heterohyrax brucei*) have been incriminated as the reservoir hosts of *L. aethiopica,* while two closely related sand fly species, *Phlebotomus longipes* and *P. pedifer* had been incriminated as its vectors (Ashford *et al.,* 1973). The distribution of CL in Ethiopia was found to be restricted between 1400 and 2700m (Hailu and Frommel, 1993) due to the restriction of the sandflies in highlands (Lemma *et al.,* 1969; Ashford, 1977). *P. longipes,* the principal vector of CL in highlands of Ethiopia, was first discovered from Addis Ababa (2348m altitude) by Parrot and Martin (1939). Bryceson (1969), however, estimated the lower limit for CL to be 1200m, which implied the vector potential of other sandflies in lowlands. The recent isolation of *L. aethiopica* from *P. sergenti* in Awash vallies (Gebre-Michael, *et al.*, 2004) and from a squirrel (*Xerus rutilus*) in lowland of Southern Ethiopia (Abebe *et al.*, 1990) proved the existence of the parasite in lowlands. Isolation of the parasite from humans and other reservoir hosts requires further investigations.

The current epidemics of CL in South Eastern Addis Ababa and Silti seem recent phenomena in the highlands. Addis Ababa was thought to be as CL free area of the highlands (Lemma *et al.,* 1969; Wilkins, 1972; Foster, 1972; Ashford, 1977). However, Sarojini *et al.*, (1984), however, listed all highlands of Ethiopia including Addis Ababa as *Leishmania* foci. The existence of suitable ecology for *L. aethiopica* in Entoto Mountains (North Western Addis Ababa) was pointed out by Ashford (1977).

VL caused by *L. donovani* s.l. in Ethiopia mainly occurs in the lowlands (<1300m. a.s.l.) and lowlands that surrounded the central highlands of the country (Hailu and Frommel, 1993). Major foci occurring in the Northwest and Southern Ethiopia. The Humera lowlands and the Belessa highland valley in the North West (Fuller *et al.,* 1976; Ashford *et al.,* 1973; WHO, 1996), the Aba Roba (in the Segen valley) of the lower Omo valley in the South West (Fuller *et al.,* 1979; WHO, 1996) and Woyoto, Gelana and Dawa Valleys of Southern Ethiopia (Lindtjorn and Olafsson, 1983; WHO, 1996). The vectors of the disease are *P. martin* for Aba Roba focus (Gebre Michael and Lane 1996) and *P. orientalis* for the Belessa (Ashford *et al.*, 1973) and Setit Humera (Gemetchu, 1975).

Co-infection of HIV and visceral leishmaniasis was reported in Ethiopia (Berhe *et al.*, 2001). Nothing definite is yet known about the animal reservoir hosts.

1.6. PROBLEM STATEMENT

Almost all CL cases in Ethiopia is due to *L. aethiopica* (Saroijini *et al.*, 1984). The pentavalent antimonial drugs with many side effects, some time, ineffective for *L. aethiopica* (Hailu and Frommel, 1993). Human-made and natural changes to the environment have been reported to increase the density of sandflies and reservoir hosts which were related to high rate of anthroponotic *Leishmania* transmissions (Dujardin, 2006) as well as zoonotic transmission (Ben Rachid *et al.*, 1987; Neouimine, 1996). Treatment of patients may be useful for the control of anthroponotic transmissions but not for zoonotic transmission of *Leishmania* like *L. aethiopica*. In the absence of any form of vaccine for clinical use, the control of *L. aethiopica* or any other zoonotic forms require interventions of the natural cycle. To programme *Leishmania* control strategy in Ethiopia, information about the reservoir hosts (hyraxes) and sandflies is very important. The aims of this study were to study the ecology of hyraxes and sandflies in relation to human *Leishmania* infections and determine those factors that affect *Leishmania* transmission cycle.

8. HYPOTHESIS

Seasonal and age dependent infections in hyraxes might exist which determined the natural infection rates of hyraxes.

1.8. OBJECTIVES

1. 8.1. General objective

Epidemiological study of cutaneous leishmaniasis in Addis Ababa, Silti, Merabete, and Awash valley.

1.8.2. Specific objectives

- To relate the distribution of human infections with the presence of hyraxes and sandfies.
- To isolate *Leishmania* parasites from hyraxes and sandfies and determine the natural infection rates.
- Identification and characterization of the *Leishmania* isolated from hyraxes and sandflies.
- To see if there is seasonality and age dependant infection in hyraxes.
- To work on the taxonomy of hyraxes in the study areas

2. MATERIALS AND METHODS

2.1. Study areas

Since leishmaniasis in Ethiopia is better understood clinically than epidemiologicaly, Addis Ababa, Silti, Merabete and Awash valley study areas were selected based mainly on prevalence of the *Leishmania* infections in humans (Addis Ababa, Silti and Merabete) and identifications of *Leishmania* infected sandflies from the Awash valley.

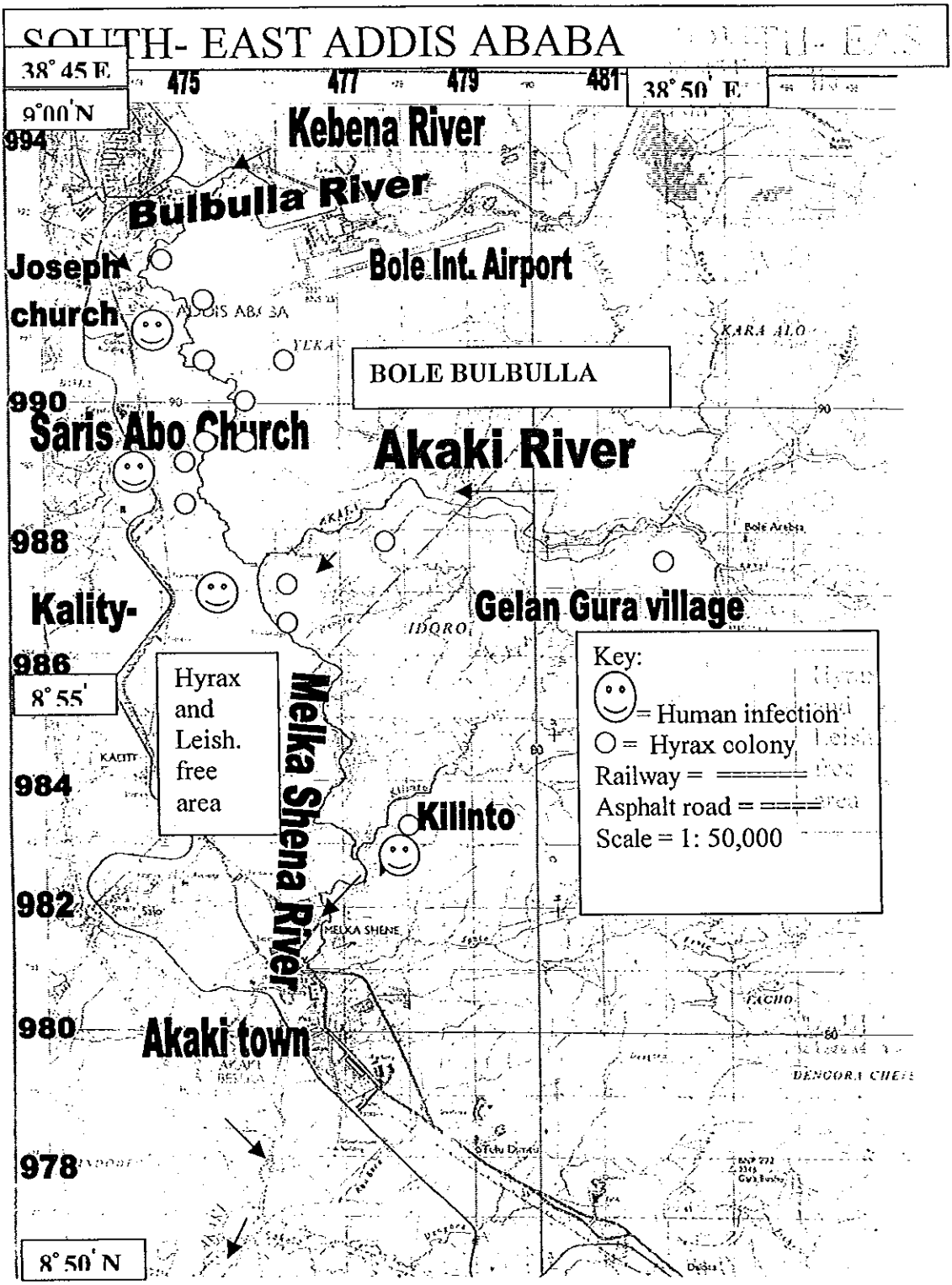


Figure 2: Map of Bulbulla - Akaki river gorges where hyraxes, sandflies and human *Leishmania* infections co-existed (source: Ethiopian map authority).

2.1.2. Silti

Silti is located 140km South of Addis Ababa at altitude of 2020m a.s.l and 8.01˚N latitude and 38˚, 2'E longitude. The mean average rainfall of Silti in 2006 was around 1115mm.The center of the town is bisected into two by the Kerate gorge with about 6 -10m depth. The gorge started from lower side of the mountain found towards the western side of the town and extends across Weleya-Sidist and then divided the center of the town (Kibet) into two until the end of the town. Houses are located on the either sides of the Kerate gorge in the Kibet area (Figure 3).

