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STUDIES ON OCCURRENCE, TRANSMISSION AND POTENTIAL MECHANICAL VECTORS OF CAMEL TRYPANOSOMIASIS IN NORTHERN KENYA

BY

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A Thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy in the University of Nairobi.

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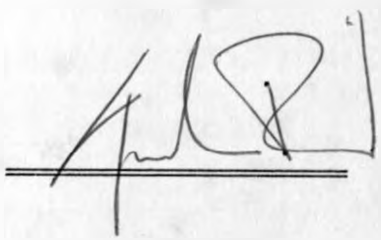
Signature 

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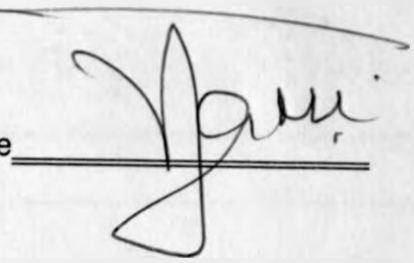
Signature 

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LIST OF ABBREVIATIONS.

E A T R O.. East African Trypanosomiasis Research Organization

F A O..... Food and Agriculture Organization of the United Nations.

I A E A.... International Centre for Insect Physiology and Ecology.

I L C A.... International Livestock Centre for Africa.

I P A L.... Integrated Project on Arid Lands.

I L R A D.. International Laboratory for Research on Animal Diseases.

K E T R I.. Kenya Trypanosomiasis Research Institute.

O A U..... Organization of African Unity.

U N E P.... United Nations Environmental Programme.

U N E S C O. United Nations Educational Scientific and Cultural Organization.

W H O..... World Health Organization of the United Nations.

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ABSTRACT

STUDIES ON OCCURRENCE, TRANSMISSION AND POTENTIAL MECHANICAL VECTORS OF CAMEL TRYPANOSOMIASIS IN NORTHERN KENYA.

Trypanosoma evansi ,Steele 1885 infection in northern Kenya camels was diagnosed by an enzyme-linked immunosorbent assay (ELISA), mouse inoculations and blood smears. The ELISA results indicated a current or past trypanosome prevalence rate that ranged from 73% to 95% in the sampled camel herds. The blood smear test revealed a maximum of 11.5% and a mean of 3.9% infection rates while the mouse inoculations revealed a maximim of 19.2% and a mean of 11.3% infection rate in the sampled herds. The mouse inoculation test (MIT) was found to be the most sensitive and suitable means of diagnosis of camel trypanosomiasis in northern Kenyan camels. Trypanosome infection rates were elevated during the wet season months as diagnosed by MIT. The mean trypanosome infection rate was 13.7+/-5% for the wet season and 4.55+/=2% for the dry season.

Absence of *Glossina* spp. in northen Kenya suggests mechanical transmission of trypanosomiasis in this region. Mechanical transmission is the mode of transmission whereby a parasite is transferred from one host to another without undergoing development within a vector. Mechanical transmission of camel trypanosomiasis within northern Kenya was ascertained by regular monitoring of 10 sentinel camels. Three of these animals became infected 7-9 months after introduction, in the absence of *Glossina* spp. *Glossina* spp. were not identified in the study area following monitoring by biconical traps.

Biting flies identified in the study area using sweep-nets and biconcal traps comprised of six tabanids, two hippoboscids and two muscids. Results of this study showed a seasonal abundance of these flies during and after the rains, which coincided with outbreaks of camel trypanosomiasis. This coupled with the fact that the major camel trypanosome *T.evansi* , is transmitted only mechanically, and infection of sentinel camels in the absence of *Glossina* spp., suppport and confirm the occurrence of mechanical transmission in nature.

Laboratory experiments showed that a tabanid (*Haematopota brunnescus,* Ricardo), *Stomoxys calcitrans* Linnaeus, and *Glossina morsitans morsitans* Westwood , could mechanially transmit *T. evansi* from infected to non-infected mice . Dissection and inoculation of fly mouthparts into mice revealed that these fly mouthparts could contain viable trypanosomes for several minutes after infective blood meals. Dissection of mouthparts of field collected *H.camelina, H.minuta.* and, *T.taeniola* after infective blood meals on camels revealed mean trypanosome infection rates of 14.5%, 23.3% and 27.4% respectively. Inoculation of triturated mouthparts of *H.camelina, H.minuta* and *T.taeniola* (following blood meals on infected camels) infected 6%, 12% and 53.3% of mice inoculated respecively. These dissection and inoculation experiments further support the concept of mechanical transmission of trypanosomes by haematophagous insects.

CHAPTER ONE

GENERAL INTRODUCTION, GENERAL LITERATURE REVIEW AND OBJECTIVES OF THE STUDY

1.0 : GENERAL INTRODUCTION

1.1: Overview of trypanosomiases with special reference to infection, particularly infection in camels.

Trypanosomiases are diseases resulting from infections by flagellate protozoans of the family Trypanosomatidae. These diseases afflict man, domestic livestock and wildlife. Thus trypanosomiases are one of the most important medical and veterinary problems that have had an impact upon the economic and agricultural developments of tropical Africa, as reported by numerous workers including Curasson (1943); Hornby (1952); Buxton (1953); Nash (1960) Willet (1970); Finelle (1974) and Jordan (1986). The disease currently posses serious problems to the development of the livestock industry in Africa. It is estimated that about 10 million square kilometres of land which could support 125 million head of cattle are greatly restricted for such usage due to the presence of trypanosomiases (Harwood and James, 1979). In trypanosomiasis endemic areas, infections in cattle results in poor milk and meat yields, abortions and death (Richard, 1979; Godfrey and Killick-Kendrick, 1963; Wilson *et al.*, 1981; Field, 1985; Olaho *et al.*, 1987 and Jordan, 1986).

Trypanosomiases in cattle is commonly known as nagana. It should be noted however that the same trypanosomes that cause nagana in cattle can cause fatal diseases to other domestic animals (Harwood and James, 1979). Trypanosomiasis due to infection by *Trypanosoma evansi* is referred to as surra. This disease is widespread in Africa as well as outside Africa. Although surra affects many domestic animals such as cattle, sheep, goats, horses, donkeys and dogs, (Chand and Singh, 1971; Khasanov and Ivanitskav, 1974; Ilemobade, 1971; Malik and Mahmoud, 1978), it predominantly affects camels (Gatt-Rutter, 1967). The distribution of surra outside tsetse (*Glossina* spp.) belts is unusual because most trypanosomes in Africa are cyclically transmitted by *Glossina* spp. vectors. *T. evansi* on the other hand, is not cyclically transmitted by *Glossina* spp. as it has been demonstrated (Kurnett and Krausse, 1934; Mukiria ,1980) not to develop in *Glossina* spp. It is therefore speculated that *T. evansi* is mechanically transmitted by haematophagous (blood-sucking) insects such as members of the families Hippoboscidae (e.g. *Hippobosca* spp.), Tabanidae (e.g *Tabanus* spp.) and Muscidae (e.g. *Stomoxys* spp. and *Haematobia* spp.). The role of *Glossina* spp in the mechanical transmission of *T. evansi* is poorly understood. Soft ticks (*Ornithodoros* spp.) have also been incriminated as possible mechanical vectors of *T. evansi*, but all these have not yet been confirmed (Gitatha, 1981).

Trypanosomiasis due to *T. evansi* has been reported to be a major constraint in camel production in Africa as well as other parts of the world such as Asia (Luckins, 1988), South America, West Indies, Panama and Mauritius (Moutia, 1928b; Lucas, 1955; Hoare, 1972; Wells, 1982). According to reports by Gitatha (1981), Wilson *et al.* (1984), Olaho *et al.*

(1987) and Rutagwenda (1982,1985), trypanosomiasis presumably due to *T. evansi* is a major constraint to camel production in Kenya.

The Kenyan camel population is concentrated in the semi-arid region of northern Kenya.These camel keeping areas are inhabited by nomadic pastoralists including the Rendille, Samburu, Gabra and Borana. Camels form the mainstay of these pastoralists lifestyle; camel milk and meat is a source of protein, and in addition, camel milk, meat and hides can be sold for cash or exchanged for other goods. Camels are the major transport animals in these areas and may well remain as the only means of transport in this region for a long time. As a result of the significant growth in world camel production and the current increase in desertification, the economic importance of camel health should be recognized. Trypanosomiasis due to *T.evansi*, which affects camels, has had less scientific attention than the *Glossina* spp.-transmitted trypanosomiases. In view of reported outbreaks of camel trypanosomiasis worldwide, coupled with the fact that the mode of transmission of camel trypanosomiasis is not clearly understood, there is a clear need to intensify research on this disease. Questions relating to camel trypanosomiasis that need to be investigated include the following:

1. Epidemiology of camel trypanosomiasis; results of epidemiological studies would lead to better understanding of trypanosome species that are involved. Knowledge of peak periods when camels are challenged most by trypanosomes can be useful to control campaigns and targeting of chemotherapy at the most appropriate time.

2. Diagnosis of camel trypanosomiasis; there is need to develop existing methods for diagnosing camel trypanosomiasis. Suitably sensitive tests require to be refined and adapted for field application.

3. Investigations on the mode(s) of transmission of camel trypanosomiasis.
4. Identification of the potential mechanical vectors of camel trypanosomiasis.
5. Investigation on the ecology and biology of the potential vectors of camel trypanosomiasis.

Research on the above listed areas are important as they have a direct bearing on present and future control programmes directed against camel trypanosomiasis. Control measures aimed at eliminating the parasite currently include chemotherapy (Jordan, 1986), use of trypanotolerant animals (Murray *et al.* 1979; I.L.C.A., 1979) and recently, research has been intensified in the field of immunocontrol to produce a vaccine (Vickerman, 1978 ; Turner, 1982) for trypanosomiases, although research in this area remains inconclusive. Chemotherapy, although the most widely used method of control of animal trypanosomiases, has drawbacks such as drug resistance (Gray and Roberts, 1971), toxicity of some drugs to certain animals, (Jordan, 1986) as well as the high cost of the majority of drugs to small scale farmers (Jahnke, 1976). As a result of the persistent problems of animal trypanosomiases in Africa, research on the diseases and entomological surveys on its vectors has drawn worldwide attention. This is exemplified by current research activities of international organizations such as International Centre for Insect Physiology and Ecology (I.C.I.P.E.), International Laboratory for Research on Animal Diseases (I.L.R.A.D.), World Health Organization (W.H.O.), International Livestock Centre for Africa (I.L.C.A.), International Atomic Energy Agency (I.A.E.A.), United Nations Environmental Programme (U.N.E.P.), and national governmental institutes such as

Kenya Trypanosomiasis Research Institute (K.E.T.R.I.) For as lon g as trypanosomiasis persists, research on epidemiology, transmission, and control has to continue. In this respect, entomological surveys on trypanosomiasis vectors which forms part of this study, are important as they can facilitate interpretations of epizootiology and epidemiology of the disease. Questions concerning camel trypanosomiasis in northern Kenya such as its mode of transmission and potential mechanical vectors have therefore been tackled in this study.

1.2: LITERATURE REVIEW

1:2:1: Diversity of trypanosomes with respect to their modes of transmission.

There are about 125 species of mammalian trypanosomes. These affect man, primates, rodents, bats, reptiles and many other animals. A detailed classification of mammalian trypanosomes is depicted on Fig. 1 below. Different species of trypanosomes undergo one of the two different modes of transmission broadly referred to as cyclical and mechanical transmission of trypanosomes.

1.2.1.1: Cyclical transmission of trypanosomes:

The major and best understood mode of transmission of trypanosomes is cyclical by *Glossina* spp. in tropical Africa and by reduviid bugs (*Rhodnius* spp, *Triatoma* spp. etc.) in Central and South America. In cyclical transmission of trypanosomes, the insect vector picks the parasite from the infected vertebrate host via a blood meal. These parasites then undergo a series of morphological and biochemical changes within the vector to form the infective metacyclic trypanosomes (Noble and Noble,

KINGDOM----------(PROTISTA) Levine et al., 1980.

SUBKINGDOM----------(PROTOZOA) Levine et al., 1980.

PHYLUM---------(SARCOMASTIGIPHORA) Levine et al., 1980.

SUBPHYLUM----------(MASTIGOPHORA) Levine et al., 1980.

CLASS----------(ZOOMASTIGOPHORA) Calkins, 1909.

ORDER----------(KINETOPLASTIDA) Honigberg et al., 1963.

SUBORDER--------(TRYPANOAOMASTINA) Kent, 1880.

FAMILY----------(TRYPANOAOMATIDAE) Doflein, 1901, Grobben, 1905.

GENUS----------Trypanosoma Gruby, 1843.

.....SECTION.....

SALVARIA

SUBGENERA

STERCORARIA

SUBGENERA

PCYNOMONAS
Hoare, 1964

T. suis.

NANNOMONAS
Hoare, 1964.

T.congolense.
T. simiae.

DUTTONELLA
Chalmers, 1913.

T. vivax.

TRYPANOZOON
Lühe, 1906.

T.brucei brucei.
T.brucei gambiense.
T.brucei rhodesiense.
T.evansi.
T.equiperdum.

HERPETOSOMA
Doflein, 1909.
T.lewisi
T.rangeli.

MEGATRYPANUM
Hoare, 1964.
T. theileri.

SCIZOTRYPANUM
Chagas, 1909
T. cruzi.

Fig.1 :Classification of mammalian trypanosomes after Hoare, 1972 and Levine et al. 1980.

1976; Harwood and James, 1979; Hoare, 1972). The infective metacyclic trypanosomes gain entry into the susceptible vertebrate host either through the salivarian route (i.e. transmission is inoculative via the bite) or stercorarian route (i.e. transmission is contaminative via the faeces).

The insect vector of salivarian trypanosomes is *Glossina* spp.except in *T. equiperdum* which is transmitted sexually and *T. evansi* which is transmitted mechanically. The insect bites the vertebrate host and the parasites develop in the insect intestine to form epimastigotes and trypomastigotes. After about two weeks depending on prevailing temperatures, these enter the salivary glands where they develop into infective metacyclic trypamastigotes. These gain entry into the vertebrate host through a bite by the insect vector (Noble and Noble, 1976).

Insect vectors of stercorarian trypanosomes are reduviid bugs. These trypanosomes enter the posterior part of the insect gut, after having been ingested in the blood meal. The parasites develop to give rise to infective metacyclic trypamastigotes in about two weeks. The insect vector usually defecates while blood-feeding, and thus deposits the infective metacyclic forms on the vertebrate hosts skin. Often the irritation produced causes the host to rub the bitten area; so there is considerable opportunity for the parasites to be rubbed into the wound made by the feeding insect. Other modes of infection other than via abraded skin are through ocular and oral mucosae. Man and other animals also get infected by eating infected bugs, bug faeces or eating other infected animals (Noble and Noble, 1979).

Salivarian trypanosomes, cyclically transmitted by *Glossina* spp. in tropical Africa include *T.brucei brucei* Plimmer and Bradford 1899, *T. brucei gambiense* Dutton 1902. *T.brucei rhodesiense* Stephens and Fantham, *T. congolense* Broden 1904, *T. simiae* Bruce *et al.*, *T. suis* Ochman 1905, and *T. vivax* Ziemann. With the exception of *T. b. gambiense* and *T.b. rhodesiense*, which cause human trypanosomiasis (FAO, 1979; Ford, 1972), all these trypanosomes cause animal trypanosomiasis in tropical Africa (Pollock, 1980; Woo, 1977; Buxton, 1955). Salivarian trypanosomes that occur within as well as outside tropical Africa include *T. vivax, T. evansi* and *T. equiperdum. T. vivax* and *T. evansi* cause animal trypanosomiasis and are believed to be transmitted mechanically outside Africa (Lucas, 1955; Wells, 1980; Moutia, 1928b. Hoare, 1972) by haematophagous insects other than *Glossina* spp., because there are no alternative cyclical vectors outside Africa in the absence of *Glossina* spp. A cyclical vector is a vector in which a parasite undergoes a cycle of development before it is transmitted to the vertebrate host. *T. equiperdum* causes trypanosomiasis in horses (dourine) worldwide and is transmitted sexually (Hoare, 1972). Stercorarian trypanosomes include *T. theileri* Laveran 1902, *T. lewisi* Kent 1880 *T. rangeli* Tejera 1920 and *T. cruzi* Chagas 1909. *T. theileri* is a parasite of domestic animals with a worldwide distribution and cyclically transmitted by tabanids (Harwood and James, 1979; Noble and Noble, 1976). *T.lewisi* is a cosmopolitan parasite of rats cyclically transmitted by the rat flea, *Ceratophylus fasciatus* (Noble and Noble, 1976). *T.rangeli* (*T.ariari*) occurs in man, monkeys, dogs and opossums in Central, and South America, and the insect vector is the reduviid bug,

Rhodnius prolixus (Noble and Noble, 1976). Most Stecorarian trypanosomes are non pathogenic except *T. cruzi.* , which causes Chagas disease in man and domestic animals in South America. Vectors of *T. cruzi* are reduviid bugs notably *Rhodnius prolixus, Triatoma infestans, T. dimidiata* and *Panstrongylus megistus. T. cruzi* organisms gain entry into the vertebrate host during the process of a blood meal. Infective forms of *T. cruzi* are subsequently present in the faeces of the insect vector(s). Defecation by the insect vector(s) takes place during blood-feeding on the vertebrate host thereby making contamination of the vertebrate host (by trypanosomes in the faeces) possible. Contamination occurs through wounds produced by the insect vector on the hosts skin during the blood meal or through any other wounds present on the hosts skin (Harwood and James, 1979; Noble and Noble, 1976; Hoare, 1972).

1.2.1.2: Mechanical transmission of trypanosomes:

Mechanical transmission of trypanosomes is a mode of transmission whereby parasites are transferred from one host to another without undergoing any development or multiplication in the vector. Mechanical transmission of trypanosomes can take place in various ways including:-

(a) during the process of castration of animals;

Mechanical transmission can occur if trypanosomes survive in the blood that remains on the equipment, thereby making the equipment contaminative or infective. Subsequent use of such equipment may cause infection to spread to previously uninfected animals.

(b) during the process of bleeding animals;

If a needle used to inject or to draw blood from infected animals is immediately applied to another animal in a herd before sterilization or

cleaning, trypanosomes that survive in the needle may be introduced into the blood stream of the second animal (Oldroyd, 1954; Adam *et al.*, 1971).

(c) intrauterine transmission of trypanosomes;

Intrauterine infection by trypanosomes is another mode by which trypanosomes can be mechanically transmitted. Trypanosomes do not undergo any development in the host but simply penetrate uterine membranes of the infected pregnant animal and gain access into the foetus (Lucas, 1955). Prenatal infection is likely to occur when maternal blood mix with foetal blood due to damages of foetal membranes or at the time of birth.

(d) sexual transmission of trypanosomes;

Trypanosomes may penetrate membranes from infected to non-infected animals during copulation. The only sexually transmitted trypanosome, *T. equiperdum* is therefore also mechanically transmitted.

(e) infection via food chain;

Viable trypanosomes in the blood of an infected animal may be ingested by a carnivore. Such trypanosomes may subsequently penetrate the mucous membranes of the carnivore and finally get into the blood to cause an infection (Woo, 1977; Harwood and James, 1979; Hornby, 1952; Sachs, *et.al.,* 1967). According to reports by Woo (1977), dogs, wolves and foxes have been found to become infected by eating freshly killed trypanosome-infected animals.

(f) mechanical transmission of trypanosomes by insect vectors;

The most important mechanical vectors of trypanosomes are haematophagous insects. This type of mechanical transmission of

trypanosomes occurs when an insect feeding on a trypanosome infected animal is interrupted so that it immediately goes to feed on another animal which may or may not be infected by trypanosomes. The insect vector thus carries the trypanosomes that survive on proboscis or other parts of its body to the next host, into whose body the trypanosomes enter through a bite or wound (Oldroyd, 1954). All blood-sucking insects including *Glossina* spp. (Jordan, 1986) are therefore potential mechanical vectors of trypanosomes whereby the proboscis may be likened to a syringe which has not been sterilized before application to the second patient (Adam *et al*., 1971).

Trypanosome species which are believed to undergo mechanical transmission by haematophagous flies in nature are primarily *T. evansi* and *T.vivax*. All *Glossina* spp.- borne trypanosomes may in some cases be transmitted mechanically (Harwood and James, 1976). This can take place when an infected animal or man is in close proximity to a susceptible host (man or animal) and when biting flies including *Glossina* spp. are numerous and actively biting these hosts.

In mechanicaltransmission of trypanosomes , the important point is how much blood containing parasites the insect carries on its proboscis, other body parts after blood-feeding on an infected animal (Oldroyd, 1954). Factors which may determine mechanical transmission of trypanosomes by insects are therefore ;

(1) Longevity (survival) of trypanosomes on the fly mouthparts;

The longer the parasites can stay alive on the fly mouthparts, the greater are the chances of mechanical transmission occurring (Whitnall,

1934; Taylor, 1930; Oldroyd, 1954; Luckins, 1988; Foil,1989). The survival time of trypanosomes in a vector has been reported to be influenced by the mammalian host (Dixon *et al.*, 1971) or the strain of trypanosome (Taylor, 1930; Lucas, 1955; Nieschulz, 1926, 1930, 1941). Dixon *et al.*, (1971) reported that the survival time of *T.brucei* appeared to be influenced by the donor hosts. Nieschulz (1939), found that differences in donor capacity were due to varying degrees of parasitaemia that each animal experiences. For instance more trypanosomes can be picked from the rat which experiences an intense parasitaemia. Theoretically a single trypanosome can result in subsequent infection but obviously if a large number of parasites are picked (Cross, 1923; Whitnall, 1934; Luckins, 1988), the potential for the infection to become established is greater. Taylor (1930) reported that there appeared to be a difference in transmissibility of three strains of *T.brucei* that he used, but Mwambu (1969) found no significant difference in the transmissibility between the two strains of *T.congolense*.

(2) Time interval between the infective and infecting blood meals; If a fly transfers rapidly from one host to another the potential for mechanical transmission is greater. According to a report by Leclerq (1952), the probability (P) of mechanical transmission declined rapidly from 0.05 within 5 minutes of an infective feed to 0.004, 0.003, 0.001 and 0.0003 after 1 hr., 3 hrs., 6 hrs., and 24 hrs. respectively.

(3) Abundance of haematophagous flies; The chances of mechanical transmission of trypanosomes increase with the number of biting flies attacking both donor and recipient animals at any given time. There are

numerous reports (Moutia, 1928; Gruvel and Balis, 1965; Cazalboui, 1905; Yagi and Razig, 1972a, 1972b; Razig and Yagi, 1975; Mohamed, 1983; Krug, 1971; Kirby, 1962; Roeder *et al.*, 1984; Luckins, 1988) of positive correlations between increased biting fly numbers and seasonal outbreaks of animal trypanosomiasis in the absence of *Glossina* spp. Reports by Foil (1987,1989) suggest that large numbers of biting flies are required to effect mechanical transmission of trypanosomes.

(4) Nature of the fly mouthparts; It has been reported (Oldroyd, 1954) that the larger and fleshier the labella, the greater the chances of mechanical transmission. This is probably because a larger mouthpart provides a larger surface area that may retain more parasites. In tabanids, when the fascicle (the parts comprising labrum, mandibles and maxillae) is withdrawn from the host, the labellae come together to trap a film of blood. This facilitates mechanical transmission by protecting the enclosed blood against the effects of drying (Luckins, 1988). Although it appears that a large quantity of blood may be necessary for successful mechanical transmission, according to Nieschulz (1926,1941), flies were able to transmit *T.evansi* when only small amounts of blood (0.01ul) were retained on the proboscis, several hours after the infective feed.

(5) Interrupted blood-feeding behaviour; The nuisance caused to hosts by tabanids and other biting flies ensures that the feeding is interrupted and this increases the chances of mechanical transmission. Nieschulz (1927), reported that biting flies, subjected to interrupted blood-feeding on a *T.evansi*-infected host were able to transmit the parasite to two other animal hosts during subsequent feeds.

(6) Herding regime; When animals are herded close together with some of them infected the chances of mechanical transmission are greater. This is because flies dislodged from the donor host do not take long to seek the next host on which to complete their blood meal but immediately probe on the nearest available host (Wells,1982; Luckins,1988).

(7) climatic factors;

There are no reports on the influence of climatic factors (temperature, humidity, wind, dust etc.) on survival of trypanosomes in fly mouthparts. These factors probably also influence survival of trypanosomes in fly mouthparts.

Although the occurrence and spread of the African trypanosomiasis is primarily by cyclical transmission by *Glossina* spp., mechanical transmission may also be important than previously realized due to the following facts:

There are certain trypanosomes such as *T.evansi* which are only transmitted mechanically. According to Hoare (1972), Mukiria (1980), Kurnett and Krausse (1934), there is no evidence to suggest that *T. evansi* is capable of cyclical development in *Tabanus* spp. or *Glossina* spp. Absence of genes needed for formation of the mitochondrion (Giron *et al.*, 1984 ,cited by Luckins,1988), the prerequisite for cyclical development in other members of the *brucei* group would seem to preclude this.

Outbreaks of *T.vivax*, and *T.evansi* have been reported in Africa in the apparent absence of *Glossina* spp. (Wells, 1972). Such instances, although only circumstantial, suggest that mechanical transmission of

trypanosomes may be more significant in nature than previously realized.

1.2.1.3: Importance of mechanical transmission of trypanosomes and current problems.

The concept of mechanical transmission of trypanosomes is not new as it was demonstrated as far back as 1895 by Bruce. With the discovery of cyclical transmission of *T.brucei* by Kleine (1909) the concept of mechanical transmission of trypanosomes became controversial. None the less, discussions on mechanical transmission of trypanosomes have continued up to now. The role of haematophagous Diptera in mechanical transmission and resulting epidemiological parameters continued to be controversial from 1950 onwards to an extent that W.H.O. expert committee stated in its 1962 report that - "apart from the problems of cyclical transmission there is need for work on mechanical transmission; to what extent and under what circumstances it occurs and to define the species of the biting flies responsible".

Studies of mechanical transmission of trypanosomes have not been encouraging due to a number of problems; one such problem in Africa has been the difficulty in selecting suitable experimental areas which are absolutely *Glossina* spp. free.

1.2.1.4 : Mechanical transmission of T.evansi and T.vivax and other trypanosomes by insects in nature based on circumstantial evidence.

The occurrence of *T.evansi* and *T.vivax* in *Glossina* spp.-free areas in Africa as well as outside Africa (Mauritius, Panama, West Indies, Brazil, Colombia, Venezuela and Mexico) suggests mechanical transmission of

these trypanosomes by haematophagous flies other than *Glossina* spp. Authors including Lucas (1955), Hoare (1972), Wells *et al.* (1970) and Wells (1982) have reported that transmission of *T.evansi* and *T. vivax* in *Glossina* spp. free areas depend on *Stomoxys* spp., tabanid flies, hippoboscid flies and other haematophagous flies. According to reviews by these authors, *T. evansi* and *T. vivax* have adapted to mechanical transmission in the absence of *Glossina* spp. Outbreaks of *T. evansi* and *T. vivax* outside Africa have quite often coincided with seasonal increases in populations of tabanid flies and *Stomoxys* spp. For instance, Moutia (1928a), reported outbreaks of *T. evansi* infection in domestic animals which coincided with seasonal increase of *Stomoxys* spp. in Mauritius. According to Moutia (1928b), the major suspect vector of *T. evansi* in Mauritius was *Stomoxys nigra* Macquart.

In Africa, epidemiological reports on *T. evansi* and *T.vivax* implicating mechanical transmission of the trypanosome by biting flies other than *Glossina* spp. are those by Cazalboui (1905), Gruvel and Balis (1965), Yagi and Razig (1972a, 1972b), Razig and Yagi (1975), Mohamed (1983), Jordan (1986) and Roeder *et al.* (1984).

Gruvel and Balis (1965) reported that *T. evansi* outbreaks in Chad coincided with seasonal increases in tabanids. According to reports by Cazalboui (1905), tabanids (*Tabanus dorsivitta* Walter and *T. unimaculatus* Macquart), and *Stomoxys* spp. were quite numerous during an outbreak of *T. evansi* infection in domestic livestock in Macina, Sudan. During another outbreak of *T. evansi* infection in camels in Sudan, *Tabanus taeniola* Paliost de Beauvios., *Atyolotus agrestis* Wiedemann, *Ancala fuscipes* Ricardo, *Ancala latipes* Macquart, and *Philoliche magretti* Bezzi were numerous in the vicinity of camels according to

reports by Yagi and Razig (1972a, 1972b) and Razig and Yagi (1975). These authors suggested that the above mentioned biting flies were the most likely mechanical vectors of camel trypanosomiasis in the region. Mohamed (1983), reported that outbreaks of *T.evansi* in Somalia coincided with the seasonal increase in tabanid flies and that the tabanids were possible mechanical vectors of camel trypanosomiasis in Somalia. Roeder *et al.*, (1984), reported an outbreak of acute cattle trypanosomiasis due to *T. vivax* infection in Ethiopia some 400 Kms away from the nearest known focus of *Glossina* spp. and considered that while the disease was probably brought by cattle, which acquired the disease elsewhere from *Glossina* spp., transmission within the herd was via the mechanical route by haematophagous Diptera.

In Kenya (Marsabit District), camel trypanosomiasis probably due to *T.evansi* has been reported by Wilson *et al.* (1984), and Gitatha (1981). The mode of transmission of camel trypanosomiasis in northern Kenya is poorly understood. It is speculated that biting flies such as *Stomoxys* sp., tabanids, *Lyperosia* (=*Haematobia*), hippoboscids and even soft ticks (*Ornithodoros* sp.) could be mechanical vectors of the trypanosome in camels (Gitatha, 1981).

Although *T.vivax* can be cyclically transmitted by *Glossina* spp. in tropical Africa, authors such as Folkers and Jones-Davies (1966), Ford and Clifford (1968) and Ford (1964) have shown that the ratio of *T.vivax* to *T.congolense* in cattle increases with the distance away from the *Glossina* spp. belts. Based on such data these authors have put forward the hypothesis that *T.vivax* is probably mechanically transmitted by haematophagous flies other than *Glossina* spp. inside as well as outside the *Glossina* spp. belts.

In Nigeria, reports by Kirby (1963) implicated mechanical transmission of trypanosomiasis by biting flies other than *Glossina* spp. He reported cattle trypanosomiasis in the Shika region of Nigeria, an area definitely free of *Glossina* spp. due, to control programs by Tsetse and Trypanosomiasis Division of the Ministry of Animal and Forest Resources. The notion of mechanical transmission of trypanosomes in the absence of *Glossina* spp. has been further supported by Krug (1971), who also reported *T.vivax* in the region in the absence of *Glossina* spp. According to Krug (1971), tabanids were numerous in the vicinity of cattle during the outbreak of *T.vivax* infection in the region.

A survey of literature has provided references that implicate mechanical transmission of trypanosomes other than *T.evansi* and *T. vivax*. Duke (1919), reported that the epidemic of sleeping sickness (*T. b. brucei*) that swept over Uganda at the end of the 19th Century was largely due to mechanical transmission from man to man by *Glossina palpalis*. Thereafter outbreaks of animal trypanosomiasis have been reported in the apparent absence of *Glossina* spp., implying mechanical transmission of various trypanosome species by other haematophagous flies. Some of these reports are as follows:

In Rhodesia, Jones (1915), Owens (1914), Rupert *et al.* (1917) and Hornby (1912), reported incidences of animal trypanosomiasis due to *T. congolense* in the absence of *Glossina* spp. Tabanids and *Stomoxys* spp. were abundant during these outbreaks and could have played the role of mechanically transmitting the parasite. Similar outbreaks of *T. congolense* in cattle were reported also in a *Glossina*-free region (Evans, 1948; 1950; Buxton, 1955) of southern Sudan. During these outbreaks, tabanids, particularly *T.taeniola, Lyperosia* sp. and *Stomoxys* sp. were

numerous. These reports indicate the possibility of mechanical transmission of trypanosomiasis.

Animal trypanosomiasis due to *T.vivax* and *T.congolense* was reported by Barnet (1947) at a Ruiru farm, Kenya (presumably *Glossina*-free), during which *Stomoxys* spp. was exceedingly numerous on the grazing cattle. *Stomoxys* spp. may have played a role of mechanically transmitting the trypanosomes during this outbreak.

The above reports point to the possibilities of mechanical transmission of trypanosomes by haematophagous flies in nature. It is quite possible that in *Glossina* spp.free areas of Africa and outside Africa, haematophagous flies such as tabanids and *Stomoxys* spp. mechanically transmit trypanosomiasis. Mechanical transmission of trypanosomes is also possible in *Glossina* spp.endemic areas. In such areas, it is possible that the widespread transmission between different species of animals or different herds is initiated by *Glossina* spp.via cyclical transmission while mechanical transmission by other haematophagous flies as well as by *Glossina* spp., probably takes place within a herd. These other haematophagous flies could be auxiliary or amplifying mechanical vectors that aggravate trypanosomiasis that has been initiated by *Glossina* spp. within a herd (Jordan, 1986).

Other workers who have reported mechanical transmission of trypanosomes include Harwood and James (1979), Jordan (1986), Riordam (1972) and Luckins (1988). Tabanids, especially members of the genus *Tabanus*, particularly appear to be the most likely efficient mechanical vectors of trypanosomes. Many other species of biting flies including *Stomoxys* spp. and even *Glossina* spp. (Jordan, 1986) are also possible mechanical vectors of trypanosomes but all these reports are

based on circumstantial evidence and need further investigation.

Literature on the subject of mechanical transmission of trypanosomes is vast but inconclusive, circumstantial, and lacks hard experimental evidence hence the need for further research on the subject. Mechanical transmission of trypanosomiasis has not been conclusively researched due to limitations which include the following:

1. The suspected mechanical vectors such as *Stomoxys* spp. tabanids and other biting flies are seasonal, (Wiessenhutter,1975; Dixon *et al.*; 1971 Okiwelu,1975), thereby not allowing all year-round investigation of their involvement in mechanical transmission of trypanosomiasis. Studies involving these flies can therefore be undertaken only during certain seasons when flies are available.

2. Cases of animal trypanosomiasis are usually promptly treated by livestock owners thereby interfering with experimental mechanical transmission studies.

3. Cyclical transmission by *Glossina* spp. may interfere with studies on mechanical transmission of trypanosomiasis in tropical Africa where *Glossina* spp. are widespread. Mechanical transmission studies of trypanosomiasis can only be undertaken in areas known to be absolutely *Glossina* spp.-free, and yet where trypanosomiasis also occurs. Such areas are rare in tropical Africa except for few isolated foci such as the arid camel keeping regions which are unfavourable to *Glossina* spp. existence (Jordan, 1986). Low densities of *Glossina* spp. may remain undetected thus complicating the subject further.

The above reports imply that *T.evansi, T.vivax* and other trypanosome species are probably mechanically transmitted when they occur in the absence as well as in the presence of *Glossina* spp. These

observations are however not conclusive as they are based on circumstantial evidence. The mode of transmission of *T. evansi* and *T. vivax* in the absence of *Glossina* spp. is therefore not clearly understood but is presumed to be mechanical by haematophagous insects (Hoare, 1972; Harwood and James, 1979; Jordan, 1986; Luckins, 1988). It is therefore necessary to investigate the mode(s) of transmission of these trypanosomes particularly with *T.evansi* which does not develop cyclically in the *Glossina* spp. vector (Kurnett and Krausse, 1934; Mukiria, 1980).

1.2.2 : Typanosoma evansi

T.evansi was the first pathogenic trypanosome shown to be pathogenic to animals. Griffith (1880) first detected *T.evansi* in the blood of equines and camels in India. Steele (1885) reported an outbreak due to *T.evansi* infection in mules in Burma. The disease due to *T.evansi* was later described by Yakimoff (1923, 1927) in Russia.

Since these early discoveries of *T.evansi* infection in camels, the disease has been reported under various names in different parts of the world affecting a cross-section of mammalian hosts. Thirty three different names ascribed to *T.evansi* include the following;

T.equinum causing "mal de caderas" in South America; *T.hippicum* causing "murrina" in Panama; *T.venezuelenses* causing "derrengadera" in Venezuela; *T.ninaekohlyakimovi* causing "suauru" in Russia, *T.soudanense* causing "el debab" in Algeria, Egypt and North Africa and *T.cameli*. The disease is known as "mbori" in Sudan, "aino" in Somalia and "gafur" in arabic. In Kenya the disease is known as "saar", "omar" or "gandi" amongst the pastoralists (Woo, 1977; Mahmoud and Gray, 1980; Gatt-Rutter, 1967; Omara Opyene, 1986). However, the widely accepted name

for the disease caused by *T.evansi* is "surra". The principle hosts of *T.evansi* are dromedary camels, equines and dogs but the infection has been reported in bovines and other domestic animals (Hoare, 1972; Woo, 1977).

T.evansi epidemics tend to involve different animal hosts in different parts of the world. In Indochina, horses are mainly affected, followed by camels, bovines and buffaloes, whereas in USSR and Middle Asia, the main hosts are camels and to a lesser extent horses. In Africa, (Somalia, Kenya, Ethiopia, Sudan, Chad, Nigeria, and French West Africa), camels are affected most. In Central and South America, horses are the main hosts followed by cattle. Natural infections of *T.evansi* have also been reported in mules and donkeys. Sheep, goats, cattle and donkeys may act as reservoir hosts (Ilemobade, 1971b; Khasanov and Ivanitskav, 1974; Malik and Mahmoud, 1978; Chand and Singh, 1971).

Although camels are largely kept far apart from other animals, none the less, they do come into contact with donkeys, goats, sheep and horses in some countries such as Kenya and Sudan. The existence of reservoir ("carrier") animals in the vicinity of susceptible camels may further facilitate transmission by biting flies (Mahmoud and Gray, 1980).

The clinical signs of *T.evansi* infections are similar to those of other animal trypanosomiases, and are characterized by anaemia, fever, oedema and cachexia, and enlargement of lymph nodes and spleen. In some cases, the liver and kidneys may also enlarge (Hoare, 1956, 1972). Secondary complications affecting the respiratory, gastro-intestinal and nervous system may occur during acute infection (Gatt-Rutter, 1967). The course of *T.evansi* infections may be acute or chronic leading to death or it may be mild and symptomless. Animals most severely affected are

camels horses and dogs. Bovines and equines are also affected but they could be latent carriers of the disease (Hoare, 1956, 1972).

In camels that are infected, trypanosomes can be detected in the blood a week after infection but clinical signs do not become obvious until a month later. The coat becomes rough and hairless, the animal loses appetite and shows progressive weakness and incoordination. Death may occur in a few weeks, months or even several years after infection (Mohamed, 1984).

1.2.3: Geographical distribution of T.evansi

T.evansi is one of the most widely distributed trypanosomes and is found in countries with warm climates and semi arid regions (Mukiria, 1980). Figure 2 is a diagrammatic representation of the geographical distribution of *T.evansi* infection (surra) in animals, after Hoare (1972).

In the old world, *T.evansi* extends in longitude from about 15°W to 125°E. In Africa, surra is prevalent in Morocco, Algeria, Tunisia, Chad, Libya, Mali, Egypt, Senegal, Sudan, Somali and northern Kenya. *T. evansi* infection has also been reported outside Africa in places such as Israel, Lebanon, Syria, Arabic Peninsula, Asia minor, Iran and Iraq. In Europe, the Transvolga region (except Turkey and Bulgaria) is an area chiefly affected by *T.evansi* infection (su-aura) in animals (Hoare, 1972). *T.evansi* also extends into Burma, Malaya, Indonesia, Mauritius, Philippines, Southern China and Vietnam. *T.evansi* has also been reported to be prevalent in Mexico, Central America, Venezuela, Brazil, and Colombia (Hoare, 1972; Woo, 1977).

The distribution of *T.evansi* overlaps with the distribution of its major host, the camel (Fig.2). *T.evansi* is found outside the area of distribution

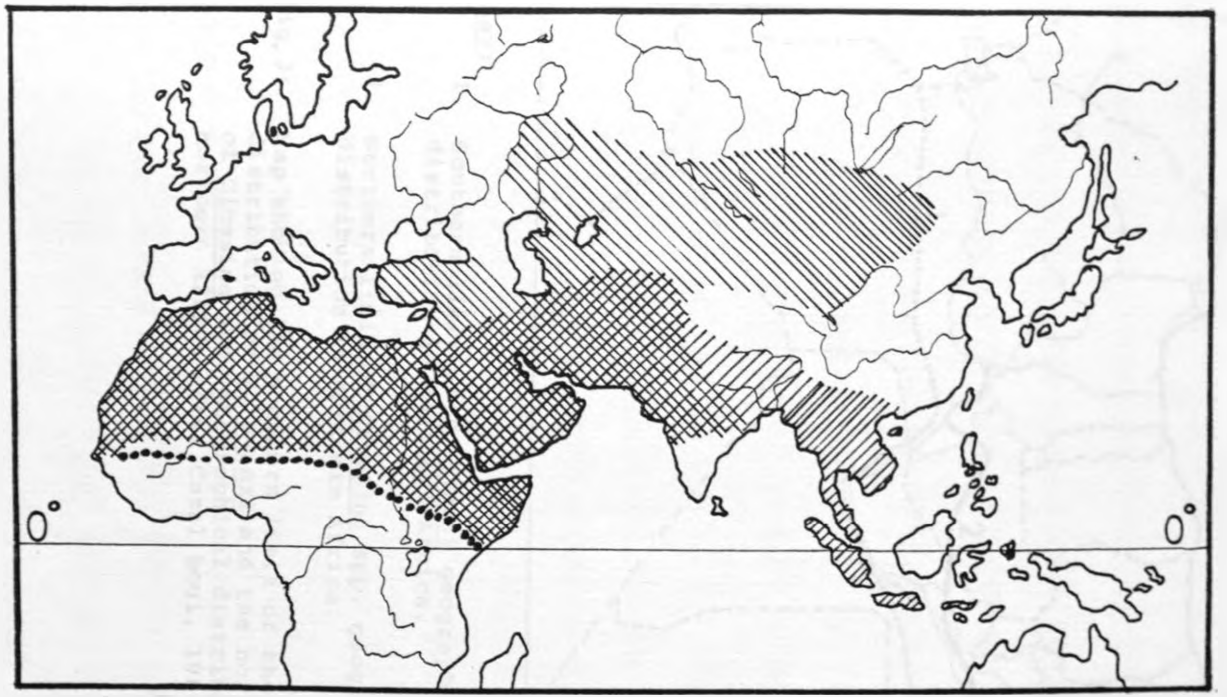
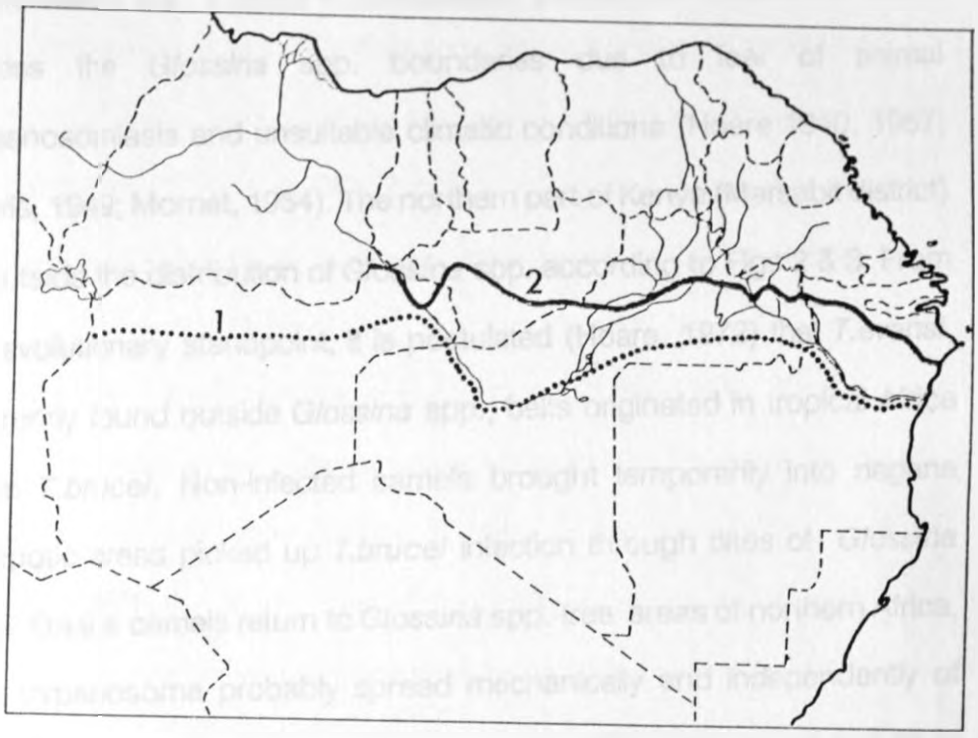


Fig. 2 :Showing some overlap between geographical distribution of T.evansi and the camel



Key:

1. Southern limit of T. evansi geographical distribution in northern Africa.

2. Northern limit of Glossina spp. geographical distribution in northern Africa.

Fig. 3: Map showing the southern limit of the geographical distribution of T. evansi and the northern limit of Glossina spp. geographical distribution in northern Africa after Cazal boui, 1905.

of *Glossina* spp. (Figs. 2 & 3). According to Figs. 2&3, the southern border of cameline *T.evansi* is above the northern border of *Glossina* spp. (Tsetse zone) distribution in northern Africa. Although these lines of demarcation are subject to fluctuations, camels are usually not taken across the *Glossina* spp. boundaries due to fear of animal trypanosomiasis and unsuitable climatic conditions (Hoare 1940, 1957; Lewis, 1949; Mornet, 1954). The northern part of Kenya (Marsabit district) is outside the distribution of *Glossina* spp. according to Figs.2 & 3. From an evolutionary standpoint, it is postulated (Hoare, 1972) that *T.evansi*, currently found outside *Glossina* spp., belts originated in tropical Africa from *T.brucei*. Non-infected camels brought temporarily into nagana enzootic areas picked up *T.brucei* infection through bites of *Glossina* spp. On the camels return to *Glossina* spp.-free areas of northern Africa, the trypanosome probably spread mechanically and independently of *Glossina* spp. and lost its ability to develop in *Glossina* spp. as well as its pleomorphism. The infection that had spread to North Africa, further spread into the Western Hemisphere as a result of movement of infected animals. This is however a hypothetical explanation for the evolution of *T.evansi* from *T.brucei* and its spread in the absence of *Glossina* spp. The notion that *T.evansi* evolved from *T.brucei* has been supported further by the fact that the two species are morphologically indistinguishable. Furthermore, when *T. brucei* is maintained by direct passages in small animals, it becomes monomorphic like *T.evansi*, and loses its ability to develop in *Glossina* spp. (Hoare,1972).

The vampire bat has also been found to be naturally infected with *T.evansi* in South and Central America (Hoare, 1972) where it could be a cyclical vector.

1.2.4: T.evansi infection as a constraint to animal production particularly in camels.

T.evansi infection has been reported to be a problem to animals both in the past as well as at the present time. Past epidemiological information on *T.evansi* is vast, particularly concerning Asia. For instance, according to reports by Luckins (1988), *T.evansi* infection caused the death of thousands of animals in Asia; in Punjab, over 12,000 deaths were reported between 1940 and 1942, of which about 50% were horses, 42% were camels and 8% were bovines. In Burma 6,500 bovines died of the disease in 1885. In Indonesia, between 1920-1927, some 25,000 cases of *T.evansi* infection were reported in domestic animals and 25% of these animals died. Severe problems due to *T.evansi* infection have been reported in other Asian countries such as Indochina, Thailand, Sumatra, Malaysia, Java, and the Philippines in the past (Hoare, 1972; Luckins, 1988). Current problems due to *T.evansi* infection in animals are not quite so well documented but there have been reported cases in Asia and Africa as follows; in Baluchistan between 1985-1986 (Panezaei, cited by Luckins, 1988) reported that over 5,000 camels were treated for *T.evansi* infection. Between 1985 and 1987, buffaloes and dairy cattle in India were diagnosed and found to be infected by *T.evansi* (Luckins, 1988). In Indonesia, according to reports by Luckins (1988), epidemics due to *T. evansi* infection affecting cattle and buffaloes still do occur on most Islands. An outbreak of *T.evansi* infection in Java killed 3,000 animals recently, as reported by Luckins (1988). In Vietnam, *T.evansi* infection killed an estimated 20,000 buffaloes. *T.evansi* infection is also currently affecting bactrian camels in Soviet Asia and northern China and buffaloes

in southern China. Recent studies have also shown a parasitaemia rate of 20% of *T.evansi* infection in buffaloes in Thailand (Luckins, 1988).

Trypanosomiasis due to *T.evansi* infection has been reported to be a constraint to livestock industry not only in Asia, but in other parts of the world such as South America (Hoare, 1972; Luckins, 1988); Mauritius (Moutia, 1928a, 1928b); West Indies and Panama (Hoare, 1972; Luckins, 1988).

T.evansi affects domestic animals such as cattle, dogs, donkeys, horses, sheep and goats (Ilemobade, 1971; Khasanov and Ivanitskav, 1974; Malik and Mahmoud, 1978; Chand and Singh, 1971), and has been reported to be a major constraint to camel production worldwide (Gatt-Rutter, 1967). In all camel populated countries, morbidity rates of up to 30% and mortality rates of 3% or more is caused by trypanosomal infection (Gatt-Rutter, 1967), the most important of which is *T.evansi* infection.

In Africa's camel keeping areas, there have been reports of *T.evansi* infection as a constraint to camel production. In Somalia, the Hargesia Veterinary Laboratory reported 30% morbidity and 100% mortality in camels as a result of *T evansi* infection in the Lafarung and Darragodle areas of Berbera district (Mohamed, 1984). In Sudan, prior to the availability of Naganol®, *T. evansi* infection was responsible for 90% of the trypanosome-infected camels (Knowles, 1924).

In northern Kenya, trypanosome stocks affecting camels was investigated by Gibson (1981, cited by Wilson *et al.*,1981) based on starch gel isoenzyme electrophoresis. The majority of these trypanosome stocks were found to be of the *evansi* type. Camel trypanosomiasis in northern Kenya is therefore attributed to *T.evansi* infection.

Elsewhere in Kenya where there are camels, trypanosomiasis probably due to *T.evansi* has been reported to be a constraint to camel production. Olaho *et al.*, (1987), reported that based on body weight measurements, haematology and reproductive parameters, trypanosomiasis was responsible for an abortion rate of 62.5% and mortality rate of 76.5% in addition to other non-quantified poor health factors of camel herds in Galana Ranch, coastal Kenya.

The camel population in Kenya is concentrated in the semi-arid regions of northern Kenya. Integrated Project on Arid Lands (IPAL), scientists have undertaken projects relating to camel ecology, nutrition and general health in these semi-arid areas of northern Kenya. IPAL Technical reports (1976-1985), reveal trypanosomiasis as one of the major diseases and a fundamental constraint to camel production within the IPAL study area. These camel keeping areas are inhabited by nomadic pastoralists and camels form the mainstay of these nomadic pastoralists life style. Heavy losses of camels are therefore unaffordable to the nomadic pastoralists in northern Kenya. Losses in terms of abortions, emaciation, and even death are presumably due to *T.evansi* infection (Gitatha, 1981). Rutagwenda (1982, 1985), Wilson *et al.*, (1984) have also documented trypanosomiasis as a major constraint to camel production in northern Kenya but the mode of transmission of camel trypanosomiasis is not understood, neither are the vector(s) known. The role of *Glossina* spp. in the transmission of camel trypanosomiasis in northern Kenya is also not established as this is an area considered to be outside the main *Glossina* spp. area of distribution in Kenya. There are still many unanswered questions concerning camel trypanosomiasis, regarding its diagnosis, control strategies, mode(s) of transmission and

vector(s).

Currently, in Kenya and elsewhere, research on camel trypanosomiasis has been extremely difficult and minimal due to the nomadic lifestyle of camel owners and their suspicions of veterinary surveys. As a result of the significant growth in world camel production and the current process of desertification, the economic importance of camel health should be recognised. More of the areas previously inhabited by cattle are gradually suffering from desert encroachment, leaving the camel as the only animal suitable for such habitats. In view of severe outbreaks of camel trypanosomiasis reported in northern Kenya, and other parts of the world, it is necessary to intensify research on camel health particularly camel trypanosomiasis.

1.3: AIMS AND OBJECTIVES OF THE STUDY.

The aims of this study were to investigate the mode of transmission of camel trypanosomiasis and to identify possible mechanical vectors of the disease in northern Kenya. This study comprised both field and laboratory components as outlined below.

Field studies: The objectives of the field studies were:

1: Determination of occurrence of trypanosomiasis in northern Kenyan camels.

2: Determination of active transmission of camel trypanosomiasis in northern Kenya.

3: Collection and identification of haematophagous flies and confirmation of the absence of *Glossina* spp. in the study area.

4: Investigation of biting fly seasonal variation and abundance in relation to occurrence of camel trypanosomiasis with a view to identifying possible mechanical vectors of this disease in the study area.

5: Determination of survival and viability of trypanosomes in biting fly mouthparts after fly blood meals on infected camels in order to asses the vector potential (mechanical) of these biting flies.

6: Determination of diurnal biting fly activity in the study area.

Laboratory studies: The laboratory component of this study was to experimentally effect mechanical transmission of a strain of *T.evansi* from experimentally infected mice to non-infected mice using selected biting flies and to define parameters that determine successful mechanical transmission of trypanosomes. Laboratory objectives were:

1: To effect mechanical transmission of *T.evansi* using a variety of haematophagous flies.

2: Determination of trypanosome survival and viability in fly mouthparts after infective blood meals on infected mice.

3: Assessment of parameters that determine mechanical transmission of trypanosomes such as the time interval between

infective and infecting blood meals and the effect of the number of flies used in transmission experiments.

CHAPTER TWO

OCCURRENCE AND TRANSMISSION OF CAMEL TRYPANOSOMIASIS IN NORTHERN KENYA

2.1: INTRODUCTION AND LITERATURE REVIEW

2.1.1: Diagnostic methods for trypanosomiasis with special reference to *T. evansi* infection.

For epidemiological studies of diseases such as trypanosomiasis, specific, sensitive and inexpensive diagnostic tests should, whenever possible be employed. In the diagnosis of trypanosomiasis, both direct and indirect methods have been used. The direct methods depend on demonstration of parasites by microscopic examination of blood or lymph. The indirect methods involve biochemical, serological tests or demonstration of parasitaemia following inoculation of blood or lymph into susceptible rodents.

The direct diagnostic methods for trypanosomiasis include wet blood films (wf), thick and thin blood or lymph smears, centrifugation and examination of cerebrospinal fluid (CSF). All these direct methods are designated as standard trypanosome detection methods (STDM) (Molyneux, 1975). The wet blood film technique is usually unsuitable when large numbers of animals or patients are to be sampled as it is both labour intensive and time consuming and stock owners do not normally cooperate for such long hours, with their animals kept away from grazing. The thick and thin blood and lymph smears have the advantage in that the smears can be stained in the laboratory and examined later exhaustively, but it is not as reliable as other tests

particularly in detecting low level infections. Lymph examination is generally only applied in cases of suspected human trypanosomiasis (Evans *et al*. 1963 cited by Mulligan,1970). Lymph is usually obtained via a puncture of the posterior cervical lymph glands, which are often swollen during the early stages of infection (Mulligan, 1970).

Wet blood films revealed up to 7.9% trypanosome infection rates in camels in northern Kenya (Rutagwenda, 1985). In a subsequent study, Rutagwenda (1985 , unpublished data) detected up to 20.4% trypanosome infection rates in a camel herd in northern Kenya using the wet film technique. In subpatent trypanosome infections, this technique appears not to be sufficiently sensitive and ought to be carried out in conjunction with other diagnostic techniques.

The Microhaematocrit Centrifuge Technique (MHCT) (Bennet, 1962; Woo 1971; Murray and McIntyre, 1977) is commonly used in the field to diagnose animal trypanosomiases. This technique offers the advantage over wet films and blood smears in that it is both sensitive, fast and both packed cell volume (PCV) and degree of parasitaemia can be estimated from one sample.(Murray,1977). MHCT have been applied in field studies in the Gambia and Kenya with success (Kelly and Schillinger, 1983). Rutagwenda (1982) and Waitumbi (1986), diagnosed up to 10% camel trypanosomiasis in northern Kenya using the MHBC technique. Woo and Rogers (1974), determined the sensitivity of the MHCT for detection of various trypanosome infections including *T.evansi* and reported that the method detected up to 85% of trypanosomes present in each capillary tube of blood sample. Murray and McIntyre (1977)

found that the MHC technique detected up to 50% or more cases of trypanosome infection than the wet films.In addition, Omara-Opyene (1986) detected a 1.5% incidence of bovine trypanosomiasis in northern Kenya using the MHC Technique.

Other centrifugation techniques for the diagnosis of trypanosomiasis include the Silicone Centrifugation Technique (SCT) after Ogbunde and Magaji (1982) and the Mini-Anion Exchange Chromatography Technique (MAECT) followed by centrifugation after Lumsden *et al.* (1977). Ogbunde and Magaji (1982) used the SCT and diagnosed 19 out of 20 cases of mice infected experimentally with *T.b.brucei* and *T.b gambiense* under laboratory conditions. The SC test has given comparable results to the MHC technique (Ogbunde & Magaji 1982).

The MAECT for detection of trypanosomes in blood, was tested as a field diagnostic method for human trypanosomiasis in the Gambia (Lumsden *et al.* 1979) and C^ote d'Ivoire (Lumsden *et al.* 1981). This technique gave up to 88% positives, while MHBC and thick blood smear techniques gave 46% and 30% positives respectively in C^ote d'Ivoire. The MAECT may be more reliable than other centrifugation techniques but less applicable for field use because of its complexity and the requirements for special buffer solutions in order to run the assay. For field use, most of these centrifugation methods have the following disadvantages: (1) they involve carrying a lot of equipment such as a generator or battery powered MHC to the field and (2) they involve labelling a series of samples more than once which may lead to confusion. Centrifugation techniques are, however, more sensitive than wet films and thick and thin smears.

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In advanced infections, trypanosomes may not be demonstrable in the blood or lymph and diagnosis may depend on the examination of cerebrospinal fluid (CSF). Examination of CSF is a direct test for trypanosomiases,particulary human trypanosomiases. It is an important technique that can be employed to determine the stage to which the disease has progressed or the manner in which it is responding to treatment. (Mulligan,1970).

In some trypanosome infections, parasitaemia may be so low that trypanosomes cannot be detected by any of the above mentioned direct tests, even after attempting to concentrate the organisms present in the blood. It therefore becomes necessary to use indirect methods to supplement the direct methods. These indirect methods involve either inoculation of blood or lymph into a susceptible laboratory animal host (cultivation *in vivo*) or inoculation into a suitable culture medium (cultivation *in vitro*). Failing these, evidence of the parasites may be sought by serological tests as reviewed by Mulligan (1970).

Rodent inoculation:

This is an indirect trypanosome diagnostic technique that involves inoculation of small quantities of blood or lymph fluids into a susceptible laboratory animal (mouse,rat etc). This technique has also been used with success under field conditions. Godfrey and Killick-Kendrick (1962) found that rat inoculations detected *T.evansi* infection in 27.6% of 143 camels in Nigeria while blood smears detected only 12.4%. Rodent inoculations have been reported to detect subpatent infection of *T.b.gambiense* in man (Hawgood *et al.* 1965) and *T.evansi* infection in camels and horses (Pegram and Scott 1976). According to studies by

Wilson *et.al.* (1981), mouse inoculation gave up to 71.4% positives for camel trypanosomiasis in northern Kenya. Boyd (1986, unpublished data), reported that rodent inoculation is sensitive for diagnosis of both *T.evansi* and *T.brucei* in camels. Other workers who have reported the diagnostic value of rodent inoculation in relation to trypanosomiasis include Henning (1956), Killick-Kendrick (1986), Geigy *et al.* (1975), Robson and Rickman (1973) and Omara-Opyene (1986).

A batch of mice (6-10) may be used for each sample in order to increase the sensitivity of the test and also to increase the chances of detecting potential mixed infections (Geigy *et al.* 1973). Rodent inoculation has its limitations in that it is labour intensive and certain trypanosomes such as *T.simiae* which do not infect rodents, cannot be detected by this technique. Even for trypanosomes that can infect rodents, there is the long prepatent period of several days before infection is evident. For field work however, rodent inoculation is one of the most practical diagnostic method that has been used with success.

In vitro Culture Technique.

Inoculation of blood or lymph into suitable culture media is unsatisfactory for the diagnosis of pathogenic trypanosomiasis in animals because of the high risk of contamination by bacteria (Lumsden, 1977). This technique was used by Weineman (1963), to detect trypanosomes in the blood and CSF of suspected cases of human trypanosomiasis when STDM had failed. Mathur (1971), cultivated *T.evansi* NS stock in NS medium (Baker, 1966). *T.evansi* NS stock has also been cultivated in a medium that comprised of 20% foetal calf bovine

serum, penicillin, streptomycin, and dissected gut of *G.morsitans* at 20 degrees Celcius (Cunningham,1977, cited by Mahmoud and Gray, 1980). According to Woo (1977), *T.evansi* has been cultured in chick embryos.

