MECHANICAL TRANSMISSION OF *TRYPANOSOMA EVANSI* AND *TRYPANOSOMA CONGOLENSE* BY AFRICAN *STOMOXYS* SPECIES.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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ABSTRACT

Simulated mechanical transmission rates of *T. congolense* and *T. evansi* were studied using wild and blood-fed *S. n. niger*, *S. taeniatus,* and laboratory reared teneral *S. n. niger*. The flies were caught in the Nairobi National Park forest and in the thickets and woodlands at Nguruman, Kenya. Direct microscopy and mouse inoculation techniques were able to detect live *Trypanosoma congolense* only up to 210 minutes, and *T. evansi* up to 480 minutes after an infective bloodmeal. Individual flies were interrupted after 1-2 minutes of feeding on infective bloodmeal and immediately transferred to non-infected mice to complete their meal. Wild and teneral *S. n. niger* transmitted *T. congolense* at transmission rates of 7.5% and 10.0% respectively and *T. evansi* at transmission rates of 20.0% and 35.0% respectively. Blood fed and wild *S. taeniatus* were not able to transmit *T. congolense* but they transmitted *T. evansi* at transmission rates of 17.5% and 13.3% respectively. There was no significant difference in the transmission of either *T. evansi* or *T. congolense* by teneral, wild and blood-fed *S. n. niger*.

Laboratory rearing of wild stable flies was attempted using the following media: rhinoceros dung; mice pellets, saw dust and water; wheat bran, saw dust and water. The latter was the best medium for rearing *S. n. niger*. *S. inornatus, S. taeniatus, S. n. bilineatus* did not develop well in all the three media. The life cycle for *S. n. niger* was approximately 23.5 days.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction.

African trypanosomes are parasitic hemoprotozoans that cause diseases in both man and animals. Trypanosomes belong to the order Kinetoplastida, family Trypanosomatidae and the genus *Trypanosoma*. The family Trypanosomatidae is divided into two sections based on the development of the trypanosomes in the insect vector. The section Stercoraria includes species that develop in the hind-gut of the insect. Among these are *T. lewisi*, *T. theileri* and *T. cruzi*. Metacyclic forms are found in the faeces of the insect and hence their transmission is contaminative. With the exception of *T. cruzi*, stercorarian trypanosomes are typically not pathogenic. In the section Salivaria the trypanosomes complete their development in the mouthparts or salivary glands of the vector. Transmission of these parasites is therefore inoculative. The salivarian section is divided into subgeneric groups namely, *Duttonella*, *Trypanozoon*, *Pycnomonas* and *Nannomonas* (Hoare, 1972). All pathogenic African trypanosomes belong to these subgenera and are represented by *T.(D). vivax*, *T.(T). brucei*, *T.(T). evansi*, *T.(T). equiperdum*, *T.(P). suis*, *T.(N). congolense*, *T.(N). godfreyi*, *T.(D). uniforme* and *T.(N). simiae*.

Most of these parasites are not host specific. *T. vivax* parasitises ruminants and equines. *T. congolense* has the same hosts as well as carnivores and suids. *T. simiae* is primarily a parasite of pigs and wild suids but may also infect other animals such as camels. *T. evansi* is pathogenic in camels and equines. *T. equiperdum* is found only in equines. Diseases caused by the classic African trypanosomes (*T. vivax*, *T. congolense*, *T. simiae*, *T. brucei* and others) are cyclically transmitted by the tsetse fly. This group of diseases is collectively referred to as `nagana'. It occurs between 14° N and 30° S, south of the Sahara, in

an area referred to as the 'Tsetse Belt'. Beyond this area, animal trypanosomiasis occurs in the form of 'Surra' caused by *T. evansi*, and it is transmitted mechanically by flies. 'Dourine' is a contagious disease of equines caused by *T. equiperdum*. The area affected by non tsetse-transmitted trypanosomes is about three times greater than that of the tsetse-borne trypanosomes (Woo, 1977). This shows that non-cyclical transmission has an important role in the occurrence and spread of trypanosomiasis.

In Africa, a variety of wild animals are infected with trypanosomes. Some species harbour trypanosomes without showing the disease symptoms, whereas others are affected to a varying degree and may succumb to the disease (Ashcroft, 1959). Many animals tolerate infection and hence serve as reservoirs.

Transmission of trypanosomes by insects may occur through cyclical or mechanical means. In cyclical transmission the parasites go through specific life-cycle stages in the vector before they develop the ability to infect the next host. Mechanical transmission occurs when the parasite is transferred directly from one host to another in its original form without having to develop in the insect.

Trypanosomiasis is considered to be economically the most important disease affecting livestock in Africa (Jawara, 1990). The prevalence of the disease in vast areas of potentially productive lands of tropical Africa impedes economic development by rendering the rearing of livestock in these areas difficult. The disease causes reproductive disorders, morbidity and more importantly, death to domestic animals (Ikede *et al.*, 1988). The actual impact of trypanosomes on livestock production is hard to estimate with precision. However, losses occur in meat production, traction power, milk, hide, skin and dung production. Tsetse flies, the principal vectors of trypanosomiases, infest

approximately 10-11 million km^2 of the African continent. One half of this area is potentially productive and would be suitable for livestock production or mixed farming if tsetse flies could be eliminated (Finelle, 1974).

The occurrence of trypanosomiasis in tsetse-free areas, has prompted work on other biting flies. Wells (1972), summarised the few experimental studies on mechanical transmission of trypanosomes. In most historical experiments, *Stomoxys calcitrans*, a common pest of cattle throughout the world, was used. In some cases (Jowett, 1911), either the fly or the parasite was inadequately identified, leading to muddled conclusions. Apart from *S. calcitrans*, Zumpt (1973) described seventeen other *Stomoxys* species occurring in Africa.

Most of these species have little or nothing known of their biology. The present study concentrated on two wild species *(S. niger niger*, and *S. taeniatus*) which are common in Nairobi National Park, Kenya (Mihok *et al.*, 1995b). The study of these flies will enable us to assess the contribution of non-cyclical transmission to trypanosome epizootiology.

1.2 Literature review.

1.2.1 *Trypanosoma (Nannomonas) congolense* (Broden, 1904).

Trypanosoma congolense was first observed by Broden (1904) in the blood of a sheep in the Congo. It is a small salivarian trypanosome which varies in length between 8 and 24 μm, with a mean length of 12.2-17.6 μm. The mammalian host may have an effect on the size of the trypanosome. The typical *T. congolense* is usually monomorphic; the pleomorphic posterior end is blunt; the undulating membrane is not prominent; a free flagellum is absent and the cell tapers towards the end. The cell has a medium-sized kinetoplast which occupies a marginal position (Adam *et al.*, 1971; Hoare, 1972).

1:2:2 *Trypanosoma (Trypanozoon) evansi.*

This was the first parasite found to be pathogenic to animals. It is probably a complex of species including several variants such as *T. equinum* and *T. venezuelensis*. It has generally been described as monomorphic. Stumpy and intermediate forms have also been reported (Hoare, 1972). The length varies from 15-33 μm with a mean length of 24 μm. The width ranges from 1.5-2.0 μm. *T. evansi* has a free flagellum. The kinetoplast is small and is usually rod-shaped and typically sub-terminal or marginal in the cell (Adam *et al.*, 1971).

1.3 Epizootiology of African trypanosomes.

Epizootiology of African trypanosomes is based on the analyses of many different factors. These factors influence the relationship between the elements that determine the occurrence and spread of the diseases in a given area. These elements are the definitive host(s), the parasites, and the vectors. Understanding the role of each element with respect to the

others requires us to know the distribution of potentially and actually infected animals and the vectors, their behavior, the dynamics of trypanosome transmission and the role of the parasite's reservoirs (C.A.B.I., 1989).

T. congolense occurs throughout tropical African regions wherever tsetse flies are found (Hoare, 1972). The high temperatures on the edges of the Sahara and Kalahari deserts and the low temperatures in the Natal and tropical highlands set the limits of fly distribution. Man and animals in Africa south of the Sahara (14°N and 30°S) are therefore exposed to infection with African trypanosomes.

Of all the trypanosomes that cause disease in domestic animals, *T. evansi* has the widest distribution. The disease is known by various names in different countries: 'Surra' in India, is the name used most; 'El debab' in Northern Africa; 'Dioujar' in Chad; 'Tahaga' in Mali; 'Doukane' in Ethiopia (Woo, 1977).

The presence of pathogenic trypanosomes in a specific animal does not necessarily lead to clinical symptoms. Pathogenicity depends on many factors, among which are the type of infected individual and its nutritional status, the strain of the parasite, the degree of resistance or immunity acquired due to prior exposure to infection, and the conditions under which the animal has been raised. In most ungulates, as in dogs, the disease due to *T. congolense* may be acute, chronic or mild, whereas in pigs, it is usually mild. The disease caused by *T. evansi* is characterised by fever and anaemia followed by oedema and cachexia. It is particularly virulent in camelines and equines. Cattle are not very susceptible (C.A.B.I., 1989).

Important reservoirs of trypanosomes include both domestic and wild animals. Most wild animals are tolerant to infection with trypanosomes (Geigy & Kauffman, 1973). This tolerance has evolved

through long association of the trypanosomes with animals, which were their original and natural hosts. Reservoir animals constitute a source of parasites that can infect man and livestock (Ashcroft, 1959).