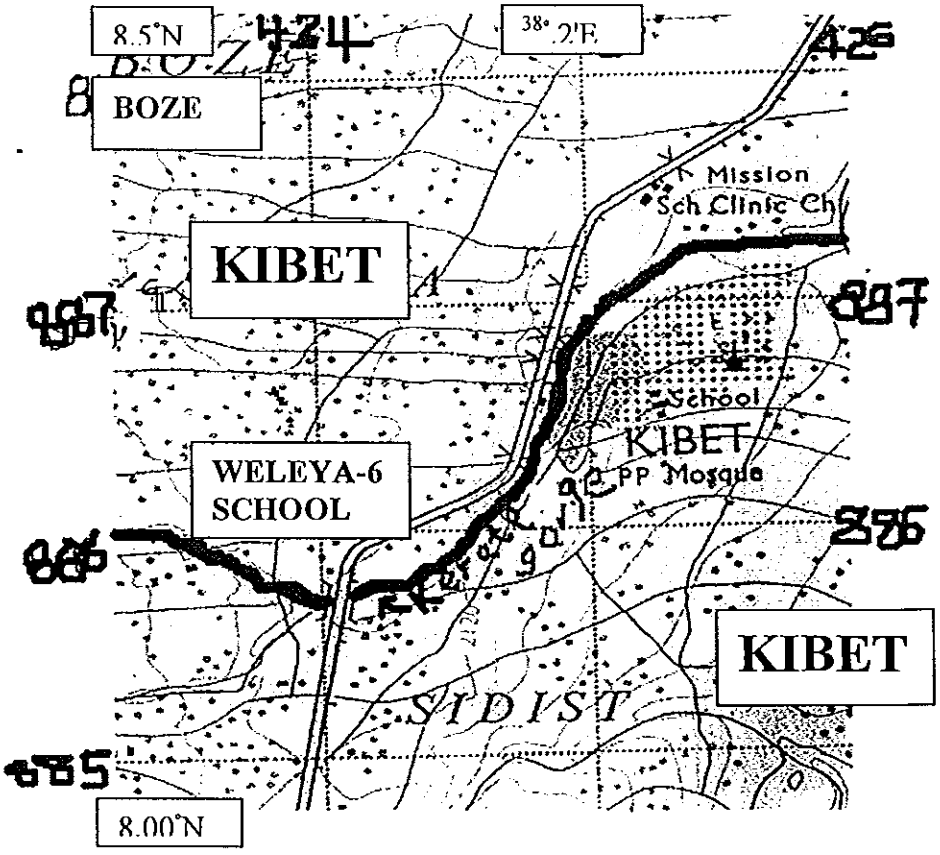


Figure 3: Map of Silti town around Kerate gorge where hyraxes, sandflies and *Leishmania* patients were found together. (source : Ethiopia map authority)

2.1.3. Merabete (North Shewa)

Alem Ketema (the largest town in Merabethe wereda) is located at 10° 0.4'N, 38°59'E and 2116m altitude a.s.l. It is located 190 km north of Addis Ababa. About 10km North of Alem Ketema was Kumamba village. The people of Kumamba are living by farming the hillsides and the flat plain near Wonchit river (1400m a.s.l) located about 10km from the village. They also raise cattle, goat and sheep. The livestock are mostly guarded at night near Wonchit river and adults and boys stay the night in the low land to keep the animals. Except the kids almost all residents go to the lowland for farming, fire wood collecting or to provide food for member of the family who spend the night in the low land. Lijagba village is located between Alemketema and Kumamba.

2.1.4. Awash - 7

Awash - 7 is located in middle Awash valley of Ethiopian rift valley at 9 °04'N and 38 °9'E at altitude around 1000m a.s.l. in the North Eastern Ethiopia in the Afar region.

2.2. Resting sites, behaviours, abundance and natural *Leishmania* infections of sandflies

Search for sandflies were made in all possible resting sites. Sandflies were collected using aspirators and CDC light trap (Model512, Hock & co., Gainseville, Florida, USA). High human biting behaviour inside hyrax caves or near hyrax holes enabled sandfly collection with aspiraters. In accessible areas, sandflies were also aspirated from their resting sites. The conditions which increase human biting rates and outdoor activities of sandflies were noted. The seasonal abundance of sandflies was determined from the number of sandflies trapped per given time each month by aspirators and CDC-traps. Domestic resting sites were searched near human dwellings. Sandflies visiting human houses at night were observed by staying and watching around mid-night when they are attracted to light and white paper. Some times CDC-traps were used to see if sandflies visit human dwellings at night. The sandflies collected knocked down by shaking before

sterilization of the surface in 1.5% savlon. Then the male and female sandflies were sorted after other insects were discarded. Female sandflies were dissected in physiological saline, for searching of *Leishmania* parasites in the gut. After removing the head, and then drawing out the gut by pulling on the posterior abdominal segments, the gut was covered with a cover slip, and then examined at x100 and x400 for *Leishmania* promastigotes. The head of the dissected female sandflies were mounted in gum on slide for latter sandfly identification.

2.3. Ecology, taxonomy, population structure and natural *Leishmania* infection of hyraxes.

The range of habitats used and the behavior of hyraxes including feeding habit were observed in selected areas. Observations of the population of hyraxes were carried out throughout 2005/2006. The months when newborn juveniles, pregnant and lactating mothers trapped were noted to find out breeding season. Hyraxes were aged and grouped as juveniles, subadult, and adults from their comparative sizes and sequential growth changes of the skull and teeth. The species were identified from teeth and skull morphology in addition to physical characters

Locally made snare traps were set up at the entrance of hyrax caves where hyraxes are found more frequently. Rodents and carnivores were trapped using collapsible traps (Bio Quip Products, USA). The traps were baited with peanut butter for the rodents while piece of rotten meat were used for carnivores. Bats were captured from the caves by hand after the gates of the caves were closed with spiny shrubs. Animals were trapped in less than 1km distance from the nearest micro-*Leishmania* foci.

A biphasic NNN (Novy-MacNeal-Nicolle) medium with an over lay of Lockes solution that is autoclaved at 15 lbs and 122 C° for 20 minutes is used for culturing *Leishmania* parasites. Samples from nose, blood, liver, spleen and bone marrow of hyraxes and other possible reservoir hosts were transferred under sterile condition to the liquid phase of NNN slants and kept in screw capped glass vials. The vials were then kept at room

temperature. A search for promastigotes was made each week by examining wet mounts under100x magnification before discarded four weeks later.

2.4. Co-existance of human *Leishmania* infections and the presence of hyraxes and sandflies

The presence of hyraxes and other potential reservoir hosts and sandflies were noted in the areas where there were human infections. In addition the possible factors that determine the transmission of the *Leishmania* parasites were studied.

2. 5. The impression smear method

Impression or touch smear of bone marrow, spleen, liver and skin were made together with thin blood smear for all animals caught to see amastigotes of *Leishmania.* The samples on the slides were fixed in absolute methanol and stained with giemsa and examined under an oil immursion objective.

2.6. Parasites inoculation into hamsters

0.1ml culture (about 10^{6} promastigotes) from stationary phase of parasite strains isolated from different hyraxes (LH2/SK, LH11/SK and LH32/SK) were inoculated subcutaneously into the nose and on the surface of pads of hind legs on 16 golden hamsters (*Mesocricetus auratus*), in order to see the formation of lesion and its progress to ulcerate after some time. Finally parasites were re-isolated in culture and stained on a slide with Giemsa from the hamsters' lesions.

2.7. *Leishmania* species identification by amplification PCR-product and restriction digestion of the internal transcribed spacer (ITS) sequence

2.7.1. DNA isolation from cultured *Leishmania* promastigote samples

Cultured promastigotes of *Leishmania* strains (LH02/SK, LH2/SK, LH11/SK and LH32/SK) isolated from hyraxes were harvested during exponential growth phase. LH02 and LH2/SK were the same and obtained from a hyrax (H-2) and the former was maintained in the laboratory through subculturing while the later was recovered from hamsters as the rest of the strains. The genomic DNA was isolated from the promastigotes by the phenol-chloroform-isopropanol extractions and ethanol precipitation method (Gadisa, 2005). Approximately 10^7 promastigotes /ml were pelleted by centrifuging at 2060xg rpm and washed three times with PBS (pH 7.2) and transferred to 1.5 ml eppendrof tube. Parasites were lysed in 500-µl lysis buffer (100mM Tris-HCl (pH 8), 50mM EDTA (pH 8.3), 1% SDS, 1% Triton X-100). The lysate was then incubated with RNase to final concentration of 100µm/ml (5µl of 10mg/ml solution at 37^0C for 1 hr to destroy contaminating RNA and further incubated overnight with proteinase K (5µl of a 10 mg/ml solution) added to final concentration of 100 µg/ml) at 42^0C to remove proteins that would interfere with further analysis of DNA. Then, 300 µl of a 25:24:1 mix of phenol: chloroform: isoamyl alcohol was added to each sample and vortexed for 5 seconds. After centrifugation at 1200xg for 5 minutes at room temperature, the upper face (layer) was carefully removed and transferred to new 1.5 ml eppendrof tube. Three hundred and fifty micro liters (0.7xvolumes) isopropanol was added to the sample and kept at -20^0C for 30 minutes. Subsequently, the samples were centrifuged at 1200xg for 15minutes. The isopropanol was removed and ice - cold 70% ethanol (1 ml) was added. After 5 minutes of incubation at -20^0C, the samples were centrifuged for 5 minutes. The ethanol was removed and the DNA samples were then suspended in 20 µl 1 TE - buffer and heated for 10 minutes in 68 ^{0}C water. Fifty fold dilutions of the DNA were used to determine concentration spectrometrically at absorption of 260nm and dilutions of 1 µg/ µl were prepared. DNA samples were stored at -20 ^{0}C until processed further.

blood meal. The total sandflies trapped from Addis Ababa were 1307 (663 males and 644 females) between March 2005 to March 2006. In Addis Ababa high sandfly abundances were observed in April, September, October and November (Table 1; Figure 4). Five hundred and eighty female sandflies collected from around hyrax holes in Addis Ababa were dissected and none of them found with *Leishmania* parasites in their gut.

Table 1: Phlebotomus sandflies collection by different methods in Addis Ababa.

(A) Phlebotomus sandflies collected by search from their resting sites in Addis Ababa (April, 2005 to March, 2006) using aspirators.

Locality	Month	Avarage sandflies/hour	Total	Male	Female
Saris	April 2005	11	44	34	10
Saris, Kality	May	7	21	12	9
Kality	June	10	50	32	18
Kilinto	July	9	45	30	15
Saris, Kality	September	12	24	15	9
Saris, Kality	October	15	30	19	11
Saris,	November	10	40	28	12
Saris	December	4	8	4	4
Kality	March 2006	7	7	3	4
Total		85	269	177	92

(B) Phlebotomus sandflies collected in Addis Ababa (April 2005 to March 2006) using CDC-traps at night.