Serological tests.

Efforts to improve indirect methods for the detection of (*T.evansi*) over direct methods, led Plantereux (1923) and Knowles (1923) to develop the Formal-Gel Test for *T.evansi* infection. This test is based on an elevation of serum proteins during infection. According to reports by Gatt-Rutter (1967), some Russian authors found this test to be reliable.

The Mercuric Chloride Test, which is also based on elevation of serum proteins during infection was developed by Bennet and Kenny in 1928. Bennet (1933) recommended the Mercuric Chloride Test as a replacement for the Formol-Gel Test. Bennet (1929) applied this test to 250 camels; and all infected camels (numbers of which were not reported), gave positive reaction. Leach (1961) confirmed the diagnostic value of this test but Receveur (1938), Pegram and Scott (1976) have found it unreliable under field conditions.

The Thymol-Test is similar in principle to the Mercuric Chloride Test but has the advantage that it can be used with haemolysed blood samples (Gatt-Rutter, 1967).

Immunodiagnostic tests.

Immunodiagnostic tests are based on the detection of specific antibodies for specific antigens in the samples to be tested. The test sample may be crushed tissues, blood, serum, mucous or any other

body fliuds.

Recently, more sensitive serological tests have been developed based on detection of trypanosome-specific antibodies in serum. These are the Compliment Fixation Test (CFT), Precipitin Tests (PT), Passive Haemagglutination Test (PHT), Capillary Agglutination Test (CAT) by Jaktar and Singh (1977), Indirect Haemagglutination Test (IHT) by Clarkson *et al.* (1971), Immunofluorescent Antibody Test (IFAT) and Enzyme-Linked-Immunosorbent Assay (ELISA) by Luckins *et al.* (1979). Further description and review of some these tests are provided by Mulligan (1970).

The CFT was used extensively by early workers for the diagnosis of *T.b.gambiense* in man. CFT has also been used to detect the presence antibody in camel trypanosomiasis (Schoening, 1924; Sabanishiev, 1973). However, the CFT is prone to interference by anti-complimentary activity in sera from several animal species and there can be difficulties in the preparation of satisfactory compliment fixing antigens (Gill, 1970, Sabanishiev, 1973). For field application, the CFT may not be practical because several preliminary titrations are necessary to determine the optimum amount of compliment required (Mulligan, 1970). Among the range of precipitin tests are the Gel-Diffusion Test (GDT) and the Ring Precipitin Test (RPT) (Mulligan, 1970) Although the GDT has been used to demonstrate trypanosomal antigen and antibody in sera of camels, horses and buffaloes (Bansal and Panthak 1971), this test is mainly of value in estimating the purity of trypanosome antigens or their respective antibodies under laboratory conditions (Weitz, 1964; Matter, 1962; Williamson and Brown, 1964; Lumsden, 1963). The other disadvantage

of precipitin tests is that precipitation is not readily produced in the serum of certain species of animals infected naturally or artificially with trypanosomes (Mulligan, 1970). Gill (1966), used the Passive Haemagglutination Test (PHT), with *T.evansi* and the test proved to be more sensitive for diagnosis of *T.evansi* infection in cattle and buffaloes than the CFT (Mulligan, 1970).

Assesments of other agglutination tests such as the Indirect Haemagglutination Test (IHT), have shown that IHT is sensitive, specific and easy to perform with experimental *T.evansi* infections in rabbits (Gill, 1964). Rutagwenda (1985) used IHT to diagnose camel trypanosomiasis in northern Kenya and the test detected a 79.4% trypanosome infection rate in the camels examined.

According to Jaktar and Singh (1971), the third agglutination test, namely Capillary Agglutination Test (CAT), can be used for diagnosis of natural and experimental *T.evansi* infections in camels and as a screening test for *T.evansi* infection in control programmes (Shen,1974). The usefulness of CAT has been demonstrated in antigenic analysis of variants of *T.brucei* (Gray, 1965) and for the study of antigenic relationships between trypanosome species (Cunningham and Vickerman, 1962; Weitz, 1962). Unfortunately, this technique is not applicable to many species of trypanosomes which do not give a sufficiently high parasitaemia in infected animals to provide a suitable source of antigen (Mulligan, 1970).

Indirect Immunofluorescent Antibody Test (IFAT).

In this test, purified antisera conjugated with suitable flourochromes (flourescein or rhodamine compounds), are applied to sections of fixed trypanosomes on glass slides.The antibodies specifically combine with antigens *in situ* and flouresce when examined under the UV microscope. Weitz (1963) used this test to demonstrate the specificity of antigens to *T. brucei* and *T.vivax*. Sudan *et.al*. (1963) also used this test to demonstrate antibodies in the sera of human patients with a history of trypanosomiasis. These tests have been used to determine levels and specificity of antibodies in sera from animals infected with *T.ninaeikohl-jakimovi* (= *T.evansi*) and seem to provide a rapid and reliable indication of infections with this trypanosone in camels (Hoare, 1972; Sabanishiev, 1973; 1977). Malik and Mahmoud (1978) were able to follow the development of *T. evansi* infections and antibody response in goats by means of IFAT. IFAT has been used extensively in the past decade for the diagnosis of both human and animal trypanosomiases under field conditions (Wery *et al*.,1970; Ashker and Ochilo, 1972), but it has its limitations. IFAT requires expensive equipment, interpretation of results are subjective, requiring skilled operators and there have been conflicting findings on differentiation between infections resulting from different trypanosome species. While workers such as Schindler and Sachs (1970), Mehlitz (1975) and Wilson (1969) have differentiated trypanosome species using IFAT, others such as Zwart *et al*. (1973) have failed to do so.

Enzyme Linked Immunosorbent assay (ELISA).

Recently, antibodies labelled with enzymes, have been used in the diagnosis of a number of diseases including trypanosomiasis. Luckins *et al.* (1978) compared the diagnostic value of ELISA and IFAT in experimental *T.evansi* infections of rabbits and found that both tests have a useful diagnostic value and are more reliable and superior to the Mercuric Chloride Test and the Formol-Gel Test. ELISA has been used successfully for the diagnosis of animal and human trypanosomiases. Omara-Opyene (1986) employed this test to diagnose bovine trypanosomiasis in Kenya and detected 57.9%, 19.7%, 27.8% infection rates in cattle with *T.brucei, T.congolense* and *T.vivax* respectively. The ELISA test offers the advantage over IFAT in that it is sensitive, simple, objective and requires only simple equipment and is therefore suitable for large scale screening of samples. ELISA results can also be assessed visually or photometrically, thus eliminating subjective bias in observer interpretation of assays (Luckins and Mehlitz, 1978; Voller *et al.*, 1975; Luckins, 1977; Ruitenberg and Buys, 1977). However, studies in both laboratory and naturally infected livestock have shown that antibody levels decline slowly following trypanocidal drug therapy and remain higher than pre-infection levels for many weeks after the parasites have been eliminated (Luckins, *et al.*, 1978;1979). This persistence of trypanosomal antibodies following chemotherapy therefore complicates the determination of the infection status of individual animals and diagnosis based on the presence of antibodies alone, in a host, is merely indicative of current or past infections. Hence, any new serodiagnostic test which is developed, should improve on existing

methods by enabling active infections to be diagnosed (Luckins *et al.* 1984). In cases of mixed infections and when antigens used are of limited specificity, results of serological tests become difficult to interpret (Cunningham and Van Hoeve, 1965). Serological reactions by themselves cannot yet be relied upon for certain diagnosis of trypanosomiasis in man and animals. At present these serological tests are regarded chiefly as experimental or epidemiological tools to be used in conjunction with the direct trypanosome diagnostic methods described previously.

DNA Probes for identification of trypanosomes.

Due to the difficulties of identifying trypanosomes in mixed infections, research has been undertaken at I.L.R.A.D by Massamba & Williams,1984; Kukla, Magiwa, Young, Moloo & Ole -Moiyoi,1987) to develop species-specific DNA probes which can be used to identify whole trypanosomes directly form tse-tse flies in the field. Others who have developed DNA probes for African trypanosomes include Gibson, *et. al,* (1987). So far, the available DNA probes can only be used to distinguish trypanosomes of the subgenus *Nannomonas* from trypanosomes of the subgenus *Trypanozoon* and not to differentiate specific species within each subgenus. According to results of Gibson *et.al,* (1987), the *T.brucei* probe tested against DNA from a range of available trypanosome stocks by southern or dot blot analysis hybridized to every *Trypanozoon* stock tested, including *T.b.gambiense,* and *T.evansi,* but, showed no cross-hybridization with *T.congolense* or *T.simiae*. Similarly, the *T.congolense* and *T.simiae* probes did not hybridize with trypanosomes of the other species. However for the subgenus

Trypanozoon, a single probe does not suffice for the identification of all members. (Gashumba, 1986; Young & Godfrey, 1983; Gibson *et.al*, 1987).

2.2: MATERIALS AND METHODS.

2.2.1: General description of the study area in northern Kenya.

Northern Kenya was chosen as a study site because of the following reasons:

(i) the camel population, presumably *T.evansi*-infected, is concentrated in northern Kenya.

(ii) northern Kenya is an area that has been reported to be *Glossina* spp.-free, and is therefore a suitable study area for studies on camel trypanosomiasis which is presumably mechanically transmitted by haematophagous insects other than *Glossina* spp.

The study area covered 23,000 km^2 (Fig.4) and included the Chalbi desert (Fig.5) and its surrounding watersheds. It lies betwwen 1° 50' and 3° 3' North and 35° 10' and 38° 00' East (Lewis, 1977). This area comprises about one third of Marsabit district. The Chalbi desert is an old lake basin which is normally dry, barren and saline. Koroli, Hed-ad and Kaisut (Fig.5) are subdesert communities supporting *Acacia* species and annual grasses. The highest point in the area is Mt. Kulal (Fig.5)

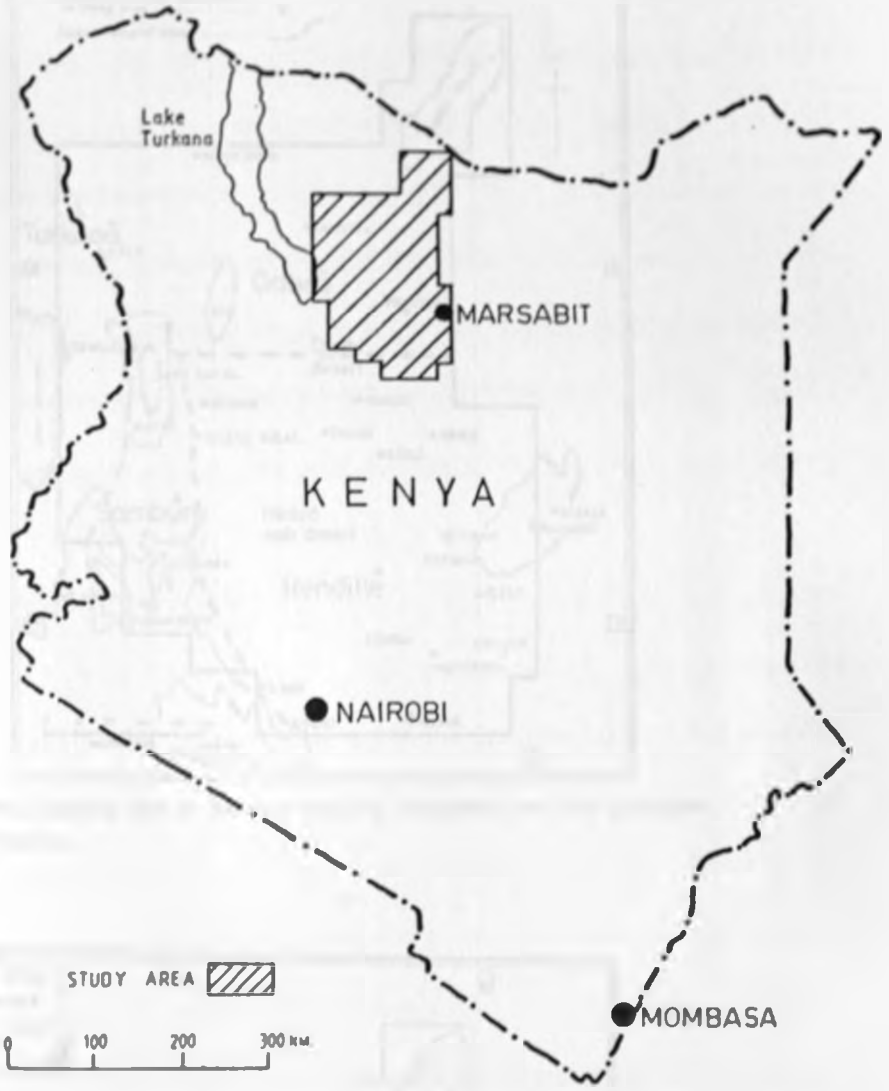

Fig. 4 :Map showing the general location of the study area in northern Kenya.

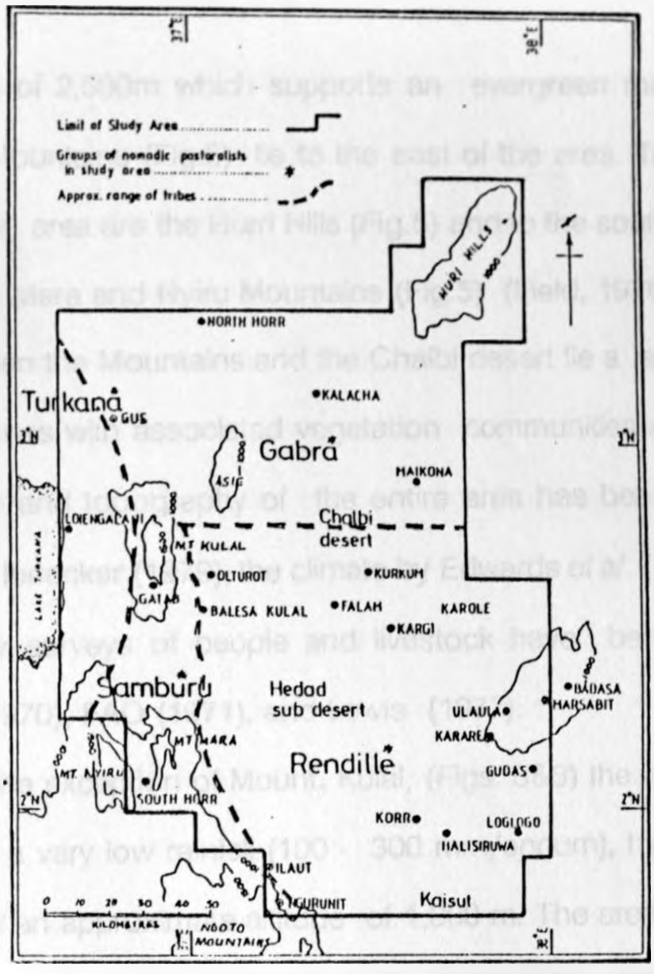

Fig.5 : Map showing limit of the study area, its inhabitants and some geographic features.

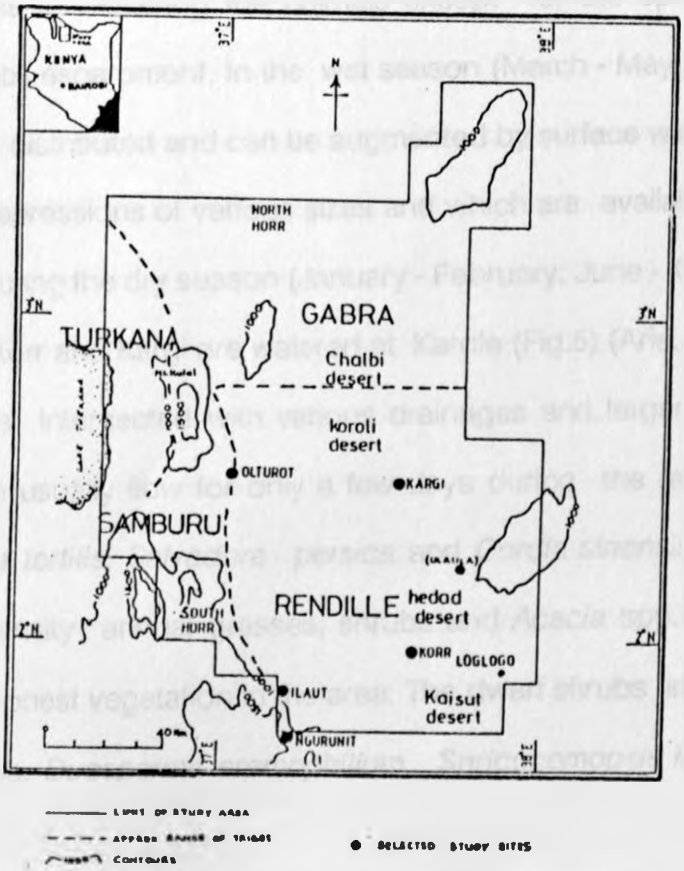

Fig. 6 :Map showing selected study sites following preliminary screening of camel herds for trypanosomiasis by ELISA

an altitude of 2,500m which supports an evergreen mist forest. The Marsabit Mountains (Fig.5) lie to the east of the area. To the extreme north of the area are the Hurri Hills (Fig.5) and to the south east border the Ndoto, Mara and Nyiru Mountains (Fig.5) (Field, 1978).

Between the Mountains and the Chalbi desert lie a series of climatic zones with associated vegetation communities and soil types. Vegetation and topography of the entire area has been described in detail by Herlocker (1979), the climate by Edwards *et al.* (1979), and the preliminary surveys of people and livestock have been reported by Watson (1970), FAO (1971), and Lewis (1977).

With the exception of Mount. Kulal, (Figs. 5&6) the area is characterized by a very low rainfall (100 - 300 mm/annum), high evaporation and lies at an approximate altitude of 1,000 m. The area is hot and the vegetation comprises subdesert scrub. Open surface water is rare over the entire area during the dry season, and there are no reliable water sources for watering the animals except for the springs around the Marsabit escarpment. In the wet season (March - May), water is slightly better distributed and can be augmented by surface water lying in pans and depressions of various sizes and which are available to livestock.

During the dry season (January - February; June - October), camels from Korr and Kargi are watered at Karole (Fig.5) (Arie, *et al*, 1984). The area is intersected with various drainages and larger water courses (which usually flow for only a few days during the rains) fringed with *Acacia tortilis, Salvadora persica* and *Cordia sinensis*. Dwarf shrubs, low density annual grasses, shrubs and *Acacia* spp. bushland is the commonest vegetation in the area. The dwarf shrubs include *Indigofera spinosa, Duosperma eremophilium, Sericocomopsis hildebrandtii* with

Salsola and *Suedda* spp. The sand plains between Mt. Kulal and Mt. Marsabit contain various vegetation communities dominated mainly by *Acacia reficiens, A. senegal* and *A. nubica* with *Aristida* spp. and *Cenchrus* spp. as the common grasses. The gravel plains in the north west of the area support a much sparser vegetation with *Daysphaera (volkensinia) prostrata* and *Seddera* spp. as important perennial forage plants. Some areas however are completely devoid of plants (Field, 1978).

The mean monthly temperature within the study area ranges from maximum of 30-47 degrees Celcius and minimum of 11-26 degrees Celcius. However at the extremely arid sites such as Kargi (Fig.6),minimum mean monthly temperatures become higher than 11-26 degrees Celcius. More details on the temperature regime of the study area are given by Edwards *et.al* (1979).

About 30,000 nomadic pastoralists live in the study area (FAO,1971). They are mainly of the Gabra and Rendille ethnic groups but Borana and Samburu are also found in the area.There are about 12 permanent settlements in the area situated mostly on the periphery, where there is permanent water.The people in the study area own about 400,000 head of livestock; sheep and goats are most numerous but the biomass of camels is similar to that of small stock. The milk of camels and meat of small stock form the mainstay of the pastoralists' diet (Field,1979).

Other animals (wildlife) in the area include zebra,oryx, hyena, cheetah,jackal wild dog, lion, elephant, dik, porcupine giraffe, gazelle, leopard,gerenuk, wild cat, hare and caracal (Kruok,1980).

2.2.2: Screening camels from selected study sites in northern Kenya for T. evansi infection using ELISA test.

Camel sera was harvested from blood samples taken from several herds within the study area in northern Kenya at Olturot, Ngurunit, Korr, Kargi, loglogo and Ula-ula. The ELISA test was used to determine whether trypanosomal antibodies in camel sera was specific to *T.evansi* (KETRI 12429) antigen and thus indicate current and/or past *T.evansi* infection in camel herds in the study area. The ELISA test was also employed to screen camel herds for *T.evansi* and subsequently identify suitable herds for further epidemiological studies of the disease in the study area.

At each of the above mentioned study sites, camels were bled in December 1985 and/or February 1986.Harvested sera were screened for trypanosomiasis using the ELISA test.

Bleeding of camels: Prior to bleeding, camels were made to kneel down. A rope was then tied around the hind legs to bring the camel to a resting position. A second rope was tied around the camel's neck in order to make the jugular vein visible. Using a gauge 16 x 1.5 hypodermic needle and a 20 ml. syringe, camel blood was drawn from the jugular vein (Plates 1 & 2) and immediately emptied into a 25 ml. universal bottle.

Plate 1 : Bleeding of camels at Olturot study site.

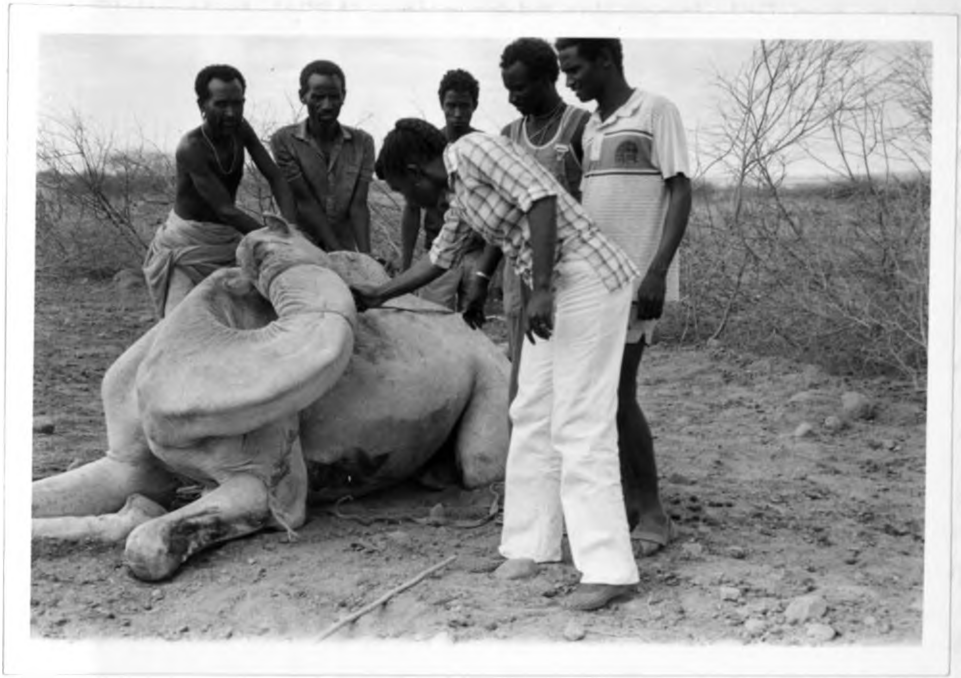

Plate 2 : Bleeding of a camel at Olturot study site: note the resting position of the camel and the drawing of blood from the jugular vein.

Serology: 20 ml. of non anticoagulated camel blood, obtained as described above, was emptied into 20 ml. universal bottles. The bottles were left to stand on a bench overnight at room temperature. The following day, approximately 2 ml. of sera could be extracted from each bottle and aliquoted into nunc tubes, frozen in liquid nitrogen and transported to Nairobi for ELISA tests. The enzyme conjugate was developed after the method of Nakane and Kawaoi [1974], as modified by Olaho *et al.* (1984), using rabbit-anti-camel IgG and peroxidase enzyme type VII (sigma grade) as described in Appendix 1. Soluble trypanosome antigen was prepared from a K.E.T.R.I. stabilate of *T. evansi* (K.E.T.R.I. 12429) as described by Luckins (1977). Details of the procedure are contained in Appendix 1. Protein concentration of all the immunoglobulin and antigen preparations was determined by the method of Lowry *et al.* (1951) as modified by Peterson (1979) and is fully described in Appendix 1.

ELISA was developed using disposable flat bottomed polystyrene 96 well plates (Linbro-Flow laboratories, U.S.A.) Optimal antigen, serum and conjugate concentrations were determined by checkerboard-titration, ELISA methodology was as follows:

(i) 200 microlitres of soluble antigen dissolved in coupling buffer (0.05 ml carbonate/bicarbonate buffer pH. 9.6) were pipetted into each well of the microplate at an optimal concentration of 10 micrograms per ml. The plates were incubated at 37°C for 3 hours and left overnight at 4°C.

(ii) Camel serum optimally diluted at 1/100 in 0.01M Phosphate Buffered Saline (PBS)/ Tween* 20 at a pH. of 7.2 was added to the antigen

coated wells (200 microlitres per well) and incubated for 1 hour at 37°C. Plates were washed and dried as above.

(iii) Rabbit-anti-camel IgG Peroxidase(RAclg G- PO) conjugate optimally diluted at 1/1000 in 0.01M PBS Tween 20 pH 7.2, was added to quantify amount of antibody connected to the enzyme (200 microlitres per well) and incubated for one hour at 37°C. Plates were washed and dried as above.

(iv) 200 microlitres of O-phenylene diamine (OPD)- 350 microlitres of 1% OPD per 10 ml of OPD diluent (0.2M Na_2HPO_4 - 126.3 mls 0.1 citric acid - 73.17 mls: 30% H_2O_2 - 334 microlitres) were added as substrate specific to the enzyme and to maintain correct pH media for the reaction respectively. The plates were incubated in the dark for 30 minutes at room temperature. The enzyme substrate reaction was stopped by adding 100 microlitres of 0.3M sulphuric acid and the plates read on an ELISA Reader (MR 580, MICRO ELISA AUTO READER, DYNATCH), programmed at wavelength 490 nm, and 570 nm, to reduce the effect of smudges, scratches and occlusions in the plastic plate on the measured absorbency of the samples.

Controls

(v) Controls for ELISA included:

(a) negative control serum: pooled from 50 camels showing negative for Mercuric Chloride Test (M.C.T) reaction, negative haemagglutination on Indirect Haemagglutination Test (IHT) and negative on mouse inoculation test (MIT)

(b) Positive control serum: pooled from 50 camels showing positive MCT, IHT and MIT.

(c) Foetal calf serum (FCS): from a camel calf, bled just after birth before suckling.

(d) Other controls included: omission of serum, omission of conjugate and omission of antigen, for routine testing; negative and positive control sera from adult camels were used and wells without serum included as blanks (denoted "B"). All sera from the field were tested in duplicates. Six negative and six positive control sera were included per plate with four blanks.

Calculations of results

(vi) At the end of each test, the mean absorbency of the positive and negative sera ($\bar{x}$+ve,$\bar{x}$-ve) were read. For each test sample, the mean absorbence of the duplicate was determined as $\bar{X}$ s and expressed as a percentage of the known $\bar{X}$ +ve as given in the formula below:

$$\frac{(\bar{X}s - \bar{X}\text{-ve})}{(\bar{X}\text{ +ve } -\bar{X}\text{-ve})} \times 100$$

Routinely, duplicates varying by more than +15% or -15% of their mean were rejected and the test repeated, for that serum sample. The same standard control sera (+ve or -ve) were used throughout, thereby making each test comparable with the previous one. Samples showing means above x-ve were regarded as positive even if they were below $\bar{x}$+ve control. Micro ELISA values of 0.222 and above were regarded as positive for batch No.1 of sera analysed in March 1986 as this was the cut of point or level of physiological significance that was close to the background reading of controls. Micro ELISA values of 0.550 and above were regarded as positive for sera batch No. 2, analysed in June, 1986. For this second batch the background reading was higher hence the difference in values of 0.222 and 0.555.

Following screening of camel herds for trypanosomiasis using the ELISA method, five suitable study sites were identified for further investigations on incidences of camel trypanosomaisis. The five study sites chosen for this purpose were Kargi, Korr, Ilaut, Olturot and Ngurunit (Fig.6). These sites were within the IPAL study area and are described below:-

(a) KARGI: This is an area that shows desertification at various stages, with man-made deserts around permanent settlements. Directly around the settlement (Plate 3), almost no vegetation is left, having been removed for firewood, grazing, building and boma construction. The elevation at this area is 450 m with an average rainfall of 200 mm annually.

(b) **KORR:** Like Kargi, this area has undergone desertification due to human settlement. It lies at an altitude of 500 m with an average rainfall of 180 - 190 mm/anum. Its wind direction are constant easterly winds (120° - 130°) and prevail throughout the year at an average speed of 16 km/hr. There is currently no vegetation around settlements and vegetation is scarce towards the periphery of the settlement.

(d) **OLTUROT** : Olturot lies on the plains of the lowlands of the IPAL study area. It has the commonest climatic subtype in the study area. The commonest vegetation comprises *Acacia* spp. and *Duosperma* spp. This area has several strips of riverine woodland lining the tributary water courses which flow from Mt. Kulal and Balesa Kulal. The woodlands are narrow but consist of dense shrub and tree vegetation which are of great importance to livestock. The area lies at an altitude of 660 m. It receives an average of 265 mm of rainfall annually and is used substantially for

Plate 3 : Kargi study site to emphasis scarcity of vegetation.

Plate 4 : Olturot study site.

Plate 5 : Olturot study site.

grazing, especially during the rainy season. There are shallow wells in this area. The wind system is influenced by the nearby Mt. Kulal, and details of this are reported by Edward *et.al*. (1979). A general view of Olturot study site can be seen on Plates 4 and 5.

(e) **NGURUNIT** This area is on the foothills of the Ndoto and Ol Donyo Mara Mountains and lies at an altitude of 700 m. The vegetation consists mainly of *Acacia tortalis* woodland with *Duosperma eremophilium*. It was originally a woodland forest of *Acacia* spp. mixed with an understorey of shrubs, but like other sites, it has undergone erosion. This area receives rain in March-May and November-December. After the long rains (March-May), Ngurunit River, arising in the Ndoto Mountains, flows until August and then again in December. In the mountain valleys, surface and subsurface run-offs occur. There is soil moisture to support woodland which could not exist here if dependent on rainfall only. This is an area of transition between lowlands and uplands with an average annual rainfall of 500 mm. This area has a high biomass and is the most important woodland unit in the IPAL study area. It is heavily utilized by cattle, small stock and camels. A general view of Ngurunit study site can be seen on plate 6.

(f) ULA-ULA Ula-Ula (Fig.6) is a settlement about 20 kms from Marsabit town. It receives a mean annual rainfall of 800 - 900 mm. The vegetation around Ula-Ula is dominated by perennial grasses such as *Pennisetum mezianum*. There are also annual grasses such as *Aristida adscensionis*, Dwarf shrubs such as *Acalypha fruiticosa* and trees such as *Acacia brevispica*. It is an area utilized by all kinds of livestock in all seasons. Due to the high altitude (900 m), the temperatures around Ula-Ula are not as high as in other study sites and range from 20°C - 27°C (Field 1980).

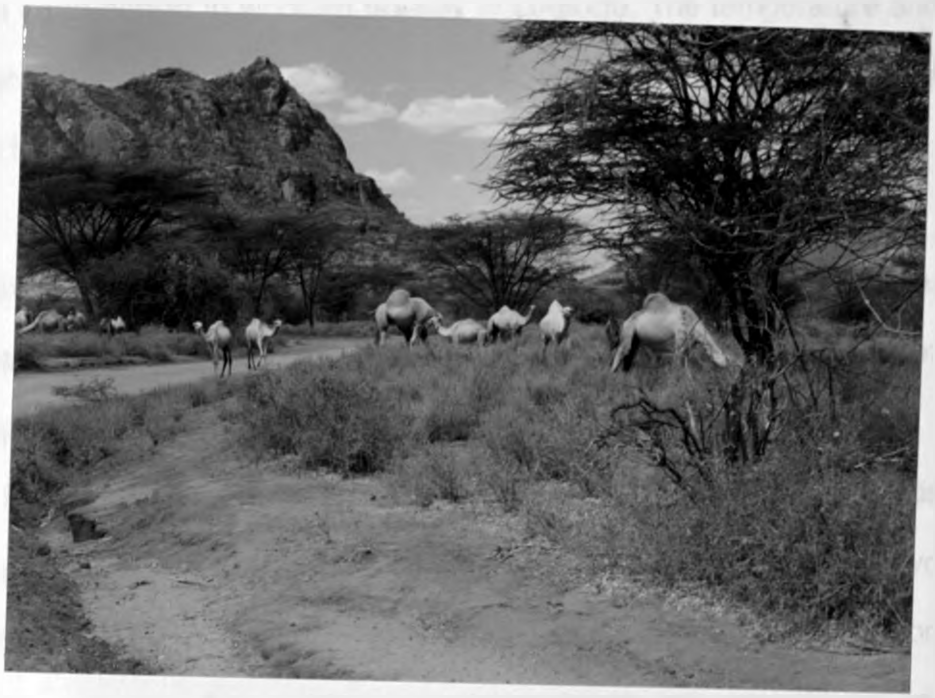

Plate 6 : Ngurunit study site: note the vegetation dominated by Acacia spp. and Duosperma spp. shrubs.

(g) LOGLOGO: Like Korr and Kargi, Loglogo (Fig.6) is an area that has been degraded heavily by overgrazing and wood-cutting. It receives slightly higher rainfall (400 mm annually) than Korr and Kargi. Large numbers of livestock are common at Loglogo due to the presence of many bore holes. Owing to efforts of the missionaries, some pastoralists have been settled in wooden houses in Loglogo. The temperature and vegetation types in Loglogo are similar to those described for Korr above (Field 1980).

2.2.3: Diagnosis and determination of seasonal fluctuations in trypanosome infection rates in selected camel herds in northern Kenya.

Following screening of camel herds using the ELISA test, two herds were identified for further investigation of camel trypanosomiasis. The two herds were the Olturot and Ngurunit study site herds. A second reason why these two herds were chosen concerned their non-nomadic nature. Available herds other than these two were also bled when possible.

Diagnosis of camel trypanosomiasis was based on blood smears and mouse inoculations as described below. Camels were bled quarterly with effect from January, 1986 up to and including November, 1987 such that blood samples were obtained during the wet and dry seasons for comparative purposes.

(a) Blood Smears: Thick and thin blood smears were prepared from samples of camel blood and stained with Giemsa. For thick smears, a drop of blood on a microscope slide was spread "slightly" with the edge of another slide placed at right angles over the drop. For thin smears, the second slide was placed at a 30° angle over the drop. By gentle movement of the second slide, the drop was spread to give a thin smear. The thin smears were fixed by flooding with absolute methanol for about 30 seconds. Both smears were stained with Giesma stain (1 to 10 parts of Giesma and distilled water) for 60 minutes. The stain was thoroughly flushed with tap water and allowed to dry. Slides were examined under the X40 objective for trypanosomes and positive cases recorded for each herd bled.

Mouse Inoculations. Mice (Balb C Strain) were transported to the study sites either by air or road. For each camel that was bled, 0.5 ml. of anticoagulant blood was inoculated intraperitoneally into two marked mice (Plate 7). Anticoagulant blood was obtained by emptying freshly-bled camel blood into Bijoux bottles containing 10 g. ethylene diamine tetracetic acid (EDTA) as an anticoagulant. The Bijoux bottles were then shaken to allow mixing of the blood with anticoagulant. The inoculated mice were transported to Nairobi where they were examined daily for trypanosomes until the standard day 60 post- inoculation using the wet-film method (Rutagwenda,1985). To prepare a wet film, the tip of the mouse tail was snipped with a sterilized pair of scissors and a drop (0.05 ml.) of blood was collected on a microscope slide and covered with a cover of slip. The film was viewed at a magnification of X 400 and parasitaemia for positive cases recorded according to the following

Plate 7 : Mouse inoculation carried out under field conditions

scheme:

[0]---No parasites in a 1 minute observation period.

[+]---Less than 10 parasites in a 1-minute observation.

[++]--Intermediate between [+] and [++].

[+++]-Parasites abundant.

Subsequent infection in one or both members of the pair of mice inoculated, was regarded as a single count of an infected case. Mice that were positive for trypanosomes were killed for stabilation of the isolates. Stabilates were prepared after the method of Lumsden (1963) and 1 ml. dimethyl sulfoxide (DMSO) was added to 1 ml. of blood containing trypanosomes to give a final concentration of 8% DMSO as a cryoprotectant. Percent trypanosome infection rates for each herd was recorded based on total positive cases divided by the total number of camels bled.

2.2.4: Use of sentinel camels to determine transmission of camel trypanosomiasis in northern Kenya.

Ten four-year old male camels were purchased after screening them for trypanosomiasis using the blood smear and mouse-inoculation tests and finding them to be trypanosome-free. All camels purchased were also negative for trypanosomes based on the ELISA test, as well except for two camels "Chamaa" and "Ale", which were positive via ELISA test and which were, purchased at Kargi station. Five sentinel camels, all negative for trypanosomes, were introduced into a U.N.E.S.C.O. owned camel herd at Olturot in April 1986. The Olturot herd was a permanently settled herd that remained in the same locality during the entire study period. The other 5 sentinel camels (including the two positive for trypanosomes

according to the ELISA test) were introduced into a local camel herd at Kargi in May, 1986. These two "positive sentinel camels were each treated with Trypacide (R) (quinapyramine-sulphate) at the recommended dose of 5 mg/kg body weight. Camel body weight was calculated according to the method of Schwartz (1980) as outlined in Appendix 2. Movement records of the Kargi camel herd were kept in terms of distances (Kms) moved, reasons for movement and dates of movements. Sentinel camels, both at Olturot and Kargi herds, were checked quarterly for trypanosome infections based on blood smears, mouse- inoculations and ELISA tests.

2.3 : R E S U L T S.

2.3.1: T. evansi infection status in camel herds following screening by ELISA technique.

Table 1 and Fig. 7 contain summarized ELISA results for camel sera obtained from Olturot, Ngurunit, Korr, Logologo Ula Ula and Kargi camel herds. The actual ELISA values obtained for all test sera are contained in Appendices 3 - 19.

According to ELISA results, all camel herds sampled had individual animals with elevated trypanosomal antibody levels specific to *T.evansi* KETRI 12429 antigen as shown on Table 1 and bar charts on Figure 7. Incidences of current or past *T.evansi* infection in camel herds sampled were relatively high and ranged from 72% - 95% as shown on Table 1 and Figure 5. Out of a total of 1,097 camel sera tested, 952 were found positive for trypanosomes, making the overall percent trypanosome infection rate 87.1% as summarized at the bottom of Table 1.

Table 1: Trypanosome infection rates in camels at selected study sites in northern Kenya based on ELISA.

STUDY SITE	HERD NO.	DATE BLED	NO.CAMELS BLED	NO.+ve/-ve		%+ve
				+ve	-ve	
Olturot	1	12/1985	60	52	8	86.6
Olturot	2	12/1985	120	101	9	84.1
Olturot	3	02/1986	60	51	9	85.0
Olturot	4	02/1986	60	51	9	85.5
Olturot	5	12/1985	120	104	6	86.7
Olturot	6	12/1985	60	57	3	95.0
Olturot	7	02/1986	95	87	8	91.5
Olturot	8	12/1985	100	93	7	93.0
Ngurunit	1	12/1985	52	48	4	92.3
Ngurunit	2	12/1985	60	54	6	90.0
Ngurunit	3	02/1986	50	47	3	94.0
Ngurunit	4	02/1985	40	37	3	92.5
Ngurunit	5	12/1986	60	57	3	95.0
Korr	1	12/1985	50	39	11	78.0
Korr	2	02/1986	50	38	12	76.0
Korr	3	02/1986	50	40	10	80.0
Ula-ula	1	12/1985	50	38	12	76.0
Loglogo	1	12/1985	50	36	14	72.0

A summary of ELISA results.

Mean %infection =952/1097=87.1 %

s.d=7.26 Var. =49.4

s.e=1.6 C.V =8.3%

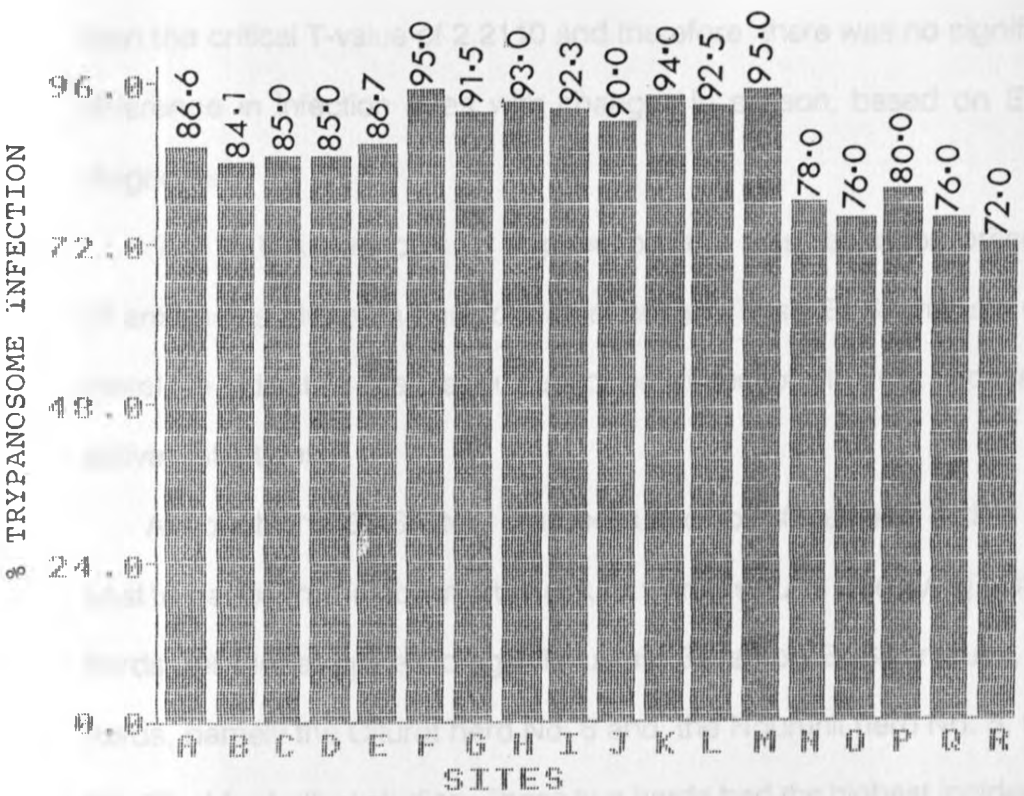

Fig. 7 : Trypanosome infection rates in camel herds at selected study sites in northern Kenya.

A - H = Olturot
I - M = Ngurunit
N - P = Korr
Q = Ula-Ula
R = Loglogo

Test sera obtained in the wet season (December, 1985) and the dry season (February, 1986) gave almost the same ELISA results. The mean trypanosome infection rates were 86.43 $\pm$7.6% and 87.25 $\pm$6.1% for the wet and dry seasons respectively as shown on Table 2. ELISA results for the wet and dry seasons were subjected to the Student's T-test to test for any significant differences. At P = 0.05, with 16 degrees of freedom, a T-value of 0.043 was obtained. This T-value is much lower than the critical T-value of 2.2110 and therefore there was no significant difference in infection rates with changes in season, based on ELISA diagnosis

The ELISA diagnosis for trypanosomiasis was based on presence of antibodies alone, in sera of camel hosts. These ELISA results were therefore indicative of either current or past infections but not necessarily active infections.

Although the ELISA test was not used to differentiate active from past trypanosome infections, the results were useful in identifying suitable herds for further epidemiological studies. Based on ELISA results, Two herds, namely the Olturot herd No. 6 and the Ngurunit herd No. 5, were identified for further studies. These two herds had the highest incidences of trypanosome infection (95%) and therefore considered to potentially have the highest percentage of active trypanosome infections. Movements of these two herds were also limited to the study area, and this was an obvious advantage for any epidemiological investigations.

Table 2: Comparative camel trypanosome infection rates in wet and dry seasons at selected sites in northern Kenya based on ELISA.

W E T S E A S O N [12/1985]			D R Y S E A S O N [2/1986]		
HERD NO.	SITE.	%INFECTION	HERD NO.	SITE.	%INFECTION
1	Olturot	86.6	3	Olturot	85.0
2	Olturot	84.1	6	Olturot	85.0
4	Olturot	87.6	7	Olturot	91.0
1	Ngurunit	92.3	3	Ngurunit	94.0
2	Ngurunit	90.0	4	Ngurunit	92.5
1	Korr	78.0	3	Korr	76.0
		$\bar{X}$=86.43+/-7.6%			$\bar{X}$=87.25+/-6.1%

Summary of t-test

At =0.05, t=0.043<2.210

df =16.

2.3.2: Trypanosome infection rates in camels based on the Blood Smear Test (BST).

Results of trypanosome infection rates for the five different camel herds sampled in northern Kenya, based on BST, are presented on Table 3. Data on Table 3. depict numbers and percent of camels positive for trypanosomiasis, based on the blood smear diagnostic technique. The Korr, Ula-ula and Loglogo camel herds were not sampled after September,1986 due to the nomadic herding regimes of the of these animals. The Ngurunit herd was also not sampled for the year 1987 due to transport problems. Of these five herds, the best sampled herd was the Olturot herd which was sampled quarterly for a period of 24 months beginning in January, 1986 to November 1987.

A total of 1,097 individual animals were examined out of which there were 43 positive cases, making the overall percent trypanosome infection in all herds samples to be 3.9%, as summarized on Table 3. Trypanosome infection rates for all herds sampled ranged from 0 - 11.5%. Of all these, the Ngurunit herd had the highest percent infection rates ranging from 3.3% - 11.5%. Infection rates for the Olturot herd ranged from 1.7% - 8.3%. Elevated trypanosome infection rates recorded from the Olturot herd were 6.6% and 8.3% and these were detected during the wet months of November,1986 and May,1986 respectively. Trypanosome infection in camel blood was not detected in the Korr and Ula-ula herds based on the blood smear technique although, according to the mouse inoculation and ELISA test, there were some infected camels in these two herds.

Table 3: Trypanosome infection rates in camels at selected study sites in northern Kenya based on the Blood Smear Test [B.S.T.].

STUDY SITE	DATE BLED	NO. ANIMALS EXAMINED	RESULT +VE	RESULT -VE	PARASITAEMIA LEVELS IF +VE +	++	+++	%+VE
Olturot	01/1986	60	1	59	1	-	-	1.7
Olturot	03/1986	101	4	97	1	1	2	3.9
Olturot	05/1986	60	5	55	1	3	2	8.3
Olturot	07/1986	95	2	93	-	1	1	2.1
Olturot	09/1986	120	4	116	-	4	-	3.3
Olturot	11/1986	60	4	56	1	2	1	6.6
Olturot	05/1987	100	3	97	-	2	1	3.0
Olturot	11/1987	100	2	98	-	1	1	2.0
Ngurunit	01/1986	50	1	49	-	1	-	2.0
Ngurunit	03/1986	60	2	58	-	2	1	3.3
Ngurunit	05/1986	52	6	46	2	2	2	11.5
Ngurunit	07/1986	50	1	49	-	1	-	2.0
Ngurunit	09/1986	40	1	39	-	-	1	2.5
Ngurunit	11/1986	60	4	56	-	1	3	6.7
Korr	05/1986	50	0	50	-	-	-	0.0
Korr	07/1986	50	0	50	-	-	-	0.0
Korr	09/1986	50	0	50	-	-	-	0.0
Ula-ula	05/1986	50	0	50	-	-	-	0.0
Loglogo	05/1986	50	3	57	-	-	3	6.0

KEY;

+++ high parasitaemia [18]

++ moderate parasitaemia [21]

+ low parasitaemia [6]

SUMMARY

mean % infection =45/1097=3.9%

s.d = 3.0 s.e = 0.6

var. =7.4 C.V =78%

Levels of parasitaemia in camels positive for trypanosomes are also depicted on Table 3. In these positive cases, parasitaemia ranged from low [+] to moderate [++] and finally high [+++]. As depicted on Table 3, 21 of positive cases had moderate ,6 had low and 18 had high parasitaemia.

2.3.3: Trypanosome infection rates in camels based on the mouse inoculation test (MIT).

Data presented on Table 4 show trypanosome infection rates in the five herds sampled. A total of 1,097 camels were sampled and blood samples from each of these animals were inoculated into mice [two mice per sample]. The mouse inoculation technique revealed patent parasitaemia in all the herds sampled as shown on Table 4. From a total 1,097 mice pairs inoculated with camel blood, 122 became infected, making the overall percent trypanosome infection rates in camels as diagnosed by this technique, 11.3% as summarized at the bottom of Table 4. Table 5 and barcharts on Fig. 8 contain data of camel trypanosome infection rates in the wet and dry seasons, as diagnosed by mouse inoculation [M.I.T.]. According to M.I.T. results, there were significant differences between the mean trypanosome infection rates in camels in the wet and dry seasons. (Table 5). The mean percent trypanosome infection rates were 13.78 + / - 5% for the wet season and 4.55 + /- 2.2% for the dry season. Statistical analysis [T-test] of data presented on Table 5 resulted in a T- value of 5.634. At $p = 0.05$ with 16 degrees of freedom, a T- value of 5.634 is much greater than the critical value of 2.210. The mean percent trypanosome infection rates in the wet season, which was 14.45%, is therefore significantly different from the

Table 4 :Trypanosome infection rates in camels at selected study sites in northern Kenya based on the Mouse Innoculation Test [M.I.T.].

STUDY SITE	DATE BLED	NO.CAMELS BLED	NO.MICE ~~PRS.~~ INNOCULATED	NO. CASES POSITIVE	%CASES POSITIVE
Olturot	01/1986	60	120	4	6.7
Olturot	03/1986	101	202	16	15.8
Olturot	05/1986	60	120	11	18.3
Olturot	07/1986	95	190	8	8.4
Olturot	09/1986	60	120	5	8.3
Olturot	11/1986	120	240	17	14.2
Olturot	05/1987	60	120	11	16.7
Olturot	11/1987	100	200	11	11.0
Nguruni	01/1986	50	100	1	2.0
Nguruni	03/1986	60	120	12	18.3
Ngurunit	05/1986	52	104	10	19.2
Ngurunit	07/1986	50	100	1	2.0
Ngurunit	11/1986	60	120	9	6.7
Korr	05/1986	50	100	4	8.0
Korr	09/1986	50	100	1	2.0
Korr	11/1986	50	100	2	4.0
Ula-ula	05/1986	50	100	6	12.0
Loglogo	05/1986	50	100	5	10.0

SUMMARY

N.B 2 mice innoculated with one camel blood sample.

Mean % infection = 122/1097=11.3%

s.d =4.97, Var. =24.7,

s.e =1.09, C.V = 41%.

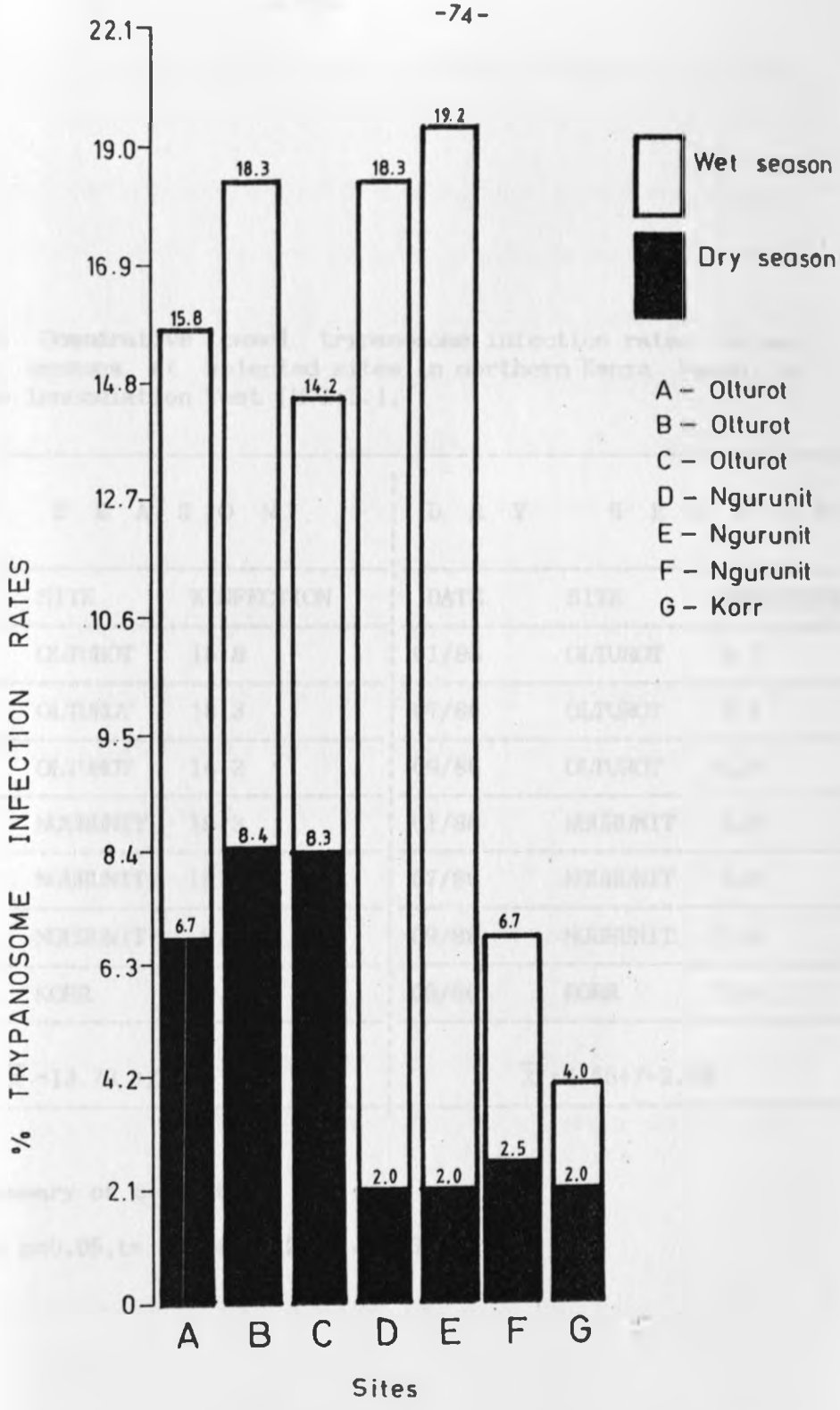

Fig.8 :Comparative camel trypanosome infection rates during wet and dry seasons at selected study sites in northern Kenya based on MIT

Table 5: Comparative camel trypanosome infection rates for wet and dry seasons at selected sites in northern Kenya based on the Mouse Innoculation Test [M.I.T.].

W E T S E A S O N			D R Y S E A S O N		
DATE	SITE	%INFECTION	DATE	SITE	%INFECTION
03/86	OLTUROT	15.8	01/86	OLTUROT	6.7
05/86	OLTUROT	18.3	07/86	OLTUROT	8.4
11/86	OLTUROT	14.2	09/86	OLTUROT	8.3
03/86	NGURUNIT	18.3	01/86	NGURUNIT	2.0
05/86	NGURUNIT	19.2	07/86	NGURUNIT	2.0
11/86	NGURUNIT	6.7	09/86	NGURUNIT	2.5
11/86	KORR	4.0	09/86	KORR	2.0
$\bar{X}$ =13.78 +/-5%			$\bar{X}$ =4.55+/-2.2%		

Summary of t- test

At $p=0.05, t= 5.634 > 2.210$. $df=16$.

mean percent infection rate in the dry season which was 5.48% based on M.I.T.

Table 6 contains comparative data of camel trypanosome infection rates in the wet and dry seasons, based on blood smears. These results show higher camel trypanosome infection rates in some of the wet seasons months particularly May, 1986 (Olturot); November,1986 (Olturot); May ,1986 (Ngurunit) and November,1986 (Ngurunit) when infection rates were 8.3%, 6.6%, 11.5% and 6.7% respectively. The mean trypanosome infection rate in the wet season which was 5.62 $\pm$3% was higher than the mean infection rate in the dry season which was 1.94 $\pm$0.6%. Statistically, there was also a difference between mean trypanosome infection rates for the two seasons as was diagnosed by the blood smear technique. The obtained T- value of 2.352 was greater than the critical value of 2.179 at $p = 0.05$ with 12 degrees of freedom. The blood smear test, like the M.I.T., revealed some differences in infection rates in camels with seasons. Furthermore, there were some wet seasons such as May ,1986 at Ngurunit, when infection rates in camels rose as high as 11.5%, as was diagnosed by the blood smear technique.

2.3.4: Some effects of trypanosome infection on camel herds in northern Kenya.

It was noted that during two wet seasons, [May, 198 at Ngurunit and May,1986 at Olturot] when infection rates in camels were particularly high based on M.I.T. and well as B.S. tests, there were reported deaths and abortions in some herds in the study areas. For instance, there were 4 deaths of male camels and 3 pregnant females

Table 6: Comparative camel trypanosome infection rates for wet and dry seasons at selected sites in northern Kenya based on the Blood Smear Test [B.S.T.].

W E T S E A S O N			D R Y S E A S O N		
DATE	SITE	%INFECTION	DATE	SITE	%INFECTION
03/85	OLTUROT	3.0	01/86	OLTUROT	1.7
05/86	OLTUROT	8.3	07/86	OLTUROT	2.1
11/86	OLTUROT	6.6	09/86	OLTUROT	3.3
03/86	NGURUNIT	3.3	01/86	NGURUNIT	2.0
05/86	NGURUNIT	11.5	07/86	NGURUNIT	2.0
11/86	NGURUNIT	6.7	09/86	NGURUNIT	2.5
11/86	KORR	0.0	09/86	KORR	0.0
$\bar{X}$ =5.62+/-3%			$\bar{X}$ =1.94+/-0.6%		

Summary of t- test.

At p=0.05 , t=2.353>2.210. d.f=16.

aborted in the Olturot herd. Similarly, 2 male camels died and 2 aborted in the Ngurunit herd. All the affected camels had previously been found positive for trypanosomes, based on blood smears.

2.3.5:Trypanosome infection of sentinel camels.

Table 7 contains data on infection status of the 10 sentinel camels that were introduced into the Kargi and Olturot herds and subsequently checked for trypanasome infection. Out of the 5 sentinel camels introduced into the Olirot herd in April 1986; one camel (No.93) become infected in December 1986 after a duration of 9 months. Infection of the sentinel camel was detected based on ELISA and M.I.T. diagnostic techniques. On a subsequent check in March 1987, all three tests [ELISA, M.I.T. and B.S.] revealed trypanosome infection in camel no. 93. The other four camels [nos. 67, 63, 98 and 58] were not infected. At Kargi study site, 2 camels out of the introduced 5 became infected in December 1986 after a period of 7 months. Infection in one camel, namely "Ale", was detected by ELISA, M.I.T. and B.S.T. tests by March 1987, while the second camel, namely "Kurach" was positive for trypanosomes based on ELISA, M.I.T. but not the B.S.T. These infected camels were still positive by March 1987 after which time the experiment was brought to an end. The objective was to determine the "timing" of transmission of camel trypanosomiasis in the study area. Infection of sentinel camels confirmed active transmission of camel trypanosomiasis within the two herds in northern Kenya during the period of study. It is also important to note that infection was picked by the sentinel camels in both herds during the short rains in December 1986. Worth noting also is the fact that throughout the course of the study, the camel herds

TABLE 7: Transmission of camel trypanosomiasis as detected through infection of sentinel camels

Camel No. or name	Date Screened and results				Date Introduced into herds	DATES CHECKED FOR INFECTION AND RESULTS											
						June 1986			August 1986			December 1986			March 1987		
	Date	ELISA	MI	BS		ELISA	MI	BS	ELISA	MI	BS	ELISA	MI	BS	ELISA	MI	BS
93	3-86	(-) .211	(-)	(-)	4-86	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(+)	(+)	(+)
67	3-86	(-) .174	(-)	(-)	4-86	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
63	3-86	(-) .186	(-)	(-)	4-86	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
98	3-86	(-) .134	(-)	(-)	4-86	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
58	3-86	(-) .093	(-)	(-)	4-86	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Daye	4-86	(-) .214	(-)	(-)	5-86	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Chamaa	4-86	(+) .0.360	(+)	(-)	5-86*	(+)	(-)	(-)	(+)	(-)	(-)	(+)	(-)	(-)	(+)	(-)	(-)
Ale	4-86	(+) .384	(+)	(-)	5-86*	(+)	(-)	(-)	(+)	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
Bamai	4-86	(-) .074	(-)	(-)	5-86	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Kurach	4-86	(-) .213	(-)	(-)	5-86	(-)	(-)	(-)	(-)	(-)	(-)	(+)	(+)	(-)	(+)	(+)	(-)

Key: * Date treated with Trypacide®

(-) Negative for trypanosomes

(+) Positive for trypanosomes.

under investigation were not herded into the known *Glossina* spp. zones in the area, as depicted on Fig.9 & 10. Furthermore, the Olturot herd was a stationary herd that remained in the same area for the total period of this study. The Kargi herd was nomadic but its movements were monitored and were limited to areas shown on Figs. 9 & 10 which were entirely within the *Glossina*-free study sites.

