RESERVOIR HOST STUDIES OF VISCERAL LEISHMANIASIS IN ABA ROBA, SOUTH-WEST ETHIOPIA

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TABLE OF CONTENTS

	Page
List of Tables.....	i
List of figures.....	ii
Acknowledgements.....	iii
Abstract.....	iv
I. Introduction.....	1
1. VL in Ethiopia and scope of the problem.....	1
2. General background.....	5
II. Materials and methods.....	18
1. The study area.....	18
a. Geography.....	18
b. Climate.....	18
c. Vegetation.....	20
d. Fauna.....	21
e. Human population and habitation.....	22
2. Field procedures.....	22
a. Trapping sites.....	22
b. Trapping animals.....	23
c. Other activities.....	23
3. Laboratory procedures.....	24
a. Maintenance of animals.....	24
b. Inspection of animals for Leishmania parasites.....	24

i. the culture method..... 25

ii. the smear method..... 25

iii. animal inoculation..... 25

iv. experimental inoculation of wild caught animals..... 26

III. Results..... 27

IV. Discussion..... 35

V. Conclusion and recommendations..... 40

VI. References..... 42

Appendix

Climatological data for Bekawele (Konso).

LIST OF TABLES

PAGE

1. Reservoir hosts of VL in known endemic areas of the world..... 13a
2. Number and types of animals collected and examined from March 1988 to April 1989 from the Aba Roba Peasant Association area..... 29a
3. Total number and types of animals surveyed from the Aba Roba area (Dec. 1986 - Apr. 1989)..... 30
4. Species of rodents identified from the Aba Roba Peasant Association area..... 31
5. Flagellate and Leishmania isolates from ground squirrels 32
6. Intraperitoneal inoculation of tissue suspensions into laboratory rodents..... 33
7. Summary of experimental infection of captive wild rodents infected intraperitoneally with 5 X 10^6 promastigotes of L. donovani (Aba Roba human strain)..... 34

LIST OF FIGURES

FIGURE PAGE

1 Sketch map showing the study villages in the Aba Roba peasant Association.....19

2 Monthly rain fall (Bekawole-Konso) and Abudance of Animals Trapped in the Aba Roba Area.....29b

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ABSTRACT

From March 1988 to April 1989 investigation of possible animal reservoirs of visceral leishmaniasis were carried out in the Aba Roba peasant association, one of the known endemic foci of visceral leishmaniasis in Southwest Ethiopia (Northern Omo Administrative Region). In this study a total of 280 individuals of different species of rodents, small and large carnivores and few domestic animals were examined. Tissues from the various organs viz., spleen, bone-marrow, liver, skin and a few drops of cardiac blood were cultured in NNN medium overlayed with Locke's solution for the isolation Leishmania. Tissue smears from the above organs were prepared from all the animals and checked for the amastigotes of L. donovani. Attempts were also made to isolate leishmania parasites indirectly, by the intraperitoneal inoculation of spleen suspension into susceptible laboratory rodents (hamsters, Balb/c mice and white mice). To see the possible potential reservoir hosts, selected species of rodents (born and reared in the animal house) were inoculated with L. donovani promastigotes isolated from a patient in Aba Roba. A Leishmania sp. was isolated from the spleen culture of a ground squirrel (Xerus rutilus) and two flagellates which were considered to be trypanosomes were also recovered from the blood cultures of two other ground squirrels. The results for the rest of the animals were negative.

I. INTRODUCTION

111. Visceral Leishmaniasis in Ethiopia and the Scope of the problem

The existence of kala-azar (KA) or visceral leishmaniasis (VL) in Ethiopia was first reported by De Marzo (in Pucco, 1965) as early as 1914. In 1926, Symes (cited in Ayele, 1982) reported 30 cases of KA in the King's African Rifle possibly contracted in the Kelem and Omo-Rate areas in Gamo-Gofa Administrative Region. The speculation that British-African soldiers returning from the Sudan or Ethiopia introduced the disease to Kenya (Mbugya and Siangok, 1981) led to the belief that VL might have established itself in Ethiopia long before the Second World-War. Since then, several cases were reported from the different geographical areas of the country. Major endemic areas include the Metema- Humera lowland areas in the Northwest (Tekle et al., 1970; Fuller et al., 1979; Haile and Lemma, 1977; Mengesha and Abuhay, 1978; Maru, 1979) and the Segen-Woito river valleys and the Omo river valley in the Southwest and the Hoyale and Gelana areas in the South (Cole and Cosgroove, 1942; Anderson, 1943; Haile and Lemma, 1977; Fuller et al., 1979; Lindtojrn, 1980; Lindtjorn and Olaffson, 1983; Lindtjorn, 1984; Ayele and Ali, 1984).

Fuller et al. (1979) reported one infected Konso while Lindtjorn and Olaffson (1983) described 31 cases of VL from the Segen-Woito river valleys and Ayele and Ali (1984) documented 27 other cases from the Aba Roba Peasant Association in Konso (all from the Gamo-Gofa Administrative Region in

Southwest Ethiopia). According to Ayele (1988), of the 173 confirmed cases of VL all over Ethiopia (1982/88) 120 to 130 cases were from the Gamo-Gofa Administrative Region. In this respect Southwest Ethiopia forms an important endemic focus where active transmission and high mortality is still taking place.

Ashford and Smith (1985) have pointed out that Southwest Ethiopia is part of the second most important VL endemic focus in East Africa. This region occurs midway between the endemic foci of Northern Kenya, Southeastern Sudan and Northwest Ethiopia (the Metema-Humera area). From their geographical location, it was suggested that these foci have a great deal in common. Commonness in the strain of the parasite viz., Leishmania donovani var archibaldi, similarity in the habits and habitats of the sandfly vectors and the human population, as well as some degree of similarity in the species composition and distribution of vertebrate hosts (Ashford and Bettini, 1989). Irregularity in the incidence of infection in man, and the unreliability of he low yearly rainfall, which causes variation in the rodent and vector pulations, are a few of the epidemiological features shared between these ndemic foci of VL in East Africa.

In Aba Roba, where three localities or villages were recognized as important endemic foci of VL (see description of the study area), it is common o see adults and young children sit on eroded termite hills, especially in he early evening hours during which transmission could be effected by the andflies inhabiting these termite hills. It is also a normal practice for he peasants to construct their huts near and around termite mounds byclearing

away the thorn bushes. Eroded termite hills have been indicated as favourable breeding sites for Phlebotomus martini and P. celiae, the sandfly vectors of VL in Kenya (Heisch et al., 1958; Minter, 1963; Nijers, 1963; Manson- Bahr and Southgate, 1964). Various rodent species and some small carnivores also inhabit these termite hills.

In Ethiopia, known endemic foci of VL occur in the agriculturally fertile lowland regions. In their surveys, Fuller et al., (1979) reported a high positive skin test rates of 84% for people who lived at lower altitudes (about 500m). It is in these fertile lowlands that large proportions of the rural population are pursuing their agricultural activities. It should also be noted that in these lowland areas large governmental development projects are in progress in which very many of the rural population will be involved (as in the Sile Cotton plantation scheme and the Ethio-Korean agricultural project in the Omo river valley). Unfortunately, as pointed out by Ayele (1982), the people in these areas, especially the productive age group may become infected with VL. As the development projects lead to population migration into these areas, there would definitely be an outbreak of VL among the new, non-immune migrant labourers or settlers.

As is the case in many developing nations, the rural areas of Ethiopia suffer from inadequate communication facilities, poor or no medical services and recurring drought periods. I have seen people walk 10 -15 kms to get some kind of treatment in the Aba Roba Peasant Association, where a small clinic is located. Coupled with malaria, VL incapacitates the very productive sector of the population. This in a way paralyses the source of subsistence and makes

the people more vulnerable to famine. The vulnerability of the people to famine retards not only the agricultural sphere, but also the economic sector. It has to be noted that, southwest Ethiopia (where endemic foci of VL are found) is one of the nation's important agricultural regions and the site of the largest game park (Zahar, 1981).