Locality	Month	Average sandflies/ night	Total	Male	Female
Saris	April-2005	56	168	104	64
Saris	May	10	10	7	3
Kality	June	15	15	10	5
Kilinto	July	12	12	8	4
Saris	September	59	177	107	70
Saris, Kality	October	72.5	145	98	47
Saris	November	74	148	84	64
Saris	December	14	14	10	4
Saris	March- 2006	25	75	50	25
Total		337.5	764	478	286

C) Phlebotomus sandflies collected using aspirators on human bait in Addis Ababa (March, 2005 to March, 2006).

Locality	Month	Avarage sandflies/hour	Total	Male	Female
Saris	March	10	30	0	30
Saris	April	18.5	37	2	35
Kality	May	4	8	0	8
Kality	June	15	30	1	29
Kilinto	July	10	24	0	24
Saris	Sebtember	24	48	4	44
Saris	October	29	29	0	29
Saris	November	30	30	0	30
Saris	December	15	15	0	15
Saris	January	16	16	1	15
Saris	March	7	7	0	7
Total		178.5	274	8	266

Table 2: Phlebotomus sandflies collection by different methods from Merabete, Silti and Awash region.

A) Phlebotomus sandflies collected by search from their resting sites using aspirators and CDC-traps (August, 2005) in Merabete.

1. Search traps using aspirators

Region	Locality	Month	Avarage sandflies/hour	Total	Male	Female
Merabete	Kumamba	August	84	84	44	40

2. CDC- traps

Region	Locality	Month	Average sandflies/ night	Total	Male	Female
Merabete	AlemKetema; Kumamba; Wonchit	August	71	284	180	104

3. 2. 2. Taxonomy

First lower premolar (P_1) was always found in the hyraxes from Addis Ababa, Merabete and Silte like any *H. brucei* but those from Awash, lose P_1 after the emergence of the inner most molars (M3). The upper triangular tusk like incisors of male adult hyraxes grows continuously in both groups and thicker and longer in hyraxes from Awash-7, *P. capensis* (Figure 6A and 6B). The molars of adult hyraxes from Addis Ababa, Silti, and Merabete were brachydont as opposed to the hypsodont of the hyraxes from Awash-7. The combined length of upper premolars (P^{1-4}) of the hyraxes found in Addis Ababa, Silti, and Merabethe ranged from 1.7 to 1.8cm and greater than the combined length of upper molars (M^{1-3}) (1.4 - 1.5). The hyraxes from Awash - 7, however, have P^{1-4} (1.9 - 2cm) less than M^{1-3} (2 - 2.5cm). The range of the cranial and mandibular length of hyraxes from Addis Ababa, Merabethe, and Silti were between 8 - 9.5 and 6 - 7.2cm respectively unlike 9.4 - 10 cm and 7 - 7.9cm of the hyraxes from Awash - 7. All of the hyraxes from the study areas were found to have dorso-ventrally flattened cranium.

The mean weight of juveniles (>5months) and subadults of *H. brucei* were 0.9 $\pm$ 0.4 (n = 25) and 1.39 $\pm$ 0.3kg (n =7) respectively while adults were with mean weight 1.9 $\pm$ 0.5 kg (n = 20). The subadult *P. capensis* from Awash valley, had a mean weight of 1.6 $\pm$ 0.4 kg (n = 4). The range of masses of the adult *P. capensis* was between 2.2 - 3.1 kg with mean weight of 2.7 $\pm$ 0.1kg (n = 4). *P. capensis* were slightly heavier than *H. brucei* for the same age and body length. The length of hind feet and fore feet of the adult *H. brucei* hyraxes from Addis Ababa, Merabethe, and Silti were between 6 - 6.5 cm and 4.1 - 4.7 cm while for those *P. capensis* from Awash, the ranges of their hind and fore feet were 7.2 - 7.9 cm and 4.9 – 5.5 cm. The black tipped guard hairs in *H. brucei* were dirty white and gray based as opposed to yellow and black based of the *P. capensis* in Awash-7. The under hairs of *H. brucei grey* while those of *P. capensis* have black. Hyraxes from Awash were yellowish in color. The white eyebrows of *H. brucei* from Addis Ababa, Merabete and Silti were markedly identified at a considerable distance as opposed to the more diffused and yellowish of *P. capensis* from Awash. There were no marked difference observed for penis size (around 6 cm) and distance between anus and perpetual opening (around 5 cm) of the two groups of hyraxes trapped. The dorsal gland is

3.2.3. Population structures

The very young hyraxes were seen and trapped only between September and November, 20005 in Saris and Kality with body weight ranged between 0.23-0.3kg and body length 21 to 26cm. Fully pregnant hyraxes were found in June, September and late November (2005) in Addis Ababa. The weight and body length of the smallest hyrax trapped from Addis Ababa were 230g and 21cm respectively. Only one breeding season was observed in hyraxes between August and December with peak birth event during September and October. Young juveniles (0.4 – 0.65 kg weight and 28 - 30 cm body length) were also trapped in Awash area in January and February, just after two or three months after peak birth events in Addis Ababa, indicating hyraxes in both areas have similar breeding season. Mating season of hyraxes in Awash might be in February when a male hyrax was found producing a mating sound for two days and chasing other males until trapped by the snare trap. All subadults were found pregnant (single fetus) in Silti and Addis Ababa while normal adult *H. brucei* were found with two fetus. Three fetuses also found in old *H. brucei* in Silti. Hyraxes were born with incisors and premolars, which are replaced at later stages. The permanent molars are erupted at different ages of the hyraxes after birth. Hyraxes replace incisors during their juvenile stages while premolars replaced at subadult stage. First upper molars (M^1) begin to erupt soon after birth (Figure 8A). First lower molars (M_1) and second upper molars (M^2) erupted to full size when the hyraxes were around 7 month (Figure 8B). The hyraxes started to replace incisors when they were around 9 months old (Figure 8D) and completed at age around 12 months old (Figure 8E). In subadults, second lower molars erupted (M_2) at age around 13 months. The inner third molars started to erupt when hyraxes were around 22 months old.

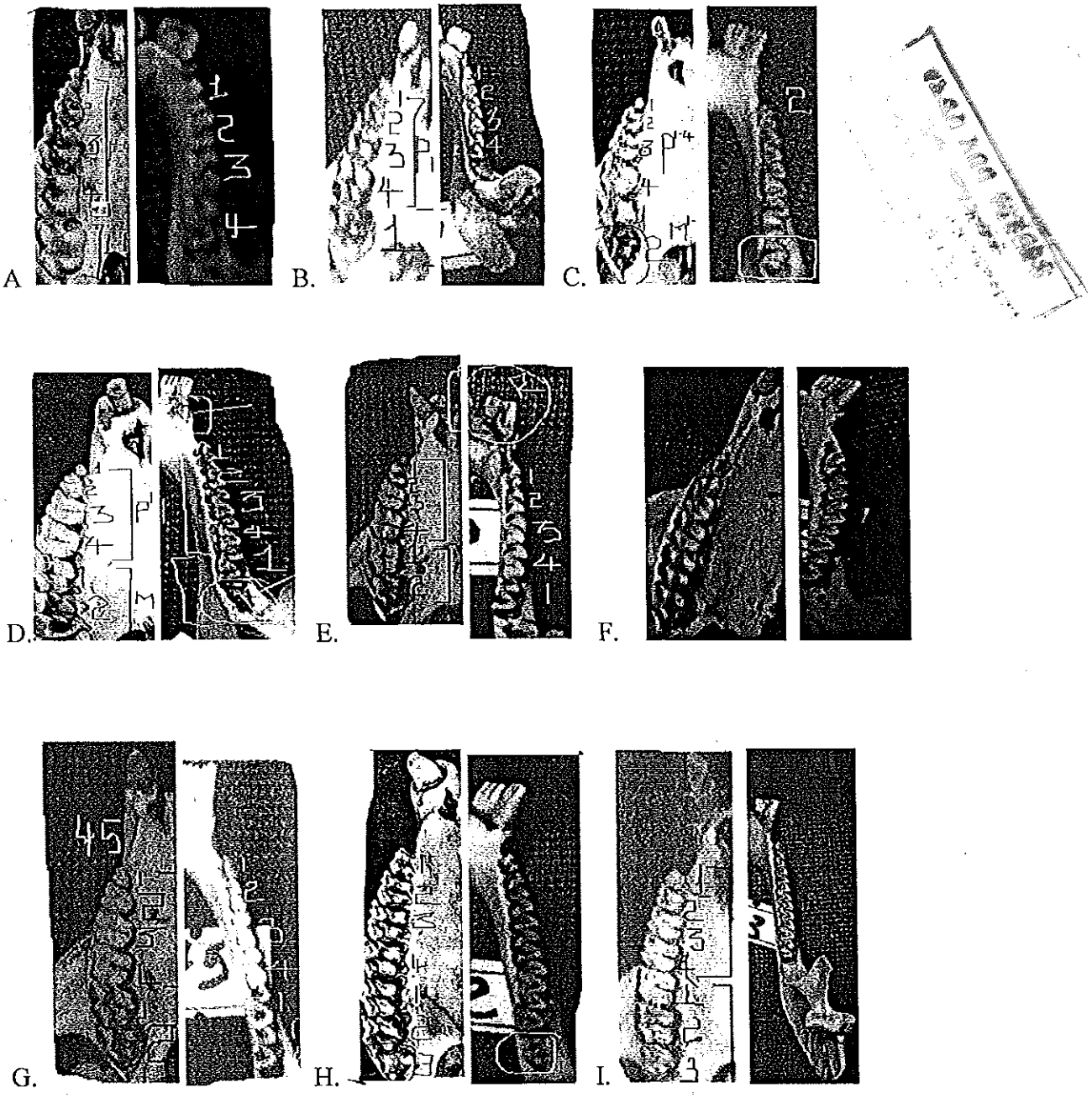
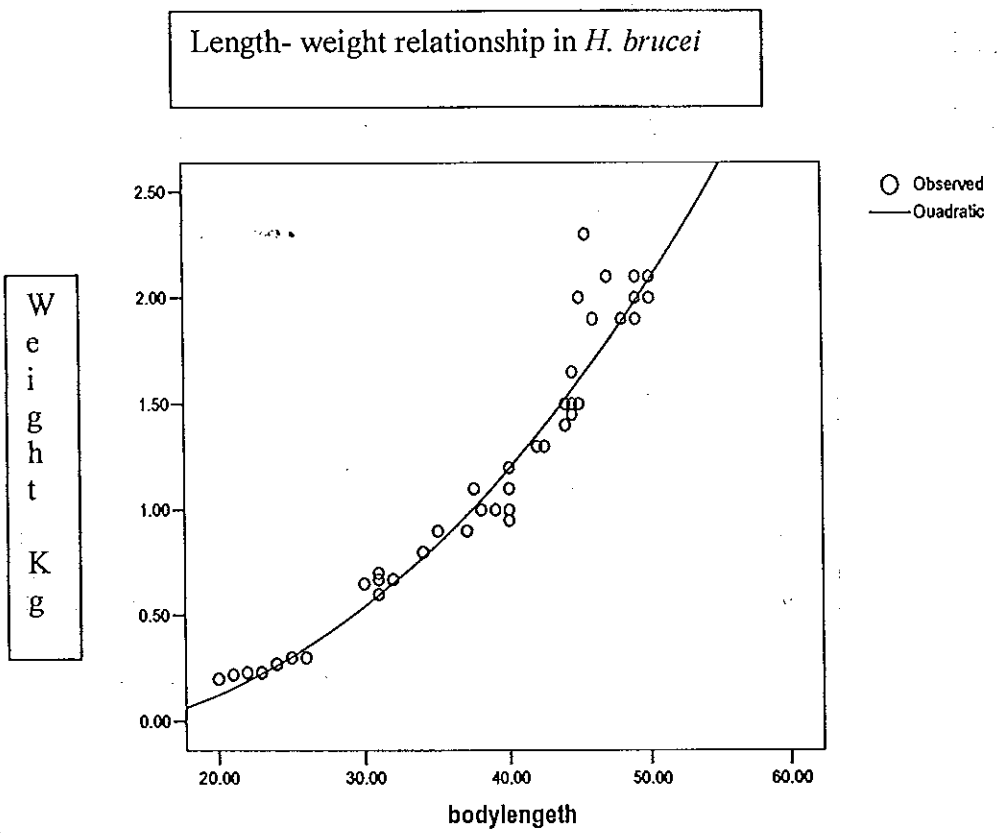