1.4 Diagnosis of African trypanosomiasis.

Clinical diagnosis of trypanosomiases is hindered by the fact that there are no pathognomonic signs of infection. In their chronic forms, trypanosomiases resemble other parasitic or infectious diseases that cause physiological stress or anaemia. Since trypanosomiasis is difficult to diagnose clinically, laboratory confirmation of a trypanosome is necessary. Methods for diagnosis of the disease include microscopic observation of the parasites, serological tests that detect trypanosome antigens and anti-trypanosome antibodies and molecular techniques that detect trypanosomal deoxyribonucleic acid (DNA) (Chaesbrough, 1987).

Trypanosomes can be detected by observation of wet films or smears made from peripheral blood, lymph or cerebro-spinal fluid of infected animals. Centrifugation of blood using microhaematocrit centrifuge tubes concentrates trypanosomes at the interface among the packed red cells, the buffy coat and plasma (Mac Innis & Voge, 1970). The movement of trypanosomes can then be detected microscopically. Blood from suspect animals can be inoculated into susceptible experimental animals such as mice and rats. From a few days to about a month, blood from the animals is observed for the presence of trypanosomes.

The Indirect Immunofluorescent Antibody Test (IFAT), Enzyme Linked Immunosorbent Assay (ELISA) and Indirect haemagglutination are some of the serological techniques used in detecting anti-trypanosomal antibodies. Since these techniques rely on antibody detection, they often fail to distinguish between animals with clinical infection and those that

have been treated recently or undergone self-cure. This problem has been solved partly by the development of Antigen-detection Enzyme-linked Immunosorbent Assays (ELISAs) which detect trypanosomal antigens in animal sera (Nantulya, 1991; Nantulya *et al.*, 1992). Antigen-ELISAs are capable of detecting chronic infections in which the parasitaemia is too low for visual detection (Nantulya and Lindqvist, 1989).

Trypanosome DNA probes, which detect repetitive DNA sequence specific for species, have also been developed. (Majiwa, 1989; Nantulya, 1991). This technique is specific and sensitive. Therefore closely-related trypanosomes are distinguished, especially in mixed infections (Majiwa *et al*,. 1993).

1.5 Control of trypanosomiasis.

Programmes to control trypanosomiasis have been in operation for a long time. Initial attempts involved the control of the tsetse vector by bush clearing and game destruction. These methods were aimed at destroying the tsetse fly habitat, limiting the sources of bloodmeal available and eliminating trypanosome reservoirs. These methods proved to be very destructive to the environment and hence became unacceptable. By the 1970s the use of insecticides had largely replaced bush clearing and game destruction, but insecticides also had undesirable environmental effects. Currently, the control of trypanosomiasis in livestock relies upon newer, safer and environmentally-acceptable techniques. These techniques include the treatment of the infected host and vector control with baited traps and screens. Raising of either trypanotolerant livestock or trypanotolerant game animals in tsetse infested areas can be an alternative to trypanosomiasis management (ILCA, 1979). Biological and genetic control methods have been advocated, but as a large scale practical solution this seems unlikely to be

of use. However, these control tools are still under review (Jordan, 1986).

Attempts to develop effective vaccines are still in progress. However, antigenic variation in trypanosomes has frustrated the effort (Cheesbrough, 1987).

1.6 The vectors and transmission of African trypanosomes.

With the exception of *T. equiperdum*, all mammalian trypanosomes have two hosts; an intermediate insect host or vector and a definitive host. Tsetse flies are considered to be the principal vectors of trypanosomes, however, the species differ in their abilities to transmit the parasites. Tabanids, *Stomoxys*, hippoboscids and other biting flies are also thought to transmit trypanosomes mechanically in the absence of tsetse flies (Wells, 1972).

Cyclical transmission occurs when parasites undergo a cycle of development in a vector. Dissections of wild tsetse flies carried out by different workers have revealed different developmental stages of trypanosomes in many *Glossina* species (Buxton, 1955). *T. congolense* has been isolated specifically from *G. palpalis, G. swynnertoni, G. pallidipes, G. morsitans, G. austeni, G. vanhoofi* and others. Tsetse flies become infected with bloodstream trypomastigotes when they take a bloodmeal from an infected host. The blood passes through the pharynx, the esophagous and the proventriculus before entering the crop. The proventriculus extrudes a chitinous tube, the peritrophic membrane. This membrane extends from the anterior part of the proventriculus to the hind-gut. The muscular proventriculus controls the release of blood from the crop to the mid-gut. The cycle of trypanosome development starts in the endoperitrophic space of the mid gut and takes about two to three weeks. The transformed parasites multiply as elongated promastigotes for

some days, and then migrate within the endoperitrophic space from the mid-gut to the labrum where they transform into epimastigotes. When they reach the hypopharynx they transform further into metatrypanosomes, which are infective to the mammalian host. In the mammalian host the parasites multiply in the lymphatic tissues as metatrypanosomes. These are later ingested by other flies to complete the cycle.

Attempts to demonstrate cyclical transmission of *T. evansi* in insects have failed (Hoare 1940, 1972). This is because the trypanosomes are killed within six hours and digested together with the bloodmeal in the tsetse flies.

These are the events that lead to natural or experimental mechanical transmission of parasites: the vector initiates feeding upon an infected host; the vector's feeding is interrupted; it moves to another susceptible host, transporting the parasites on or within its body parts. Lastly, the vector feeds upon the susceptible host and introduces the parasites. Factors that may therefore ensure the success of transmission include: the time interval between the infective and the infecting bloodmeal; the proximity of the infected and susceptible animals; the abundance and feeding behavior of the potential vectors; the environmental stability of the parasites; the titre of the disease agent; the amount of blood transferred between the hosts, and the nature of the mouthparts.

Dixon *et al.* (1971a, b) found that the longest survival time for *T. brucei* in *S. calcitrans* was thirty minutes. He detected no trypanosomes in the mouthparts after one hour. He therefore concluded that although *S. calcitrans* was not an efficient vector for *T. brucei*, it carried the parasite in its infective form for a while and hence could be considered as a

potential vector. Boreham and Geigy (1976) also showed that *T. brucei* could remain viable in *Auchmeromyia* larvae for about twenty one hours.

The distance between the infected and the susceptible host is important; the closer they are, the higher the probability of the parasites being passed from one to the other, irrespective of the parasite involved (Foil, 1989). Several species of blood-sucking insects belonging to the genera, *Tabanus, Stomoxys, Atylotus* and *Lyperosia* are considered to be important mechanical vectors. This is because they are interrupted frequently during feeding, are highly mobile and abundant, and they have mouthparts that are adapted for transmission (Lun *et al.*, 1993; Foil, 1989).

As stated before, work on artificial/laboratory experimental mechanical transmission of trypanosomes using assorted biting flies has been recorded by various workers. According to Wells (1972) transmission of *T. congolense* by *Stomoxys* species was achieved only by Bouet and Raubaud (1912). Few others like Baird (1930), Mihok *et al.* (1995a) also tried unsuccessfully to transmit *T. congolense*. Artificial transmission of *T. evansi* by *Stomoxys* has been achieved (Mihok *et al.*, 1995a; Ogonji, 1983).

Field transmission observations have also been reported in tabanids (Dirie *et al.*, 1989; Foil, 1989; Foil *et al.*, 1987; Luckins, 1988; Otte and Abuabara, 1991; Wells, 1972; Wiesenhutter, 1976), *Stomoxys* (D'Amico *et al.*, 1996; Dixon *et al.*, 1971a, b; Wells, 1972) and a few other biting flies as reported by Wells (1972).

1.7 STOMOXYS (GEOFFROY, 1762).

Stable flies are placed in the family Muscidae. *S. calcitrans* and the seventeen others listed below comprise the genus *Stomoxys* (Zumpt, 1973).

Stomoxys calcitrans Geoffroy

Stomoxys ochrosoma Speiser

Stomoxys omega Newstead

Stomoxys transvittatus Villeneuve

Stomoxys bouati Roubaud

Stomoxys taeniatus Bigot

Stomoxys sitiens Rondani

Stomoxys niger niger Macquart

Stomoxys varipes Bezzi

Stomoxys uruma Shinonaga & Kano

Stomoxys bengalensis Picard

Stomoxys niger bilineatus Grünberg

Stomoxys indica Picard

Stomoxys pulla Austen

Stomoxys pallidus Roubaud

Stomoxys stigma Van Emden

Stomoxys xanthomelas Roubaud

Stomoxys luteola Villeneuve

Most of these muscoid flies are found in Africa. They are thought to have originated from the old world but one species, *S. calcitrans* is cosmopolitan (Foil & Hogsette, 1994). *S. niger niger* is found throughout Africa and is characterised by dark legs and linear markings on the abdomen. In nature, *S. niger niger* develops in decaying vegetation e.g. sugar-cane residues (Foil and Hosgette, 1994). The fly has been a

considerable pest of cattle in certain parts of Africa. *S. n. bilineatus* is another subspecies of *S. niger*. *S. taeniatus*, a large all-yellow stable fly has a localised distribution. It also occurs as the form *S. brunnipes*, only differing in colour of the femur (Zumpt, 1973). *S. taeniatus* also feeds on cattle. Mihok *et al.* (1995b) reported large numbers of this fly in Nairobi National Park. *Stomoxys species* show differences in their activity patterns. For instance, *S. n. niger*, *S. taeniatus* and *S. brunnipes* are active in the morning and evening. *S. calcitrans* is active in the early afternoon (Harley, 1965; Kangwangye, 1973; Kunz and Monty, 1976).