Despite VL's profound economic, social and medical importance, the study of its epidemiology is still incomplete. As in Kenya, and to a lesser degree in the Sudan, the question of possible reservoir hosts and their role in the transmission and maintenance of the disease has also remained unanswered in Ethiopia. In the last 10 years, new information has been obtained regarding human cases and sandfly population of the Aba Roba focus. Nevertheless, this information is insufficient, without corresponding work on the mammals of the area. In this regard, very few attempts have been made to investigate the natural hosts of this disease in various parts of the country. The brief investigations of Haile and Lemma (1977) and Ayele and Ali (1984) on rodents as the possible reservoirs of VL in the south and southwest of Ethiopia, were he pioneer studies. Since the early 80's, priority has been given by TDR/WHO the study of VL in Ethiopia (Gemetchu, 1988). As a result, intensive ies of human VL cases, sandfly vectors and reservoir hosts are in progress he staff of the Institute of Pathobiology and the Department of Biology, Ababa University, in the Aba Roba focus. However, the question whether e is an animal reservoir in Aba Roba or not, has not yet been answered. Some have suggested the presence of animal reservoirs in all East African VL foci, with the exception of central Kenya where it is thought to be anthroponotic (TDR, 1980; Ashford and Smith, 1985; Le Blancqu and Peters,

1986).

As described earlier, Aba Koba is one of the major East African VL foci and therefore, the incrimination and identification of any mammal as the natural host of VL would illuminate its epidemiology and help to pave the way for feasible control measures to be taken in this and other East African foci, with similar disease transmission patterns.

2. General Background

The leishmaniases are a spectrum of diseases caused by the protozoan parasites of the genus Leishmania. These disease are recognized by three main clinical forms: visceral, cutaneous and mucocutaneous leishmaniasis. They are a major cause of disfigurement and mortality in tropical and subtropical regions of the world, where about 400,000 people are victimized each year (Marinkelle, 1980; Dennis et al., 1986). The visceral form is the most dangerous and fatal diseases of man.

Human VL is caused by leishmania parasites grouped under Leishmania donovani complex (Lainson and Shaw, 1987). The geographical distribution of these parasites extends from the Pacific coast of China to parts of Asia including India, parts of Africa and large parts of Central and South America (TDR/WHO, 1980). The leishmania parasites exist in two different morphological forms, viz;, a non-motile amastigote form within the vertebrate cells and tissues and a flagellated motile promastigote form in the gut of the

sandfly vectors or in culture media at temperatures below 30°C (Gray, 1974; Das Siddhartha, et al., 1986).

According to WHO (1984) and Lainson and Shaw (1987) there are three subspecies of L. donovani. They are L. d. donovani, L.d. infantum and L.d. chagasi. L.d. donovani prevails in the Far- East, India and East Africa. On the other hand, L.d. infantum is the well known agent of VL in the mediterranean region, Middle- East, South and Eastern Europe and the USSR. Finally, L.d. chagasi is said to be the agent for New World visceral leishmaniasis.

There are differences of opinion on the taxonomic criteria used to subdivide the L. donovani parasites into separate subspecies. However, from the available literature, it is clear that a consensus is developing that differences observed among the strains of L. donovani are due to intrinsic factors, viz;, biochemical features, behaviour of the parasite in the vertebrate host (laboratory animals) and invertebrate hosts, or in culture, and also in their host specificity (TDR/WHO, 1980). The distribution of the strains of the parasites into geographically isolated areas is also influenced by the distribution of their respective sandfly vectors (Lainson and Shaw, 1987). The distribution of the various sandfly vectors is limited by environmental factors such as humidity, temperature, altitude and host distribution. The intrastrain parasite variations are also related to the adaptation of the parasites to a wide variety of vertebrates in the different geographical regions of the world. Citing the works of Hoogstraal and Heyneman in the Sudan, Fuller et al., (1976) stated that the isolation of L. donovani

from various animals (e.g. rodents, carnivores and man), in a given locality, indicates how genetic separation and development of strain variation, can come about. Hence, vertebrate hosts do play an important role not only in the distribution of the parasites, but also in bringing about strain variation.

As suggested by Lysenko (1971) visceral leishmaniasis was initially enzootic, covering large areas in the Mediterranean basin, Asia and Africa; with jackals, wolves, foxes and later dogs involved in the natural cycle of the disease. The same author remarked that, subsequently, the parasite evolved into various "Serodemes and zymodemes, species and subspecies" depending on the biological and biochemical variations observed in each"strain" of these organisms. It seems that man is not a natural host for L. donovani, until a given strain secondarily is established in man, who then acts as a reservoir (Fuller et al., 1976).

The feeding habits of sandfly vectors limit the role of the vertebrate hosts in the distribution and maintenance of VL in the different geographic zones. Gemetchu (1982) explained that some sandflies may be zoophilic feeding mainly on wild and domestic animals and secondarily on man while others live mainly in or around human habitation and may be anthropophilic. The mammalian hosts thus affect the distribution of VL directly or indirectly i.e. through their importance as blood meal sources for the sandfly vectors. Therefore, the incrimination of certain species of sandfly vectors and reservoir animals in endemic areas of VL is a task of primary importance.

A reservoir animal of a parasite is defined as an ecological system

consisting of vector (s) and animal(s) that can transmit and sustain the parasite population for an unlimited time (WHO, 1984; Ashford and Bettini, 1987). Actually, reservoirs are of two types - natural and accidental. Those animals that provide the parasite long-term survival are referred to as natural reservoirs while the accidental reservoirs harbour the parasite under particular or altered environmental conditions. The latter types have no significant part in the maintenance of the disease in nature, since infection is often scanty or cryptic and they therefore, represent "dead ends" (Killick-Kendrick, 1988).

According to Bray (1983), WHO (1984), Mann (1986) and Killick-Kendrick (1988), an animal is considered as a "good" reservoir host of Leishmania if it fulfils the following conditions;-

- present in large numbers so that it will be the chief blood source for the sandfly vectors,
- have an adequate parasitemia (in the peripheral blood and/or the skin) such that the sandfly vectors can easily pick-up the parasites;
- be in habitats which it shares with the vector, and is in close contact with man; and
- be resistant to the effects of infection and harbour the parasite for a fairly long period of time.

In view of the last criterion, Garnham (1971) postulated that maintaining the parasite by some animals for long periods of time results in a natural selection, which renders these animals resistant to the parasitic infection.

In fact, there are exceptions to such general conclusions. In some vertebrates, for instance dogs (which are the known reservoirs of infection to man in the Mediterranean areas and South-America), could harbour high parasitaemia and the disease is symptomatic resulting in severe disease of the dogs (WHO, 1984). But in most situations, Garnham's postulate holds true. For example, where the supposed natural reservoirs of infection is asymptomatic say rodents, search for the possible reservoirs of infection is mandatory. Such tasks, therefore, follow some laboratory protocols,. Thus, demonstration of parasites from wild-caught and experimentally infected animals can normally be achieved either through culture of tissues and blood or by animal inoculation of tissue suspensions or by both methods. Impression smears of tissues is an alternative, but its limitation is that parasites can not be identified to the species level. Hence, investigators do not apply this method as a sole or main way to identify reservoir hosts. Generally, the method of choice differs from place to place and depends on the availability of facilities.