Figure: 8. Half of upper cranium and lower mandibular jaws of hyraxes brought together to show eruption of molars and replacement of incisors and premolars at different ages of hyraxes.

- A = Hyrax - 29 or H-29 , the smallest hyrax with 0.21cm body length and 0.23kg weight , only upper molar erapted and trapped in September.
- B = H - 41 (around 3 months old) with 0.3kg weight and 25cm length that sampled in November

- C = H-2 - juvenile (5-6 months old) with 0.67kg weight and 31cm long and sampled in April. Second molars on upper jaw and first lower jaw were erupting. This was the first hyrax found *Leishmania* infected.
- D = H-11 - juvenile (around 10 months old) with 1.1kg weight and 38cm length that sampled in June. Second inscisors began erupting on both jaws to replace the old. This was the hyrax found *Leishmania* infected from Kality area.
- E = H-19 = Juvenile (around 12 months old) sampled in July. All incisorse replaced but the second molars on the lower jaw were not yet errupted.
- F = 77, subadult with 42.5cm and 1.3kg sampled in May, has replaced first and second premolars on both jaws and was around 16 months old.
- G = Subadult (around16 months old) - sampled in December with second molars on both jaws was weighed 1.3kg and 43cm long.
- H = Subadult (around 22 months old) found pregnant with fully developed faetus and sampled in September. The inner most molars started erupting. The third hyrax found infected with Leishmania.
- I = Adult H-73 sampled in April and might be more than 36months

A) *H. brucei*



B)

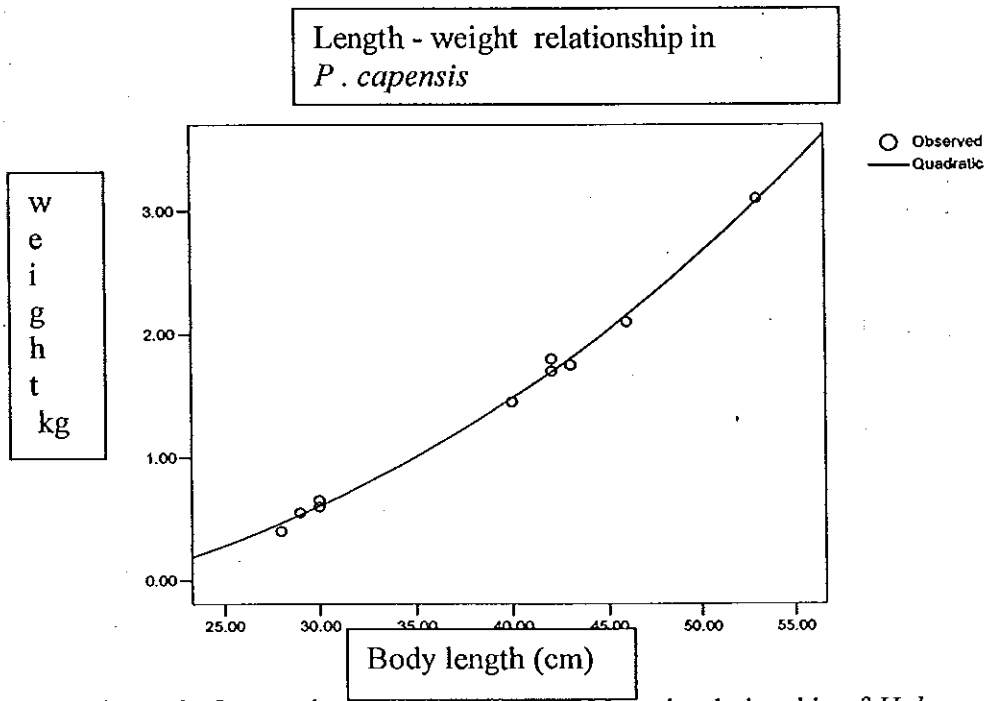


Figure 9: Comparison of the weight and length relationship of *H. bruce* and *P. capensis*

3.3. Co-existance between human *Leishmania* infections, hyraxes and sandflies

In Addis Ababa, 8 hyrax colonies in Saris, 4 in Kality and 3 in Kilinto (Akaki) were found. Thirty-five CL cases (active and healed/with past scar) were encountered from inhabitants living around hyrax colonies. Of the 16 human adults (>20 years) infected, four were guards. In Addis Ababa, high infection rates were found in SarisAbo and Bole-Bulbulla near the Bulbulla gorge and Kilinto areas where people were living very close to hyrax colonies and sandfly resting sites (Figures 10, 11 and 12; Table 3). The other area where high human infections were associated with hyraxes and sandflies existences was Silti. The highest human infections were found in central Kibet, where large number of sandflies and hyraxes were found in the Kerate gorge. In this area, people live in houses on both sides of the gorge. The market and the known shopping houses of the town were located about 5 to 10 m from the gorge. According to report of Silti Woreda health office, 75 CL cases were registered during the first outbreak of the disease in the area during 2005. Among the 75 cases, 51 were from Kibet. The next area secondly affected was Weleya - Sidist with 20 cases. The rest of the infections were from Alenso, Munteso and Boze areas (Figure 3). Human infections were less as one moves away from hyrax – sandfly habitats in Silti. A similar result was obtained from Addis Ababa. No CL patients were found outside the flight range of sandflies. All age groups seemed not be affected at the same rate. Boys and girls between 10 and 19 ages were heavily infected (Table3 and 4). This age group was known for frequent visit to hyrax - sandfly habitats.

Figure 10: Hyraxes - sandflies habitat in Kilinto (Akaki) where human *Leishmania* infections were found very close to it.

Figure 11: Hyraxes - sandflies habitate in around Saris Abo Church where human *Leishmania* infections were found very close to it.

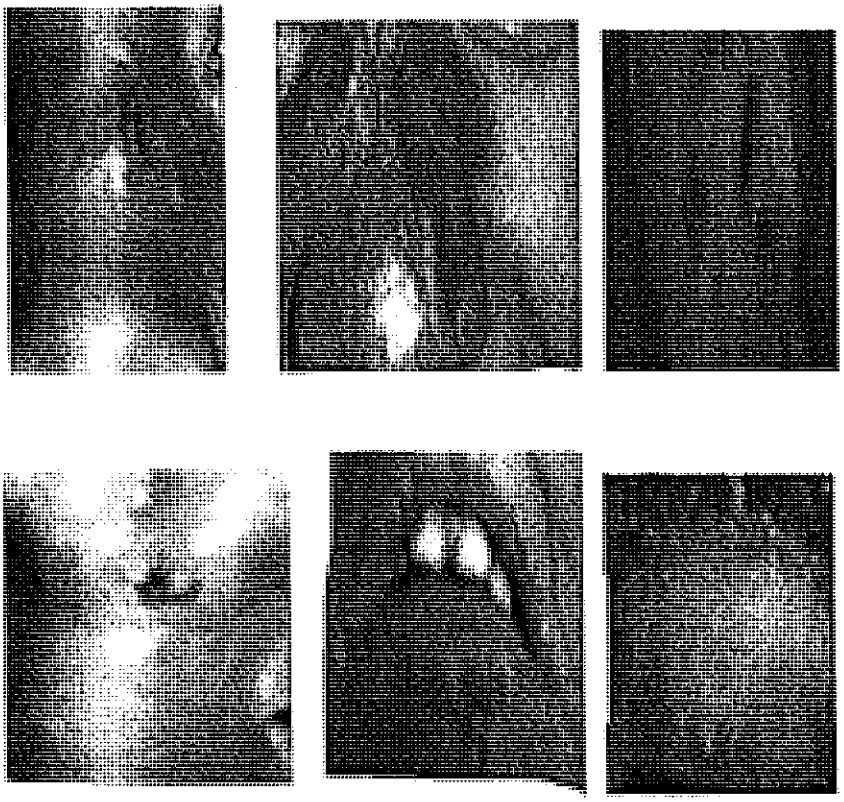


Figure 12: Cutaneous leishmaniasis in patients living around Bulbulla-Akaki river gorges in Addis Ababa.

