ROLE OF THE MONITOR LIZARD *VARANUS NILOTICUS* LAURENTI IN THE EPIDEMIOLOGY OF TRYPANOSOMIASIS ALONG THE SHORES OF LAKE VICTORIA, KENYA.

BY

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DECLARATION

This thesis is my original work and has not been submitted for a degree to any other university.

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We certify that this work was carried out by Zipporah Waithera Njagu under our supervision and submitted with our approval as supervisors.

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ABSTRACT

Trypanosomiasis is one of the major factors that hinder development of rural economies in Africa. The disease is endemic in some of the most potentially-productive lands of tropical Africa where it severely constrains livestock production and human settlement. The Lake Victoria shores in Kenya and Uganda have been endemic foci for human sleeping sickness since the early 1900's. *Glossina fuscipes* has been responsible for these epidemics. Reptiles especially the monitor lizard are preferred food sources for *G. fuscipes*. Studies were carried out to determine the role played by the monitor lizard, *Varanus niloticus* in the natural transmission cycle of trypanosomiasis between tsetse flies, livestock and man. The objective was to determine the natural occurrence of trypanosomes in lizards. This survey was further supplemented by experimental infections of lizards with *Trypanosoma brucei* and *Trypanosoma congolense*.

A combination of diagnostic techniques was used to determine the natural occurrence of trypanosomes in monitor lizards at Busia (Kenya) and Rusinga Island along the shores of Lake Victoria. None of the lizards caught from Rusinga Island (n=27) was infected while 5.3 % (n=19) of the lizards caught from Busia, were infected with trypanosomes. The trypanosome parasite isolated from Busia was characterised as *Trypanosoma brucei* using the polymerase chain reaction (PCR) and the identity was confirmed by hybridisation using a *Trypanozoon* specific probe. This is the first record of isolation of *T. brucei* from any reptile. The *T. brucei* parasite was infective to laboratory rats and to tsetse.

Experiments were carried out to infect lizards with *Trypanozoon* and *Nannomonas* trypanosomes by cyclical passage using tsetse. It was possible to infect monitor lizards with *T. brucei* parasites without showing any clinical symptoms of the disease. However, infection of monitor lizards with savannah and riverine *T. congolense* was unsuccessful. The *T. brucei* parasite was infective to *G. m. centralis* (19.4%) and to laboratory rodents. These results indicate that the monitor lizard facilitates the developmental cycle of *T. brucei* and forms an important link in the transmission cycle of the parasite between the tsetse vector and mammals.

Comparative studies were further carried out to determine the influence of monitor lizard, crocodile and goat blood on infections with *T. brucei* and *T. congolense* in six *Glossina* species. Goat blood supported high infections (52-78.4%) whereas monitor lizard and crocodile blood had depressive effects (18.4-39.9%) on infections in all tsetse. The *morsitans* group of tsetse (*G. m. centralis* and *G. m. morsitans*) was more susceptible to infections with *T. brucei* and *T. congolense* than the *palpalis* tsetse (*G. fuscipes, G. p. gambiensis* and *G. tachinoides). Palpalis* flies maintained totally on monitor lizard blood in the laboratory had low maturation rates (4.6-12.4%) of *T. brucei* organisms. On the other hand, flies maintained on interrupted feeds of goat and lizard, had higher maturation rates of *T. brucei* (8.4-16.3%). This could represent the natural situation where lizards harbour parasites that never reach maturation sites in flies utilising lizards solely as a food source. In the event of an occasional tsetse feed on a favourable host, e.g goat, parasites acquired from lizards mature and can be transmitted to other hosts marking the beginning of an epidemic.

Results of this study indicate that monitor lizards (*Varanus niloticus*) act as reservoirs of *T. brucei* parasites as evidenced by the isolation of *T. brucei* from 5.3% of wild monitor lizards caught from Busia area. This was further verified by infectivity experiments which demonstrated that lizard blood is not toxic to *T. brucei*. In undisturbed areas, monitor lizards act as natural cryptic (hidden) reservoirs of trypanosomiasis. Along the shores of Lake Victoria, man has encroached on the natural habitat of monitor lizards and *G. fuscipes*. A close contact therefore exists between man, livestock, monitor lizards and *G. fuscipes* near water along the lake shores. Results of this study suggest that monitor lizards are an important link in the fly-livestock-man cycle of *T. brucei* parasites. These findings are important in the control of trypanosomiasis. In the past, control efforts have not considered reptiles as important hosts in the disease cycle and yet these animals are prevalent in trypanosomiasis endemic foci. Trypanosome parasite circulation between *G. fuscipes* and monitor lizards serves as natural cryptic pools of infective trypanosomes. These could be important in the sporadic resurgence of trypanosomes after periods of quiescence at the Lake victoria shores. This in part explains the inability to eradicate the disease from many endemic foci across tropical Africa.

CHAPTER ONE

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 General Introduction

Trypanosomiasis is one of the major factors that impedes development of rural economies in Africa. The disease is endemic in some of the most potentially-productive lands of tropical Africa where it severely constrains livestock production and human settlement. Trypanosomiasis is a parasitic disease caused by several species of flagellate protozoa of the genus T*rypanosoma* that inhabits the blood, various body tissues and fluids. The disease affects man and many species of domestic and wild animals. *Trypanosoma congolense* Broden 1904 and *Trypanosoma vivax* Ziemann 1905, usually infect cattle, sheep and goats whereas *Trypanosoma brucei* Plimmer and Bradford 1899, (which includes *Trypanosoma brucei gambiense* Dutton 1902 and *Trypanosoma brucei rhodesiense* Stephen and Fantham 1910), infects man and causes gambian and rhodesian sleeping sickness, respectively. These African trypanosomes are transmitted cyclically by various tsetse flies (*Glossina* spp.) in a wide range of climatic and ecological zones.

Animal trypanosomiasis (nagana) causes enormous losses in livestock production, resulting in shortage of natural organic fertilisers for crop production, and a shortage of dairy products, animal proteins and animal traction. Approximately 3 million cattle in Africa are estimated to be lost each year due to the disease (Gyening, 1990). It has been estimated that the total area infested with tsetse flies, and precluded from productive cattle husbandry has the potential of supporting about 125 million heads of cattle (Odhiambo, 1986).

Human African trypanosomiasis (sleeping sickness) occurs in 36 African countries between latitudes 14° North and 29° South, following the geographic distribution of tsetse. Approximately 50 million people living in some 200 foci are at risk of acquiring the infection. About 25,000 new cases are reported annually, but this is acknowledged to be a gross underestimate in view of poor reporting, difficulty of diagnosis and inaccessibility of affected areas (WHO, 1986; Kuzoe, 1993). Recently, an alarming upsurge in sleeping sickness has been reported in Central Africa where at least 250,000 people are estimated to be infected (WHO, 1994). The gambiense form of the disease occurs in West and Central Africa and the rhodesiense form is found in East and Southern Africa. Human trypanosomiasis results in poor health and low industrial output. It also causes depopulation by death or by migration of people from tsetse-infested areas. This results in large areas being relinquished to tsetse flies and wild animals (Ford, 1971). Sleeping sickness has been the major cause of depopulation of large tracts of Africa, thus fear of the disease has led to abandonment of fertile lands (Kuzoe, 1991).

The importance of tsetse as vectors of trypanosomes stems from their feeding habits: they suck blood from wild hosts which harbour trypanosomes, and then feed on domestic animals, and occasionally on man. Wild animals are the natural reservoir hosts of some species of trypanosomes (Ashcroft, 1959). Although many of the wild animals do not appear to develop the disease, some species are susceptible to infection (Molyneux, 1982). In some cases domestic animals can also harbour chronic trypanosome infections and can act as reservoirs, particularly in the case of *T. brucei* (Koerner *et al.*, 1995).

Host-feeding patterns, which are the outcome of host and the ecology of the fly, largely determine the patterns of disease transmission and hence the overall epidemiology of trypanosomiasis. Tsetse hosts influence the prevalence and the type of infections (Moloo, 1973). Infection rates in tsetse are also modified physiologically by host-species factors ingested with the infective meal (Mihok *et al.,* 1993). Of the three main groups of tsetse, the *palpalis* group are the most opportunistic feeders, utilising a wide variety of available hosts (Weitz, 1963). However, their natural preference is reptiles (Moloo, 1993). On the shores of Lake Victoria for example, monitor lizards provide the bulk (73-98%) of the food for *Glossina fuscipes* (Mohammed-Ahmed & Odulaja, 1997). This region has been a historical focus of sleeping sickness with major epidemics recurring at irregular intervals since the early 1900's (Mbulamberi, 1989). These epidemics have been related to the increase and subsequent spread of *Glossina fuscipes* (Okoth and Kapaata, 1986). Along Lake Victoria shores in Uganda, the problem has been exacerbated by changes in agricultural practices, probably owing to recent political turbulence. This has encouraged the growth of bush in which flies have established (Enyaru *et al.,* 1997).

Man and livestock come into contact with tsetse and lizards near water in the lake region. *G. fuscipes,* like other riverine tsetse, has adapted to the many new peridomestic habitats that have resulted from rapid population growth in this area. Given this species' opportunistic food habits, there is always considerable potential for transmission of parasites among diverse groups of animals and humans.

The role played by monitor lizards in the disease cycle in the Lake Victoria region is unknown. For instance, it is not known whether monitor lizards act as reservoirs of pathogenic trypanosomes; as no surveys have been conducted. Similarly,

the influence of reptilian bloodmeals on trypanosome development in *Glossina fuscipes* and other riverine species of tsetse has also not been studied. The present study describes investigations carried out on trypanosomiasis in the monitor lizard, aspects of which may be important in the understanding the role of reptiles in the transmission cycle of trypanosomes between tsetse, man and livestock and also in designing control strategies for the disease.

1.2 Literature Review

1.2.1 Systematics of tsetse flies

Tsetse flies are placed in the genus *Glossina,* family Glossinidae. The genus is subdivided into three subgeneric groups on the basis of the male and female genital armature characters. These divisions are: *fusca* (subgenus *Austenina*), *palpalis* (subgenus *Nemorhina*) and *morsitans* groups (subgenus *Glossina* s.s.) (Potts,1970). These groups which were initially established on the basis of morphology, have since been confirmed by more refined genetic techniques (Jordan, 1986; Carlson *et al.,* 1993; Gooding, 1996;). Altogether, there are 23 extant species and 8 further subspecies of *Glossina* Wiedmann (Table 1.1).

1.2.2 Distribution of tsetse flies.

Ford (1971) described the zoogeography of the genus *Glossina.* At present, tsetse are found mainly in Sub-Saharan Africa. A small population representing a much wider ancient distribution of tsetse exists in Saudi Arabia (Elsen *et al.,* 1991). Fossil records of tsetse date back to 40 million years ago from the Oligocene shales of Colorado in North America.