1.7.1 General morphology of *Stomoxys* species.

The adult fly is about 6-8 mm long and resembles a common house fly. The mouth parts are modified into a piercing organ. The labium, hypopharynx and labrum are sclerotised and form the proboscis which is prominent and directed horizontally forward. The slender palps may be the same length as the proboscis, or slightly shorter. The aristae of the antennae have setae on the dorsal side only. The medial vein M_{1+2} is curved forward and the apical cell is open.

1.7.2 Life history and habits of *Stomoxys* species.

Stomoxys prefer out-door light and are not seen in dark stables or houses. Both males and females are blood feeders. In the absence of blood they feed on sugar solution for glycogen synthesis (Venkatesh & Morrison, 1982).

S. calcitrans breeds near stables and in straw heaps. It has also been found breeding in the manure of cows, sheep and other animals when mixed with a good deal of straw. The material must be moist, since dryness prevents larval development (Parr, 1962). Wild species have been found breeding in a variety of wildlife dungs (Zumpt, 1973) but their main

breeding sites are unknown. Coastal areas, marine and freshwater grasses and algae washed ashore also form a natural media for large scale development of stable fly larvae (Kunz & Monty, 1976).

At present, the information on the stable fly life cycles is based almost entirely on *S. calcitrans* and *S. n. niger* (Zumpt, 1973). Before the female lays eggs, she needs several blood meals. The eggs are about 1 mm long, curved on one side and straight and grooved on the opposite side. They are laid in batches of about 25-50. The incubation period varies from 2 to 5 days at a temperature of 26°C. Higher temperatures result in shorter incubation periods. The eggs hatch into larvae, which reach full growth in about 14-26 days. Before pupation the larvae move to the drier parts of the material. The pupal stage lasts about 5 days. The imago emerges, crawls away, unfolds its wings and is ready to fly in less than half an hour. The size of the adult fly is dependent on developmental conditions such as nutrition, temperature, and moisture. Mating takes place within a week and egg deposition begins at about 18 days after emergence. Mating is not required for oogenesis, but it is necessary for oviposition (Venkatesh and Morrison, 1982).

1.7.3 The economic importance of *Stomoxys*.

The flies are a constant source of irritation as a result of their painful bites. Bites from *Stomoxys* can cause hypersensitivity and formation of intradermal blisters leading to bleeding sores (Moorehouse, 1972). Favoured feeding sites are the back of the knee and the elbow. Landing rates of 80-100 flies per minute are not uncommon during outbreaks. Swarms of stable flies have reportedly caused the evacuation of beaches and tourist resorts in Northwest Florida. This has led to a decline in the tourist industry. Fosbrooke (1963) while investigating the *Stomoxys* plague in the Ngorongoro crater found that *Stomoxys* pestered

cattle and wildlife alike. Lions climbed up trees and hid in hyena holes to avoid the flies.

In agricultural areas, the stable fly is mainly a pest of livestock; livestock feeding is interrupted leading to decreased milk yields and loss of weight. Bloody legs are often seen on cattle during periods of heavy infestations (Kunz and Monty, 1976). In 1993, the cattle industry in the USA reported a 28% weight loss in cattle due to *S. calcitrans* (Foil and Hogsette, 1994).

The stable fly is thought to be important in the mechanical transmission of trypanosomes, infectious anaemia of horses, summer mastitis and of the anthrax bacillus. On rare occasions the stable fly becomes involved in accidental myiasis in man (Greenberg, 1973).

1.7.4 Colonisation of *Stomoxys*.

Colonisation attempts are recorded as early as the mid 1920s, but most developments have occurred since the 1950s. The colonisation of stable flies was necessary to support control efforts with chemicals and for biological investigations. Whereas many workers have contributed to the colonisation of *S. calcitrans* very little has been recorded on the biology of other stable flies (Zumpt, 1973). Various culture media have been recommended (Bailey *et al.*, 1975; Kunz and Schmidt, 1978). Bloodmeals have been found to be obligatory for the development of *Stomoxys* (Moobola & Cupp, 1978; Parr, 1962).

1.8 Justification of the study.

It is apparent from the literature review that the role of stable flies in the transmission of trypanosomiasis should be updated. This study was carried out to expand on and verify previous historical investigations; specifically to try to ascertain whether transmission takes place, and, if it

does, to determine the frequency at which the flies transmit parasites. Previous attempts at laboratory transmission of *T. evansi* and *T. congolense* were done with *S. calcitrans*. Bearing this in mind, the present study was initiated to investigate the role of wild stable flies, specifically *S. taeniatus* and *S. niger niger*. The flies feed on livestock and are easily-available in the Nairobi park. The trypanosomes were chosen arbitrarily from those known to grow well in mice and were therefore convenient to work with.

1. 9 Objectives of the study.

The specific objectives were as follows:

1)To elucidate the life cycles of *S. niger niger* and *S. taeniatus*.

2)To verify whether the above flies can transmit *T. congolense* and *T. evansi*.

3)To compare the abilities of the flies to transmit the different parasites.

4)To investigate whether the feeding history of the fly facilitates or blocks transmission, i.e. teneral versus non-teneral, wild flies direct from the field versus previously blood-fed or honey-fed flies.

CHAPTER 2

MATERIALS AND METHODS.

2.1 Study areas and trap descriptions.

Stable flies were collected from the forest in Nairobi National Park, in the thickets and woodlands of Nguruman and the cattle boma at the International Centre of Insect Physiology and Ecology (ICIPE), Kenya. Flies were captured in Vavoua (Laveissiere & Grébaut, 1990) and Nzi traps (Mihok, unpublished) baited with 1-octen-3-ol. The Vavoua trap (Plate 1) is made from blue/black cotton cloth and white polyester mosquito netting. It has three radial blue/black hanging screens suspended from the inside of an upper netting cone. It is highly effective for Stomoxyinae (Mihok *et al.*, 1995b). The Nzi trap (Plate 2) is a trap for biting flies. The trap is an improvement on many kinds of traps e.g. Siamese (Kyorku *et al.*, 1995) and NG2G (Brightwell *et al.*, 1991) now used around the world for the control of biting flies. It is also made from blue/black cotton cloth and white polyester mosquito netting. Flies are attracted to the large blue and black surface and enter the trap through the base. They finally collect in the collecting bag at the top of the trap.

***Plate 1**: A photograph of Vavoua trap in the Nairobi park forest*

***Plate 2**: A photograph of Nzi trap in Nguruman thickets*

2.2 Rearing of *Stomoxys*.

Flies were brought from the field and kept in cages (Plate 3) on the bench in the laboratory. *S. n. niger*, *S. taeniatus*, *S. n. bilineatus*, *S. boueti*, *S. inornatus*, *S. transvittatus*, *S. calcitrans*, *S. brunnipes* and *S. pallidus* were represented in the catch. The flies were maintained according to the method of Bailey *et al.* (1975) and Kunz and Schmidt (1978). They were fed daily on citrated (5g of sodium citrate dissolved in 5 ml of water for 1 litre blood) goat or cattle blood and a 10% sucrose solution. The blood and the sucrose solution were soaked on separate pieces of cotton wool and placed in a petri dish at the bottom of the fly cage or on top of the cage and left overnight. The blood was stored at 4°C and used for up to 2 weeks. A moist black cloth was provided for oviposition. The eggs were removed daily and seeded in the larval media.

Initially, different larval media (1:1:2 parts by volume of wheat bran, saw-dust and water; 1:1:2 mice pellets, saw dust and water; sterilised black rhinoceros dung [*Diceros bicornis* L.]) were used in attempts to rear the flies. Later, the best larval media was used to rear *S. n. niger* and *S. taeniatus*. Mice pellets contain protein and fibre supplements that are essential in stable fly diets. The larval media were put in plastic containers [17 cm height; 8 cm diameter (Plate 4)] whose tops were covered in black cloth to prevent excessive drying and contamination of *Stomoxys* eggs by those from stray flies. The containers were left on the benches at room temperature (22-23°C). After pupation, the containers were uncovered and transferred into fly emergence cages where F_1 generation flies emerged. The cages consisted of a wire frame (30 x 45 x 30 cm) covered with white netting.

Experiments were conducted randomly using groups of either wild *S. n. niger* and wild *S. taeniatus* (obtained directly from the field) or *S. n. niger* tenerals (The F_1 generation flies).

2.3 Trypanosomes.

The trypanosomes consisted of one stock each of *T. congolense* and *T. evansi* (IL 1934). *T. congolense* (K/60) is a cloned isolate from a cow at Kilifi, Kenya (Masake *et al.*, 1987). *T. evansi* (IL 1934), was derived from a stock of *T. evansi*, originally isolated from a South-American Capybara (Magnus *et al.*, 1982). The parasites were grown and maintained in Balb/c mice. Experiments were conducted randomly using one parasite stock at a time and at a parasitaemia of about 10^7/ml.

2.4 Mice.

Balb/c mice were bred at ICIPE. They were kept in rectangular plastic boxes with a removable wire mesh top. They were provided with a bedding of wood shavings and were fed on mice pellets (Unga Feeds, Nairobi brand) and water.