Table 3: Cutaneous leishmaniasis in people living near hyrax colonies around Bulbulla and Akaki river gorges (2005).

. Abbrevation: F=Female; M= Male; S= Saris; K= Kality; A=Akaki

AGE	SEX		AREA			CL
	F	M	S	K	A	Active / Healed
0-9	3	5	6	1	1	1 / 7 = 8
10-19	7	4	4	5	2	4 / 7 = 11
20-29	2	2	3	1	0	0 / 4 = 4
30-39	4	2	4	2	0	2 / 4= 6
40-49	1	1	0	1	1	1 / 1 = 2
>50	1	3	3	0	1	1 / 3 = 4
TOTAL	20	15	18	10	7	4/23=35

Table 4: Silti woreda health office report of *Leishmania* infections in different age group in Silti town (2005).

AGE (Yrs)	FEMALE	MALE
1-9	13	5
10-19	19	10
20-29	4	1
30-39	7	1
40-49	3	0
>50	3	2

CL was not common in Merabete although hyraxes were common. Infront of Arbegna high school, South of the town, was a gorge of basalt rock with active sliding stones with high concentration of hyraxes. Sandflies were also collected from the cracks inside the cliffs of this area. But there were no human infections in the areas. The other areas where sandflies and hyraxes were found were Lijagba, Kumamba and the areas around Wenchit river. In these areas there were no human CL cases. In Lijagba, hyraxes were found close to human dwellings (< 50 m) in piles of stones collected at one side of the farm. No sandfly was trapped using CDC-trap from the inside of the piles of the stone. In Awash - 7, many hyraxes were found in the cliffs and boulder falls about 800m away from Awash - 7 rail way station where sandflies were collected using CDC-traps from around hyrax holes. No human infections were found in the area.

The only animals other than hyraxes in the studied areas that were found in large number and expected to be possible reservoir hosts of *Leishmania* were Baboons in Awash, grivet monkey in Silti and *Ratus rattus* and *Praomys* spp. in Addis Ababa. The baboons spent the night on top of hyrax hole on the cliffs in Awash, while the grivet monkeys stayed on the exposed roots of the trees on the cliffs of the Kerate gorge in Silti.

3.4. Animals and sandflies trapped and *Leishmania* natural infections

A total of 79 hyraxes (27 males and 52 females) were trapped from Addis Ababa (n = 51) , Awash valley (n = 13), Silti (n = 11) and Merabete (n = 4) (Table 7). Hyraxes from Merabete were trapped in August 2005 while those from Awash-7 and Silti were trapped in January- February and March-April respectively (Appndix-I). In addition 41 bats, 3 Mongoose and 3 Genet cats and the rodents (12 *Rattus ratus* and 14 *Praomys* spp) were trapped and examined (Table 5). Samples from 6 hyraxes were contaminated and discarded. *Leishmania* parasites were found in the NNN medium only from the juveniles and subadults *H. brucei* collected from Addis Ababa in April, June and September. A total of 6.3 % (3/48) *H. brucei* in Addis Ababa were found positive. Infection rate of hyraxes of Saris was 11.1 % (2/18), while that of Kality was around 5.9 % (1/17). No hyraxes from Kilinto were found positive for *Leishmania* parasites. Only samples from

nose produced *Leishmania* positive in NNN culture. None of the bats, Mongooses and Genet cats and the rodents were found *Leishmania* positive in NNN medium (Table 5). The sample from the civet trapped by the snare trap from Kality was contaminated. Hyraxes (6 from Kilinto and 2 from Kality in Addis Ababa; 1 from Silti) and a genet cat from Merabete were found with immobile flagellates from blood and liver samples that looked like trypanosomes and failed to grow up on sub-culture. But in Saris, where hyrax habitats were rarely visited by cattle, these flagellates were not found.

Table 5: The different mammals examined for *Leishmania* parasites in culture and their natural infection rates.

Animals	Area of the animals sampled	*Leishmania* positive in NNN-medium	*Leishmania* negative in NNN - medium	Number of Contaminat-ed Samples	Total
Hyraxes	A.A	3	48	3	51
Hyraxes	Merabete	0	1	3	4
Hyraxes	Awash-7	0	13	0	13
Hyraxes	Silti	0	11	0	11
Bats	Merabete	0	41	0	41
Mongoose	Merabete, Awash-7	0	3	0	3
Genet cat	Merabete, Awash-7	0	3	0	3
Civet	A.A	0	0	1	1
Black rat	A.A	0	12	0	12
Praomys spp.	A.A	0	14	0	14
Total		3	145	8	148

Table 6: Number of naturally *Leishmania* infected hyraxes in different age groups (Addis Ababa, Silti and Awash-7) in 2005- 2006. A) Number of naturally *Leishmania* infections in juveniles and subadults (from Addis Ababa, Silti and Awash-7. Abbreviation : CI = body cofficient index ; CLe = cranial length ; ML= mandibular length; Av. = average; Leish.= *Leishmania*

Hyrax	Month years	Weight (kg)	Length (cm)	CI	CLe/ ML (cm)	Estimated. Age (month)	No. *Leish.* Infected hyraxes
1. *H. brucei*	Nov.2005	0.2	20	0.01	4.5 / 3	0 (fetus)	0
2. *H. brucei* n = 8	Sep.- Nov.2005	0.23 - 0.3 (av. = 24)	21 - 26 (av = 24)	0.01	5 - 5.5 / 3.7 - 4	0-2	0
3. *P. capensis* n = 4	Jan. – feb.2006	0.4 - 0.6 av. = 0.5	28 - 30 Av. = 29	0.02	5.5 - 6.2 / 4.2 - 4.8	3-5	0
4. *P. capensis*	Jan.2006	0.65	30	0.02	6.2 / 4..5	5.5	0
5. *H. brucei* n = 7	March- Apri 2005,2006	0.6 - 0.7	30 - 32 Av. = 31	0.02	6..5 – 6.6 / 4.9 - 5	5-6	1
6. *H. brucei* n = 7	March- may 2005,2006	0.8 - 1 A V. = 0.9	34 - 38 AV.= 36	0.03	7 - 7.5 / 5.2 - 5.4	7-9	0
7. *H. brucei* n = 10	May- Aug. 2005,2006	1 - 1.2 AV. = 1	38 - 40 AV = 39	0.03	7.5 - 7.7 / 5.4 - 5.8	9-12	1
8.P. *capensis* n =1	Jan. 2006	1.45	40	0.03	7.5 / 6	7.5 / 6	0
9. *H. brucei* n =1	Apr. -June 2006	1.1 - 1.3	40 - 43	0.035	7.8 - 8.3 / 6 - 6.3	16 -17	0
10.*P. capensis* *n=4*	Jan - Feb. 2006	1.7 - 1.8	42 - 43	0.03	8.4 - 8.5 / 6.5 - 6.7	16 -18	0
11. *H. brucei* n = 5	Mar.- Sept. 2005,2006	1.4 - 1.5 AV. = 1.45	44 - 45	0.03	7.9 - 8 ..5 / 6.2 – 6.5	18 - 22	1
12. *H. brucei* n= 5	Oct.- Nov. 2005,2006	1.65 - 2 AV. = 1.7	45 - 45.5 Av.= 45	0.04	8.4 - 8.5 / 6..5 - 6.6	22-24	0

3.5. The outcome of *Leishmania* strains isolated from hyraxes on golden hamsters

All the three strains of *Leishmania* isolated from hyraxes caused lesions in golden hamsters on their snout and pads (Table 8). Of 16 hamsters inoculated only one remained normal. Formation of lesion began between weeks 18 to 21. The hamsters inoculated first were not ulcerating until one year. During re-isolation, 60% of samples taken from the lesions of hamsters were found *Leishmania* positive in NNN-culture for all the three strains (Table 9; Figure 13). In addition, giemsa stains were positive for amastigotes in the smears.

Table 8: The outcome of *Leishmania* strains isolated from hyraxes on golden hamsters.

Leishmania strains (Passage)	Number of hamsters inoculated	Date of inoculation	No. weeks for appearance of lesion
H2/Sa/Sk (2)	5	14/5/2005	23
H2/Sa/Sk (5)	3	15/6/2005	19
H11/Ka/Sk (2)	4	12/7/2005	18
H32/Sa/Sk (2)	4	8/11/2005	21

Table 9: Re-isolations of leishmania from nose and feet pad of hamsters using NNN-media and giemsa stains

Strain	NNN-media		Giemsa stain of touch smear from lesion
	No. of samples from skin lesion	No. of +ve skin samples	
H2/Sa/Sk	4	2	+
H11/Ka/Sk	3	2	+
H32/Sa/Sk	3	2	+

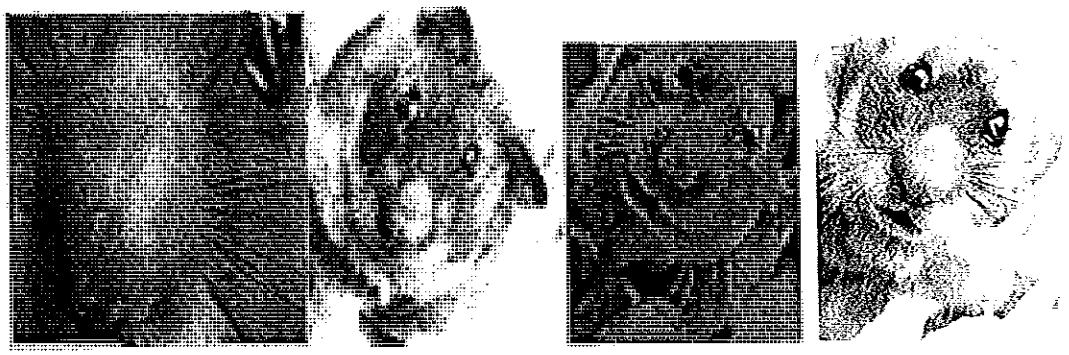


Figure 13: Formation of lesions in golden Hamsters after strains isolated from hyraxes were inoculated into noses and feet pads.