2.4: D I S C U S S I O N.

2.4.1: Screening of animals for trypanosomiasis using the ELISA method.

The Presence of *T.evansi* in camel herds within the study area was confirmed following screening of sera using the ELISA test.Infection rates in camel herds ranged from 72-95% an indication of either past or current prevalence of trypanosomiasis in the herds that were screened. The Olturot herd no.6 and Ngurunit herd no. 5 which had the highest infection rates based on ELISA results were thus identified for further investigations. ELISA was therefore quite useful for large scale screening of sera samples for past and or current trypanosomiasis challenge within a camel herd. The ELISA method was simple, objective and required only simple field procedures and equipment; sera was obtained using a simple procedure and readily preserved by freezing in portable liquid Nitrogen containers. Observable colour change in positive cases was another added advantage to the ELISA test.In addition to observations in this study on the suitability of ELISA for mass screening of animal trypanosomiasis, there is support for this method by other workers such as Askar and Ochillo (1972), Momire *et al.* (1980), Luckins (1977), Luckins and Mehlitz (1978) and Wilson (1969) all of whom reported on

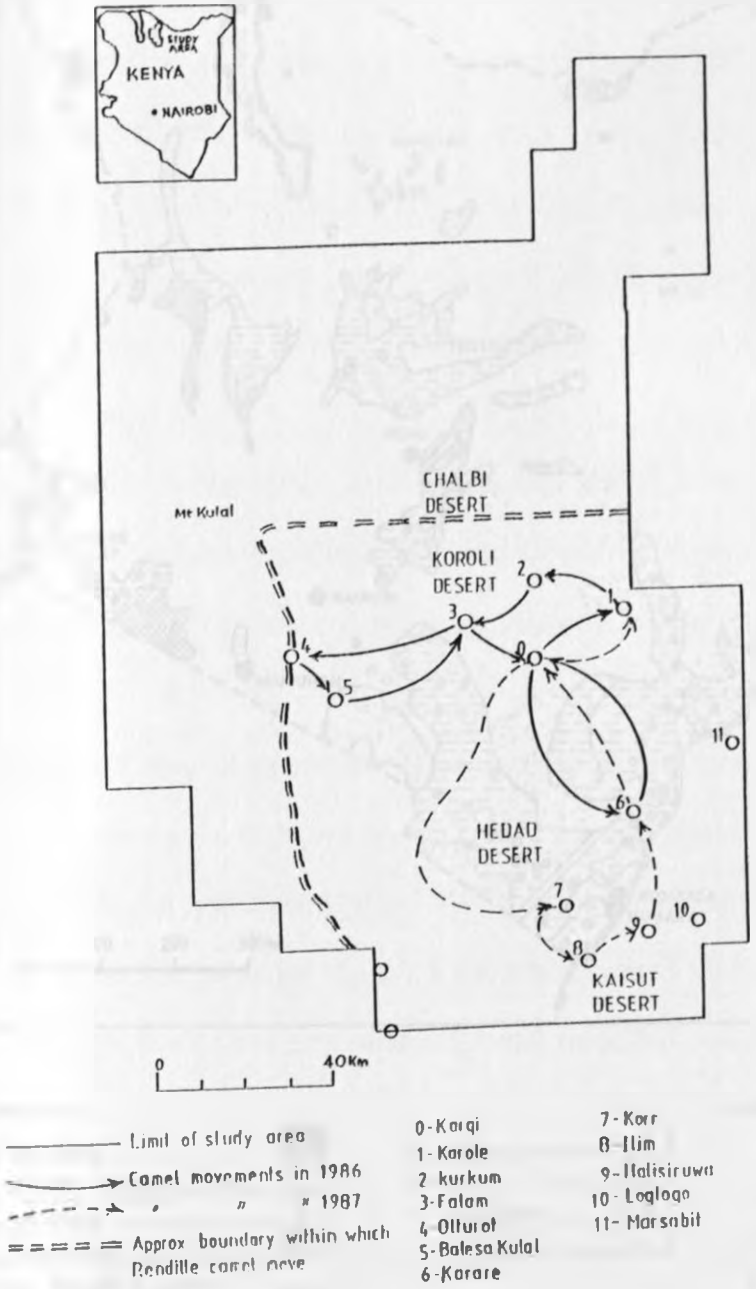

Fig. 9 :Map showing movements of the nomadic Kargi camel herd into which five sentinel camels were introduced between April 1986 and March 1987

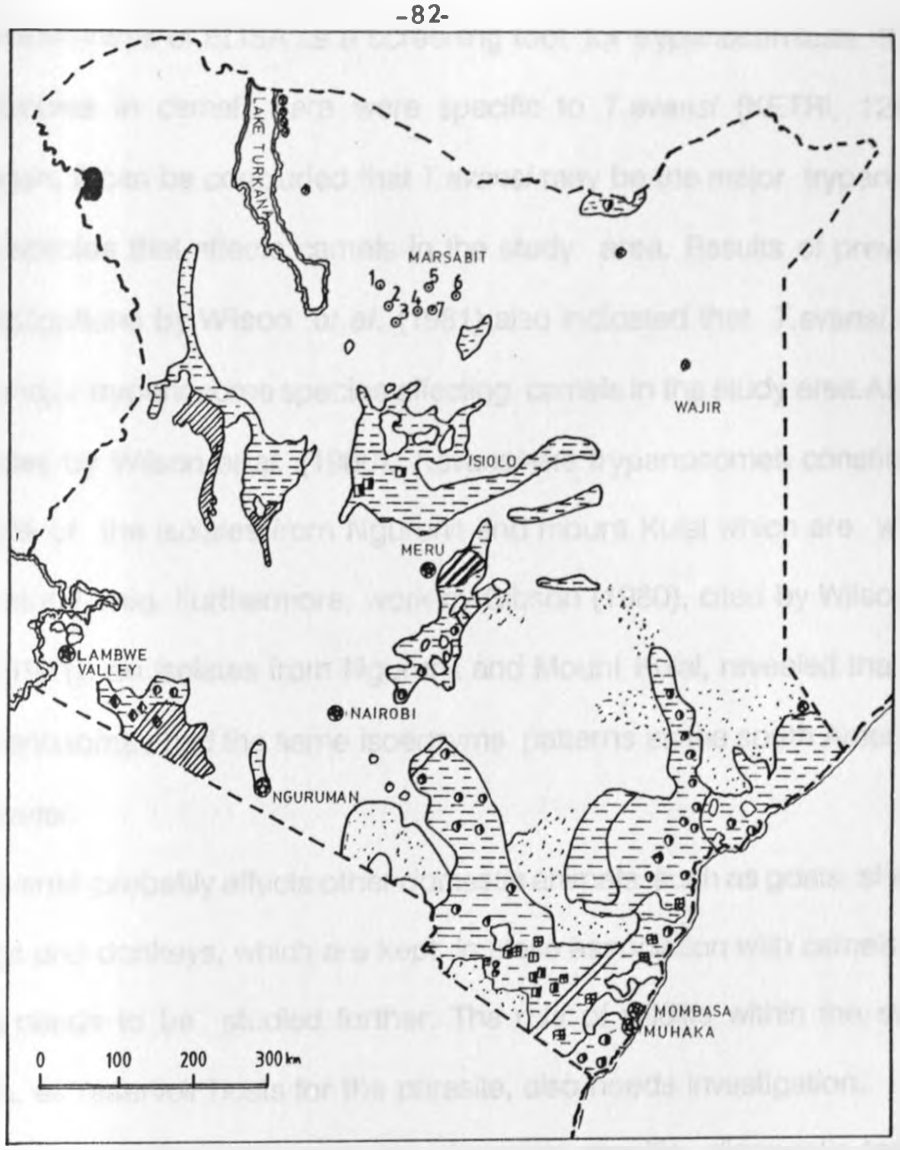

Fig.10

:Distribution of tse-tse (Glossina spp.) in Kenya. Atlas of Kenya 1970 (Survey of Kenya).

the usefulness of ELISA as a screening tool for trypanosomiasis. Since antibodies in camel sera were specific to *T.evansi* (KETRI, 12429) antigen, it can be concluded that *T.evansi* may be the major trypanosome species that affects camels in the study area. Results of previous investigations by Wilson *et al.* (1981) also indicated that *T.evansi* was the major trypanosome species affecting camels in the study area.As per studies by Wilson *et al.* (1980), *T.evansi*-like trypanosomes constituted 95.5% of the isolates from Ngurunit and mount Kulal which are within the study area. Furthermore, work by Gibson (1980), cited by Wilson *et al.* (1981), on isolates from Ngurunit and Mount Kulal, revealed that the trypanosomes had the same isoenzyme patterns as the south American *T.evansi.*

T.evansi probably affects other domestic animals such as goats, sheep, dogs and donkeys, which are kept in close association with camels but this needs to be studied further. The role of wildlife within the study area, as reservoir hosts for the parasite, also needs investigation.

Although the ELISA test was the most specific diagnostic test of camel trypanosomiasis, it was least sensitive in differentiating active from past trypanosome infections in camels that were screened. The ELISA test did not detect seasonal changes in trypanosome infections although there may have been such seasonal fluctuations. This was so because diagnosis using ELISA was based solely on detection of antibodies in camel sera. Trypanosomal antibodies have been reported (Luckins *et al.*)to persist following drug therapy. As a result of this, differentiation of active infections from previous infection status of individual animals by ELISA becomes difficult. In epidemiological studies of trypanosomiasis, where active infections require to be differentiated from previous

infections, the ELISA cannot be used. This relies on other diagnostic tests which can detect active infections.such as the MIT and BST.

2.4.2: The MIT as a diagnostic method for trypanosomisis.

The MIT proved to be the best diagnostic method to diagnose active trypanosome infection in camels in northern Kenya, not only because it was sensitive but because it detected only patent infections at the time of bleeding. Since MIT detects only active infections,it becomes an important diagnostic test to determine which individual infected animals need to be treated as opposed to mass treatment which is expensive. The reliability of mouse inoculation as a diagnostic test for animal trypanosomiasis has been reported by many workers [Boyd, 1986; Wilson *et al.*, 1984; Baker, 1996 and Killick- Kendrick, 1962]. In this study, the reliability of this test has been further confirmed although previous workers such as Wilson *et.al.* (1980) reported higher trypanosome infection rates [71.4%] in camels in the same study area. A 71.4% trypanosome infection rate is strikingly high as compared to 11.9% obtained in this study.In another report by Wilson *et.al.*(1981) MIT revealed only 6.3% camel trypanosome infection in samples from Ngurunit,mount Kulal, Ol maisor and Galana ranch. A possible explanation for this discrepancy might be the consistent trypanocidal drug therapy the northern Kenya camels have been receiving over the years,both from the Government Veterinary Officers and individual researchers, ever since there were reports of camel trypanosomiasis in the region by Wilson *et.al* [1981]. The other possibility could have been due to high biting fly population which mechanically transmitted camel trypanosomiasis during the particular year of study by Wilson *et.al.*

[1981]. Almost similar to the findings of this study, are results reported by Godfrey and Killick-Kendrick [1962], who detected up to 27.6% (40/145) trypanosome infection rate in 145 camels sampled in Nigeria. Rodent inoculation has also been shown by Pegram and Scott (1976); Schillinger (1982); Godfrey and Killick-Kendrick (1963) to be a sensitive diagnostic method for both patent and subpatent infections of camel trypanosomiasis.Subpatent infections are cases in which trypanosomes cannot be detected by examination with a X40 objective, of 200 microscope fields of a stained thick blood smear (Lumsden,1977). Work by Paris *et al.*(1984) at I.L.R.A.D., showed that inoculation into laboratory mice was more sensitive for detection of *T.brucei* than the BST and centrifugation.

Although the mouse inoculation test was found to be the best diagnostic test, a number of technical problems were encountered with this technique in this present study. Transport of mice by road resulted in several deaths as a result of heat stress due to the arid conditions of the study area. Water and food for the mice had to be carried to the study site to maintain them during field trips. This technique was however the best in that it was inexpensive, reliable and simple and could detect active infections. Until cheap immunodiagnostic methods which can detect antigen and distinguish past from active infections are developed, MIT may remain the best available diagnostic tool for active camel trypanosome infections.

2.4.3:BST as a diagnostic method for animal trypanosomiasis.

The blood smear technique was the least sensitive method to diagnose camel trypanosomiasis in this study. It was least sensitive because it missed on infections that were detected by other two diagnostic tests. Other workers such as Rutagwenda [1985] have reported camel trypanosome infection rates as high as 20.4% using the wet film technique which is similar in principle to the blood smears. The BST has been used in Baringo,Kenya by Wilson and Njogu (1981) to detect *T.evansi* infection in camels and it revealed 7.7% infection rate for the 65 camels tested. Godfrey and Killick-Kendrick [1962] detected 12.4% infection rates in camels in Nigeria suggesting that it is a useful diagnostic test for trypanosomiasis but ought to be used in conjunction with more sensitive tests such as mouse inoculations because it misses cases of low parasitaemia (subpatent infections). In infected animals with high parasitaemia, blood smears can be useful in determining active trypanosome infections. It should be noted that detection of camel trypanosomiasis even using one of the least sensitive tests such as the blood smears successfully determined the occurrence of camel trypanosomiasis in northern Kenya which was one of the objectives of the study.

2.4.4: Comparative trypanosome infection rates during the wet and dry seasons.

A general trend of elevated trypanosome infection rates during the wet months (March - May, October - December) was noted in this study. During the dry months (June - September) infection rates were lower.

Fluctuations in infection rates for the two seasons were most noticeable based on mouse inoculation than on blood smears. A fall in trypanosome infection rates during the dry season [June-September] has been reported by Wilson *et.al.* [1981] but based on the Mercuric Chloride Test and Indirect Haemagglutination Test. Field [1985], also reported a rise in trypanosome infection rates in camels during the wet seasons and noted that these elevations in trypanosome infections coincided, not only with the rains but, also with an increase in biting fly numbers at Ngurunit. Prevalence of camel trypanosomiasis during the wet season was also observed by Olaho and Wilson (1981) in Kenya and by Lesse (1914) in some parts of East Africa. These workers also noted that during the wet seasons, biting flies were abundant. Statistical analysis of data relating to infection rates in camels for the wet and dry seasons based on blood smears and mouse inoculation revealed higher infection rates in the wet than in the dry seasons. These confirm previously reported differences in infection status of the northern Kenya camels during the wet and dry seasons by Field [1985] and Wilson *et. al.* [1981]. and implicate mechanical transmission of the parasite by these biting flies.

2.4.5: Trypanosome infection of sentinel animals.

In this study,three out of the 10 (30%) sentinel or contact camels employed in this study became infected after a duration of 7-9 months (210-270 days) in the apparent absence of *Glossina* spp.

Trypanosome infection of sentinel camels is indicative of mechanical transmission of camel trypanosomiasis in northern Kenya by biting flies other than *Glossina* spp. because this survey was conducted in a

Glossina spp. free area. According to the available tse-tse distribution maps of Kenya, the study area is *Glossina* spp. free. Lack of *Glossina* spp. in the study area further supports the concept of mechanical transmission of trypanosomiasis in the region.In a similar study ,Lucas (1955) put 16 healthy cattle along with 4 *T.congolense* infected cattle in a presumed *Glossina* spp.-free field situation in western Uganda. In 211 days, 5 out of the 16 (31.25%) previously healthy contact (sentinel) cattle became infected. At the same site using a different strain of *T. congolense*, 3 out of 12 (16.7%) of the sentinel cattle became infected in 61 days. Bouffard (1907) put a healthy calf in close contact with a *T.vivax*-infected calf in a fly proof stable in the presence of 40 wild caught *Stomoxys* spp. The previously healthy calf became infected 12 days later. Use of sentinel animals is therefore a useful tool in assessing transmission of trypanosomes under field conditions.It should be noted however, that sentinel animals may not always pick up infection as was revealed by studies by Folkers and Mohamed (1965) in Nigeria.It is possible that infection of sentinel animals is likely only when biting flies are abundant at a time of high parasitaemia in the infected animals. An extended experimental period of several months is also necessary before making conclusions as to whether the sentinel animals can pick up the infection from infected animals.It is important to note that the 3 sentinel camels used in this study, became infected during the wet season in December,1986 when biting flies were available.

CHAPTER THREE

IDENTIFICATION, AND BITING ACTIVITY OF POTENTIAL MECHANICAL VECTORS OF CAMEL TRYPANOSOMIASIS

3.0 INTRODUCTION AND LITERATURE REVIEW

3.1.1: Collection of haematophagous insects of medical and veterinary importance.

The purposes of insect collection in general range from collection for taxonomic studies, ecological studies as well as collection as a means of reducing and thereby controlling harmful insect populations. In this study, a review of insect collection methods will be restricted to adult haematophagous insects of medical or veterinary importance. A thorough review of sampling of all kinds of insect populations has been given by Southwood (1966).

In the study of insect vector groups, sampling methods have been designed to determine geographical distribution, seasonal variation and abundance, association of vectors with different types of habitats such as vegetation in the case of *Glossina* spp., and aquatic breeding grounds in the case of mosquitoes and *Simulium* spp.

Other objectives for sampling insect vectors are to collect sufficient material for dissections for associated studies on parasites, viruses or

other pathogens, or to study transmission related behaviour such as biting and blood-feeding activity , or degree of contact between vector and host. Dissections of vectors vary depending on the vectors and pathogens involved. Dissections of the salivary gland, gut and proboscis of *Glossina* spp. for instance is usually aimed at determining infection rates by trypanosomes and to identify species of trypanosomes involved. Similarly, salivary gland dissections in Anopheline mosquitoes may show rates of sporozoite infections (Venters *et al.*,1974). Dissections of *Glossina* spp. ovaries using the ovarian age-grading technique may provide information on age of a given population (Snow,1980). The significance of age-grading is that it provides information related to adult mortality patterns of a given population.

In order to asses the source of insect blood meals,stomach contents of freshly killed or recently engorged blood-sucking insects, smeared on filter paper, may be subjected to immunological precipitin tests. Since 1967, the Imperial College Field Station in England has provided the test centre for identification of blood meal source for blood-sucking insects from all over the world. (Muirhead-Thomson,1982).

As a result of the great diversity in insect life cycles and habitats and the varied reasons for sampling insects, sampling methods have been greatly developed and in some cases trap development has culminated in such traps being used as a means of vector control as is the case with *Glossina* spp. where biconical traps impregnated with Deltamethrin has been implemented as a control measure (Laveissiere *et al.*,1978).

Insects of medical or veterinary importance whose past and current sampling techniques are reviewed in this study include Dipterans of the families;Psychodidae (sand flies),Culicidae (mosquitoes),Simulidae (black flies),Ceratopogonidae (midges and gnats), Muscidae (e.g *Glossina* spp., *Stomoxys* spp,and *Haematobia* spp.) and Tabanidae (deer flies and horse flies). There exists a great diversity of insect trapping (capture) methods which cannot all be reviewed except for a few examples.

The two major types of haematophagous trapping methods are broadly categorized into direct and indirect methods. The direct methods involve collection of samples from bait animals, humans or any devices attractive to insects. Insects that land on baits or any attractant devices are subsequently collected by sweep-nets, forceps, aspirators or simply by hand following knock down by insecticides (Morris, 1960; Service,1977a). A wide variety of flies such as blackflies (Service,1977a); mosquitoes (Muirhead-Thomson,1982; Service,1977b; Jupp,1978); midges (Jamback and Wathews,1963; Turner,1972; Kettle,-1969a); sandflies (Disney,1969; Ovazza *et al.*,1965; Le Berre *et al.*,1964); *Glossina* spp. and tabanids (Nash,1930; Potts,1930; Harley,1965; Barras,1959) have been sampled using the direct collection **method from bait animal hosts.**

There have been several modifications to the direct collection method from animal bait hosts; for example animal baits may be secured in an enclosed structure which may be periodically opened to allow insects to enter. Insects thus trapped may be subsequently collected by sweep-nets, aspirators etc. Traps that operate on this principle take the

form of an artificial stable and are usually constructed of wire or mosquito gauze on a wooden framework. Such traps have been given a variety of names including Magoon, Stable and Egyptian traps (Muirhead-Thomson,1968), and bed-nets (Service.1977a). Such traps have been used extensively to estimate population changes and seasonal population trends in vector densities by workers such as Harley (1965) who sampled *Stomoxys* spp and tabanids in Uganda. Traps of this type are however considered satisfactory only in areas of very high vector densities as their performance at low vector densities is still debatable (Muirhead Thomson,1968).

Another variation of Stable-type traps is the use of bait animals under suspended large collapsible nets (Muirhead-Thomson,1968) or tents (Service,1977 a). The device may be periodically lowered and thereby it encloses insects attracted to the bait animal.John *et al.*(1984) used such a collapsible tent, baited with a cow and managed to collect simuliids, ceratopogonids, culicids and tabanids in Newfoundland, Canada.

Haematophagous insects may be captured directly by placing a bait animal inside an artificial pit shelter or placing the bait animal inside a cage in an existing pit shelter. This method has been employed in sampling studies of Anopheline mosquitoes (Wharton *et al.*,1963) and *Glossina* spp.(Hargrove,1976).

A different kind of direct trapping that has been employed particularly in *Glossina* spp. sampling , is the use of a mobile crush, baited with an animal. This method is based on the principle that

haematophagous flies are attracted to moving objects. The bait animal may be replaced by a trapping cage or electrical grid of wires mounted on the back of a vehicle. The grid wires thus electrocute insects that land on it following attraction to the moving vehicle (Swynnerton,1936).

As opposed to direct methods, the indirect methods involve the use of specially designed traps placed at strategic points with or without animal baits to collect flies independently of the observer. Whereas some of these indirect trapping devices are based on the principles of either visual or odour as stimuli for the flies, others simply obstruct insects in flight and these are subsequently trapped in collecting cages within the traps.

Attraction of insects to light has led to the use of battery operated light traps to sample a wide variety of haematophagous insects. Some of the indirect trapping devices have been developed to eliminate the presence of man which may be repellent to flies (Jordan,1986). Most of the indirect trapping devices however have a common advantage in that they have been specially designed to efficiently capture specific insect vectors under specific conditions and they are not labour intensive as they only require periodic checking.

Some of the indirect trapping devices that have been employed in the capture of haematophagous insects include light traps, electric nets or screens, suction or fan traps, sticky traps, simulated animal models or silhouettes and other mechanical devices which incorporate bait host animals, odour and visual attractants.

Battery operated light traps such as CDC light traps and Disney traps are generally used to collect night-biting insects such as midges and mosquitoes (Breyev, 1958 ; Fox,1953 ; Muirhead-Thomson,1982 ; Service,1977c). Light traps have also been reported to successfully catch blackflies by Breyev (1963), Davies and Williams (1962), Williams (1962,1965) and some nocturnal tabanids (Corbet,1964).

Electric nets and screens have been used by many workers to sample haematophagous insects, among them Koch and Spielberger (1979),Randolph and Rogers (1978), Rogers and Smith (1977) and Vale and Phelps (1974) but these are rather expensive, bulky and require constant supervision and maintenance,and present problems in remote areas.

Tubular fan traps (after Fallis *et al.*,1967) that use a 6-volt power supply, and measure 4 inches in diameter by 5 inches long,with four 3 inch blades, have been used to sample biting insects. The traps are suspended from the ends of 12 ft. arms extending from a turntable which moves through 90 degrees every 2 minutes. Flies sucked into the trap are collected in attached gauze bags. Carbon dioxide or hot water bags may be incorporated in the traps as odour and heat attractants respectively. Fan traps baited with carbon dioxide (Fallis *et al.*,1967) or carbon dioxide plus a black bait cloth (Thompson,1976) have been used successfully to sample blackfly species. Suction traps which operate on the same basic principles as fan traps have been used by Bidlingmayer (1974) and Service (1971) to sample *Culicoides* spp. and mosquitoes with much success.

Sticky surfaces have been routinely used to capture sandflies (Davies,1957; Dergacheva *et al.*,1979: Disney,1966), blackflies (Bellec,1976),*Glossina* spp.(Vale,1969) and tabanids (Okiwelu,1977). Adjustable sticky sandfly traps for indoor trapping were found efficient in capturing sandflies in Machakos, Kenya by Mutinga (1981). Blackfly and mosquito species were also found in these traps.

Further details of trapping techniques for various insects groups have been reported by the following authors; collection methods for adult mosquitoes have been reviewed by Muirhead-Thomson (1982) and Service (1977c). A comprehensive review of sampling methods of adult blackflies has been given by Disney, (1766); Fallis *et al.*, (1967) ; El-Bashir *et al.*; (1976); Service (1977b) and Thompson (1976).

Sampling methods for midges and sandflies have been reported by Kettle (1969a,1969b,1969c); Service (1974); Tanner and Turner, (1975); Thatcher and Hertig (1966) and Ward *et al.*(1973) respectively.

It should be noted that some traps originally designed to sample a particular group or genus of haematophagous flies have demonstrated an efficiency in sampling other biting flies as well. Also worth noting is the fact that most reports on mechanical traps or animal silhouettes (models) have been aimed at trapping *Glossina* spp. because of the tremendous research attention *Glossina* spp. have received in tropical Africa. Collection of other haematophagous flies such as tabanids and *Stomoxys* spp. has therefore depended on *Glossina* spp. collection techniques.

Silhouettes that assume the shape of animals that have been used to collect *Glossina* spp. include the Morris trap after Morris (1963); the Langridge's Box Screen and Moloo's Awning Screen Skirt as described by Moloo (1973).

The Morris trap is cylindrical, assuming the shape of a goat-sized animal. The curved body is open at the bottom. Flies attracted to this opening from the undersurface are directed to a slit along the upper surface of the body, over which a cage is superimposed. A non return device thus traps all insects that enter the cage. The Morris trap was originally designed in West Africa for studies on *Glossina palpalis* and *G.tachinoides*. Later a modified form of the Morris trap was found to be very effective for trapping *G.pallidipes* in East Africa (Muirhead Thomson,1982). Work by Morris (1963) in Liberia revealed that a double sized Morris trap (4x4 ft.) could attract not only *Glossina* spp. but many species of tabanids and these have been used routinely to sample tabanids in north Africa. Morris traps have also been found to attract tabanids in Uganda (Muirhead Thomson,1982).

The Langridge's box screen was once established by the Kenya Tsetse Survey and Control Department in Kibwezi area as the most efficient trap for *G.pallidipes* , while work by Van-Etten (1977) in Nkuruman, Kenya revealed that the Awning screen skirt was the most efficient trap for sampling *G.pallidipes* in the area. This trap has a major drawback in that it is not collapsible and therefore not easy to transport.

Box shaped traps have also been used to sample *Glossina* spp. The earliest trap of this kind was the Harris trap after Harris (1938). This trap

is box-shaped (2x1x1 ft.) with a non-return "V" shaped entrance underneath, and is suspended about 0.7 m above ground. The Harris trap was found very efficient in collecting *G.pallidipes* in Zululand and even led to great reductions in numbers of this fly species when used for control purposes (Harris,1938). More recently, a box trap of about 1 m cube with a collection cage on top, has been found efficient for collecting *G.m.morsitans* and *G.pallidipes* in Zimbabwe (Vale,1982).

In recent years, development trends to improve *Glossina* spp. trapping have been exemplified by developments of the vertical vane traps (Hargrove,1977); monoconical traps (Lancien,1981) and the biconical trap (Laveissiere and Challier,1973). These traps differ from most other mechanical traps in that they do not attempt to assume an animal shape (Owaga,1981).

The vertical vane trap has been used extensively in *Glossina* spp. studies in East Africa and Zimbabwe (Hargrove,1977). The monoconical trap has been used more as a control trap when impregnated with Deltamethrin in Zaire (Lancien,1981). The monoconical trap is essentially a target, as the attracted insects are not retained. This trap has been used to control *G.fuscipes quanzensis* (Lancien,1981) in Zaire.

The biconical trap was originally designed for riverine *Glossina* spp. of West Africa, particularly *G.palpalis gambiensis*, Vanderplank. The attractive element in its design is the contrast between the black and blue colours. The biconical trap has proved to be one of the most efficient *Glossina* spp. traps in East Africa (Owaga,1981) and other parts of Africa. The biconical trap has been used extensively for *Glossina* spp. trapping

in Kenya (Dransfield *et al.*,1983; Owaga,1980,1981; Turner,1981; Van-Etten,1977), in the Niger and Mozambique (Turner,1980) and Ivory Coast (Jordan,1986)

Work by I.C.I.P.E. scientists (Owaga,1980; Turner,1981) has shown that incorporation of natural host odour attractants such as buffalo urine with the biconical trap increases the overall efficiency of the trap. Other odour attractants, usually incorporated into the biconical trap or other traps include, artificial odours such as carbon dioxide and acetone (Vale,1980), or both the natural host odour plus carbon dioxide (Vale,-1977; Hargrove and Vale,1979). Currently the biconical trap is widely used to trap *Glossina* spp.in many parts of Africa (Jordan,1986).

The latest development in *Glossina* traps is the Ngu trap at I.C.I.P.E. by Brightwell *et al.* (1985). The Ng2B trap has proved to be an efficient trap for sampling *Glossina* spp. at Nguruman, Kenya.(Otieno, 1989). Kabaterine (1989, Personal communications on unpublished data), however found the biconical trap to be more efficient than the Ng2B trap in Lambwe Valley, Kenya.

Although tabanids have often been captured in traps designed for *Glossina* spp., there have been some specific developments geared to specific tabanid trapping such as the Morris trap in Liberia by Morris (1963). Another development in tabanid trapping was the Manitoba Horsefly Trap designed by Thomson (1969). This comprises of a tripod with a collection container fitted at the apex. Suspended beneath the cone is a visual attractant in the form of a black sphere, which has been found to attract these flies. This trap has been used to sample tabanids in New Jersey ,U.S.A. by Thompson and Pechuman (1979) but has not

been exploited in tropical Africa probably because tabanids have been caught exclusively in *Glossina* spp. traps.

Another development in tabanid trapping was the Malaise Trap designed by Malaise (1937) and further modified by Townes (1962). The Malaise trap operates on the principle that flying insects are obstructed by baffles and fly upwards into the collection apparatus. The Malaise trap has been modified to include carbon dioxide emission to simulate the presence of a host animal. The Malaise trap has been used successfully to collect tabanids by many workers including Smith *et al.* (1965); Roberts (1970,1971a,1971b, 1972, 1974, 1975); Blume *et al.* (1972) and Okiwelu (1975).

A canopy trap (Catts,1970), was another development in tabanid trapping. This trap has combined features of the Malaise trap and the Manitoba Horsefly trap. The canopy trap was found effective for collecting a variety of blood-sucking flies including mosquitoes, blackflies and stable flies in addition to tabanids in Delaware,U.S.A. According to Catts (1970), the canopy trap captured as many as 1,000 tabanids per hour.

Although different traps have been developed to suit different fly species, it is important to note that in situations where populations of *Glossina* spp. and other biting flies are to be monitored concurrently, the biconical trap is a suitable choice. This is because although originally designed to collect *Glossina* spp., the biconical trap has been shown to attract other species of biting flies such as tabanids (*Atylotus agrestis, A.fuscipes, Tabanus taeniola, Stomoxys nigra, Haematobia minuta, Haematobosca unisraiata* and *Musca crassirotris*) by Dransfield *et al.*

(1983). In addition to being capable of attracting biting flies other than *Glossina* spp., the biconical trap offers the advantage of being easily assembled, portable and sturdy (Owaga,1981; Turner,1981). The biconical trap is also not likely to be stolen due to the holes (slits) on the cotton material part of the trap. It is for this reasons that the biconical trap was adopted as a means of collecting biting flies in northern Kenya, together with the hand-net collection method. Presence or absence of *Glossina* spp. had to be monitored in this study, hence the use of this trap as a single efficient means to sample both *Glossina* spp.and other biting flies in the study area.

3.2: MATERIALS AND METHODS.

3.2.1:Collection of biting flies from the study area for taxonomic and morphological appraisal.

Biting flies were collected from the entire study area at Kargi, Korr, Ilaut, Olturot and Ngurunit sites using two basic methods, the direct sweep-net and the indirect Challier (biconical) trapping methods. The different study sites were chosen because each had permanent U.N.E.S.C.O. (I.P.A.L.) camp sites with accommodation facilities, water, field transport and field assistants who could be trained to assist in routine insect collection work. Since the objective of this study was to capture and identify a wide variety of biting flies, it was necessary to sample areas with different ecological habitats. This was in line with the concept that different biting fly species may be found in the different ecological habitats. These five sites had different climatic and vegetation

attributes as already mentioned in section 2.2.3 above. It should be noted, however, that site differences was not an objective under investigation, the aim of the exercise was to collect or trap sufficient samples from a wide sampling area in order to obtain enough material for taxonomic studies. Collection of biting flies was designed to be as random as possible; in undertaking collections from camel hosts using sweep-nets, camel hosts were randomly chosen regardless of sex, age or health status of the animal. Similarly, the biconical traps were placed on randomly selected transects at all study sites as described below.

3.2.1.1: Sweep-net collections.

Insect sweep-nets (Plate 8) were used to collect biting flies directly from camels between 0600 h and 1600 h from January 1986 to December 1987. The morning collections were undertaken between 0900 h within the animal enclosures after which, camels left immediately for grazing sites. Collections of biting flies were continued at grazing sites up to 1600 h when camels returned to the enclosures. Biting flies were located principally from the camel belly and legs. Collected specimens were emptied into kilner jars and killed, within the jars, using chloroform soaked in cotton wool. Specimens were then preserved in 70% alcohol and transported back to the laboratory. All flies collected were identified using standard taxonomic keys (Borror and De Long, 1976; Oldroyd, 1952, 1954, 1957). Following identification in the laboratory, samples of Tabanidae and Hippoboscidae were sent to the Entomology section of the British Museum, London, U.K. for confirmatory taxonomic diagnosis.

Plate 8 : Hand-net used to collect biting flies from camels at Olturot study site.

3.2.1.2: Challier (biconical) trap collections

Challier, (biconical) traps were constructed at I.C.I.P.E. using specifications of Challier and Laveissiere (1973) shown on Plate 9 and Fig.11. The main components of a biconical are the upper and lower cones, a supportive pole , an additional smaller upper cone and a collecting cage. The upper part of the larger cone is made out of an outer white insect netting material ("c" on Fig. 11) and an inner black cotton material ("e1" on Fig.11). The lower cone consists of an outer blue cotton material ("d" on Fig. 11) and an inner black cotton material ("e2" on Fig.11). The lower cone has slits ("f" on Fig.11) to expose the black cotton cloth underneath, and through which the insects enter into the trap. The lower and upper cones are separated by a circular metal rim ("r" on Fig.11) of a diameter of 80 cm. The trap is supported by a pole ("g" on Fig.11) which is 203 cm long. The lower end of the pole is pointed and is pegged into the ground to hold the trap upright. The upper end of the pole is hollow and the smaller upper cone ("b" on Fig.11) fits into this hollow part of the pole. The smaller upper cone is made out of a metal framework and covered with white cotton cloth. The collecting cage ("a" on Fig.11) fits into the smaller upper cone as shown on Fig.11. The collecting cage is made out of a rectangular metal framework and is covered by a removable white cotton material when in use.

The traps were baited with camel and cow urine as olfactory attractants. The reason for baiting the traps was to optimize the size of the catch following I.C.I.P.E. reports that buffalo urine (Owaga, 1983) and cow urine and acetone (Dransfield *et al*. 1985) incorporated with these

Plate 9 : A biconical trap to collect biting flies and monitor Glossina spp. at Ngurunit study site.

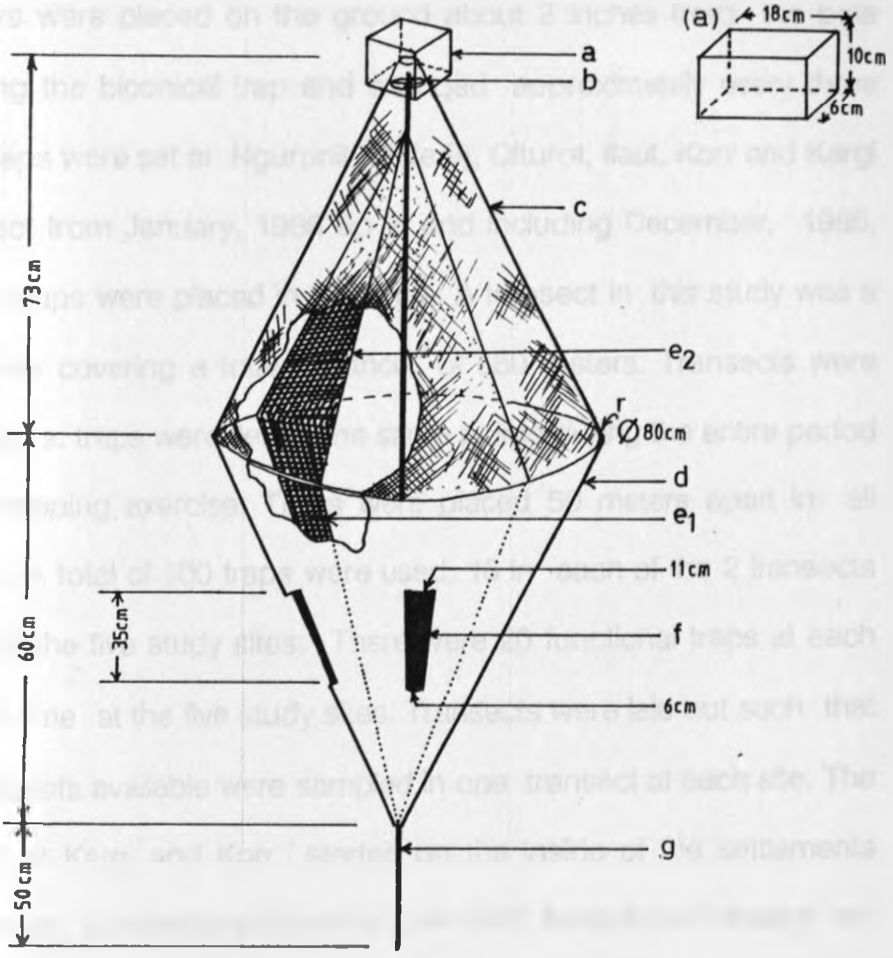

Fig 11 Diagram showing different components and dimensions of the biconical trap. (After Challier and Laveissiere, 1973).

traps, increased the size of the catch of *Glossina* spp. In this study, camel and cow urine were obtained locally within the study area. These were placed in plastic containers with perforated covers. The plastic containers were placed on the ground about 2 inches from the pole supporting the biconical trap and changed approximately every three days. Traps were set at Ngurunit (Plate 9), Olturot, Ilaut, Korr and Kargi with effect from January, 1986 up to and including December, 1986. Biconical traps were placed in transects. A transect in this study was a straight line covering a total distance of 550 meters. Transects were stationery i.e. traps were left at the same points during the entire period of the trapping exercise. Traps were placed 50 meters apart in all transects. A total of 100 traps were used, 10 in each of the 2 transects at each of the five study sites. There were 20 functional traps at each particular time at the five study sites. Transects were laid out such that mixed habitats available were sampled in one transect at each site. The transects at Kargi and Korr started on the inside of the settlements running in an outward direction into open land. At Ilaut one transect ran northwards from the main settlement, passing through both open land and areas with scattered *Acacia* spp trees. At Ngurunit one transect started from a pastoralist homestead running into the surrounding *Duosperma* spp and *Cordylobia* spp dominated bushland. This transect faced the foothills of the Ndoto Mountains (Fig. 5) from the homestead. The second transect at Ngurunit ran parallel to the Ngurunit River starting from the U.N.E.S.C.O. campsite. This transect ran through thick bushland and *Acacia* spp woodland area. At Olturot,one transect ran

through an open grassland area from the U.N.E.S.C.O. bore hole to the nearby airstrip. The second transect started from the U.N.E.S.C.O. campsite and ran into a nearby bushland and woodland area. The traps were emptied daily. Whenever traps were torn they were either replaced with new ones or repaired. Five field assistants who were local pastoralists, were seconded, one at each of the five study sites. Following initial training of these assistants, they emptied biconical traps daily. Trap contents were subsequently counted and counts entered into field data sheets. Samples were then stored in labelled jars for respective traps and trap sites. All traps were similar in size and colour. The effect of the attractant was not assessed as this was outside the objectives of the study.

3.2.1.3: Box Trap collections

The Box trap (Plate 11) was not originally designed for use in this study but a K.E.T.R.I. team of scientists visited one of the study sites (Ngurunit) and set two box traps for two consecutive days at Ngurunit in May 1987, to specifically capture *Glossina* spp.

3.2.1.4: Morphological appraisal of collected biting flies.

After completion of field collection, pictorial sketches of the specimens were made in order to emphasis some of the external morphological features useful in distinguishing the collected flies.This was accomplished by viewing specimens under a dissecting binocular microscope and sketching the entire fly, antennae, wings and head regions where appropriate. Original sketches were transferred onto tracing

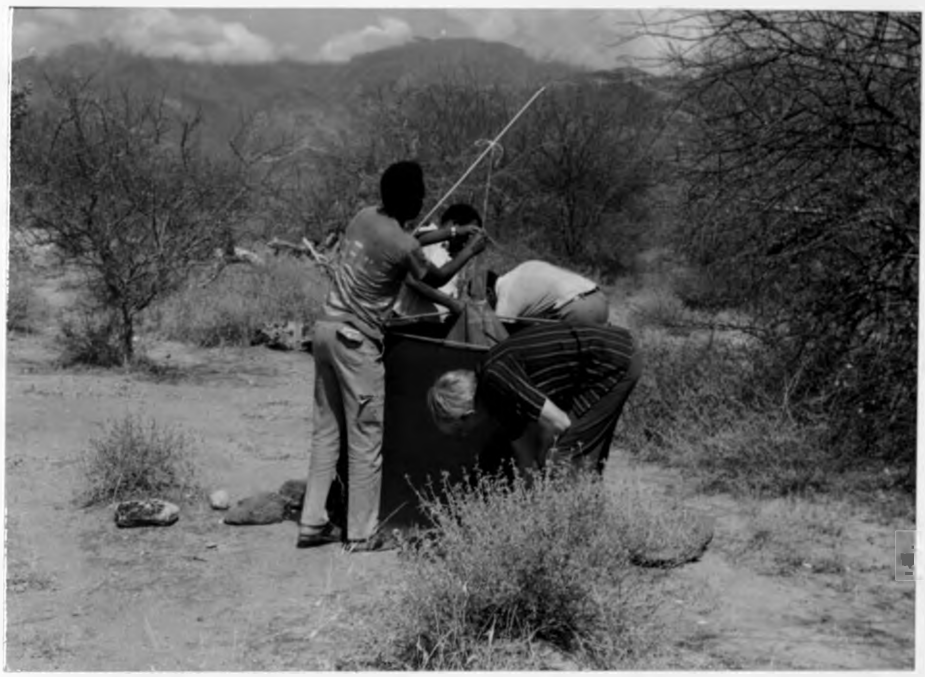

Plate 10 : A box trap to monitor Glossina spp. and collect biting flies at Ngurunit study site.

paper. Detailed measurements of certain dimensions of the fly body parts such as body length and width, wing size and proboscis length were taken and averaged.

3.2.2: Assessment of diurnal biting activity of biting flies in the study area

Observations on blood-feeding patterns (biting periodicities) of flies were made at Ngurunit site after the long rains in May and June 1986. Observations on blood-feeding activity of *H.camelina* and *H.minuta* were however made at Korr study site in September 1986 because these flies were not entirely seasonal and were present on camels all year round. Parameters that were examined in relation to biting fly diurnal blood-feeding activity included peak blood-feeding hours of the day, and preferred biting (predilection) sites on the host animal. Resting sites of the flies following blood meals was not an objective in this study but was noted when possible. To determine peak blood-feeding hours of the day, numbers of biting flies attacking the camel host were quantified at hourly intervals in the course of a 12 hour observation period. Peak biting periods of *S.calcitrans, T.taeniola*, and *A.agrestis* which landed on camels only to blood-feed, were based on maximum hourly counts of flies per animal. Since *H.camelina* and *H.minuta* tended to be permanently ectoparasitic on camel hosts and did not land on the hosts specifically to blood-feed, peak blood-feeding hours of these flies were not based on numbers on hosts but on host reaction to the fly bites.

3.2.3: Assessment of seasonal variation and abundance of biting flies in the study area

These studies were undertaken at all the five study sites (Ngurunit, Olturot, Ilaut, Korr and Kargi) for a duration of 24 months beginning in January 1986 to December 1987. To determine seasonal variation and relative abundance of biting flies, two methods, namely sweep-net and biconical trapping were employed. The main objective in this case was to collect large enough samples of biting flies to warrant quantification of any changes (differences) in numbers of respective flies with seasons(monthly changes). This was done with the aim of arriving at a monthly relative index of biting fly population density in the study area. In view of the fact that trypanosome infection rates in camels was concurrently being monitored, it was necessary to determine monthly (hence seasonal) changes in biting fly population densities and relate such changes to incidence of camel trypanosomiasis. Collection or trapping of biting flies was therefore undertaken with two appropriate definitions, which were counts of flies per camel per month and catch per trap per month so as to arrive at a relative population index for each fly species in each month of sampling. Details of the two collection methods used to satisfy the objective are given below:

3.2.3.1: Direct fly counts based on sweep-net collections.

Direct fly counts of biting flies available on camel hosts were undertaken at all the five study sites for 24 consecutive months,(January,1986 to December,1987). Fly counts per camel were arrived at by making counts

of the total number of flies on the camel . The daily counts were consolidated to arrive at monthly counts per camel The biting fly relative population density was therefore based on monthly mean counts per camel. A total of 80 camels were sampled within a period of one month.In order to determine whether monthly fluctuations in fly counts were due to seasonal changes, the monthly means (from 80 camels sampled) were plotted for the 12 months of the year 1986 and 1987. Also presented alongside the plot of fly monthly means were the rainfall profiles of each specific study site to show the relationship between fly numbers and rainfall patterns prevailing during the period of study.

3.2.3.2: Biting fly counts based on biconical traps.

Biting flies were trapped at all five study sites using biconical traps set as described under section 3.2.1.2 above. Daily trap collections were consolidated and averaged to finally provide mean numbers per trap per month for each individual biting fly species collected. Trapping commenced in January 1986 up to and including December,1986. The mean monthly fly data was plotted over the 12 months of the year 1986 to depict any fluctuations in fly abundance with the monthly changes.

In order to determine statistically, whether the monthly fluctuations in biting fly numbers were due to seasonal changes, a completely randomised design for Analysis of Variance (ANOVAR) after Norman (1981) and Bailey (1981) was found suitable and was utilized whenever the raw data was normally distributed.

In this study the null hypothesis (H_o); "all monthly means of biting flies per camel or per trap are equal" was to be tested against the alternative hypothesis (H_a); "all monthly means of biting flies are not equal". Results of the Variance ratio (F) test wherever applicable were summarised in the form of ANOVAR tables. Raw data that was not normally distributed was simply averaged as monthly means of flies per camel or per trap and presented in tables along with respective standard deviations (SD) and in bar charts.

3.2.3.3: Statistical considerations in the study.

In this study the independent variables were the 12 calender months and the number of observations were the fly counts per camel per trap per month. Table 8 below is a simulated ANOVAR table that was followed for data that was subjected to the F-test.

TABLE 8: A generalized ANOVA table after Bailey,1981.

source of variation	d.f.	SS	Error MS	F-value	Probability
Between months	k-1	T^2/n_i-C	M	M/S^2	
Within months	N-k	by subtraction (-)	S^2		
Total	N-1	X_i^2j-C			

In this study N = No. of camels sampled x 12 calender months

k = 12

C = correction factor computed as $G^2/N = T_i^2$

where T^2 = sum of total fly counts squared

The variance ratio (F) = M/s^2

where S^2 = is the estimate of variances of the distribution of fly counts from different camels within 12 common months or the pooled sum of squares within monthly means.

S^2 = residual sum of squares/(N-k)

M, the measure of the spread of monthly means was computed as $M = (T_i^2/n_i - C)/(k-1)$

When the value of M is significantly greater than the value obtained for S^2, results are regarded as significant and the null hypothesis H_o is rejected in favour of the alternative hypothesis H_a. In the variance ratio test fi = k-1 represent the degrees of freedom for the numerator; f2 = N-k represent the degrees of freedom for the denominator. A reference was made to tables of the F-distribution e.g (Norman,1981, Statistical

methods in Biology) using the significance levels as they are at the 5% point.

To test the homogeneity of variances a suitable method, or test, the Bartlett's test was followed. The quantity calculated in the Bartlett's test are distributed approximately like X^2 with k-1 degrees of freedom. Final values of X^2 obtained,if greater than the critical value at $p=0.05$ are regarded as significant. The variance ratio test was applied to 1986 data only and only to monthly variances of biting flies per animal and not per trap. This was so because the monthly fly data per camel for 1987 were similar in trend to 1986 data. Monthly variances of flies per trap were obviously significantly different from each other based on summary tables therefore the F-test was not necessary.

3.3: R E S U L T S.

3.3.1 : Appraisal of collected biting flies and their vectorial capacities.

A variety of biting flies of the insect Order Diptera were collected from the study area using sweep-nets and Challier (biconical traps). A list of these biting flies, methods of collection and animal hosts (where applicable) is given on Table 9. According to information on Table 9 some biting flies collected by means of biconical traps were not observed directly on animal hosts. These included *Haematopota albihirta, Philoliche distincta, Tabanus atrimanus* and *T.leucostomus*. Likewise two fly species, namely *Hippobosca longipennis* and *Haematobia minuta* were collected only by means of sweep-nets directly from animal hosts.

TABLE 9 : A species list of possible mechanical vectors of camel trypanosomiasis in northern Kenya based on sweep-net and Challier (biconical) trap collections.

Collection Dates	Collection Sites	Collection method(s)	Biting fly species,	Major host animal
12/85 - 12/87	Kargi Korr Ilaut Olturot Ngurunit	Sweep-net	Hippobosca longipennis	dog
12/85 - 12/87	Kargi Korr Ilaut Olturot Ngurunit	Sweep-net	Haematobia minuta*	camel cattle donkeny sheep goat
05/86	Ngurunit	biconical trap	Tabanus atrimanus** Tabanus leucostomus**	unknown
03/86 - 06/86	Ngurunit	biconical trap	Philolicne distincta**	unknown
03/86 - 06/86	Ngurunit	biconical trap	Haematopota albihirta**	unknown
03/86 - 07/87 03/87 - 07/87	Ilaut Olturot Ngurunit	Sweep-net biconical trap	Tabanus taeniola***	camel cattle
03/86 - 07/86 03/87 - 07/87	Ilaut Olturot Ngurunit	Sweep-net biconical trap	Atylotus agrestis***	camel cattle
12/85 - 12/87	Ilaut Olturot Ngurunit	Sweep-net biconical trap	Stomoxys calcitrans***	camel cattle
12/85 - 12/87	Kargi, Korr Ilaut Olturot Ngurunit	Sweep-net biconical trap	Hippobosca camelina***	Man camel donkeny sheep goat

* Direct sweep-net collections only.
** Indirect biconical trap collections only.
*** Sweep-net and biconical trap collections.

Hippobosca camelina, Stomoxys calcitrans,Tabanus taeniola and *Atylotus agrestis* were collected from biconical traps as well as directly from animal hosts using sweep-nets. It is evident from these collections that some study sites such as Ngurunit and Olturot had a greater species diversity compared to study sites such as Korr and Kargi. While all nine fly species listed on column 4 of Table 9 were collected at Ngurunit study site, only three fly species (*H.camelina, H.longipennis*, and *H.minuta*) were collected from Korr and Kargi study sites. Next to Ngurunit in terms of species diversity was Olturot and Ilaut study sites where a total of five different biting fly species namely *H.camelina, H.longipennis, S.calcitrans T. taeniola* and *A.agrestis* were collected.

While some biting fly species such as *H.longipennis* were host specific parasitising mainly dogs, others such as *H.camelina* and *H.minuta* had a wide range of animal hosts. The major animal host for *H.camelina* was the camel but goat,sheep, cattle,donkey and even man were bitten by the fly (Table 9). *T.taeniola* and *A.agrestis* were specifically collected from the camel host although they were noted to bite nearby animals such as cattle as well, when these were available. Animal hosts for *H.albihirta, P.distincta, T.atrimanus* and *T.leucostomus* were not determined because these biting flies were available only in the biconical traps and not on the animals during the period of study. The above named biting flies collected from the study area belonged to three different insect families as described below:

(1) Family Hippoboscidae

The adult flies of this family are flattened in appearance, with wings which are either well developed, shortened or absent. The legs are short and strong and broadly separated. The adults are external parasites of birds and mammals and range in size from 2.5 to 10 mm. Members of this family are larviparous. Hippoboscids are commonly referred to as louse flies due to their resemblance to lice. Hippoboscids of mammals comprise 4 genera; *Melophagus Lipotena, Neolipotena* and *Hippobosca* (Harwood and James,1979).

Two species of *Hippobosca* were collected from the study area and are described below:

(a) Hippobosca camelina

This fly is commonly known as the camel fly . It is a large stout fly that measured 9-12 mm (mean of 11.68+/-0.32) in body length based on the examined 50 specimens. The fly is brown in colour.The body is flat and horny which is probably an adaptation towards an ectoparasitic mode of life. The proboscis is extremely slender and protrusible as shown in Fig. 12b. The proboscis is composed of the same parts as those of blood sucking Muscidae (i.e. labella, hypopharynx,labrum, epipharynx. labium and rostrum).The tip of the labella of *H.camelina* is armed with chitinous teeth (Fig. 12d) to pierce the skin of the host. The sizes of the examined 30 proboscis ranged from 0.6 to 1.2 mm (mean of 0.96+/-0.16) in length. The wing of H.camelina (Fig.12c) is well developed and ranged from 8 to 10 mm (mean of 9.86+/-1.5) in length.

Hippobosca camelina

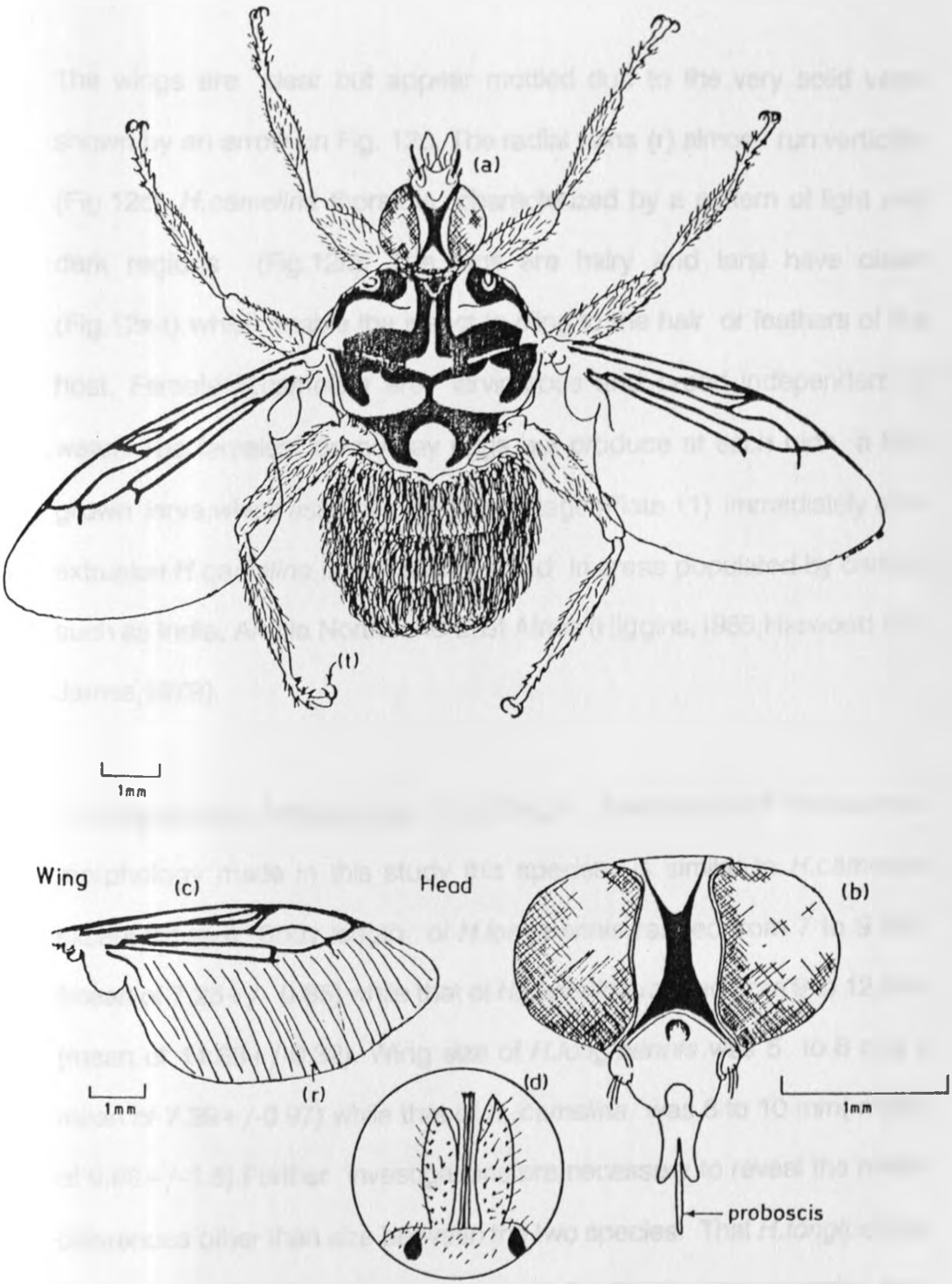

Fig. 12 :Showing general morphology(a), head and proboscis (b), wing(c), and chitinous teeth on labellae(d), of H. camelina .

The wings are clear but appear mottled due to the very solid veins shown by an arrow on Fig. 12c. The radial veins (r) almost run vertically (Fig.12c). *H.camelina* thorax is characterized by a pattern of light and dark regions (Fig.12a). The legs are hairy and tarsi have claws (Fig.12a-t) which enable the insect to cling to the hair or feathers of the host. Female *H.camelina* are larviparous and breed independent of water. The females do not lay eggs but produce at each birth, a fully grown larva,which assumes the pupal stage (Plate 11) immediately after extrusion.*H.camelina* is commonly found in areas populated by camels such as India, Arabia North and East Africa (Higgins,1986;Harwood and James,1979).

(b) **Hippobosca longipennis**. According to observations of the external morphology made in this study this species is similar to *H.camelina* except for size. Body length of *H.longipennis* ranged from 7 to 9 mm (mean of 7.25+/- 0.85) while that of *H.camelina* ranged from 9 to 12.mm (mean of 11.68+/-0.32). Wing size of *H.longipennis* was 5 to 8 mm (mean of 7.39+/-0.97) while that of *H,.camelina* was 8 to 10 mm(mean of 9.86+/-1.5).Further investigations are necessary to reveal the major differences other than size between the two species. That *H.longipennis* is commonly found on dogs in Asia and the Mediterranean region,has been reported by Harwood and James, (1979). *H.longipennis* has been introduced to America (Westcolt,1973) but apparently is not established there.

Plate 11 : Puparium of H.camelina.

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Vectorial capacity of hippoboscids

All adult hippoboscids are blood sucking ectoparasites of mammals. Although there is no experimental evidence to associate hippoboscids with disease transmission,they are potential mechanical vectors of pathogens. The blood sucking activity of *H.camelina* and *H.longipennis* coupled with their painful bites (Harwood and James,1979) makes them potential vectors of blood parasites such as trypanosomes. Cyclical development of blood parasites in *H.camelina* and *H.longipennis* was not investigated in this study but must not be ruled out. Hippoboscids are not intermittent blood feeders but attack animal hosts in large numbers thereby increasing their chances of being mechanical vectors of parasites if they probe on infected and subsequently on non-infected hosts.

(11) Family Muscidae

This is a family to which the non blood sucking housefly (*Musca domestica*) belongs. Important genera in this family in terms of disease transmission of blood parasites include *Glossina* spp., *Stomoxys* spp.and *Haematobia* (=*Lyperosia*) spp.Haematophagous members of this family collected in the study area were *Stomoxys calcitrans* and *Haematobia minuta*.