To use the culture method for isolation of parasites,a suitable media is prepared based on the growth requirements of a particular strain of a leishmania parasites. Different modifications of the biphasic NNN media have been suggested for primary isolation purposes (Walton et al., 1977; Lainson, 1981, Jaffe et al., 1984; Schnur and Jacobson, 1987). Using modified NNN medium with Locke's overlay, Rassam and Al-Mudhaffar (1979) isolated L. donovani successfully in Iraq. The modification of the NNN medium involves alteration of the bacto-agar by nutrient rich agar such as Difco blood agar (Walton et al., 1977) and Nutrient agar or BHI agar (Marin et al, 1982). The

NNN medium is preferred not only for its suitability for primary isolation of Leishmania parasites but also for its low cost and availability (Hockmeyer,1981). It should, however, be remembered that successful isolation is not dependent only on the kind of media utilized, but also on factors such as the nature of a given stock of parasite and the burdens of parasites the animal under investigation harbours (Schnur and Jacobson, 1987). Insensitivity to low parasitaemia and long delay in the appearance of promastigotes are some of the drawbacks of the biphasic NNN medium. Such limitations could obscure results and may lead to wrong conclusions. However, whenever parasites are not isolated from wild caught animals, it should not be related solely to such limitations of the media. Other important factors involved in the transmission pattern of the disease should also be considered. The activity and abundance of the sandfly vectors, their intimacy and feeding preference to the local mammals and the abundance of the possible reservoir animals need to be studied since these mainly govern the prevalence of infection among wild animals.

The number of animals caught and examined determines the prevalence rate of infection (Killick-Kendrick, 1983). In order to have a large sample of suspected animals, trapping areas must be chosen carefully and the trapping period must be fairly long. Focus on areas where human activities coincide with the habitats of suspected reservoirs should yield fair numbers of wild animals (Travi, 1988).

When the search for infected mammals fails to show any natural infection, attempts should be made to establish experimental infections in animals

(brought from non-endemic areas of the same but of the same species as those in the study area) (Stauber et al., 1958). These animals should be infected with a human Leishmania parasite isolated from cases in the area under investigation. This helps to establish the susceptibility of the animals to the "target" leishmania (Killick-Kendrick, 1988) and predict the potentiality of these animals as sources of human infection in the endemic area. Stauber et al., (1963) did a careful laboratory investigation on the susceptibility of Arvicanthis niloticus (the Nile grass rat) for a Sudanese isolate of L. donovani. A. niloticus was found to be resistant to symptoms of infection, harboured the parasite for long periods of time and was highly susceptible. Similarly, Gradoni et al., (1983) experimentally verified that Rattus rattus (the black rat) was the probable reservoir host of L. donovani infantum in Italy. The results obtained from the experimental infection of suspected mammalian hosts depended on some important parameters.

Stauber (1966), Poulter (1981) and Sacks and Perkins (1984) indicated that variables such as route of inoculation,dose of infection, source of parasites and age of promastigotes play a significant part. Although wild-animals may not be exposed to high doses of parasites under natural conditions, injecting as many as five million parasites into an experimental animal will help overcome the loss of promastigotes when inoculation is intraperitoneal or intradermal (Stauber, 1966). In nature, relatively few parasites are injected by the bite of the sandfly vectors (Lainson and Shaw, 1987). With respect toroute of inoculation, Stauber (1966) indicated that intracardiac infection is preferable to intraperitoneal, intravenous or intradermal routes and that influences the patency of the parasites and their

density in the predilected organs. It was also demonstrated that splenic amastigotes from human cases are more infective than promastgotes from cultures, even when the route of inoculation is the same (Stauber, 1969; Poulter, 1981). Stauber (1958), Zuckerman (1975) and Poulter (1981) were of the opinion that promastigotes from old cultures were less infective than from fresh cultures. But Sacks and Perkins (1984) experimental result indicated that infective promastigotes predominate only in the stationary phase (from old culture) a fact nowadays taken as a common feature of all Leishmania species. They stated that seven-day old mid-gut promastigotes from sandflies (analogous to stationary phase of culture promastigotes) produced more infective stages than three-day-old mid gut promastigotes (equivalent to log phase culture promastigotes), which were infective to Balb/c mice. Generally, in experimental investigations of the susceptibility of mammalian hosts to leishmania parasites, due attention must be paid to the above key factors.

Another line of approach to discover whether a mammal is a potential reservoir host is to determine the attractiveness of the animal in question to the known sandfly vector in the locality (Killick-Kendrick, 1988).

As explained earlier the epidemiology of VL in many countries of the world is far from complete. More information is available on the distribution of the parasite species and their insect vectors than of the reservoir hosts involved. Even in areas where incrimination of the reservoir hosts is successfully achieved; the status of such animals as foxes and rats is not yet fully understood (WHO, 1984). Thus, to bridge such a gap intensive and detailed studies have been made in many endemic areas of VL to know its

natural hosts. However, the effort is too little to be compared to the distribution of the disease. Systematic study has been done only in 18 out of 112 VL endemic countries (De Raadt, 1986). Of the accumulated factors hindering reservoir host studies, the following are the common ones:remoteness of many of the endemic foci and their inaccessibility by road, economic constraints in many developing nations, lack of technically trained personnel and shortage of facilities. The latter ones are the outcome of economic constraints. Moreover, the peculiar nature of leishmania parasites in general, and that of L. donovani in particular has made life difficult for many researchers. These include the failure of the parasites to grow in slightly contaminated cultures, the high specificity of the sandfly vectors to a limited number of mammalian hosts and the incapacity of the parasite to develop in any vertebrate (Lainson, 1981). In this respect, Chandler (as cited in Heyneman, 1961) stated the following: "few problems in parasitology have caused more fruitless efforts, more blasted hopes, more false conclusions or more unfounded speculation than the transmission of leishmaniasis." Hence, few attempts were successful in the past in incriminating the natural mammalian hosts of VL.

In some endemic foci, relatively few animals may be found harbouring leishmania parasites. To incriminate such animals as reservoirs of infection, the parasites must be isolated from them repeatedly and the isolate must be identical to that recovered from man in the same locality (Stauber, 1966; WHO, 1984; Lianson and Shaw, 1987; Killick- Kendrick, 1988). In addition the infection rate should not be below 1% since such values i.e.; below 1% would only tell incidence of infection (Bray, 1983). Table 1 summarizes the

Table 1
Reservoir hosts VL in Known Endemic Areas of the World

Vertebrate Host	Country	Reference
Man	India Southern China ? Central Kenya	WHO (1984), Le Blancq and Peter (1985)
Arvicanthis niloticus	Sudan	Hoogstraal and Heyneman (1969)
Rattus rattus	Egypt, Italy	Azab *et al.*, (1984), Bettini *et al.*, (1980), Gradoni *et al.*, (1983)
Dog	Algeria	Belazzoug (1986)
Foxes	Brazil	WHO (1984)
Cerdocyon thous *Lycolopes vetulus*		
Vulpes vulpes	Italy USSR	Bettini *et al.*, (1980) Lysenko (1971)

? not yet confirmed.

reservoirs of VL in the New and Old-Worlds.

Bettini et al. (1980) have recorded L.d. infantum from Rattus rattus in Tuscany, (Italy) and Gradoni et al. (1983) verified this experimentally. Positive cultures of L. donovani from the blood and liver of the black rat (R. rattus) was also recorded in Iraq (El-Adhami, 1976).

Owing to the variable nature of East African strains of L. donovani, the study of the reservoir hosts of VL faced with difficulties and uncertainties. Opinions on the regional variations in the clinical and epidemiological features of East-African VL and on the identity of the parasite are in discordance. Le Blancq and Peters (1986) suggested that isolates from the Sudan, Kenya and Ethiopia are biochemically similar to each other. But variations as in the species of sandfly vectors i.e., P. orientalis in the North (The Sudan and N-Western Ethiopia) and P. martini in the South (Northern Kenya and Southwestern Ethiopia) cause differences in the local epidemiology. The infection rate in the sandfly vectors is low whereas the disease may be common in man (Ashford and Bettini, 1987).