2.5 Examination of mice for parasites.

Examination of live trypanosomes in the tail blood of mice was effected by means of the wet film method. This was suitable for rapid screening of infected mice. The tip of the tail was snipped off using a pair of sterilised scissors. The blood exuding from the tail was then picked up with a cover slip and placed on a slide and examined under the microscope at a magnification of 400x. The presence or absence of trypanosomes was then noted.

Plate 3: *A photograph of a Fly cage on the laboratory bench. The cage was used to rear Stomoxys.*

Plate 4: *A photograph of Larval media containers with saw dust & wheat bran*

2.6 Cryopreservation of parasites.

A quantity of heparinised blood was obtained from an infected mouse. Glycerol was added dropwise while shaking the blood to give a final concentration of 10%. The glycerolated blood was left for 15 minutes to equilibrate and then put in capillary tubes whose ends were then sealed with plasticine. The capillary tubes were transferred to a plastic test tube and labelled. The test tube of capillaries was then inserted into an insulated plastic bottle and then covered with a thick piece of cotton wool. The bottle was then suspended in the vapour phase of liquid nitrogen and allowed to cool for 2 hours. After cooling, the tube of capillaries was transferred from the cooling device to a storage canister in the liquid nitrogen container. When the parasites were needed, a few capillary tubes were withdrawn, thawed and mobility and viability checked microscopically. The test blood was then inoculated intraperitonealy into mice which were later bled to obtain the parasitaemic blood that was used in the experiments.

2.7 Dissection of flies.

A fly which had earlier on been fed on infected blood was placed on a slide under a binocular dissecting microscope in a few drops of normal saline solution. The head was severed and placed on a separate slide. Using a pair of forceps the proboscis was pulled out and covered with a cover slip separating the labrum and the hypopharynx. The first sternal sclerite of the fly was held firmly and slowly pulled backwards tearing away the other sternal sclerites up to the tip of abdomen. The digestive tract was then removed with a pair of fine forceps placed on a slide. Both the mouthparts and the gut were then examined microscopically and the presence or absence of trypanosomes noted.

2.8 Determination of the time taken for *Stomoxys* to feed to repletion.

Individual flies were allowed to take blood from a piece of cotton wool soaked in mouse blood. The sex, species of the fly and the time taken for it to feed were then noted.

2.9 Survival of trypanosomes.

In order to determine how long trypanosomes remained alive in *Stomoxys*, groups of 40 flies were fed on parasitaemic blood. Five flies from each group were then killed at intervals, dissected and their mouthparts examined for trypanosomes. The number of proboscis with live trypanosomes was recorded. Similarly, guts from 50 flies which had earlier on been fed on infected blood were examined at intervals for trypanosomes. The result was scored as either positive or negative.

2.10 Imitation of mechanical transmission using a needle.

A 25 gauge syringe needle was inserted in a small piece of cotton wool soaked in parasitaemic blood which had been obtained freshly from an infected mouse and immediately used to prick the skin of an uninfected mouse. The blood on the needle contained approximately 5 x 10^6 parasites/ml. This experiment was done to imitate mechanical transmission. Trypanosome growth in mice was then monitored for one month afterwards by microscopic examination of wet smears of tail blood. The presence or absence of trypanosomes was noted as before. Infection by each stock of parasites was attempted 20 times.

2.11 Inoculation of infected fly homogenates into mice.

Groups of thirty flies were fed on parasitaemic blood as described before. At 0, 5, 10, 20, 30, 45, 60, 120, 150, 180, 210, 300, 480

minutes and 24 hours after the infective feed, individual flies were placed in tubes each containing 0.5 ml of normal saline and homogenised. The resulting mixture was injected into two mice using a 25 gauge syringe needle. Trypanosome development in the mouse was monitored for one month afterwards and the results noted.

2.12 Determination of transmission rates.

Mechanical transmission was attempted with individual flies. Before transmission the flies were treated as follows: **(a)** obtained directly from the field (wild), **(b)** fed on cattle blood the previous day, **(c)** not fed at all (tenerals), and **(d)** fed on pure honey the previous day.

Prior to transmission attempts with wild flies, twenty flies were dissected and observed under the microscope to ensure absence of parasites. Each fly was then held in a vial (Plate 7) with one end consisting of nylon mesh. The fly was allowed to take a partial blood meal from a piece of cotton wool. Feeding was interrupted after 1-2 minutes when the observer was sure that blood had been taken. Partial bloodmeal represents an intake of about 22,000 parasites (Mihok *et al.*, 1995a). The fly was then transferred to an uninfected mouse to complete its meal (Boreham and Geigy, 1976). The experiment was recorded if the fly was seen to feed or probe. Trypanosome growth in the mouse was afterwards monitored for one month by microscopic examination of wet smears of tail blood. Time for first appearance of the parasites in the blood was noted and the presence or absence of trypanosomes recorded.

2.13 Statistical analysis.

The stable flies were sexed and identified to species using the keys of Zumpt (1973). Differences in feeding time among the stable flies were tested using the t-test. The chi-square test was carried out separately to

test the differences in the transmission rates of *T. congolense* and *T. evansi* by wild flies, blood-fed flies, and teneral flies. Also, the total transmission rates (total number of mice infected with *T. congolense* versus those infected with *T. evansi*) was tested.

CHAPTER 3

RESULTS

3.1 Life cycles of *Stomoxys* species.

Table Ia, Ib and Ic show results obtained after rearing *Stomoxys* in different media. Out of the hundred eggs seeded in the rhino dung, thirty five *S. n. niger*, nine *S. inornatus* and one *S. n. bilineatus* adults emerged. One hundred and five eggs placed in mice pellets, sawdust and water medium yielded twenty *S. n. niger*, five *S. taeniatus*, three *S. n. bilineatus* and four *S. inornatus*. One hundred and two eggs placed in wheatbran, saw-dust, and water medium yielded forty *S. n. niger*, eight *S. taeniatus* and ten *S. bilineatus* adults. The development of the flies in the various media took 21, 27.4 and 24 days respectively.

Out of four attempts to rear *S. taeniatus* in 1:1:2 Wheatbran, sawdust and water medium, only one resulted in complete development from eggs to adult. The rest of the time, larvae died after about four days. Only two adult flies emerged from one hundred and fifty eggs in the one successful replicate. Adult *S. n. niger* were obtained from all five attempts at rearing. Development of *S. n. niger* from egg to larvae took an average of 4.6 days; the larval stage lasted 10.5 days and the pupal stage 8.5 days. Adults lived for 12 to 14 days after eclosion. Three hundred and seventy adults were obtained from a total of six hundred and sixty one eggs. Both species laid similar eggs. The eggs were white in colour, 1 mm length and curved (banana shape) with a depression on the inner side. A one-day-old larvae (Plate 5) was 1-1.5 mm long and white in colour. The mature larvae were light brown and their lengths ranged from 12 to 12.5 mm. The pupae (Plate 6) were dark to light brown measuring between 4-4.5 mm.

Table Ia: Development of *Stomoxys* species in various media 22-23°C

	Mean				
Media	Eggs	larvae	Pupae	Adults	Species
1	100	50	*40	35	*S. n. niger*
				9	*S. inornatus*
				1	*S. n.bilineatus*
2	105	70	*35	20	*S. n. niger*
				5	*S. taeniatus*
				3	*S. n.bilineatus*
				4	*S. inornatus*
3	102	64	*43	40	*S. n. niger*
				8	*S. taeniatus*
				10	*S. n.bilineatus*

1- Rhino dung
2- Mice pellets, saw-dust and water
3- Wheat bran, saw-dust and water
* - Adults more than pupae, this was due to error in counting of larvae.

Table Ib: Development of *S. n. niger* 22-23°C

	average for five replicates			
	Eggs	Larvae	Pupae	Adults
Total	661	506	452	370
Mean days	132.2	102.2	90.4	74.0
Range	75-170	56-153	44-144	40-135
S.D	41.2	42.8	47.0	41.0
S.E	18.4	19.1	21.0	18.3

Incubation Period = 4.6
Larval " = 10.5
Pupal " = 8.5
Life cycle
Eggs to Adult (days) = 23.6

Table Ic: Development of *S. taeniatus* 22-23 °C

	average for five replicates			
	Eggs	Larvae	Pupae	Adults
Total	606	35	10	2

Incubation Period = 7.3
Larval " = 18.0
Pupal " = 9.0
Life cycle
Egg to Adult (days) = 34