(a) Stomoxys calcitrans; This fly is commonly known as the stable fly and is quite similar to the house fly. *Stomoxys* is however more robust with a broader and shorter abdomen. The abdomen has three dark spots on each of the second and third segments (Fig.13a). Whereas the house

Stomoxys calcitrans

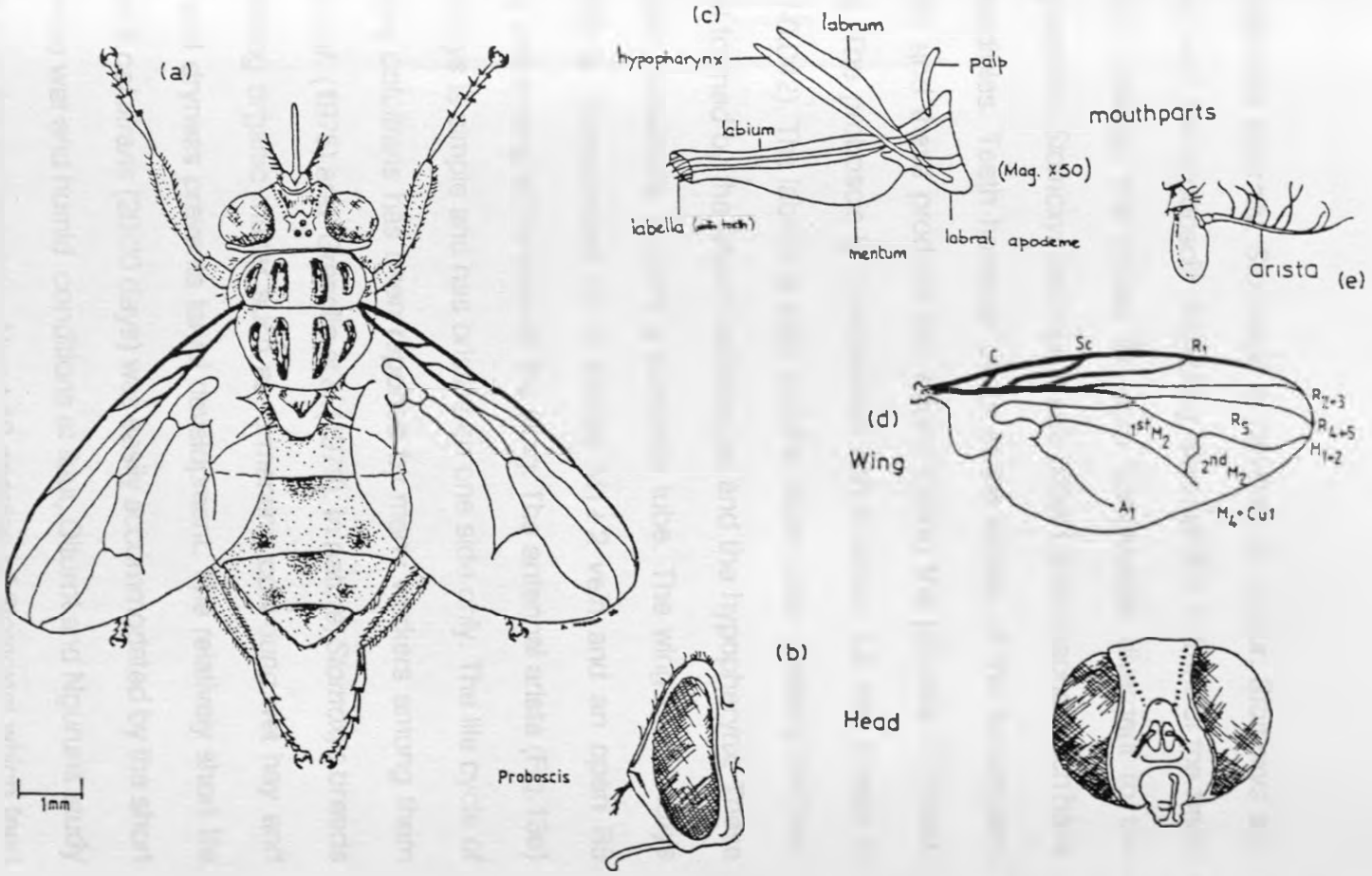


Fig.13 :Showing general morphology(a), head (b), mouthparts (c), wing (d), & antennal arista (e) of S. calcitrans

fly is yellowish ventrally, *Stomoxys* is ash-grey in colour. *Stomoxys* sits upwards with the proboscis projecting bayonet-like in front of the head (Fig.13b) whereas the house fly faces downwards divergent to the resting position. *Stomoxys* belongs to sub-order Cyclorrhapha which have lost mandibles. Teeth however occur in the labella of the mouthparts (Fig.13c) and these produce the wound during the process of blood-feeding. The proboscis is conspicuous and is about 1.5 mm (mean of 1.45+/-0.042). The labium is rigid and the labial gutter contains the food channel formed by the labrum-epipharynx and the hypopharynx. These are fused proximally to form a complete tube. The wing of *Stomoxys* (Fig.13d) is characterized by a curved M1+2 vein and an open R5 (apical) cell ending at the apex of the wing. The antennal arista (Fig.13e) of *Stomoxys* is simple and has bristles on one side only. The life cycle of *Stomoxys calcitrans* has been reported by many workers among them Bailey *et al*. (1975) and Grasella *et al*.(1979). In nature, *Stomoxys* breeds in decaying organic matter such as fermenting cow-dung,wet hay and straw and dryness prevents larval development. The relatively short life cycle of *S.calcitrans* (20-30 days) was easily accommodated by the short favourable wet and humid conditions at Ilaut, Olturot and Ngurunit study sites during the wet seasons. About 18 species of *Stomoxys* which feed on mammalian blood have been identified. *Stomoxys* is found world wide under a variety of climatic conditions both in the tropics and temperate regions (Zumpt,1950; Harley,1965; Kangwagye,1973).

(b) ***Haematobia minuta***

Members of the genus *Haematobia* belong to the subfamily Stomoxyinae along with *Stomoxys*. *Haematobia* (=*Lyperosia*) are commonly referred to as hornflies in North America. These flies closely resemble *Stomoxys* but are much reduced in size and are brown in colour. The body length of *H.minuta* is 3 to 4 mm (mean of 3,54+/-1.6) and unlike *Stomoxys*,the mouthparts (Fig.14a&14c) are ensheathed by palpi which are as long as the proboscis. The antennal arista (Fig.14b) is simple with hairs projecting from one side only as in *Stomoxys* spp. The wing of *H.minuta* (Fig.14d) is similar in structure to that of *Stomoxys* but is greatly reduced measuring 2.5 mm (mean length of 2.43+/-0.26). *H.minuta* can be distinguished from *S.calcitrans* based on size and the length of the palpi in relation to the proboscis; palpi of *Stomoxys* are extremely slender and short and cannot be seen when the fly is viewed from above. *Haematobia* on the other hand have palpi that are flattened from side to side and form a complete sheath for the proboscis, which they almost equal in length.

Vectorial capacity of *S.calcitrans* and *H.minuta*.

Stomoxys spp. was observed to be an intermittent blood feeder in this study. The fly may seek a blood meal 6 to 8 hours after emergence (Mitzmain,1913). It is a viscious biter,inflicting painful bites before finally drawing blood (Simmon,1944) and may feed several times in a day. These facts in association make *Stomoxys* a potential mechanical vector of blood parasites such as trypanosomes. Survival of

Haematobia minuta

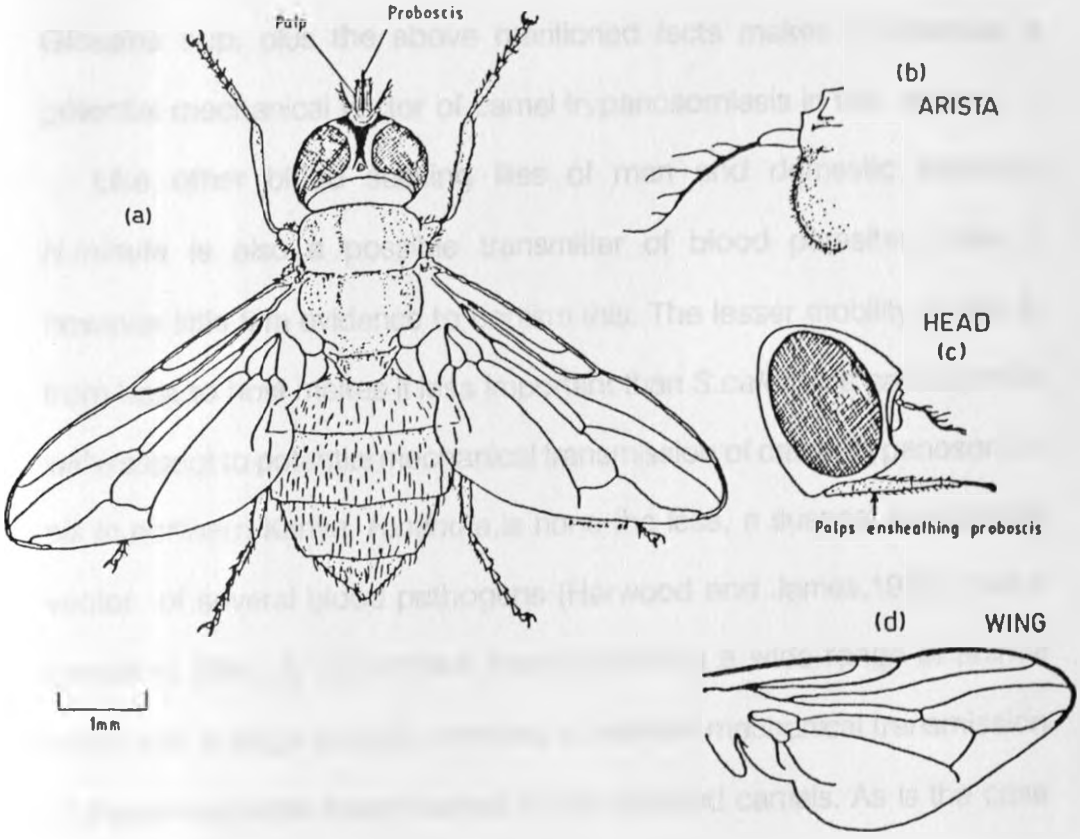


Fig. 14 : Showing general morphology(a), antennal arista (b), head region (c), and wing (d), of H. minuta.

trypanosomes, particularly *T.evansi* in mouthparts of *S.calcitrans* has been confirmed (Mitzmain,1912; Moutia,1928b; Beck,1928; Ogonji,1983). Presence of *S.calcitrans* in northern Kenya in the apparent absence of *Glossina* spp. plus the above mentioned facts makes *S.calcitrans* a potential mechanical vector of camel trypanosomiasis in the region.

Like other blood sucking flies of man and domestic livestock, *H.minuta* is also a possible transmitter of blood parasites.There is however little firm evidence to confirm this. The lesser mobility of this fly from host to host makes it less important than *S.calcitrans* and tabanids with respect to potential mechanical transmission of camel trypanosomiasis in northern Kenya. *H.minuta*,is none the less, a suspect mechanical vector of several blood pathogens (Harwood and James,1979). It is a prevalent biting fly in northern Kenya,attacking a wide range of animal hosts and in large enough numbers to warrant mechanical transmission of trypanosomiasis from infected to non-infected camels. As is the case with hippoboscids, cyclical development of trypanosomes in *H.minuta* is a subject that needs to be examined in future.

(iii) Family Tabanidae . Members of this family are characterized by the following features; a short 3-segmented antenna, the last segment usually bearing a style. These flies belong to suborder Brachycera and are commonly known as horse flies, deer flies, clegs or mango flies. They are large flies measuring 6 - 10 mm in smaller species and up to 25 mm in the larger species. Tabanids are notorious pests of domestic livestock and only females suck blood. The eyes are extremely large, usually

holoptic in males, while females are dichoptic. The wing venation is characteristic in that the branches of vein R_{4+5} diverge broadly,thereby enclosing the apex of the wing between them. The mouthparts of the females are blade-like and function as cutting instruments although the labella are fleshy and fitted for sponging.Information about the life cycle of tabanids is limited to those of Neave (1915); King (1910;1926) and Oldroyd (1954). Eggs are laid in masses on wet ground, leaves, stems,decaying organic wood or on rocks and reeds overhanging water. Egg masses may contain up to 450 eggs (King,1909). Larvae in an egg mass hatch simultaneously and fall directly into water. Larvae are carnivorous and prey upon worms, snails, beetle larvae etc. and are also cannibalistic. The pupal stage is normally found upright in mud after the water dries up. This stage is stationery either in water or mud. The duration of these early stages are 4-7 days from egg to larvae,4-6 weeks from larvae to pupae and 6-8 days from pupae to adult. The wet season (March-May) in northern Kenya was accompanied by temporary streams, and stagnant water holes; these provided breeding habitats for tabanids and explains the sudden appearance of these flies during the wet months. Tabanids have a cosmopolitan distribution and are only absent at very high altitudes. Tabanids are among the largest of all families of Diptera. Tabanidae of the Ethiopian region have been described in detail by Oldroyd (1952, 1954 and 1957) There are about 3,000 species distributed through 30 to 80 genera,the number depending upon the interpretation of the author. Classification of Tabanidae to subgenera level is depicted on Fig.15. Four genera of Tabanidae were collected in

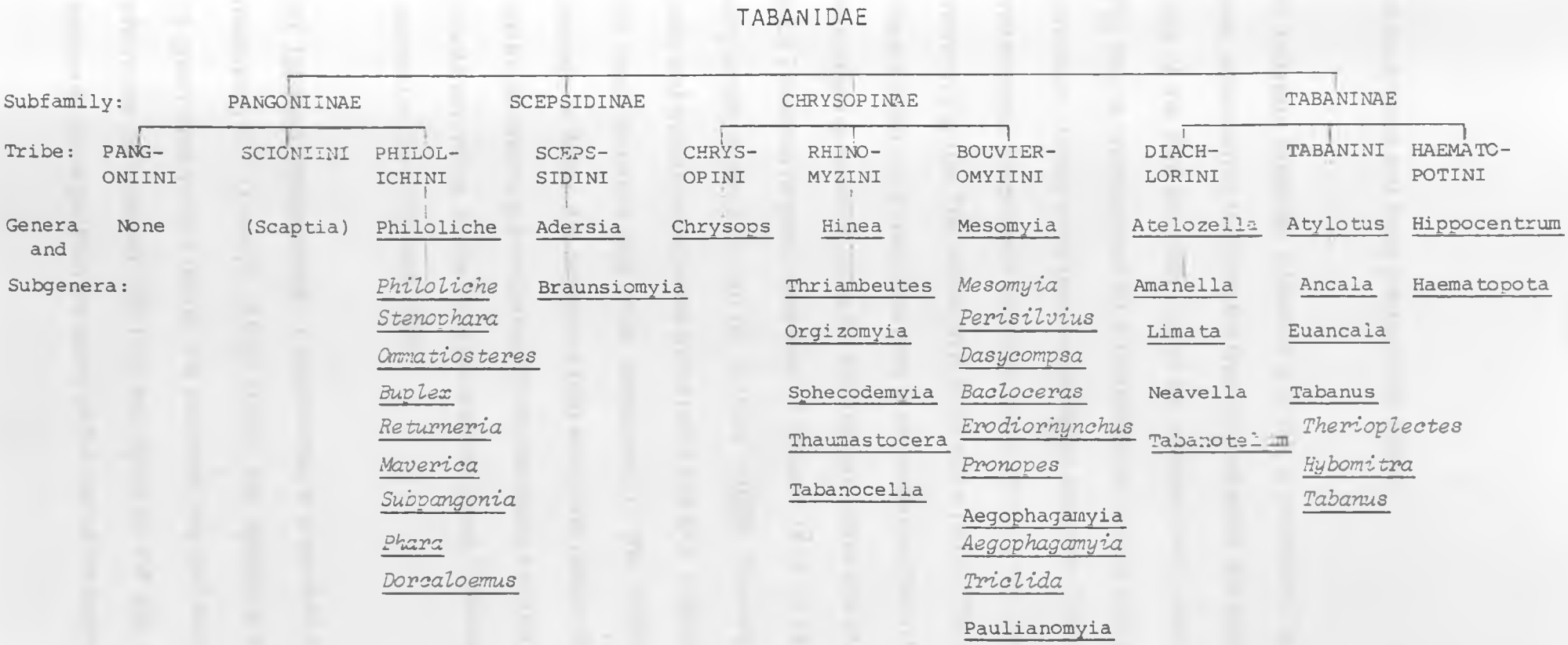


FIG. 15 : Classification of Tabanidae to genera and subgenera after Oldroyd. 1957.

the study area and these are reported below;

(a) Tabanus taeniola: *T.taeniola* (Fig.16a) is recognized by unbanded eyes, antenna (Fig.16c) with the first segment white, the second and the base of the third red; with rest of the antenna being black. The frons (Fig.16b) is characterized by a reddish brown colour with a thin white tomentum . Mostly white hairs occur on the antennae. The thorax has a characteristic pattern with the mesonotum having three grey stripes as shown in Fig.16a. The scutellum is black with a red tip. The abdomen is characterized with three yellow rows of stripes as depicted in Fig.16a. The femora are grey with a yellow tip, the tibiae are yellow and tarsi are black. Adult *T.taeniola* ranges in size from 17 - 19 mm ($\bar{X}$ of 17.13 $\pm$2) and its wing length is about 15 mm ($\bar{X}$ of 15.55 $\pm$0.29). The wing (Fig.16d)is clear and without an appendix to the radial fork (R_4). *T.taeniola* is one of the most abundant and most widespread of the Tabanidae in the lowlands of Africa. It is distributed both within and outside the equatorial rain forest stretching from Senegal to the Nile Valley including Ethiopia up to southern Africa. Inside the equatorial rain forest, *T.taeniola* has been reported in Zaire (Oldroyd,1954).

(b) Tabanus leucostomus: *T.leucostomus* is a medium-sized tabanid measuring 10 - 11 mm ($\bar{X}$ of 9.62 $\pm$0.84). The species is characterized by green eyes without bands; the abdomen has bold sublateral spots which are well defined (Fig.17a) and spots of the first and second segments are larger than the spots on the rest of the segments. Spots

Tabanus taeniola

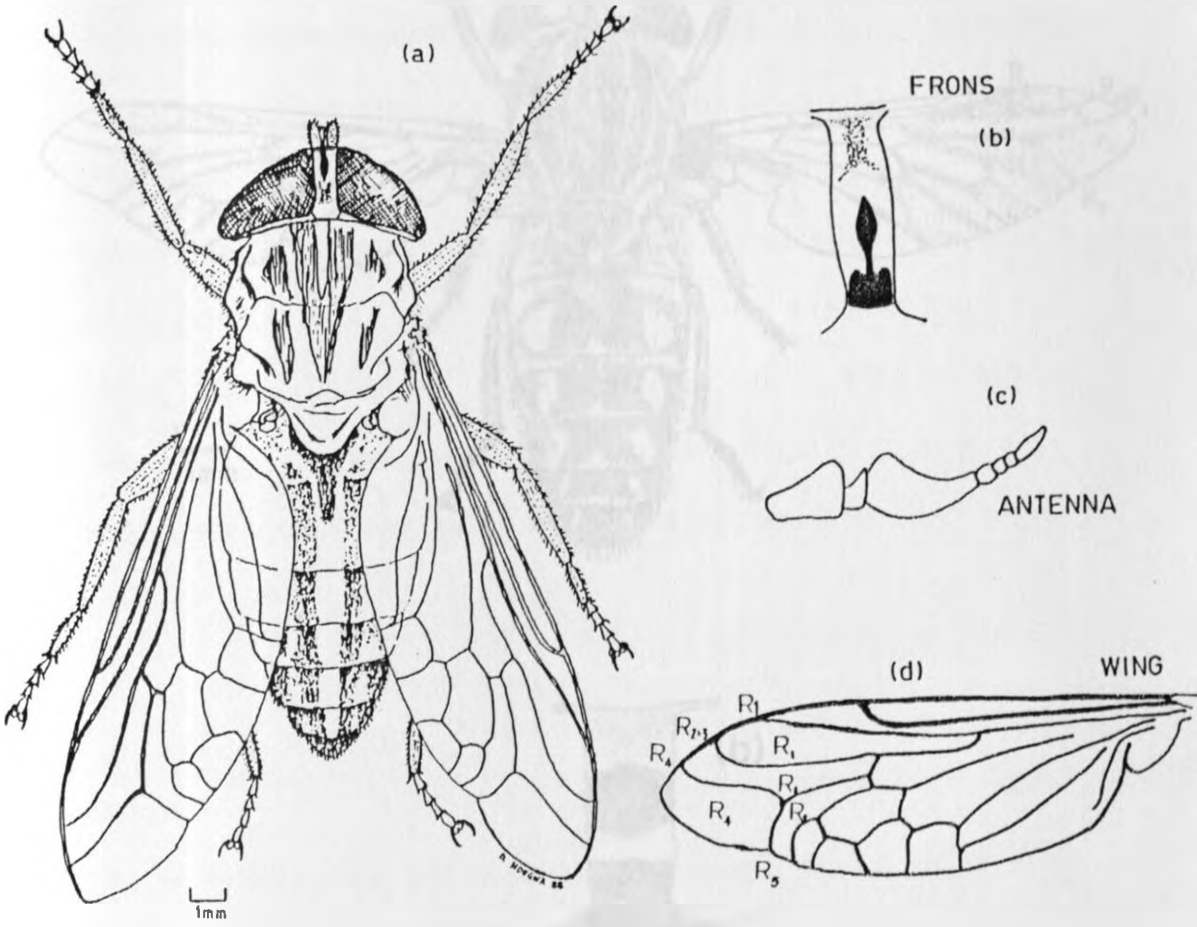


Fig. 16 : Showing general morphology (a), frons (b), antenna (c), & wing (d) of T. taeniola

Tabanus leucostomus

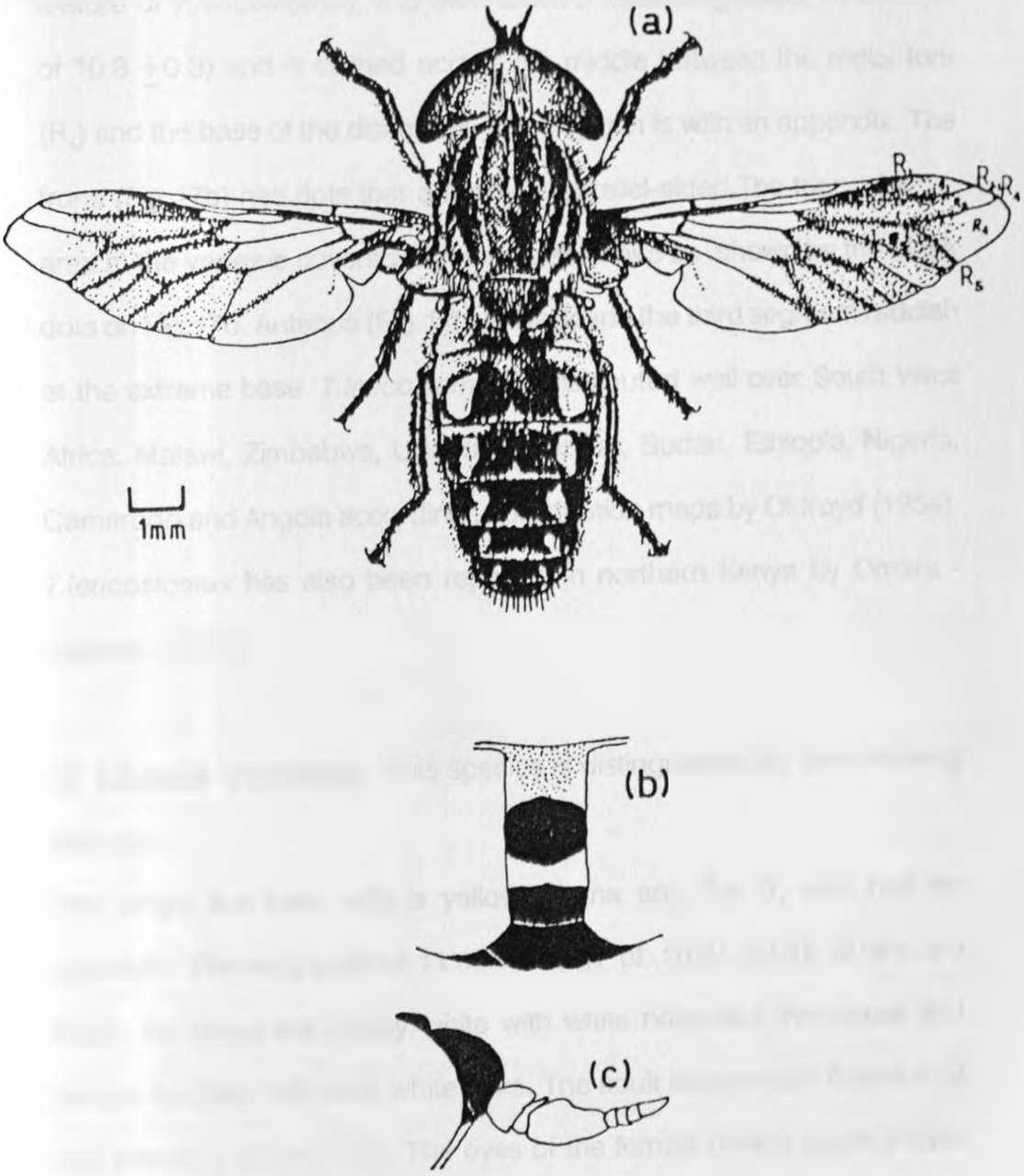


Fig. 17: General morphology and wing (a), frons (b), and antenna (c) of T. leucostomus.

diminish in size posteriorly. The wing (Fig.17a) is a distinct characteristic feature of *T.leucostomus*. It is dark brown, measuring about 11 mm ($\bar{X}$ of 10.8 $\pm$0.3) and is stained across the middle between the radial fork (R_4) and the base of the discal cell. The (R_5) vein is with an appendix. The frons (Fig.17b) has dots that are almost parallel-sided.The triangular area at the vertex is covered with long black hairs as shown by the black dots on Fig.17b. Antenna (Fig.17c) is black and the third segment reddish at the extreme base. *T.leucostomus* is distributed well over South West Africa, Malawi, Zimbabwe, Uganda, Zanzibar, Sudan, Ethiopia, Nigeria, Cameroon and Angola according to distribution maps by Oldroyd (1954). *T.leucostomus* has also been reported in northern Kenya by Omara - Opyene (1986).

(c) Tabanus atrimanus: This species is distinguished by the following features:-

The wings are clear with a yellow stigma and the R_4 vein has no appendix. The wing is about 11 mm long ($\bar{X}$ of 10.81 $\pm$0.3). All tarsi are black, the tibiae are mostly white with white hairs and the coxae and femora are grey with thick white hairs. The adult body length is about 13 mm (mean of 23.1+/-0.4). The eyes of the female have a single purple band (Fig.18a). The frons (Fig.18b) is slightly convergent; the calli are brown or light brown: lower callus nearly square,and jointed to a long ovate upper callus of slightly darker colour and the second antennal segment (Fig.18c) is somewhat swollen near the base. *T.atrimanus* is distributed in South Africa, Tanzania, Malawi, Zaire, Zimbabwe and Kenya

T. atrimanus

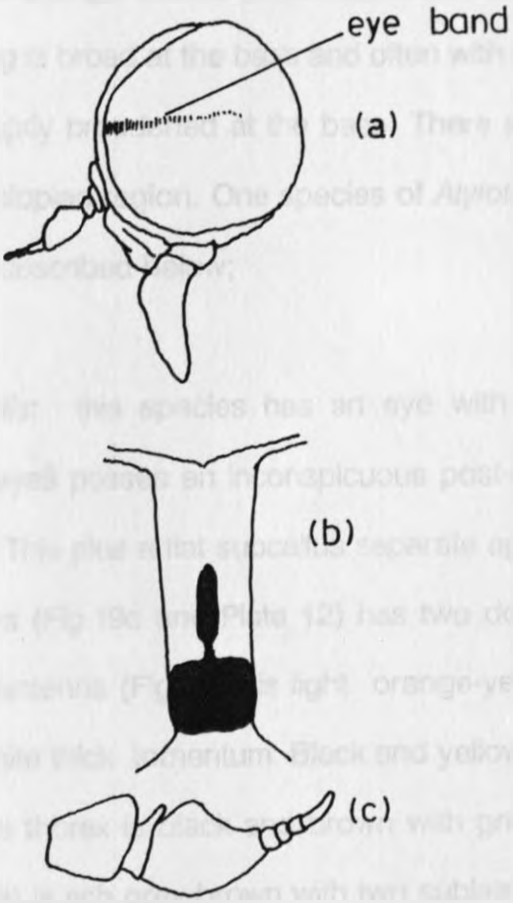


Fig.18 : Showing female eye and its single purple band(a), frons (b) and antenna (c) of T. atrimanus

(Oldroyd,1954).

Genus *Atylotus* Osten-Sacker

This genus is easily recognized by the large,strongly rounded head, and the eyes show a single narrow purple band. The wings are clear.The R4 vein of the wing is broad at the base and often with an appendix and the costa is abruptly broadened at the base. There are six species of *Atylotus* in the Ethiopian region. One species of *Atylotus* collected from the study area is described below;

(d)*Atylotus agrestis*: this species has an eye with a purple band (Fig.19b-p). The eyeš posses an inconspicuous post-occipital fringe of hairs (Fig.19b-h). This plus a flat subcallus separate *agrestis* from other *Atylotus*. The frons (Fig.19c and Plate 12) has two dots and is slightly convergent, The antenna (Fig.19d) is light orange-yellow and the first segment has a white thick tomentum. Black and yellow hairs appear on the antennae. The thorax is black and brown with green patches. The abdomen (Fig 19a) is ash grey-brown with two sublateral yellow stripes which are broader anteriorly but taper towards the rear hind margin of each segment. The palpi are swollen at the base, the proboscis is orange, the femora yellow and the fore tibiae are light brown. Wings, including the costa, are pale yellow, the R_4 has an appendix. The wings are about 11 mm ($\bar{X}$ of 10.97 $\pm$0.2) in length. The body length of *A. agrestis* is about 14 mm ($\bar{X}$ of 13.89 $\pm$0.5). It is a widely distributed tabanid species stretching from Senegal to Natal and westwards to

Atylotus agrestis

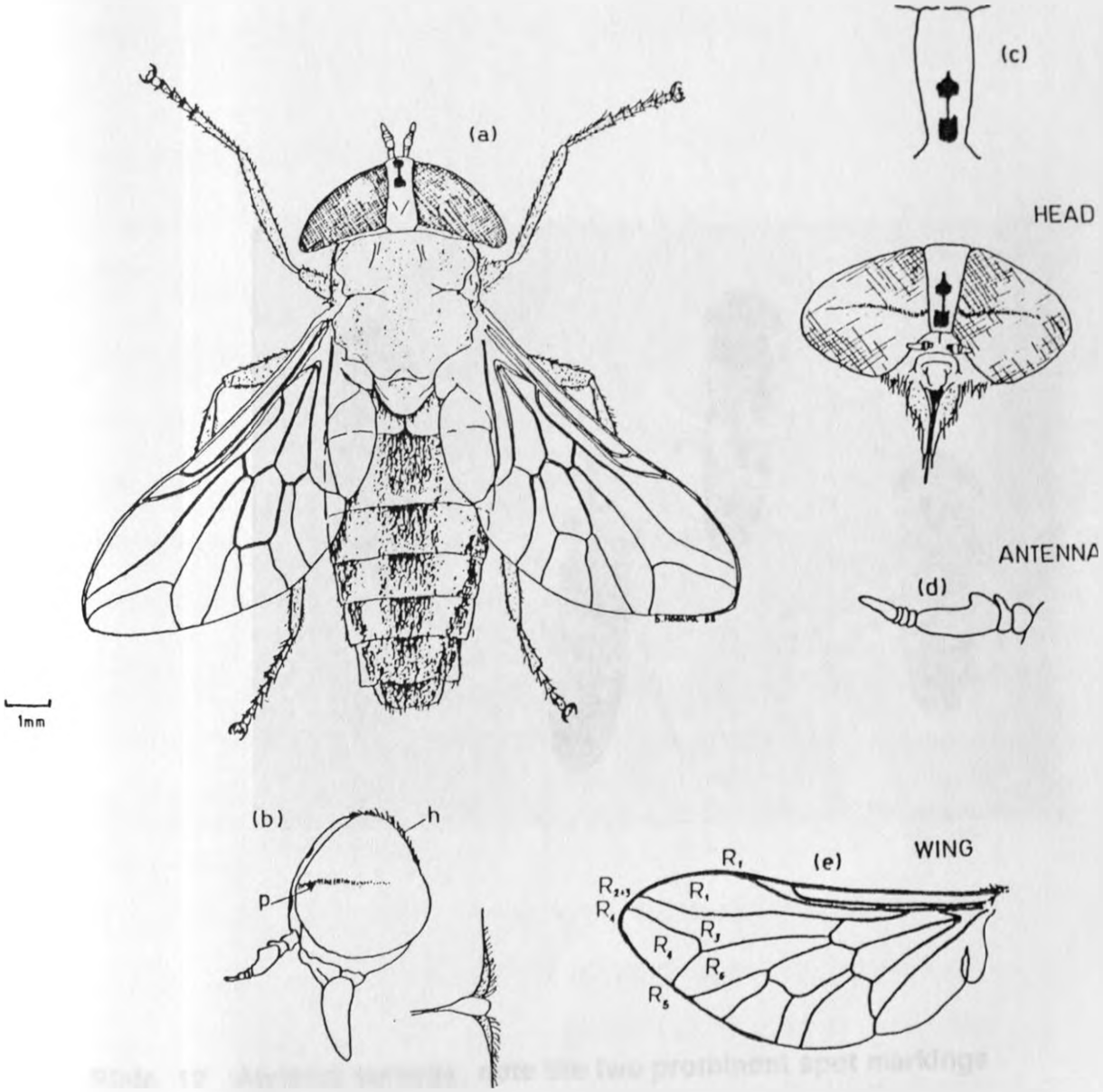


Fig. 19 : Showing general morphology (a), eye (b), frons (c), antenna (d), and wing (e) of A. agrestis

Plate 12 :Atylotus agrestis: note the two prominent spot markings on the frons.

Angola. It is also very common in Tanzania, Zimbabwe, Egypt, Arabia and Mauritius in open thorn bushlands and open grasslands.

Genus Haematopota Meigen.

Haematopota flourish mainly in the Ethiopian region where there are about 171 species.This genus is characterized with a wing pattern of light spots and streaks forming rosettes; palpi are slender with pale and black white hairs; the frons have a pair of velvety brown or black spots and the antennae are segmented, the last three relatively small, the second always small and cup-like, with a dorsal projection. The single species of *Haematopota* collected from the study area (Ngurunit) is described below;

(e) Haematopota albihirta. *H.albihirta* is a relatively small tabanid measuring about 12 mm ($\bar{X}$ of 12.04 $\pm$0.1). *H.albihirta* is black and white in appearance and is distinguished by the following features; The wing is black and white with rosettes (Fig.20a) grouped into two irregular transverse pale bands. The wing is about 11 mm ($\bar{X}$ of 10.6 $\pm$ 1.98). The frons (Fig. 20b) is narrow and the paired spots are velvety black/brown and close to the eye margin. A distinct third spot is present. The antenna is as depicted in figure 20c with an elongated third antennal segment and short first segment. *H.albihirta* can also be distinguished by a single very white band of the fore tibiae. *H.albihirta* is a widely distributed tabanid in the Ethiopian region where it is also referred to as *H.mactans*, Austen. It occurs in Kenya, Tanzania, Malawi, Zimbabwe, Lesotho,

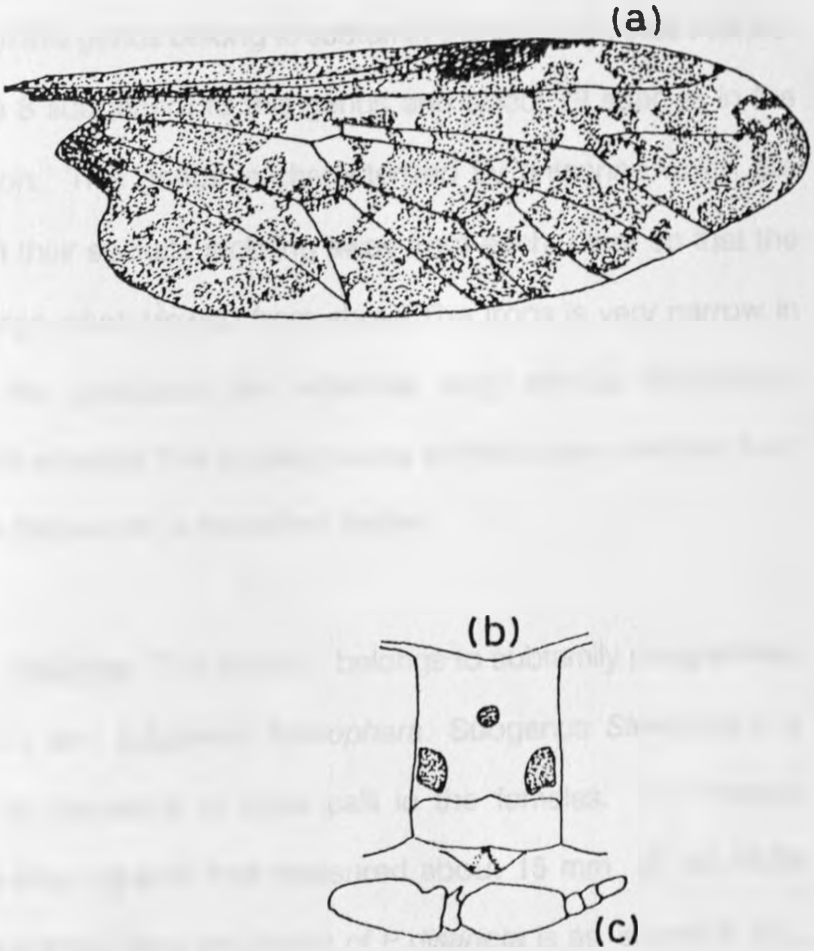


Fig. 20: Wing (a), frons (b) and antenna (c) of H. albihirta.

Ethiopia, Sudan, Uganda and South Africa (Oldroyd,1954).

Genus Philoliche. Wiedemann

Members of this genus belong to subfamily Pangoninae, tribe Philolichini. There are 8 subgenera to this genus and about 79 species in the Ethiopian region. This genus is characterized by antennae which are separated with their sockets inclining away from each other so that the antennae diverge when viewed from above.The frons is very narrow in females and the proboscis are extremely long without filamentous outgrowths of the labella.The single species of *Philoliche* collected from the study area (Ngurunit) is described below;

(f) Philoliche distincta: This species belongs to subfamily pangoninae, tribe Philolichini and subgenus *Stenophara*. Subgenus *Stenophara* is differentiated by presence of facial calli in the females. *P. distincta* (Fig.21a) is a large tabanid that measured about 15 mm ($\bar{X}$ of 15.06 $\pm$0.25). The extremely long proboscis of *P.distincta* is as shown in Fig. 21a and Plate 13. The proboscis is as long as about 10 mm ($\bar{X}$ of 9.96 $\pm$0.16. The antennae (Fig.21b) has greyish brown with black and yellow hairs, on the first two segments while the other segments are bright orange. The palpi are red with black hairs. The thorax is ash-grey with obscured yellow sublateral stripes . The abdominal dorsum is grey/brown and the ventrum is ash grey. The legs are yellow/brown, the fore and mid tibiae yellow and the hind tibiae dark brown. The wing (Fig.21d) is uniformly smoky grey with an open first posterior cell. The

Philoliche (stenophara) distincta

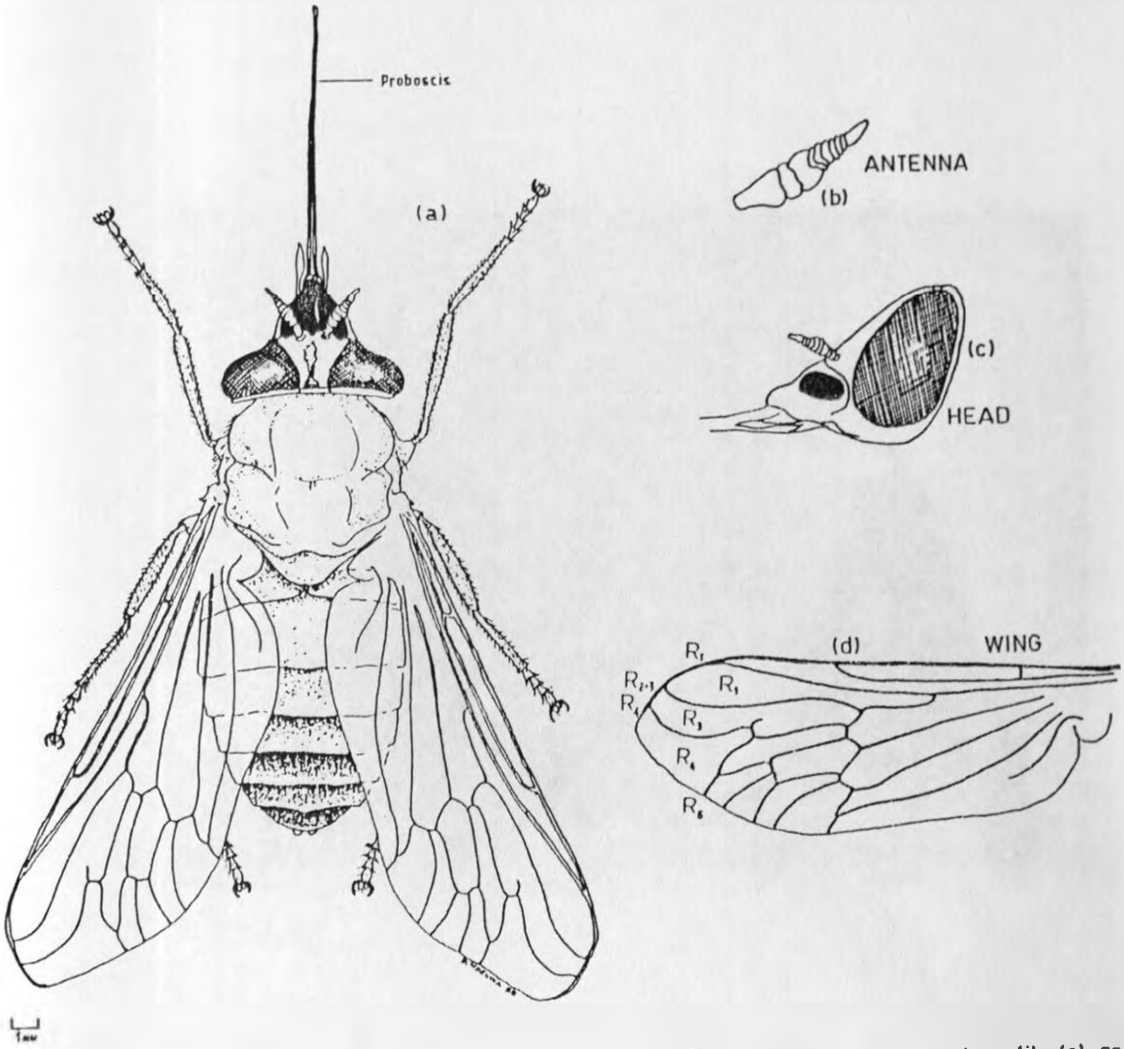


Fig. 21 Showing general morphology and proboscis (a), antenna (b), head profile (c) and wing (d) of P. distincta

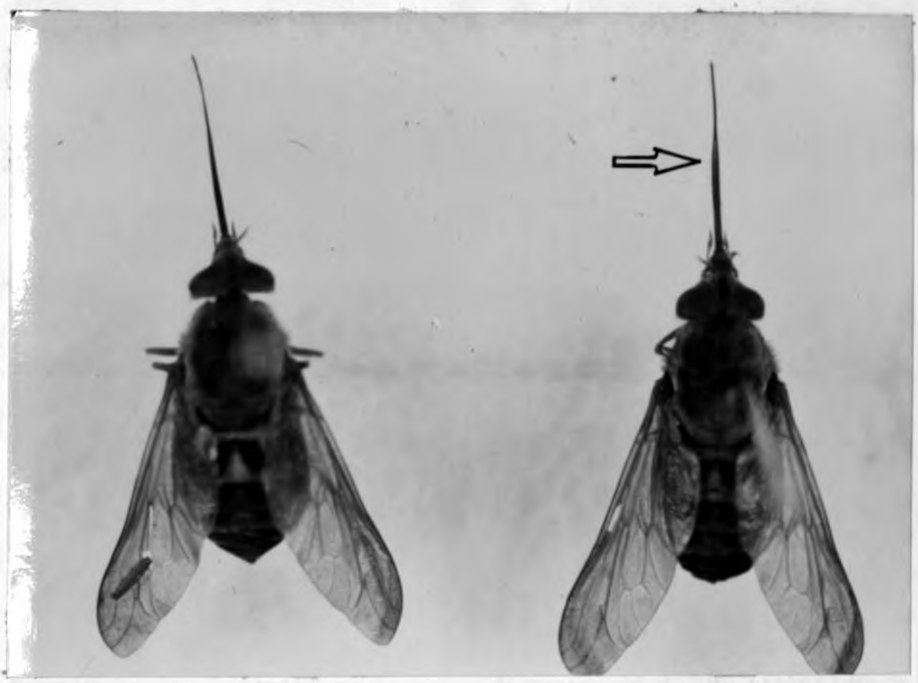


Plate 13 : Philoliche distincta: note the extremely long proboscis.

wing length is about 15 mm ($\bar{X}$ of 14.87 $\pm$0.15). *P.distincta* occurs in the Ethiopian region particularly Ethiopia, Somalia, Zaire, Kenya and Tanzania (Oldroyd, 1957).

3.3.2.1:Peak blood-feeding hours of biting flies in the study area.

Results presented on Table 10 and Fig.22a-22c show peak diurnal blood-feeding hours of the selected biting flies at Ngurunit study site based on observations made in June,1986.

Stomoxys calcitrans landed on the camel host only when seeking blood meals and did not remain on hosts after their completion of blood meals. Biting hours of *S.calcitrans* ranged between 0900 h and 1700 h but definite peaks of blood-feeding activity were observed. The highest numbers attacking the camel host was recorded around midday (1200 h) and this was 185 flies per camel as shown on Table 10 and Fig. 22a. A second peak of blood-feeding activity of *S.calcitrans* with 127 flies per camel was between 1500-1600 h.The morning peak created a greater disturbance to the camels than the afternoon peak.

Blood-feeding activity of *T.taeniola* was observed between 1000 h and 1600 h and was more pronounced between 1100-1300 h as shown on Table 10 and Fig.22b. The maximum number of *T.taeniola* recorded on the camel host during the peak blood-feeding period (1200 h) was 29 flies per camel.

A.agrestis was observed biting camel hosts for only short periods of time throughout the day ranging from 1100-1500 h. The highest number

Table 10: Maximum hourly counts of biting flies on camels at Ngurinit study site, June, 1986

Time of day (hours)	maximum counts		
	S.calcitrans	T.taeniola	A.agrestis
0600-0700	0	0	0
0700-0800	0	0	0
0800-0900	5	0	0
0900-1000	36	6	0
1000-1100	117	16	7
1100-1200	185	29	13
1200-1300	120	18	11
1300-1400	90	15	5
1400-1500	51	11	2
1500-1600	127	5	0
1600-1700	40	0	0
1700-1800	0	0	0
TOTAL	171	100	38

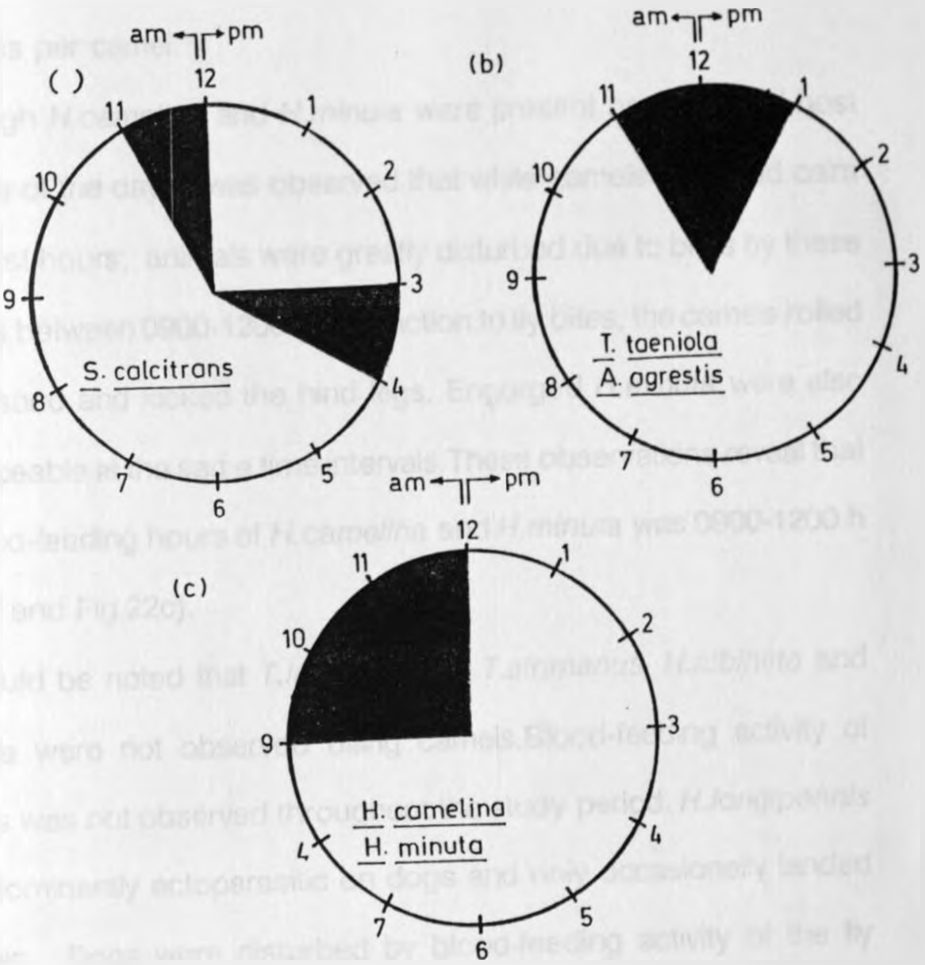


Fig 22 a,b,c : Showing diurnal peak blood-feeding hours of biting flies on camels at Ngurunit study site

of *A.agrestis* recorded at 1200 h which was the peak blood-feeding hour was 13 flies per camel.

Although *H.camelina* and *H.minuta* were present on the camel host at all times of the day, it was observed that while camels remained calm during most hours; animals were greatly disturbed due to bites by these fly species between 0900-1200 h. In reaction to fly bites, the camels rolled onto the sand and kicked the hind legs. Engorged *H.minuta* were also most noticeable at the same time intervals.These observations reveal that peak blood-feeding hours of *H.camelina* and *H.minuta* was 0900-1200 h (Table 10 and Fig.22c).

It should be noted that *T.leucostomus, T.atrimanus, H.albihirta* and *P.distincta* were not observed biting camels.Blood-feeding activity of these flies was not observed throughout the study period. *H.longipennis* was predominantly ectoparasitic on dogs and only occasionally landed on camels. Dogs were disturbed by blood-feeding activity of the fly between 0900 h and 1200 h, and it was during these hours when *H.longipennis* driven off by the dog apparently landed on the camel host.

3.3.2.2: Detailed diurnal biting activity of T.taeniola at Ngurunit study site.

Table 11 and Fig.23 show results of detailed observations of *T.taeniola* peak biting activity on camels at Ngurunit. *T.taeniola* was the biting fly selected for this particular study because it was the predominant tabanid present in large enough numbers for quantitative data collection. *S.calcitrans* was also abundant but studies on biting activity of *Stomoxys*

Table 11: T.Taeniola blood feeding hours on camels at Ngurunit study site, May, 1986.*

Time of day [hours]	Mean no.of flies/camels	standard deviation
0600-0700	0	-
0700-0800	0	-
0800-0900	0	-
0900-1000	2	0.8
1000-1100	9	2.3
1100-1200	18	2.6
1200-1300	15	3.4
1300-1400	13	2.1
1400-1500	7	1.8
1500-1600	4	0.9
1600-1700	3	0.5
1700-1800	0	-

* Sampled on all 30 days from a total of 150 different camels

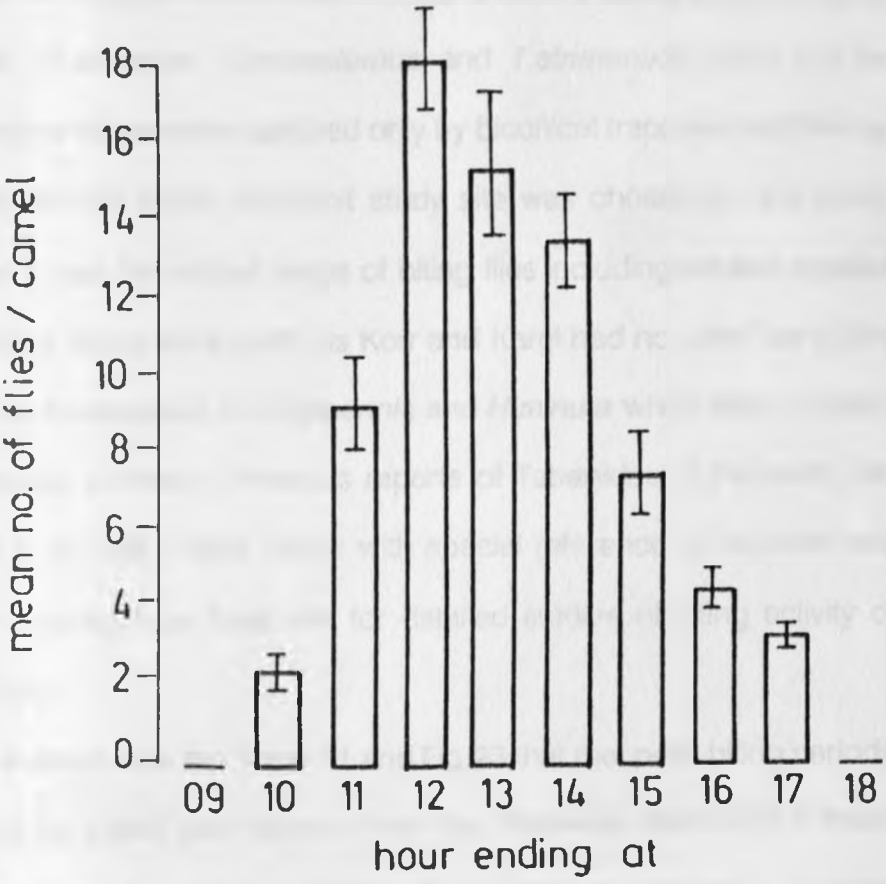
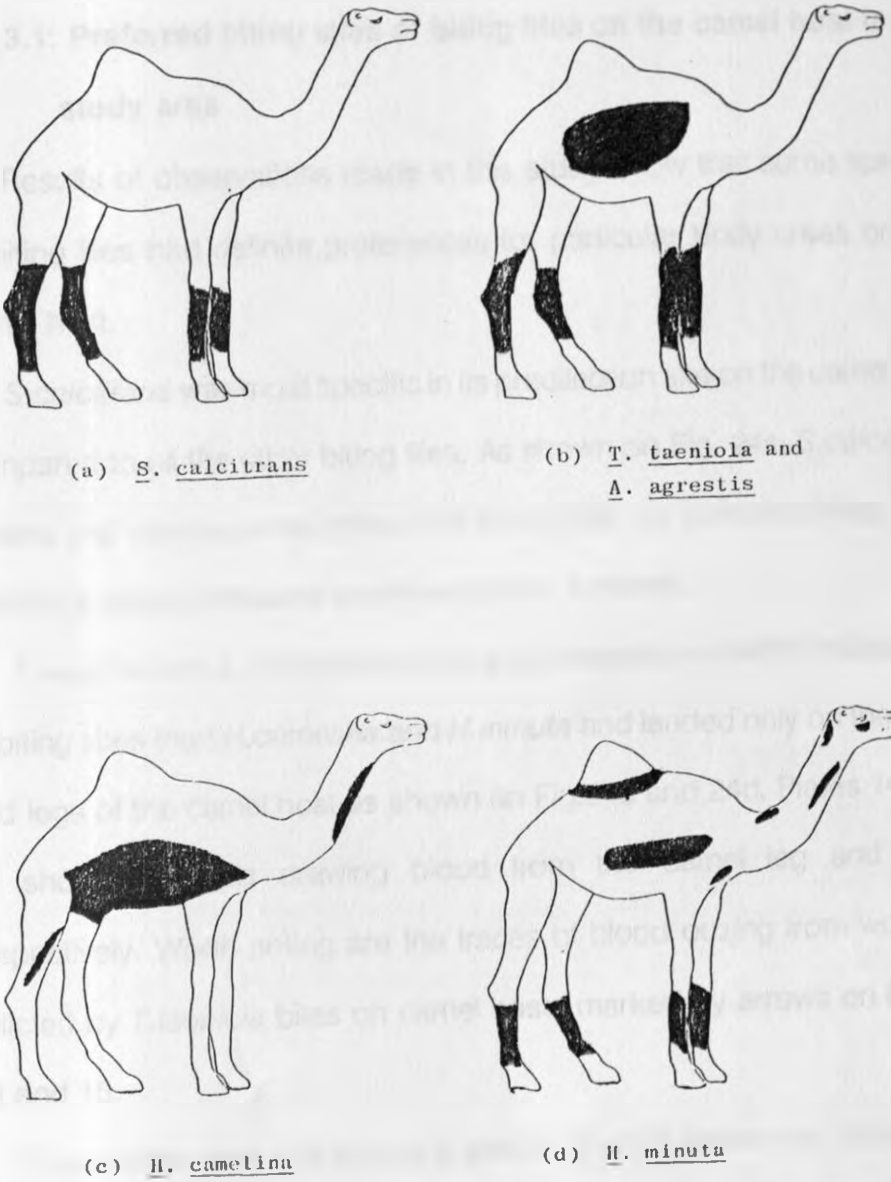


Fig. 23: Blood-feeding hours of T. taeniola at Ngurunit study site; May, 1986.

spp.are numerous and thus need not be repeated. Biting activity of other tabanids (*P.distincta, T.leucostomus* and *T.atrimanus*) could not be assessed as these were captured only by biconical traps and not directly from the animal hosts. Ngurunit study site was chosen for this study because it had the widest range of biting flies including several species of tabanids. Study sites such as Korr and Kargi had no other biting flies except for *H.camelina, H.longipennis* and *H.minuta* which were entirely ectoparasitic on hosts. Previous reports of Tabanidae in the study site (Wilson *et al.*,1981) were made with special reference to Ngurunit site thereby making it an ideal site for detailed studies of biting activity of Tabanidae.

It is evident from the Table 11 and Fig.23 that the peak biting periods of *T.taeniola* varied with hours of the day. Between 0600-0900 h there were no biting flies on the camels. From 0900 h onwards *T.taeniola* started landing on and took blood-meals from camels. Average numbers of *T.taeniola* on a camel host between 0900-1000 h were 2+/-0.8. Between 1000-1100 h the average number of *T.taeniola* per camel was 9+/-2.3. Between 1100-1200 h the number went up to 18+/-2.6. Between 1200-1300 h and 1300-1400 h the mean number of *T,taeniola* per camel went down to 15+/-3.4 and 13+/-2.1 respectively. Mean numbers of *T.taeniola* per camel continued to diminish from 7+/-1.8,4+/-0.9, 3+/-1.0 to 0+/-0.0 between 1500 h and 1600 h. Fig.23 is a pictorial representation of the peak biting periods of *T.taeniola* and also shows the peak blood-feeding hour to be the hour ending at 1200 h.



(a) S. calcitrans

(b) T. taeniola and A. agrestis

(c) H. camelina

(d) H. minuta

Fig 24: Biting fly predilection sites on the camel host at Ngurunit study site.

3.3.3.1: Preferred biting sites of biting flies on the camel host in the study area.

Results of observations made in this study show that some species of biting flies had definite preferences for particular body areas on the camel host.

S.calcitrans was most specific in its predilection site on the camel host compared to all the other biting flies. As shown on Fig. 24a, *S.calcitrans* landed and consequently blood-fed specifically on the camel legs. The hind legs were particularly preferred to the forelegs.

T.taeniola and *A.agrestis* were comparatively more specific in selection of biting sites than *H.camelina* and *H.minuta* and landed only on the belly and legs of the camel host as shown on Fig.24c and 24d. Plates 14 and 15 show *T.taeniola* drawing blood from the camel leg and belly respectively. Worth noting are the traces of blood oozing from wounds inflicted by *T.taeniola* bites on camel hosts marked by arrows on Plates 14 and 15.