Reservoir host studies in East Africa have been undertaken mainly in the Sudan and Kenya. Pioneer investigations made in the Sudan in the early 1960's increased our knowledge of VL but were unsuccessful as related to the reservoir hosts (Heyneman, 1961). Despite the heroic efforts of workers in Kenya (Heisch, 1957; Manson-Bahr and Southgate, 1964; Ngoka and Mutinga, 1978; Githure et al., 1986) and in the Sudan (Hoogstraal et al., 1963) no definite statements of confirmation were made as to which animals were actually

responsible for maintaining the parasites causing VL in these countries.

After the epidemics in Kenya in the 1950's, the attention of many workers was focused on the task of identifying the reservoir hosts of human VL in this region. In the late 1950's many animals were examined for L. donovani but without success. Heisch (1957) examined many animals. Using hamsters he revealed leishmania parasites from ground squirrel (Xerus rutilus). These isolates where later identified as L. major (Chance, 1978). Following the epidemic outbreaks of 1950's, Manson-Bahr and Southgate (1964) examined hundreds of rodents in Kenya, all with no success. Heisch et al., (1959) recovered leishmania parasites from gerbils (Tatera robusta which were once more identified to be L. major (Chance, 1978). Peters et al., (1977) using biochemical methods, demonstrated that the Leishmania sp from Tatera sp in the Baringo district and Kerio valley of Kenya was in fact L. major not L. donovani. Ngoka and Mutinga (1978) in Baringo and Mutinga and Ngoka (1983) in West-Pokot (both in Kenya), isolated Leishmania from gerbils and from a dog, respectively. The isolates from the gerbils were typed as L. major whereas the parasites from the dog in West-Pokot (and two other isolates from dogs in MachaKos) by Mutinga et al., (1980) were L. donovani. These findings suggest that dogs may be the natural reservoirs of VL in Kenya. However, since infection in dogs was rare (2 out of 288 in Machakos and 1 of 80 in West-Pokot) and the Machakos dogs were from the home of a VL patient. It was concluded that dogs are probably accidental hosts of VL in Kenya Ashford and Bettini, 1987). In other instances, Mutinga et al., (1982) recovered "leishmania-like" parasites from a genet cat (Genetta genetta) and from four mongoose (Helogale pervualis) in the Kitui district using NNN medium.

Recently, Githure et al.,(1986) did not find L. donovani in the rodents they examined, they found Mastomys (Praomys) natalensis , Taterillus emini and Aethomys kaiseri as new hosts of L. major in Kenya.

A less intensive but pioneering investigation of animals as reservoir hosts of VL was made in the Sudan in the 1960's. Three of one hundred eighty-eight (3/188) rodents (Arvicanthis niloticus) examined were positive for L donovani parasites in the Southern Sudan (Hoogstraal and Heyneman, 1969). These parasites were considered identical to human isolates of L. donovani in the area. In their extensive epidemiological report on VL in the Sudan, Hoogstraal and Heyneman (1969) noted that the same parasites were also recovered from a black rat (R rattus) and spiny mouse (Acomys albigena) from the Malakal town, where human cases of VL were unknown. In addition, they found similar parasites in organs of two wild carnivores (Genetta sp and Felis serval). However, they were doubtful of the role of these carnivores as reservoirs of VL. They assumed that these animals may have become infected by eating rodents they preyed upon. Lately, Sixl et al., (1987) reported the isolation of Leishmania sp from a jackal in South Sudan and said that this animal is a reservoir host of leishmaniasis in this area.

The objectives of the present study were to undertake a year round study to find out the possible reservoir host(s) of VL in Southwest Ethiopia; particularly in the Aba Roba area in the Segen river valley. The presence or absence of an animal reservoir within this region would provide relevant information as to whether VL is zoonotic or anthroponotic in southwest Ethiopia. The study was also aimed at rendering ideas on the species

composition and abundance of the possible mammalian hosts of VL in the area.

II. MATERIALS AND METHODS

1. The Study Area

a. Geography

The Aba Roba Peasant Association is located in the southeastern part of the Northern Omo administrative region, bordering the southwestern part of the Sidamo administrative region. Actually, the Segen river forms the natural boundary between the two administrative regions (Fig. 1). Aba Roba is about 515 kms southwest of Addis Ababa. It is located at latitude $5^{0}15"$ N and longitude $37^{0}35"$E. The elevation of the area ranges from 900m at the Segen valley floor to 1480m at Foro.

b. Climate

According to Gemetchu (1977) and the Ethiopian Mapping Agency (1988), this region occupies the intermediate zone between the dry subhumid and semiarid parts of the country. Meteorological data for Aba Roba are not available. In regard to rainfall, in tropical Africa, there is no extreme localization of rainfall between places a few kilometres apart (Delany and Happold, 1979). Thus, the Aba Roba Peasant Association, 14 kilometres southeast of Bekawele (altitude of 1460m) and for which meteorological data is available, is expected to receive a more or less similar amount of rainfall as Bekawele (Konso). Field observation during the study periods confirmed this. Based on this assumption, the annual rainfall of the area ranged from about 750mm to 870mm for the years 1987/88 (Appendix 1). The short rains are in November and December while the main rains occur from March to May. Heavy