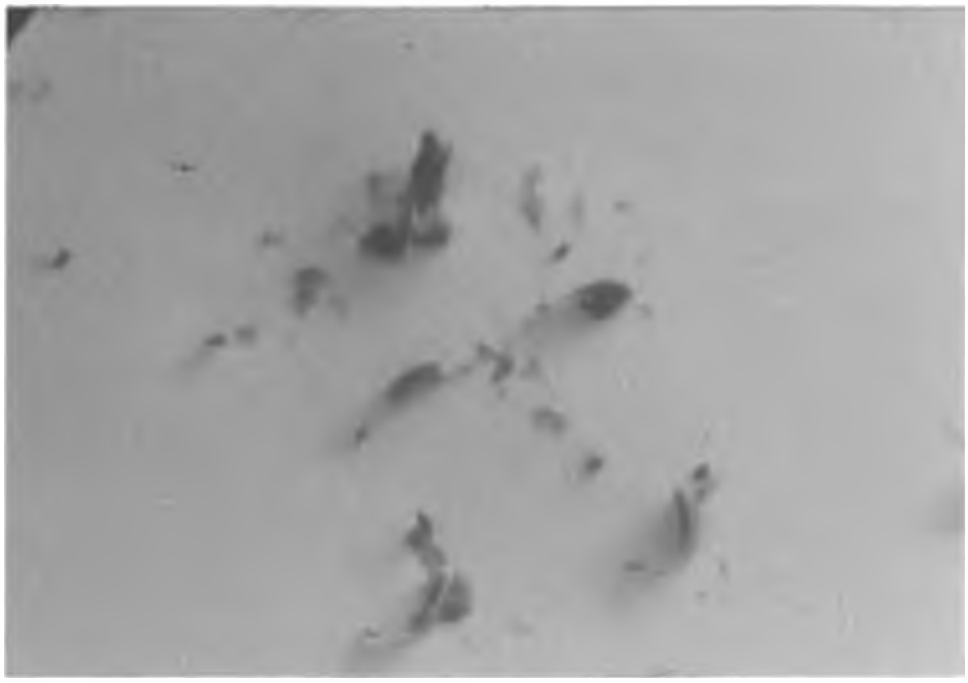

***Plate 5**: A photograph of one day old larvae (1-1.5 mm) of S. n. niger.*

Plate 6: A photograph of pupae (4-4.5 mm) and pupae cases of S. n. niger

Plate 7: A photograph of feeding vials used in mechanical transmission experiments

3.2 Feeding time in *Stomoxys*.

Table II shows the mean time taken by the flies to feed to repletion. The flies were seen to feed several times on blood during one feeding period. The flies showed no significant differences in the time they took while feeding. ($p = 0.05$).

Table II: Feeding of *Stomoxys species*

Species	Sex	N	Mean time(sec)	Range	S.D	S.E	F-Value
S. n. niger							
Wild	M	5	129	65-180	50.8	22.7	
	F	5	127	55-209	67.8	30.3	0.02
S. n. niger							
tenerals	M	5	199	121-242	50.4	22.5	
	F	5	220	168-314	65.9	29.5	0.32
S. taeniatus							
wild	M	5	188	125-251	45.0	20.1	
	F	5	169	120-225	38.3	17.2	0.51

No significant differences among the sexes. For each species (N = 10, df = 1,8, P = 0.05)

3.3 Survival of trypanosomes.

Tables IIIa shows the survival of *T. evansi* and *T. congolense* in the proboscis of stable flies. For *T. evansi* 50% wild *S. n. niger*, 37.5% *S. n. niger* tenerals and 60% wild *S. taeniatus* had live trypanosomes up to the 7th, 5th and 5th minute respectively. *T. congolense* survived in 50% wild *S. n. niger*, 47.5% *S. n. niger* tenerals, 45% wild *S. taeniatus* up to the 5th, 7th, 5th minute respectively. Parasites were mainly found at the

base of the proboscis and in the surrounding normal saline solution used in the dissection.

Table IIIa: Survival of trypanosomes in the mouthparts of *Stomoxys* species after a bloodmeal

Parasite	*T. congolense*			*T. evansi*		
Fly	1	2	3	1	2	3
Time(min)						
0	5	5	5	5	5	5
1	1	3	4	4	3	4
2	5	3	2	5	4	5
4	4	3	5	3	1	1
5	5	5	2	2	2	1
7	0	1	0	1	0	0
9	0	0	0	0	0	0
10	0	0	0	0	0	0

Fly 1- *S. n. niger* wild
2- *S. n. niger* tenerals
3- *S. taeniatus* wild
Five proboscis observed in each time interval

Tables IIIb and IIIc show the survival of *T. evansi* and *T. congolense* respectively in the guts of the stable flies. *T. evansi* survived in the guts of wild *S. n. niger*, *S. n. niger* teneral and *S. taeniatus* for 125, 140 and 105 minutes respectively. *T. congolense* survived in wild *S. n. niger*, *S. n. niger* tenerals and wild *S. taeniatus* for 105, 105 and 125 minutes respectively. In both cases as the time progressed the trypanosomes became sluggish and later disappeared. No fly had any parasites after 24 hours.

Table IIIb: Survival of *T. evansi* (IL 1934) in *Stomoxys* species guts after a bloodmeal

Time	*S. n. niger* wild	*S. taeniatus* wild	*S. n. niger* tenerals
0 min	++++	++++	++++
15 "	++++	++++	++++
30 "	++++	++++	++++
60 "	++++	+++-	++++
75 "	+++-	+++-	++++
90 "	+++-	++--	++++
105 "	+++-	+---	++++
125 "	++--	----	+++-
140 "	----	----	+---
24 hrs	----	----	----

++++ = > 20 active trypanosomes
+++ = 3 - 20 active trypanosomes
++ = 1 - 2 active trypanosomes
+ = occasional trypanosome
— = dead or sluggish trypanosomes

Note: Five flies were dissected in each time interval

Table IIIc: Survival of *T. congolense* in *Stomoxys* species guts after a bloodmeal

Time	*S. n. niger* wild	*S. taeniatus* wild	*S. n. niger* tenerals
0 min	++++	++++	++++
15 "	++++	++++	++++
30 "	++++	++++	++++
60 "	++++	++++	++++
75 "	++++	++++	++++
90 "	+++-	+++-	+++-
105 "	+++-	+++-	+++-
125 "	----	----	+---
140 "	----	----	----
24 hrs	----	----	----

++++ = > 20 active trypanosomes
+++ = 3 - 20 active trypanosomes
++ = 1 - 2 active trypanosomes
+ = occasional trypanosome
— = dead or sluggish trypanosomes
Note: five flies dissected in each time interval

3.4 Infection of mice using contaminated needles.

Table IVa and IVb show results obtained after mice were pricked with a needle contaminated with *T. evansi* or *T. congolense*. For *T. evansi*, infection was produced in all mice (N = 20). The Prepatent period was 4 days and all mice were dead by day 12. A *T. congolense* contaminated needle produced infection in 15 mice (N = 20). The Prepatent period was 12 days. All the mice were still alive 29 days after infection.

Table IVa: Infection of mice after pricking with a needle contaminated with *T. evansi*

	Days after infection					
Mouse no.	2	4	6	8	10	12
1	-	+	+	+	dead	
2	-	-	+	+	dead	
3	-	+	+	+	dead	
4	-	-	-	-	+	dead
5	-	+	+	+	dead	
6	-	+	+	+	dead	
7	-	+	dead			
8	-	+	dead			
9	-	-	-	+	dead	
10	-	-	+	+	dead	
11	-	+	dead			
12	-	-	+	+	dead	
13	-	-	-	+	dead	
14	-	-	+	+	dead	
15	-	-	+	+	dead	
16	-	-	-	+	dead	
17	-	+	+	+	dead	
18	-	-	-	+	+	dead
19	-	-	-	+	+	dead
20	-	+	+	+	dead	

\+ = mouse infected
\- = mouse not infected

Table IVb: Infection of mice after pricking with a needle contaminated with *T. congolense*

	Days after infection											
Mouse no.	4	6	8	10	12	14	16	20	22	24	26	29
1		-	-		+	+	+	+	+	+	+	+
2	-	-	-	-	+	+	+	+	+	+	+	+
3	-	-	-	-	-	-	+	+	+	+	+	+
4	-	-	-	-	+	+	+	+	+	+	+	+
5	-	-	-	-	+	+	+	+	+	+	+	+
6	-	-	-	-	-	-	-	-	+	+	+	+
7	-	-	-	-	-	-	-	-	-	+	+	+
8	-	-	-	-	-	-	-	-	-	+	+	+
9	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	+	+	+
11	-	-	-	-	-	-	-	-	-	+	+	+
12	-	-	-	-	-	-	-	-	+	+	+	+
13	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-		-	+	+
15	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	+	+	+	+	+	+	+
18	-	-	-	-	-	-	-	-	+	+	+	+
19	-	-	-	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-	-	+	+

\+ = mouse infected
\- = mouse not infected

3.5 Infection of mice with infective fly homogenate.

Tables Va and Vb show results obtained after mice were inoculated by infective fly homogenate. For *T. evansi*, homogenate from infected wild *S. n. niger*, *S. n. niger* tenerals and wild *S. taeniatus* produced infection in 19, 17 and 16 mice (each N = 28) up to 180, 480 and 180 minutes after the bloodmeals, respectively. *T. congolense* infected wild *S. n. niger*, *S. n. niger* tenerals and wild *S. taeniatus* produced infection in 15, 16, and 15 mice (each N = 28) up to 150, 210 and 210 minutes after the bloodmeal, respectively.

For both trypanosome species, the main transition in survival occurred between 60 and 120 minutes.