H.camelina were slightly more specific than *H.minuta* on biting site selection. This fly was predominantly on the camel belly (Plate 16),hind legs (Plate 17), perianeal region and neck region as shown on Fig.24c.

H.minuta was least specific on its predilection sites on the camel host. It landed on the belly (Plate 18),around the eyes, ears, neck, hump, legs and torso (Fig.24d).

Preference for specific sites on the animal host was also observed for *H.longipennis* although in relation to the dog and not the camel host. *H.longipenns* was found solely on the back region immediately below the

Plate 14 : T.taeniola drawing blood from the belly of a camel host.

Plate 15 : T.taeniola blood-feeding on the legs of a camel host. Note the blood oozing from bitten areas (arrowed).

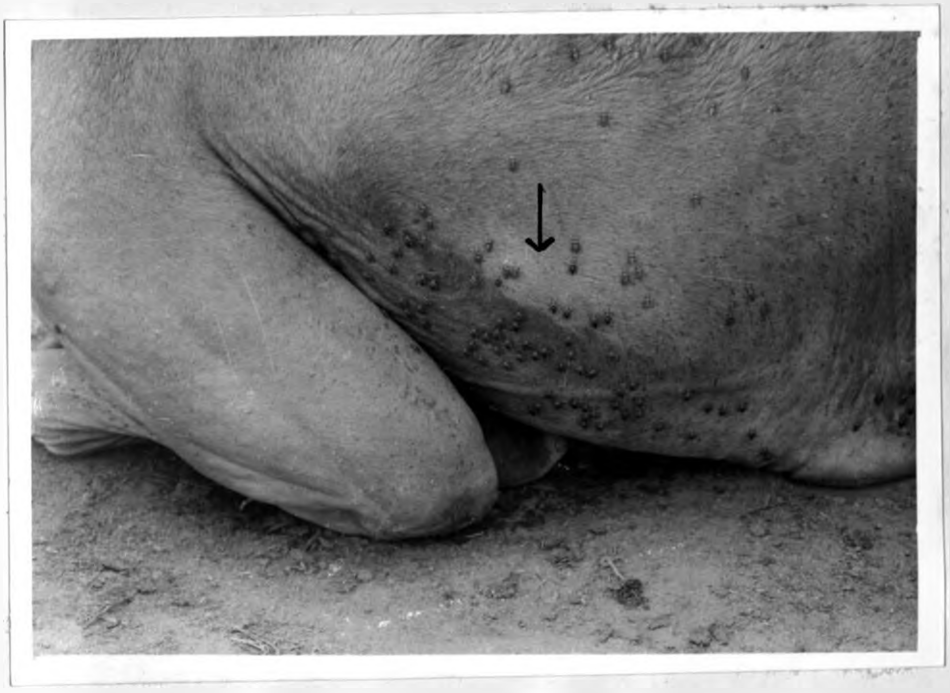


Plate 16 : The camel belly, a preferred biting site of H.camelina.

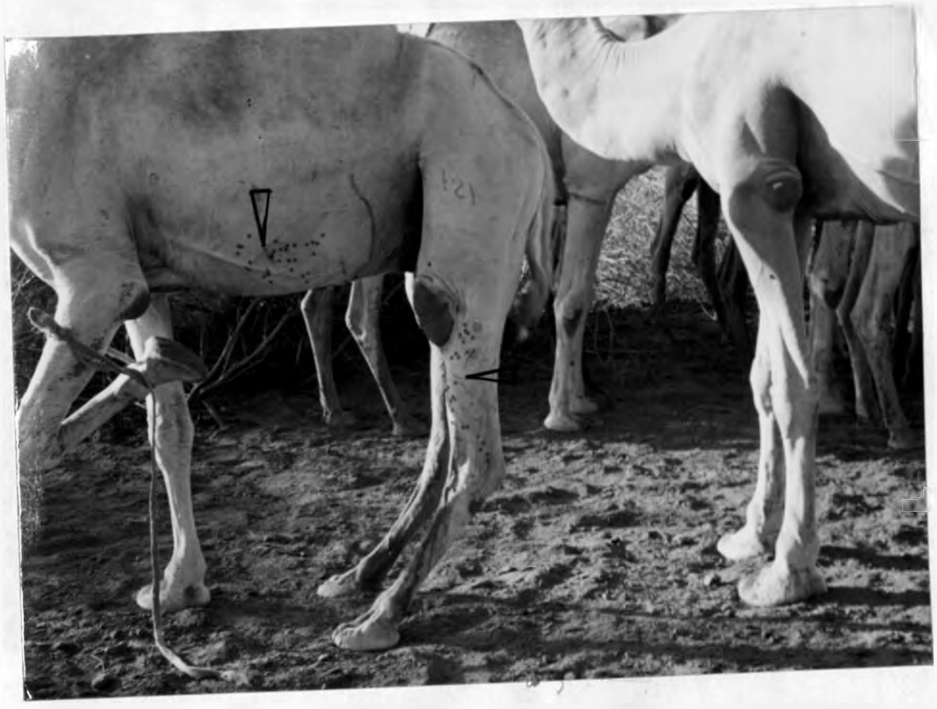


Plate 17 : Camel belly and legs, the preferred biting sites of H.camelina.

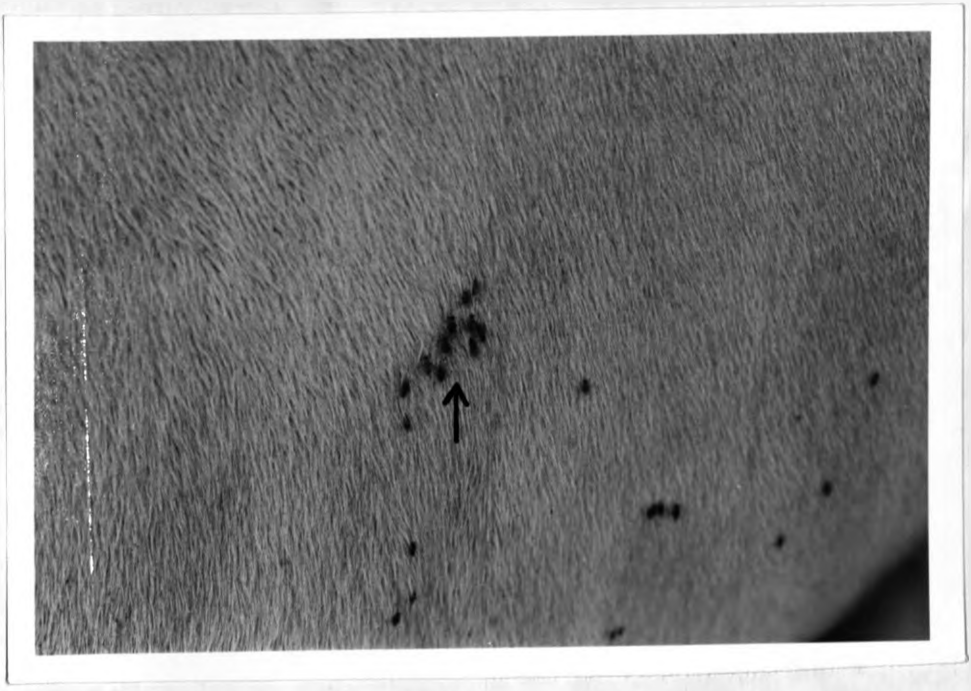


Plate 18 : H.minuta blood-feeding on the camel host belly.

Plate 19 : H.longipennis on the back of a dog host.

head as shown on Plate 19 .

3.3.3.2: Resting sites of biting flies in the study area

After completion of blood meals, some biting flies, particularly *T.taeniola* and *A.agrestis* sought resting sites to digest the blood meal and extrude the water from the blood meal. Flies that were more or less ectoparasitic on camels such as *H.minuta* and the hippoboscids remained on the host after blood meals. The engorged biting fly species that were most noticeable on the surrounding vegetation was *T.taeniola*, probably due to its relatively large size. This fly species was sited resting on branches of shrubs of *Acordia sinensis* (Plate 20&21) which appeared to provide shade.

3.3.4:Seasonal variation in biting fly abundance based on monthly counts per camel.

Numbers of all biting flies fluctuated with the different months of sampling. Changes in fly abundance for all fly species coincided with the rainfall patterns prevailing in the study area during the period of study. Peaks of abundance of these flies were noted to occur from March-May which was during the long wet season . Fly numbers generally declined from June -October,which was during the dry season. Apart from *H.camelina* and *H.minuta* which were present on camels all year round, *S.calcitrans, T.taeniola* and *A.agrestis* were strikingly seasonal, and occurred in large numbers only during or after the wet seasons (March-May and October-December).The seasonal flies were absent or

Plate 20 : Engorged female T.taeniola resting on a lower branch of Acordia sinensis at Ngurunit study site.

Plate 21 : A.sinensis bushes the major resting sites for engorged female T.taeniola at Ngurunit study site.

present in extremely low numbers during the dry season months (June -September and January-February).

3.3.4.1: Hippobosca camelina

Table 12.contain means and standard deviations (S.D) of *H.camelina* per camel per month based on data compiled from the entire study area for years 1986 and 1987.*H.camelina* was the most predominant biting fly in the entire study area being present at all study sites the whole year round and in the highest numbers based on monthly collection results. Monthly mean counts of *H.camelina* ranged from a minimum of 83.1 in January to maximum of 298.7 in May as shown on Table 12. Higher numbers, reflected in higher means of *H.camelina*, were recorded during and after the rains (March-June of 1986 &1987). At the very beginning of the year (January-February) and towards the end of the year (August-December) *H.camelina* were present on camels at all sites but in reduced numbers compared to the wet months (Table 12 & Fig.25) Fig.25 is a further representation of data on Table 12 to show the relationship between *H.camelina* monthly mean counts per camel (abundance) and the prevailing rainfall pattern in the study area during the period of study.According to these results, there was a definite major peak of abundance of monthly mean counts per camel between March and June which coincided with the long wet season (March-May).Monthly mean numbers of *H.camelina* rapidly declined after the long rains from July to September, but slightly increased during the short wet rains (November-December). The most noticeable and hence major peak

Table 12 monthly mean counts of H. camelina per animal in the study area (1986 & 1987).

	Dry season		Wet season			Dry season				Wet season		
$\bar{x}$	83	120	277	297	298	251	186	105	78	100	104	101
sd.	5.24	18.63	17.65	19.57	18.35	15.09	16.78	8.8	8.01	9.02	12.22	10.61

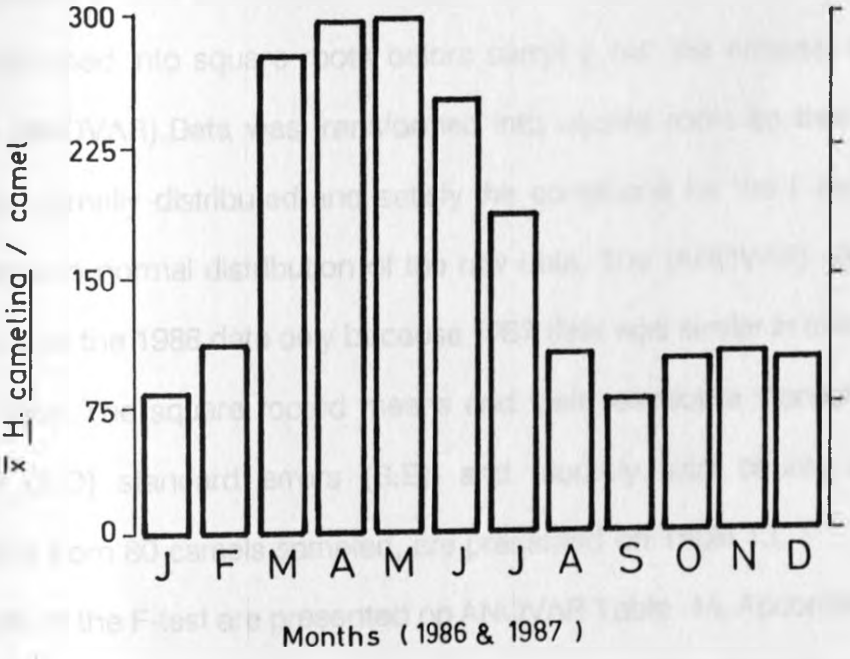


Fig. 25 Monthly fluctuations in H. camelina numbers per camel in the study area based on direct counts.

of abundance of. *H.camelina*, however, was during and after the long rains (March-June).

Raw data pertaining to monthly counts of *H.camelina* per camel contained in Appendix 21 was subjected to the variance ratio (F-) test to detect whether the monthly fluctuations in numbers of these flies were due to chance or due to seasonal change. The raw data for *H.camelina* was transformed into square roots before carrying out the Analysis of variance (ANOVAR).Data was transformed into square roots so that it would be normally distributed and satisfy the conditions for the F-test, which requires normal distribution of the raw data. The (ANOVAR) was carried out on the 1986 data only because 1987 data was similar in trend to 1986 data. The square rooted means and their respective standard deviation (S.D) standard errors (S.E), and monthly total counts of *H.camelina* from 80 camels sampled, are presented on Table 13.

Results of the F-test are presented on ANOVAR Table 14. According to the F value of 1253.176 (Table 14), the seasonal peaks depicted on Fig.25 are not due to chance but due to some external effect because this F-value (1317.938) is much less than the critical value of 1.83 at $p=0.05$.Furthermore according to Table 14, the S2, the estimated variances within monthly means of *H.camelina* is 63.6095 and much less than the value obtained for M (83833.3777). M is the measure of the spread of monthly means. The results of the F-test on data pertaining to *H.camelina* is therefore regarded as significant and HA (monthly means are not equal) accepted in favour of the alternative hypothesis Ho.

Table 13: Number [total counts and square rooted means] of H.camelina per month per camel in the study area for the year 1986

variable motnhs	total no. flies	mean no. flies/camel*	S.D.	S.E.
Jan.	2168.6	27.232	4.75	0.810
Feb.	2867.2	35.668	7.058	0.812
Mar.	6197.8	77.498	9.584	0.812
Apr.	7641.2	95.514	8.034	0.812
May.	7028.0	87.848	6.828	0.812
Jun.	3792.0	47.408	6.904	0.812
Jul.	3240.8	40.510	8.540	0.886
Aug.	1284.2	16.052	7.178	0.886
Sep.	1145.2	14.316	5.830	0.886
Oct.	1369.4	17.120	5.594	0.754
Nov.	2064.4	25.806	6.902	0.754
Dec.	2282.6	28.468	5.946	0.74
total within	41080.4	513.440	83.148 6.929**	9.79

Table 14: ANOVAR table of H.camelina per camel per month based on transformed square rooted data in appendix 21a-21e.

source of variation	d.f.	SS	error MS	F-value	Prob.
between months	11	533286.5782	M(83833.3777)	1317.938	0.000
within months	4740	30137.7230	S (63.6095)		
total	4751	563424.3012			

C.V = 4.5963%

Key...* means after transformation of original data into square roots.
** mean of means.

3.3.4.2: Haematobia minuta

Monthly mean counts, and S.D of *H.minuta* for 1986 and 1987 are presented on Table 15. *H.minuta* was next to *H.camelina* in terms of abundance in the entire study area. *H.minuta* was present in all months of 1986 and 1987 and at all study sites and the monthly mean counts ranged from a minimum of 14.14+/-4.365 in September to 64.3+/-8.214 in May. Bar charts (Fig.26) show variations in numbers of *H.minuta* with months as well as the relationship between such variations to the rainfall pattern prevailing in the study area during the period of the study. As shown on Fig.26, there was an increase in numbers of *H.minuta* per camel with the onset of the long rains in March. The increment reached a peak in May with a mean of 64.3+/_8.214 *H.minuta* per camel. After the long rains, the monthly mean counts of *H.minuta* drastically decreased from 64.3+/-8.214 in May to 14.4+/-4.365 in September. During the short rains (November -December), there was a slight increase in monthly mean numbers of *H.minuta* per camel. It is evident on bar charts on Fig.26 that fluctuations in *H.minuta* monthly mean counts are related to rainfall such that a peak of abundance occurred immediately after the rains particularly in May.

Presented on Table 16 are square rooted means, S.D, S.E and monthly totals of H.minuta.Results of the F-test were significant with an F-value of 42560.12> 1.83 at p=0.05. The significance of these results are further reflected in values of S^2 (estimated variances within months) and M (measure of the spread of monthly mean counts per camel). The value of 0.52 obtained for S^2 is much less than the M value of 1217.69

Table 15 monthly mean counts of H. minuta per camel in the study area (1986 & 1987).

	Dry season		Wet season			Dry season				Wet season		
$\bar{x}$	22	24	49	61	64	44	29	16	14	16	24	25
sd.	2.8	4.5	8.8	7.4	8.2	7.3	5.9	5.0	4.3	3.7	4.4	5.7

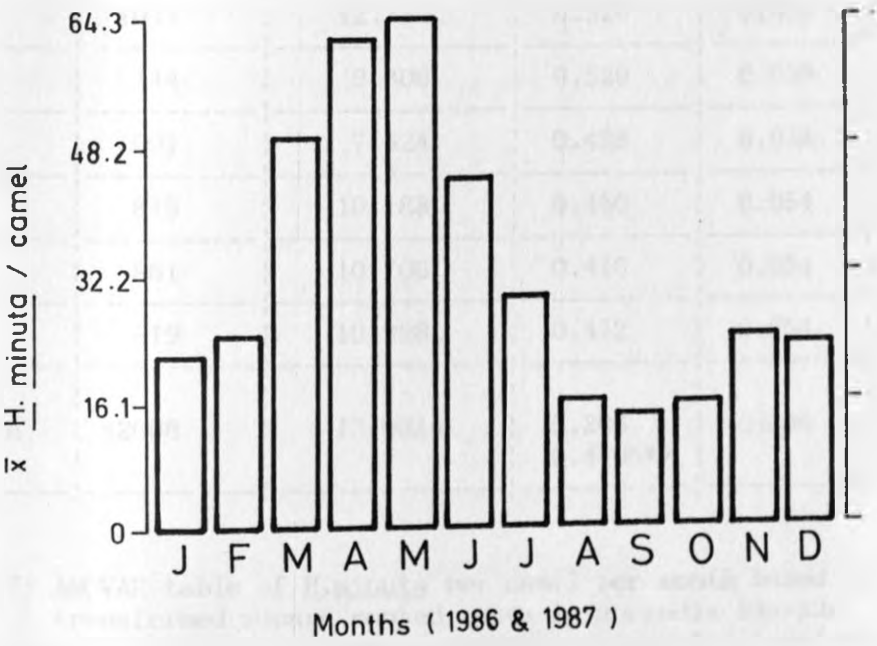


Fig. 26 Monthly fluctuations in numbers of H. minuta per camel in the study area based on direct fly count.

Table 16:Number [total counts and square rooted means] of H.minuta per month per camel in the study area for the year 1986

variable motnhs	total no. flies	mean no. flies/camel*	S.D.	S.E.
Jan.	756	9.450	0.498	0.042
Feb.	948	11.856	0.336	0.042
Mar.	1522	19.032	0.324	0.042
Apr.	1641	20.524	0.330	0.042
May.	1512	18.912	0.430	0.052
Jun.	1255	15.696	0.686	0.062
Jul.	1041	12.710	0.326	0.064
Aug.	744	9.300	0.520	0.038
Sep.	601	7.524	0.498	0.038
Oct.	815	10.188	0.450	0.054
Nov.	861	10.706	0.416	0.054
Dec.	819	10.398	0.472	0.054
total within	62608	13.0315	5.286 0.4405**	0.585

Table 17: ANOVAR table of H.minuta per camel per month based on transformed square rooted data in appendix 22a-22e.

source of variation	d.f.	SS	error MS	F-value	Prob.
between months	11	5499.01	M (1217.69)	42560.1	0.000 0.000
within months	4740	167.17	S (0.52)		
total	4751	5664.18			

C.V = 4.5045%

Key..* means after transformation of original data into square roots.

** mean of means.

(Table 17) an indication that the monthly fluctuations in mean numbers of *H.minuta* per camel are real and are due to factors other than chance alone. Data pertaining to *H.minuta* was square rooted for normal distribution before ANOVAR was carried out.

3.3.4.3: Stomoxys calcitrans

S.calcitrans unlike *H.camelina* and *H.minuta* were not present on camel hosts for all the 12 months in the years 1986 and 1987 as shown on Table 18, which contains zero entries in August and September. Other than during the peak periods of abundance (April-June), monthly mean counts of *S.calcitrans* were relatively low compared to *H.camelina* and almost comparable to *H.minuta*. Monthly mean counts of *S.calcitrans* ranged from a minimum of 8.4725+/-3.09 in February to a maximum of 125.695+/- 10.59 in May. Peak periods of abundance as well as the relationship of *S.calcitrans* abundance to rainfall in the study area is depicted on bar charts on Fig.27. According to Fig.27 there were two peaks of abundance of *S.calcitrans* per camel. The major peak of abundance occurred in May and a minor peak occurred in October,and both peaks coincided with the availability of rainfall. *S.calcitrans* was particularly abundant immediately after the long wet season in May, absolutely absent during the dry spell in August and September and present in low numbers with the onset of the short rains in October. Statistical analysis (F-test) was not carried out on data pertaining to *S.calcitrans* because this data was not normally distributed as evidenced by some zero entries for the months of August and September.

Table 18 Monthly mean counts of S. calcitrans per camel in the study area (1986 & 1987)

	Dry season		Wet season			Dry season				Wet season		
$\bar{x}$	8.6	8.4	54	119	125	70	19	0	0	55	51	13
sd.	1.5	3.0	3.6	13.9	10.5	6.8	2.0	–	–	9.8	10.9	4.7

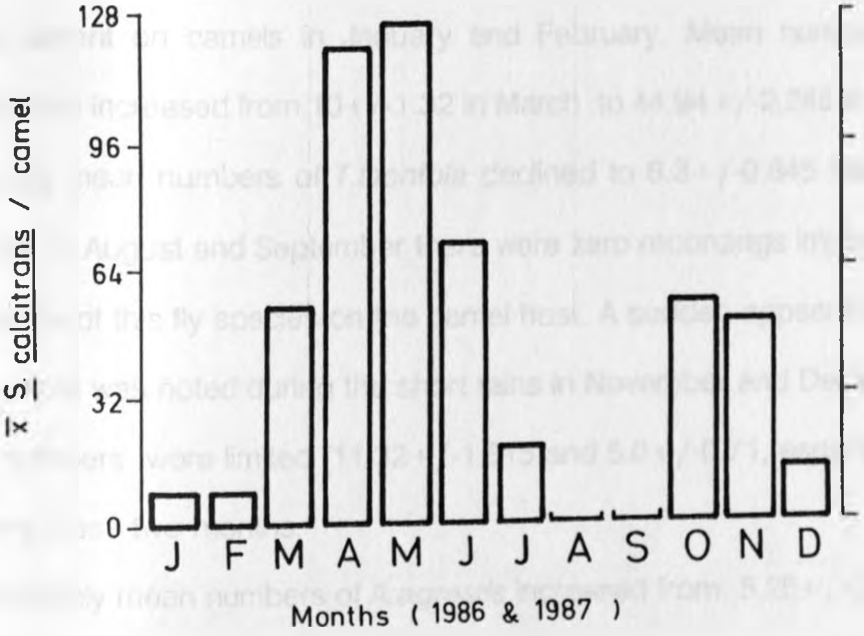


Fig 27 Monthly fluctuations in S. calcitrans numbers per camel in the study area based on direct fly count.

Seasonal fluctuations in numbers of *S.calcitrans* however is satisfactorily depicted on bar charts on Fig.27. The relationship between monthly (seasonal) changes in numbers of *S.calcitrans* per camel to the prevailing rainfall pattern in the study area is also quite evident on Fig.27.

3.3.4.4: Tabanus taeniola and A.agrestis

Tables 19 and 20 show monthly means and S.D of *T.taeniola* and *A agrestis* respectively.These flies were entirely seasonal and occurred on camels only during certain months of the years 1986 and 1987. *T.taeniola* was absent on camels in January and February. Mean numbers of *T.taeniola* increased from 10+/-1.32 in March to 44.94+/-2.245 in May. By July mean numbers of *T.taeniola* declined to 6.3+/-0.645 flies per camel. In August and September there were zero recordings implicating absence of this fly species on the camel host. A sudden appearance of *T.taeniola* was noted during the short rains in November and December but numbers were limited (11.32+/-1.615 and 5.0+/-0.71,respectively) during these two months.

Monthly mean numbers of *A.agrestis* increased from 5.25+/-0.95 in March to 16.0+/-2.0 in May, followed by a decrease between May to August. By September and October there was complete absence of *A agrestis* on camels (Table 20). There were very low numbers (3.5+/-1.45 and 2.0+/-0.21) in November and December. Both *T.taeniola* and *A.agrestis* had two peaks of abundance depicted on bar charts on Figs. 27 and 27 respectively. The major peaks of abundance of these two species of tabanids occurred in May. The minor peaks occurred in

Table 19 Monthly mean counts of *T. taeniola* per camel in the study area (1986 & 1987)

	Dry season		Wet season			Dry season				Wet season		
$\bar{x}$	0	0	10	18	44	21	6	0	0	0	11	5
sd	–	–	1.3	1.5	2.2	1.8	0.6	–	–	–	1.6	0.7

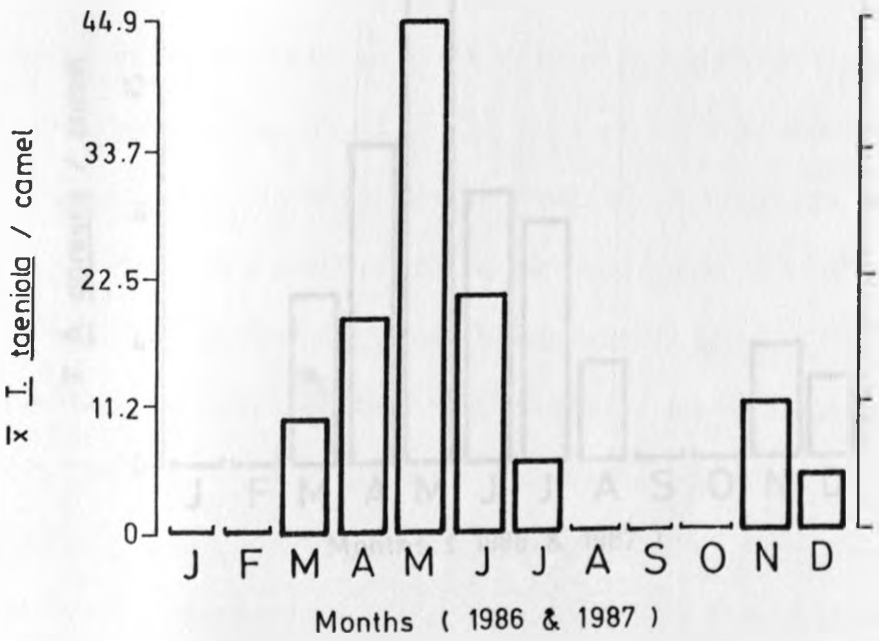


Fig. 28 Monthly fluctuations in *T. taeniola* numbers per camel in the study area based on direct fly counts.

Table 20 Monthly mean counts of A. agrestis per camel in the study area (1986 & 1987)

	Dry season		Wet season			Dry season				Wet season		
$\bar{x}$	0	0	5.2	10	16	8.5	7.5	3	0	0	3.5	2
sd	—	—	0.9	2.2	2.0	0.7	0.6	0.4	—	—	1.4	0.2

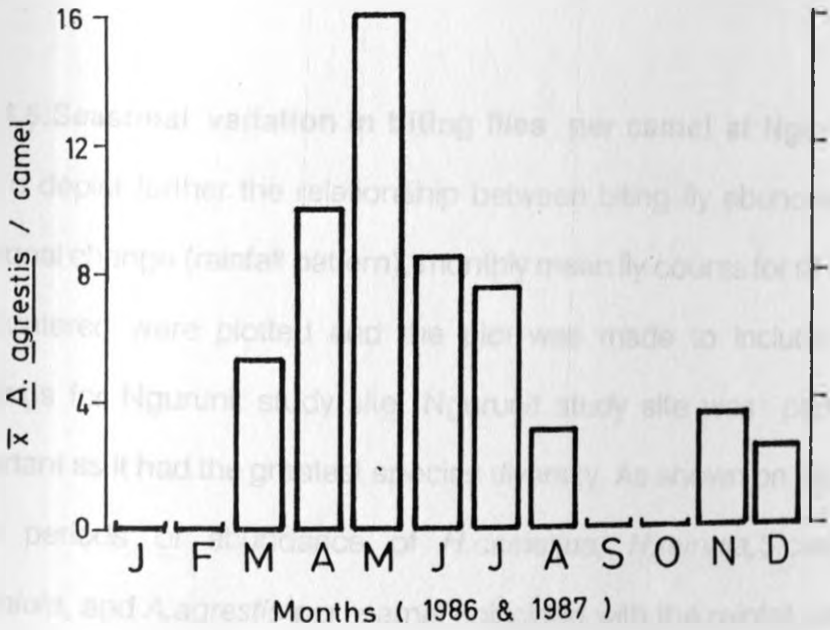


Fig. 29 Monthly fluctuations in A. agrestis numbers per camel in the study area based on direct fly counts

November. As with other biting flies already mentioned,it is evident (Figs.28 and 29) that peaks of abundance of *T.taeniola* coincided with rainy seasons such that peaks occurred either immediately after the long wet season in May or during the short wet season from October to December.Since the assumption of normality of raw data and homogeneity of variances are essential in ANOVAR, data pertaining to *T.taeniola* and *A.agrestis* was not subjected to ANOVAR because they were not normally distributed when taking into account zero entries during the dry seasons.

3.3.4.5:Seasonal variation in biting flies per camel at Ngurunit.

To depict further the relationship between biting fly abundance and seasonal change (rainfall pattern), monthly mean fly counts for all species encountered were plotted and the plot was made to include rainfall readings for Ngurunit study site. Ngurunit study site was particularly important as it had the greatest species diversity. As shown on Fig.30 the peak periods of abundance of *H.camelina, H.minuta,S.calcitrans, T.taeniola*, and *A.agrestis* per camel coincided with the rainfall patterns. For most flies peaks of abundance occurred in May. This was interesting to note because there was plenty of rain at Ngurunit in May,1986. With the exception of *H.camelina* and *H.minuta* the entirely seasonal flies such as *S.calcitrans, T.taeniola* and *A.agrestis* rapidly appeared with the appearance of the rains and disappeared with the disappearance of the rains.

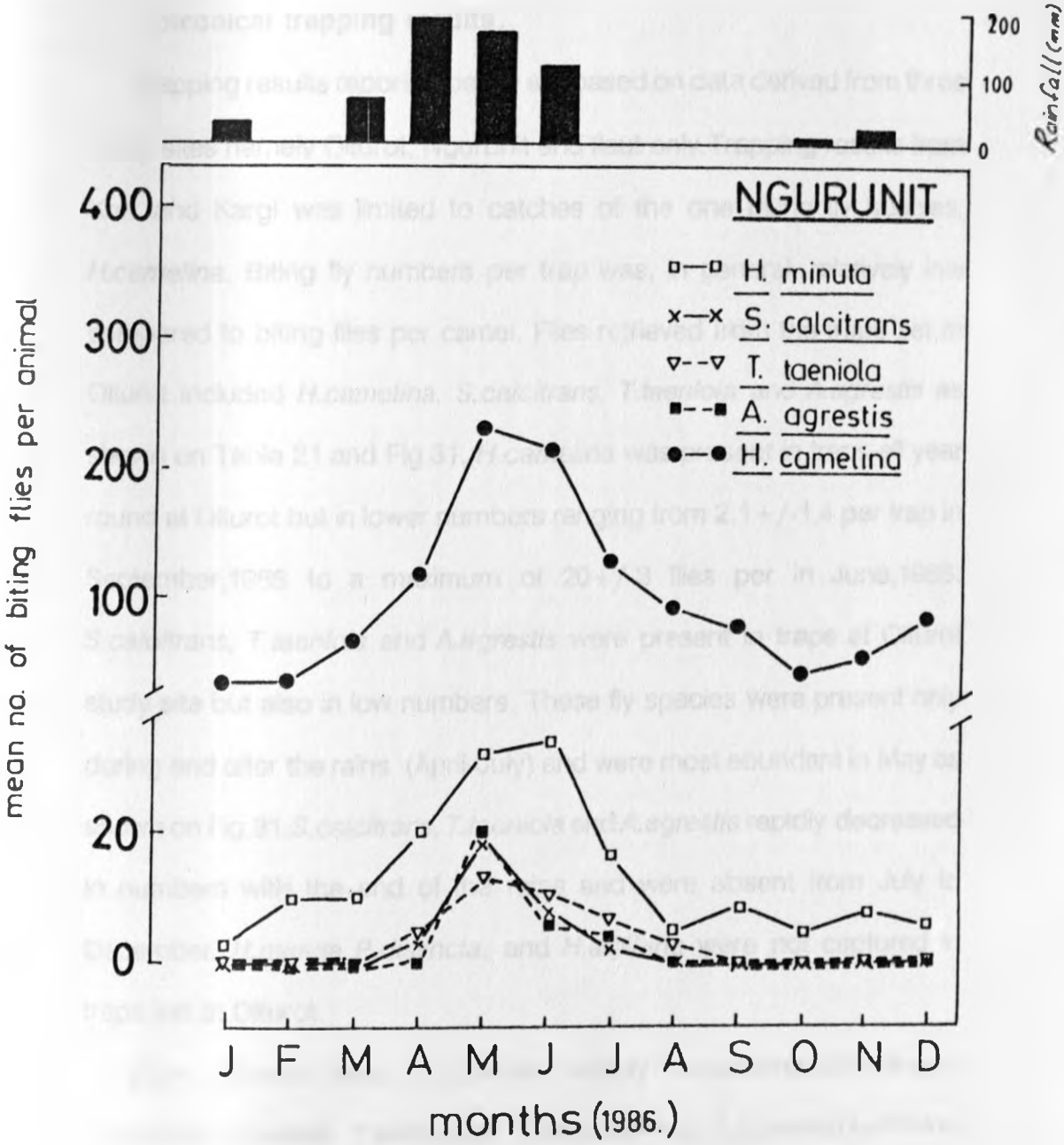


Fig. 30: Seasonal fluctuations in numbers of H. camelina, H. minuta, S. calcitrans, T. taeniola and A. agrestis at Ngurunit study site based on direct fly counts.

3.3.5:Seasonal variation in abundance of biting flies based on biconical trapping results.

Trapping results reported below are based on data derived from three study sites namely Olturot, Ngurunit and Ilaut only.Trapping results from Korr and Kargi was limited to catches of the one biting fly species, *H.camelina*. Biting fly numbers per trap was, in general, relatively low compared to biting flies per camel. Flies retrieved from the traps set at Olturot included *H.camelina, S.calcitrans, T.taeniola* and *A.agrestis* as shown on Table 21 and Fig.31. *H.camelina* was present in traps all year round at Olturot but in lower numbers ranging from 2.1+/-1.4 per trap in September,1986 to a maximum of 20+/-3 flies per in June,1986. *S.calcitrans, T.taeniola* and *A.agrestis* were present in traps at Olturot study site but also in low numbers. These fly species were present only during and after the rains (April-July) and were most abundant in May as shown on Fig.31.*S.calcitrans, T.taeniola* and *A.agrestis* rapidly decreased in numbers with the end of the rains and were absent from July to December. *H.minuta P.distincta*, and *H.albihirta* were not captured in traps set at Olturot.

Eight different biting fly species namely *H.camelina,S.calcitrans, T.taeniola.T.taeniola, T.atrimanus, T.leucostomus, A.agrestis,H.albihirta* and *P.distincta* were collected from Ngurunit study site as shown on Table 22 and Fig.32. With the exception of *H.camelina* which was present in the traps for all the 12 months of the year 1986, the other biting flies were entirely seasonal and occurred only during and immediately after the rains. *S.calcitrans,T.taeniola, P.distincta* and *H.albhirta* were captured in

Table 21 :Mean monthly number of biting flies per trap at Olturot for 1986.

months	J	F	M	A	M	J	J	A	S	O	N	D
fly spp.												
H. camelina												
mean	8.4	3.6	4.5	7.4	12.0	20.0	4.3	8.7	2.1	2.8	9.5	11.1
sd	1.8	2.2	1.8	2.3	1.7	3.0	0.9	2.2	0.3	1.4	1.7	2.5
S. calcitrans												
mean	-	-	-	3.4	11.8	8.8	-	-	-	-	-	-
sd	-	-	-	1.4	2.7	2.5	-	-	-	-	-	-
T. taeniola												
mean	-	-	-	11.9	23.7	16.9	9.3	-	-	-	-	-
sd	-	-	-	1.3	2.4	3.0	2.1	-	-	-	-	-
A. agresitis												
mean	-	-	-	3.8	6.5	5.0	2.6	-	-	-	-	-
sd	-	-	-	0.2	1.7	1.1	0.4	-	-	-	-	-
P. distincta												
mean	-	-	-	-	-	-	-	-	-	-	-	-
sd	-	-	-	-	-	-	-	-	-	-	-	-
H. albihirta												
mean	-	-	-	-	-	-	-	-	-	-	-	-
sd	-	-	-	-	-	-	-	-	-	-	-	-

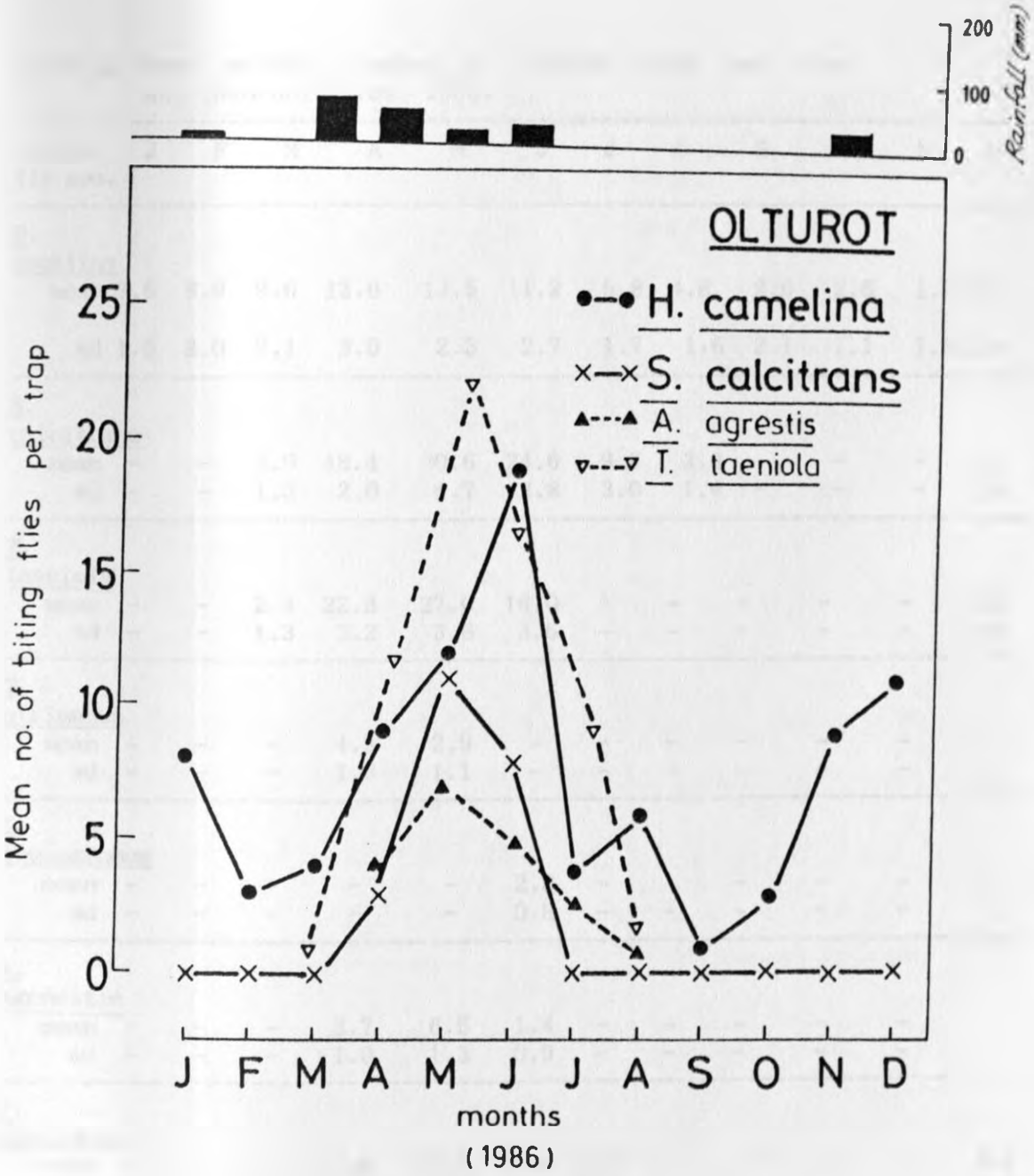


Fig. 31: Seasonal fluctuations in biting fly numbers at Olturot study site based on biconical trapping.

Table 22 :Mean monthly number of biting flies per trap at Ngurunit for 1986.

months fly spp.	J	F	M	A	M	J	J	A	S	O	N	D
H. camelina												
mean	7.6	8.9	9.6	12.8	13.5	11.2	5.8	4.8	9.6	2.6	1.7	7.7
sd	1.5	2.0	2.1	3.0	2.3	2.7	1.7	1.6	2.1	1.1	1.3	2.4
S. calcitrans												
mean	-	-	3.0	18.4	30.6	24.6	9.6	3.9	-	-	-	4.5
sd	-	-	1.3	2.0	4.7	2.8	3.0	1.4	-	-	-	1.6
T. taeniola												
mean	-	-	2.4	22.3	27.0	16.0	-	-	-	-	-	2.3
sd	-	-	1.3	3.2	3.6	3.6	-	-	-	-	-	1.2
T. atrimanus												
mean	-	-	-	4.3	2.9	-	-	-	-	-	-	-
sd	-	-	-	1.0	1.1	-	-	-	-	-	-	-
T. leucostomus												
mean	-	-	-	-	-	2.8	-	-	-	-	-	-
sd	-	-	-	-	-	0.8	-	-	-	-	-	-
A. agresitis												
mean	-	-	-	2.7	6.5	1.4	-	-	-	-	-	-
sd	-	-	-	1.0	1.3	0.9	-	-	-	-	-	-
P. distincta												
mean	-	-	-	2.8	17.5	22.4	8.7	-	-	-	-	2.2
sd	-	-	-	1.8	3.0	5.4	0.6					1.9
H. albihirta												
mean	-	-	-	-	8.2	12.9	4.8	-	-	-	-	-
sd	-	-	-	-	2.6	4.2	1.6	-	-	-	-	-

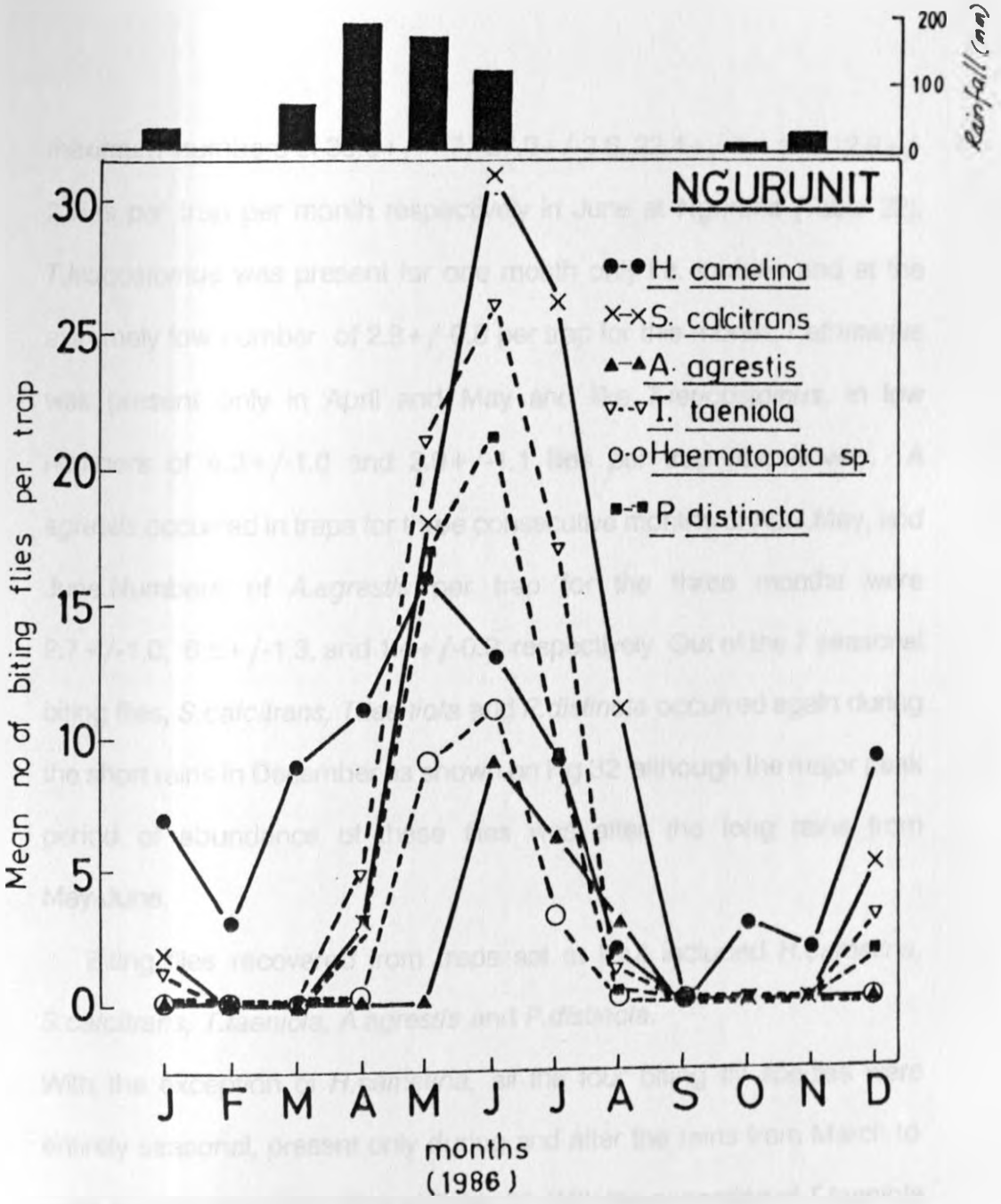


Fig. 32: Seasonal fluctuations in biting fly numbers at Ngurunit study site based on biconical trapping.

maximum numbers of 30.6+/-4.7, 27.0+/-3.6, 22.4+/-5.4 and 12.9+/-2 flies per trap per month respectively in June at Ngurunit (Table 22). *T.leucostomus* was present for one month only i.e. in June and at the extremely low number of 2.8+/-0.8 per trap for this month. *T.atrimanus* was present only in April and May and like *T.leucostomus*, in low numbers of 4.3+/-1.0 and 2.9+/-1.1 flies per trap respectively. *A agrestis* occurred in traps for three consecutive months of April,May, and June.Numbers of *A.agrestis* per trap for the three months were 2.7+/-1.0, 6.5+/-1.3, and 1.4+/-0.9 respectively. Out of the 7 seasonal biting flies, *S.calcitrans, T.taeniola* and *P.distincta* occurred again during the short rains in December as shown on Fig.32 although the major peak period of abundance of these flies was after the long rains from May-June.

Biting flies recovered from traps set at Ilaut included *H.camelina, S.calcitrans, T.taeniola, A.agrestis* and *P.distincta*.

With the exception of *H.camelina,* all the four biting fly species were entirely seasonal, present only during and after the rains from March to June as depicted on Fig.33 and Table 23. With the exception of *T.taeniola* which peaked in April, *S.calcitrans, A.agrestis* and *P.distincta* peaked in May. *H.camelina*, although present all year round also peaked in May as shown on Fig.33.

Based on biconical trapping results at Olturot, Ngurunit and Ilaut, all flies except *H.camelina*, occurred only either during and after the rains. The relationship between biting flies per trap and the rainfall patterns at specific study sites is depicted on Figs.31,32 and 33

Table 23 : Mean monthly number of biting flies per trap at Ilaut for 1986.

months	J	F	M	A	M	J	J	A	S	O	N	D
fly spp.												
H. camelina												
mean	7.4	6.4	8.7	9.2	13.6	11.4	6.1	4.9	9.0	3.2	2.2	7.4
sd	1.7	2.0	2.1	1.6	2.7	2.5	1.9	1.6	2.4	1.4	1.0	1.7
S. calcitrans												
mean	-	-	3.4	12.7	25.3	13.6	7.2	-	-	-	-	-
sd	-	-	1.1	2.4	4.3	3.3	2.8	-	-	-	-	-
T. taeniola												
mean	-	-	1.3	4.7	3.8	1.8	-	-	-	-	-	-
sd	-	-	0.1	2.1	1.8	0.6	-	-	-	-	-	-
A. agresitis												
mean	-	-	-	2.0	8.9	2.5	-	-	-	-	-	-
sd	-	-	-	0.6	1.4	1.3	-	-	-	-	-	-
P. distincta												
mean	-	-	-	2.1	3.6	-	-	-	-	-	-	-
sd	-	-	-	0.3	0.2	-	-	-	-	-	-	-
H. albihirta												
mean	-	-	-	-	-	-	-	-	-	-	-	-
sd	-	-	-	-	-	-	-	-	-	-	-	-

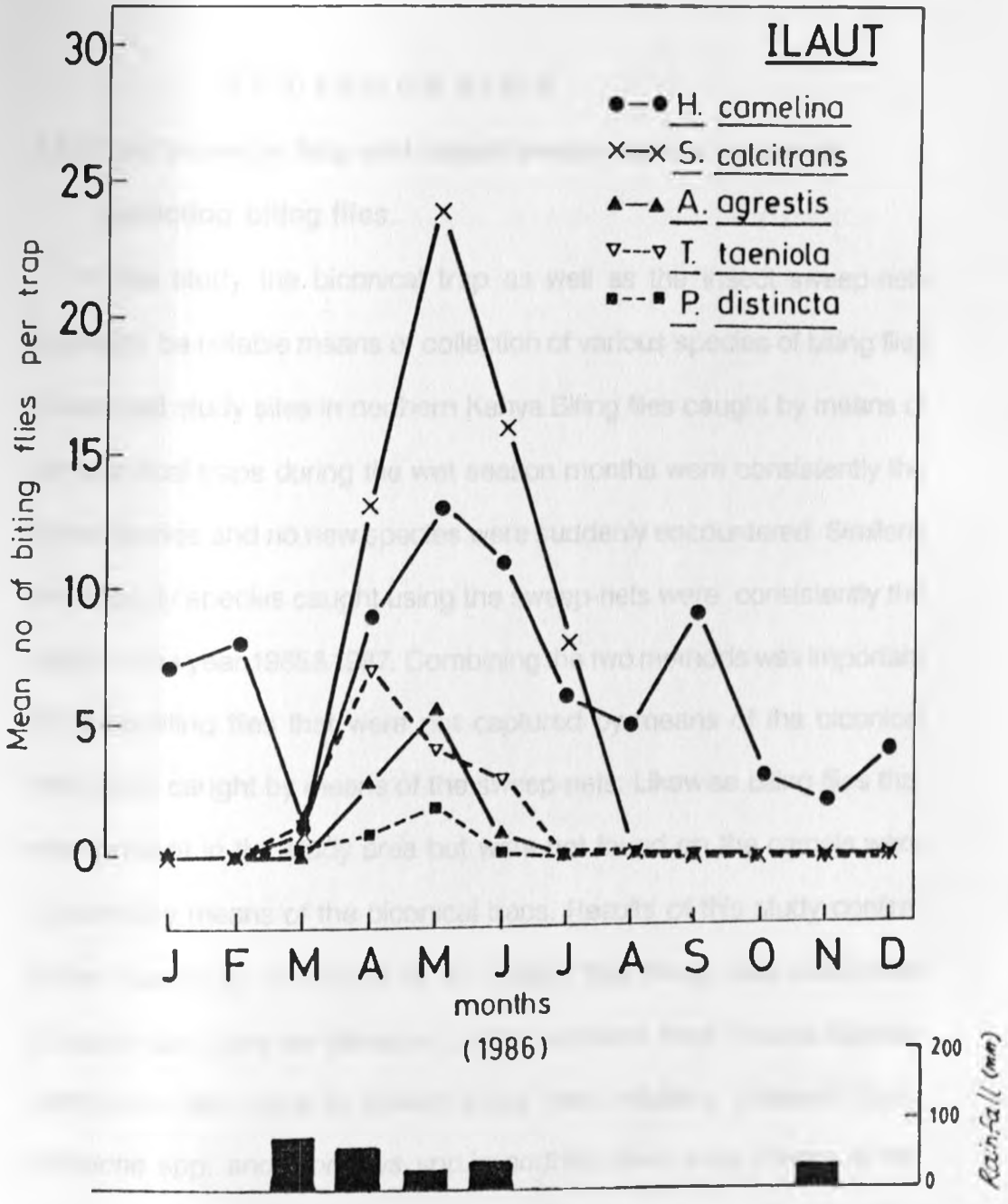


Fig. 33: Seasonal fluctuations in numbers of biting flies at Ilaut study site based on biconical trapping.

3.4: D I S C U S S I O N

3.4.1:The biconical trap and insect sweep-nets as means of collecting biting flies.

In this study the biconical trap as well as the insect sweep-nets proved to be reliable means of collection of various species of biting flies at selected study sites in northern Kenya.Biting flies caught by means of the biconical traps during the wet season months were consistently the same species and no new species were suddenly encountered. Similarly the biting fly species caught using the sweep-nets were consistently the same for the year 1986&1987. Combining the two methods was important because biting flies that were not captured by means of the biconical traps were caught by means of the sweep-nets. Likewise biting flies that were present in the study area but were not found on the camels were captured by means of the biconical traps. Results of this study confirm earlier reports by Dransfield *et al.* (1983), that biting flies other than *Glossina* spp. may be attracted to the biconical trap. Omara-Opyene (1986) was also able to collect biting flies including *Tabanus* Spp., *Philoliche* spp. and *Stomoxys* spp.in northern Kenya by means of the biconical trap.The direct sweep-net collection method was limited to biting flies available on the camel host at the time of collection. The indirect trapping method i.e. the use of biconical trap was therefore more efficient than the direct sweep-net collection method. This was so because not all biting flies captured by means of the biconical trap were captured by means of the sweep-nets. Only one biting fly species (*H.minuta*) was

caught by means of the sweep-nets and not by the biconical traps whereas four biting fly species (*T.atrimanus, T.leucostomus, H.albihirta* and *P.distincta*) were caught strictly by means of the biconical trap. The box trap caught mainly *S.calcitrans* although it is specifically designed to capture *Glossina* spp. This indicated the absence of *Glossina* spp. in the study area.

3.4.2: Seasonal variations in abundance of biting flies in the study area.

Seasonal fluctuations in the size of monthly catches and in the occurrence of individual fly species has been confirmed in this study.Highest incidences of seasonal fluctuations in numbers was observed for *S.calcitrans* and tabanids (*T.taeniola, T.atrimanus, T.leucostomus, A.agrestis, H.albihirta* and *P.distincta*). Seasonal fluctuations in all biting flies showed a dependence on the annual rainfall pattern similar to those described by Reid (1957) in Sudan; Harley (1965) in Uganda; Goodier (1966) in Zimbabwe; Clarke (1968) and Okiwelu (1975,1977) in Zambia; Glasgow (1946), Wiessehutter (1975), Chapman (1960) and Vanderplank (1944) in Tanzania; Folkers and Mohamed (1965) in the Shika region of Nigeria.

Wet conditions are a prerequisite for breeding for some biting flies, hence the sudden appearance of *S.calcitrans* and tabanids with the onset of the rains. Seasonal occurrence of tabanids has drawn the attention of some authors in view of the suspected role of mechanical transmission of trypanosomes by these flies in the absence of *Glossina* spp. As far

back as 1905 Cazalboui (1905) reported numerous *T.unimaculatus* Macq. and *T.dorsivitta* Walker during the rainy season (June-October) in the Sudan, when cattle and horses were virtually covered by these tabanids. According to reports by Lewis (1949), tabanids in inland Sudan were abundant in years of heavy rain during the rainy season. Reports of increased numbers of *T.taeniola, T.biguttatus, A.agrestis, A.fuscipes,Ancala latipes* and *P.magretti* in the Sudan during the rainy season have also been given by Yagi and Razig (1972a,1972b,1975) and Razig and Yagi (1975).

Results of this study are comparable to studies by Harley (1965) in Uganda, in which human bait catches revealed a seasonal occurrence of tabanids; the greatest abundance was in May-August which was during and after the rainy season. A second peak of abundance of tabanids was noted towards the end of the second rainy season (November-December) by Harley (1965). Similarly, in Zambia, Okiwelu (1975) reported abundance of *T.taeniola,T.suis, T.conformis, T.sandersoni, T.variabilis, H.pallidimarginata, H.pertubans, H.decora, H.stimulans,H .hirsutitarsis* and *H.distincta* during the rainy season (November-April) in Wenya Game Reserve.

Although seasonal fluctuation of biting flies is closely associated with rainfall, it is interesting to note that this is not always the rule for some species of tabanids. Studies by Wiessenhutter (1975) revealed that *T.gratus* Loew and *H.decora* were not wet season but dry season species, while *T.taeniola* increased during and after the rains in Tanzania.

In this study, the tabanids (*T.taeniola, T.atrimanus, T.leucostomus, A.agrestis, H.albihirta* and *P.distincta*) were all entirely wet season flies with *T.taeniola* being the predominant tabanid.Predominance of *T.taeniola* among tabanid collections has been observed by Harley (1965) who reported that this species constituted over 50% of the total tabanid collection following his studies in Uganda.

Tabanids, although abundant during the wet season, were persistent and most species were available in May,towards the end of the rains rather than in March, at the beginning of the rains. Studies by Yagi and Razig (1972a) revealed that some tabanids (*T.taeniola, A.agrestis* and *A.fuscipes*) peaked at the end of the rains while *Ancala latipes* and *P.magretti* were seen in low numbers only during the rains in Sudan. It appears therefore that peak periods of abundance of tabanids are not uniform but occur at different times for different species within the rainy seasons.

Occurrence of tabanids only during the rainy season or their increased numbers during the rainy season is best explained by the nature of their life cycle; tabanids are semi-aquatic in their life cycle (Oldroyd,1954; King,1909; Neave,1915). Food requirements for adult tabanids are also best met in the wet seasons, when plants are available, even in the normally arid areas like northern Kenya. Carbohydrates are necessary to provide energy for adult tabanid, which visit flowers for nectar and honeydew during the rainy season.

The vicious blood-feeding behaviour of female tabanid accounts for their adaptation to arid areas such as northern Kenya which although dry

is continually habited by livestock such as camels. Protein from blood meals, needed for egg production and the animal hosts are particularly plenty during the wet seasons. Cattle, which are usually absent in the study area are driven into the area during the wet season, thereby providing an extra source of blood meals in addition to the camel host blood. Male tabanid have no mandibles and are not able to pierce the skin to suck blood. Males rely on the carbohydrates diet readily available during the wet season and probably take blood from open wounds produced by other blood-sucking insects to supplement the plant-derived diet.

It was noted that tabanids were absent at Kargi and Korr study sites even during the wet season. Lack of vegetation at these sites,hence lack of plant diet source and resting sites may be a possible explanation for this observation.

As with tabanids, the seasonal pattern of *S.calcitrans* may be related to the life cycle and the readily available protein diet from extra animals in the study area during the wet season. *S.calcitrans* breeds in wet organic decaying matter such as cow-dung and straw (Harwood and James,1979). Since dryness prevents larval development of *S.calcitrans*, it was not surprising that the fly was not captured during the dry season. *S.calcitrans* was also absent at Kargi and Korr sites, which remained extremely hot and dry even during the wet season with limited vegetation.

The relatively short life cycle of *S.calcitrans* (20-30 days) was easily accommodated by the short favourable wet and humid environmental conditions during the wet season months at Olturot, Ngurunit and Ilaut

study sites.

Seasonal occurrence of *Stomoxys* spp. although not previously documented in Kenya has been studied in Uganda, Zambia, Mauritius, Sudan and Mali. Studies in Zambia by Okiwelu (1975,1977) using sticky adhesives to catch flies, revealed that *S.calcitrans* and *S.nigra* occurred over two rainy seasons. *Stomoxys* spp. were reported to be numerous during the rainy season (June-October) in Mali by Cazalboui (1905) and the fly season coincided with outbreaks of trypanosomiasis. Abundance of *Stomoxys calcitrans* and *S.nigra* in Uganda coincided with the rains (Harley,1965). According to reports by Moutia (1928b) *S.nigra* abundance in Mauritius was closely associated with rains. Studies by Kunze and Monty (1976) revealed seasonal abundance of *S.nigra* and *S.calcitrans* in Mauritius. Populations of *S.nigra* of up to 200 flies per animal were observed during the warm wet season. Surprisingly in studies by Moutia (1928b) and contrary to findings of this study, *S.calcitrans* were abundant during the dry seasons (145 flies per animal) and virtually absent during the wet season.