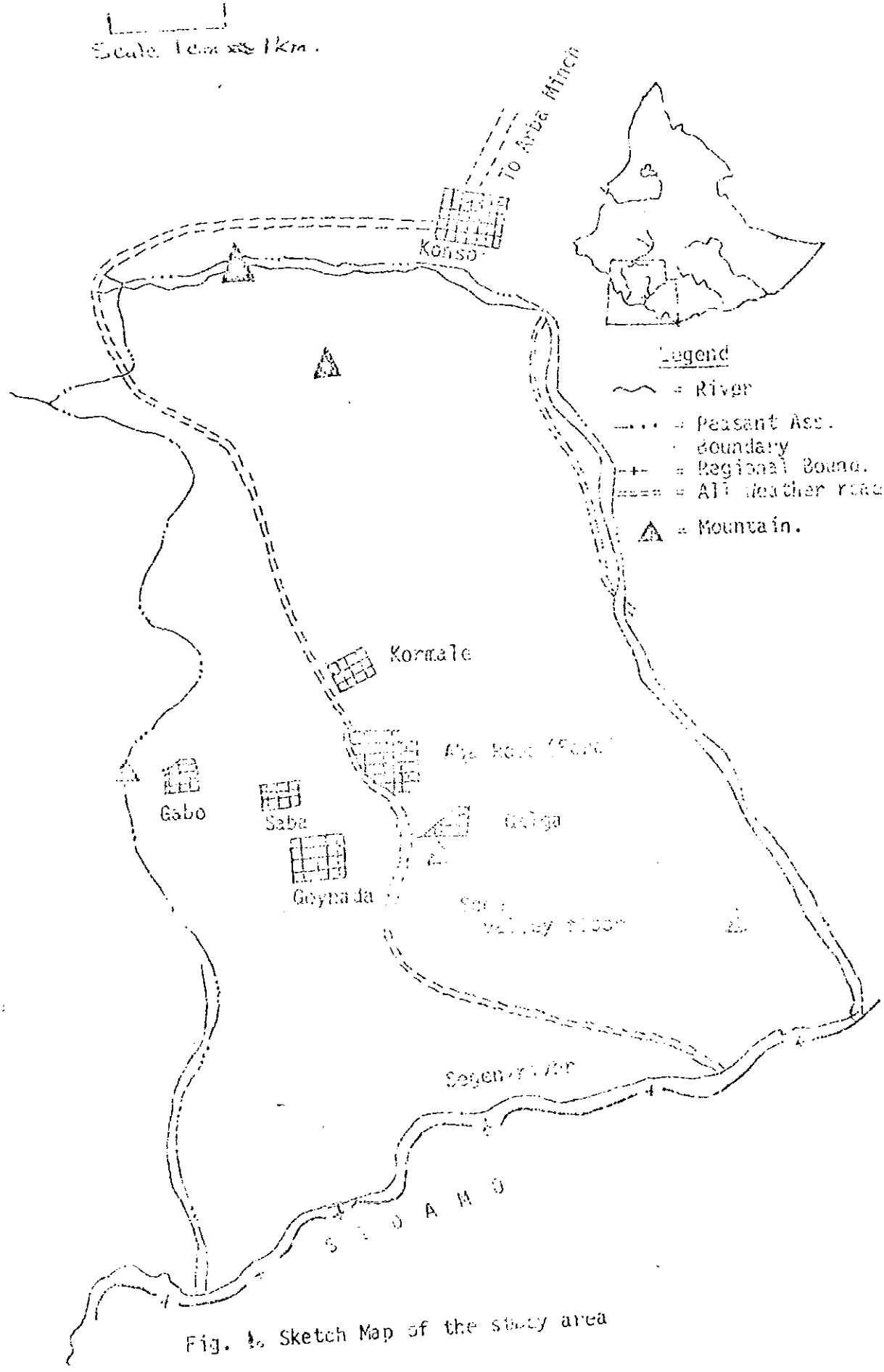

Fig. 1. Sketch Map of the study area

rains occur mainly in the month of April.

With respect to temperature, Bekawele has an average of 31.5^{o}C and 15.9^{o}C mean maximum and mean minimum temperatures respectively. By employing a temperature decrease of 1^{o}C for any 180m increase in altitude (Fantoli in Demissew, 1980), the mean maximum and mean minimum temperatures will respectively 34.6^{o}C and 19.0^{o}C for the Segen valley floor, at 900m altitude, 34.1^{o}C and 17.5^{oC} for Galga at 1000m; and 31.4^{o}C and 15.8^{o}C for Foro at altitude of 1480m.

These villages and areas of the Aba Roba Peasant Association constituted an important focus of VL and thus were chosen as suitable study areas for reservoir host studies of the disease in Southwest Ethiopia.

c. Vegetation

As in the other parts of the country, the vegetation of this area is disturbed by human activity. Patches of shrub, thorn scrubs interspersed with grasses and some scattered Acacia and other trees are the dominant vegetation. Favourable rains allow a seasonal cover of grasses and herbs to grow which provide suitable food and habitat for several species of small mammals especially rodents. The dense herb material formed immediately after the rain, enables rodents such as the grass rats (Arvicanthis sp), elephant shrews (Elephantulus sp), the gerbils (Tatera robusta) and the spiny mouse (Acomys sp) to construct their burrows by digging the soft earth. The animals are presumed to feed on the fruits, seeds, stems and roots of some of vegetation the area. In Aba Roba the land is very rugged and hilly and is often unfit

for crop cultivation except in the open plain on the valley floor along the Segen river. Here, the people cultivate sorghum, maize and legumes (such as beans) as the main food crops. The Konso people are very well known for their terrace-farming. Hence, they grow cotton, millet and sorghum on the steep slopes near the various villages. Banana and papaya are planted as horticultural cash crops along the Segen river banks. They also keep goats, sheep and cattle and use the open fields and grass covered hill sides for grazing.

d. Fauna

Various types of small and large animals were observed in the Aba Roba area. Animals such as baboon, aardvack, dik-dik, hyenas occur but are uncommon although, bats and various small rodents and carnivores are common. The animals trapped during this study will be considered later in the Results section.

Species identification for some of the animals trapped in the areas under investigation was attempted. Such an attempt was made by:

- referring to characteristics of the animals complied in Yalden et al. (1976), Kingdon (1974);
- using cytotaxonomic approach made in collaboration with the Ethio-Soviet Biological Expedition team;
- comparing the anatomy of some of the animals to specimens in the Natural History Museum of the Addis Ababa University.

e. Human Population and Habitation

The population in the 6 villages of the Aba Roba Peasant Association is 4612 (Ahmed Ali, pers. comm.). About one-third of the total population live in Foro, Galga and the Segen valley floor. Homesteads are associated with small farms and sometimes with eroded termite hills.

The close proximity of the human dwellings to eroded termite hills may enhance the transmission cycle of VL between man, suspected sandfly vectors and possible mammalian reservoirs of the disease.

2. Field Procedures

a. Trapping Sites

Small animals, especially rodents, were trapped around Foro, Galga and the Segen valley floor from March, 1988 to April, 1989.

Selected trapping sites and areas were:

- within and around family homesteads;
- near the mouth of rodent burrow and rodent runs under the thorn bushes;
- near and around termite hills and rock-cliffs; and

- along animal tracks and human footpaths around small farms.

b. Trapping of Animals

Rodents were trapped using conventional and/or collapsible traps (Bio Quip Products, USA). Traps were set at dusk and left overnight to trap nocturnal rodents. These were checked in the morning for trapped animals. Empty traps were then left the whole day to trap diurnal rodents. Traps were checked intermittently to see if they had caught rodents. The traps were baited with peanut butter, crushed sunflower seeds and pieces of freshly cut pineapple. Animals caught were then transferred into larger cages and transported by vehicle to Addis Ababa within 5 to 8 days capture.

c. Other Activities

Dogs from the study villages and Goynada (another village within the Association) were caught and examined by taking fluid from the popliteal lymph glands and aspirates from the spleen and inoculated into NNN culture media and checked for the presence of leishmania parasites after arrival in the laboratory at Addis. Impression smears of the fluids were also made on clean slides and examined for amastigotes.

Sheep and goats slaughtered in Foro market were examined for leishmania infection by impression smear of liver and spleen tissues.

3. Laboratory Procedures

a. Maintenance of Animals

Upon arrival in Addis Ababa, the caged animals were placed in the animal house (Science Faculty, Addis Ababa University), where the room temperature was kept fairly high (22 to 26^{o}C) by warming the room with an electric heater. The animals were provided with sorghum, laboratory animal feed and tap water.

Small carnivores were provided with meat. The animals were repeatedly checked for any external lesion by looking on their bodies until they were killed.

b. Inspection of Animals for Leishmania Parasites

Examination of animals for the presence of leishmania parasites (L. donovani) was made in the laboratory (Science Faculty), in the locally made glass cabinet or hood, within 9 to 12 days of capture. The cabinet was thoroughly swabbed with 70% alcohol and lit with a UV-light throughout (except when dissecting the animals) to provide an aseptic environment. Animals were etherized in a killing jar, weighed, measured and sexed. They were then washed with disinfectant (Savlon and detone) and then rinsed in tap-water before dissection.

Samples of cardiac blood, bone-marrow, liver, spleen and skin from lips, nose and ears were taken, using the methods described by Hoogstraal and Heyneman (1969) and Jaffe et al. (1984). The remains of each animal was labelled and kept in a deep freeze for a later identification. The following procedures were adopted to isolate parasites from the animals.

i. The Culture Method

For the in vitro cultures, modified biphasic NNN medium was used (sterilized by autoclaving at 15 lbs and 121^{o}C for 20 minutes) and overlayed with Locke's solution. Two hundred units of penicillin and 200ugm of streptomycin were added to each 1.0ml of the overlay. The medium was prepared as described in WHO (1984), Jaffe et al. (1984) and Schnur and Jacobson (1987) and enriched with pooled rabbit-blood. The modification of the medium was made by replacing the Difco blood agar with nutrient agar (Oxoid) and blood agar bases (Oxoid).