Table Va: Infection of mice with *T. evansi* after inoculation with infective fly homogenate

Time after bloodmeal	*S. taeniatus* wild	*S. n. niger* wild	*S. n. niger* tenerals
0 min	++	++	++
5 "	++	++	++
10 "	++	++	++
20 "	++	++	++
30 "	++	++	++
45 "	++	++	++
60 "	++	++	++
120 "	+-	++	--
150 "	--	-+	+-
180 "	+-	++	+-
210 "	--	--	--
300 "	--	--	--
480 "	--	--	+-
24 hrs	--	--	--

++ = Infection produced in both mice
-- = No infection produced
-+ = infection produced in only one mouse

Table Vb: Infection of mice with *T. congolense* after inoculation with infective fly homogenate

Time after bloodmeal	*S. taeniatus* wild	*S. n. niger* wild	*S. n. niger* teneral
0 min	++	++	++
5 "	++	++	++
10 "	++	++	++
20 "	++	++	++
30 "	-+	++	++
45 "	++	++	++
60 "	++	-+	--
120 "	--	--	+-
150 "	-+	++	++
180 "	--	--	--
210 "	+-	--	+-
300 "	--	--	--
480 "	--	--	--
24 hrs	--	--	--

++ = infection produced in both mice
-- = no infection produced
-+ = infection produced in only one mouse.

3.6 Transmission of trypanosomes.

Table VI shows the results of attempts to transmit trypanosomes mechanically to mice by wild *S. taeniatus*, *S. n. niger* tenerals and wild *S. n. niger*. For *T. evansi*, 13.3% (N = 30) transmission was obtained using *S. taeniatus* fed on blood 24 hours prior to the experiment. Wild *S. taeniatus* obtained 17.5% (N = 40) transmission. *S. taeniatus* did not transmit *T. congolense*. Wild *S. n. niger* had transmission rates of 20% (N = 30) and 7.5% (N = 30) for *T. evansi* and *T. congolense* respectively. *S. n. niger* previously-fed on blood had 16.7% (N = 35) and 3.3% (N = 40) transmission rates for *T. evansi* and *T. congolense* respectively. *S. n. niger* tenerals had a transmission rate of 35% (N = 40) and 10% (N = 30) for *T. evansi* and *T. congolense* respectively. Flies fed on honey died within 24 hours of their feeding. Therefore transmission could not be attempted.

There was no significant difference in the transmission of *T. evansi* by teneral, wild and blood-fed *S. n. niger* (Chi-square = 3.73, df = 2, p = 0.16). There was also no significant difference in the transmission of *T. congolense* by wild, blood-fed and teneral *S. n. niger* (Chi-square = 1.05, df = 2, p = 0.59). Using Fisher Exact Test, blood-fed and wild *S. taeniatus* showed no significant difference in their transmission rates for *T. evansi* (p = 0.75). Total transmission rates of *T. congolense* and *T. evansi* by *S. n. niger* varied significantly (Chi-square = 10.67, df = 1, p = 0.001).

Table VI: Fly transmission experiments.

Fly species	Treatment	% Transmission rates			
		T. evansi	N	*T. congolense*	N
G. taeniatus	blood	13.3	30	0.0	40
	Wild	17.5	40	0.0	40
S. niger niger	blood	16.7	35	3.3	40
	Wild	20.0	30	7.5	30
	tenerals	35.0	40	10.0	30

CHAPTER 4

DISCUSSION

4.1 Transmission experiments.

Mechanical transmission of parasites by biting flies depends on several factors: the feeding of the vectors, the ability of the parasites to survive in the fly and their adaptability to the new host.

4.1.1 Feeding in *Stomoxys*.

Stable flies were observed to feed several times during each artificial feeding trial until they were satiated. This manner of repeated feeding is conducive to parasite transmission in nature, where the fly may continue feeding on another host. Interrupted feeding may be common when bites by the flies irritate the animals.

The initial and final destination of blood is important in determining the possible route followed by parasites within the fly. In my study, both adult and teneral flies dissected immediately after feeding contained blood in their midgut only. Earlier reports regarding the destination of blood and sugar solutions in stable flies are contradictory. Champlain and Fisk (1956) reported that blood went to the crop first and then to the midgut for digestion. A more recent report by Lee and Davis (1979) suggests that the destination of blood is dependent on the age of the fly and the source of the blood. Most of the meal taken from a warm blood-soaked pad was found in the midgut, with a small amount in the crop. Blood taken from the human hand went directly to the midgut (presumably the natural situation). Flies older than 54 hours took blood directly to the midgut regardless of the feeding technique. The fact that bloodmeals in stable flies seem to go directly to the midgut, suggests that if trypanosomes are ingested together with the bloodmeal, then they can be transferred while still alive to the next host by regurgitation of the gut contents (Straif *et al.*, 1990). Any differences in transmission rates by different flies could

not be explained by their feeding behaviour since they all fed in a similar way.

4.1.2 Survival of trypanosomes in *Stomoxys*.

T. congolense and *T. evansi* survival in/on the mouthparts of *Stomoxys* species was limited to about 7 minutes (Table IIIa). Other reports on survival times of trypanosomes in stable flies are poorly documented (Wells, 1972). In a few experiments, live parasites were demonstrated in the proboscis and guts of stable flies. According to Wells (1972), *T. vivax* (a single organism) survived in the proboscis of *S. calcitrans* for 40 minutes. No *T. congolense* survived in the proboscis of *Stomoxys* following ingestion of parasitaemic blood. Examples of other insects that can keep trypanosomes alive for some time in their mouthparts include *Glossina, Tabanus, Anopheles* and *Lyperosia* species. My results indicate that *T. congolense* and *T. evansi* can survive in the mouthparts of the stable flies for only a short time. Likewise, Dixon *et al.* (1971b) and Ogonji (1983), found the survival of trypanosomes in the mouthparts of *Stomoxys calcitrans* to be limited, e.g., only 10 to 20 minutes. These observations suggest that mouthparts of *Stomoxys* species do not favour survival of trypanosomes. There could be many possible explanations. In particular the structure of their mouthparts is such that only a small number of parasites can be retained after a bloodmeal. Similarly, the direct passage of blood to the mid-gut during feeding leaves very little blood in the proboscis. No dead trypanosomes were observed in the proboscis, ruling out the possibility of toxic saliva as suggested by Greenberg (1973). However, as time progressed dead parasites could be seen in the saline used to mount the proboscis. This implied that the parasites on the outside of the proboscis would have contributed little to the total number of parasites transferred to the next

host. The fact that parasites survived at all in/on the proboscis suggests a simple mechanism for mechanical transmission through contaminated mouthparts.

Direct microscopy and mouse inoculation techniques, were able to detect live *T. congolense* only up to 210 minutes and *T. evansi* up to 480 minutes after an infective bloodmeal. *T. evansi* and *T. congolense* survived in the guts for up to 140 and 125 minutes respectively. Reports in older literature summarized by Wells (1972) show different survival times for various trypanosome species in different biting flies. The maximum survival time for *T. congolense* in the gut of *Stomoxys* was 24 hours. The parasites were dead by the second day after the infective feed. *Anopheles, Glossina, Tabanus* and *Haematopota* species also kept trypanosomes alive in their guts for modest lengths of time. In all these experiments, the trypanosomes were not able to establish themselves in the stable flies, therefore ruling out cyclical transmission.

According to Molyneux (1977), the establishment and development of trypanosomes in the guts of insects, specifically *Glossina* is dependent on numerous factors. These include endogenous factors: age, sex, genetic composition, behavior, concurrent infection (viral, bacterial, fungal), biochemical and physiological state of the fly; ecological factors: climate and availability of infected hosts; and parasite factors which include the numbers of the parasites available to the fly and the strain of the parasite. A number of studies on the digestive physiology of haematophagous insects have also revealed various defense mechanisms in insects. Among these are: lectins (Maudlin & Welburn, 1987), proteolytic enzymes (Champlain & Fisk, 1956; Deloach & Spates, 1979; Gooding & Rolseth, 1976; Lehane, 1976; Schneider *et al.*, 1987) and haemolysins (Spates *et al.*, 1982). Lectins kill trypanosomes and prevent their establishment in the gut; similarly proteolytic enzymes may

digest the trypanosomes together with the blood. Since only a few studies have been carried out with stable flies, the failure of trypanosome-establishment can presumably be attributed to any number of trypanocidal factors in the guts of the flies. Although parasites eventually die, their ability to survive in the gut for about two hours allows for a second possible mechanism of transmission i.e., regurgitation. Straif *et al.* (1990) used forced capillary feeding to demonstrate regurgitation in *Stomoxys* species. If their results are generally applicable, regurgitation could provide a basis for mechanical transmission in the wild.

4.1.3 Transmission rates of trypanosomes by *Stomoxys*.

The successful transmission by a contaminated needle implied that it is possible to transfer *T. congolense* and *T. evansi* from one host to another through simple surface contamination of mouthparts. Transmission through interrupted feeding was demonstrated in 44 of 355 attempts. Mihok *et al.* (1995), Ogonji (1983), Wells (1972) and others have also recorded artificial mechanical transmission of trypanosomes. Previous historical research demonstrated mechanical transmission by *Stomoxys* species in 7 of 17 attempts (Wells, 1972) and 16 of 347 attempts (Mihok *et al.*, 1995a), using various *Trypanosoma* species. In all these experiments, different fly species and stocks of parasites were used. Altogether, it is clear that mechanical transmission is possible with most Stomoxyinae, but the phenomenon is sporadic.