The distribution of tsetse flies in Africa falls mostly between latitude 14°N and 29° S, representing an area of about 11 million Km^2 (Seed & Hall,1992). Distribution limits are sensitive to temperature,whereas abundance is a complex function of rainfall, vegetation and host availability. The distribution of tsetse has changed greatly from the summarised record produced by Ford and Katondo (1977). These changes have been due to human population growth and the resulting environmental changes. The current

Table1.1 Species and subspecies of *Glossina* Wiedmann 1830 (Adapted from Moloo, 1993)

***Morsitans* group**

G. morsitans morsitans Westwood 1850
G. morsitans centralis Machado 1970
G. morsitans submorsitans Newstead 1910
G. swynnertoni Austen 1923
G. pallidipes Austen 1903
G. longipalpis Wiedmann 1930
G. austeni Newstead 1912

***Palpalis* group**

G. palpalis palpalis Robineau-Desvoidy 1830
G. palpalis gambiensis Vanderplank 1949
G. fuscipes fuscipes Nestead 1910
G. fuscipes martinii Zumpt 1935
G. fuscipes quanzensis Pires 1948
G. pallicera pallicera Bigot 1891
G. pallicera newstead Austen 1929
G. caliginea Austen 1911
G. tachinoides Robineau-Desvoidy 1850

***Fusca* group**

G. fusca fusca Walker 1849
G. fusca congolensis Newstead & Evans 1921
G. tabaniformis Westwood
G. longipennis Corti 1895
G. brevipalpis Newstead 1910
G. nigrofusca nigrofusca Newstead 1910
G. nigrofusca hopkinsi Van Emden 1944
G. fuscipleuris Austen 1911
G. medicorum Austen 1911
G. frezili Gouteux 1987
G. severini Newstead 1913
G. schwezi Newstead and Evans 1921
G. haningtoni Newstead and Evans 1922
G. vanhoofi Henrard 1952
G. nashi Potts 1955

approach to contemporary tsetse distribution mapping is based on the use of matched environmental and fly distribution data to define areas of suitability for each tsetse species. These data are then used to make predictions for other areas. Recently, advances in micro-computing technology, availability of remotely-sensed data and the development of image-processing and analytical techniques have permitted more accurate predictions of the current distribution of several species of tsetse (Rogers & Williams, 1993; Rogers *et al.,* 1996; Hay *et al.,* 1996). The *fusca* group are mainly forest inhabiting species. Although this group inhabits vast areas of West and Central Africa, its forest habitat is rarely occupied by cattle. Hence, the group as a whole has rarely been implicated in the transmission of trypanosomes to livestock (Jordan, 1986). *G. tabaniformis* has however been shown to be an important vector of trypanosomiasis to cattle in West Africa (Leak *et al.,* 1991). In East Africa two species of this group, *G. brevipalpis* and *G. longipennis,* occupy different typical habitats. *G. brevipalpis* occupies islands of forest often associated with water courses, whereas *G. longipennis* inhabits semi-arid areas. *G. longipennis* has been implicated as a vector of trypanosomiasis (Makumi *et al.,* 1996).

Species of the *palpalis* group, like those of the *fusca* group, occupy the lowland rain forests, but are also found in the drier savannah areas along rivers and streams. The group occurs along the river systems draining into the Atlantic Ocean, Mediterranean Sea and the inland drainage systems of some of the big African lakes, but not along river systems draining into the Indian Ocean (Jordan, 1986).

G. p. gambiensis occurs in humid and subhumid zones between Senegal and Benin. It is common in gallery forests, or in humid savannah areas with a fringing forest. *G. tachinoides* occurs from Guinea to the Central African Republic, east from

9th-20th degrees of longitude and north from 6th-14th degrees of latitude. Some isolated areas infested with *G. tachinoides* exist in Ethiopia. It mainly occupies areas along rivers and streams in the savannah areas of West Africa outside the lowland rain forests, but it can invade areas of cleared forest, peridomestic habitats and degraded forest areas.

G. fuscipes mostly occupies the Congo Basin, but the distribution extends further North to Chad, the Central African Republic, Cameroon, Gabon, Sudan, Ethiopia, Uganda, Kenya and Tanzania (Ford, 1971). In Kenya, the distribution of *G. fuscipes* is restricted to the area around Lake Victoria and along the main rivers and tributaries that flow into it (Jordan, 1986). *G. tachinoides, G. p. gambiensis* and *G. fuscipes* are efficient vectors of sleeping sickness; they are characteristically associated with the transmission of the chronic gambiense form of the disease typical of West and Central Africa. However, *G. fuscipes* along the shores of Lake Victoria and possibly *G. tachinoides* (in the western lowlands of Ethiopia) also transmit the acute rhodesiense disease.

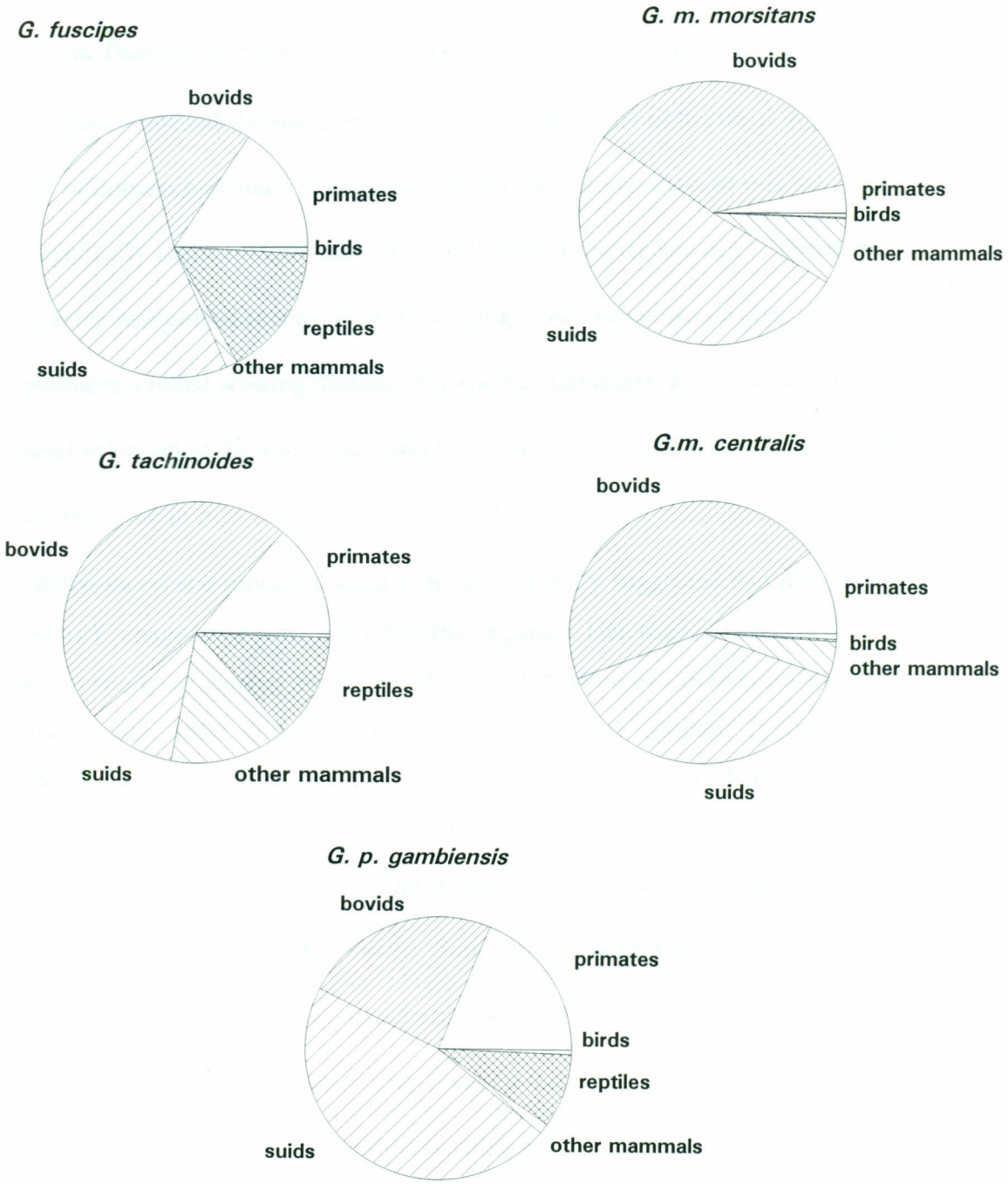
The species of the *morsitans* group occupy savannah woodlands and have the widest distribution in Africa. They are widely distributed in wetter areas; and in drier areas they are concentrated in patches of mesophytic vegetation near water. *G. m. morsitans* ranges from Mozambique and Zimbabwe in the south to Tanzania in the north. According to Jordan (1986), this subspecies infests the 'miombo' woodlands of East Africa and the 'mopane' woodlands in the Zambezi valley. *G. m. centralis* occurs from Botswana and Angola to southern Uganda. *G. m. submorsitans* has an east to west distribution from Ethiopia to Senegal inhabiting acacia woodlands. It also occurs in the Guinea Savannah and parts of Sudan (Jordan, 1986). *G. swynnertoni* occurs in northern

Tanzania and the adjoining parts of Kenya. *G. longipalpis* and *G. pallidipes* occur mainly in thickets and forest edges in West and East Africa, respectively. *G. austeni* occupies secondary shrub, thicket and islands of the forest along the East African coast from Mozambique to Somalia.

1.2.3 Feeding behaviour of tsetse flies

Tsetse flies are obligatory haematophagous insects feeding on blood obtained exclusively from wild vertebrate fauna, domestic animals and man.. If the bloodmeal contains trypanosomes, these may, depending on the trypanosome and the tsetse species, multiply in the fly and transform into infective metacyclic forms in the salivary glands or proboscis (Hoare, 1972). Host preference of *Glossina* can be determined through the identification of bloodmeals from recently-fed flies (Weitz, 1963). Feeding habits of *Glossina* are characteristic for each species. There is enormous evidence that available hosts are actively selected as a source of food. Tsetse flies have a preference for specific hosts, even though other species may be available in large numbers (Moloo *et al.,* 1971). Moloo (1993) summarised feeding habits of the three groups of flies (Fig. 1.1). Species of the riverine group are largely confined to the vicinity of water. Their sources of food are extremely variable. A characteristic of these species is that they can readily adapt themselves to whatever sources of food available (opportunistic feeders). They are known to feed largely on aquatic reptiles (*Varanus, Crocodylus* spp*).* In undisturbed areas, for instance along the river Nile, these reptiles provide the only source of food for *G. fuscipes*.

Fig. 1.1 Food habits of four species of tsetse
(Data adapted from Moloo, 1993)



1.2.4 Trypanosomes

African trypanosomes are hemoprotozoans that cause diseases in both man and animals. They belong to the order Kinetoplastida, family Trypanosomatidae and the genus *Trypanosoma*. The genus includes five well-differentiated subgenera, namely *Trypanozoon, Duttonella, Nannomonas, Pycnomonas* and *Schizotrypanum*.

Trypanosomes of the subgenus *Nannomonas* are some of the most important pathogens of domestic animals in sub-Saharan Africa. All members of this subgenus develop cyclically in the midgut and in the proboscis of the tsetse vector. There are currently three recognised species within this subgenus. *Trypanosoma congolense* which produces a lethal wasting disease of domestic ruminants, *T. simiae* which causes acute lethal infection in domestic pigs, and a new species, *T. godfreyi*, which causes a sub-acute infection in pigs (McNamara *et al.*, 1994).

Behavioural and morphological analysis originally suggested that trypanosomes classified as *T. congolense* were a heterogeneous group. Heterogeneity has been confirmed recently by isoenzyme and DNA characterisation of stocks from all over Africa (Gashumba, 1990; Gibson *et al.*, 1988). The nominal "species" *T. congolense* represents at least 4 subgroups of uncertain taxonomic status (Kilifi, Riverine/Forest, Savannah and Tsavo) (Garside & Gibson, 1995).

The subgenus *Trypanozoon* is represented by *T. b. brucei, T. b. gambiense, T. b. rhodesiense, T. equiperdum* and *T. evansi.* Some members of this subgenus, (*T. b. brucei, T. b. rhodesiense* and *T. b. gambiense*) are morphologically indistinguishable, but differ in the nature of the disease they cause in man and domestic animals. *T. b. gambiense* and *T. b. rhodesiense* are the causative agents of human sleeping sickness. The disease occurs in two main forms,the chronic and acute forms attributed to *T. b. gambiense* and *T. b. rhodesiense* respectively. Sleeping sickness occurs exclusively in

tropical Africa where the vector (*Glossina*) is present. *T. b. gambiense* is transmitted by riverine flies (*palpalis* group), *G. p. gambiensis* and *G. tachinoides* in West Africa and by *G. fuscipes* in East Africa. The *morsitans* group are the vectors of *T. b. rhodesiense*, e.g. *G. morsitans, G. swynnertoni* and *G. pallidipes. T. b. brucei* infects most domestic mammals, but is not often pathogenic. The main vectors of *T. b. brucei* are *G. morsitans* and *G. pallidipes* (*morsitans* group), *G. brevipalpis* (*fusca* group) and *G. fuscipes* (*palpalis* group). Animal trypanosomiasis is also encountered outside the tsetse fly belt, where the most important pathogenic trypanosome, *T. evansi,* a parasite of camels, is transmitted mechanically by biting flies. *T. equiperdum,* a parasite of horses, is transmitted sexually.