A few drops of cardiac blood and small pieces of tissues from the organs mentioned above (3.3.2) were aseptically transferred to the liquid phase of the NNN slants kept in screw-capped glass vials. These were then incubated at 24 - 26^{o}C. A search for promastigotes was made each week under the x40 phase objective. Cultures were examined (weekly) for four weeks and then discarded.

ii. The Impression Smear Method

Impression smear of bone-marrow, spleen, liver and skin were made and thin blood films were prepared on clean slides from all animals caught. Slides were fixed in absolute methanol and stained with 25% Geimsa for 20 minutes. The slides were then examined for amastigotes with the oil immersion objective. A minimum of 10 minutes of examination was given to each slide.

iii. Animal Inoculation

Spleen tissues were aseptically removed and ground with a sterile glass pestle and mortar in normal saline from selected species of rodents killed at

various periods of this investigation. About 0.5ml of this suspension was drawn into a sterile disposable 1 ml tuberculin syringe and inoculated intraperitonially into hamsters, Balb/c mice and white mice. These laboratory rodents were sacrificed at 60 and 90 days post inoculation. Specimens from the blood, bone-marrow, liver and spleen of these animals were processed and examined as described above.

iv. Experimental Inoculation of Wild-Caught Rodents

Experimental animals, seven Nile grass-rats, two gerbils, four black-rats and four spiny mice were inoculated by intraperitoneal injection of 5 $x10^6$ four day- old culture promastigotes (stock isolated from a VL patient in Aba Roba in June 1988; isolate L 399/88. Kindly provided by Ato Asrat Haile of the Institute of Pathobiology, Addis Ababa University). The promastigotes were collected by the method of Jaffe et al., (1984) and counted in a Neubauer haemocytometer. The animals inoculated were born and reared in the animal house, the progeny of wild caught rodents in the study area. They were closely examined for any sign of prior infection and sacrificed at 8, 60, 90 and 127 days post inoculation and examined for infection as described in sections II.3.b.i.and II.3.b.ii.above. Spleen material from one of the spiny mice, and one of the grass rats was injected into two hamsters. One of the hamsters was later found dead in the animal house, and thus only survivor was sacrificed and examined as described.

III. R E S U L T S

In this study 280 wild and domestic animals were investigated (rodents, insectivores, hyrax, and small and large carnivores). The surveys were carried out in the Aba Roba Peasant Association from March 1988 to April 1989 and the animals examined are listed in Table 2. Among the animals trapped and examined, the gerbils predominated followed by ground squirrels and Nile grass rats. In general, an increase in the number of rodents was observed following periods of seasonal rainfall (August, October, November, December and January) and a decrease in the population size was observed immediately after the rains (March, April, May, June, July and September) (Fig.2).

In the three villages (Goynada, Galga and Foro), there were 50 dogs recorded. Forty-one of these were caught and examined for Leishmania infection.

The total number of animals sacrificed and examined to find the reservoir host (s) of VL in the Aba Roba endemic areas since Dec. 1986 (Gemetchu, unpublished data, pers.comm.) up to the present study are shown in Table 3. Using the available sources of information, the wild-caught animals, the rodents in particular, were identified. The results of such an attempt is indicated in Table 4.

The present findings record the isolation of flagellates from tissue and cardiac blood cultures of two ground squirrels and a Leishmania sp from the spleen culture of another ground squirrel (Table 5). No parasite were

recovered or isolated from the rest of the animals examined by both the culture method and/or by smear preparations. Subinoculation of ground tissue suspensions from some of these wild-caught animals into a few laboratory rodents did not show any natural infection either (Table 6). Similarly, all the experimentally inoculated rodents showed no L. donovani infection (see Table 7).

Table

Number and types of animals collected (out of bracket) sacrificed and examined (in bracket) from March 1988-April 1989

From the Aba Roba peasant association (Southwest Ethiopia)

Common name	Species name	March	April	May	June	July	August	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	Total
Gerbil	Tatera robusta	5(3)	7(5)	7(4)	5(4)	5(6)	4(4)	6(5)	5(4)	10(9)	6(7)	7(4)	-(3)	8(5)	8(5)	81(68)
Ground squirrel	Xerus rutilus	2(2)	2(2)	3(2)	5(3)	5(5)	2(2)	3(3)	5(2)	5(4)	5(3)	4(3)	-	6(5)	2(2)	48(40)
Spiny Mouse	Acomys cahirinus	3(1)	3(2)	1(2)	4(3)	2(3)	3(1)	-	3(3)	-(1)	4(4)	7(4)	-(3)	1(1)	-(1)	31(25)
Grass rat	Arvicanthis sp	-	-	-	-	-	7(3)	1(4)	3(2)	4(5)	8(4)	8(3)	-(3)	3(3)	4(8)	38(35)
Black rat	Rattus rattus	-	1(1)	-	2(2)	1(1)	4(1)	1(-)	5(3)	-(2)	2(2)	5(3)	-(2)	-	1(1)	22(18)
Multimammate rat	Mastomys natalensis	3(3)	-	-	3(3)	1(1)	-	-	4(2)	2(1)	2(-)	-(2)	-	-	-	15(12)
Elephant shrew	Elephantulus sp	-	1(1)	-	-	3(3)	2(2)	1(-)	-	1(1)	1(1)	4(2)	-	-	-	13(10)
Genet cat	Genetta sp						2(-)	-(2)								2(2)
Mongoose	Helogale sp	-	-	1(1)	-	-	-	-	-	-	-	-	-	2(2)	1(1)	4(4)
Hyrax	Heterohyrax sp		2(2)													2(2)
Dog	Canis familiaris														50(41)	50(41)
Sheep and goats					19											19(19)
Total/Month		13(9)	16(13)	12(9)	17(15)	17(19)	24(13)	12(14)	25(16)	22(23)	28(23)		35(21)	-(11)	17(18)	32(28)