4. DISCUSSION

4.1. Co-existance between human *Leishmania* infections, hyraxes and *P. longipes*

The sandflies found in Addis Ababa were *P. longipes* (Quate, 1964). The sandfly species that were found in Silti were also be *P. longipes*. In Merabete the populations of sandflies were composed of *P. longipes* and *P. gibensis* (Balkew, Pers. Comm., 2005). The main sandflies found in the Awash valley are *P. orientalis*, *P. sergenti, P. arabicus, P. gemetchi*, *P. bergeroti*, *P. martini* and *P. seavus* (Gebre-Michael *et al.*, 2004).

The existence of specific *Leishmania* species in an area depends on the close existence of specific reservoir host (s) and specific vector species which in turn is affected by the existence of suitable ecological and biological conditions (Ashford, 1977; Ashford, 2000). *L. aethiopica* transmission cycle is maintained in different parts of Ethiopia only if hyraxes and *P. longipes* and/or *P. pedifer* live close enough for parasite cycle (Ashford *et al.*, 1973; Ashford, 1977). The favored *P. longipes* resting sites such as cracks and crevices inside basalt rocks, ground burrows and inside bridge in the studied areas were found to be shared by hyraxes, indicating the way how the presence of hyrax (source of blood meal) affect the distribution of sandflies and CL. Human cutaneous leishmaniasis was consistent with the presence of hyraxes and *P. longipes* in all the studied areas. Along the sides of Melka Shena/Akaki river between Kality and Akaki (Kilinto), there was an area (about 6 km) with sandflies but no cutaneous leishmaniasis and hyraxes. In the rest of hyrax free part of Addis Ababa, also there was no CL although *P. longipes* were common. In Silti *Leishmania* focus, almost all human infections were from Kibet and Weleya-Sidist, where the Kerate gorge (habitat of hyraxes and sandflies) was located very close to human settlements. In Boze area, which is slightly away from the gorge, human infections were rare (Figure 3). Similar result was observed in Saris Abo, Bole Bulbulla areas and Kilinto (Addis Ababa) near hyrax and sandfly habitat (Figures 10 and 11). Long flight range of *P. longipes* (about 1 km) from their day resting sites was estimated in Sebeta, which was the longest distance traveled by *P. longipes* to get the

appropriate animal for blood source, including man (Foster, 1972). In Bole – Bulbulla, *P. longipes* were found to regularly visite a house located 2 km from the possible Bulbulla gorge resting sites. In the area, however, there were many vacant houses that might serve as day resting site. *P. longipes* visited houses after midnight in all parts of Addis Ababa where a potential day resting sites found in the areas. Martin (1939) and Foster,(1972) found *P. longipes* in Addis Ababa as a pest. Humal infetions might be the result of biting by infected sandflies visiting houses at night. Human infection might also be dependent on ecological relationship between human activity and reservoir/sandfly system. The age group between 10 - 19 years that was observed visiting hyrax - sandflies habitats frequently in Addis Ababa and Silti for various activities were found with high infection rates. Similar situations were observed in different *Leishmania* foci in Ethiopia (Ashford, *et al.* 1973; Asrat and Formell, 1993; Mengistu, 1992; Gadisa, 2005). Sex linked infections were seen in Silti. Females for age group 1-9 and 30 - 39 were more infected (Table 4). This might be due to longer time females spent in the houses, which are located near hyrax holes. Especially during chilly days when sandflies become more active and travel longer distance away from their resting sites at day (Ashford, *et al.*, 1973), females who spend most of their time in the house probably were more vulnerable. Sylvatic vectors usually cause more cases among adult males who work in forest while domestic vectors are more of a threat to women and children (Davis *et al.*, 2000). Hyrax - sandfly - man transmission was, therefore, dependent on a combination of the flight range and human habits.

The finding of young adult sandflies in the caves of Kumamba (Merabete) showed the existence of sandfly breeding sites in the caves. Searchs for the most expected sandfly breeding sites such as cracks and crevices of basalt rocks were impossible as these areas were inaccessible.

The only wild mammals that were in large numbers and shared common holes or lived near the holes of hyraxes and *P. longipes* were *Rattus rattus* and *Praomys* spp. (in Addis Ababa); grivet monkeys (in Silti) and baboons (in Awash). The *R. rattus* and *Praomys* spp sampled were found *Leishmania* negative in NNN - medium. In addition, these

rodents were proved to be not a preferred host for *P. longipes* to serve as a source of blood meal in laboratory experiments and were not found infected with *L. aethiopica* (Lemma *et al.*, 1969; Foster, 1972; Ashford *et al.*, 1973). The mammals that were reported to be infected in the laboratory, such as baboons and monkeys (Hailu *et al.*, 1995) were found in large numbers in the study areas except Addis Ababa. These animals were not sampled due to the difficulity of trapping. Bats, porcupine, mongoose, civet, hyena, and domestic animals that can be found in different study sites had never been reported to be the reservoir hosts of *L. aethiopica* in Ethiopia. These animals were again too few to maintain the parasite cycle in the wide range where the human infections were observed. Mongooses and genet cats were found in large number in lowland areas of Marabethe, along the sides of Wonchit river where visceral leishmaniasis has been identified from children (Hailu, unpublished data). These carnivores were probably the reservoir hosts of visceral leishmaniasis in this area as they have been reported to be infected by *L.* donovani in Sudan (Hoogstraal and Heynenman, 1969; Chance *et al.*, 1978). Since many bats were found together with sandflies in Kumamba, where there were no cutaneous leishmaniasis cases, it is possible to suggest bats to serve as the source of blood meal for sandflies as domestic animals and humans. All the bats sampled from the area were found to be negative for *Leishmania* in NNN - medium.

The epidemics of cutaneous leishmanisis in South East - Addis Ababa seem to have began around 1990 when people started to settle around the Bulbul –Akaki river gorges (Pers. Comm. with local people), however, Silti seemed a recent phenomenon. But some patients without travel history to *Leishmania* endemic areas were found in Addis Ababa for almost two decades (Hailu, Pers. comm.). It seemed that, in Addis Ababa, the disease started when people began to settle near hyrax colonies, due to the expansion of the city, where zoonotic cutaneous leishmanisis had been maintained between sandflies and hyraxes along Bulbulla - Akaki river gorges. This might be like many epidemics in areas such as the Middle East following settlment of non - immune people in areas where ZCL was maintained (Neouimine, 1996). In Silti, fewer hyraxes were known to exist in Kerate gorge for many years as indicated by local people. The cutaneous leishmaniasis epidemics in Silti, which started in 2005, was most probably related to the recent increase

in number of hyraxes in the area. The increase in number of hyraxes may be related to habitat shift of the animals from the nearby mountain area (South West of the town) to peridomestic areas as a result of synanthropic adaptations of hyraxes. The ecological changes of Kerate gorge (due to erosion and construction of the bridge) might creat an ideal condition for increase in number of both hyraxes and sandflies. The walls of the Kerate gorge was actively sliding as a result of gully erosions and burrows were being formed in the rooting systems of the trees on the sides of the gorge. The inside of burrows was humid and shared by hyraxes and very suitable for sandfly populations to increase. Hyraxes adaptations always tend to minimize predations (Barry and Shoshani, 2000). In Kerate gorge no carnivorous were found including eagles which feed almost exclusively on hyraxes in Zimbabwe (Barry and Mundy, 1998) and as observed in Kilinto and Saris. The emergence of Silti *Leishmania* focus was an ideal situation to explain how man made (the construction of bridge) and natural environmental changes could result in the creation of new focus by affecting the size of both vectors and reservoirs. Synanthropic behaviour of Meriones *shawi* where sandflies existed increased the incidence of zoonotic cutaneous leishmaniasis in Morocco (Neouimine, 1996). Similarly if hyraxes adapt in the rest part of Addis Ababa (other than SE Addis Ababa) where sandflies are common, there could be *Leishmania* epidemics. Openings left for flood passage inside footing of a bridge that were seen serving as gates for hyraxes in Silti which lead to the central inside of the bridge with loosely packed area where hyraxes and sandflies perfectly adapted to live. Hyraxes were found living inside the building of a Church in Addis Zemen (Teshome Personal communication, April-2006). *Leishmania* epidemics following man made environmental changes were observed in central and Southern Tunisia after the construction of irrigation dams that have changed the temperature and humidity of the soil and vegetation. The ecological changes in the area were increased in number of sandflies and rodents as well as leishmania epidemics (Ben Rachid *et al.,* 1987). Another out break of zoonotic leishmaniasis due to human environmental change was in northern Iran where plantation of trees in the region, to prevent erosion, have increased the number of *Rhobomys opimus* and resulted in *Leishmania* epidemics in the area (Neouimine, 1996).