The subgenus *Duttonella* is represented by *T. vivax.* The parasite is pathogenic to various domestic ungulates (sheep, cattle, horses and camels). *T. vivax* is transmitted by tsetse and also mechanically by blood sucking *Diptera,* especially tabanid flies. The development of *T. vivax* in tsetse is confined to the proboscis (Hoare, 1972).

1.2.5 Life cycle of trypanosomes

Trypanosomes can be divided broadly into two groups; the Salivaria and the Stercoraria, which differ in their modes of transmission. Salivarian trypanosomes undergo cyclic development in the insect before being transmitted with the saliva, or are transmitted mechanically. Stercorarian trypanosomes also undergo development in the insect but the infective forms are deposited in the faeces of the vector (Hoare, 1972). African trypanosomes have well organised life cycles that span the tsetse and the vertebrate hosts. The life cycle stages refer to morphological characteristics, identified by shapes of the parasites as well as the organelles, the position of the

kinetoplast in relation to the nucleus and the extent of the flagella apparatus (Vickerman, 1985). When a tsetse fly feeds on an infected host, it ingests trypanosomes in the trypomastigote form. The trypomastigotes pass into the midgut where they are contained within the peritrophic membrane. The trypanosomes undergo developmental changes in the midgut and then migrate to the salivary glands *(Trypanozoon)* or the proboscis (*Nannomonas*) as infective metacyclics. When the tsetse feeds the infective metacyclics are injected into the host, and if the host is susceptible, another infection is started (Hoare, 1972). Transmission of salivarian trypanosomes is therefore inoculative. Stercorarian trypanosomes undergo similar development but metacyclics are found in the hindgut and passed out with faeces. If metacyclics are deposited on the skin of a susceptible vertebrate host, they penetrate either through abrasions or the intact mucous membrane, develop in muscle cells and release trypomastigotes into the blood where they are available to a vector. Transmission of stercorarian trypanosomes is therefore contaminative (Hoare, 1972).

1.2.6 Human trypanosomiasis (sleeping sickness)

Two forms of sleeping sickness are recognised in man; gambiense and rhodesiense sleeping sickness. The causative agents are *T. brucei gambiense*, and *T. brucei rhodesiense* (Hoare, 1972). *Trypanosoma b. gambiense* causes Gambian sleeping sickness endemic in West, Central and East Africa. This is usually a chronic human infection with fluctuating waves of parasitaemia. As the disease progresses, parasites disappear from peripheral circulation. The disease occurs mainly during the dry season. During this time shades for tsetse flies are restricted to water pools or lakes where people frequently visit. Transmission of *T. gambiense* is, therefore by the lacustrine and

riverine species *(palpalis)* of *Glossina* (Okoth & Kapaata, 1986*)*. Rhodesiense sleeping sickness is caused by *T. b. rhodesiense.* The disease is usually acute and is spread by the *morsitans* or savannah group tsetse. The disease occurs in East and Southern Africa (Jordan, 1986). The early stage of rhodesiense sleeping sickness is characterised by fever, headache and joint pains followed in the late stage by neurological symptoms and endocrine disorders.

1.2.7 Vectorial capacity of tsetse flies.

Pathogenic trypanosomes that infect tsetse flies follow specific routes during the course of their development (Hoare,1972; Pollock, 1980). These routes or sites have been used to categorise trypanosomes into broad groups. Thus, trypanosome infection of the hypopharynx and labrum only are attributed to the trypanosome subgenus *Duttonella* (or the *vivax* group), of the hypopharynx and gut to the subgenus *Nannomonas* (or *congolense* group), and that of the hypopharynx, salivary glands and gut to the subgenus *Trypanozoon* (or *brucei* group). The duration of trypanosome development in the fly also differs in relation to the life cycle of each subgenus, those of the *vivax* group generally taking the shortest time and the *brucei* group the longest (Pollock, 1980).

Knowledge of the vectorial capacity of any vector is important in order to understand the epidemiology of the disease as well as for control strategies. The infection rate is not only a function of the type of the trypanosome, but also a function of the medium in which the trypanosome is presented to the fly (Jordan, 1976). Field and laboratory studies on the infection rates of salivarian trypanosomes have shown that some species or subspecies are better vectors than others (Buxton, 1955; Harley

and Wilson, 1968; Roberts and Gray, 1972; Moloo and Kutuza, 1988 a, b). Trypanosome infection rate may therefore be used as an index of vectorial capacity of a particular tsetse species.

Harley and Wilson (1968) compared the ability of *Glossina morsitans, G. pallidipes* and *G. fuscipes* to transmit *T. congolense* in a controlled study in the laboratory. They found that *G. fuscipes* had a significantly lower infection rate than either *G. morsitans* or *G. pallidipes* and they concluded that *G. fuscipes* was less susceptible to infection with trypanosomes of the subgenus *Nannomonas* than the other species.

Roberts and Gray (1972) compared the efficiency of *G. m. submorsitans* and *G. tachinoides* as vectors of *T. congolense, T. simiae* and *T. vivax*. They showed that *G. m. submorsitans* was a more efficient vector of trypanosomes of the subgenus *Nannomonas* than *G. tachinoides.* Nearly all infections in *G. m. submorsitans* were mature, with trypanosomes in the hypopharynx and other organs of the flies, whereas the majority of the infections in *G. tachinoides* were immature, with trypanosomes present only in the gut of the flies. Both species were equally efficient vectors of *T. vivax.*

Under field conditions it is rare to find *G. fuscipes* with mature *T. brucei* infections even though wild animals from which flies derive their meals may be infected. Gibbins (1941) working in the Nile District of Uganda dissected 2,122 *G. fuscipes* but found none infected with trypanosomes, despite the fact that 215 cases of sleeping sickness had been diagnosed in the same area. The poor vectorial capacity of the *palpalis* group of flies has been reviewed by Stephen (1986). Moloo and Kutuza (1988a) showed that *G. p. palpalis*, G. *p. gambiensis, G. fuscipes* and *G. tachinoides*,

all *palpalis* group tsetse, are poor vectors of Nigerian and Tanzanian *T. congolense* compared with *G. m. centralis*.

1.2.8 Vector-parasite-host interactions

During the life cycle of the African trypanosomes the parasites undergo various developmental changes within the mammalian host and the tsetse vector (Hoare, 1972). Within the vector, these changes start immediately upon ingestion of an infected bloodmeal and result in the formation of procyclics from the bloodstream forms of the parasite (Englund *et al.,* 1982). This process involves a series of complex morphological and physiological changes that enable the parasite to adapt to a radically different environment within the fly's midgut.

The establishment of infection is a complex process with many infections lost after the initial establishment. Determination of the many factors that influence the establishment of trypanosomes in *Glossina* has been the subject of extensive research reviewed by many authors (Jordan, 1974; 1976; Molyneux, 1980; Maudlin, 1991; Molyneux & Stiles, 1991). Molyneux grouped all the factors involved under three broad headings: These include. (1) endogenous factors associated with the fly (fly's age at infective feed, sex of fly, genetic differences between and within fly's species, biochemical and physiological state of the fly and concurrent infections of the fly); (2) ecological factors (climate, availability of infected hosts, hosts available for subsequent feeding); (3) parasite and host (parasite numbers available to fly, type of parasite and its infectivity to the fly, immune state of the host and its' attractiveness to the fly).

The trypanosome infection rates of *Glossina* have been shown to be related to their hosts. Hosts are therefore major determinants in the epidemiology of African trypanosomiasis. Lloyd and Johnson (1924) found a higher infection rate in *G. tachinoides* caught where wild ungulates were common than in an area where they were rare. Jordan (1965) found that infection rates in *Glossina* in Nigeria were related to the percentage feeding on Bovidae. In a study on the relationship between hosts and trypanosome infection rates, Moloo (1973) found that variation in the feeding patterns of *G. swynnertoni* in six different areas within the Ikoma-Serengeti area of Tanzania resulted in differences in the level of trypanosome infection and in the proportions of *T. vivax*-type organisms. He further showed that infection rates with *T. vivax* decreased as the percentage of meals derived from Suidae increased, but increased with increasing number of feeds from Bovidae. *Congolense*-type infection remained the same regardless of the contribution from either of these two families. These results suggest that the source of the bloodmeal is an important factor in determining trypanosome infection level in natural populations. Host blood type, particularly at the time of the infective feed has been shown experimentally to influence the subsequent trypanosome infection rates in tsetse (Mihok *et al.,* 1993; Olubayo *et al.,* 1994).

Local variations in the level of trypanosome infection in a tsetse population may result from differences in the wildlife populations (Ford and Leggate, 1961), since some wildlife species are better reservoirs of infection than others (Ashcroft *et al.,* 1959). A number of environmental factors have also been examined for their effects on the susceptibility of *Glossina.* Fairbairn and Culwick (1947) showed that raising the incubation temperature of puparia and the maintenance temperature of adult *G. morsitans* increased the mature infection rate with *T. b. rhodesiense.* Fairbairn and

Watson (1955) showed that *T. vivax* infection rates in *G. palpalis* increased with increasing incubation temperature of puparia but decreased with increasing fly's maintenance temperature. In the field, a close relationship exists between infection rates, particularly with *vivax*-type infections, and mean annual temperatures (Ford and Leggate, 1961). The effect of puparial incubation temperature has been confirmed experimentally by Ndegwa *et al.,* (1992).

Breeding experiments have demonstrated large differences in *T. congolense* infection rates between the genotypes of *G. m. morsitans*; these differences are dependent solely on the genotype of the female parent (Maudlin, 1982). Maudlin and Dukes (1985) showed that differences between reciprocal crosses of *G. m. morsitans* in susceptibility to *T. congolense* infection persist over many generations and concluded that susceptibility/refractoriness is an extrachromosomally inherited character.

Field data have shown that trypanosome infection rates typically increase with fly's age (Tarimo *et al.,* 1985; Woolhouse *et al.,* 1993; Leak and Rowlands, 1997). However, lower susceptibility to infection among older flies has been shown for laboratory maintained tsetse exposed to *T. brucei* and *T. congolense* (Welburn and Maudlin, 1992). Some of these trends have however been attributed to the late maturation of *T. b. brucei* (Dale *et al.,* 1995).

The sex of the fly is also important in determining infection rates. Under laboratory conditions, salivary gland infection rates of *T. brucei* subspecies are higher in male than in female tsetse (Maudlin and Dukes, 1985; Dale *et al.,* 1995). On the other hand, Moloo (1981) found that female *G. m. morsitans* had higher infection rates than males when infected with *T. congolense.*

Another factor to consider in determining infection rates are the differences among trypanosome stocks in their establishment in tsetse. Mature infection rates of some *T. congolense* stocks (Masake *et al.,* 1987) are quite low in *G. m. centralis* which is normally an efficient vector (Moloo & Kutuza, 1988a). A given trypanosome population probably also plays an important role in its' establishment in a tsetse vector. Genetic exchange between trypanosomes occurs in tsetse. The frequency of genetic exchange may differ among different trypanosome serodemes (Schweizer *et al.,* 1988). It is therefore possible that factors in different *Glossina* species and sexes may influence the frequency of genetic exchange as well as maturation patterns. This may explain inconsistencies in the vector's competence of different *Glossina* spp. to different populations of *T. vivax, T. congolense* and *T. brucei* (Moloo and Kutuza, 1988a, b).