Although *S.nigra* has been reported in other parts of Kenya such as Kibwezi area, the species was not among collections from the study area. Zumpt (1973) noted that *S.nigra* was the most common species in the Ethiopian region. Coaker and Passmore (1958) and Harley (1965) also found *S.nigra* to be more abundant than *S.calcitrans* in Uganda. In northern Kenya on the other hand, the situation was different in that only *S.calcitrans* was available from the traps and on the animals during the wet season months.

Although seasonal occurrence of biting flies in the study area was most noticeable for tabanids and *Stomoxys calcitrans*, it was also noted for *H.camelina* and *H.minuta*. *H.camelina* was the most prevalent biting fly in the study area. With a life cycle independent of water it was therefore the best adapted fly to the arid environment. Female *H.camelina* do not lay eggs but produce fully grown larvae which assume the pupal stage immediately after extrusion. The puparium is hard and protected by the heavy coat, another adaptation to harsh arid conditions. This fly is laterally flattened and possesses prominent claws, an adaptation to an ectoparasitic mode of life on the animal host. Since *H.camelina* is well adapted to an ectoparasitic mode of life particularly, on the camel host, this enhances it's survival and establishment in the arid environment along with the camel host. Physiological adaptation(s) of *H.camelina* to the desert environment is another important possibility that needs to be studied. The food requirement of *H.camelina* is probably limited to a blood meal diet only. Due to limited flight activity, *H.camelina* may not require plant (carbohydrate) diet, hence it's dependence on a complete blood meal diet. *H.camelina* was present at all study sites all year round but with the largest numbers during and after the wet season months. Fluctuations in numbers of *H.camelina*, like tabanids and *S.calcitrans*, followed the prevailing rainfall pattern in the study area. Although the life cycle of *H.camelina* is independent of water, it is likely that wetness prolongs the adult life span or speeds up the life cycle leading to the observed abundance of these flies during and after the wet season.

H.minuta was the second prevalent biting fly in the study area but in slightly lower numbers than *H.camelina*. *H.minuta* appeared well adapted not only to the camel host but to all animals in the study area. Physiological adaptations of *H.minuta* to the arid situation may be similar to those of *H.camelina* and need further studies. *H.minuta* was not host specific to the camel host but appeared to be an opportunistic feeder, taking blood meals on any animal host, including the donkey, which appeared to have been avoided by the other biting flies. Morphologically, the reduced size of *H.minuta* is suitable for it's observed ectoparasitic mode of life on animal hosts in the study area. Like *H.camelina*, it was limited in flight activity and it also probably does not require a source of energy (Plant diet) and thus survives solely on a protein blood meal diet. Seasonal fluctuations in numbers of *H.minuta* were similar to *H.camelina* such that flies increased during and after the rains.

3.4.3: Diurnal biting behaviour of biting flies.

Diurnal biting periodicities of flies reported in this study extended from 0900-1500 h, there being noticeable specific peaks of blood-feeding activity for individual species. Definite peaks of blood-feeding activity of haematophagous insects is a well documented phenomenon for tabanids, *Stomoxys* spp, mosquitoes and other biting flies. Studies by Harley (1965) in Uganda showed peak blood-feeding activity of *T.taeniola, T.par, T.thoracinus, Atylotus fuscipes, Ancala africana* and *Ancala fasciata nilotica* to be 1200-300 h during the wet season. Peak blood-feeding hours of tabanids was observed to be around midday (1200 h) in Zambia

(Okiwelu,1975) and Tanzania (Vanderplank,1944). Woo (1977) also reported that tabanids usually feed during the warm sunny hours of the day. These observations are similar to results of this study, whereby peak blood-feeding periods for tabanids (*T.taeniola* and *A.agrestis*) was midday (1200 h). Based on human bait catches, some tabanids (*Chrysops silacea* and *C.didmidiata*), exhibited a peak blood-feeding activity at 1700 h (Duke,1958). This was a much later peak of blood-feeding activity compared to findings of this study, whereby *T.taeniola* and *A.agrestis* exhibited a peak blood-feeding activity at 1200 h.

Stomoxys calcitrans, unlike the tabanids had two peaks of blood-feeding activity; the morning peak occurred at 0900 h and the afternoon peak occurred at 1500 h. *S.calcitrans* has been shown to be mainly active between 1000-1500 h (Woo,1977). The late afternoon blood-feeding activity noted in this study, was also observed by Harley (1965) in Uganda. Kunze and Monty (1976) noted a morning (0800-0900 h) and an afternoon (1400-1500 h) peak of blood-feeding activity of *S.calcitrans* and *S.nigra* in Mauritius.These findings are quite similar to observations made in this particular study . Kunze and Monty (1976) also noted that peaks of blood-feeding activity were more pronounced for the female *S.calcitrans*, a factor that was not investigated in this study.

It was interesting to note from studies by Harley (1965) that peak blood-feeding hours of tabanids varied with seasons. In the dry season the peak period of blood-feeding activity was 1100-1200 h whereas in the wet season the peak blood-feeding period was 1200-1300 h in Uganda. In this study, tabanids and *Stomoxys calcitrans* were not available in the

dry season, therefore peak blood-feeding hours during the wet and dry seasons could not be compared.

Reports on diurnal blood-feeding activity on *Glossina* (*G.pallidipes*) showed that biting activity occurred in the early morning (0500-0600 h) and again later in the afternoon (1600-1800 h) at Kilifi, Kenya (Moggridge,1949). It is likely that blood-feeding hours of *Glossina* spp. are different from blood-feeding hours of tabanids and *Stomoxys* spp.

Important environmental factors that appeared to determine blood-feeding activity of flies observed in this study, included time of day, temperature and light intensity because the flies were noted to blood-feed during bright sunny hours of the day when temperatures were higher than early morning or late afternoon temperatures.

Light intensity has been reported to influence flight activity of tabanids (Duke,1958; Corbet,1964; Roberts,1974). With changes in light intensities in the course of the day, blood-feeding hours of tabanids are therefore not likely to remain uniform. Kunze and Monty (1976) noted that temperature enhanced blood-feeding activity of *Stomoxys* spp. more than any other environmental factor. Work by Alverson and Noblet (1977) revealed that important meterological factors that determined activity of tabanids (*Chrysops* spp *Tabanus* spp.) in S.Carolina, U.S.A. were temperature, barometric pressure, relative humidity, time of day and cloud cover.It is possible that blood-feeding activity of haematophagous flies is determined by a combination of several factors . It is also important to note that the relative importance of meterological factors that may affect biting fly activity is dependent upon the location of sampling and the

insect species involved.

The importance of understanding peak hours of biting activity of haematophagous flies cannot be over-emphasised. Such knowledge makes collection of these flies easier especially using the direct collection methods from the animal hosts. Secondly, for effective control measures against these flies, information on their biting activity is useful in cases where topical insecticides are to be applied.

In this study, some biting flies showed a remarked host specificity although *H.camelina* and *H.minuta* were not quite host specific. *H.longipennis* for instance was remarkably host specific and took blood meals specifically from the dog host. Tabanids (*T.taeniola* and *A.agrestis*) and *S.calcitrans* preferred the larger animal hosts such as camels and cattle as opposed to goats, sheep and dogs. Species of tabanids have been reported to bite camels, cattle, horses, donkey, buffalo and man in the Sudan (Lewis,1949). It is probable that tabanids will feed on most large mammals available to them. The donkey, although a large mammal, was not attacked by tabanids and *S.calcitrans* in this particular study.

Preference for some animals hosts over others is a phenomenon commonly reported for haematophagous flies (Muirhead-Thomson,1982). For instance, *G.pallidipes* and *G.morsitans* have been shown (Pilson *et al.*,1978) to prefer the ox over goat and sheep.

Preferred predilection site studies in relation to the camel host showed that different fly species preferred specific camel body regions. *H.minuta* however,which was not host specific, was also not site specific on the

camel host. Preference of different body regions by tabanids was observed in studies by Folkers and Mohamed (1965) in Nigeria, whereby *Haematopota* spp. and *Tabanus* spp. preferred the legs. Mullens and Gerhardt (1979) showed that different species of tabanid selected specific areas of the body when blood-feeding on cattle in U.S.A. In this study, *T.taeniola* and *A.agrestis* preferred the legs and belly while in studies by Mullens and Gerhardt (1979), *T.sulcifrons, T.attratus, T.americanus* and *T.anulatus* preferred the upper torso or back regions of cattle hosts while *T.quinquevittatus* preferred the lower body regions including the legs.

In this study, *Stomoxys calcitrans* preferred the hind legs,a phenomenon previously observed at Dagorretti slaughter house, Kenya (Ogonji,1983).

Factors that have been put forward as determinants of blood-feeding site selection by haematophagous flies include hair length, tail action, head swinging, kicking and stampeding (Muirhead-Thomson,1982). In this study, however, *S.calcitrans* preferred the hind legs of the camel host in spite of the kicking action by the camel in response to the bites, suggesting that factors other than the above mentioned may influence site selection by haematophagous flies. Tail action may not be particularly important in relation to the camel host, whose tail is relatively short to influence flies landing on the hind quarters of the animal.

Determination of resting sites of biting flies following blood meals was not an objective in this study. Resting sites of one species of Tabanidae (*T.teaniola*), was however, observed to be the lower branches of *Acordia sinensis*. The preponderance of this tabanid at lower levels such as tree

branches may be related to its host preferences. It is likely that hosts of *T.taeniola* are not arboreal. By resting at lower levels *T.taeniola* is thus able to locate animal hosts which appear to be predominantly domestic livestock. Studies by Okiwelu (1977) in Zambia also showed that *Tabanus* spp. and *Haematopota* spp. rested at lower levels i.e. only up to 4 m. above ground in a woodland area. Haddow (1945) and Haddow *et al*. (1950) also reported that tabanids rest close to the floor of the forest in Uganda. Worth noting is the fact that understanding of resting sites of insect vectors or suspect vectors in general is important for the effective use of residual insecticides.

CHAPTER FOUR

FIELD AND LABORATORY MECHANICAL TRANSMISSION EXPERIMENTS

4.0:INTRODUCTION AND LITERATURE REVIEW

Several species of biting, non-biting flies and ticks have been implicated in mechanical transmission of various trypanosome species, particularly *T.evansi*. Since early reports by Nieschulz (1927,1928,1929, 1930), Taylor (1930) and Moutia (1928b), only a few reports have been published on experimental mechanical transmission of trypanosomes by biting flies. Most of the early work on mechanical transmission of trypanosomes lacks full details of the procedures followed and other details such as parasitaemia of donor animal hosts, numbers of flies used and time intervals between infective and infecting blood meals by the biting flies. A survey of the literature has revealed conflicting experimental evidence on mechanical transmission of trypanosomes by biting flies. For instance while Chaudhuri *et al*., (1960), Mwambu (1969) and Fraser and Symonds (1908) failed to mechanically transmit *T.evansi* using *Stomoxys* spp. others such as Moutia (1928b), Duke *et al*., (1934) Schuberg and Kuhn (1911), successfully transmitted trypanosomes using *Stomoxys* spp. There is therefore a need for further experimentation (laboratory and field-orientated) to update information on mechanical transmission of trypanosomes.

Mechanical transmission of trypanosomiasis by biting flies other than *Glossina* spp is generally an accepted phenomenon (Wells, 1982; Hoare,

1947, 1957; Weitz; 1973 Luckins, 1988), to explain outbreaks of trypanosomiasis particularly, *T.evansi*, in the absence of *Glossina* spp. There remains a need to demonstrate mechanical transmission of trypanosomes experimentally and to define parameters that determine its occurrence in order to conclusively confirm mechanical transmission of trypanosomes by biting flies and to comprehend the implications under field conditions.

One important parameter that affects mechanical transmission of trypanosomes concerns the survival and viability of trypanosomes in the biting fly mouthparts after an infective blood meal. Knowledge of survival time and infection rates within mouthparts of wild biting fly populations, in relation to epidemiology and epizootiology of trypanosomiasis, cannot be over-emphasised. This is so because the ability of trypanosomes to utilize biting flies as mechanical vectors depends primarily on the ability of the parasites to survive and remain viable in fly mouthparts. This parameter has been investigated in this study, both in the field and in the laboratory.

4.1.1: Tabanids in experimental mechanical transmission of trypanosomes.

Compared with *Stomoxys* spp. and other biting flies, tabanids, especially *Tabanus* spp. have generally been implicated as the most likely mechanical vectors of trypanosomes both in the field and in the laboratory. The first scientific evidence of mechanical transmission of *T. evansi* by tabanids was provided by Rogers (1901), in India in a classic experiment. He demonstrated that when horseflies (=tabanids) which had just fed on a *T.evansi*-infected dog were interrupted in their blood-

feeding and at once allowed to bite a healthy dog or rabbit, the latter became infected. Mechanical transmission of trypanosomes was later reported by other workers including Sergent Ed and Et (1905, 1906). Using *T.nemoralis* Meigen, 1820, they effected mechanical transmission of *T.brucei* but did not report the full details of their experiments. Fraser and Symonds (1908) found that *Tabanus* spp. could transmit *T.evansi* if not more than 5 minutes elapsed between the two feeds. Lesse (1909),in India, reported positive mechanical transmission of *T.evansi* with *Tabanus* spp. and *Haematopota* sp. Transmission was possible even when as few as 4 flies per trial were used. Mitzmain (1916) reported that *Tabanus striatus* Fabricus, could transmit *T.evansi* mechanically and was probably the main vector in the Phillippines. Cross and Patel (1921) reported the mechanical transmission of *T.evansi* using *Tabanus albimedius* Walker. Subsequent work by Cross (1923), showed that *T. albimedius* mechanically transmitted *T.evansi* but with fewer successes when the donor animal had a low parasitaemia. Experiments by Nieschulz (1926, 1941) in the East Indies showed that *T.immanis* Wiedemann, *T minmus* Walp., *T.rubidus* Wiedemann, and *T.striatus* could mechanically transmit *T.evansi*. Maramorosch (1962) has listed all tabanid species that are possible mechanical vectors of *T.evansi*. Kaneveld (1931), cited by Krinsky and Pechuman (1975), reported experimental transmission of *T. theileri* from tabanids by the stercorarian route. Although most experimental mechanical transmission experiments indicate that tabanids are the most likely mechanical vectors of *T.evansi*, other biting flies must not be ruled out because epizootics of *T.evansi* (Greenberg, 1973) have occurred in the absence of tabanids but in the presence of other flies such as *Stomoxys* spp. and *Haematobia* spp.

4.1.2: Stomoxys spp. in experimental mechanical transmission of trypanosomes.

Nieschulz (1926,1927,1928,1930,1941) can be credited with the most thorough contribution to the study of mechanical transmission of *T.evansi* using *Stomoxys* spp. and other flies. Working in Java and Sumatra, he performed 116 experiments with *S.calcitrans* and *S.brunipes* Billeneuve on various animals and obtained 44 successful transmissions when he used 10 - 25 flies per trial. Musgrave and Clegg (1903) in the Phillippines, mechanically transmitted *T.evansi* from infected monkeys to dog, horse, rat and guinea pig by means of *S.calcitrans*. Sergent Ed and Et (1905, 1906), using *Stomoxys*, sp., mechanically transmitted *T.brucei*. Schuberg and Kunh (1906) reported the transmission of *T.brucei* using *Stomoxys* sp. Edington and Coutts (1907) transmitted *T.evansi* using *S.nigra* in 50% of the 12 trials they conducted in Algeria. Lesse (1909) reported only one positive result in three trials with *Stomoxys* sp. in the mechanical transmission of a trypanosome species which he did not specify. Martin *et al*., (1909), using *Stomoxys* sp. successfully transmitted *T.cazalboui* Laveran, 1905 mechanically. Jowett (1911) reported only one success in transmitting trypanosomes mechanically by both *Stomoxys* sp. and *Haematopota*. sp. Kuhn and Schuberg (1911) mechanically transmitted *T.gambiense* using *Stomoxys* sp. Bouet and Roubaud (1912), using *Stomoxys* sp. mechanically transmitted *T.vivax* and *T.brucei*. Sergent and Donatien (1921, 1922) mechanically transmitted *T.berberum* and *T.evansi* among guinea pigs using *Stomoxys* sp. Systemic experiments in Mauritius (Moutia, 1928b) indicated that *Stomoxys nigra* transmitted *T.evansi*. Transmission was successful when the interval between the two feeds

was less than 10 minutes. Positive results from experiments by Poulton (1934) cited by Lucas (1955), led him to the conclusion that *Stomoxys* sp. and tabanids could mechanically transmit *T.congolense* from infected to healthy cattle. Mwambu (1969) conducted a study of mechanical transmission of *T.congolense* by *Stomoxys* sp. but failed to transmit the parasite using this fly. In a study by Dixon *et al*., (1971) in Uganda, the role of *Stomoxys* sp.in mechanical transmission of trypanosomes was evaluated and it was found to be a possible but poor mechanical vector of *T.brucei*. Although some researchers such as Taylor (1930), Duke *et al*. (1934) and Schuberg and Kuhn (1911) have successfully transmitted trypanosomes using *Stomoxys* spp., other workers such as Mwambu (1969), Fraser and Symonds (1908) and Hornby (1928) have failed to do so, making the role of this fly in the mechanical transmission of trypanosomes uncertain. These inconsistencies in experimental and natural mechanical transmission of trypanosomes calls for further investigations on the subject.

4.1.3: Glossina spp.in experimental mechanical transmission of trypanosomes .

Although the role of *Glossina* spp.in mechanical transmission of trypanosomes in nature is poorly understood; under laboratory conditions, there have been reports indicating that *Glossina* spp. can mechanically transmit several species of trypanosomes. According to a report by Dutton *et al*., (1907), *Glossina palpalis* Rob. - Desv. mechanically transmitted *T.gambiense*. In a study by Taylor (1930), *Glossina tachinoides* mechanically transmitted *T.brucei* between guinea pigs, when the interval between the two feeds was 10 minutes. Bailey (1965), reported successful mechanical transmission of *T.rhodesiense* using *Glossina morsitans* from infected to clean mice. In a study by Mwambu (1969), *G.morsitans* and *G.austeni* Austen mechanically transmitted *T. congolense*. Using *Glossina* sp., Poulton (1934) mechanically transmitted *T.congolense* when the interval between the two feeds was 15 seconds. Bruce *et al*., (1911) mechanically transmitted *T.gambiense* using *G. palpalis* when the interval between the two feeds was up to 2 hours.

4.1.4: Mosquitoes and other flies in experimental mechanical transmission of trypanosomes .

Species of mosquitoes have been implicated in the mechanical transmission of trypanosomes (Kazanskii, 1959, cited by Gatt-Rutter, 1967). According to reports by Wenyon (1926), *Aedes* spp. mechanically transmited *T.gambiense*. Roubaud and Lafont (1914) also reported that *Aedes* sp., *Culex* sp. and *Stegomyia fasciata* Theobold, 1901 could mechanically transmit *T.gambiense* and *T.brucei*. Contrary to these

reports, Lesse (1909) and Taylor (1930) failed to transmit *T.evansi* with *Culex* sp., *Stegomyia* sp. and *Aedes* sp. respectively. The role of mosquitoes in mechanical transmission of trypanosomes is therefore still uncertain but evidently their role is very small because Nieschulz (1933) cited by Curasson (1943) achieved transmissions of *T.evansi* in only 3 out of the 3,200 specimens.

Apart from biting flies, non-biting flies have also been implicated in the mechanical transmission of trypanosomes by Musgrave and Clegg (1903). In their experiment, *Musca domestica* Linneaus successfully transmitted *T.evansi* from infected to non-infected dog after contact with an exposed wound. Similarly Darling (1912) also reported that he mechanically transmitted *T.evansi* using the housefly (*Musca domestica*). Thompson and Lamborn (1934) showed that the faeces of non-biting flies which had fed on blood infected with *T.brucei*, contained viable trypanosomes. If such faeces were deposited on a fresh wound they could potentially cause infection. Lamborn and Howatt (1939) reported an infection of a dog by *Musca sorbens*, Wiedemann, 1830, when the fly was transferred after feeding on the blood of a *T.rhodesiense*-infected patient to a fresh cut on the ear of the dog (Lucas, 1955). Other workers such as Nieschulz (1930), however, failed to effect mechanical transmission of trypanosomes using *Musca* spp.

4.1.5: Ticks in experimental mechanical transmission of trypanosomes.

The possible role of ticks in mechanical transmission of trypanosomes, particularly *T.evansi*, has been studied by Cross and Patel (1921, 1922) who transmitted *T.evansi* from camels to rabbits by means of soft ticks, *Orthonidoros crossi* and *O.lahorensisis*. The most recent investigation as to whether ticks may be involved in the transmission of *T.evansi*, has been done by Krimse and Taylor (1978) who conducted studies with *Hyalomma anatocilum excavatum, H.dromedarii, Dermacentor andersoni* and *Rhiphpcephalus sanguineus*. These workers obtained results that led them to the conclusion that ticks cannot be efficient mechanical transmitters of trypanosomes.

4.1.6: Survival and viability of trypanosomes in the mouthparts of putative mechanical vectors.

Survival of trypanosomes in insect mouthparts has been studied extensively (Harley,1966; Clarke,1966,1969; Wijers,1958; Bruce *et al.*, 1911; Buxton,1955; Jordan,1964; Ryan,1982; Simmons and Leggate, 1962; Dutton *et al.*,1907) but only in relation to the cyclical vector, *Glossina* spp. Studies of infection of mouthparts of biting flies other than *Glossina* spp. are scarce and the few that have been reported are not recent.

The capability of haematophagous flies as mechanical vectors of trypanosomes depends primarily on the survival of the trypanosomes in the fly mouthparts (Oldroyd, 1954) and in the case of tabanids and *Stomoxys* spp., the morphological adaptation of the mouthparts for a

combination of blood-sucking and lapping modes of feeding (Luckins, 1988). The most recent work on the survival of trypanosomes in the mouthparts of *Stomoxys* sp. was given by Dixon *et al.*(1971) and Ogonji (1983). In studies by Dixon *et al.* (1971), *Stomoxys calcitrans* were allowed to bite cattle experimentally infected with *T.brucei* and the survival of trypanosomes in the fly mouthparts was assessed by inoculating triturates of the fly into mice after measured time intervals. The longest recorded survival time of trypanosomes in *Stomoxys* spp. mouthparts on this basis was 30 minutes. Beck (1910) observed viable *T.gambiense* in the proboscis of *Stomoxys* sp. for 10 - 20 minutes. Clarkson *et al.* (1971) found that *T.vivax* could survive in the proboscis of *Stomoxys calcitrans* for up to 40 minutes after the infective blood meal. Moutia (1928b), reported active *T.evansi* in the proboscis of *Stomoxys nigra* from 1 - 10 minutes after the infective blood meal at a room temperature of 25 - 27°C. According to reports by Taylor (1930), out of 13 *Stomoxys* sp. dissected immediately after an infective blood meal, 3 had motile trypanosomes in their proboscis. Flies dissected after a lapse of 5 minutes however, had no trace of trypanosomes in the proboscis. According to studies by Ogonji (1983), *T.evansi* survived in the proboscis of *S.calcitrans* for as long as 20 minutes after the infective blood meal. Van Saceghem (1921) however, reported that *T.vivax* and *T.congolense* failed to survive in the proboscis of *Stomoxys* sp. immediately after infective blood meals.

Dissection and examination of proboscis of *Glossina tachinoides* (Taylor 1930), revealed that motile trypanosomes may survive in the proboscis for as long as 3 hours; and that the maximum number of trypanosomes contained in the proboscis immediately after an infective meal varied up to a maximum of 600, when trypanosomes were abundant

in the peripheral blood of the infected animal. In the same study, trypanosomes taken up by *Stomoxys* sp., *Anopheles costalis, A. funestus* and *Lyperosia* (*Haematobia*) sp., survived in the mouthparts for shorter periods of times (up to 10 minutes) and in smaller numbers as compared to *Glossina*. In a study by Dutton *et al.* (1907), mouthparts of *G.palpalis* contained motile trypanosomes for up to 1.75 hours after an infective blood meal.

Possible but unproven mechanical vectors of camel trypanosomiasis in northern Kenya, based on results of this study, include several species of tabanids, *Stomoxys* sp. *Haematobia* sp. and *Hippobosca* sp. In view of this and to clarify the conflicting reports on mechanical transmission of trypanosomes in the literature, this study was conducted to assess the role of local candidate biting flies in the mechanical transmission of trypanosomiasis. This study, was therefore a follow up of these field observations as well as of a previous laboratory study. In the previous laboratory study (Ogonji 1983), only one biting fly, *Stomoxys calcitrans* was used as the fly vector in mechanical transmission studies of *T. evansi*. In this particular survey however, a variety of available biting flies including *Glossina m.morsitans, Aedes aegypti, Haematopota brunnescus* and *Stomoxys calcitrans* were all employed in mechanical transmission studies under laboratory conditions. Survival and viability of trypanosomes in the mouthparts of these flies were also assessed. Under field conditions, actual mechanical transmissions were not effected. Survival and viability of camel trypanosomes in the mouthparts of the

biting flies collected from infected camels was assessed. This was done with a view to investigate the role of such flies in the mechanical transmission of camel trypanosomiasis in nature, and particularly in the *Glossina* spp. free regions of northern Kenya.

4.2: MATERIALS AND METHOD.

4.2.1: Field studies.

4.2.1.1:Dissections of field-collected fly mouthparts.

This exercise was undertaken in order to establish that biting fly mouthparts retain trypanosomes after infective blood meals under field conditions. Secondly the exercise aimed at determining the longevity of the trypanosomes in the fly mouthparts after an infective blood meal under field conditions. Collected biting flies were dissected between 1 - 10 minutes after the infective blood meal. This exercise was therefore intended to ascertain whether trypanosomes could survive for as long as 10 minutes in the biting fly mouthparts after infective blood meals, as this is considered an adequate time interval for mechanical transmission of trypanosomes to be effected in nature.

Procedure: Biting flies, namely *H.camelina, H.minuta*, and *T.taeniola* were collected from infected camels using a hand-held insect sweep-net. Collected flies were placed individually into Kilner jars containing cotton wool soaked in ether to immobilize them. Each fly head was severed from the rest of the body and placed on a microscope slide containing a drop of 10% foetal calf serum (FCS) in physiological saline. Using a portable field microscope (Kyowa optical 711340), the mouthparts were dissected out and viewed at magnifications of X20, X40, and X100 on a

field compound microscope (WILD) to detect for the presence of trypanosomes. In positive cases, the numbers of motile trypanosomes was recorded.Dissections of *H.camelina* and *H.minuta* were carried out at Olturot study site from January,1986 to December,1986 as these flies were available all year round. Dissections of *T.taeniola* were undertaken at Ngurunit study site only in May-June and November-December,1986 when these flies were available.

4.2.1.2:Inoculation of field collected fly mouthpart triturates into clean mice.

Procedure: A batch of 5 flies collected from infected camels were anaesthetized using ether as previously described, and their mouthparts quickly crushed in a suspension of 10% FCS in physiological saline. The resulting suspension was then inoculated intraperitoneally (I.P.) into clean Balb C mice. Mice were checked thereafter until the standard day 60 post-inoculation, as this is the upper time limit for incubation of trypanosomes in mice (Rutagwenda, 1985). Mice were checked for trypanosomes using the wet film, thin and thick blood smear techniques described in section 2.2.2 above.

4.2.2: Laboratory studies.

4.2.2.1: Laboratory animals.

Trypanosomes: One stabilate of *T.evansi* (KETRI 1188) was initially inoculated into several mice. Parasites were thereafter maintained in mice throughout the research period by subsequent passages from infected to non-infected mice via a sub-inoculation procedure.

Mice: White male ICR Walter Reed Strain of mice were obtained weekly from Veterinary Laboratories, Kabete. These were maintained in plastic cages covered with galvanized wire, placed in an animal house at 25 - 27°C and 60-70% relative humidity. Mice were fed on commercial mouse pellets and water was supplied *ad libitum*.

Guinea Pigs, Rabbits: Guinea pigs and rabbits, bred at the Zoology Department animal house, were used as the source of blood meal for the flies (*S.calcitrans*). The animals were bred under the same conditions as the mice but in addition to the rabbit pellets, they were supplemented with vegetables (carrots and lettuce). Again, water was supplied *ad libitum*.

Maintenance of putative mechanical fly vectors for laboratory mechanical transmission experiments with T.evansi.

To effect laboratory mechanical transmission experiments with *T.evansi* from infected to non-infected mice, putative fly vectors were used. The selection of these putative vectors was dependent on their availability locally and their adaptation for laboratory rearing. With the exception of a tabanid (*Haematopota brunnescus*), which could not be reared in the laboratory, the other putative vectors including *Stomoxys calcitrans, Glossina m.morsitans* and *Aedes aegypti*, were reared in the laboratory as described below:-

(a) S.calcitrans

S.calcitrans were originally collected from cattle at Dagoretti Market, using a long-handed insect-sweep net. Dagoretti market is situated about 19 kms from Nairobi. Cattle driven to major slaughter houses within this market are constantly available in crushes outside the slaughter house. The collected flies were kept in insect cages of 45 x 45 x 45 cm. dimensions with plywood floor and covered with white cotton insect net screen . Into one side of the insect cage, a round opening of about 20 cm. in diameter, was made and the frame of this opening was arranged to hold one end of a removable sleeve made of black cloth. The wild-collected flies were brought back to the laboratory inside these cages and offered blood meals on guinea pigs daily as well as being provided with 10% sugar solution.

For blood feeding, guinea pigs were first anaesthetized with Sagatal (Sodium Pentabarbotone) administered intraperitoneally, at a dose of 28 units or 0.44 mls/Kg of body weight per animal. The belly of the guinea pig was then shaved to facilitate fly feeding and the shaved anaesthetized guinea pig was placed upside down to expose the shaved belly inside the fly cage containing the adult flies. The flies were given access to the host animal for 1 - 2 hours after which it was removed from the fly cage. The supplement diet of 10% sucrose solution, soaked in cotton wool within in a petri dish, was placed at the bottom of the cage. The sugar solution was removed 3 - 4 hours before the flies were allowed a subsequent blood meal. Flies were offered a blood meal on a daily basis and always between 0900-1100 h of the day. The flies were exposed to artificial light (60 Watt electrical bulbs) during the night to encourage

oviposition. Before transmission experiments, the flies were given water only, or starved for several hours to maximize the chances of them taking a blood meal.

Oviposition Media:

Eggs of adult flies were oviposited on an oviposition medium which consisted of crushed dried cow-dung, crushed dried blood,and water. The cow-dung and blood were mixed and wrapped in a piece of black muslin cloth. The cloth was made wet by dipping it into water. The media was placed inside a petri dish and the dish was placed on the floor of the insect cage, and onto which, white eggs were deposited by the adult female flies.

Incubation of Eggs and Larval Media: Eggs were removed daily from the oviposition medium using a soft camel brush. The eggs were gently brushed onto the prepared larval rearing media. The larval rearing medium was prepared from 1: 1: 2: parts by volume of wheatbran ,sawdust and distilled water respectively as described by Bailey *et al*., (1975). The mixture was placed in kilner jars or 150 ml beakers with depths of 8 cm. and diameters of 6.5 cm., after Stone (1976). To prevent excessive drying or contamination of *S calcitrans* eggs with eggs of stray flies the, jars were covered with muslin cloth or fine cotton material. Trays containing larval media and eggs were placed in incubators at a temperature of 26 $\pm$ 1°C and relative humidity (R.H) of 60 - 70% with a photoperiod of 12 hours dark and 12 h.light. After pupation, covers were removed from the jars and they were transferred into fly cages, inside which adult F1 generation flies emerged. Adults that emerged were fed as previously outlined above and used for experimentation by 3 - 5 days post emergence.

(b) *Glossina m.morsitans*:

Laboratory bred *G.m.morsitans* were obtained from International Centre for Insect Physiology and Ecology (I.C.I.P.E.). *G.m.morsitans* were reared at I.C.I.P.E. The I.C.I.P.E. colony of *G.m.morsitans* originated from flies obtained from Langford, Bristol,England in 1968. The colony in Langford in turn originated from Zimbabwe. The flies were bred at a temperature of 25+/-0.5 degrees Celcius and 70-80% R.H. (Wanyonje,1986). Both sexes were used in experimentation.

(c) *Haematopota brunescus*:

Haematopota brunescus were collected from a baited goat at Karura Forest, Nairobi area, using a sweep-net. Since these flies are not easily reared in the laboratory, the field collected flies themselves were used in transmission experiments immediately after collection. Like *G.m.morsitans* they were placed inside feeding tubes in batches of 5 flies per feeding tube.

(d) *Aedes aegypti*:

Aedes aegypti mosquito larvae were reared in the Department of Zoology of the University of Nairobi at 28+/-2 degrees Celcius and a 14-h photoperiod.Larvae were fed *ad libitum* on a pinch of finely ground dog biscuits (Mwangi and Rembold,1986) until they entered the pupal stage. Rearing of *A.aegypti* larvae was undertaken in a dilute (0.6% NaCl) solution in 250ml beakers to synchronize larval instars after the method of Zebitz (1984). Newly emerged mosquitoes were placed inside the feeding containers shown on Plate 22 and used in *T.evansi* mechanical transmission experiments.

4.2.3. Laboratory procedures.

4.2.3.1: Mechanical transmission experiments.

Mechanical transmission experiments were conducted using selected available biting flies as vectors and Balb C mice as hosts. To effect this, biting flies, namely *G.m.morsitans, H.brunnescus, S.calcitrans* and *A. aegypti* were blood-fed on *T.evansi* (K.E.T.R.I 1188 strain) infected donor mice and subsequently on non-infected recipient mice within a 10 minutes period. The recipient mice were thereafter checked for the presence of trypanosome infection using the wet film method. Blood-feeding of the biting flies was conducted by placing 5 flies inside feeding containers with a screen mesh on one end of the stoppered tube (Plate 22) to permit fly feeding on *T.evansi*-infected anaesthetized mice. Mice were anaesthetized with Sagatal administered at a dose of 0.44 ml/kg body weight. This required dosage per mouse was prepared by adding 0.44 ml of the stock Sagatal into 33 ml of physiological saline to give 0.0133 ml of the former solution per 1 ml of saline: 1 ml of this preparation was administered to each mouse via the intraperitoneal route. Flies that had probed or partially engorged on the infected mouse had their blood-feeding interrupted by removing the feeding container from the infected donor mouse and replacing it immediately on the shaved belly of an anaesthetized non-infected recipient mouse. Due to the availability of large numbers of *G.m.morsitans, S.calcitrans* and *A. aegypti*, these transmission experiments were repeated as many as 100 times. There were only 23 trials with *H.brunnescus* which was not available in large

Plate 22 : Feeding tubes used for laboratory mechanical transmission experiments.

numbers. Parasitaemia in mice that became infected as a result of the transmission experiments was monitored until death of the host animals.

4.2.3.2: Dissections of fly mouthparts after infective blood meals on mice.

Dissections of biting fly mouthparts were undertaken in order to assess the survival (longevity) of *T.evansi* (K.E.T.R.I.1188) in the mouthparts of selected biting flies (*G.m.morsitans, S.calcitrans, H. brunnescus* and *A.aegypti*). These flies were fed individually on *T.evansi*-infected mice following the feeding method described above. Engorged or partially engorged flies were immediately anaesthetized with ether and their mouthparts dissected on a microscope slide containing a drop of 10% FCS in physiological saline. These mouthparts were viewed under a microscope at x40, x100 and x400 magnification for the presence of parasites. The procedure was repeated 100 times with *G.m.morsitans, S. calcitrans* and *A.aegypti* and 30 times with *H.brunnescus*, at increasing intervals of time after infective blood meals. These time intervals in minutes were 5, 10, 15, and 20 for *A.aegypti*; 5, 10, 15, 20, 25 and 30 for *S.calcitrans* and *H.brunnescus*, and 20, 30, 40, 50, 60, 70, 90 and 120 for *G.m.morsitans*. In positive cases, the number of trypanosomes that survived in the mouthparts was recorded.

4.2.3.3: Inoculation of infected fly mouthpart triturates into mice.

This exercise was undertaken with the objective of assessing not only the survival of trypanosomes but also the viability of these trypanosomes in fly mouthparts after infective blood meals. Flies were

fed on *T.evansi* (KETRI 1188)-infected mice as described above. After infective blood meals, fly mouthparts were crushed using a pestle and mortar in a suspension of 10% physiological saline. The suspension was inoculated intraperitoneally into clean mice at increasing intervals of time (i.e 5,10,20,30 and 60 mins.) after infective blood meals. For each time interval, a total of 50 mice were inoculated. Each inoculum of each fly species contained 5 mouthparts and 10 mice were inoculated with the same inoculum containing 5 mouthparts. Biting fly species whose mouthparts were inoculated into mice comprised *G.m.morsitans, H. brunnescus, S.calcitrans* and *A.aegypti*. Inoculated mice were individually marked using picric acid and subsequently checked for trypanosome infection using the wet-film method. Mice were routinely maintained for 60 days, and if infected, until death of the animal.

4.3: R E S U L T S.

4.3.1: Field dissections of biting fly mouthparts.

Table 24 contains results of field dissections of *H.camelina,H.minuta* and *T.taeniola* mouthparts after blood meals on trypanosome-infected camels at Ngurunit study site. On the first column of Table 24 is a list of biting fly species whose mouthparts were dissected after infective blood meals on camels. On the first row are the 12 months of the year 1986. For each fly species, there are four rows corresponding to each of the 12 months. The first of these rows,contain figures pertaining to numbers of mouthparts found positive for trypanosomes following dissections. On the second row after each fly species, are the percentages (%) found positive for trypanosomes following dissections. On the third row after each fly species, are the mean number of motile trypanosomes counted

Table 24: Trypanosome infection rates in biting fly mouthparts following bloodmeals on infected camels based on dissection

months / fly spp	Jan	Feb	Mar	Apr	May	June	July	Aug.	Sept	Oct.	Nov	Dec.
H. camelina No. + VE	0	0	0	0	0	62	65	56	90	0	0	34
% + VE	0	0	0	0	0	15.5	16.25	14.0	22.25	0	0	17
Mean	-	-	-	-	-	5±2.8	6±3.4	3.6±1.8	3.7±1.9	-	-	4.7±2.1
Max/Min	-	-	-	-	-	10,1	11,2	6,2	7,2	-	-	7,1
H. minuta No. + VE	0	0	0	0	13	63	41	50	30	42	26	50
% + VE	0	0	0	0	13	63	41	50	30	42	26	50
Mean	-	-	-	-	2±1.0	3.9±1.9	4.3±2.0	5.3±2.7	3.1±1.6	4.1±1.8	4.7±2.6	9.1±2.3
Max/Min	-	-	-	-	9,1	19,2	11,1	6,1	7,1	9,1	6,2	11,1
T. taeniola No. + VE	ND	ND	ND	6	70	23	ND	ND	ND	ND	20	10
% + VE	ND	ND	ND	6	70	23	ND	ND	ND	ND	20	10
Mean	ND	ND	ND	10±5.4	13.6±1.8	3.3±1.8	ND	ND	ND	ND	14.1±3.1	5.9±2.1
Max/Min	ND	ND	ND	18,2	17,3	6,1	ND	ND	ND	ND	21,7	9,1

ND = Not done, Overall % positives; H. camelina = 14.5% (349/2400), H. minuta = 22.4% (269/1200)
T. taeniola = 23.8% (143/600)

in mouthparts for the positive cases . The figures in brackets are the maximum (first bracket) and minimum (second bracket) number of motile trypanosomes counted in fly mouthparts of all positive dissections.

Examined mouthparts of *H.camelina* were negative for trypanosomes following dissections in January, February,April, March, and May. Dissections of *H.camelina* mouthparts carried out in June, August, September and December were found positive. For dissections carried out in these four months, *H.camelina* mouthpart infection rates were 15.5%, 16.25%, 14.0%, 22.25% and 17.0% respectively. For these positive cases the mean number of trypanosomes found in *H.camelina* mouthparts were 5.7+/-2.8, 6.0+/-3.4, 3.6+/-1.8,3.7+/-1.9 and 4.7+/-2.1 respectively. The total number of *H.camelina* mouthparts found positive for trypanosome infection were 349 out of 2400 dissections (14.5%). The maximum number of trypanosomes recorded in the *H.camelina* mouthparts was 10.

Dissections of *H.minuta* mouthparts from January to April were all negative. Infection rates of *H.minuta* mouthparts were 13%, 63%, 41%, 50%, 30%, 42%, 26% and 50% in June, July, August, September, October, November and december respectively. Mean numbers of trypanosomes found in the *H.minuta* mouthparts were 2.5+/-1.0, 3.9+/-1.9, 4.6+/-2.0, 5.3+/-2.7, 3.1+/-1.6, 4.1+/-1.8, 4.7+/-2.6, and 9.1+/-2.3 respectively. The maximum number of trypanosomes recorded in *H.minuta* mouthparts was 19. A total of 1200 *H.minuta* mouthparts were dissected. Out of theses 269 or 22.4% were found positive for trypanosome.

Mouthparts of *T.taeniola* were not dissected in the months of January, February, July, August, September and October because these flies were

not available. Out of 600 mouthparts of *Tabanus taeniola* dissected after infective blood meals on camels, there were 143 or 23.8% positive cases. as summarized at the bottom of Table 24. For these positive cases, carried out in April, May, June, November and December infection rates in *T.taeniola* mouthparts were 60%, 70% 23%, 20%, and 10% respectively. A maximum number of 21 trypanosomes was recorded in *T.taeniola* mouthparts.

4.3.2: Field inoculation of fly mouthpart triturates into mice.

Results presented as pie-charts on Figs. 34,35, and 36 show infectivity (to mice) of *H.camelina, H.minuta* and *T.taeniola* mouthparts after inoculation of these mouthpart triturates into mice.The infectivity of these fly mouthparts varied with the fly species. *H.camelina* mouthparts were least infective and infected only 6% of the 50 mice inoculated (Fig.34). Mouthparts of *H.minuta* were moderately infective to mice and infected 12% of the 50 mice inoculated (Fig.35). Mouthpart triturates of *T.taeniola* were most infective to mice and infected 53.3% of 30 mice inoculated (Fig.36).

Time intervals between infective blood meals and inoculation of mouthpart triturates into mice was 1-3 minutes throughout the experimentation. This implies that mouthparts of *T.taeniola, H.minuta* and

H. camelina

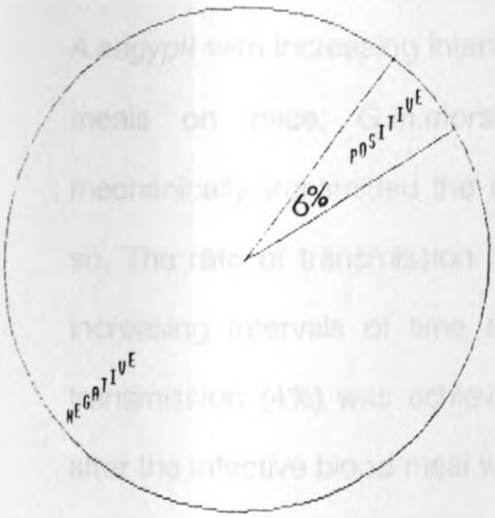


Fig. 34

H. minuta

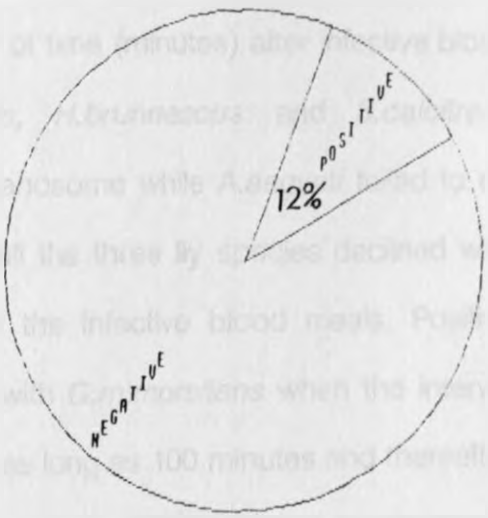


Fig. 35

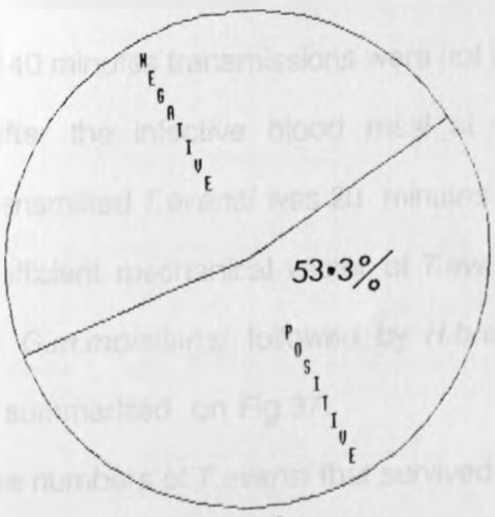


Fig. 36

T. taeniola

Figs. 34-36: Diagrammatic representation of percentage of mice infected following inoculation with triturated mouthparts of H. camelina (fig. 34), H. minuta (fig. 35) and T. taeniola (fig. 36).

H.camelina may contain viable trypanosomes, which if inoculated into mice 3 minutes or more after the infective blood meals can cause an infection in the mice.

4.3.3:Laboratory transmissions, and dissections of fly mouthparts.

Fig.37 is a representation of results of mechanical transmissions of *T.evansi* (KETRI 1188) by *G.m.morsitans, H.brunnescus, S.calcitrans* and *A.aegypti* with increasing intervals of time (minutes) after infective blood meals on mice. *G.m.morsitans, H.brunnescus* and *S.calcitrans* mechanically transmitted the trypanosome while *A.aegypti* failed to do so. The rate of transmission for all the three fly species declined with increasing intervals of time after the infective blood meals. Positive transmission (4%) was achieved with *G.m.morsitans* when the interval after the infective blood meal was as long as 100 minutes and thereafter , there were no positive transmissions. *H.brunnescus* mechanically transmitted *T.evansi* but when the interval after the infective blood meal exceeded 40 minutes transmissions were not effected. The longest time interval after the infective blood meal at which *S.calcitrans* mechanically transmitted *T.evansi* was 20 minutes.

The most efficient mechanical vector of *T.evansi* under laboratory conditions was *G.m.morsitans*, followed by *H.brunnescus* and finally *S.calcitrans* as summarized on Fig.37.

Approximate numbers of *T.evansi* that survived in the mouthparts of *A.aegypti, S.calcitrans, H.brunnescus* and *G.m.morsitans* are depicted in bar-charts in Figs. 38, 39, 40 and 41. Mean numbers of parasites in the mouthparts of these flies decreased with increasing intervals of time after the infective blood meals on mice. For all dissections carried out after 25

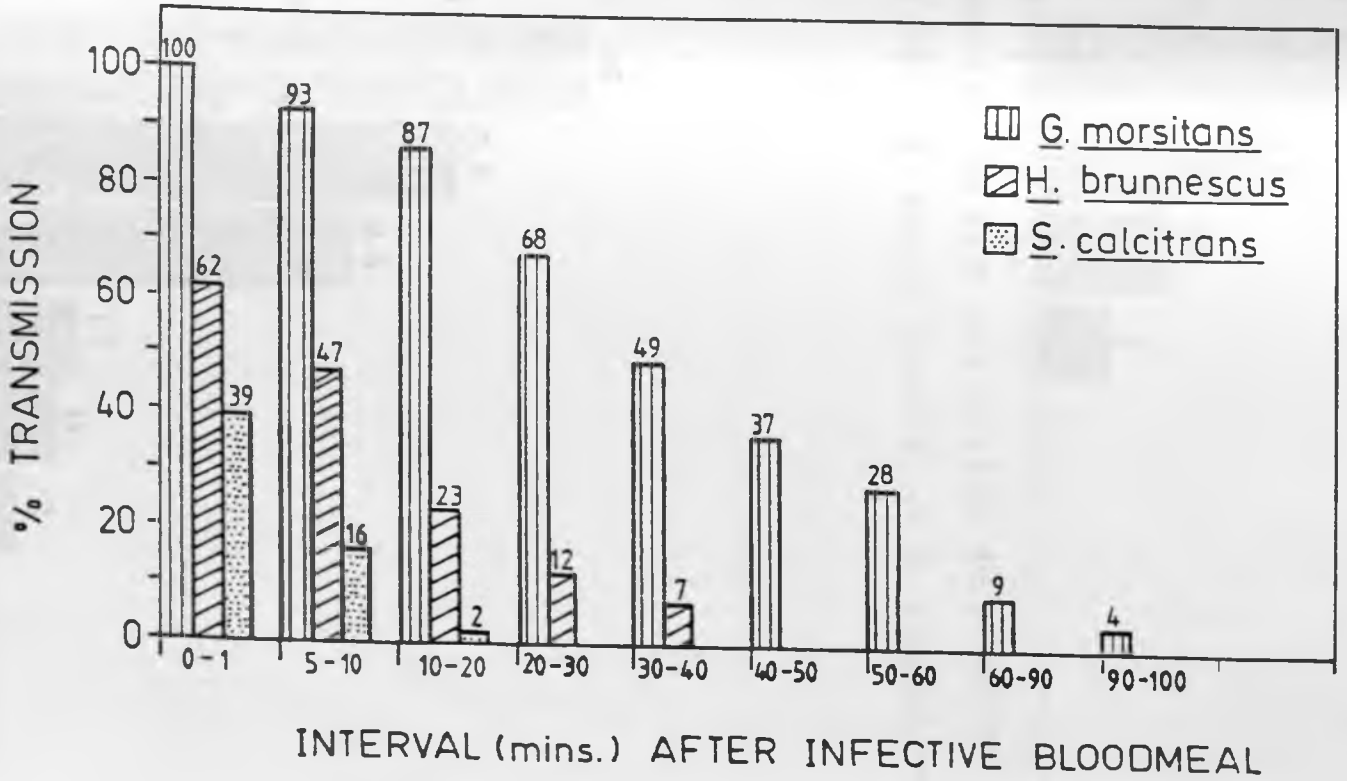


Fig.37 : Transmission rates of T. evansi by biting flies with increasing time intervals after infective bloodmeals.

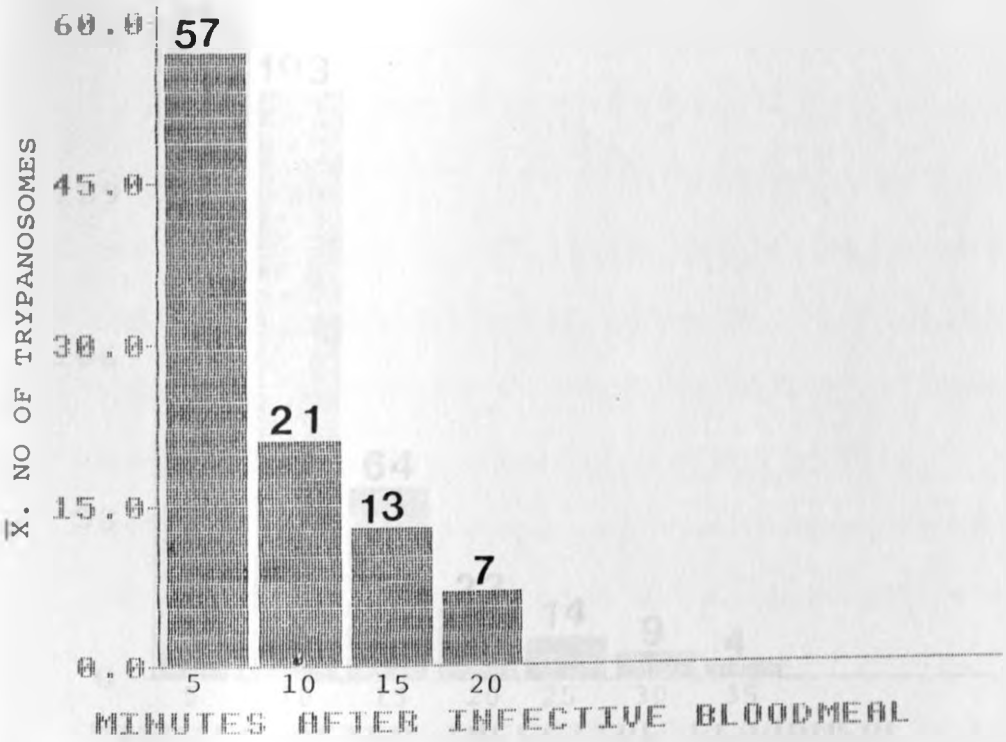


Fig. 38: T. evansi survival in A. aegypti mouthparts

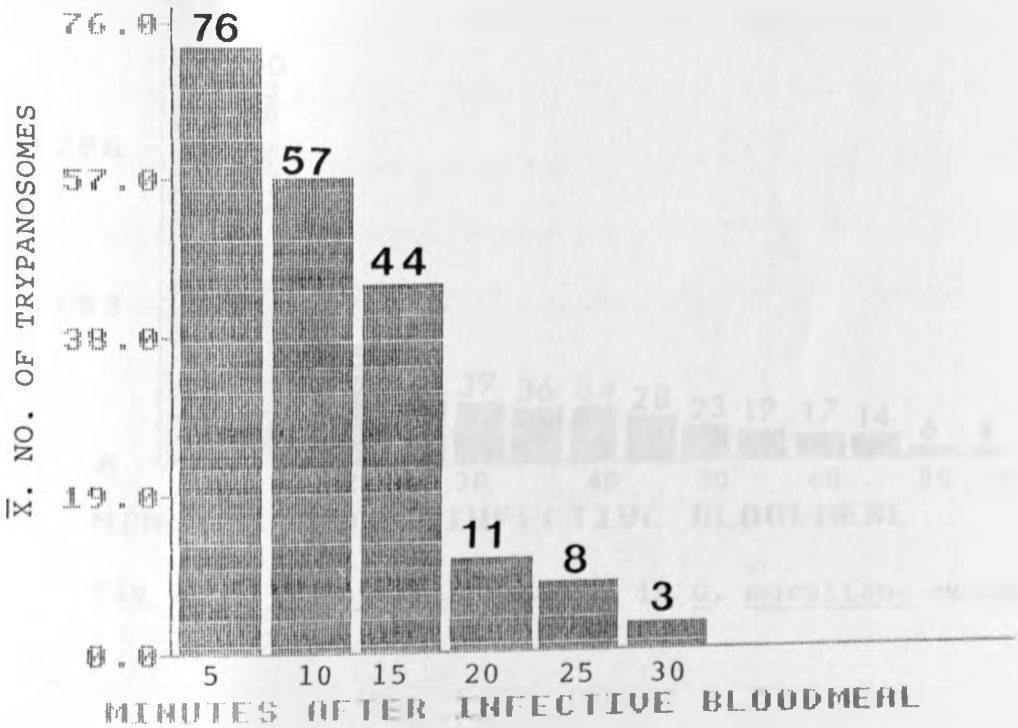


Fig. 39 : T. evansi survival in S. calcitrans mouthparts.

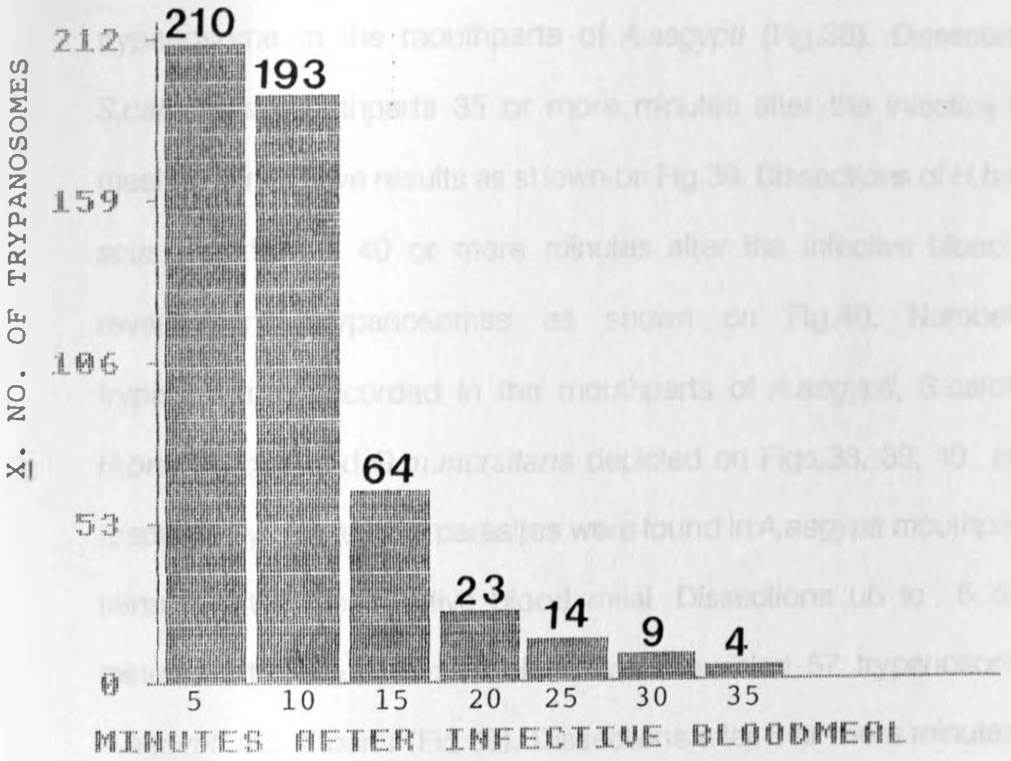


Fig. 40 : T. evansi survival in H. brunnescus mouthparts

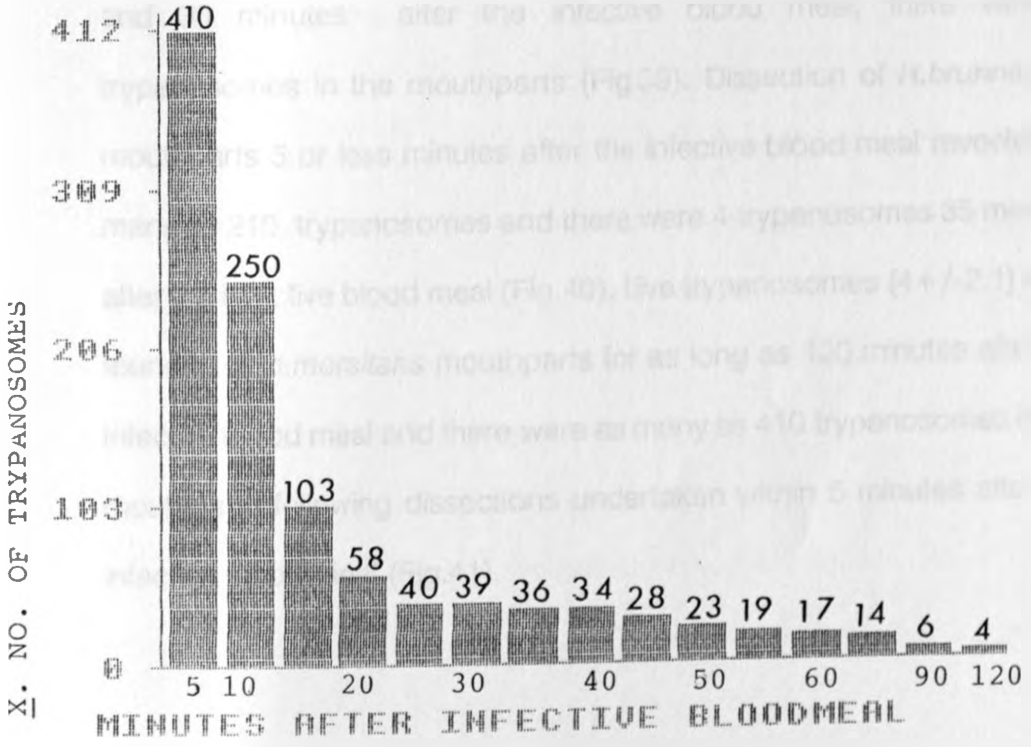


Fig. 41 : T. evansi survival in G. morsitans mouthparts.

or more minutes following the infective blood meal, there were no live trypanosome in the mouthparts of *A.aegypti* (Fig.38). Dissections of *S.calcitrans* mouthparts 35 or more minutes after the infective blood meal gave negative results as shown on Fig.39. Dissections of *H.brunnescus* mouthparts 40 or more minutes after the infective blood meal revealed no trypanosomes as shown on Fig.40. Numbers of trypanosomes recorded in the mouthparts of *A.aegypti, S.calcitrans, H.brunnescus* and *G.m.morsitans* depicted on Figs.38, 39, 40 and 41 respectively. Seven live parasites were found in *A.aegypti* mouthparts 20 minutes after the infective blood meal. Dissections up to 5 or less minutes after the infective blood meal revealed 57 trypanosomes in *A.aegypti* mouthparts (Fig.38). Dissections after 5 or more minutes after the infective blood meal revealed 73 parasites in *S.calcitrans* mouthparts and 30 minutes after the infective blood meal, there were 3 trypanosomes in the mouthparts (Fig.39). Dissection of *H.brunnescus* mouthparts 5 or less minutes after the infective blood meal revealed as many as 210 trypanosomes and there were 4 trypanosomes 35 minutes after the infective blood meal (Fig.40). Live trypanosomes (4 + /-2.1) were found in *G.m.morsitans* mouthparts for as long as 120 minutes after the infective blood meal and there were as many as 410 trypanosomes in the mouthparts following dissections undertaken within 5 minutes after the infective blood meal (Fig.41).