4.2. Hyraxes

4.2.1. Age determination in hyraxes

According Barry, (1994), hyraxes were grouped as juvenils (<12 months old), subadults (12-18 months old) and adults (> 24 months). Fairall (1980) estimated the weight of 5 month old *Procavia* to be 0.7kg while 0.3 kg for *H brucei*. In this study 0.65kg *P. capensis* was estimated to be 5 months but 0.3 kg *H. brucei* were found to be around 3 months. The estimation of age of hyraxes in this research was done by comparing the skull and comparative sizes of hyraxes. *P. capensis* was estimated to be 5-6 months old before first molars erupted on the lower jaw (Lieberman *et al.*, 2004). *H. brucei* trapped from Addis Ababa and Silti with sign of first lower molar eruption was estimated to be 5-6 month old. Since it was only September – November peak breeding season, it was possible to estimate age of juveniles and subadults born in the last and previous breeding season, respectively. For example if 0.4 kg and 1.3 kg hyraxes trapped in December, the former hyrax was born recently and its age was most probably between 2-4 months while the second subadult hyrax that were born in the previous breeding season most probably aged between 14-16 months. The precision of the estimate can be increased by looking at the changes of the skulls from different samples which showed slight variation depending the month they born. Subadults were found with first and second molars on both upper and lower jaws. Corbet (1979) used this character to distinguish subadults. Subadults were found pregnant in March and April in Silti and Addis Ababa when they were around 16-18 months old. These showed hyraxes reach sexual maturity at around 12 months unlike the 16-18 months mentioned by Hoeck (1982). The field observation of male hyraxes in Awash-7 with long lasted calling sound and aggressive behaviour during February might indicate that the hyraxes have their mating season around this month. The evidences for this suggestion were the fact that all matured female hyraxes give birth after 7 or 8 months (gestation period) between August and December. The reason for wide mating season was indicated due to the ability of hyraxes to have many estrous

cycles (Hoeck, 1982). Female biased hyraxes samples were found in all the study areas, which was due to dispersal of male hyraxes after reached sexual maturity (Hoeck, 1982).

4.2.2. Hyrax taxonomy

P_1 is always found in the hyraxes from Addis Ababa, Merabethe and Silte like that of a *H. brucei* (Barry and Shoshani, 2000) as opposed to those from Awash, which lost P_1 after the emergence of M^3 like that of *P. c. pallida* in Ogaden area (Corbet, 1979). The hyraxes from Addis Ababa, Merabete and Silte were *H. brucei* and have the following characterstics of the species : brachydont dentitions (Hoeck,1975; Deniro and Epistein,1978) combined length of premolars (P^{1-4}) greater than the combined length of molars (Corbet, 1979); narrow muzzle (Skinner and smithers, 1990), smaller in size and adults mostly not greater than 48cm (Corbet, 1979); browsing habit (Hoeck,1975; Deniro and Epistein,1978); white eye brown and ventral hairs (Barry and Shoshani, 2000); white or yellow-orange dorsal spot (Corbet, 1979; Barry and Shoshani, 2000); black tiped light gray guard hairs and gray under hairs (Barry and Shoshani, 2000). On the other hand, hyraxes from Awash were *P. capensis* had the following characterstics of *Procavia*: hypsodont dentitions (Hoeck, 1975; Deniro and Epistein, 1978), combined length of premolars less than the combined length of molars (Corbet, 1979), adults mostly greater than 3 kg (Barry and Mundy, 1998), yellow eye brown and ventral hairs (Corbet, 1979; Barry and Shoshani, 2000) and yellow dorsal spot with black based or none recognizable spot (Corbet, 1979). The weight and length of the different age groups of hyraxes from Addis Ababa, Merabethe and Silte were almost the same with length weight measurements for different age groups of *H. brucei* from Zimbabwe while those samples from Awash valley were similar to *P. capensis* of Zimbabwe (Barry and Mundy, 1998), (Figure 9). The cranial, mandibular and hind feet lengths of Addis Ababa, Merabethe and Silte were similar with *H. brucei* from South Africa (Barry and Shoshani, 2000). Unlike Dendrohyraxes, all hyraxes sampled had dorsoventrally flat cranium (Jones, 1978).

4.3. Seasonal and age dependent *Leishmania* natural infections in hyraxes

The low chance of sandflies infection following blood meal from infected hyraxes was expected (Ashford, 1977). *P. sergenti* were allowed to feed on *P. capensis* that was positive for *L. tropica* (using PCR) but no infection was found (Jacobson *et al.* 2003). On the otherhand, experimental *L. tropica* infection of golden hamsters by the bite of *P. sergenti* in Isreal was possible (Svobodova and Votypka, 2003). Such experimental *L. aethiopica* infection has not yet been demonstrated. Naturally, hyraxes were estimated to be bitten by infected sandflies at least once in a week (Ashford, 1977). But, infection in hyraxes was found to be rare. Infection in hyraxes probably was the result of repeated biting of infected sandflies. It is, therefore, more likely to get infected hyraxes after the two peak sandflies abundance and biting in spring and autumn. But, seasonal and age dependent infection in hyraxes was observed in this research and Ashford *et al.* (1973) where almost all infected hyraxes were juveniles and subadults (< 2 years) which were found in spring and summer. No infections were found in spring and winter (Table 6). The natural infection rate of *H. brucei* in Addis Ababa was 6.3 % (3/48). Ashford *et al.* (1973) were found infected hyraxes only in spring and summer. The reason for the absence of *Leishmania* positive *H. brucei* in Akaki and Silti, where human infections were common, might be related to the age of the hyraxes, the season hyraxes sampled and the sensitivity of the NNN-medium and smear staining. Age and season dependent *L. major* infections of *Psammomys obesus* in Tunisia were demonstrated where peak transmission was in late summer and autumn (70%) infection rate and low transmission (almost 0% infection rate) in dry season (Fichet- Calvet *et al.*, 2003). Sandflies emergence and hyrax birth season in autumn probably coincided and non-immuned new born juveniles might have the chance of being infected with *Leishmania*. But no infection was found in autumn even if enough juveniles, subadults and adults were sampled. The low infection rate in hyraxes in autumn were thought to be compensated by high infection rate in sandflies to maintain stable *Leishmania* parasites population (Ashford, 1977). In spring and summer, the non-immuned juveniles have passed at least one peak sandflies abundance and biting and had a more chance of being infected. The remaining might

infected in autumn or in another spring peak. By the time they become adult, they have passed at least three peak sandfly biting and high chance of being immuned due to previous exposure.

It might be possible to make a comparision between human age dependent infections and hyraxes although there was no previous work on immunity of hyraxes. Hyraxes are more exposed to sandfly bite compared to humans. Infection rate in children generally is high compared to adults (Hailu and Frommel, 1993; Mengistu *et al.*, 1992; Gadisa, 2005). This does not mean adults are not exposed to *Leishmania* infections as children but adults acquired immunity during previous exposure. The juvenile hyraxes born without immunity showed infection rate more than adults even if all hyraxes exposed to the bite of infected sandflies at the same rate. The only possible difference was that the adults have developed immunity during the previous exposures. Hyraxes live, almost throughout the day, with sandflies and there was high chance of being bitten by infected sandflies compared wih humans who are rarely bitten by sandflies visiting houses at night. If the sand fly population is high in an area then most of hyraxes most probably infected and immuned before they became adults. In humans it was possible for adults to be infected after living for so many years in the *Leishmania* endemic areas (Ashford *et al.*, 1973; Hailu and Frommel, 1993). The lack of *Leishmania* positive hyraxes sampled in NNN- media in Kilinto (Akaki) might be related hyraxes populations which were adult dominated due to selective perdation of juveniles by eagles. In Silti, where no pressure of predation, the hyrax population was juvenile dominated. In this area, parasite transmission cycle most probably high especially in the peak transmission seasons. The reason for the lack *Leishmania* from hyrax sampled from Silti might be due to sampling in March which was some what earlier than the season when most likely to obtain infection.

No sandflies were found *Leishmania* infected from the total of 790 sandflies dissected from Addis Ababa (March, 2005 to March, 2006), Silti (April, 2006), and Merabete (August, 2005).The reason for lack of natural *Leishmania* infection in the sandflies might be due to high nulliparous rate of the sandflies sampled. The limitation of this research

was the inability to distinguish between parous and nulliparous sandflies which was essential to determine sandfly infection rate. *Trypanosoma* species might be the natural parasites of hyraxes. 21% of blood and liver samples from Kilinto and Akaki areas were found with trypanosoma like flagellates. Further investigations required to demonstrate this situation.

4. 4. Species identification of the *Leishmania* strains isolated from hyraxes

Isolation of *Leishmania* parasites in NNN- medium together with *Leishmania* positive giemsa stain on slides from lesions of human patients (Hailu, unpub. data) and isolations of the parasite in NNN- medium from hyraxes (*H. brucei*) were diagnostic for *Leishmania* parasites existence in Addis Ababa. Formation of lesion in golden hamster (*Mesocricetus auratus*) following inoculation of the *Leishmania* strains from hyraxes and re-isolation of the parasites from the hamsters both in culture and giemsa stain was also diagnostic for the parasites. Unlike *L. tropica*, which produced lesion on golden hamsters in 4 weeks (Svobodova and Vootypka, 2003), the *Leishmania* strains from hyraxes produced lesions in the hamsters with in 18-23 weeks, which was similar to the 25 weeks required for *L. aethiopica* to produce lesions (Humber *et al.*, 1989). The lesions on hamsters were progressive and were not ulcerated up to one year. Humber *et al.*, (1989) mentioned lesion formation only after 1 year for *L. aethiopica.*

Although the agents of CL other than *L. aethiopica* were not reported in the highlands of Ethiopia, it was necessary to know the identity of the species of *Leishmania* in Addis Ababa because it was a newly described *Leishmania* focus. The *Leishmania* strains from hyraxes were found out to be *L. aethiopica* due to the formation of *L. aethiopica* specific bands around 162 bp on the agaros gel (Schonian *et al.*, 2000) after restriction digestion of PCR product (amplified ITS-1 region). Similar Band was produced by the *L. aethiopica* reference strain (MHOM/ET/72/L100) (Figure15).