Establishment of trypanosomes in the tsetse fly's gut involves complex interactions between the fly, endosymbionts and the trypanosome itself (Maudlin and Ellis, 1985; Welburn & Maudlin, 1991). The process is mediated by several factors such as (1) midgut lectins (Maudlin & Welburn, 1987; 1988; Welburn *et al.,* 1989); (2) trypanoagglutinins and trypanolysins (Ibrahim *et al.,* 1984; Ingram and Molyneux, 1991) and (3) digestive proteases (Stiles *et al.,* 1991; Imbuga *et al.,* 1992a; Osir *et al.,* 1993). Some workers have proposed that the peritrophic membrane might influence the development of the gut form trypanosomes (Lehane and Msangi, 1991).

Tsetse possess at least seven gut proteolytic enzymes (Cheesman and Gooding, 1985); some of these are presumably responsible for the high mortality of bloodstream forms after ingestion by the fly. *In vitro*, bloodstream forms are susceptible to lysis by preparations from tsetse (Imbuga *et al.,* 1992a, b). In contrast, procyclic forms appear

to be rather insensitive, establishing incipient infections in the gut for a few days before experiencing mortality (Welburn *et al.,* 1989). Agglutinins (lectins) are a group of carbohydrate binding proteins with ubiquitous distribution in nature. Ibrahim *et al.,* (1984) first demonstrated in the midgut of the tsetse *G. austeni* the presence of an agglutinin which had a particular specificity for D-glucosamine and a lesser affinity for N-Acetyl glucosamine in vitro. Feeding D-glucosamine to tsetse with the infective feed was shown to have a profound effect on midgut infection rates, with more than 90% teneral *G. m. morsitans* establishing midgut infections (Maudlin and Welburn, 1987). This suggested that the normal action of the midgut lectin *in vivo* was to prevent colonisation of the fly's gut by parasites. Mihok *et al.,* (1992c) confirmed the *in vivo* action of D-glucosamine in *G. m. centralis* and *G. pallidipes*. Midgut lectin activity in *G. p. palpalis* can also be inhibited by glucosyl moieties with glucosamine being the most efficient inhibitor (Welburn *et al.,* 1994). *G. p. palpalis* and *G. pallidipes* midgut infection rates were increased by the addition of galactose alone in contrast to *G. m. morsitans* where this sugar had no effect (Maudlin and Welburn, 1987). D-glucosamine significantly increased midgut infection rates of older *G. m. morsitans* and *G. p. palpalis* to levels comparable to those of teneral flies (Welburn and Maudlin, 1992, Welburn *et al.,* 1994). This suggests that the peritrophic membrane does not present a physical barrier to infection in older flies; nor can the developmental state of the membrane be involved in the enhanced susceptibility of teneral flies. Higher titres of glucosyl lectin in fed flies compared with tenerals indicate that a single bloodmeal greatly increases the rate of trypanosome killing in the midgut (Welburn *et al.,* 1989). Super infections have been produced in flies infected with procyclic forms by

providing serum free infective feeds (Maudlin &. Welburn, 1984), suggesting trypanoagglutinating activity is switched on by bloodmeal serum in *G. m. morsitans*.

According to a model proposed to explain this process, the lectins mediate both lysis and differentiation of the parasites (Maudlin, 1991). Many tsetse species harbour intracellular endosymbiotic micro-organisms associated with gut tissue. Phylogenetic characterisation has shown that these organisms constitute a distinct lineage of Proteobacteria and have evolved concordantly with their insect host species, suggesting an evolutionary ancient association for this symbiosis (Aksoy *et al.,* 1997). This close evolutionary association confirms the obligatory functional role these organisms play in the biology of tsetse. In susceptible tsetse the action of an endochitinase produced by the micro-organisms leads to an accumulation of glucosamine in the fly's midgut, which in turn blocks the lectin-mediated trypanocidal activity (Molyneux and Stiles, 1991). In contrast, refractory flies with few micro-organisms produce relatively less glucosamine and the parasites entering these flies are consequently more vulnerable to lysis by the lectins.

G. pallidipes and *G. p. palpalis* have been shown to be inherently more trypanocidal than *G. m. morsitans* (Welburn *et al.,* 1989). Ingram & Molyneux (1990) showed that midgut extracts of *G. p. palpalis* displayed lytic activity. Stiles *et al.,* (1990) suggested that a trypanolysin as well as agglutinin may be active in the posterior midgut of the *palpalis* group flies, adding to the innate resistance of these flies to midgut infections. Welburn *et al.,* (1994) confirmed the presence of trypanolysin in the midguts of *G. p. palpalis* and also showed that trypanocidal activity residual to that caused by the glucosyl lectin is blocked by galactose. The haemolysin and the galactosyl lectin, may well be the same molecule.

1.2.9 Trypanosomes of reptiles

The first record of a trypanosome in a reptile was by Laveran and Mesnil (1902), who described *Trypanosoma damoniae* in the tortoise. Since their observation, 43 species of trypanosomes have been described in tortoises, snakes, lizards, iguanids, chamaeleonids and crocodiles (Telford *et al.,* 1995 a, b).

Lloyd *et al.,* (1924) in Nigeria found two species of monitor lizards (*Varanus exanthematicus* and *V. niloticus)* infected with *T. varani*, as well as crocodiles infected with *T. grayi*. The vectors of these two trypanosome species were considered to be *G. palpalis* and *G. tachinoides* (Lloyd and Johnson, 1924; Lloyd *et al.,* 1924). *T. varani* has also been described from crocodiles (Lloyd *et al.,* 1924). Minter-Goedbloed *et al.,* (1983) described a trypanosome obtained from *G. pallidipes* in an area where monitor lizards were frequent. The trypanosome underwent development in the hindgut of the fly. It is still not clear whether the parasite was *T. grayi* or *T. varani* since both parasites undergo stercorarian development being extruded from the posterior hind gut with faeces.

1.2.10 Wildlife reservoirs of disease.

African trypanosomiasis is endemic in wildlife, but whereas domestic animals succumb to the infection, wild animals generally show a high degree of resistance. Many species of wildlife living in tsetse-infested areas carry trypanosome infections, show no obvious disease symptoms, and so act as carriers (reservoir hosts). Wild animals are often highly resistant or even completely refractory to disease, although they often harbour trypanosome infections. Ashcroft (1959) summarised the results of several

surveys; out of 1242 wild animals examined, 19.5% were infected. *T. brucei* accounted for 4.25%, *T. vivax* for 6.3% and *T. congolense* for 10.4%. As the majority of cases were diagnosed by blood slides, it could be that these cases were underestimated. Improvements in techniques of parasite detection and identification over the years have extended the list of infected animals and indicated a different pattern. In general the number of animals harbouring cryptic trypanosomes is in excess of those showing detectable parasitaemia, even when using a combination of diagnostic methods (Dillmann and Townsend, 1979).

1.3 Conceptual basis of the study

The *palpalis* group of flies occupying riverine habitats have been responsible for the great epidemics of sleeping sickness in East, West and Central Africa. Reptiles constitute a major food source of these riverine *Glossina* (Okoth and Kapaata, 1988; Moloo, 1993; Mohammed Ahmed & Odulaja, 1997). There exists a very close contact between man, livestock and reptiles at watering points along rivers, streams and lake shores. The flies are also known to occupy peridomestic habitats. The hosts utilised by flies are major determinants in the epidemiology of trypanosomiasis. Except for the early studies of Woo and Soltys (1969) which showed that trypanosomes could survive in American reptiles (caymans) and still retain their infectivity, no work has been carried out to determine the role played by reptiles in the epidemiology of trypanosomiasis. For instance, it is not known whether trypanosomes are able to survive in a pivotal host (the monitor lizard) and for how long. It is also unknown whether these trypanosomes retain their infectivity to tsetse and laboratory rodents. The

behaviour of trypanosomes in tsetse that feed almost exclusively on monitor lizards is also unknown. Studies were therefore carried out in the Lake Victoria region, on trypanosomiasis in the monitor lizard, aspects of which may be important in the overall understanding of the role of these reptiles in the disease transmission cycle and thus help in formulating control strategies for the disease.

1.4 Objectives of the study

1.4.1 General objective

The main aim of the study was to determine the role of reptiles in the natural transmission cycle of trypanosomes between tsetse flies, man and livestock.

1.4.2. Specific objectives

(1) To examine wild monitor lizards for the presence of trypanosomes.

(2) To determine infectivity of different trypanosome stocks/species to monitor lizards.

(3) To determine the effect of monitor lizard blood on the survival of different species of flies.

(4) To determine the effect of monitor lizard blood on survival of trypanosomes *in vitro* and infectivity of different stocks of trypanosomes in tsetse.

CHAPTER TWO

2.0 THE OCCURRENCE OF NATURAL INFECTIONS OF PATHOGENIC TRYPANOSOMES IN THE MONITOR LIZARD *VARANUS NILOTICUS* LAURENTI

2.1 Introduction

The Lake Victoria region in both Kenya and Uganda has been a historic focus for sleeping sickness since the early part of this century. The first reported epidemic in Uganda was at the turn of the century when an estimated third of a million people probably died (Mbulamberi, 1989). The causative agent for this epidemic was thought to be *Trypanosoma brucei gambiense,* although this has been challenged recently (Enyaru *et al.,* 1997). In Kenya, sleeping sickness epidemics due to *T. brucei rhodesiense* have been reported in parts of Western and Nyanza provinces (Wellde *et al.,* 1989). These epidemics have been due to the growth and spread of *Glossina fuscipes* (Okoth & Kapaata, 1986; 1988) with the site of maximum man-fly contact being near water.

Although *Glossina fuscipes* is a poorer vector than other tsetse species (Stephen, 1986; Moloo & Kutuza, 1988 a, b), it readily bites man and therefore transmits trypanosomiasis due to its peridomestic habits. The flies can extend their distribution from the normal riverine and lacustrine habitat to human settlements. *G. fuscipes* colonises *Lantana* thickets and other vegetation surrounding homesteads. This was confirmed by Okoth and Kapaata (1988) who showed that *Glossina fuscipes* not only rested in vegetation surrounding homesteads in Busoga, Uganda but also bred there. This behaviour results in intense man-fly contacts even at very low fly density

and provides the basis for a man-fly-man cycle of infection. The peridomestic behaviour of *G. fuscipes* in Uganda and in Kenya does not appear to be linked to any domestic animal as in the case of West Africa for *G. tachinoides* and *G. palpalis* and pigs (Sekeketi and Kuzoe, 1984). Hence, removal of domestic animals would probably not reduce the peridomestic population of *G. fuscipes.* When natural habitats and wild hosts are destroyed, *G. fuscipes* will adapt itself to the peridomestic habitat.

In Kenya *Glossina fuscipes* is restricted to the area around Lake Victoria and along the main rivers and tributaries that flow into it (Jordan, 1986). Large populations are especially present near Rusinga Island. Species of wild animals found on the island include bushbuck, dikdik, hippopotamus, monkeys and monitor lizards. With the exception of lizards, other animals are found in low numbers (Mwangelwa, 1990). Monitor lizards are the major food source of *Glossina fuscipes* in the Lake Victoria region in Kenya (Mohammed-Ahmed & Odulaja, 1997). Okoth and Kapaata (1988), while working in sleeping sickness endemic foci in Uganda found similar food preferences. Lizards are therefore of considerable importance as maintenance hosts and are potentially a reservoir of trypanosomes.

In Africa, wild fauna harbour trypanosome species that are pathogenic to man and domestic animals, without showing symptoms of the disease. The wild fauna and some domestic animals therefore act as reservoir hosts. Their tolerance to these parasites is the result of an evolved association as original and natural hosts. Many surveys have been carried out to determine trypanosome infection rates in wildlife (Dillman and Townsend, 1979). These studies revealed general trends on the prevalence of trypanosomes, but in most cases the sample sizes were small (Wells and

Lumsden, 1968). Hence, specific mechanisms responsible for disease transmission cycles between and among wildlife and livestock species remain poorly understood.