T. congolense was transmitted successfully by *S. n. niger*, but *S. taeniatus* failed to transmit it. This pattern suggests differences in the overall feeding and probing behaviour of the two fly species, or more simply, differences in the infectivity of the parasite stocks. Unpublished anthrone tests of wild flies from Nairobi National Park indicate that 18.9%

and 2.5% (N = 243) of *S. taeniatus* caught in traps have fed recently on sugar or blood respectively. Similarly, 40% and 2% of *S. n. niger* (N = 582) contained sugar and blood respectively (Mihok, unpub). Hence most of the wild flies used in my experiments had probably fed previously on wild sources of sugar (nectar, honeydew). Although teneral flies had higher transmission rates than either blood-fed, or wild flies, these differences were not significant. In *Glossina*, a bloodmeal stimulates the release of trypanocidal substances including proteolytic enzymes, lectins, lysins, and probably other yet-uncharacterised molecules. In the case of trypanosome infected blood, lectins lyse bloodstream forms (Maudlin, 1991) but may also promote differentiation of the parasites (Abubakar *et al.*, 1995). It is presumed that species with high lectin levels prevent establishment of the parasites. Midguts of teneral tsetse flies have little lectin activity compared with fed flies (Maudlin & Welburn, 1989). Little is known about *Stomoxys*, though this possibility exists.

Certain plant diets (which may contain lectins) decrease the capacity of sand flies (*Phlebotomus*) to transmit leishmaniasis by reducing the survival of *Leishmania* in the gut (Schlein & Jacobson, 1994). These results are intriguing and may be of relevance to *Stomoxys*. The low mechanical transmission rates in previously blood-fed flies when contrasted with teneral flies suggest the presence of many complex variations underlying transmission in the field. There is clearly a need to carry out further investigations on vectorial capacity of stable flies in relation to both feeding behaviour and gut physiology.

4.2 Breeding experiments.

From about a hundred eggs seeded in the three types of media (Rhino dung; mice pellets saw dust and water; wheat bran, saw dust and water), 45, 32, and 58 adult stable flies emerged respectively. This yield

was low. Also, when wheat bran, saw dust and water medium was used to rear *S. taeniatus* and *S. n. niger, S. taeniatus* failed to develop except in one case where only two adult flies emerged. There could be many possible explanations. In particular, the media and the temperature may not have been appropriate for the proper development of the flies. Also, the results indicate that different species of stable flies require specific conditions for proper breeding. This is in agreement with Kunz and Monty (1976) who described different breeding media for *S. calcitrans* and *S. n. niger*. Since Rhino dung was found in reasonable amounts near the trapping sites, it is likely that the flies oviposit in the dung. The flies bred well in Wheat bran, saw dust and water media. This media has been used before and found to be appropriate for the laboratory rearing of stable flies, although the work was mainly with *S. calcitrans* (Bailey *et al.*, 1975; Kunz and Schmidt, 1978; Ogonji, 1983). The second media, in which wheat bran was substituted with mice pellets was found unsuitable for breeding for all the *Stomoxys* species.

RECOMMENDATIONS AND CONCLUSIONS.

The results of this study are consistent with the notion that stable flies are potential mechanical vectors in the field where animals may be found grouped together with a few of them infected with trypanosomes (D'Amico *et al.*, 1996; Dixon *et al.*, 1971a, b; Otte and Abuabara, 1991; Foil *et al.*, 1987; Wells, 1972 among others). In such cases it is believed that transmission occurs in the absence of *Glossina* and is spread and maintained by other biting flies such as tabanids and *Stomoxys* (Lucas, 1955). Though knowledge on wild Stomoxyinae in Africa is scarce (Zumpt, 1973), *S. n. niger* and *S. taeniatus* obviously feed on cattle (Kangwagye, 1973). These species as well as other Stomoxyinae are found in high densities in appropriate habitats during rainy seasons (Mihok

et al., 1995). Therefore, they could be of interest in the epizootiology of trypanosomiasis. Clearly, their role in the occurrence of trypanosomiasis, especially in tsetse-free areas cannot be ignored.

Consequently, as a contribution towards the study for the control of the stable flies, and therefore control of mechanically spread trypanosomiasis and other diseases, further investigations should be carried out in the following areas: the effect of diet on survival of trypanosomes in the fly guts; trypanocidal and lectin activity in their guts; and appropriate breeding media for wild Stomoxyinae.

REFERENCES

Abubakar, L., Osir, E. O., Imbuga, M. O., 1995. Properties of a bloodmeal induced midgut lectin from the tsetse fly *Glossina morsitans*. Parasitol. Res. **81:** 271-275.

Adam, K. M., James, P., and Zanman, V., 1971. Medical and Veterinary Protozoology. Churchill, London. pp. 66-67.

Ashcroft, M. T., 1959. The importance of African wild animals as reservoirs of trypanosomiasis. East Afr. Med. J. **36**: 289- 297.

Ashcroft, M. T., Burri, E., and Fairbairn, H., 1958. The experimental infection of some African Wild animals with *Trypanosoma rhodesiense*, *Trypanosoma brucei* and *Trypanosoma congolense*. Ann. Trop. Med. Parasit. **53**: 147-161.

Bailey, D. L., Whitfield, L. T., LaBrecque, C. G., 1975. Laboratory biology and techniques for mass producing the stable fly, *Stomoxys calcitrans* (L.) Diptera: Muscidae. J. Med. Ent. Vol. **12**, (2): 189-193.

Baird, W. H. W., 1930. Attempts to transmit trypanosomiasis by flies other than tsetse. In Wells, E. A., 1972. The importance of mechanical transmission in the epidemiology of 'Nagana': A review. Trop. Anim. Hlth. Prod. **4**: 74-88.

Bouet, G. and Roubaud, E., 1912. 'Expériences de transmission des trypanosomiases animales de l' afrique occidentale francaise, par les stomoxes'. Bulletin de la société de pathologie exotique.

Boreham, P. F. L., Geigy, R., 1976. Culverts and trypanosome in the Serengeti National Park (Tanzania) III. Studies on the genus *Auchmeromyia*, Brauer and Bergenstamm (Diptera: Calliphoridae). Acta Trop. 33:74-87.

Brightwell, R., Dransfield, R. D., Kyorku, C., 1991. Improvement of a low-cost trapping technology for *Glossina pallidepes* and *G. longipennis*. Med. Vet. Entomol. **5**:153-164.

Broden, A., 1904. Les infections à trypanosomes dans l'état du congo. Bull. soc. Etudes Colon. **11**:116-142.

Buxton, P. A., 1955. The natural history of tsetse flies. H. K. Lewis & Co. Ltd., London.

C. A. B. I. 1989. Manual of Tropical Veterinary Parasitology, London.

Champlain, R. A & Fisk, F. W., 1956. The digestive enzymes of the stable fly, *Stomoxys calcitrans* (L.) The Ohio Journal of Science **56**(1) 52-62

Cheesbrough, M., 1987 Medical laboratory manual for tropical countries Volume 1 Ed 2 PP 256. Butterworth-Heinmann Ltd., Halley court, Jordan Hill, Oxford Ox 2 8EJ

D Amico, F., Gouteux J. P., Le Gall, F., Cuisance, D., 1996 Are stable flies (Diptera Stomoxyinae) vectors of *Trypanosoma vivax* in the Central African Republic? Vet Res **27**(2). 161-170.

Deloach, J. R. and Spates, G. E., 1979. Rate of digestion of 51-Cr-haemoglobin by *Stomoxys calcitrans*. (Diptera: Muscidae). J. Med Entomology. **16** 493-496.

Dirie, F. M., Wallbanks, K. R., Aden, A. A., Bornstein, S and Ibrahim, M. D., 1989. Camel trypanosomiasis and its vectors in Somalia. Veterinary Parasitology, **32** 285-291.

Dixon, J. B., Cull, R. S., Dunbar, I. F., Greenhill, R. J., Grimsaw, C. G., Hil, M. A., Landeg, F. J. & Miller, W. M., 1971a I Non-cyclical transmission of trypanosomiasis in Uganda Abundance and biting behaviour of Tabanidae and *Stomoxys*. Vet. Rec. August. 28th, 233-235

Dixon, J. B., Cull, R. S., Dunbar, I. F., Greenhill, R. J., Grimsaw, C. G., Hil, M. A., Landeg, F. J., & Miller, W. M., 1971b. II Experimental assessment of the survival time of *Trypanosoma* brucei in *Stomoxys calcitrans* Vet Rec August 28th, 233-235.

Finelle, P., 1974 African animal trypanosomiasis economic problems. World Anim. Rev **10** 15-18

Foil, L. D., 1989. Tabanids as vectors of disease agents Parasitology Today **5** 88-96

Foil, L. D., Adams, W. V., McManus, J. M., Issel, C. J., 1987 Bloodmeal residues on mouthparts of *Tabanus fuscicostatus* (Diptera Tabanidae) and the potential for mechanical transmission of pathogens J. Med Entomol **24** 613-6.

Foil L. D., and Hogsette, J. A., 1994 Biology and control of Tabanids, stable flies and horn flies Rev Sci. Tech off int Epiz **13** (4):1125-1158

Fosbrooke, H. A., 1963. The *Stomoxys* plague in Ngorongoro (1962). E Afr Wild J **1** 124-126

Geigy, R., and Kauffman, M. 1973. Sleeping sickness Survey in the Serengeti Area (Tanzania) 1971 Acta Trop **30** 12-23

Greenberg. B., 1973. Flies and disease Volume II. Princeton Univ Press Princeton New Jersey pp 42-50, 315-331.