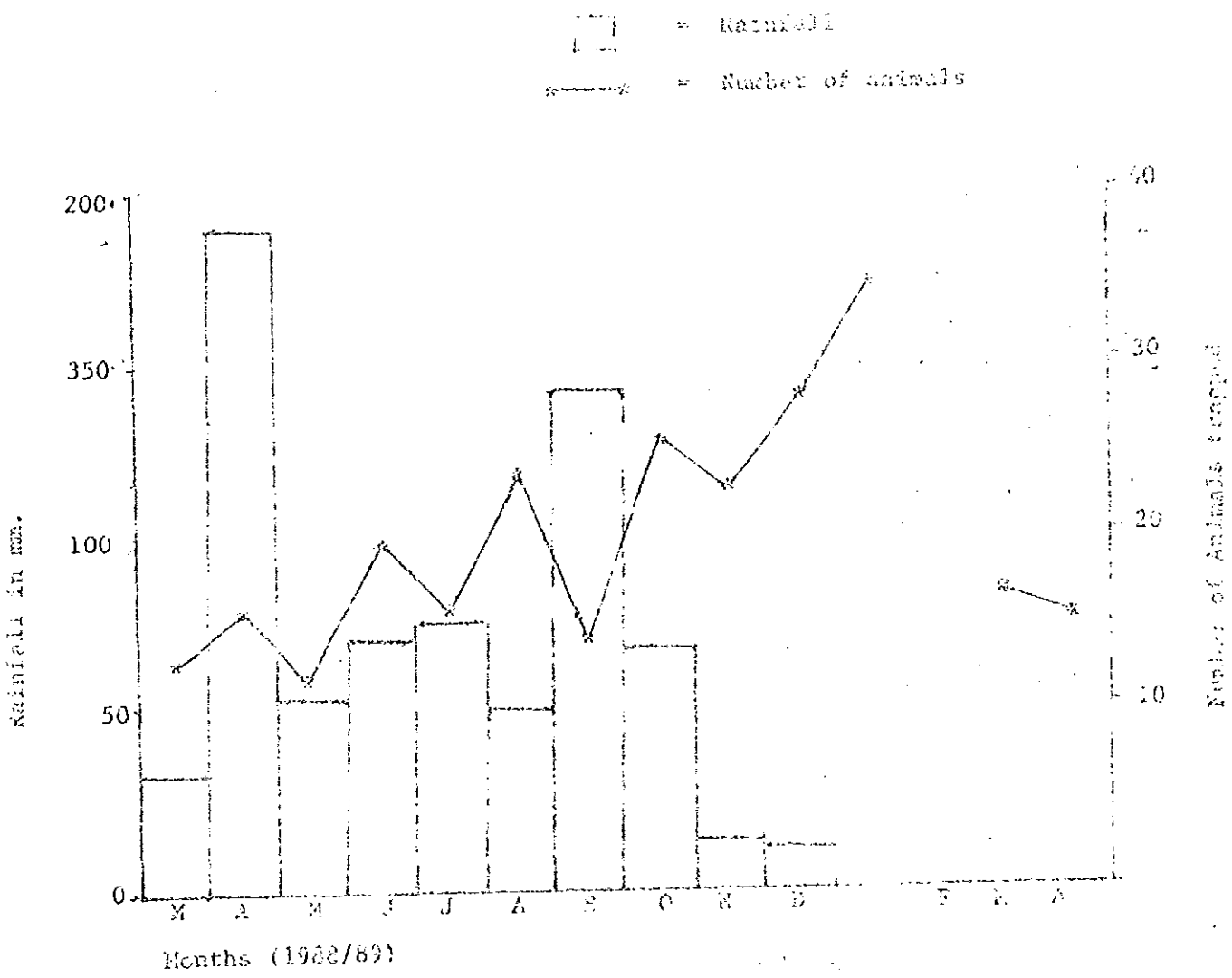

Fig. 2 – Monthly abundance of animals and rainfall distribution pattern in Aba Roba

TABLE 3

Total Number and Types of Animals Examined from the Aba Roba Area (Dec. 1986-April 1989)

Animal	Dec. 1986- Sept. 1987 *	April 1986- Feb. 1987 *	March 1988- April 1989	Total
Spiny mouse	26	12	29	67
Grass-rats	9	8	35	52
Black rats	-	6	18	24
Praomys	34	13	12	59
Gerbils	10	2	68	80
Elephant shrews	3	-	10	13
Ground squirrels	23	5	40	68
Hyrax	-	8	2	10
Mongoose	1	1	4	6
Genet cat	-	-	2	2
Dogs	-	70	41	111
Sheep and goats	-	-	19	19
Total	106	125	280	511

* Data for Dec. 1986-Sept. 1987 came from Gemetchu (Gemetchu unpublished data)

TABLE 4

Species of Rodents Identified from the

Aba Roba Peasant Association

Species Identified	Karyotype Analaysis		Remark
	2N	NF	
Ground squirrel *Xerus rutilus*	38	76	Has also been recorded from segen (Yalden *et al.*, (1976)
Spiny mouse *Acomys cahirinus*	36	68	Yalden et al., (1976) have also reported it from Konso
Gerbils *Tatera robusta*	36	70	Both large and small specimens have same Karyotype
Grass rat *Arvicanthis* sp.	60	78	Unique in localization of heterochromatin, Needs further identification
Elephant shrew *Elephantutus sp*	26	-	Only one species, *E. rufescence* is known from Ethiopia. Has also been recorded from Gamo Gofa Yalden *et al.*, (1976)
Praomys natalensis	-	-	Recorded from Konso (Yalden *et.al.*, (1976)

Note: 2N = Diploid number of chromosomes

NF = Fundamental Number.

*This species with 2N = 36 and NF = 68 was identified as *A.percivali* Dollman from the Omo valley and is very akin to A.cahirinus (Mathey,1968).

Table 5
Flagellate and Leishmania Isolates from Ground Squirrels

Host of Origin	Place of Origin	Isolate Designation	Remarks
Xerus rutilus	Galga	Trypanosomes	- Fast moving - kinetoplast posterior to the Nucleus
Xerus rutilus	Galga	Trypanosomes	
Xerus rutilus	Galga	Leishmania sp	- slow moving flagellates in culture

TABLE 6

Intraperitoneal Inoculation of Tissue Suspension into Laboratory Rodents

Type of Rodent	Number	Material Inoculated	Result	Remarks
Balb/c mice	2	Flagellates from +ve blood culture of a ground squirrel	Culture & smear -ve	Killed at 30 and 60 days post-inoculation
Balb/c mice	4	Spleen suspension from one Acomys and 3 gerbils (sickly looking)	Culture & smear -ve	Killed 60 days Post-inoculation
Hamsters	2*	Spleen suspension from laboratory, inoculated Acomys and Arvicanthis	Culture & smear -ve	Killed 30 days Post-inoculation
White mice	2	Material from spleen culture of a +ve ground squirrel	Culture and smear -ve	Killed 30 and 45 days post-inoculation

* One of the hamster was found dead in the animal house and examined only by impression smear.

TABLE 7

Summary of experimental infection of captive wild rodents infected intraperitoneally with $5X10^6$ promastigotes of L.donovani (Aba Roba human strain)

Group	Animals	Interval Days	Result	
			Smear	Culture
			*sp.BM.Lv.Bd	sp.Bm.Lv.Bd
I	1 Tatera	8 days	-ve	-ve
	1 Acomys			
	1 Rattus			
	2 Arvicanthis			
II.	1 Acomys	60 days	-ve	-ve
	1 Rattus			
	2 Arvicanthis			
III	1 Acomys	90 days	-ve	-ve
	1 Arvicanthis			
IV	1 Acomys	127 days	-ve	-ve
	3 Arvicanthis			

Note: Sp = spleen; BM = Bone-marrow; Lv= Liver; Bd = Blood.
Two Black rats died before they could be sacrificed.

IV. DISCUSSION

The population densities of the local mammals were not studied. There was no previous information on the abundance and species composition of the mammals in the study area either. However, a fair number of animals were trapped using 10 traps per trip. Availability of food and shelter, and fluctuation in temperature seem to have a direct effect on the distribution and abundance of animals in the Aba Roba area. This can be seen from the fact that following the rainy months when the vegetation cover is well developed and food is made available the number of the animals trapped increased and declined in the rest of the dry months(Table 2 and fig. 2).

Relatively very few mongooses and ground squirrels trapped compared to their obvious abundance in the field. This is because of the unsuitability of the traps to catch these animals. Moreover, the mongooses were difficult to trap since they are timid and easily alarmed by human presence. The hyraxes and the genet cats were caught by the peasants using local traps. The genet cats are very rare and the hyraxes occupy rock-cliffs which were difficult to reach.

As can be seen from Table 2, all the animals caught were not killed and examined. Some died on the way to Addis Ababa and some after arrival in the animal house. The long distance (615kms) from Aba Roba might have exhausted the animals and changes encountered in temperature, food and habitat in Addis might have been the cause for the death of some of the animals in captivity.

Wild animals in captivity often die of shock.

Generally, from the total number of animals surveyed in this study, rodents are the most abundant and are closely associated with man as village commensals next to his domestic animals (cattle for example). Domestic animals were not included other than dogs in this study (except few goats and sheep) since none have been recorded anywhere as reservoirs of VL. In this study the previous studies of Gemetchu and his associates, a total of 511 mammals were surveyed. This shows that a fair number of animals from the area have been examined surveyed or investigated.