4.3.4:Laboratory inoculation of fly mouthpart triturates into mice.

Table 25 contains results of inoculation of mouthpart triturates of *G.m.morsitans, H.brunnescucs, S.calcitrans* and *A.aegypti* with increasing time intervals after infective blood meals on *T.evansi*-infected mice. When the intervals after infective blood meals were 0-5, 10, 20, 30, and 60 minutes, mouthparts of *G.m.morsitans* infected 82%, 64%, 40%, 32% and 18% of the mice inoculated respectively. *H.brunnescus* mouthparts inoculations 0-5, 10 and 20 minutes after the infective blood meal , infected 34%, 10% and 2% of the mice respectively. Inoculations of *H.brunnescus* mouthparts into mice after time intervals that exceeded 30 minutes gave negative results as shown on Table 25. Mice inoculated with *S.calcitrans* mouthparts 30 minutes or more after the infective blood meal gave negative results. When mouthparts of *S.calcitrans* were inoculated into mice, 0-5, 10 and 20 minutes after the infective blood meal, 30%, 12% and 6% of the mice became infected respectively. Inoculations of *A.aegypti* mouthparts into mice at time intervals that exceeded 10 minutes after the infective blood meal all gave negative results. Only 10% and 4% of the mice inoculated with *A.aegypti* mouthparts 0-5 and 10 minutes after the infective blood meal became infected. As shown on Table 25 , mouthparts of *G.m.morsitans* retained the most viable and infective trypanosomes after infective blood meals on mice, followed by *H.brunnescus, S.calcitrans* and finally *A.aegypti*.

Table 25 : *T.evansi* infection [%] in mice inoculated with fly mouthpart triturates at increasing time intervals after infective bloodmeals on mice.

	TIME INTERVALS [IN MINUTES] AFTER INFECTIVE BLOOD MEALS				
Fly spp.	0-5	10	20	30	60
G. morsitans	82	64	40	32	18
H. brunnescus	34	10	2	0	0
S. calcitrans	30	12	6	0	0
A. aegypti	10	4	0	0	0

4.4: DISCUSSION.

4.4.1: Survival and viability of camel trypanosomes in mouthparts of field collected flies.

Results of the two field experiments (fly mouthpart dissections and inoculations of fly mouthpart triturates into mice) have shown that mouthparts of *H.camelina, H.minuta* and *T.taeniola* can retain viable and infective trypanosomes for periods of time. This is an essential prerequisite for mechanical transmission. A higher number of camel trypanosomes were retained in *T.taeniola* mouthparts compared to those retained in mouthparts of both *H.camelina* and *H.minuta*. Similarly, mouthparts of *T.taeniola* were more infective to mice than *H.camelina* and *H.minuta* mouthparts. Retention of viable trypanosomes in *T.taeniola* mouthparts may be attributed to the morphological features of the fly mouthparts or the physiological components of the saliva of this fly. As is the case with most tabanids (Oldroyd,1954), *T.taeniola* mouthparts are fleshier and larger than mouthparts of most other biting flies (Luckins,1988) .Results of these field experiments have shown that the survival and viability of camel trypanosomes in biting fly mouthparts may be influenced by the parasitaemia of the donor animal, host. There were more positive results when Ngurunit-herd camels were used as donor hosts. The trypanosome-infected camels in the Ngurunit herd were noted to experience higher parasitaemias than the Olturot herd camels. Studies relating to infection of field collected flies other than *Glossina* spp. with trypanosomes are lacking, thereby limiting information upon which comparisons can be made to the results of the current study. A major factor reported to determine trypanosome infection rates in natural

populations of *Glossina* spp. is the source of the blood meal and temperature (Jordan,1963). Infection rates in *Glossina* spp. have been reported to be as high as 50% (Jordan,1963) but this is with respect to cyclical transmission as opposed to mechanical transmission. Overall trypanosome mouthpart infection rates were 14.5%, 22.45 and 23.8% for *H.camelina, H.minuta* and *T.taeniola* respectively.

In the case of *Glossina morsitans submorsitans* in Nigeria there was a significant difference between dry and wet season infection rates such that infection rates were higher during the wet season (Jordan,1963).In this study, infection of *T.taeniola* mouthparts during the dry season was not assessed as it was strictly a wet season biting fly. In the case of *H.camelina* and *H.minuta*, infection rates of mouthparts were not significantly affected by seasons, contrary to reports by Jordan (1963).

4.4.2:Laboratory mechanical transmission of trypanosomes and factors that affect transmission.

According to results of laboratory studies, *Glossina m.morsitans* appears to be the most efficient mechanical vector of trypanosomes. Tabanids are also efficient transmitters of trypanosomes but not as efficient as *Glossina m.morsitans*. *Stomoxys calcitrans* can also transmit trypanosomes mechanically but not as efficiently as tabanids and *G.m. morsitans*.The efficiency of *Glossina* spp in mechanical transmission of trypanosomes was also reported by Taylor (1930) who transmitted *T.brucei* between pigs using *Glossina* spp. Using *G.tachinoides* with as few as 4 flies. Bailey (1965),reported successful mechanical transmission of *T.rhodesiense* using *Glossina morsitans* from infected to clean mice.Positive laboratory mechanical transmission of trypanosomes by

Glossina spp. have also been achieved by Bruce *et al.* (1911); Poulton (1930) and Mwambu(1969).

Results of this study,confirm the numerous previous reports (Roger,1901; Sergent ED and ET,1905,1906; Fraser and Symonds,1907; Lesse,1909; Mitzmain,1916; Cross and Patel,1921,1923; Neschulz,1926, 1941; Maramorosch,1962; Gruvel and Balis,1965; Greenberg,1973; Luckins,1988) that tabanids are able to mechanically transmit trypanosomes under laboratory conditions.

Failure of a mosquito (*A.aegypti*) to mechanically transmit trypanosomes is not unique to this particular study. Reports by Taylor (1930), showed that *Aedes costalis, A.funestus*, and *A.vittatus* failed to mechanically transmit *T.brucei.* Heckenroth and Blanchard (1913), however reported successful mechanical transmission of *T.gambiense* using unspecified species of mosquitoes.In view of failure of transmission of *T.evansi* by *A.aegypti* despite the many trials in this study, it is unlikely that mosquitoes are important potential mechanical vectors of trypanosomiasis in nature. The rapid disappearance of trypanosomes in mosquito mouthparts and lack of intermittent feeding behaviour probably reduce chances of mosquitoes to transmit trypanosomes. Mosquitoes were present in the study area only during and after the rainy season. The role of mosquitoes in mechanical transmission of camel trypanosomiasis, if any, was therefore only possible during and immediately after the rainy season.

Although reports by Luckins (1988) indicated that flies (*Tabanus* spp.) are able to mechanically transmit *T.evansi* several hours after the infective blood meal, results of this study, show that the ability of flies to mechanically transmit trypanosomes is influenced greatly by the time

interval between infective and infecting blood meals. With increasing time intervals between the two blood meals, the rate of mechanical transmission of the trypanosomes is reduced. Dixon *et al.* (1971), also found that for effective mechanical transmission of *T.brucei* by *Stomoxys calcitrans* from infected to non-infected cattle, the interval between the two feeds had to be as short as 30 seconds or less. In this study, transmissions were achieved with intervals of up to 20 minutes with *S.calcitrans*; 25 minutes with *H.brunnescus* and 100 minutes with *G.m.morsitans*. Inconsistencies between these findings and those of Dixon *et al.* (1971) may result from differences in animals hosts and trypanosome species. *T.evansi*, which is transmitted only mechanically, may be better adapted to mechanical transmission than other trypanosome species which are normally transmitted cyclically. Nieschulz (1930); Lucas (1955); and Taylor (1930), reported that differences between trypanosome strains is also a factor that may determine mechanical transmission. Only one strain of *T.evansi* was used in this study and it was readily transmitted by all candidate flies except *A.aegyti*. Fly numbers may be another important factor that determines mechanical transmission of trypanosomes based on results of this study and reports by Luckins (1988).In preliminary mechanical transmission experiments where single flies were used, none except *G.m.morsitans,* was able to transmit *T.evansi*. The least number of *S.calcitrans* and *H.brunnescus* able to transmit *T.evansi* in this study was 5. It was also noted that a single *S.calcitrans* or *H.brunnescus* did not feed actively as soon as they landed on the host animal skin, whereas the same flies fed viciously and immediately they landed on the animal when in groups of 5 or more. A stimulus produced when flies are in groups, that enhances blood-feeding activity, is a probable explanation

for this type of observation.It is interesting to note that although Lesse (1908) used as many as 15 flies per trial, he got only one positive transmission of trypanosomes with *Stomoxys* spp., whereas with *Tabanus* spp., several trials were positive when he used only four flies. Mechanical vector capacity therefore depends also upon fly species in addition to the number feeding at any one time. To this end, the nature of biting fly mouthparts may be the important factor as reported by Oldyrod (1954).

The species of recipient and donor animals may also have a decisive influence on the outcome of mechanical transmission experiments and may even explain some of the inconsistencies in mechanical transmission results reported by different workers. Transmission experiments with *T.evansi* using mice, yielded positive results in the current work as well as in studies by Ogonji (1983). In experiments by Chaudhuri *et al.*(1966),involving larger animals such as dogs and ponies, results were negative. The differences in donor capacity are probably due to the varying degree of parasitaemia each animal experiences. Nieschultz (1930), found that the rat experiences intense parasitaemia.For this reason, more trypanosome can be picked up by the fly from the rat than from the horse which does not experience such an intense parasitaemia.

The most important factor that affects mechanical transmission is the survival of the parasites in the fly vector mouthparts. In the context of the present study, *T.evansi* survived in the mouthparts of *G.m.morsitans, H.brunnescus* and *S.calcitrans. G.m.morsitans* retained the highest number of parasites, and for as long as 1 hour 40 minutes after infective blood meals. Work by Taylor (1930) is almost comparable to these findings .He found 600 *T.brucei* in *G.tachinoides* mouthparts immediately

after the infective blood meal,and trypanosomes survived in the mouthparts for as long as 3 hours. Moutia (1928b), found that *T.evansi* survived in *S.nigra* mouthparts for up to 10 minutes at 25-27°C.In experiments by Taylor (1930), *T.brucei* survived in the mouthparts of *S.calcitrans* mouthparts for only 5 minutes.In this study, *T.evansi* survived in *S.calcitrans* for a much longer time (30 minutes) than some of these previous reports. Mouthparts of *H.brunnescus* were even more favourable to the survival of *T.evansi*, such that as many as 210 parasites were counted in the mouthparts immediately after the infective blood meal and motile trypanosome were found as long as 35 minutes after the infective blood meal.

In nature, *T.evansi* infection occurs in the absence of *Glossina* spp., therefore the only other likely vectors of the parasite are biting flies such as tabanids, *Stomoxys* spp., *Haematobia* spp., *Hippobosca* spp. and others. Tabanids and *Stomoxys* spp. feed intermittently, a prerequisite for mechanical transmission of trypanosomes, and are therefore the most likely putative vectors. That tabanids are the most likely vectors in nature has been proposed by many workers among them Moutia (1928b); Yagi and Razig,1972a,1972b; Razig Yagi,1975; and Luckins, 1988). Although workers such as Nieschultz (1930) and Chaudhuri *et al*. (1966) have reported that *Stomoxys* spp. are not involved in mechanical transmission of trypanosomes,results of this study indicate that *Stomoxys* spp. are indeed potential mechanical vectors of trypanosomiasis in nature. Results of this study also show that tabanids and *Stomoxys* spp. can harbour viable and infective trypanosome in the mouthparts after infective blood meals for as long as 20 minutes. The retention of viable and infective

trypanosome in the fly mouthparts is a major requirement for mechanical transmission of trypanosomes. These further suggest the greater possibility of tabanids and *Stomoxys* spp. as mechanical vectors of trypanosomiasis in the absence of *Glossina* spp. and particularly *T.evansi*, which does not undergo cyclical transmission in an insect vector.

CHAPTER FIVE

GENERAL DISCUSSION, RECOMMENDATIONS, TRYPANOSOMIASIS CONTROL PROSPECTS AND CONCLUSIONS

5.1: General discussion

There is evidence based on results of this study and reports by Frazil (1977), Wilson *et al.*, (1981), Rutagwenda (1982, 1985) and Olaho *et al.*, (1987) that trypanosomiasis is one of the most important diseases affecting camels in Kenya. Apart from deaths and abortions, trypanosomiasis has also been confirmed to cause anaemia and general loss of condition (Rutagwenda, 1985).

Although transmission of animal trypanosomiasis in the absence of *Glossina* spp. has been refuted by authors such as Wells (1972), results of this study show occurrence of the diseases at selected study sites in northern Kenya in the absence of the cyclical vector, *Glossina* spp. Infection of the sentinel camels in the apparent absence of *Glossina* spp. implicates mechanical transmission of the disease in the these northern Kenya camels. Other studies in which sentinel animals have become infected with trypanosomes in the apparent absence of *Glossina* spp. include those by Bouffard (1907), Lucas (1955) and Boyt (1970). There are no past or current records of presence of *Glossina* spp. in the study area. This absence has been further supported by the use of the biconical trap, which is specially designed to trap *Glossina* spp. These flies were not found at any time among the resulting insect collections. Camels within the study areas, although migratory, do not enter *Glossina*

spp. areas of distribution.

At least 70% of camel sera tested for trypanosomiasis using the Elisa test in association with *T. evansi* antigen, had trypanosomal antibodies indicating past or current trypanosomal challenge. The ELISHA test therefore confirmed presence of the diseases in the study area and was useful in selecting potentially suitable herds and sites for further epidemiological surveys of this disease.

The actual incidence of camel trypanosomiasis in northern Kenya was established by two diagnostic tests, namely the Mouse Inoculation Test (MIT) and the Blood Smear Test (BST). The MIT and BST detected upto to 19.2% and 11.5% trypanosome infection rates in camels respectively. This further confirmed the high incidence of the disease in the region. The Mouse Inoculation Test proved to be the most sensitive diagnostic technique for detection of patient and subpatent infections. This is in agreement with results of Godfrey and Killick-Kendrick (1962, 1963) and Paris *et al.*, (1966).

Based on reports by Wilson *et al.*, (1981), there is evidence that the majority of northern Kenyan camel trypanosome stocks are of the *T. evansi* type although the existence of other trypanosomes, particulary *T. brucei* cannot be ruled out completely. *T. vivax* have not been reported among the trypanosomes affecting northern Kenya camels. These later species of trypanosomes do not survive in laboratory rodents and could not be detected by the Mouse Inoculation Test even if they are present in camels. The Blood Smear Test did not reveal *T. congolese* which is morphologically distinguishable from the *T. brucei-type* of trypanosomes. Since the antigen preparation for the ELISHA analysis in this study was specific in this study was specific for *T. evansi*, the high incidence of

infection based on ELISHA further supports that *T. evansi* is the major trypanosome species affecting northern Kenya camels.

Laboratory transmission studies to determine whether the trypanosome isolates from northern Kenya can cyclically develop in *Glossina* spp. were not performed but this would conclusively confirm whether *T. evansi* is the only trypanosome stock that affects northern Kenya camels. In *Glossina* spp. endemic areas such as Galana, Ol-Maosor and Baringo, there is a possibility of *Glossina* species-cyclically trypanosomes such as *T. brucei, T. vivax* and *T. congolese* in camels.

Results of this study showed that trypanosome challenge in the selected northern Kenya camel herds peaked during the wet season, based on the Mouse Inoculation Test. Mean trypanosome infection rates for the year 1986 were 13.78+/-5% during the wet season and 5.45+/-2% during the dry season. It is apparent that transmission occurs mainly during the wet season. Results also showed a definite relationship between biting fly abundance and camel trypanosome infection rates. Highest incidence of camel trypanosomiasis (18.3% and 15.8% at Olturot site and 18.3% at Ngurunit site) were recorded in the rainy month of May, based on the Mouse Inoculation Test. Similarly biting fly abundance peaked during the wet season months (March-May), thereby coinciding with the period of camel trypanosome challenge. Hippoboscids and *Haematobia minuta,* although present all year round within the study area, increased in number during the immediately after the rains. The tabids (*T. taeniola, T. leucostomus, T. atrimanus, H. alnihirta,* and *P. distincta*] and *S. calcitrans* were entirely seasonal and occurred only during and immediately after the rains.

The striking seasonal occurrence of tabanids and *S. calcitrans* in the absence of *Glossina* spp. is significant in terms of mechanical transmission, when viewed in conjunction with the seasonally observed outbreaks of camel trypanosomiasis in the study area. Another factor that further implicates mechanical transmission of camel trypanosomiasis in area is the infection of the specifically monitored sentinel camels. Infection of sentinel camels employed in this study demonstrated active transmission of camel trypanosomiasis in the absence of *Glossina* spp. vector. This further implicates mechanical transmission of camel trypanosomiasis as opposed to cyclical transmission. Mechanical transmission of camel trypanosomiasis in nature has been proposed by a number of workers including Lewis (1947), Gruvel and Balis (1965, Mahmoud and Gray (1980), Mohamed (1983), Yagi and Razig (1972a., 1972b). Razig and Yagi (1975), Hoare (1972), Woo (1977) Gitatha (1981), and Omara-Opyene (1986). The several possible ways in which mechanical transmission of camel trypanosomiasis could take place in northern Kenya include the following; during bleeding of animals, during castration of bulls and via bites by haematophagous insects. Of these, it is highly likely that mechanical transmission of camel trypanosomiasis in northern Kenya is predominantly by haematophagous insects.

In this study, epidemiological evidence in favour of mechanical transmission of camel trypanosomiasis concerns the incidence and transmission of camel trypanosomiasis in the absence of *Glossina* spp. Experimental evidence in favour of mechanical transmission of the main camel trypanosome (*T. evansi)* is also emphasised. Results demonstrate that tabanids (*H. brunnescus*, and *T. taeniola)*, *S. calcitrans, Haematobia minuta* and *H. camelina* can harbour infective trypanosomes in their

mouthparts after infective blood meals. A tananid (*H. brunnescus)*, S. calcitrans and *G.m. morsitans* also mechanically transmitted *T. evansi* (KETRI 1188) from infected to non-infected mice under laboratory conditions. Failure to demonstrate cyclical development of *T. evansi* in the cyclical vector, *Glossina* spp. (Kurnett and Krausse, 1943; Mukiria, 1980), confirms that this parasite has adapted to a mechanical mode of transmission. Although Wells (1972), suggested that cyclical development of trypanosomes in an insect vector other than *Glossina* spp. should not be ruled out, no developmental forms of *T. evansi* have been found in *Stomoxys* spp. (Moutia 1928b) or in tabanids (Hoare, 1972, Krinsky, 1976). An interesting result in this study was the fact that *G.m. morsitans* was the most efficient mechanical vector of *T. evansi* under laboratory conditions. Based on these results, and those by Taylor (1930) and Gingrich *et al*., (1983) cited by Foil (1989), it is possible that *Glossina* spp. are also mechanical vectors of camel tryposomiasis in future, in areas, obviously, where the distribution of *Glossina* spp. overlaps with the distribution of the disease in camel. This however, was not the same in this particular study area.

The major suspect mechanical vectors of camel trypanosomiasis in northern Kenya are the tabanids (particularly *T. taeniola)* and *S. calcitrans*, based on their seasonal occurrence in relation to incidence of the disease in camels and their characteristic interrupted blood-feeding behaviour. Transmission of the disease seems to be complex such that hippoboscids and *Haematobia minuta*, which are present all year round, probably maintain the disease at low levels while *Stomoxys* spp. and tabanids are responsible for the major seasonal disease outbreaks.

Of the potential candidate mechanical vectors, *T. taeniola* appears to be the major suspect in the case of camel trypanosomiasis because it occurs in larger numbers compared with other tabanids; it requires a large blood meal (Wiessenhutter, 1975) and is a painful biter and persistent blood-feeder (King, 1910, Lewis 1947). A most important point in addition to these is that *T. taeniola* feeds in an interrupted and aggressive manner thus, enhancing, opportunities for mechanical transmission. Tabanids have been reported (Lukins, 1988) to have a combination of piercing and sucking as well as sponging mouthparts, specially adapted for mechanical transmission of trypanosomes. It is worth noting that tabanids are not only incriminated as mechanical vectors of trypanosomiasis but are also capable of mechanically transmitting other pathogenic organisms including *Anaplasma marginale*, causing anaplasmosis, *Rickettsiae (Francisella) tularebsis,* causing tularaemia, *Basillus anthracis*, causing anthrax, equine infectious anaemia, (Foil *et al*., 1984), and Hog Cholera Virus (HCV) (Harwood and James , 1979).

Direct blood-feeding effects of tabanids, hippoboscids, *Stomoxys calcitrans* and *Haematobia minuta* alone can lead to anaemia, weight loss, loss in milk yields and general loss of condition in the victimized animals and even death. Control measures against all the biting flies in the study area is therefore highly recommended.

5.2: **Immediate recommendations**

Since trypanosomiasis in general is widespread in animals in developing countries, an immediate solution to the problem has often involved the use of trypanocidal drugs, either for treatment or prophylactic

purposes. An ideal trypanocidal drug should be cheap, effective in a single dose and efficacious for more than one trypanosome species. It should have no side effects and should readily create drug-resistant strains. Drugs against animal trypanosomiasis include Berenil^R, Antrycide^R, Samorin^R, and Naganol^R. Berenil^R has been reported to be toxic to camels while Samorin^R is effective. Antrycide^R is effective but is no longer produced leaving Naganol^R as the only alternative. Unfortunately Naganol^R is expensive compared to the other trypanocides. Trypacide^R recently developed by May and Baker Co., and locally available, has demonstrated effectiveness against camel trypanosomiasis (Waitumbi, 1986) and may be administered to northern Kenya camels both as a curative and a prophylactic drug.

Such a control measure should ideally be implemented during the wet season or just before the onset of the rains. Due to the high cost of trypanocidal drugs, it becomes difficult for pastoralists to treat an entire herd of over 100 camels. When administering prophylactic chemotherapeutic drugs, priority should be given to calves, who have not been exposed to any previous trypanosome challenge. The next priority should be females since these provide milk which forms a substantial food source among the pastoralists. Since the severity of trypanosomiasis in livestock has been shown to be influenced by past history of exposure to the disease, calves or any other previously non-challenged animals are likely to die or suffer serious attacks compared to animals with previous exposure to the disease. Animals which have recovered naturally from trypanosomiasis, can be immune to challenge to that particular trypanosome strain. Incidences of trypanosomiasis that recur are usually less severe compared to original attacks (Woo, 1977).

Although chemotherapy is the most widely employed approach to control trypasonomiasis in many African countries, this alone does not provide a permanent solution to the disease due to potential drug resistance problems after prolonged use of certain drugs active against present resistant strains of trypanosomes are therefore urgently required. If treatment is relaxed, the incidence of disease soon returns due to the existence of reservoirs of the parasite (Jordan, 1986). There is a need for follow up studies to examine the role and importance of reservior animals for the particular parasite involved.

Results of this study show that trapping is effective in capturing haematophagous insects. Use of traps as a fly vector control measure has been exploited with respect to *Glossina* spp. but this method may be applicable to other haematophagous insects. The efficiency of these traps may be increased by incorporating attractants such as carbon dioxide and acetone (Vale, 1980) or both the natural animal odours such as urine (Owaga, 1980; Turner, 1981) plus carbon dioxide (Vale, 1977;, Hargrove and Vale, 1979). Traps have also been designed such that the captured insects are instantaneously electrocuted or killed with insecticides (Jordan, 1986). Trapping appears to be a practical control measure for biting flies in northern Kenya because traps are easily assembled, cheap, non-toxic to the environment and will control target insects only. The biconical trap, currently used to capture *Glossina* spp. in many parts of Africa (Jordan, 1986) may be useful not only to capture but to control haematophagous flies in northern Kenya. The most appropriate attractant that may be incorporated with the trap in the northern Kenya situation would be the inexpensive and readily available camel urine as opposed to acetone and carbon dioxide which are

expensive. The camel urine may be poured into plastic containers and placed beside the pole supporting the trap. Apart from traps, screens and targets impregnated with insecticides have been used to control *Glossina* spp. Insecticide [Deltamethrin] impregnated screens and targets, used successfully to reduce numbers of *G.m. morsitans* and *G. pallidipes* in Zimbabwe (Jordan, 1986) and *Glossina* spp. in Galana, Kenya (Opiyo *et al.*, 1987) may reduce numbers of biting flies in other areas as well and need to be exploited in addition to the biconical trap in controlling haematophagous insects in northern Kenya.

The ectoparasitic biting flies such as *Haematobia* spp. and *Hippobosca* spp. and even tabanids and *Stomoxys* spp. can be controlled and/or repelled by insecticide impregnated ear tags placed on camels and other domestic livestock. Such insecticide impregnated ear tags have been reported to be highly effective for the control of muscid flies [Kunz and Schimdt, 1985: Hogsette and Ruff, 1986], *Stomoxys calcitrans* and *Hippobosca maculata* Leach and tabanids [Wright *et al.*,: Parashar *et al.*, 1989]. Pyrethroids have often been used in impregnated ear tags, but with the rapid resistance of *Haematobia* spp. to this chemical, organophosporous compounds seem to be a reasonable starting point to control the biting flies on host animals. Direct application of repellents on the animals may also greatly reduce the attack rate and hence reduce the chances of mechanical transmission of camel trypanosomiasis. Larvae of tabanids (the major suspect vectors of camel trypanosomiasis), have been reported to be susceptible to dieldrin at levels as low as 0.2 ppp (Hansiens, 1956). Pyrethrins plus a synergist (Piperenyl butoxide) and a repellent (Butxy polypirperonyl propylene glycol) have been applied to control *Stomoxys* spp. in temperate countries. Application of these or

other insecticides to stagnant water, rivers or other biting fly breeding sites may provide an immediate solution in reducing fly numbers in northern Kenya if implemented just prior to or during the rainy seasons when biting flies are abundant. Camels dips do not exist in northern Kenya at present. Construction of camel dips may greatly reduce the numbers of *H. camelina* and *H. minuta* with the added advantage of reducing tick loads on camels. Dipping is particularly relevant to *H. camelina* and *H. minuta* because these flies tend to be permanently ectoparasitic on animals as opposed to *S. calcitrans* and tabanids which land on animals specifically to blood feed.

In the northern Kenya situation, animal husbandry measures that may reduce the incidence of camel trypanosomiasis are as follows: there should be routine testing of camels for active infections using a technique such as the Mouse Inoculation Test. Infected animals, once identified, should be treated. During treatment, syringes and needles should be sterilized before application to the next animals as this is a possible mode of mechanical transmission of these trypanosomes. Care should be exercised in the exchange or purchase of animals, particularly during the wet season when trypanosomiasis outbreaks are at peak. Cultural practices such as bleeding of animals during ceremonies or as a traditional locally practised treatment measure, should be performed carefully to reduce chances of mechanical transmission of trypanosomes from infected to non-infected animals. Another possible means of mechanical transmission of trypanosomes is during castration of animals. This should be done with care and equipment cleaned before application from animal to animal. Heavy biting fly areas should be avoided fly attack rates can be reduced by covering bellies of animals w

Communal grazing and watering of animals, currently practised by nomadic pastoralists in northern Kenya, should be discouraged during the wet seasons when inter-herd mechanical transmission of trypanosomiasis is most likely to take place due to the seasonal high incidence of diseases and biting fly bundance.

5.3 Prospects and limitations of trypanosomiases control.

Control of the main vector of animal trypanosomiasis (*Glossina* spp.) in African countries, including Kenya, is highlighted as one of the priority measures against the disease. To achieve this, however, there is a requirement for long term programmes plus continuous monitoring of fly numbers. Possible potential fly-vector control measures include chemical control, biological control and genetic control. Currently, *Glossina* spp. control is effected almost exclusively by insecticides. The first synthetic insecticide used to this effect was DDT. A successful *Glossina* spp. control operation in which DDT was used was initiated in the River Komadugu, north-eastern Nigeria in 1955. In this operation DDT applied from knapsacks pressure sprayers as a suspension reduced numbers of *G.m. submorsitans* and *G. tachnoides* to an encouraging extent that systematic campaigns against *Glossina* spp., using additional application techniques such as use of helicopters, began in Nigeria in 1956 and have continued to the present time (Davies, 1964; Jordan 1986).

Dieldrin, which has similar properties to DDT, replaced DDT in residual sprays on vegetation in Kenya (Glover *et al.*, 1958) after test trials of the two. These two compounds have been employed in treatment of vegetation with residual deposits applied from the ground.

The only changes ever since they were first used has involved application techniques. Another successful control operation against *G.m. morsitans* and *G. pallidipes* was the Sabi-Lundi campaign which covered the south-eastern region of Zimbabwe and the adjoining border region of Mozambique. The campaign was carried out in 1962-1971 and the first step was to eliminate wildlife, known to be hosts of *Glossina* spp., by shooting. This was followed by ground spraying of residual insecticides (DDT and dieldrin) using pressurized knapsacks (Robertson *et al.*, 1972). Since this time, fixed wing aircraft sequential aerosol technique has been used on a limited scale (Chapman, 1976) and may be more applicable in future. Aerial spraying of dieldrin in Lambwe Valley, Kenya carried out in 1968-1971 reduced the population of *G. pallidipes* but the fly population reached a high level again in 1980 (Coutts, 1981). Reinvasion of previously insecticide treated areas by *Glossina* spp. is thus a common problem encountered in trypanosomiasis control operations.

Endosulfan when applied as an aerosol of fine droples from aircraft has demonstrated an effectiveness against *Glossina* spp. in Tanzania (Hocking *et al.*, 1966), Zambia (Park *et al.*, 1972) and *G.m. centralis* in the Okavango Delta, Botswana (Davies and Bowles, 1979). Aerial spraying using endsulfan in Lambwe Valley, Kenya led to rapid reductions in numbers of *G. pallidipes* (Coutts, 1981) but the area is still not *Glossina* species-free.

Currently, the most potentially valuable new synthetic pyrethroid for use in *Glossina* spp. control operations is deltamethrin (Hadaway *et al.*, 1977). This compound can be sprayed from the ground (Spelberger *et al.*, 1979) or from air (Molyneux, 1978). Deltamethrin has also been used effectively for impregnating traps, targets and screens to control *G.*

palpalis gambiensis and *G. tachnoides* in Ivory Coast/Burkina Faso. (Laveissiere *et al.*, 1981), and *G.f. qunzensis* in the Republic of Congo (Lancien, 1981). Further field trials, under various conditions, are required before firm recommendations can be made as to whether deltamthrin could effectively replace well-established insecticides such as dieldrin for large scale control operations. Chemical control such aerial spraying may not be suitable in the arid northern Kenya situation due to the nomadic life style of the pastoralists and extremely vast area would have to be sprayed to cover the entire Marsabit District alone. Cost would obviously present a major consideration in such an operation

Although traps, screens and targets were originally designed to sample *Glossina* spp., recent trap developments have increased their efficiency to an extent that they have been employed in small control operations. The Morris trap reduced populations of *G.m. gambiensis* in Liberia (Morris, 1950). Similarly the Morris trap, impregnated with insecticide, reduced numbers of *G. palpalis* in the Island of Principle in 1954 (De Azevedo *et al.*, 1962). The biconical trap, impregnated with detamethrin, reduced numbers of *G.p. gambiensis* and *G. tachnoides* in Ivory Coast/Burkina Faso. (Laveissiere *et al.*, 1981). In the latter study, simple screens of blue material instead of the biconical trap also managed to reduce fly numbers at the same level of magnitude in the region. Acetone odour-baited targets, impregnated with dieldrin, achieved eradication of *G.m. morsitans* and *G. pallidipes* in the Island of Kariba. Similar targets impregnated with deltamethrin gave promising results in Zimbabwe (Jordan, 1986). Although odour attractants such as CO2, acetone and urine increase the efficiency of traps it has been suggested (Hargrove, 1971) that it would be more efficient to sterilize and release

flies rather than simply kill them. The monconical trap impregnated with deltamethrin proved to be effective in reducing numbers of *G.f. quanzensis* in the Republic of Congo (Lancien, 1981). Deltamethrin impregnated odour-baited targets have been used on the Galana Ranch, Kenya as a *Glossina* spp. control method. Targets at a density of 5 per square Km were installed and baited with acetone and octenol sprayed with deltamethrin at 0.1% strength at 3-monthly intervals. Results showed marked reductions in fly numbers and some areas remained *Glossina* species-free for several months (Opiyo *et al.*, 1987). The Ngu2B trap designed at I.C.I.P.E. by Brightwell *et al.*, (1985), baited with the an effective attractant, preferably animal urine, may provide long lasting control of *Glossina* spp. in Kenya and elsewhere in view of the fact that this trap has been reported to be more effective than the biconical trap. Trapping alone, as a means of vector elimination, offers the advantage that it is non-polluting, inoffensive to non-target organisms, does not require much manpower and is applicable in remote areas. Other types of non-chemical approaches to *Glossina* spp. control include biological control, and genetic control.

Biological control of insect vectors or potential insect vectors involves the use of pathogens (viruses, protozoa, bacteria, and fungi), predators or natural parasites to eliminate or reduce numbers of the adult fly or its immature stages. When it is possible to culture large numbers of pathogens, they may be dispersed and applied in similar ways as insecticides and are referred to as microbial insecticides. These however, require proof of vertebrate safety, effectiveness and specificity. Use of parasites or predators may involve introduction of the particular parasite or predator into the area under consideration. Efficacy of biological

control of haematophagous insects is currently inconclusive because only a few attempts have been made to release biological agents in nature to control these flies. Although *Glossina* spp. predators [Gruvel, 1977], parasitiods [Simmonds *et al.*, 1971, pathogens and nematodes [Briggs *et al.*, 1977] have been reviewed, use of such biological control agents against *Glossina* spp. have not been implemented on a large scale and not much is known about the effectiveness of these. Potential biological control agents against *Glossina* spp., include predator ants, birds and jumping spiders according to reports by Kiragu and Dransfield (1987) following an I.C.I.P.E. supported project at Nguruman, Kenya. Reports by Simmonds *et al.*, [1977], indicate that parasitoids may represent future *Glossina* spp. biological control agents and this needs to be explored. Information on parasites or pathogens of tabanids, hippoboscids, *Stomoxys* spp. and *Haematobia* spp. is still lacking and requires further investigation. Although chalcid wasps are parasitic on eggs of tabanids (Lesse, 1927), this method of control is not always immediate as it requires ass rearing of the parasite prior to introduction into field conditions. It is worth noting that although tabanids, *Haematobia* spp. and hippoboscids are potential mechanical vectors of several pathogens and parasites, the paucity of contemporary research on these flies arises from the lack of established laboratory colonies. Future research on biological control agents of these flies will greatly benefit from the establishment of laboratory colonies although this is clearly difficult.

The use of chemosterillants [genetic control] may be relevant in the future control of fly vectors and potential vectors of animal trypanosomiases. The major draw back with chemosterillants, however is that they are presently prone to affect a wide range of non-target

organisms and cannot be put into general field use. Use of insect attractants to concentrate biting flies on targets, traps or screens followed by chemosterilization, knock down insecticide, or electrocution may provide a highly specific method for eliminating specific trypanosomiases vectors. Many dipteral females posses sex pheromones in the cuticle which induces copulatory behaviour in males of the same species, but because of the lack volatility of these pheromones, they have not been exploited as attractants to bring flies into the vicinity of traps or targets [Langley *et al.*, 1982]. A combination of pheromones and suitable chemosterillants or/and insecticides need to be investigated for future control of *Glossina* spp. and other haematophagous flies.

The other type of genetic control that has led to the eradication of the screw worm [*Cochliomyia hominivorax*] in California is the Sterile Male Release Technique (SRMT). Adult flies are reared in large numbers and males are irradiated by exposure to gamma irradiation and released back into the wild population to overwhelm the natural population over several generations. A SRMT pilot project initiated in 1971 in Tanzania showed efficay for this method for control of *G.m. morsitans* (Bakuli *et al.*, 1981). The initial stages of SRMT oriented *Glossina* spp. control operation, i.e. the mass rearing of flies was successfully effected for *Glossina p. palpalis* by the Biological Control (BICOT) Centre in Vom, Plateau State, Nigeria and may successful in future (Tenabe *et al.*, 1981).SMRT may be feasible for *Stomoxys* spp. which is easily reared under laboratory conditions but the economic impact of this fly on domestic livestock has not been sufficiently immense to warrant such a costly and tedious exercise. This method of control is clearly not feasible for tabanids, *Haematobia* spp. and hippobocsids which have not yet been reared under laboratory

conditions.

In view of the current limitation of fly vector control and trypanocidal drug resistance, a current research area has been on the mechanisms of mammalian immunity to trypanosomes with the ultimate goal of producing a vaccine. Immunisation of livestock against trypanosomiasis would provide a permanent solution to the disease worldwide. Unfortunately, immunisation prospects are so far being complicated due to the phenomenon of antigenic variation. The use of variable surface glycoproteins [VSC] as a basis of immunoprophylaxis remain uncertain in view of the complexity of processes regulating antigen variation [Turner, 1985]. Despite this, Ryu (1975), cited by Mahoud and Gray (1980), studied the protection imparted to mice by killed *T. evansi* vaccine containing Carbol-PRS or 10% Lugol's solution gave the best protection. So far no vaccines have produced for trypanosomiasis but biochemical research on trypanosomes, currently going on at ILRAD and other centres, have to be maintained as a priority area for potential future control of animal trypanosomiases.

Another limitation to animal trypanosomiases control operations has been the fact that the diseases is not at the top of the list of priorities in developing countries, where other diseases such as malaria and schistosomiasis cause serious problems to man. Insecticides, drugs and transport which are necessary in implementing control programmes, have to be purchased from developed countries, thereby requiring adequate foreign currency exchange and yet most developing countries cannot afford this. Most African countries lack sufficient personnel specifically trained for trypanosomiasis control. There is need for more training and

information centres such as the F.A.O./I.E.M.V.T./G.T.Z. specialist training programme in France. Activities of trained veterinary officers are quite often limited due to lack of adequate funds especially in remote regions such as the semi arid lands of Africa. Efforts to eradicate *Glossina* spp. vectors are in some cases thwarted because of incomplete coverage of fly infested regions as a result of political boundaries. Incomplete coverage of fly infested areas subsequently leads to reinvasion of treated areas by flies from nearby untreated regions. International donors plus experts have embarked on short term trypanosomiasis control projects which are consequently valueless because results are never made meaningful or applied by traditional livestock owners who keep most of the African livestock. To this end, on-going work by I.C.I.P.E. scientists, whereby the local livestock-keeping pastoralists within Nguruman, Kenya are also involved in research activities, as part of an intergraded *Glossina* spp. control campaign, must be commended.

With respect to the mechanically transmitted trypanosomiases, *Glossina* spp. control is important because it is also potential mechanical vector. Control of other haeatophagous flies is also important, particularly with reference to camel trypanosomiasis. Vector control and chemotherapy alone may not provide a long term solution to the problem because other modes of mechanical transmission of trypanosomes exist: These include transmission from the infected pregnant animal to the foetus, transmission during bleeding and castration. Theses were not investigated in the current study but there is need to investigate them and to assess how much they contribute to the overall animal trypanosomiasis

epidemiological pattern. An equally important factor that requires further studies is the characterization of trypanosome species that have been isolated from camel hosts and whether such isolates are cyclically transmitted by *Glossina* spp. under laboratory conditions.

In view of the limitations of each individual control measure against animal trypanosomiasis, an intergraded control strategy (i.e. chemotherapy, vector control and improved animal husbandry measures) are inevitable. Since biological and genetic control of vectors and immunisation of animals against trypanosomiases, are still in experimental phases, there is a likelihood that the use of trypanocidal drugs and insecticides may increase in the foreseeable future. To this end, new curative drugs active against present resistant strains of trypanosomes need to be introduced. An intergraded control strategy for camel trypanosomiasis in northern Kenya would therefore comprise of use of drugs to treat infected animals, use of camel urine baited biconical traps, screens and targets to reduce biting fly numbers, use of insecticide impregnated ear tags to repel and control biting flies, application of insecticidal fly-repellents on animals and improved animal husbandry measures.

5.4 Conclusions.

Results of this study have provided new information relating to the mode of transmission and putative mechanical vectors of camel trypanosomiasis in northern Kenya.

1. Occurrence of camel trypanosomiasis in the study area has been established based on the Mouse Inoculation and Blood Smear Diagnostic Techniques. The parasite involved is most likely *T. evansi*.

2. The efficiency of inoculation of blood into susceptible laboratory rodents, in conjunction with the blood smear test, has been demonstrated as a sensitive diagnostic method for camel trypanosomiasis.

3. Tryponosomiasis in northern Kenya camels is most prevalent during the rain seasons, based on the Mouse Inoculation Test. It is also during this period that numbers of biting flies reach maximum levels.

4. Use of sentinel camels in the field, has shown that camel trypanosomiasis can be transmitted in the absence of *Glossina* spp. and implies mechanical transmission by flies other than *Glossina* spp.

5. The biconical trap, although originally designed to trap *Glossina* spp. proved to be efficient in capturing other biting flies.

6. Results of laboratory transmission experiments and examination of biting fly mouthparts, after infective blood meals, have confirmed the ability of tabanids, *Stomoxys* spp. and *Glossina* spp. to mechanically transmit trypanosomes.

7. In view of the fact that the major camel trypanosome, *T. evansi*. is transmitted only mechanically and the infection of sentinel camels in the absence of *Glossina* spp., there is no doubt that transmission of camel trypanosomiasis is mechanical in northern Kenya. Other factors that

further support the concept of mechanical transmission are the positive laboratory transmission experiments and the relationship between biting fly abundance and outbreaks of trypanosomiasis.

8. Identified possible biting fly mechanical vectors of camel trypanosomiasis in northern Kenya include Tabanids (*T. taeniola, T.leucostomus, T. atrimanus, A. agrestis, H. albihirta* and *P. distincta*), hippoboscids (*Hippobosca, P. distincta*).

9. Tabanids and *S. calcitrans* appear to be the more important potential mechanical vectors on the basis of their seasonal occurrence in relation to the incidence of camel trypanosomiasis and their characteristic intermittent blood-feeding behaviour. Of the tabanids, *T. taeniola* probably plays a major role as a mechanical vector of camel trypanosomiasis because it is the predominant tabanid in the region, has a large and fleshy mouthpart, inflicts painful bites and blood-feeds bot intermittently and aggressively.

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APPENDIX 1

Elisa Methodology

(1) Preparation of camel IgG (CIgG) and Rabbit anti-camel IgG (RACIgG) after the method of Nakane (1979) and Kawaoi (1974) and modified by Olaho *et al* (1984).

Normal CIgG was prepared from normal camel serum by ammonium sulphate precipitation (ASP) followed by fractitionation on DEAE-52 cellulose using standard laboratory procedures. The purity of the final CIgG preparation was checked by the double immunodiffusion (DID) and immunoelectrophoresis (IEP) techniques. A single precipitation line was evidence of purity.

RACIgG was prepared by immunizing two Newzealand-white adult rabbits with 0-5 mg of pure CIgG in complete Freund's adjuvant ($^1/_m$). After 1 month, the rabbits were boosted with the same dose ($^1/_m$) of CIgG in incomple Freund's adjuvant, followed by two more boots (with the same dose of CIgG in incomplete Freund's adjuvant) at two weeks' interval. The rabbits were finally boosted with the same dose of antigen ($^1/_v$) without Freund's adjuvant and bled to death after 4 days of boosting. Before each boosting, the rabbits were always bled for serum to test for the level of anti-CIgG antibodies using DID and IEP techniques. The IgG fraction of the rabbit immune sera was prepared by the ASP

followed by frationation on DEAE-52 cellulose 52, using standard laboratory procedures. The purity of the final preparations was checked by DID and IEP using whole camel serum and the purified camel IgG. Only fractions giving a single precipitation line were used in the enzyme conjugation.

2. RACIgG-Peroxidase RACIG-PO conjugate

RACIgG-PO conjugate was prepared using Horse radish Peroxidase (Sigmatype vi). The method employed was modified after Nakane and Kawaoi (1974) as outlined below:

(i) To 50 mg of peroxidase was added 9 ml of deionized distilled water and 1 ml of sodium iodoperiodate (32mg/ml of distilled water). The mixture was stirred slowly at room temperature for 30 minutes, (strictly so!) and then dialysed against acetate buffer (1.0 mm pH 4.4) at 4^0C, overnight with many changes).

(ii) 150 of RACIgG was dissolved in 10 mls of 1.0 M Carbonate buffer pH 9.0. The oxidised periodate mixture was then added to the anti-body solution and the pH adjusted to 9.0. The enzyme antibody mixture was stirred slowly at room temperature for two hours, the pH adjusted to 7.6 and mixture left

unstirred overnight at 4^0C.

(iii) 20 mg of glycine were added to the mixture (at this stage 10 mg of sodium cyanoborohydride is supposed to be added but this was not found to be necessary). The mixture was stirred slowly at room temperature for two hours and diaysed at 4^0C against 0.01 M PBS pH 7.2 (with several changes).

(iv) The final preparations was centrifuged at 10,000 g for 20 mins. at 4^0C to remove precipitates.

(v) An equal volume of glycerol was added and the conjugate stored at -20^0C (at these conditions the conjugate keeps for quite long).

3. Preparation of soluble trypanosome antigen for ELISA

Soluble trypanosome antigen was prepared from a KETRI stabilate of *T. evansi* KETRI 12429 after Luckins (1977) as described below:

Trypanosomes were harvested from mice or rates parasitemic after the method of Lanham and Godfrey (1970). The harvested trypanosomes were washed three times in cold phosphate saline glucose (PSG) buffer pH. 8.0 and then once in cold 0.01M phosphate buffered

saline (PBS(pH. 7.2. Trypanosomes were then suspended in cold PBS and subjected to twenty-seconds ultrasonication at maximum amplitude on an MSE Ultrasonic Disintergrater (Safam Electrical Instrument Co. England). The resulting suspension was centrifuged at 10,000g for 30 minutes at $+4^0$C. The protein of the supernatant was determined as described below and stored in liquid Nitrogen (-196^0C) until ready for use.

4. Protein determination

Protein concentration of all the immunoglobulin and antigen preparations was determined by the method of Lowry *et al* (1951) as modified by Peterson (1979). The procedure was as follows:

Material and equipment

Protein solution to be measured
Buffer in which protein is dissolved
Any standard protein with known extinction (e.g. Bovine Serum Albumin = 6.7)
2% (W/) copper sulphate hydrate ($5H_20$)
4% (W/) Sodium potassium tartrate
3% (w/) Sodium carbonate in 0.2m sodium hydroxide
Folin and ciocalteus phenol reagent (BDH, England
Visible light spectormeters (Bechman USA model)

Procedure

4(a) Img/ml solution of the standard protein Bovine Serum Albumin (BSA) is made and the extract concentration from its absorbence at 280 nm calculated.

4(b) An aliquot of unknown solution containing 5-50 mg protein, the same volume of the buffer, blank and 0, 2, 5, 10, 20, 35 and 50 ml of standard protein solution (the zero tube is the water blank) is put into separate tubes.

4(c) Distilled water is added to bring contents of each tube to the same volume (ideally less than 200 ml but the assay will take up to 1 ml).

4(d) 1 ml of copper sulphate solution and 1 ml of tartrate solution with 48 ml of carbonate solution (freshly prepared are mixed). I ml of mixture are put into test tubes and incubated for 10 minutes at room temperature (RT^0).

4(e) 50 ml of phenol reagent is added to each tube mixed and incubated at RT^0 for 25 minutes.

4(f) The contents of each test-tube are mixed again and after 5 minutes the absorbence of each tube read at 640 nm using the water blank to zero the spectrophotometer.

4(g) The absorbence of the protein standard is plotted against the protein concentration and from this the amount of protein in the unknown is read off. Subtract any absorbence of the bufer blank and calculate the protein concentration in the original solution from the volume used for assay in step 2 above.

APPENDIX 2

Calculation of Camel body weight

CAMELS

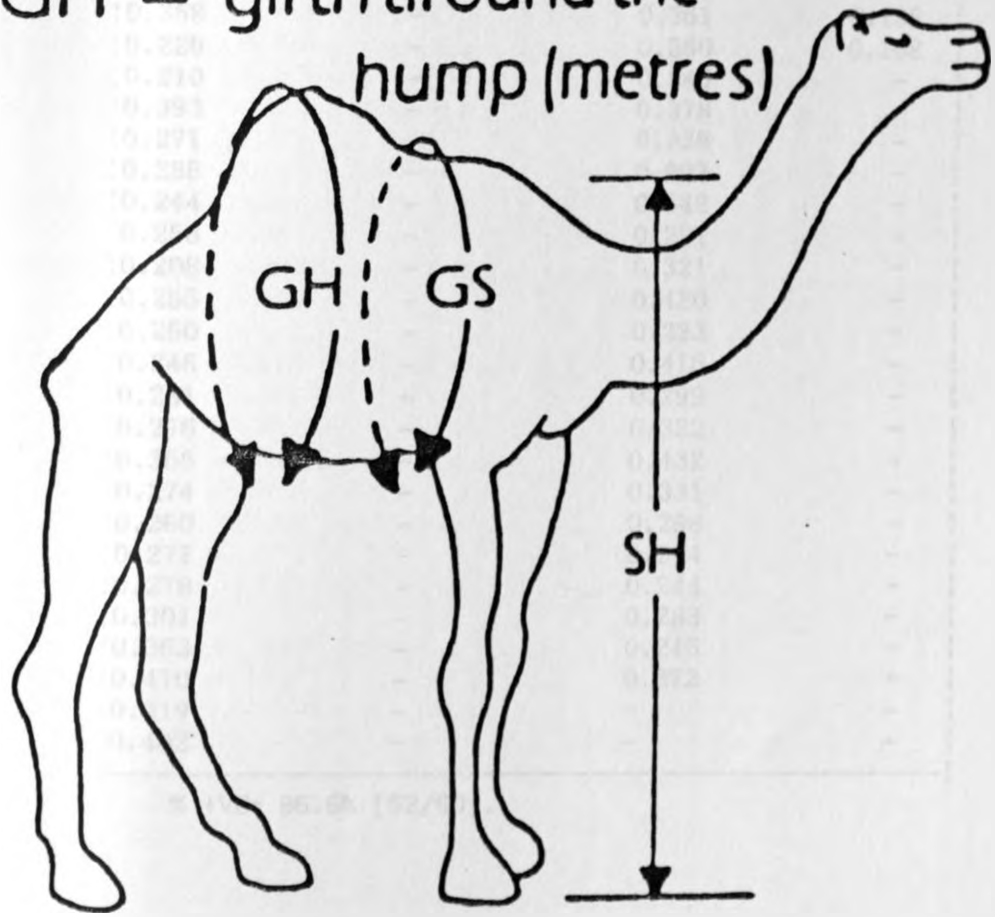
Bodyweight (kg) =

$$SH \times GS \times GH \times 50$$

where SH = shoulder height (metres)

GS = girth of shoulder (metres)

GH = girth around the hump (metres)



APPENDIX 3

Camel trypanosome infection rates based on ELISA values for Olturot camel herd no.1 bled on Dec 1985.

ELISA VALUE		>0.220=positive <0.220=negative	
MALE		FEMALE	
POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
0.316	0.209	0.258	0.167
0.432	0.203	0.288	0.210
0.412	0.130	0.229	0.166
0.358	-	0.361	0.136
0.226	-	0.260	0.108
0.210	-	0.245	-
0.393	-	0.378	-
0.271	-	0.338	-
0.285	-	0.293	-
0.244	-	0.342	-
0.258	-	0.291	-
0.208	-	0.321	-
0.255	-	0.420	-
0.250	-	0.223	-
0.246	-	0.416	-
0.281	-	0.299	-
0.276	-	0.322	-
0.355	-	0.432	-
0.274	-	0.331	-
0.260	-	0.298	-
0.277	-	0.314	-
0.278	-	0.244	-
0.301	-	0.283	-
0.363	-	0.246	-
0.410	-	0.372	-
0.319	-	-	-
0.402	-	-	-

% +VE= 86.6% [52/60].

APPENDIX 4

Camel trypanosome infection rates based on ELISA values for Olturot herd no.2 bled on Dec.1985.

ELISA VALUE	>0.221=positive <0.221=negative				
MALE			FEMALE		
POSITIVE		NEGATIVE	POSITIVE		NEGATIVE
0.295	0.404	0.148	0.460	0.441	0.071
0.258	0.242	0.197	0.480	0.366	
0.328	0.241	0.114	0.400	0.293	
0.328	0.470	0.124	0.456	0.299	
0.400	0.273	0.108	0.231	0.285	
0.284	0.362	0.217	0.361	0.267	
0.361	0.279	0.213	0.263	0.418	
0.420	0.329		0.285	0.501	
0.248	0.365		0.245	0.260	
0.265	0.367		0.229	0.290	
0.331	0.233		0.345	0.431	
0.315	0.593		0.317	0.387	
0.237	0.431		0.516	0.221	
0.350	0.413		0.520	0.277	
0.475	0.351		0.597	0.251	
0.244	0.305		0.432	0.502	
0.260	0.296		0.431	0.419	
0.430	0.305		0.529	0.378	
0.360	0.430		0.338	0.267	
0.446	0.292		0.274	0.291	
0.475	0.368		0.355	0.284	
0.451	0.265		0.318	0.382	
0.305	0.390		0.358	0.312	
0.281	0.453		0.518	0.422	
0.298			0.291	0.412	
0.397			0.408	0.430	
0.354			0.336	0.292	
0.268			0.412	0.306	
0.301			0.283	0.332	
			0.418		

%+VE=84.1% [101/120].

APPENDIX 5

Camel trypanosome infection rates based on ELISA values for Olturot herd no.3 bled on Feb. 1986.

ELISA VALUE			>0.550=positive <0.550=negative	
MALE			FEMALE	
POSITIVE		NEGATIVE	POSITIVE	NEGATIVE
0.919	0.753	0.100	1.027	0.260
0.825	0.826	0.291	1.019	0.317
0.732	1.027	0.472	0.603	0.210
0.632		-	0.993	0.166
0.929		-	0.788	0.136
1.281		-	0.621	0.108
0.672		-	0.936	-
0.848		-	0.604	-
0.576		-	0.982	-
0.616		-	1.023	-
0.814		-	1.011	-
0.689		-	0.943	-
0.590		-	0.766	-
0.913		-	0.674	-
0.709		-	0.818	-
0.802		-	1.030	-
0.691		-	1.019	-
1.033		-	0.970	-
1.002		-	0.593	
0.580		-	0.696	
0.599		-	0.742	
0.608			1.009	
1.028			0.982	
0.698			1.003	

%+VE=85.0% [51/60].

APPENDIX 6

Camel trypanosome infection rates based on ELISA values for Olturot herd no.4 bled on Feb.1986.

ELISA VALUE	> 0.221=positive < 0.221=negative

MALE			FEMALE		
POSITIVE		NEGATIVE	POSITIVE		NEGATIVE
0.245	0.255	0.108	0.425	0.356	0.152
0.272	0.319	0.136	0.331	0.314	0.168
0.313	0.286	0.159	0.362	0.229	0.208
0.254	0.263	0.219	0.277	0.361	0.210
0.400	0.316	-	0.260		0.195
0.285		-	0.319		-
0.274		-	0.281		-
0.355		-	0.285		-
0.225		-	0.293		-
0.244		-	0.253		-
0.378		-	0.267		-
0.368		-	0.284		-
0.421		-	0.412		-
0.256		-	0.290		-
0.316		-	0.283		-
0.337		-	0.291		-
0.228		-	0.258		-
0.361		-	0.394		
0.292		-	0.240		
0.308		-	0.227		
0.425			0.287		

%+VE=85.0% [51/60].

APPENDIX 7

Camel trypanosome infection rates based on ELISA values for Olturot herd no.5 bled on Dec.1986

ELISA VALUE			>0.550=positive <0.550=negative			
MALE			FEMALE			
POSITIVE			POSITIVE			NEGATIVE
1.029	0.957	0.634	0.856	0.667	0.693	0.345
1.033	0.958	0.623	0.572	0.607	0.982	0.333
0.685	0.922	0.863	0.853	1.005	1.127	0.450
0.668	0.936	0.781	0.708	0.813	0.748	0.448
0.588	0.818	0.779	0.676	0.632	0.697	0.291
0.712	0.744	0.772	1.039	0.909	0.631	
0.624	0.696	0.781	0.723	0.669	0.567	*0.462
0.561	0.582	1.314	0.667	0.898	0.594	
0.594	0.850	0.773	0.706	0.725	0.844	
0.830	0.698	0.795	0.675	0.566	0.833	
0.718	0.638	0.581	0.943	0.770	0.675	
0.826	0.630	0.949	1.213	1.209	0.787	
0.564	1.073	0.998	0.638	0.783	0.914	
0.584	0.646	0.737	0.773	1.010	-	
0.744	0.582	0.788	0.961	0.997	-	
0.658	0.933	0.851	0.719	0.588	-	
0.667	0.755	0.782	0.639	0.694	-	
0.768	0.914	-	0.610	0.856	-	
0.706	0.866	-	1.030	1.078	-	
0.555	0.778	-	0.923	0.802	-	
0.779	0.634	-	0.760	0.594	-	
						* Male

%+VE=86.7% [104/120].

APPENDIX 8

camel trypanosome infection ratesbased on ELISA values for Olturot herd no.6 bled on Dec. 1986

ELISA VALUE			>0.220=positive <0.220=negative		
MALE			FEMALE		
POSITIVE		NEGATIVE	POSITIVE		NEGATIVE
0.597	0.352	-	0.244	0.414	0.210
0.529	0.345	-	0.237	0.292	0.204
0.433	0.480	-	0.315	0.470	0.183
0.431	0.401	-	0.593	0.241	-
0.591	0.329	-	0.372	0.247	-
0.398	0.320	-	0.431	0.413	-
0.520	0.361	-	0.416	0.261	-
0.317	0.248	-	0.472	0.321	-
0.345	0.321	-	0.475		-
0.313	0.292	-	0.350		-
0.479	0.391	-	0.305		-
0.453	-	-	0.313		-
0.451	-	-	0.416		-
0.265	-	-	0.260		-
0.340	-	-	0.430		-
0.281	-	-	0.362		-
0.455	-	-	0.286		-
0.294	-	-	0.431		-
0.450	-	-	0.298		-

%+VE=95.0% [57/60].

APPENDIX 9

Camel trypanosome infection rates based on ELISA values for Olturot herd no.7 bled on Feb.1986.

ELISA VALUE	>0.220=positive <0.220=negative	
MALE		FEMALE
POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
0.424	0.413	0.166	0.501	0.413	0.201
0.413	0.750	0.134	0.439	0.264	0.197
0.482	0.403	-	0.526	0.417	0.213
0.420	0.369	-	0.377	0.226	0.214
0.393	0.496	-	0.365	0.238	0.164
0.490	0.488	-	0.384	0.245	0.117
0.382	0.396	-	0.394	0.309	-
0.558	0.462	-	0.389	0.374	-
0.386	0.517	-	0.442	0.358	-
0.370	0.391	-	0.400	0.268	-
0.381	0.577	-	0.419	0.287	-
0.552	0.441	-	0.399	0.314	-
0.682	0.450	-	0.394	0.387	-
0.407	0.384	-	0.477	0.422	-
0.531	0.573	-	0.378	0.390	-
0.500	0.306	-	0.504	0.308	-
0.362	0.570	-	0.465	0.311	-
0.399	0.380	-	0.397	0.590	-
0.476	0.417	-	0.284	0.439	-
0.388	0.374	-	0.413	-	-
0.337	0.470	-	0.297	-	-
0.414	0.390	-	0.364	-	-
0.418	-	-	0.466	-	-

%+VE=91.5% [87/95].