Geofroy, E., 1762. Histoire abrégée des insects qui se trouvent aux environs des paris **2**:690 Pp. Paris.

Gooding, R. H., and Rolseth, B. M., 1976 Digestive processes of haematophagous insects XI Partial purification and some properties of six proteolytic enzymes from tsetse fly-*Glossina morsitans morsitans*. Westw (Diptera: Glossinidae) Can J Zool **54** 1950-1959

Harley, J. M. B., 1965. Seasonal abundance and diurnal variations in activity of some *Stomoxys* and tabanids in Uganda Bull. Ent. Res. **56** 318-332.

Hoare, C. A., 1940. Studies on the behaviour of *Trypanosoma evansi* in the tsetse flies, with special reference to its phylogeny. Parasitology **32** 105.

Hoare, C. A., 1972. The Trypanosomes of Mammals. Blackwell Scientific Publications. Oxford

Ikede, B. O., Elhassan, E., and Alpavie, S. O., 1988. Reproductive disorders in African trypanosomiasis A review. Acta trop **45** 5-10.

ILCA, 1979. "Trypanotolerant livestock in West and Central Africa". Volume 1 "General study" Volume 2 "Country Studies" International Livestock Centre For Africa. Addis Ababa (Monograph No. 2)

Jawara, A. S. D. K., 1990. Animal diseases as a factor limiting economic development in Africa.

Jordan, A. M., 1986. "Trypanosomiasis Control and African rural development" Longman, London.

Jowett, W., 1911. 'Further note on a cattle trypanosomosis of Portuguese East Africa' in Wells, E. A., 1972 The importance of mechanical transmission in the epidemiology of 'nagana': A review Trop Anim. Hlth Prod **8** 195-201

Kangwagye, T. N., 1973 Diurnal and nocturnal biting activity flies (Diptera) in Western Uganda Bull Ent Res **62** 17-29

Kunz, S. E. and Monty, J., 1976 Biology and ecology of *Stomoxys nigra* Macquart and *Stomoxys calcitrans* (L) (Diptera Muscidae) in Mauritius. Bull Ent Res 66: 745-755.

Kunz, S. E. and Schmidt, D C., 1978 A report from U S. Livestock insects Laboratory, Agricultural Research Service, U. S. Department of Agriculture

Kyorku, C A , Machika, C Otieno, L H , Mwandandu, J., 1995 An improved odour -baited trap for a mixed population of *Glossina* spp. In the Kenya coast In proceeding, 10th scientific meeting of the African Association of Insects Scientist, Mombasa, Kenya (In press).

Laveissière, C and Grébaut, P , 1990 Recherche sur les pieges a glossines (Diptera Glossinidae) Mise au point d un modèle economique Le piege "vavoua". Trop Med Parasitol **41**.185-192

Lehane, M J , 1976 The digestive enzyme secretion in *Stomoxys calcitrans* Diptera Muscidae Cell Tiss Res **170**:275-287.

Lee M K W., and Davies, D M , 1979 Feeding in the stable fly, *Stomoxys calcitrans* (Diptera Muscidae) I. Destination of blood, sucrose solution and water in the alimentary canal, the effects of age on feeding and blood destination J Med Entomol **15** (5-6):541-554

Luckins, A G , 1988. *Trypanosoma evansi* in Asia Parasitology Today **4** 137-142

Lucas, J. M. S., 1955 Transmission of *Trypanosoma congolense* in cattle under field conditions in the absence of tsetse fly Veterinary record, **67** 403-406

Lun, Z R , Fang, Y , Wang, C J , Brun, R , 1993. Trypanosomiasis of domestic animals in China Parasitology Today **9** 41-45

Mac Innis, J A , & Voge, M , 1970. Experiments and Techniques in Parasitology W H Freeman Company

Magnus, E , Vervoort, T , Meirvenne, V N , 1982 Serological cross-reactions among trypanosome variable antigen isotypes of subgenus *Trypanozoon* Ann Soc belge Med Trop **62**:25-39

Majiwa, P A O , 1989 Recombinant DNA probes as tools for epidemiological studies of parasitic diseases Discovery and innovation **1**:35-40

Majiwa, P A O , Ole-Moi Yoi, K O., and Nantulya, M V., 1993 New techniques for diagnosis of the African trypanosomiases. Agbiotech News and Information Vol 5 No.3 115N-120N

Masake, R A , Nantulya, V M., Musoke, A J., Moloo, S K , and Nguli, K., 1987. Characterization of *Trypanosoma congolense* serodemes in stocks isolated from cattle introduced onto a ranch in Kilifi, Kenya Parasitology **94** 349-357

Maudlin, I , 1991 Transmission of African trypanosomiasis Interactions among tsetse immune systems, symbionts, and parasites Adv Dis. Vec. Res **7** 117-148

Maudlin, I and Welburn, S C., 1987 Lectin mediated establishment of mid-gut infections of *Trypanosoma congolense* and *Trypanosoma brucei* in *Glossina morsitans* Trop. Med Parasitology **38** 167-170.

Mihok, S , Maramba, O., Munyoki, E , Kagoiya, J., 1995a. Mechanical transmission of *Trypanosoma* spp by African Stomoxyinae Trop. Med. Parasitol **46** 103-5

Mihok, S., Kang'ethe, k , and Githaiga, K , 1995b Trials of traps and attractants for *Stomoxys* spp (Diptera Muscidae) J. Med Entomol **32**(3) 283-289

Molyneux, D H , 1977. Vector relationships in Trypanosomatidae Adv. Parasitol **15** 1-82

Moobola, S. M and Cupp, E W , 1978. Ovarian development in the stable fly, *Stomoxys calcitrans* in relation to diet and juvenile hormone control Physiol **18** 105-107

Moorehouse, D E , 1972 Cutaneous lesions on cattle caused by stable fly Australian Veterinary Journal **48** 643-644

Nantulya, V M and Lindqvist, K J , 1989 Antigen-detection enzyme immunoassay for the diagnosis of *Trypanosoma vivax*, *T. congolense* and *T brucei* infections in cattle Trop Med Parasitol **40** 267-272.

Nantulya, V. M , 1991 Molecular diagnosis of parasites. Experimentia **47** 143-145.

Nantulya, V M , Lindqvist, K. J., Stevenson, P., Mwangi, E. K., 1992 Application of a monoclonal antibody-based (antigen-ELISA) for field diagnosis of bovine trypanosomosis. Ann Trop. Med Parasit **86** 225-230

Ogonji, F A , 1983 Mechanical transmission of *Trypanosoma evansi* Steel by *Stomoxys calcitrans* Msc Thesis, University of Nairobi.

Otte, M J and Abuabara, J Y, 1991. Transmission of South American *Trypanosoma vivax* by the neotropical horse fly *Tabanus nebulosus*. Acta Trop **49**:73-76.

Parr, H. C. M., 1962. Studies on *Stomoxys calcitrans* (L.) in Uganda. East Africa Bull Ent Res **53**:437-443.

Straif, S., Maier, W., Seitz, H. M., 1990. Regurgitation as a potential mechanism of pathogen transmission in the biting fly, *Stomoxys calcitrans*. Zeitschrift fur Angewandte Zoologie **77**:357-365.

Schlein, Y., and Jacobson, L. R., 1994. Mortality of *Leishmania major* in *Phlebotomus papatasi* caused by plant feeding of the sand flies. Am. Trop. Med. Hyg. **50**(1):20-27.

Schneider, F., Houseman, G. J., and Morrison, P. E., 1987. Activity cycles and regulation of digestive proteases in the posterior midgut of *Stomoxys calcitrans* (L.) Diptera: Muscidae. Insect Biochem. **17**(6):859-862.

Spates, G. E., Stipanovic, R. D., Williams, H., Holman, G. M., 1982. Mechanism of haemolysis of in a blood-sucking dipteran, *Stomoxys calcitrans*. Insect. Biochem. **12**:707-712.

Venkantesh, K. and Morrison, P. E., 1982. Bloodmeal as a regulator of triglycerol synthesis in the haematophagous stable fly, *Stomoxys calcitrans*. J. Comp. Physiol. **147**:49-52.

Welburn, S., Maudlin, I., Ellis, D. S., 1989. Rate of trypanosome killing lectins in midguts of different species and strains of *Glossina*. Med. Vet. Ent. **3**:77-82.

Wells, E. A., 1972. The importance of mechanical transmission in the epidemiology of 'Nagana': A review. Trop. Anim. Hlth. Prod. **4**:74-88.

Wiesenhutter, E., 1976. Research into the relative importance of Tabanidae (Diptera) in mechanical disease transmission. IV. A contribution to the epidemiology of bovine trypanosomiasis in Africa. Trop. Animal. Prod. **8**:195-201.

Woo, P. T. K., 1977. Salivarian trypanosomes producing diseases in livestock outside of sub-Saharan Africa. In Parasitic Protozoa. Volume I (Ed. Kreiser, J. P.) pp. 269-296.

Zumpt, F., 1973. The Stomoxyine Biting flies of the World. (Diptera: Muscidae) Taxonomy, biology, economic and importance control measures. Gustav Fisher Verlag, Stuttgart, pp. 97.