The history of sleeping sickness in East Africa has provoked controversy not only about the origins and spread of the disease, but also the identity of the causative organisms (Hide *et al.,* 1994; 1996). The occurrence of outbreaks in isolated pockets along the Lake Victoria shores after periods of quiescence suggests that the nature of the disease, its' epidemiology, and control strategies are still not fully understood. In Uganda, vector control, when sustained, has been effective at reducing levels of trypanosomiasis in humans. Epidemic types of *T. brucei* have nevertheless persisted for many years and can still be found in areas not under control like Buvuma island (Enyaru *et al.,* 1997). These residual foci could be the origins of sporadic epidemics on the mainland. There is also a possibility that cryptic hosts may be acting as reservoirs of the disease. Cattle are also clearly capable of acting as hidden reservoirs (Maudlin *et al.,* 1990).

Lizards have received little attention as potential cryptic reservoirs as they are thought to harbour trypanosomes not infective to man. Except for the historic study of Woo and Soltys (1969) no concerted effort has been made to assess the importance of reptiles as reservoirs of any pathogenic trypanosome. It is not known whether lizards naturally harbour trypanosomes infective to man and livestock. In this study, experiments were carried out to determine the occurrence of trypanosome infections in wild monitor lizards, (important food sources of 'riverine' *Glossina,* the vectors for human sleeping sickness) at the Lake Victoria shore.

2.2 MATERIALS AND METHODS.

2.2.1 Study area

Surveys were carried out at two locations along the shores of Lake Victoria (Fig. 2.1). (1) Rusinga Island is located in the Mbita Division, Suba District in Nyanza Province, Kenya. This region is in the eastern part of Lake Victoria. It is located between latitudes 0 20' and 0 30' South and longitudes 34 06' and 34 15' East. Rusinga Island joins the mainland at Mbita Point by a causeway about 50 m long. (2) Busia District at Sio Port in Western Province, Kenya. The area is at the border of Kenya and Uganda and is in close proximity to villages in S. E. Uganda (endemic foci for sleeping sickness).

2.2.2 Collection of monitor lizards

Monitor lizards (*Varanus niloticus*) were obtained from Sio Port in Busia District, (Kenya) and the Rusinga Island area of Lake Victoria. Hooks and traps baited with fish were used to catch the lizards. The traps were set at strategic points about one to two metres from the water edge at the lake shore (Plate2.1 a, b). Lizards attracted to the fish bait were caught in either the hooks or the traps. The lizards were caught in the morning hours between 10.00 am and 12.30 pm as they came out of the water to bask.

2.2.3 Goats

East African goats were used in this study. These had been diagnosed previously as trypanosome-free using xenodiagnosis (this involves feeding teneral *G. m. centralis* on goat blood followed by dissection of flies six days post feeding to determine the

Fig 2.1 Map of the Lake Victoria shores in Kenya and Uganda showing Rusinga Island and Sio Port study areas.

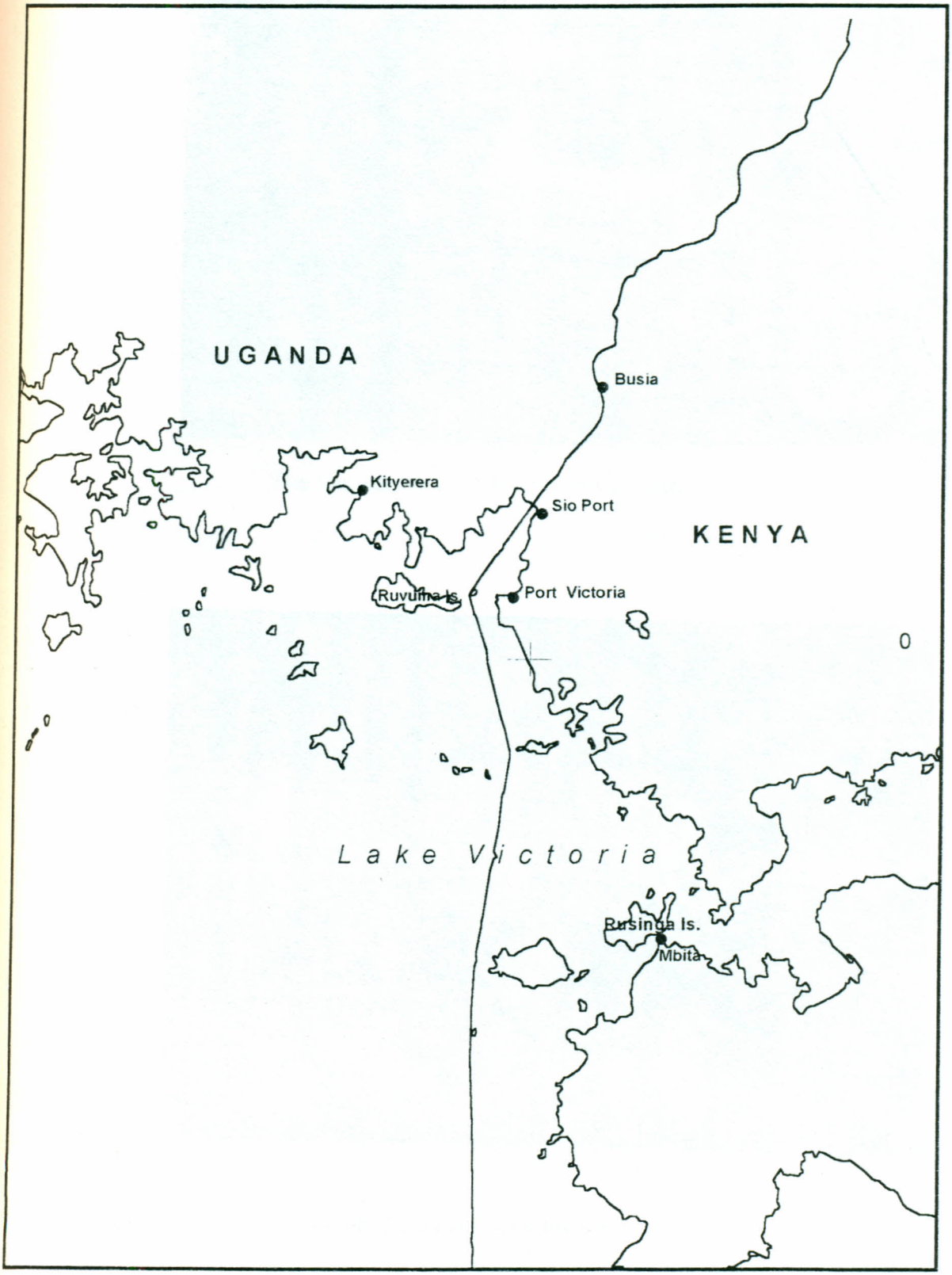


Plate 2.1a

The trap used for catching monitor lizards

Plate 2.1b

A monitor lizard caught by the hook method

presence or absence of midgut procyclic forms of trypanosomes). The goats were kept in fly-proof pens and maintained on a diet of hay, protein supplements and water.

2.2.4 Rats used to grow trypanosomes

Male laboratory-bred Wistar rats were used. These were obtained from the Animal Rearing and Quarantine Unit, at the International Centre of Insect Physiology and Ecology (ICIPE).

2.2.5 Tsetse flies used to raise parasites

Teneral male *G. m. centralis* were obtained from colonies bred at ICIPE. The flies were maintained under controlled conditions at 25^0 C and a relative humidity of 65-80% in holding rooms with 12 h of light and 12 h of darkness. The *G. m. centralis* colonies were reared on NewZealand White rabbits.

2.2.6 Bleeding of monitor lizards

Blood from lizards was obtained from the ventral tail vein as described by Esra *et al.*, (1975). Monitor lizards were restrained manually, the tail cleaned in 70% alcohol and a 19 G (1½") needle inserted into the caudal vein at the tail (Plate 2.2). The blood was allowed to flow into evacuated tubes containing heparin as an anticoagulant. The blood was either used immediately or cryopreserved in liquid nitrogen until required.

Plate 2.2

Bleeding a monitor lizard from the ventral tail vein

2.2.7 Bleeding of goats

Blood from goats was obtained by jugular venipuncture. This was done by introducing a Gauge 18 needle into the jugular vein and allowing the blood to flow into evacuated tubes containing heparin as anticoagulant.

2.2.8 Cryopreservation of blood

Blood was cryopreserved in aliquots of 2 ml. Fresh blood was mixed with 15% glycerol, and left for 25 minutes at room temperature to equilibrate before storage in 2 ml cryogenic tubes. The mixture was pre-cooled in the vapour phase of liquid nitrogen for 2-6 hours, and then immersed in the liquid phase for permanent storage.

2.2.9 Diagnostic techniques for detecting trypanosomes in lizards

2.2.9.1 Preparation of thin, thick and wet blood smears

Thin, thick and wet blood smears were prepared using small amounts of blood as described by Baker (1970). For each of the tests 5 µl of blood was used. Following rapid air drying, the thick film preparation was placed in distilled water for 5 min. to dehaemoglobinise, whereas thin films were fixed in methanol. Thick, and thin smears were then stained in 10% Giemsa for 30 min. Subsequently, 200 fields were viewed under oil immersion. The wet film was examined by phase contrast microscopy at 400 X to detect bloodstream trypanosomes.

2.2.9.2 Dark ground/ phase contrast buffy coat technique (DG)

The buffy coat zone, prepared in a micro-haematocrit capillary tube filled with 70 µl blood and centrifuged for 5 min at 1200 rpm, was examined for trypanosomes as follows: the capillary tube was cut with a diamond pointed pen 1 mm below the buffy coat to incorporate the uppermost layer of the erythrocytes and 1 cm above to include some plasma. The contents of the tube were gently released on to a clean microscope slide, covered with a 22 x 22 mm coverslip and observed under phase contrast and dark ground illumination. The packed cell volume (PCV) of the lizards was read on the microhaematocrit reader before carrying out the parasitological examination.

2.2.9.3 The kit for *in vitro* isolation technique (KIVI) for trypanosomes.

Monitor lizard blood (10 mls) was drawn into a syringe containing 5% Liquid Roche (sodium polyanetholesulphonate) anticomplementary anticoagulant (Le Ray *et al.*, 1970). The blood mixture was then dispensed equally into two vials, each containing 10 ml of GLSH-DCA and sealed with parafilm. The culture medium (GLSH-DCA) includes glucose, lactalbumin, serum and haemoglobin (Le Ray, 1975) diluted with an equal volume of Hanks solution (Le Ray *et al.*, 1970) and complemented with 3mM cis aconitate (Brun and Schonenberger, 1981). KIVI was carried out as described by Truc *et al.*, (1992). The vials were mixed by gentle manual agitation and kept in a dark cupboard at 27 °C. The inoculated vials were examined for trypanosomes at least twice a week by microscopic examination of samples of the culture medium. If the KIVI was negative 4 weeks following inoculation it was discarded.

2.2.9.4 Inoculation of blood into laboratory-bred rats.

Monitor lizard blood was inoculated into immunosuppressed rats in order to facilitate infections. Immunosuppression was done one day before infection by injecting freshly prepared cyclophosphamide solution into the peritoneal cavity of rats at a dose of 200 mg/kg. About 0.5 ml of lizard blood was inoculated intraperitoneally into each rat. Five rats were used for each lizard sample. Wet film smears of rat tail blood were examined 3 times a week for 30 days for the presence of trypanosomes (before the animals were assumed to be free of infection). If the rat was infected it was bled through cardiac puncture and the parasites cryopreserved in liquid nitrogen for characterisation studies.

2.2.9.5 Xenodiagnosis with susceptible tsetse.

Xenodiagnosis with *G. m. centralis* was used to isolate and characterise infections in lizards. Monitor lizard blood was mixed with goat blood (which facilitates infection) in the ratio 1:4 and used to membrane feed 50 male teneral *Glossina morsitans centralis* on day 0. The flies were subsequently fed on day 3 on goat blood. On day 6 the flies were immobilised by brief chilling, wings removed and dissections of the midguts carried out to detect procyclic forms.