APPENDIX 10

Camel trypanosome infection rates based on ELISA values for Olturot herd no. 8 bled on Dec.1985

ELISA VALUE >0.220=positive <0.220=negative					
MALE			FEMALE		
POSITIVE		NEGATIVE	POSITIVE		NEGATIVE
0.480	0.392	0.209	0.531	0.433	0.119
0.623	0.396	0.217	0.400	0.597	0.123
0.297	0.671	-	0.322	0.282	0.159
0.319	0.558	-	0.310	0.318	0.074
0.277	0.470	-	0.690	0.438	0.187
0.311	0.391	-	0.703	0.347	-
0.289	0.407	-	0.611	0.456	-
0.465	0.578	-	0.390	0.360	-
0.497	0.340	-	0.418	0.291	-
0.384	0.278	-	0.363	0.446	-
0.494	0.411	-	0.401	0.413	-
0.380	0.464	-	0.455	0.295	-
0.398	0.478	-	0.533	0.475	-
0.342	0.479	-	0.395	0.328	-
0.423	0.504	-	0.472	0.441	-
0.387	0.433	-	0.324	0.409	-
0.501	0.388	-	0.396	0.238	-
0.319	0.390	-	0.267	0.283	-
0.499	0.561	-	0.279	0.329	-
0.314	0.720	-	0.238	0.410	-
0.284	0.563	-	0.361	0.237	-
0.240	0.493	-	0.284	-	-
0.369	0.248	-	0.273	-	-
0.499	0.369	-	0.529	-	-

%+VE=93.0% [93/100].

APPENDIX 11

Camel trypanosome infection rates based on ELISA values for Ngurunit herd no.1 bled on Dec.1985.

ELISA VALUE		>0.220=positive <0.220=negative	
MALE		FEMALE	
POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
0.294	0.136	0.290	0.195
0.308	-	0.400	0.176
0.283	-	0.282	0.200
0.378	-	0.273	-
0.225	-	0.293	-
0.355	-	0.312	-
0.274	-	0.275	-
0.421	-	0.224	-
0.431	-	0.244	-
0.358	-	0.378	-
0.404	-	0.338	-
0.441	-	0.285	-
0.319	-	0.414	-
0.291	-	0.248	-
0.400	-	0.316	-
0.321	-	0.276	-
0.319	-	0.263	-
0.290	-	0.273	-
0.298	-	0.255	-
0.308	-	0.260	-
0.250	-	0.294	-
0.267	-	0.315	-
0.277	-	0.426	-
0.387	-	-	-
0.284	-	-	-

%+VE=92.3% [48/52].

APPENDIX 12

Camel trypanosome infection rates based on ELISA values for Ngurunit herd no.2 bled on Dec. 1986.

ELISA VALUE		>0.220=positive <0.220=negative			
MALE			FEMALE		
POSITIVE		NEGATIVE	POSITIVE		NEGATIVE
0.433	0.400	0.147	0.404	0.423	0.212
0.432	0.529	0.128	0.237	0.229	0.190
0.284	0.480	-	0.450		0.217
0.597	0.435	-	0.294		0.215
0.315	-	-	0.455		-
0.520	-	-	0.281		-
0.285	-	-	0.390		-
0.516	-	-	0.306		-
0.317	-	-	0.365		-
0.518	-	-	0.451		-
0.345	-	-	0.329		-
0.263	-	-	0.248		-
0.231	-	-	0.365		-
0.412	-	-	0.330		-
0.347	-	-	0.315		-
0.456	-	-	0.593		-
0.413	-	-	0.332		-
0.475	-	-	0.431		-
0.350	-	-	0.297		-
0.305	-	-	0.222		-
0.269	-	-	0.361		-
0.340	-	-	0.279		-
0.446	-	-	0.239		-
0.360	-	-	0.317		-

%+VE=90.0% [54/60].

APPENDIX 13

Camel trypanosome infection rates based on ELISA values for Ngurunit herd no.3 bled on Dec. 1986.

ELISA VALUE	>0.550=positive <0.550=negative				
MALE			FEMALE		
POSITIVE		NEGATIVE	POSITIVE		NEGATIVE
0.719	0.708	-	1.326	0.988	0.419
0.610	1.013	-	1.090	0.866	0.470
0.639	1.291	-	0.747	0.877	0.344
0.789	0.664	-	0.683	1.334	
1.039	0.926	-	0.950	0.723	-
0.688	1.075	-	0.798	1.011	-
0.760	0.975	-	1.076	-	-
0.689	1.027	-	1.065	-	-
0.813	0.882	-	0.835	-	-
0.723	-	-	0.850	-	-
0.662	-	-	0.680	-	-
0.995	-	-	0.711	-	-
0.930	-	-	0.707	-	-
0.818	-	-	0.736	-	-
0.967	-	-	0.854	-	-
0.879	-	-	0.708	-	-
0.551	-	-	0.957	-	-
0.806	-	-	1.054	-	-
0.015	-	-	0.602	-	-
0.860	-	-	0.858	-	-
0.868	-	-	1.014	-	-

%+VE=94.05 [47/50].

APPENDIX 14

Camel trypanosome infection rates based on ELISA values for Ngurunit herd no.4 bled on Feb. 1986.

ELISA VALUE	>0.220= positive <0.220= negative		
MALE		FEMALE	
POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
0.285	0.210	0.263	0.195
0.396		0.345	0.204
0.324		0.516	0.210
0.347		0.480	
0.398		0.460	
0.279		0.361	
0.238		0.510	
0.361		0.455	
0.284		0.390	
0.410		0.317	
0.247		0.236	
0.244		0.471	
0.328		0.347	
0.430		0.413	
0.446		0.261	
0.475		0.309	
0.451		0.331	
0.231		0.298	
0.294			

%+VE=92.5% [37/40].

APPENDIX 15

Camel trypanosome infection rates based on ELISA values or Ngurunit herd no.5 bled on Feb.1986.

ELISA VALUE			>0.550= positive <0.550= negative		
MALE			FEMALE		
POSITIVE			POISITIVE		NEGATIVE
0.839	0.674	0.410	1.034	1.004	0.431
0.596	1.230	-	0.698	0.883	0.291
0.586	-	-	0.753	-	-
0.708	-	-	0.603	-	-
1.233	-	-	1.011	-	-
0.029	-	-	1.033	-	-
1.018	-	-	0.828	-	-
0.674	-	-	0.594	-	-
0.943	-	-	0.724	-	-
0.808	-	-	0.669	-	-
0.607	-	-	0.800	-	-
0.786	-	-	0.923	-	-
1.260	-	-	0.623	-	-
1.010	-	-	0.588	-	-
1.043	-	-	0.693	-	-
0.770	-	-	0.944	-	-
0.603	-	-	1.039	-	-
1.284	-	-	1.009	-	-
0.690	-	-	0.787	-	-
0.558	-	-	0.672	-	-
0.849	-	-	1.280	-	-
0.823	-	-	1.004	-	-
0.613	-	-	1.012	-	-
0.668	-	-	0.615	-	-
0.592	-	-	0.598	-	-
1.003	-	-	0.558	-	-
0.925	-	-	-	-	-

%+VE=95.0% [57/60].

APPENDIX 16

Camel trypanosome infection rates based on ELISA values for Korr herd no.1 bled on Dec. 1985.

ELISA VALUE		>0.220=positive <0.220=negative	
MALE		FEMALE	
POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
0.396	0.207	0.245	0.128
0.285	0.206	0.291	0.160
0.334	0.180	0.323	0.178
0.402	0.199	0.278	0.136
0.290	-	0.253	0.168
0.267	-	0.289	0.203
0.342	-	0.254	0.208
0.258	-	0.259	-
0.229	-	0.253	-
0.279	-	0.276	-
0.285	-	0.286	-
0.260	-	0.319	-
0.279	-	0.406	-
0.264	-	0.425	-
0.362	-	0.311	-
0.339	-	0.329	-
0.271	-	0.299	-
0.289	-	0.295	-
0.387	-	-	-
0.308	-	-	-
0.421	-		

%+VE=78.0% [39/50]

APPENDIX 17

Camel trypanosome infection rates based on ELISA values for Korr herd no.2 bled on Dec. 1986.

ELISA VALUE		>0.550= positive <0.550= negative	
MALE		FEMALE	
POSITIVE	NEGATIVE	POISITIVE	NEGATIVE
1.091	0.400	0.960	0.416
1.026	0.516	0.900	0.407
1.033	0.294	0.831	0.368
0.981	0.303	0.742	0.298
0.570	0.291	0.588	0.488
0.609	-	0.563	0.211
0.948	-	0.701	0.285
0.675	-	0.809	-
1.381	-	1.040	-
1.007	-	1.066	-
0.717	-	1.021	-
0.759	-	1.043	-
0.883	-	0.597	-
0.912	-	0.926	-
0.744	-	0.828	-
1.200	-	0.703	-
1.095	-	0.609	-
0.801	-	-	-

%+VE=76.0% [38/50].

APPENDIX 18

Camel trypanosome infection rates based on ELISA values for Korr herd no.3 bled on Feb. 1986.

ELISA VALUE	>0.220= positive <0.220= negative		
MALE		FEMALE	
POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
0.258	0.216	0.378	0.192
0.263	0.097	0.241	0.118
0.316	0.198	0.236	0.208
0.271	0.206	0.227	0.094
0.314	-	0.253	0.134
0.389	-	0.225	0.187
0.283	-	0.410	-
0.326	-	0.319	-
0.284	-	0.233	-
0.254	-	0.238	-
0.426	-	0.246	-
0.221	-	0.471	-
0.294	-	0.384	-
0.239	-	0.390	-
0.318	-	0.434	-
0.288	-	0.251	-
0.330	-	0.360	-
0.378	-	0.317	-
0.311	-	0.377	-

%+VE=80.0% [40/50].

APPENDIX 19

Camel trypanosome infection rates based on ELISA values for Ula-ula herd no.1 bled on Dec. 1986.

ELISA VALUE		>0.220=positive <0.220=negative	
MALE		FEMALE	
POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
0.419	0.126	0.488	02137
0.442	0.117	0.365	0.214
0.681	0.204	0.386	0.197
0.500	-	0.398	0.164
0.399	-	0.411	0.214
0.386	-	0.504	0.211
0.490	-	0.374	0.217
0.482	-	0.380	0.209
0.413	-	0.399	0.174
0.558	-	0.397	-
0.375	-	0.392	-
0.407	-	0.419	-
0.597	-	0.387	-
0.570	-	0.424	-
0.750	-	0.365	-
0.206	-	0.367	-
0.512	-	-	-
0.540	-	-	-
0.440	-	-	-
0.387	-	-	-
0.432	-	-	-
0.360	-	-	-

%+VE=76.0% [38/50].

APPENDIX 20

Camel trypanosome infection rates based on ELISA values for Log-logo herd no.1 bled on Dec.1985.

ELISA VALUES	<0.220=positive <0.220=negative		
MALE		FEMALE	
POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
0.371	-	0.415	0.110
0.529	-	0.279	0.129
0.424	-	0.284	0.148
0.578	-	0.242	0.210
0.520	-	0.371	0.147
0.258	-	0.244	0.071
0.345	-	0.359	0.111
0.354	-	0.282	0.116
0.238	-	0.363	0.204
0.231	-	0.458	0.189
0.374	-	0.298	0.137
0.465	-	-	0.206
0.451	-	-	0.210
0.292	-	-	0.190
0.474	-	-	-
0.359	-	-	-
0.281	-	-	-
0.406	-	-	-
0.450	-	-	-
0.440	-	-	-
0.316	-	-	-
0.249	-	-	-
0.211	-	-	-
0.233	-	-	-
0.300	-	-	

%+VE=72.0% [36/50].

APPENDIX 21a

H.camelina/ camel at Olturot

JANUARY/1986
049 052 053 047 058 057 047 048 048 048 049 046 050 051 050 048 052 050
050 049 047 050 049 047 048 049 050 050 047 053 047 054 050 047 058 046
050 051 052 053 054 050 053 052 00 047 046 046 054 050 047 049 047 047
048 053 049 053 051 050 051 050 051 054 053 054 050 051 051 045 051 044
057 056 044 050 051 051 043 051.

FEBRUARY/1986
087 088 084 088 089 086 088 085 084 085 085 084 085 088 086 088 085 083
085 087 086 085 086 085 086 085 090 087 086 085 094 085 090 084 086 089
086 089 084 086 085 087 085 084 083 089 088 088 089 085 084 083 089 086
086 087 084 085 087 087 088 083 084 087 087 087 086 087 088 086 085 083
082 088 081 090 091 083 085 083.

MARCH/1986
211 210 211 209 210 208 207 211 209 210 212 211 210 209 208 208 211 209
209 211 213 206 213 210 214 213 213 212 210 211 211 210 208 213 215 207
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213 210 207 214 213 215 212 211 214 210 206 207 207 210 212 215 214 214
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APRIL/1986
287 288 287 290 291 290 290 290 291 290 292 290 289 290 291 287 286 288
290 286 291 288 286 290 288 290 291 290 289 290 291 291 291 292 286 285
281 293 290 289 290 291 287 288 287 290 286 290 290 288 287 294 285 291
290 288 286 292 292 285 288 298 295 292 292 294 284 289 290 290 286 291
290 287 292 290 290 287 290 290.

MAY/1986
129 131 128 130 134 131 131 130 129 131 128 128 131 127 132 130 131 130
130 131 132 131 130 131 128 127 129 132 130 133 131 132 129 128 132 127
130 128 131 130 129 132 130 131 132 130 133 132 127 131 132 132 133 126
131 131 127 126 134 131 132 129 130 132 131 127 123 129 133 131 135 134
132 124 130 132 131 126 129 132.

JUNE/1986
076 078 074 074 074 073 077 075 074 076 075 077 077 077 076 078 080 079
075 077 075 073 076 075 073 076 078 079 077 075 076 075 074 076 074 071
070 074 075 074 078 078 073 079 075 076 077 074 074 075 074 078 074 077
079 073 077 076 071 072 072 068 066 079 079 076 080 065 076 078 077 073
075 078 071 075 077 079 080 076.

APPENDIX 12a cont.

075 081 083 080 079 075 077 079 079 074 076 078 073 078 075 076 076 077
076 074 073 074 075 079 078 077 077 076 078 079 077 076 076 077 074 077
075 074 075 076 078 078 071 080 077 075 074 073 078 076 075 076 080 082
072 083 079 077 076 075 074 073 083 080 077 071 078 079 077 075 074 073
076 067 075 073 073 077 078 076.

AUGUST/1986
051 053 050 051 049 053 048 054 053 050 050 052 051 050 051 053 054 052
053 050 049 050 051 052 053 054 053 053 050 051 052 053 050 051 052 047
049 048 049 050 048 051 053 052 053 050 051 051 054 053 051 052 046 055
047 053 054 053 051 050 050 051 044 046 053 045 047 055 049 055 054 053
044 052 050 051 047 053 050 052.

SEPTEMER/1986
044 043 044 040 041 044 041 042 042 040 041 040 039 041 041 040 043 044
041 043 042 041 040 038 039 041 049 040 036 043 045 045 041 042 041 040
045 041 044 043 034 045 046 040 038 044 045 040 042 042 037 041 040 033
036 046 041 040 042 044 043 040 041 041 043 034 038 046 033 047 040 045
041 043 040 041 044 040 041 042.

OCTOBER/1986
056 057 056 050 051 055 057 056 052 050 052 051 053 052 050 051 047 055
057 054 050 053 052 052 053 054 050 052 050 051 051 057 051 050 048 056
058 055 052 053 050 051 045 047 050 053 052 052 050 051 057 056 050 053
050 052 051 054 051 053 055 044 046 052 053 057 058 050 052 055 050 051
055 051 053 050 048 056 050 051.

NOVEMBER/1986
061 066 058 060 061 060 060 060 064 063 061 059 064 063 057 063 060 062
063 065 063 062 060 061 067 063 060 061 063 062 058 060 057 060 066 067
065 061 064 060 062 065 064 055 057 059 060 060 060 061 061 064 060 061
063 068 056 064 062 061 067 068 064 064 063 065 060 057 052 060 062 063
061 054 055 060 067 061 061 053.

DECEMBER/1986
052 050 053 051 049 050 054 048 051 052 053 050 053 052 051 053 052 055
056 054 053 056 057 051 052 050 054 052 054 052 054 053 057 059 051 053
046 047 049 054 057 058 055 044 052 050 051 053 052 056 052 055 054 051
048 048 049 050 051 056 053 052 054 050 051 052 052 058 049 057 051 051
050 047 044 050 053 054 050 051.

APPENDIX 21b

H. camelina/camel at Ngurunit

JANUARY/1986

086 089 089 086 088 090 090 089 090 091 089 085 088 089 088 087 092 086
087 085 089 088 086 089 087 085 088 091 092 093 087 090 090 091 092 092
090 089 090 090 089 091 090 090 091 093 090 092 089 088 087 090 091 087
088 089 089 086 090 090 093 094 090 901 089 087 090 090 089 094 094 088
090 090 093 093 091 095 088 079.

FEBRUARY/1986

230 231 230 230 230 232 231 231 234 230 234 230 235 233 231 230 232 230
231 230 231 230 230 233 227 228 234 228 229 227 229 231 230 229 234 235
234 232 228 230 231 230 230 231 230 232 231 230 232 228 227 230 228 229
230 231 232 231 228 233 230 230 231 230 231 230 231 233 232 229 230 231
233 232 231 231 235 230 231 226.

MARCH/1986

552 547 551 550 552 550 547 548 547 549 551 550 550 553 554 551 553 557
551 556 547 549 550 558 552 551 553 550 550 551 547 546 544 553 557 551
550 551 543 559 557 551 550 548 552 553 551 550 554 557 551 549 538 547
547 541 543 549 556 550 552 552 551 550 543 549 547 556 548 541 550 551
547 548 547 542 541 550 554 558.

APRIL/1986

534 535 534 530 534 532 530 530 531 533 527 528 534 536 530 533 531 530
527 529 533 530 537 535 528 527 532 532 534 534 535 538 531 532 522 526
536 530 531 537 533 526 528 528 539 530 530 531 534 530 531 532 537 539
521 529 527 531 530 534 534 530 531 528 522 521 536 540 531 530 538 520
531 534 534 538 530 530 531 530.

MAY/1986

573 571 570 572 570 573 547 570 548 573 577 571 573 573 570 573 576 578
570 572 573 570 573 570 576 573 575 573 573 574 570 573 572 570 573 571
572 570 574 570 578 546 541 547 570 571 570 572 571 570 573 574 570 570
574 647 578 576 571 570 573 570 571 570 576 577 576 544 549 570 576 578
570 543 546 570 545 547 540 579.

JUNE/1986

374 370 367 368 370 376 370 372 371 369 366 368 372 370 375 368 375 370
375 370 371 372 372 370 373 376 374 370 371 369 365 378 376 370 371 379
371 374 378 367 366 361 371 370 376 380 379 371 372 375 371 374 372 370
368 366 367 360 379 380 377 376 371 370 377 376 373 372 370 371 369 379
372 374 365 370 371 370 374 377.

APPENDIX 21b cont.

JULY/1986
209 211 210 296 210 213 211 210 212 213 210 208 210 209 210 206 214 210
211 210 213 210 210 213 208 214 214 210 215 212 210 212 297 210 211 208
210 214 209 214 208 215 210 211 210 209 210 209 207 204 206 210 213 207
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211 217 213 211 209 207 209 215.

AUGUST/1986
088 090 087 088 090 089 086 091 087 091 088 087 089 090 089 087 085 087
087 089 091 088 078 086 088 087 084 083 081 090 088 089 090 903 090 089
089 095 090 091 092 088 089 084 082 088 087 090 094 097 092 086 090 085
093 090 091 089 086 087 084 082 090 091 088 089 093 094 092 091 094 095
084 088 085 083 086 089 090 091.

SEPTEMBER/1986
021 017 018 018 019 018 016 020 021 017 018 023 022 017 021 023 022 021
019 023 021 021 020 018 018 019 018 017 021 019 017 016 018 017 016 019
018 020 020 018 024 021 021 019 021 023 019 020 023 022 028 022 021 020
017 014 020 025 021 020 017 019 015 016 024 021 022 018 025 019 023 021
013 028 022 021 020 015 017 014.

OCTOBER/1986
209 203 207 206 202 203 208 207 208 206 209 202 203 202 205 206 207 202
204 203 202 202 203 201 204 202 205 202 204 203 205 207 201 210 201 202
207 206 203 206 204 209 203 207 206 204 201 202 207 208 201 211 203 210
206 204 201 210 212 211 219 108 201 209 203 201 208 207 204 208 206 205
201 210 214 211 209 204 201 206.

NOVEMBER/1986
222 230 229 226 227 231 231 223 230 231 227 226 225 226 229 230 230 229
228 230 227 226 224 221 222 230 231 235 232 228 226 225 228 224 229 226
235 233 230 231 225 222 233 230 231 227 228 223 221 229 236 231 235 240
232 230 231 236 233 234 231 230 223 228 225 227 230 231 235 221 229 227
231 231 234 237 230 221 224 226.

DECEMBER/1986
213 212 211 213 212 217 215 211 216 219 211 218 215 210 211 214 216 197
209 205 213 210 211 219 217 214 212 210 210 214 215 212 211 216 219 218
210 208 207 210 211 216 215 211 218 215 214 211 210 217 219 208 213 211
205 203 213 217 215 219 210 206 211 209 204 205 209 210 211 217 216 214
211 205 209 216 211 213 219 217.

APPENDIX 21c

H.camelina/camel at Ilaut.

JANUARY/1986
050 041 062 063 060 040 043 040 041 059 030 033 035 060 044 040 050 053
055 046 061 037 030 080 070 074 055 052 051 044 039 058 051 053 060 040
053 030 050 048 045 047 056 057 056 050 052 050 051 045 049 041 069 031
033 035 061 066 062 057 053 041 053 059 060 067 043 042 040 041 052 050
056 050 059 033 060 067 060 053.

FEBRUARY/1986
089 090 097 090 091 087 098 078 077 076 090 098 095 084 079 071 096 089
080 094 079 078 096 095 099 078 080 080 081 078 093 088 081 084 089 089
090 099 092 077 075 098 093 092 090 091 088 087 088 097 099 085 078 087
078 086 088 081 089 096 094 095 095 090 089 089 088 086 082 085 080 089
088 089 091 079 080 099 094 086.

MARCH/1986
215 209 213 199 206 209 201 204 234 198 201 235 221 210 207 200 239 211
224 209 202 220 230 216 210 197 200 230 216 211 218 200 205 206 200 219
189 253 199 185 217 220 225 200 218 205 203 210 215 211 209 206 200 241
225 214 210 210 201 241 233 239 210 213 225 229 220 200 207 211 219 202
200 210 237 216 200 214 222 215.

APRIL/1986
246 214 210 250 200 230 225 220 216 200 199 190 265 200 249 230 240 235
228 231 245 220 225 229 220 210 254 220 210 246 220 224 228 231 240 245
205 240 215 224 200 195 253 207 201 210 220 250 245 270 189 186 180 250
200 236 214 232 269 200 203 249 237 213 209 205 200 201 200 271 266 200
209 222 236 231 200 249 240 238.

MAY/1986
250 255 249 260 255 253 230 239 245 256 240 231 259 250 250 240 233 239
245 236 230 233 237 230 229 240 247 241 245 255 250 259 259 268 260 255
234 230 219 220 269 276 214 225 256 250 245 244 249 248 240 241 239 230
237 237 245 240 210 229 220 234 246 256 267 251 250 257 240 243 240 246
200 207 209 200 210 230 274 266.

JUNE/1986
126 140 113 123 130 139 129 130 110 140 141 167 100 123 126 135 149 150
137 120 134 130 145 140 146 140 130 132 160 107 100 132 115 126 122 137
119 146 167 140 128 120 107 110 129 130 159 146 110 132 138 130 131 130
145 127 120 116 119 121 126 135 133 130 128 122 120 133 148 140 110 145
130 148 139 120 136 139 127 136.

APPENDIX 21c cont.

JULY/1986
110 130 124 130 136 115 128 123 117 130 120 110 110 117 129 119 117 121
122 121 129 116 115 110 110 100 121 122 123 110 119 110 130 133 138 126
126 100 114 110 125 134 150 141 130 130 128 122 120 123 116 110 101 102
110 111 107 109 132 105 110 123 129 145 127 122 121 120 140 132 110 117
120 128 131 122 136 130 100 108.

AUGUST/1986
082 086 090 111 085 123 093 098 090 073 131 148 088 075 125 088 071 107
070 130 112 077 063 148 104 087 098 131 102 110 115 081 090 097 107 112
086 051 136 098 084 108 097 066 101 118 100 125 077 089 099 095 149 075
081 094 107 111 115 109 100 078 090 099 132 122 109 120 114 106 115 100
063 129 084 118 101 100 080 085.

SEPTEMBER/1986
085 088 058 059 066 060 077 098 077 078 084 050 076 082 080 085 088 081
066 074 072 067 055 066 059 078 071 065 083 080 071 085 070 071 068 065
098 065 064 051 050 073 070 071 056 052 050 069 070 077 075 090 089 074
077 070 063 066 061 070 074 088 082 080 063 071 060 061 053 050 070 074
077 089 092 090 087 073 055 067.

OCTOBER/1986
078 071 079 077 080 071 072 073 081 088 066 078 067 060 090 091 091 092
073 079 077 070 080 057 098 090 071 072 077 077 076 078 093 085 088 081
070 060 064 066 082 080 081 077 072 074 079 090 090 075 077 071 070 078
070 059 055 092 090 088 080 075 077 060 069 065 092 090 077 082 080 091
077 078 071 064 080 077 072 075.

NOVEMBER/1986
080 069 070 079 060 066 084 085 081 088 082 090 088 092 099 088 084 090
100 065 109 067 078 066 069 061 101 083 088 089 064 093 090 084 099 090
076 083 088 081 080 089 093 096 064 061 090 084 085 070 076 064 056 066
090 090 092 088 089 084 084 080 081 080 082 070 095 086 080 058 055 082
093 091 099 080 088 081 084 083.

DECMBER/1986
067 055 067 073 087 090 092 088 066 077 078 077 088 082 090 081 085 081
055 098 065 066 051 090 056 078 065 068 071 085 083 078 079 069 090 093
063 077 077 071 072 089 090 080 064 071 064 056 067 063 061 098 070 072
074 077 080 081 090 090 060 063 082 077 074 070 069 070 078 088 075 077
070 071 057 055 090 083 078 074.

APPENDIX 21d.

H.camelina/camel at Korr.

JANUARY/1986
180 206 198 193 169 199 170 220 230 175 198 183 190 192 199 193 190 160
225 209 220 210 220 166 150 260 198 192 190 205 230 180 187 190 199 193
241 177 217 200 208 260 190 184 199 194 190 189 150 188 184 185 172 190
199 194 198 191 190 160 197 233 215 206 210 190 196 170 173 199 196 214
194 190 189 205 231 239 167 198.

FEBRUARY/1986
230 244 200 210 184 210 233 215 190 235 202 240 221 240 237 215 210 200
229 211 204 239 210 217 243 250 180 200 217 230 218 210 226 209 205 240
220 241 217 205 203 200 201 233 238 240 243 211 210 220 226 227 241 219
204 221 228 220 231 213 187 245 221 223 220 210 195 229 251 210 240 241
218 227 247 217 206 221 220 219.

MARCH/1986
616 636 620 632 630 622 615 637 640 612 611 641 600 652 630 622 621 631
619 633 634 618 650 604 615 611 641 620 625 619 635 633 617 639 608 642
640 623 644 617 608 600 651 600 624 618 620 620 622 621 527 618 607 639
636 600 615 612 601 610 618 640 605 620 627 631 643 619 600 611 610 603
647 750 613 622 631 626 620 625.

APRIL/1986
590 650 581 600 580 660 620 640 604 638 636 612 620 623 600 607 660 641
631 629 600 607 612 650 652 610 609 620 624 644 613 640 642 610 615 611
639 620 636 631 643 640 610 592 609 657 650 645 621 620 623 648 610 613
634 635 611 617 632 608 601 649 602 611 646 651 600 601 612 610 630 633
620 630 645 610 633 638 609 634.

MAY/1986
513 543 540 526 503 560 510 520 546 555 530 535 512 510 540 560 522 544
540 521 511 539 542 510 555 509 510 527 548 530 549 551 500 569 560 514
503 536 531 534 540 548 522 529 540 517 550 541 514 533 530 518 559 560
511 543 533 527 539 560 420 528 539 520 544 547 509 503 506 659 548 540
516 560 541 538 529 550 521 537.

JUNE/1986
490 421 420 495 500 511 510 512 509 489 499 490 513 500 521 489 497 509
503 524 510 499 492 486 496 520 500 510 521 510 508 501 509 507 500 502
509 501 507 517 529 483 500 509 503 505 500 511 498 496 515 512 500 503
500 507 501 504 507 506 521 511 527 500 509 502 511 527 508 499 492 490
503 509 505 497 491 521 513 515.

APPENDIX 21d cont.

JULY/1986
211 200 220 223 213 209 226 205 200 215 219 210 221 180 197 210 218 211
229 205 200 241 204 189 190 206 205 200 201 216 218 219 210 228 220 216
205 200 217 209 206 208 206 206 214 225 201 203 210 201 207 229 201 214
215 217 200 208 209 206 210 210 224 216 210 225 210 200 219 203 230 215
186 211 205 200 212 220 214 213.

AUGUST/1986
110 090 099 120 121 114 108 100 103 118 109 107 100 115 100 108 102 108
104 106 114 113 102 103 100 095 098 116 100 103 100 102 113 109 104 112
100 105 099 094 106 105 101 102 117 120 218 097 106 111 115 107 102 116
109 112 111 100 094 089 100 117 104 108 115 100 104 106 106 114 112 107
102 102 110 115 109 105 121 103.

SEPTEMBER/1986
079 087 096 099 094 088 093 079 095 095 092 096 097 098 093 080 091 094
090 070 120 132 089 095 097 099 083 090 090 098 090 079 099 104 110 070
077 095 090 093 090 087 094 090 084 088 080 099 109 100 095 078 099 104
100 084 092 090 101 095 090 093 097 078 086 090 096 112 107 100 085 089
090 097 099 091 093 105 099 093.

OCTOBER/1986
160 167 174 172 169 166 173 170 164 160 175 180 160 159 166 173 163 161
160 150 170 155 171 174 177 169 154 160 155 167 163 164 166 163 172 170
172 170 165 180 155 153 160 168 159 169 166 162 160 169 166 163 155 152
155 150 152 163 167 169 160 164 166 160 170 178 163 160 166 163 164 160
166 178 162 170 159 153 166 163.

NOVEMBER/1986
177 180 170 179 172 170 171 174 172 177 171 175 170 169 170 174 177 175
170 156 189 177 174 176 177 178 170 171 176 172 183 176 171 188 190 177
173 166 178 177 170 175 174 170 184 188 183 182 199 167 157 160 167 190
190 174 179 180 176 180 185 183 188 170 179 174 171 173 178 176 175 170
171 168 160 169 176 170 182 174.

DECEMBER/1986
166 168 170 169 167 170 167 168 164 158 171 173 157 158 164 171 162 163
151 155 167 170 176 160 165 159 173 170 171 177 170 165 170 173 166 162
163 167 165 180 183 169 166 162 165 166 160 161 172 177 173 166 167 160
155 151 159 150 160 163 159 171 167 166 160 158 155 151 161 167 180 181
168 163 170 171 159 167 165 164.

APPENDIX 21e.

H.camelina/camel at Kargi

JANUARY/1986
101 072 090 094 101 098 099 090 087 091 093 090 087 112 109 090 096 085
100 070 114 116 070 082 104 116 089 090 091 096 095 080 085 114 119 090
090 093 097 090 089 088 081 075 109 107 090 092 097 091 090 094 096 091
077 100 090 095 092 095 097 117 105 104 110 100 116 101 090 076 070 071
089 093 090 084 101 110 079 095.

FEBRUARY/1986
111 113 109 120 128 119 108 131 103 121 117 107 096 113 106 125 107 110
127 100 089 099 094 125 130 120 112 118 129 095 112 110 117 115 128 115
139 092 106 148 112 119 110 104 128 105 120 112 119 117 106 109 110 101
089 083 109 113 132 128 120 122 095 099 091 129 121 109 116 136 112 119
116 109 123 117 105 121 096 115.

MARCH/1986
289 290 309 330 306 320 287 290 295 290 291 293 287 288 341 330 311 326
319 289 311 318 322 341 350 279 287 290 295 305 267 299 298 290 296 315
339 315 327 346 330 321 320 360 341 326 329 318 306 288 293 310 320 327
324 309 337 304 321 320 311 317 330 334 310 311 327 354 279 276 298 290
287 295 306 321 326 320 318 311.

APRIL/1986
503 511 510 519 498 490 487 520 538 561 527 512 500 520 519 506 521 502
526 518 509 536 521 539 512 517 500 528 509 501 510 511 533 518 507 551
478 490 510 524 518 512 531 520 510 500 512 529 509 510 510 540 528 500
508 480 481 489 490 492 529 501 523 514 507 511 509 502 540 543 534 513
505 513 500 510 509 486 500 519.

MAY/1986
420 426 415 400 430 413 459 540 538 530 425 439 421 400 413 420 418 409
418 400 410 422 433 440 446 437 429 422 421 429 433 441 400 409 410 430
403 422 420 428 430 415 421 438 409 405 415 433 458 450 410 407 402 411
420 536 420 430 413 400 430 427 412 418 409 401 410 411 400 438 451 518
416 444 420 429 425 401 526 410.

JUNE/1986
340 370 362 344 350 367 341 370 355 341 340 349 366 361 369 370 360 354
351 344 379 365 360 352 340 339 331 340 348 367 369 360 344 341 350 354
380 375 365 347 344 340 352 340 357 320 327 361 360 354 352 386 350 361
367 338 340 356 350 351 331 366 380 352 353 358 340 349 341 340 330 339
320 346 376 354 355 351 350 340.

APPENDIX 21e cont.

JULY/1986
188 191 209 204 189 186 199 190 211 202 211 209 213 221 230 180 186 185
241 244 231 217 200 201 210 209 197 199 206 202 205 218 211 220 213 238
215 200 240 246 231 206 201 215 186 193 204 221 228 200 219 210 231 240
220 224 200 210 245 233 215 209 200 210 221 220 226 228 250 240 244 211
200 185 198 200 207 200 210 227.

AUGUST/1986
087 099 103 114 121 100 084 085 098 076 121 109 120 077 117 096 084 111
096 076 087 098 090 091 106 090 098 094 082 091 090 075 110 090 093 087
088 090 098 095 084 081 091 072 114 077 080 093 088 085 090 092 120 115
090 094 090 092 094 078 082 090 098 094 094 090 099 063 099 097 104 100
090 101 096 090 091 095 092 089.

SEPTEMBER/1986
073 077 079 088 067 072 097 090 081 066 070 078 077 089 075 080 071 079
070 077 072 069 066 062 080 081 079 077 064 060 084 072 080 077 073 070
056 090 091 062 055 059 050 093 096 077 077 054 093 082 080 090 096 071
077 070 060 066 067 074 073 066 064 055 098 080 090 095 092 090 080 077
072 069 066 070 079 073 076 071.

OCTOBER/1986
055 066 063 061 070 051 067 069 051 053 074 060 058 061 079 075 070 067
035 090 089 052 061 060 067 066 062 051 056 050 068 060 054 051 060 057
043 084 080 041 044 056 080 070 071 061 066 063 057 051 050 032 066 097
060 067 070 055 061 066 074 063 060 059 055 050 052 071 061 060 053 058
090 091 052 058 070 065 061 060.

NOVEMBER/1986
100 108 102 090 087 078 070 068 066 078 071 077 073 070 075 080 078 073
034 085 076 077 072 071 069 068 060 092 090 066 073 078 063 098 037 079
088 070 076 072 070 090 067 062 060 074 065 070 078 073 071 077 078 076
077 077 070 080 060 063 085 070 081 073 070 076 045 090 092 078 077 075
056 068 096 075 090 092 070 056.

DECEMBER/1986
070 071 120 128 103 073 073 065 067 066 085 113 067 054 067 033 112 067
068 078 077 109 072 127 114 073 085 052 062 078 085 037 060 064 064 062
078 060 065 051 098 041 100 061 063 119 085 057 060 078 090 089 077 070
078 102 096 070 075 078 073 076 054 059 092 088 074 077 070 071 093 076
074 070 082 078 072 070 071 075.

APPENDIX 22a

H.minuta/camel at Olturot

JANUARY/1986
001 009 001 007 004 002 003 003 001 005 005 009 003 008 009 006 001 004
010 001 001 003 003 002 007 005 005 001 001 004 005 005 002 002 008 001
004 003 006 006 001 002 005 002 002 001 001 003 003 008 009 008 008 006
006 003 005 005 002 002 004 004 007 008 005 006 003 003 002 002 003 003
004 002 001 002 002 004 005 008.

FEBRUARY/1986
022 030 022 031 022 025 021 029 022 024 023 027 025 025 029 031 022 021
024 027 025 025 029 026 022 021 033 024 023 021 022 028 026 026 028 029
032 030 023 028 029 024 024 026 031 023 026 027 025 029 029 022 026 028
021 020 029 020 027 023 027 029 034 021 020 020 024 028 028 025 025 025
023 021 020 029 025 024 028 026.

MARCH/1986
040 033 030 031 033 035 033 030 032 032 031 028 028 029 030 034 041 033
031 035 036 030 031 031 033 031 030 027 022 031 030 037 033 032 032 031
030 036 034 033 033 030 035 027 029 032 030 031 033 026 034 030 033 038
025 029 034 030 031 031 042 040 028 026 033 027 033 030 034 031 030 029
029 033 031 030 028 035 026 034.

APRIL/1986
039 035 041 045 050 029 045 044 038 037 038 033 045 056 050 051 047 045
043 041 036 038 043 044 026 039 040 040 041 047 044 039 042 044 041 040
044 041 040 040 043 041 041 042 046 049 053 041 040 043 046 044 042 042
043 045 042 041 040 037 039 049 040 041 044 041 041 043 041 042 046 045
050 040 041 041 040 041 042 044.

MAY/1986
061 058 051 050 058 055 052 050 051 064 046 062 049 048 052 054 059 051
050 059 051 057 052 055 056 056 053 044 049 046 050 052 049 055 041 055
054 059 059 050 053 051 053 046 050 057 051 052 052 057 055 056 053 053
048 053 049 050 055 052 050 055 047 056 057 057 059 050 051 057 055 058
056 051 046 041 058 056 053 056.

JUNE/1986
033 025 034 029 030 028 027 029 030 033 029 031 032 025 032 031 025 040
027 039 031 029 029 032 033 031 028 031 030 029 027 026 025 027 028 032
040 031 030 032 035 029 029 031 028 026 020 029 030 035 032 030 029 028
034 033 031 032 031 028 032 033 025 028 029 029 031 024 033 028 031 036
032 025 033 027 026 035 030 029.

APPENDIX 22a cont.

JULY/1986
010 016 011 015 019 005 009 032 020 009 014 011 015 018 012 020 003 010
021 005 006 024 020 011 017 018 011 013 015 002 008 021 020 004 014 011
010 009 011 012 011 018 016 013 011 010 006 009 012 013 012 006 004 009
012 006 002 003 006 012 011 017 015 017 014 009 003 016 017 013 016 015
009 003 002 001 018 017 005 003.

AUGUST/1986
001 003 015 009 014 011 001 001 015 004 008 007 015 009 014 005 003 006
010 015 004 009 005 007 003 005 004 014 012 009 002 015 004 005 012 013
009 008 006 015 007 004 006 015 012 011 004 013 015 012 004 014 008 004
011 012 015 012 009 013 012 004 012 003 002 007 007 008 006 001 003 004
005 002 005 007 001 008 005 012.

SEPTEMBER/1986
005 001 002 005 003 006 008 007 009 010 009 009 007 005 001 004 013 012
011 003 015 013 009 007 005 008 011 010 004 003 011 015 014 010 013 012
009 004 007 003 002 006 008 004 004 001 001 005 008 014 017 011 010 007
004 007 013 012 005 008 003 005 002 017 013 011 012 001 010 013 011 014
017 006 016 014 009 011 014 012.

OCTOBER/1986
003 007 007 008 006 008 007 006 005 005 009 003 002 009 004 004 003 002
005 009 006 006 007 002 006 007 009 008 008 007 001 003 007 003 002 001
005 005 001 006 003 001 008 009 009 004 002 010 011 007 003 003 004 005
005 001 009 003 003 002 009 007 011 014 009 014 012 022 020 008 004 004
002 903 007 007 003 002 009 010.

NOVEMBER/1986
012 005 006 010 002 009 010 011 005 009 006 003 009 005 009 009 011 013
013 011 009 101 006 005 004 009 010 005 005 007 007 008 007 003 002 009
005 008 009 008 011 013 011 010 009 009 007 005 010 009 005 008 009 010
011 006 009 011 014 010 006 006 008 012 005 007 011 007 007 008 008 010
009 010 007 005 009 009 010 009.

DECEMBER/1986
008 009 008 010 011 004 013 014 011 010 009 008 006 012 003 006 013 001
005 009 009 001 003 007 005 010 011 014 016 009 007 014 013 017 010 011
005 009 005 004 005 005 009 019 014 013 011 012 012 001 009 005 003 005
010 013 016 015 016 010 011 012 005 006 005 010 013 019 018 018 004 019
013 015 009 007 005 008 011 010.

APPENDIX 22b.

H.minuta/camel at Ngurunit.

JANUARY/1986
041 037 043 049 038 040 041 033 035 050 043 044 040 047 041 039 034 047
042 044 042 038 044 035 038 040 040 058 045 041 042 044 051 045 050 041
036 028 045 058 051 052 034 051 041 045 043 042 040 035 050 045 023 058
044 042 041 040 060 052 045 044 051 043 050 038 036 033 045 046 046 050
045 042 050 051 037 033 045 041.

FEBRUARY/1986
055 059 060 051 046 045 053 046 061 045 039 056 057 059 062 045 044 056
045 034 057 057 041 050 056 054 053 063 060 050 051 059 060 058 057 058
045 038 050 056 055 059 062 051 046 048 059 055 058 059 055 054 051 050
056 068 038 056 055 059 051 066 057 055 046 049 050 053 059 063 060 054
059 052 059 051 054 047 056 051.

MARCH/1986
188 176 170 180 180 186 179 170 160 200 180 188 184 180 173 164 177 179
184 150 160 157 198 201 185 193 190 192 182 184 194 190 195 170 173 176
183 180 193 194 197 190 159 168 163 170 171 188 183 181 180 194 197 195
190 174 184 176 170 175 164 167 160 169 180 188 185 184 180 185 199 204
201 165 170 185 181 190 195 187.

APRIL/1986
200 199 196 191 190 194 197 195 187 180 184 209 201 200 210 189 194 199
190 182 178 192 190 194 196 189 182 207 211 200 206 209 192 219 190 183
183 206 201 213 205 213 207 179 184 188 182 180 194 195 190 192 199 187
193 201 205 204 209 214 219 206 192 191 190 195 183 188 170 174 180 184
204 210 229 210 200 189 180 181.

MAY/1986
140 132 139 127 120 139 131 128 149 140 145 131 130 120 122 136 139 130
133 130 134 123 129 126 124 128 126 127 120 125 130 139 133 131 130 142
135 132 130 128 120 121 134 149 142 140 125 136 131 139 137 130 134 124
146 140 142 140 142 138 138 130 128 125 131 130 140 120 129 120 151 140
145 132 138 136 130 131 139 137.

JUNE/1986
067 059 070 069 066 063 060 071 072 075 079 071 070 068 060 057 055 056
056 079 077 072 070 068 078 077 070 079 074 071 070 081 082 064 066 067
070 077 073 071 070 073 070 073 072 068 064 065 056 060 085 075 077 072
060 068 062 065 062 056 051 068 090 083 081 080 073 079 080 072 063 054
069 077 078 071 070 072 075 076.

APPENDIX 22b cont.

JULY/1986
067 056 055 060 078 071 070 054 055 052 051 070 062 060 067 061 069 070
070 072 070 065 062 066 066 069 052 055 068 069 065 066 067 053 056 059
061 064 066 067 062 069 060 074 067 063 059 068 054 067 069 066 051 062
060 076 071 072 058 069 060 064 061 066 063 061 060 068 066 061 052 069
052 050 070 071 050 067 063 061.

AUGUST/1986
032 005 022 014 012 011 130 018 019 020 015 017 008 031 002 022 030 018
006 034 005 017 030 029 017 023 024 020 005 008 031 026 023 009 011 012
018 015 013 009 004 006 011 030 027 028 020 025 023 017 015 015 018 012
034 039 030 006 014 017 013 015 015 013 018 016 011 013 011 010 013 032
013 016 018 013 028 021 020 023.

SEPTEMBER/1986
009 002 003 003 010 014 015 005 009 002 004 009 007 005 002 009 009 008
007 004 004 014 016 010 004 007 005 008 002 009 004 011 019 003 009 002
005 008 002 007 005 008 007 003 004 009 009 001 003 014 016 010 005 005
008 003 009 002 002 009 004 004 001 004 009 002 002 005 005 001 004 010
006 008 002 005 007 005 003 008.

OCTOBER/1986
007 015 009 007 005 015 008 012 010 014 012 015 012 013 008 019 008 006
012 011 010 016 013 007 006 005 011 012 010 007 008 006 013 010 011 008
004 002 015 012 010 011 008 009 008 006 002 009 010 011 010 011 010 009
014 019 009 008 017 013 011 012 005 005 014 011 012 010 015 012 009 003
012 014 019 012 009 008 009 006.

NOVEMBER/1986
038 012 044 050 036 041 040 040 017 040 019 020 015 019 012 012 015 014
043 040 041 034 036 040 037 033 035 018 014 012 019 020 016 052 009 028
036 043 015 019 014 016 012 015 024 028 025 030 042 037 052 042 047 044
032 014 044 008 012 009 023 021 029 020 012 038 016 035 039 046 045 032
030 009 017 033 015 030 033 034.

DECEMBER/1986
045 041 038 050 052 033 048 041 040 038 031 038 038 033 030 047 041 042
040 053 054 050 045 048 049 048 044 045 045 040 041 037 033 046 040 041
058 052 050 041 030 060 045 046 040 041 037 035 035 039 037 035 033 030
031 047 059 050 055 056 050 051 046 047 038 039 039 030 031 040 043 040
042 040 044 041 036 039 030 041.

APPENDIX 22c.

H.minuta/ camel at Ilaut

JANUARY/1986
003 001 003 002 002 005 007 004 003 001 005 002 002 004 004 006 004 005
005 003 003 004 002 001 001 005 004 007 003 003 004 003 003 001 008 001
004 002 005 005 005 004 005 006 003 007 001 002 005 003 007 006 002 001
002 004 005 006 004 003 002 001 001 007 005 005 003 002 003 001 008 001
006 005 001 001 004 003 003 002.

FEBRUARY/1986
006 022 007 008 016 002 021 013 005 023 009 023 012 022 023 012 008 004
020 005 003 016 021 007 009 017 010 009 022 007 012 002 013 006 010 018
008 006 009 005 008 005 004 010 012 022 006 012 013 017 018 007 024 011
008 020 013 005 019 011 007 020 020 007 019 012 023 015 009 007 012 018
016 024 012 006 017 011 016 015.

MARCH/1986
010 016 006 017 019 019 006 022 008 022 009 014 023 007 012 013 019 008
007 025 020 011 021 013 021 009 015 016 010 021 015 006 010 005 008 023
015 009 013 010 023 024 025 024 024 010 013 007 008 015 023 022 011 009
012 023 025 020 010 016 028 019 015 012 021 009 020 025 008 018 019 023
022 008 027 024 027 005 013 010.

APRIL/1986
033 026 031 022 011 039 018 010 030 019 024 021 035 013 010 029 039 016
033 039 034 021 011 016 027 017 019 026 015 031 038 022 023 021 019 020
016 015 023 022 015 017 020 019 025 021 022 023 029 038 031 015 027 019
018 027 016 011 021 034 036 012 028 032 037 035 020 023 019 030 013 018
037 010 022 030 025 032 023 027.

MAY/1986
034 031 039 034 036 033 030 028 027 209 045 034 031 030 036 030 031 027
024 026 029 028 040 030 031 024 026 023 020 021 028 025 028 039 031 024
031 020 023 033 030 037 301 023 022 021 020 026 037 031 024 029 025 026
031 033 032 037 035 021 028 036 036 033 023 025 028 028 021 029 041 023
025 922 029 031 027 035 026 039.

JUNE/1986
036 035 037 032 030 039 031 028 028 028 032 037 033 034 039 030 039 038
033 030 041 040 032 033 034 038 033 030 040 035 032 022 029 037 046 021
026 045 020 030 038 038 039 027 082 039 020 026 032 031 026 036 043 035
034 036 039 043 040 042 040 041 035 038 030 035 030 028 021 027 049 036
037 0380 030 034 031 039 035 032.

APPENDIX 22c cont.

JULY/1986
042 014 032 042 047 053 040 029 012 049 012 048 043 011 045 054 038 047
044 019 050 014 019 052 026 029 031 044 043 020 014 054 027 026 043 049
050 017 016 011 025 025 012 018 017 046 043 039 027 026 050 014 016 041
040 045 031 029 026 049 017 012 048 019 044 046 037 049 048 054 022 032
023 028 039 040 045 041 032 027.

AUGUST/1986
023 015 016 014 019 014 023 022 020 026 019 014 020 021 023 014 017 016
010 011 021 024 017 014 011 013 012 018 017 019 021 021 017 011 016 013
015 009 033 024 012 015 013 017 018 019 018 017 109 015 016 016 014 012
015 011 018 014 014 018 012 017 015 021 010 125 019 013 018 010 024 022
016 015 011 019 019 021 019 012.

SEPTEMBER/1986
012 003 017 028 025 026 003 030 028 027 021 006 003 027 030 011 010 005
005 008 010 025 013 037 025 005 008 011 029 032 031 003 020 013 006 014
011 010 026 030 029 010 013 014 006 013 020 007 030 031 011 008 029 005
037 010 008 005 013 005 010 009 030 027 003 006 021 027 029 028 007 026
027 025 002 011 025 023 021 015

OCTOBER/1986
023 006 006 020 019 026 021 033 026 031 022 007 003 038 018 003 030 018
019 024 021 023 021 035 015 007 029 038 007 029 008 039 033 020 019 026
040 035 022 008 008 011 028 005 027 032 039 023 024 009 011 006 028 008
033 025 030 025 009 038 019 018 030 026 021 026 023 035 025 008 029 038
039 028 038 007 006 027 033 028.

NOVEMBER/1986
023 023 034 034 031 030 029 026 040 026 028 038 033 031 040 046 035 026
031 037 030 028 025 021 020 036 031 026 026 042 040 027 035 038 036 038
035 033 031 030 024 028 029 045 023 038 036 035 049 044 035 039 033 030
031 038 023 022 020 027 062 031 039 034 037 039 030 027 022 026 041 019
037 034 030 031 025 027 030 032.

DECEMBER/1986
028 022 024 029 040 025 034 034 031 030 027 022 021 029 046 030 032 033
026 023 029 043 040 041 039 036 035 028 026 029 026 027 022 036 033 031
037 035 031 030 028 018 028 024 048 040 041 034 037 039 030 033 031 036
026 028 020 024 023 047 046 040 049 045 034 033 030 031 035 031 028 017
023 026 029 030 031 032 037 035.

APPENDIX 22d

H.minuta/camel at Korr

JANUARY/1986

051 056 042 041 048 051 058 055 045 053 056 059 050 050 044 042 046 037
058 059 060 048 066 059 064 063 059 060 067 047 053 049 052 052 055 048
049 049 048 053 050 051 053 048 049 040 041 056 052 954 051 048 061 059
058 042 055 053 051 057 053 051 057 052 042 041 040 046 055 049 047 049
046 048 048 039 041 044 047.

FEBRURY/1986

064 061 050 062 058 070 070 079 062 075 073 062 066 060 063 061 057 082
056 067 069 053 075 077 073 070 068 066 063 081 062 066 061 065 069 062
066 056 055 059 051 094 085 088 082 060 062 064 067 052 051 050 067 065
059 053 054 068 060 061 081 085 095 090 091 085 082 067 058 053 049 096
067 055 078 063 061 060 048 066.

MARCH/1986

099 087 100 104 013 110 094 082 078 099 093 091 090 083 087 080 085 090
107 102 116 089 094 098 090 093 091 075 072 089 088 084 081 085 083 096
090 093 091 097 099 094 086 083 070 085 081 093 100 115 110 109 105 102
086 088 083 080 078 077 073 096 092 088 084 081 080 096 093 091 099 093
104 11 102 098 093 090 085 077.

APRIL/1986

145 132 139 154 139 143 149 144 140 150 152 158 156 153 137 133 145 149
160 136 133 130 120 167 145 149 145 143 140 150 151 159 157 151 159 166
144 147 146 140 140 141 135 139 157 154 137 133 130 138 130 142 140 149
167 155 145 149 147 141 140 132 130 138 136 131 139 142 129 167 156 153
148 140 142 145 148 149 140 136.

MAY/1986

154 150 153 155 147 143 133 140 149 156 158 150 140 168 163 161 160 145
152 158 155 150 152 157 153 150 169 157 166 160 174 152 150 152 150 157
159 160 163 142 140 150 164 157 152 150 152 157 150 147 144 141 140 143
152 150 151 510 144 138 130 169 167 160 174 166 165 160 146 148 164 150
163 158 148 145 140 156 150 142.

JUNE/1986

024 019 034 037 023 021 011 020 027 026 029 031 019 011 018 030 020 021
028 024 027 022 015 018 030 025 029 029 028 021 022 027 204 029 014 011
012 017 023 021 016 019 015 025 029 022 020 023 026 028 025 028 028 031
025 030 025 017 015 010 011 017 015 015 012 109 013 023 025 026 025 029
027 022 026 018 014 011 016 012.

APPENDIX 22d cont.

JULY/1986
017 009 010 015 024 017 021 008 021 011 027 024 006 011 012 015 109 016
015 013 011 010 014 019 017 011 015 012 012 016 013 011 006 019 028 022
026 013 019 011 012 017 007 015 023 022 012 020 026 024 018 017 011 010
020 021 025 013 915 017 015 011 019 018 019 019 014 013 015 011 019 012
011 019 015 023 017 027 014 018 019.

AUGUST/1986
014 011 008 007 024 009 007 005 025 021 012 025 014 017 018 016 011 020
013 019 018 017 014 010 014 017 019 011 011 013 012 019 007 009 011 010
011 018 019 020 023 006 013 017 016 014 005 009 025 015 018 017 018 015
016 019 018 011 016 022 017 015 011 014 015 011 013 019 012 018 019 018
014 019 008 007 016 015 019 011 014.

SEPTEMBER/1986
019 020 025 017 026 023 015 017 012 019 020 018 022 027 021 020 021 029
017 011 023 027 025 029 016 024 029 020 024 026 022 016 028 020 021 025
016 016 019 028 020 012 024 028 028 027 020 023 021 020 011 025 029 028
025 016 025 028 028 023 019 023 015 013 011 010 025 018 017 019 015 018
018 019 015 015 020 023 027 025 023.

OCTOBER/1986
030 034 031 023 021 020 032 029 028 028 026 024 021 023 022 029 022 022
022 026 023 024 023 021 028 028 027 024 028 026 029 030 033 025 028 034
031 022 021 011 018 016 023 024 018 017 016 019 018 018 021 011 023 029
023 016 013 029 028 014 011 012 013 029 012 021 022 026 025 021 020 025
029 012 023 023 013 016 011 012 026.

NOVEMBER/1986
031 026 029 036 030 020 045 035 031 024 027 031 020 032 037 033 035 030
023 029 033 037 021 022 020 024 036 033 038 035 030 036 033 031 030 020
029 027 022 026 028 030 354 035 016 041 023 039 030 033 036 039 034 032
030 025 029 029 026 028 020 025 038 039 039 039 033 035 032 021 034 031
026 028 022 034 037 039 033 034 031.

DECEMBER/1986
045 041 037 033 028 036 039 034 033 031 030 034 038 035 039 030 038 039
043 031 023 038 028 029 022 041 040 038 030 034 032 037 033 039 035 038
035 036 030 037 033 031 029 025 037 036 033 030 031 035 039 037 037 025
033 031 036 039 042 034 036 039 030 031 024 038 027 022 020 040 020 038
030 035 039 040 026 034 031 030 032.

APPENDIX 22e.

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JANUARY/1986
028 034 038 039 045 040 036 030 031 033 039 035 031 032 035 030 025 029
034 033 038 037 030 020 046 039 035 038 030 031 033 038 035 035 037 030
031 035 039 039 033 036 030 027 034 046 044 041 040 034 027 037 030 029
035 030 025 031 036 039 035 033 038 035 031 023 038 032 047 049 040 041
035 039 036 030 036 035 013 033.

FEBRUARY/1986
023 028 028 026 015 018 011 012 010 024 026 021 020 020 024 023 028 029
020 013 018 018 014 017 019 016 016 015 020 020 024 025 020 022 020 021
027 025 026 020 029 024 020 021 028 017 018 017 019 011 015 014 026 012
020 023 020 017 109 014 011 015 014 012 020 023 021 010 011 014 019 016
019 016 016 025 019 015 011 010.

MARCH/1986
076 068 069 060 080 075 077 074 071 075 071 072 075 069 079 079 071 074
078 077 071 074 075 075 070 071 072 079 078 079 077 078 072 027 070 068
068 074 073 075 074 070 071 071 070 065 066 067 067 060 057 078 071 071
073 075 077 079 079 079 073 071 070 068 062 066 064 070 074 077 078 070
080 083 076 072 064 078 071 070.

APRIL/1986
067 067 065 072 073 076 075 074 072 073 075 076 072 073 065 067 063 078
070 071 076 074 072 076 071 070 073 084 066 063 062 062 065 077 066 067
086 087 080 083 063 071 069 074 075 060 063 071 076 075 072 072 073 068
071 076 077 075 069 080 087 073 072 072 074 068 065 061 065 067 066 068
070 064 086 080 076 071 066 071.

MAY/1986
076 071 075 081 085 091 064 070 071 074 075 079 072 086 087 082 078 078
076 071 070 079 072 077 073 077 079 078 072 073 070 064 061 072 069 071
066 075 070 071 064 067 057 062 060 060 067 067 070 071 066 065 063 068
069 074 067 061 064 062 068 078 075 082 081 062 065 062 067 065 061 063
079 065 075 072 071 070 073 076.

JUNE/1986
083 078 083 088 092 098 064 070 071 084 085 086 080 092 090 089 085 084
084 086 080 077 073 077 079 078 079 070 077 074 075 087 088 083 081 080
085 086 081 083 075 095 092 099 091 083 087 086 084 088 083 082 085 096
078 071 074 077 079 056 079 098 090 075 079 067 081 084 084 088 087 085
080 086 089 088 081 080 079 077.

APPENDIX 22e cont.

JULY/1986
078 070 088 072 066 074 075 071 074 071 074 081 082 080 064 069 067 070
074 075 078 077 078 079 076 075 075 074 073 075 076 066 073 072 075 066
074 075 073 076 073 067 076 075 073 071 068 072 075 089 090 079 075 094
089 081 080 077 076 071 074 075 073 069 078 090 089 085 090 097 092 090
093 098 099 078 080 090 095 098.

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021 018 019 018 011 020 029 026 209 022 028 026 029 015 019 016 022 020
021 024 028 030 012 024 023 028 016 018 014 013 019 018 018 014 017 015
023 022 029 026 025 017 019 020 012 925 028 025 023 024 027 024 027 025
029 021 020 023 021 020 022 015 018 019 019 013 011 014 022 027 021 022
025 012 018 098 019 015 013 020.

SEPTEMBER/1986
022 018 017 016 009 013 025 017 019 017 016 023 020 020 021 023 024 015
024 017 016 023 022 027 026 029 006 024 023 021 022 026 024 020 024 025
029 022 025 028 018 019 016 016 016 024 020 023 021 019 015 013 011 010
025 012 008 004 015 013 019 017 018 016 011 010 015 020 023 027 027 019
021 011 010 015 016 007 019 023.

OCTOBER/1986
029 021 028 027 031 030 026 020 030 021 028 028 029 028 023 027 021 023
027 023 021 022 021 022 026 024 028 033 031 030 021 021 022 025 027 026
024 026 021 020 033 021 025 022 024 023 029 023 022 020 020 029 022 030
025 024 026 023 027 022 021 025 020 034 028 022 024 021 027 023 028 029
021 027 029 018 025 019 022 021.

NOVEMBER/1986
028 029 034 035 030 020 028 026 028 033 031 030 038 039 024 034 025 029
921 029 025 025 029 027 034 020 030 030 035 039 031 031 030 035 036 039
022 029 042 040 035 022 032 025 040 041 032 031 033 036 039 037 039 038
030 032 041 025 026 023 022 020 029 026 024 028 028 024 022 021 020 040
035 028 023 022 027 029 028 026.

DECEMBER/1986
029 023 024 021 018 019 020 023 024 028 027 025 024 026 032 023 026 022
021 021 030 031 033 028 024 022 021 022 025 022 021 027 023 028 027 027
023 029 036 028 029 028 021 029 027 028 021 030 028 032 020 022 023 020
018 023 023 028 041 045 022 028 033 031 021 016 020 021 025 024 020 010
023 026 022 030 028 021 016 017 023.

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