5. CONCLUSION AND RECOMMENDATIONS

Cutaneous leishmaniasis in Addis Ababa and Silti is due to *L. aethiopica*. The only animal reservoir hosts of *L. aethiopica* using all the criteria in all study areas, were hyraxes. The roles of other animals like baboons, monkeys, mongoose, genet cats, bats and domestic animals, as incidental hosts require further investigations. Synanthropic adaptations of hyraxes and ecological changes of hyrax-sandflies ecosystems (man made and natural) are the important factors of the *Leishmania* epidemics in Addis Ababa and Silti. *Leishmania* infection in hyraxes most probably was age dependent and seasonal. Additional investigations are necessary especially if there are also seasonal human and sandflies infections in Ethiopia. The immunologicl responses of hyraxes to *Leishmania species* is not known, a future research on this area may give additional information about leishmaniasis. The use of more sensitive simple diagnostic methods like PCR is also important in the study of *Leishmania* epidemiology. Leishmaniasis is a dynamic disease and circumstances change the transmission pattern continuously. Education of those risk factors for leishmaniasis may help to control the diseases before causing huge destructions. Ecological modifications of hyrax habitats might be a visible direction in control of disease transmission. A future work on the population dynamics, taxonomy and behaviours of hyraxes in Ethiopia is important from *Leishmania* epidemiological point of view. A Search for reservoir hosts of *Leishmania* spp. especially in lowlands of Ethiopia should be continued as all agents cutaneous leishmaniasis in Old World were known to exist in this country.

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7. APPENDEX

Appendex - I: Data of physical characterstics of hyraxes.

Abbrebations: S=sex; B.L= body length in cm ; B.W= bodyweight in kg; H.L=hindfeet(pad) length in cm; FL= fore feet (pad) length in cm ; CL= condrobasal length in cm; ML= mandibular length in cm ; 1,2,3,...= code given to hyraxes in order of their trap

A) *H. brucei* sample from Saris areas (Addis Ababa). February 2005 - April 2006

Code	S	Area	Month	B.L	B.W	H L	FL	CL	ML	Age
43	F	Saris	Nov	20	.20	2.70	4.5	4.5	3	Juvenile
41	M	saris	Nov.	25	.30	4.60	3.10	5.3	4	Juvenile
42	M	saris	Nov.	25	.30	4.20	3.00	5.2	3.9	Juvenile
62	F	saris	Mar.	31	.60	5.00	3.40	6.7	5	Juvenile
59	F	saris	Mar.	31	.70	5.00	3.40	6.6	5	Juvenile
2	M	saris	Apr.	31	.67	5.50	3.50	6.6	4.9	Juvenile
75	F	saris	April	32	.67	5.00	4.00	6.6		Juvenile
61	M	saris	March	31	.60	5.00	3.40	6.7	4.9	Juvenile
3	F	saris	Apr.	34	.80	5.50	3.50	7	5.3	Juvenile
72	F	silti	Apr.	35	.90	6.00	4.00	7.5	5.6	Juvenile
66	F	silti	Mar.	37	.90	5.60	3.70	7.5	5.6	Juvenile
45	F	saris	Dec.	42	1.30	6.10	4.50	8.3	6.2	subadult
33	F	saris	Sep	45	1.45	6.40	4.40	8.5	6.4	subadult
32	F	saris	Sep	45	1.50	6.40	4.50	8.4	6.4	subadult
1	F	saris	Feb.	45	1.50	6.40	4.50	8.4	6.5	subadult
39	F	saris	Sep	45	1.65	6.50	4.50	8.4	6.5	Adult
43	F	saris	NOV	45	2.00	6.30	4.50	8.5	6.5	Adult
38	F	saris	Octob	45	1.50	6.30	4.40	8.5	6.5	Adult
40	F	saris	NOV	50	2.10	6.50	4.50	9	7	Adult
44	M	saris	NOV	47	2.10	6.20	4.20	9	7.1	Adult
63	F	saris	March	49	2.00	6.60	4.50	9.3	7.3	Adult
60	M	saris	March	50	2.00	6.5	4.4	9.4	7.2	Adult

B) *H. brucei* sample from Kality (May 2005 - March 2006)

Code	S	Area	Month	B.L	B.W	HL	FL	CL	ML	Age
29	F	kality	Sep.	21	0.2	4	2.4	5	3.7	Juvenil
34	F	kaliti	Oct.	22	.23	4.10	2.50	5	3.7	Juvenil
35	M	kaliti	Oct.	24	.27	4.00	2.90	5	3.7	Juvenil
36	M	kaliti	Oct.	23	.23	4.40	3.00	5.2	3.8	Juvenil
37	M	kaliti	Oct.	25	.30	4.20	3.00	5.3	3.8	Juvenil
30	M	kaliti	Sep.	26	.30	4.20	3.00	5.3	4	Juvenil
6	F	kaliti	Mar.	40	.95	5.80	4.00	7.2	5.4	Juvenil
14	F	kaliti	June	41	1.10	6.00	4.50	7.5	5.6	Juvenil
9	F	kaliti	May	38	1.00	5.80	4.00	7.5	5.6	Juvenil
7	F	kaliti	May	38	1.00	5.80	4.00	7.5	5.7	Juvenil
11	M	kaliti	June	38	1.10	5.80	4.00	7.6	5.7	Juvenil
13	F	kaliti	June	40	1.20	5.80	4.30	7.7	5.8	Juvenil
10	F	kaliti	June	44	1.40	6.50	4.50	8.7	6.7	adult
5	F	kaliti	May	44	1.50	6.30	4.20	8.5	6.6	adult
8	F	kaliti	May	45	1.50	6.20	4.40	8.4	6.4	adult
12	F	kaliti	June	46	2.30	6.20	4.20	8.5	6 .6	adult
31	F	kaliti	Sep	50	2.10	6.50	4.50	8.9	6.9	adult

C) *H. brucei* sample from Akaki (Kilinto) July 2005 and May 2006

Code	S	Area	Month	B.L	B.W	HL	FL	CL	ML	Age
20	M	akaki	July	38	1.00	5.80	4.00	7.5	5.8	Juvenil
19	M	akaki	J uly	40	1.1	6	4.5	7.5	5.6	Juvenil
21	F	Akaki	July	38	1	5.4	4	7.4	5.7	Juvenil
23	F	akaki	July	40	1	5.6	4.2	7.5	5.5	Juvenil
77	F	akaki	May	42.5	1.3	6.8	5	8.3	6.3	Juvenil
78	M	akaki	May	44.5	1.5	7.5	5.5	8.7	6.7	adult
16	F	akaki	July	46	1.90	6.30	4.50	9.1	7	adult
18	F	akaki	July	47	2.1	6.5	4.7	9.2	7	adult
22	F	akaki	July	50	2.00	6.70	4.70	9.5	7.4	adult
17	M	akaki	July	49	1.90	6.50	4.50	9.3	7.3	adult
15	F	akaki	July	50	2.10	6.50	4.50	9.2	7.1	adult
79	M	akaki	May	49	2.00	6.50	4.40	9.3	7.3	adult
24	F	akaki	July	49	1.90	6.50	5.00	9.1	7.1	adult

D) Silti *H. brucei*

Code	S	Area	Month	B.L	B.W	HL	FL	CL	ML	AGE
68	F	Silti	Mar.	30	.65	4.60	3.10	6.5	4.9	Juvenile
64	F	Silti	Mar.	31	.70	5.00	3.40	6.7	5	Juvenile
70	M	Silti	Mar.	34	.80	5.30	3.60	7	5.4	Juvenile
72	F	Silti	Apr.	35	.90	6.00	4.00	7.5	5.6	Juvenile
66	F	Silti	Mar.	37	.90	5.60	3.70	7.5	5.6	Juvenile
69	F	silti	Mar.	38	1.00	5.70	3.70	7.6	5.8	Juvenile
71	M	Silti	Mar.	38	1.00	5.90	4.10	7.5	5.7	Juvenile
67	F	Silti	Mar.	38	1.00	5.60	3.80	7.8	5.7	Juvenile
65	F	Silti	March	44	1.40	6.40	4.50	8.7	6.5	Subadult
74	F	Silti	April	48	1.90	7.00	4.70	9.3	7.3	Adults
73	F	Silti	April	49	2.10	7.00	4.60	9.5	7.5	Adults

E) **Merabete:** *H. brucei*

Code	S	Area	Month	B.L	B.W	HL	CL	ML		AGE
26	F	Merab.	Aug.	36	0.9	5.8	4	7.1	5.4	Juvenil
27	M	Merab.	Aug.	35	0.8	5.8	4	7.1	5.4	Juvenil
25	M	merab.	Aug.	39	1.00	5.70	4.00	7.4	5.8	Juvenil
28	F	merab.	Augus	44	1.40	6.00	4.30	7.6	6	sub adult

F) Awash-7: *P. capensis*

Code	S	Area	Month	B.W	B.L	FL	HL	CL	ML	AGE
56	M	Awash-7	Feb.	0.4	28	3	4.5	5.5	4.2	Juvenile
51	M	Awash-7	Jan.	0.55	29	3.1	4.5	6.1	4.4	Juvenile
54	F	Awash-7	Feb.	0.55	29	3.1	4.8	5.8	4.5	Juvenile
48	F	Awash-7	Jan.	0.6	30	3.4	5	6.2	4.8	Juvenile
50	M	Awash-7	Jan	0.65	30	3.5	5	6.2	45	Juvenile
49	M	Awash-7	Jan	1.45	40	4.2	6.2	7.5	6	subadult
58	M	Awash-7	Feb.	1.7	42	4.7	7	8.5	6.5s	subadult
53	M	Awash-7	Feb.	1.75	43	4.8	7	8.5	6.8	subadult
52	F	Awash-7	Jan.	1.8	42	4.5	6.5	8.4	6.4	subadult
55	M	Awash-7	Feb.	1.8	47	5	7.2	9.4	7	adult
47	F	Awash-7	Jan	2.2	47	4.9	7.9	9	7	adult
46	F	Awash-7	Jan.	2.2	48	5	7.3	9.9	7.4	adult
57	M	Awash-7	Feb.	3.1	5.3	4.7	7	10.2	7.8	adult

Appendex - I I. **Consent to use picture of CL patients from Addis Ababa.**

Consent were reached with Adult CL patients and parents of children with CL to use photos that do not indicate their identity.

Appendex - I I I. Permission to trap animals

This research was undertaken after Ethiopian wild life conservation and development autority has given permission to collect the animal samples from the study areas.