2.3 Characterisation of trypanosomes

2.3.1 Infection of flies

In order to establish identities of isolated parasites, 50 teneral male *G. m. centralis* were infected with 1 x 10^4 trypanosomes/ml from stored material. The flies were maintained on goat blood until day 33. The flies were starved for two days and dissections carried out between days 35 and 37 on the gut, proboscides and salivary glands for mature parasites. If parasites were found in the labrum and proboscides only,

they were attributed to the *Nannomonas* subgenus, whereas salivary gland infections were attributed to the *Trypanozoon* subgenus (Hoare, 1972).

2.3.2 Polymerase chain reaction (PCR)

2.3.2.1 Preparation of template DNA for PCR

A trypanosome stabilate from the infected lizard, initially cryopreserved in liquid nitrogen in the field, was grown in different mice until parasites reached a high parasitaemia (1×10^5 parasites/ml). The trypanosomes were separated from blood by DEAE cellulose column chromatography (Lanham & Godfrey, 1970). Trypanosomes separated in PSG, (pH 8.0) were pelleted by centrifugation at 12000g for 5 minutes. DNA was purified by extraction with phenol and recovered by ethanol precipitation (Maniatis *et al.,* 1982).

2.3.2.2 PCR amplification

Polymerase chain reaction (PCR) is an *in vitro* technique which permits amplification of a specific DNA region that lies between two regions of defined oligonucleotide primer sequences. The PCR technique allows a million-fold or more amplification of a specific DNA sequence in the presence of a thermostable DNA polymerase derived from the bacteria *Thermus aquaticus*. Development of PCR assay relies on identification of specific DNA sequences as target molecules, flanked by the selected oligonucleotide primers. The target DNA can then be amplified in the presence of the defined primers, DNA polymerase and a nucleotide mixture to supply the required bases. PCR amplification of microsatellite target DNA sequences enables low numbers of trypanosomes to be identified (Masiga *et al.,* 1992, 1996; Majiwa *et al.,* 1987; Gouteux & Gibson, 1996).

In this study a total of 5 µl of the trypanosome cell lysate was used for amplification of species specific sequences in PCR in a 100 µl reaction mixture. This mixture contained 10 mM Tris-HCl pH 7.5, 50 mM KCl, 0.02% BSA/ml, 2.0 mM $MgCl_2$, 1 µl of each of the four deoxynucleotide triphosphates (dNTPs), 1 unit of Taq (*Thermus aquaticus*) DNA Polymerase and 0.5 µl of each of the oligonucleotide primers specific for *T. brucei*, Kilifi and Tsavo *T. congolense* (Majiwa *et al.,* 1994). The reaction mixture was overlaid with light mineral oil to prevent evaporation during the repeated heating and cooling. The samples were subjected to 35 amplification cycles in a programmable DNA thermal cycler (Autogene II). Amplification conditions were as follows: 94°C for 1 minute (denaturation), 55°C for 3 minutes (annealing), 72°C for 1 minute (extension). The reaction mixture was cooled to 4°C. The amplified PCR products were thoroughly but gently mixed with 8 µl of DNA gel loading buffer (10 mM Tris/Hcl, (pH 8.0), 25 mM EDTA (pH 8.0),10% w/v Ficoll, 0.01 % w/v bromophenol blue). Seven microliters of each sample were resolved by electrophoretic fractionation in a 2% Ultra pure agarose gel, stained with ethidium bromide and photographed under an ultraviolet transilluminator using Polaroid 667 film.

2.3.3 Hybridisation with species-specific DNA probes

The underlying principle of DNA hybridisation is that a specific fragment of the DNA (the probe) complementary in sequence to the DNA to be detected (target) is cloned from the organism of interest or synthesised chemically, labelled and then used to detect its complementary copy which is usually immobilised on a solid support (Nylon or nitro-cellulose membranes). Different approaches have been used to isolate highly

repetitive DNA sequences which can identify species and subspecies of different African trypanosomes (Majiwa *et al.,* 1994).

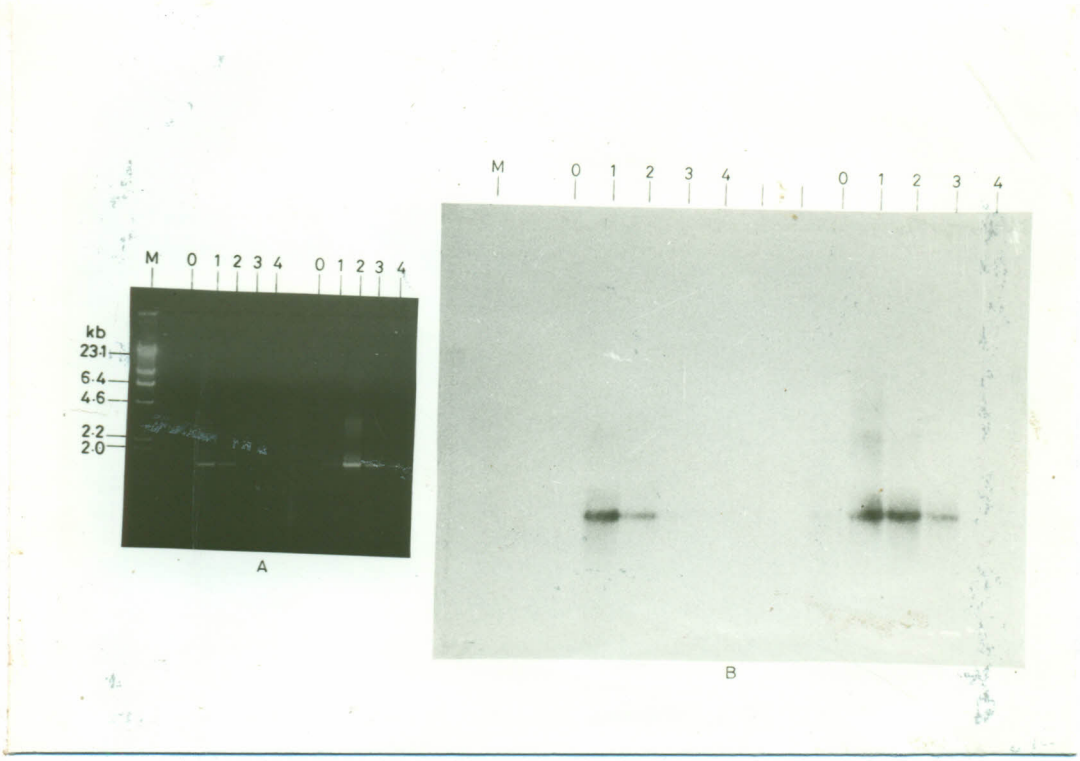
In the present study the products of PCR amplifications were fractionated on 2% agarose gels in TAE (Tris-Acetate-EDTA) buffer following standard procedures (Maniatis *et al.,* 1982). The DNA's were then transferred onto nitrocellulose sheets and hybridised for 16-20 h at 65°C with ^{32}P- labelled pgDR1 ingi, a cloned DNA probe specific for *Trypanozoon* (Kimmel *et al.,* 1987). The nitrocellulose filters were air dried and autoradiographed overnight at -70°C with X-ray film (Fuji RX- 100) and intensifying screens (Dupont Cronex)

2.4 Results

2.4.1 Diagnosis of monitor lizards for trypanosomes

Twenty seven monitor lizards (27) were trapped from Rusinga Island whereas 19 were caught from Sio Port, over a period of 1½ years. No trypanosomes were detected in any of the lizards caught from Rusinga Island. Out of the lizards examined from Busia, 5.3 % (n=19) were naturally infected with trypanosomes. These parasites were detected using inoculation into immunosuppressed rats and xenodiagnosis using *G. m. centralis* (but not through the other diagnostic techniques). Out of five rats inoculated with blood from the infected lizard, three were positive with trypanosomes 11, 15 and 17 days post inoculation. Xenodiagnosis using *G. m. centralis* revealed procyclics 6 days post infection in 6.8% (n=44) of the flies. The DNA of trypanosomes from the infected lizard was amplified with primers specific for two *T. congolense* subgroups (Kilifi and Ngulia) and *T. brucei.* No amplification product was obtained using *T. congolense* primers whereas an amplification product of 800 bp was obtained using the *T. brucei*

Plate 2.3



(A) = Agarose gel plate stained with ethidium bromide showing an 800 bp PCR amplification product typical of *T. brucei* using primer ILO342.

(Lane M: Molecular weight markers, Lane 0: Negative control, Lanes 1-3 serial 10-fold dilutions of target DNA)

(B) = Autoradiograph showing hybridisation of the *Trypanozoon*- specific DNA probe PgDR1 with the 800 bp PCR amplification product in A.

Ngulia) and *T. brucei*. No amplification product was obtained using *T. congolense* primers whereas an amplification product of 800 bp was obtained using the *T. brucei* primer (Plate 2.3A). To confirm identity, the product of PCR amplification with *T. brucei* was diluted 1:1000 and hybridised with pgDR1, a segment of the ingi probe specific for *Trypanozoon*. There was strong hybridisation with pgDR1 (Plate 2.3B). Using xenodiagnosis to characterise the isolated parasite, mature metacyclis of *T. brucei* were found in the salivary glands in 10.5 % (N=38) *G. m. centralis* dissected on day 35 post infection.

2.5 Discussion

In the past, it was assumed that wild animals, particularly bushbuck, were the most important reservoirs of sleeping sickness, presumably because the first human-infective trypanosome was isolated from a bushbuck (Heisch *et al.*, 1958). Since then, surveys for potential reservoirs for sleeping sickness in the Lake Victoria region have found trypanosomes in cattle, dogs and goats (Wellde *et al.*, 1989; Hide *et al.*, 1996). These recent findings have coincided with the decline of wild animal populations both in Kenya and Uganda (Hide *et al.*, 1997). The diagnosis of a current infection with African trypanosomes in animals can only be made by demonstrating the parasite in the blood or tissue fluids of infected animals. The sero-diagnostic techniques which are available depend on the demonstration of circulating antibodies and as a result cannot by themselves confirm an active infection. However, the reliability of many of the parasitological techniques currently in use for detecting trypanosomes in naturally occurring field cases, where parasites are often low and sporadic, is often questioned.

In this study, trypanosomes were isolated from 5.3 % of monitor lizards obtained from Busia. Although a combination of diagnostic techniques was used, parasites were detected using mouse inoculation and xenodiagnosis only. The kit for *in vitro* isolation of trypanosomes which allows direct inoculation of host's blood into a culture medium, with the subsequent transformation and multiplication of procyclic trypanosomes is a sensitive technique, but limited by a long time lag before the organisms multiply adequately in culture. Moreover, infections may be missed because of microbial contamination inhibiting trypanosome multiplication (Truc *et al.,* 1994). In this study, KIVI was not able to detect trypanosomes in the lizards. This failure of the KIVI technique has previously been reported by McNamara *et al.,* (1995) who showed that 20% of stocks from parasitaemic patients did not grow in KIVI. The failure of KIVI suggests that the technique is either unsuitable for isolating trypanosomes at certain stages of the infection or that it may be selective for some populations of trypanosomes. The results of this study have confirmed the difficulty of using simple parasitological techniques for the detection of cryptic widlife reservoirs. Even with the use of immunosuppressed rats, a long observation period was required to detect the infected lizards, presumably because trypanosomes were present at very low parasitaemia. A single trypanosome however, is sufficient to infect a tsetse fly, provided that the fly is susceptible (Maudlin & Welburn, 1989; Baker, 1991). Hence, xenodiagnosis on day 6 with *G. m. centralis* was able to reveal the infection. In practice, infections can be detected with good reliability as early as day 3 (Olubayo *et al.,* 1994).

Characterisation of the lizard isolate in this study using the polymerase chain reaction (PCR) showed it was *Trypanosoma brucei*. This was confirmed by

hybridisation with the pgDR1 probe specific for *Trypanozoon* and by maturation in the salivary glands of *G. m. centralis*. This is the first record of isolation of *T. brucei* from any reptile. The finding of *T. brucei* at an endemic focus of sleeping sickness (Busia, Kenya) suggests that lizards serve as reservoirs of human disease in addition to cattle (Maudlin *et al.,* 1990) and other wild animals (Heisch, 1958). This finding is of particular relevance to the unexplained recrudescence of sleeping sickness in specific foci after many years of absence. Lizards are more abundant than other wildlife in the lake region and are natural hosts of *G. fuscipes*.