Hundreds of rodents and other animals were examined at different times in Kenya (Heisch, 1953; Manson-Bahr and Southgate, 1964; Mutinga and Ngoka, 1983), in the Sudan (Hoogstraal et al., 1963) and to some extent in Ethiopia (Haile and Lemma, 1977) in the last 30 years. But all investigations were negative, in the sense that none of these previous workers were able to incriminate the natural host(s) of VL by isolating L donovani parasites from the animals they investigated. Such apparent absence of infection among wild-caught animals led some workers to think that VL in some areas of East Africa is anthroponosis as in that of India.

However, the isolation of L. donovani from rodents (Hoogstraal and Heitlein, 1964; Hoogstraal and Heyneman; 1969) and from Jackal (Sixl et al., 1987) in the Sudan, the revelation of this parasite in dogs in Kenya (Ngoka and Mutinga, 1978 and Mutinga et al., 1980) indicate VL may frequently be zoonotic. The demonstration of L. major from different rodents in Kenya

(Heisch, 1957; Heisch et al., 1959 Mutinga and Mogoka, 1983) and from Nile grass rats in Ethiopia (Haile and Lemma, 1977) further strengthened the assumption that VL is zoonotic in many parts of East Africa. The isolation of Leishmania parasites from ground squirrels in the present study would also support this assumption, if they are ultimately shown to be L. donovani. The possibility that the ground squirrel parasite is L. major (as in Kenya), however, can not at present be excluded.

The trypanosome-like flagellates recovered from the two ground squirrels in the present work, are not yet characterized or identified. But using characters such as the movement of the flagellates in culture and position of the Kinetoplast from their smear preparation, the parasites were designated as trypanosomes (Table 5). From morphological observations, cultural characteristic and preliminary data from DNA probes at the Armauer Hansen Research Institute (AHRI), Addis Ababa, the isolates from the third ground squirrel were identified as Leishmania sp. Culture was sent is sent to London by Dr Tamas of AHRI, for further confirmation and species identification. The flagellates designated as trypanosomes are cryopreserved in liquid nitrogen in the Institute of Pathobiology for future study and identification.

One has to give name at least, to the animal from which he has isolated a parasite. Thus, the attempt made to identify the mammals investigated in the course of this study is shown in Table 4 with the information on which such identification is based on. To clarify the matter further, the identification of the animals is based upon museum specimens (Natural History Museum, Addis Ababa). The distributional data shown in Yalden et al;, (1976) and Kingdon

(1974), of body measurements of the animals compared to the recorded values in Kingdon (1974). The cytogenetic method of identification based on karyotype analysis was made in collaboration with the Ethio- Soviet Biological Expedition team. This is used as the main basis of identifying the rodents in this study. I hope this attempt will initiate a further identification of the mammals in this area.

With respect to experimental inoculation of L. donovani parasites, I were selected a few representative species of rodents for this purpose. Some of these were elsewhere found naturally infected with L donovani (Acomy ssp., Hoogstraal and Dietlein, 1964; Hoogstraal and Heyneman, 1969; Rattus rattus, Hoogstraal et al., 1963; El-Adhami, 1976; Bettini et al., 1980; Arvicanthis niloticus, Hoogstraal and Heyneman, 1969 and infested with L. major (Tatera sp., Heisch et al., 1959; Chance et al. 1978). In addition, the Rattus and Arvicanthis sp were also found capable of supporting parasite replication in their visceral organs after experimental infection with L donovani parasites (Shatry et al, 1987; Gradoni et al., 1983 and Stauber et al, 1966). It is very difficult to state why the experimentally inoculated rodents in this study did not show infection. Probably insufficient infective forms were inoculated or the culture was too young and not yet in the late stationary phase, when infected forms are introduced. Either some of the rodents may not be susceptible to the parasite under investigation at all or as described in Stauber's experiment on gerbils, some of the rodents might have recovered and become immune to the infection (Stauber, 1958).

Some of the following, or all, could be possible reasons why more

Leishmania parasites were not detected in this study of the wild-caught animals are;-

i) The insensitivity of the technique due to scanty infection;

ii) Bacterial contamination, which inhibit leishmania growth;

[illegible] positive animals [illegible];

iii) The possibility of missing infected mammals, as the size of the traps and their low number (10 traps/trip), were inefficient for many mammals and insufficient to trap all the possible mammalian reservoirs of infection and

iv) As described by Stauber (1958) in an endemic area, all animals that are exposed may not be infected.

V. CONCLUSION AND RECOMMENDATIONS

It is not the first time Leishmania parasites were recovered from ground squirrels. Heisch (1957) isolated what later proved to be L. major from a ground squirrel using inoculation of hamsters. In the present study, Leishmania sp were isolated directly from X. rutilus using the NNN medium. It is also the first time that Leishmania was isolated from a rodent in the Aba Roba Peasant Association. Actually, this animal meets most of the criteria set out in Bray (1983) for a good reservoir host (see the introduction section). From my field experience the following can be said of this animal:

- it is found in large numbers and hence could be a good blood meal source for the suspected sandfly vectors;
- it has a good contact both with man and the suspected sandfly vectors, since it inhabits termite hills found near human habitations which are often inhabited by the probable sandfly vectors and
- it wanders through farm fields (I saw a squirrel stealing seeds of newly planted maize).

Further, the infection rate is 2.5% (one out of forty examined) in these animals. According to Bray (1983), in order to call an animal a reservoir host, the infection rate should be greater than 1%. Therefore, provided that the identity of the isolate finally [illegible] is known and be identical to the human isolate of L. donovani, we would only then be able to conclude that the ground squirrel (Xerus rutilus) is the natural host of VL in Aba Rob.

In general to resolve doubts and confusions further extensive study work

should be made on animal reservoirs in the Aba Roba focus. Highly refined laboratory and field techniques should be employed. The seasonal population dynamics of the suspected mammalian hosts should also be determined. The following are some points recommended to elucidate the status of mammals in the maintenance and circulation of VL in Southwest Ethiopia in general at the Aba Roba Peasant Association area in particular.

1. The most sensitive and refined techniques such as the DNA probe and monoclonal antibody techniques should be employed to the effect that even occult infections could be detected.
2. The use of different types and sizes of traps for both bigger and smaller mammals would guarantee effective trapping.

3. The laboratory protocol of animal sacrifice and examination should be made under strictly sterile conditions and experimental animals kept alive for a longer period (important to six months where possible).

4. The population dynamics and species composition of other possible mammalian hosts should be studied.

5. Studies should also be made to know which of the suspect animals in the area are strongly attractive to the probable sandfly vectors.
6. Blood meal analysis of the probable sandfly vectors is also important as it has some use in predicting the possible reservoir animals.

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Appendix 1

Climatological Data for Bekawele (Konso)
(Jan. 1987 - Dec. 1988)

·ıth	Mean Maximum Temperature (°C)		Mean Minimum Temperature (°C)		Rainfall in mm		Remark
	1987	1988	1987	1988	1987	1988	
ıary	29.4	30.3	17.5	18.9	21.5	18.8	
·uary	29.8	31.0	18.4	19.5	22.8	17.0	
:h	28.4	31.5	18.8	20.1	97.3	32.1	
:l	26.8	27.4	17.9	17.6	238.5	191.8	
	25.2	24.8	17.0	17.6	214.3	54.3	
	24.6	25.3	16.4	16.4	63.2	72.5	
	25.7	24.1	16.3	15.9	3.3	77.8	
ıst	27.0	25.9	16.3	16.8	9.4	50.4	
.mber	28.5	26.3	17.1	16.6	47.3	142.2	
ber	27.8	26.7	17.4	17.0	105.5	68.6	
mber	27.9	28.5	17.5	17.2	28.6	14.1	
mber	30.1	29.31	18.1	17.4	18.0	11.6	

urce - The National Meteorological Service, 1988)