Experiments in this study have further shown that lizard blood is not toxic for *T. brucei.* Therefore reptiles act as a cryptic pool of *T. brucei* infection and the parasite circulates between *G. fuscipes* and these reptiles. In the event of an occasional feed on man or livestock, *G. fuscipes* may then spark a human cycle of the disease. Lizards may have acted as hidden reservoirs for the disease since the early 1900's epidemics of sleeping sickness at the Lake Victoria shores and may be responsible for resurgence of the disease after periods of quiescence in certain foci around the lake shores.

Flies of the *palpalis* group take a high proportion of bloodmeals from lizards and may therefore harbour infections of stercorarian trypanosome species such as *T. grayi.* Gouteux & Gibson (1996) showed a high prevalence of *T. grayi* in *G. fuscipes* in the Central African Republic. Their results showed that infections with reptilian trypanosomes could reach high levels in tsetse and these may give a misleading impression of the actual numbers of immature, salivarian infections. Minter-Goedbloed *et al.,* (1983) isolated from the gut of a naturally infected *Glossina pallidipes* in Kiboko, a savannah area in Kenya, trypanosomes that underwent development in the posterior region of the alimentary tract of *G. m. morsitans.* The cultured trypanosomes

were infective to non-indigenous reptiles. Lizards infected with these organisms were negative by microscopy, but *G. m. morsitans* fed on the lizards had massive infections in the hindgut and midgut. In this study, despite the fact that a wide range of techniques were used for detecting trypanosomes, no *T. grayi* organisms were found in the lizards.

The finding of *T. brucei* in lizards at Busia is attributed to the proximity of Busia to Busoga, Bunyundo and Bukunya villages in Uganda where human sleeping sickness epidemics have occurred recently (Okoth & Kapaata, 1986) as opposed to Rusinga Island where the disease has been absent for many years. It is however necessary that a long term study be conducted in areas of differing epidemiology in order to determine transmission cycles and possible human infectivity patterns. Control strategies should also consider the possible role of these reptiles in the disease cycle.

CHAPTER THREE

3.0 EXPERIMENTAL INFECTION OF MONITOR LIZARDS WITH CLONES OF *TRYPANOSOMA BRUCEI* AND *TRYPANOSOMA CONGOLENSE*

3.1 Introduction

Trypanosomes are digenetic parasites whose life cycle is completed in two hosts; a definitive host and the vector. The definitive hosts include man, domestic and wild animals. Man and domestic animals succumb to infection with trypanosomes whereas many wild animals are tolerant. Wild animals and some domestic animals may therefore act as reservoir hosts for trypanosomiasis. The mechanisms of tolerance and resistance are not well understood.

Wide variations in susceptibility in wildlife to *T. b. rhodesiense* and *T. b. brucei* were reported by Ashcroft *et al.,* (1959). Based on these differences wild animals were divided into two groups. One group consisted of host species that died of infection, which included monkeys and Thomson's gazelles; the other group consisted of animals that resisted or tolerated infections, and these included baboons, bushpigs and warthogs. Ashcroft *et al.,* (1959) tried to infect a few animals with *T. congolense,* but in most cases, the animals could not be infected. If they did get infected their parasitaemia was of short duration and the animals recovered. Subsequently, resistance to trypanosome infection by wildlife has been reported by various researchers (Rickman and Kolala, 1982; Grootenhius *et al.,* 1990; Reduth *et al.,* 1994). Rickman and Kolala (1982) reported that serum from eland, waterbuck and to a lesser extent spotted hyena and hippopotamus killed bloodstream form trypanosomes *in vitro.*

The *palpalis* group take a variable but often high proportion of their feeds from reptilian hosts (Moloo, 1993). Mohammed -Ahmed & Odulaja (1997) reported that 73-98% of feeds of *G. fuscipes* near Lake Victoria in Kenya were derived from lizards. The Lake Victoria region has been a historic focus of sleeping sickness since the early 1900's (Mbulamberi, 1989). *G. fuscipes* has been responsible for these epidemics (Okoth & Kapaata, 1988). There is close contact between man, lizards and *G. fuscipes* near water along the lakeshore. The possibility of reptiles acting as pivotal hosts for this disease should not be ignored. For an animal to act as an effective reservoir, the trypanosomes must adapt to the animal and must retain their infectivity to tsetse.

A few attempts have been made to infect reptiles with African trypanosomes. Bruce *et al.,* (1911) and Duke (1935) were unable to establish infections of *T. brucei* in crocodiles and monitor lizards. In contrast, Watson (1960) inoculated a virulent laboratory strain of *T. b. gambiense* into a young crocodile and obtained a transient parasitaemia. Woo and Soltys (1969) showed that caymans (crocodilian family) could be infected experimentally with *T. brucei* and that the infection persisted for a prolonged period of 12 weeks without causing clinical signs of the disease. The organisms remained infective to mice by syringe challenge. This suggests that reptiles may serve as reservoir hosts for pathogenic trypanosomes. Woo and Soltys (1969) further showed that turtles from a temperate climate could be infected with *T. brucei* if acclimatised to 37°C for 15 days. Caymans from the tropics kept either at room temperature or at higher temperature could also be infected with *T. brucei*. These findings suggested that some reptiles from the tropics may be more susceptible to infection than reptiles from temperate regions.

Molyneux (1973) described the behaviour of two virulent laboratory adapted stocks of *T. b. brucei* and *T. b. gambiense* in the lizards *Varanus niloticus* and *Varanus exanthematicus*. The capacity of the stocks to adapt to reptiles and retain their infectivity to mammals through a series of blind passages when maintained at ambient temperatures was evident. Monitor lizards supported brief infections. However, other lizards (*Agama agama* and *Testudo sulcata)* maintained infections for several weeks.

No detailed studies have been carried out to investigate the potential role of lizards from the Lake Victoria region as reservoirs of trypanosomiasis. For instance, it is not known whether lizards from this region are susceptible to organisms of the subgenera *Trypanozoon* and *Nannomonas.* The effects of such infections on lizards is also not known. The ability of flies to acquire infections from lizards has also not been studied. In this study, attempts were made to infect monitor lizards from the Lake Victoria region with *T. brucei* and *T. congolense* using natural cyclical challenge with tsetse. The possibility of transmitting infections from lizards back to flies and to susceptible laboratory rodents and thus completing the life cycle was also investigated. The findings of this study may be important in understanding the epidemiology of trypanosomiasis in the Lake Victoria region.

3.2 MATERIALS AND METHODS

3.2.1 Tsetse flies used to infect lizards

Teneral *G. m. centralis, G. fuscipes, G. palpalis gambiensis and G. tachinoides* males were used . *G. m. centralis* were obtained from the Insect Rearing Unit at the International Centre of Insect Physiology and Ecology (ICIPE), Kenya. *Glossina fuscipes* were obtained as pupae from the International Atomic Energy Agency (IAEA,

Vienna) whereas *G. tachinoides* and *G. p. gambiens*is were obtained as pupae from Centre International de Recherche-Developpement sur L'Elevage en Zone Subhumide (CIRDES) (West Africa) (Plate 3.1). The riverine flies were maintained at 25°C, relative humidity 85%, 12 h period of light and 12 h period of darkness.

3.2.2 Trypanosomes used to infect lizards

Trypanosoma brucei (Ng3), savannah *T. congolense* (Ng5) both isolated from *G. pallidipes* at Ngulia in Tsavo West National Park, Kenya (Mihok *et al.,* 1992a) and riverine-forest *T. congolense* (Anr3), a west African stock described by Gibson *et al.,* (1988), were used. Cryopreserved parasites diluted in goat blood to 10^4 parasites/ml were used to infect 100 male teneral flies of each species through silicone membranes. Flies were subsequently fed on goat blood until day 35 post infection. This allowed ample time for maturation of slow growing parasites. Between days 33 and 35 post infection, flies were allowed to probe individually on a warm slide in order to determine flies extruding infective metacyclics. Only flies with metacyclics were used to infect lizards.

3.2.3 Infection of lizards

Experiments were carried out to study *T. brucei* and *T. congolense* infection in lizards using *G. m. centralis*, *G. fuscipes*, *G. tachinoides* and *G. p. gambiensis.* Flies with metacyclic infections were allowed to feed freely on trypanosome-free lizards. Flies

Plate 3.1

Photograph of riverine tsetse flies used in the survival experiments

Top row = males Bottom row = females

From left to right: *G. fuscipes* (Vienna), *G. p. gambiensis*
G. tachinoides, G. fuscipes (Mbita)

kept in a normal insectary cage did not feed well when presented with lizards. Hence, flies were released to feed naturally in a large cage holding a lizard; the cage had very fine netting (Plate 3.2). Once fed, the flies were collected by covering the feeding cage with a black cloth (Plate 3.3) which prompted upward flight. The fed flies were then dissected to confirm the presence of metacyclics in the proboscis and salivary glands. The lizards were exposed to different numbers of male flies due to differences in maturation rates among tsetse species and trypanosome stocks.

3.2.4 Examination of lizards for parasites.

Lizards were restrained manually and bled by snipping the toe nails with small sterile scissors for small amounts of capillary blood. This was done every few days for 30 days or until the lizards died. Thin, thick smears and the buffy coat were examined for trypanosomes as described in Chapter 2. The buffy coat from microhaematocrit capillary tubes was spread on a slide and examined for the presence of trypanosomes using phase-contrast microscopy (Murray *et al.*, 1977). The number of trypanosomes was estimated using the semi-quantitative scoring method proposed by Paris *et al.*, (1982). Packed cell volume (PCV), measured by microhaematocrit centrifugation was used as the index of anaemia. The rectal temperature of the lizards was also recorded by inserting a clinical thermometer in the rectum of the lizard. Once a lizard was positive with trypanosomes, it was bled from the ventral tail vein and the blood used to infect 50 teneral *G. m. centralis;* portion of the blood (0.5 ml each) was also inoculated into three laboratory-reared Wistar rats. Wet smears from rats were examined every two days. Examination of rats was discontinued when positive, or at 30 days if negative.

Plate 3.2

Cage used for feeding flies on lizards during the lizard infection experiments

Plate 3.3

Cage used for harvesting flies post-feeding on lizards in the lizard infection experiments

3.3 Results.

3.3.1 Infection of lizards with *T. brucei*

There were wide variations in maturation rates of *T. brucei* among *morsitans* and *palpalis* tsetse. Hence, the lizards were exposed to different numbers of fly bites (Table 3.1). When *G. m. centralis* was used, the lizards developed *T. brucei* infection 8 days post feeding. There was a gradual rise of parasitaemia (Fig. 3.1) and the infection was detectable with the buffy coat technique for 12 days. Thereafter, no parasites could be detected by this method and rat inoculation until day 28 when the lizard died. During the infection period there was a decline in the packed cell volume from 29% to 23% (Fig. 3.2) and a rise in the rectal temperature of the lizard (Fig. 3.3). During the infection period, blood from this lizard was infective in all the rats (N=3) 7 days post infection and xenodiagnosis was positive in 19.04% *G. m. centralis* (N=42) 6 days post infection (Table 3.1).

G. fuscipes produced an infection in the lizard 12 days post infection. The infection lasted for 9 days. Thereafter, no parasites could be detected in the lizard using the buffy coat, xenodiagnosis and rat inoculation techniques until day 30 when the experiment was discontinued. During the infection period there was very little fluctuation in parasitaemia. The packed cell volume declined from 34 to 26% and there was an increase in rectal temperature from 18°C on day 0 of infection to 27°C by day16 post infection (Figs. 3.2 & 3.3). Rat inoculation was positive in 2 of 3 rats 9 days post infection. Xenodiagnosis was positive in 18.7% *G. m. centralis* (N=32) 6 days post infection.

Table 3.1 Experimental infection of lizards with *T. brucei* and *T. congolense* using different tsetse species

Fly	no. flies	Parasite	pp lizard	Dur-inf	pp rat	xeno
Gmc	17	T. brucei	8	12	7	8/42
Gff	6	T. brucei	12	9	9	6/32
Gtach	11	T. brucei	14	10	8	8/38
Gpgamb	10	T. brucei	-	-	-	-
Gmc	12	T. congo s	-	-	-	-
Gpgamb	10	T. congo s	-	-	-	-
Gff	4	T. congo s	-	-	-	-
Gtach	8	T. congo r	-	-	-	-
Gpgamb	7	T. congo r	-	-	-	-
Gff	2	*T. brucei* (B)	-	-	-	-
Gtach	4	*T. brucei* (B)	-	-	-	-
Gpgamb	3	*T. brucei* (B)	-	-	-	-

Gmc = *G. m. centralis,* Gff = *G. fuscipes*, Gtach = *G. tachinoides* Gpgamb = *G. p. gambiensis,* Para = parasite, pp lizard = prepatent period in lizard, pp rat = prepatent period in rat, xeno = xenodiagnosis, T.congo s = *T. congolense* savannah, T. congo r = *T. congolense* riverine, *T. brucei* (B)= *T. brucei* from Busia, (-) = no infection

Fig. 3.1 Pattern of *T. brucei* (Ng3) infections in lizards infected using three tsetse species

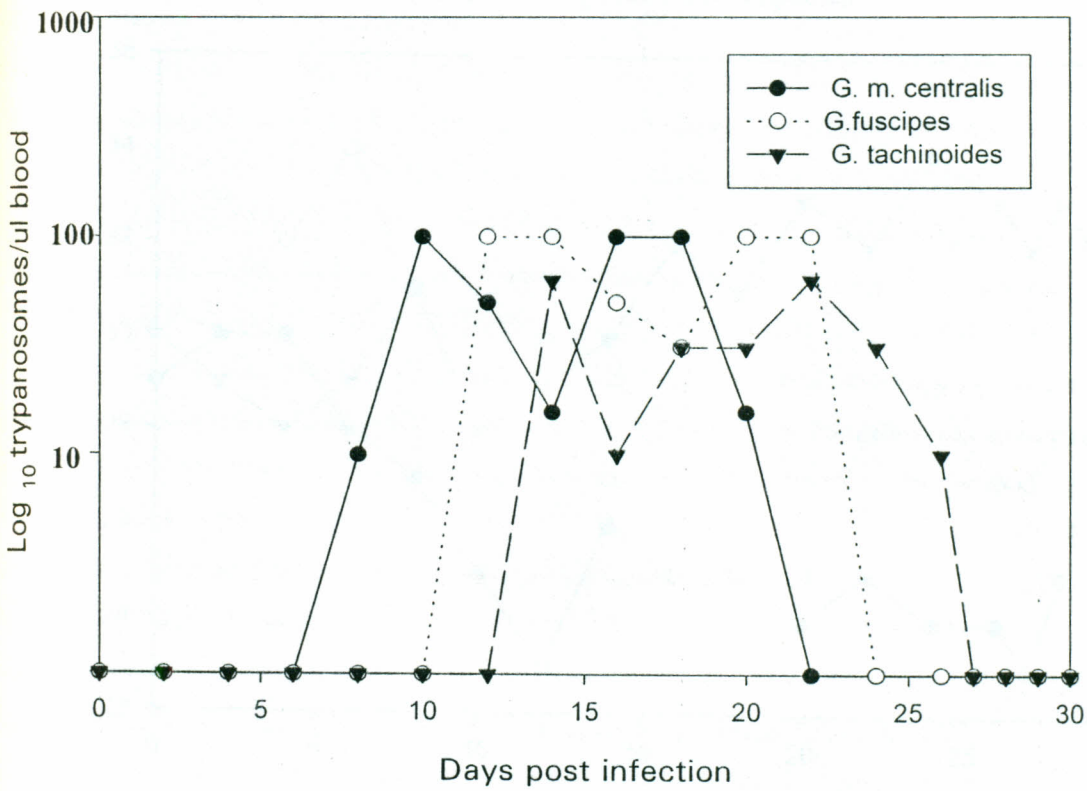


Fig 3.2 Changes in packed cell volume (PCV) in lizards infected with *T. brucei* using three tsetse species

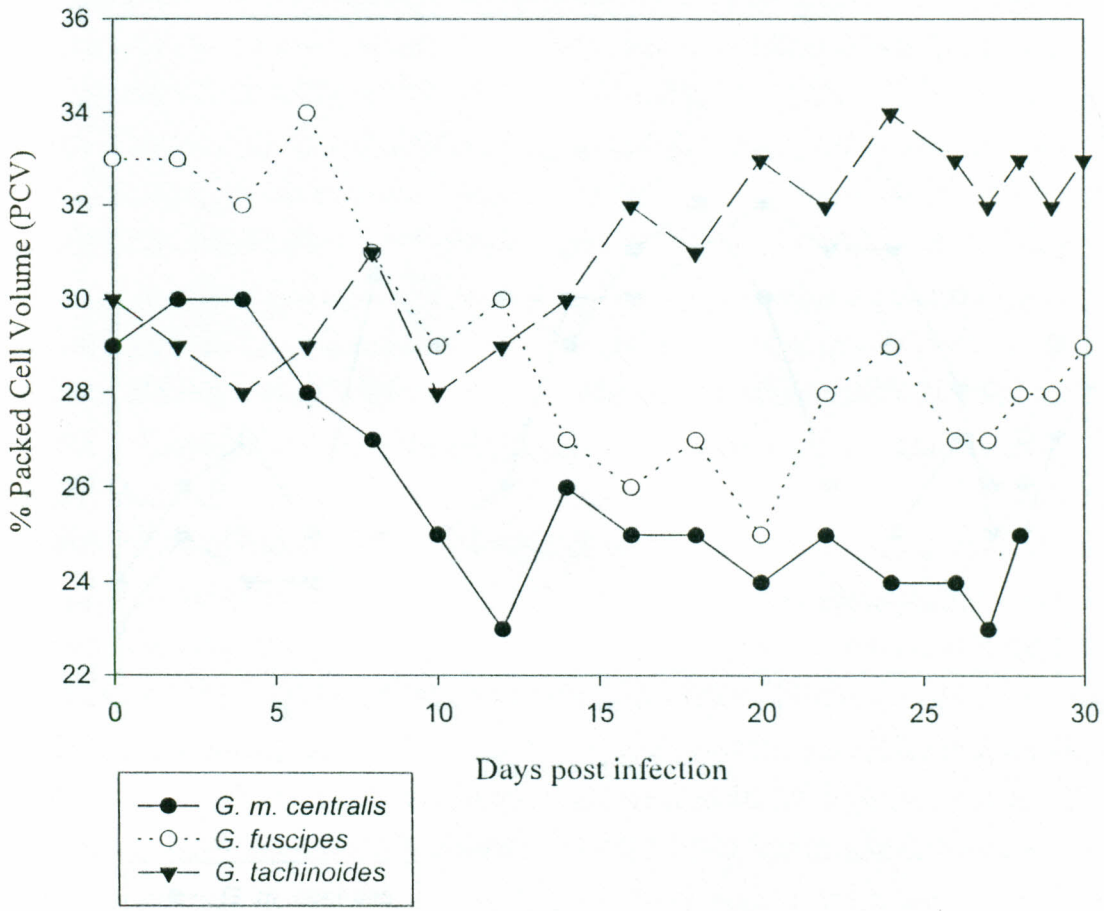
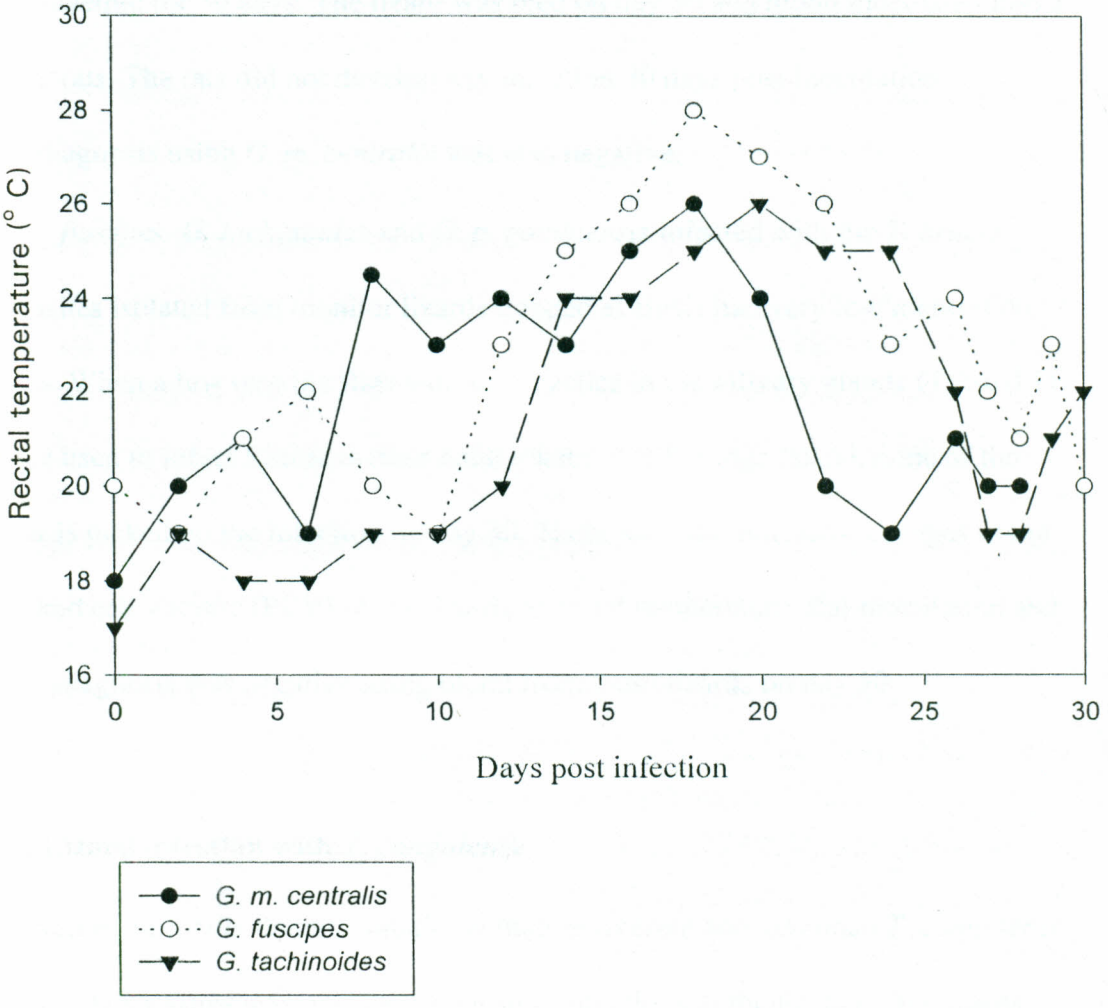


Fig. 3.3 Changes in rectal temperature in lizards infected with *T. brucei* using three tsetse species



The monitor lizards infected using *G. tachinoides* developed an infection on day 14 (Fig . 3.1). The infection was of short duration lasting 10 days. There was an initial decline in the packed cell volume on day 5 followed by a gradual increase. The infected lizard blood produced infection in 2 of 3 rats 10 days post infection and was infective to 21%(n=38) *G. m. centralis*. *G. p. gambiensis,* though containing mature *T. brucei* infection was not able to transmit the infection to the lizards. The lizards remained aparasitaemic for 30 days. The lizard was bled on day 30 and blood inoculated into 3 Wistar rats. The rats did not develop any infection 30 days post inoculation. Xenodiagnosis using *G. m. centralis* was also negative.

G. fuscipes, G. tachinoides and *G. p. gambiensis* infected with the *T. brucei* parasites isolated from monitor lizards trapped at Busia had very low maturation rates. When a few riverine flies with metacyclics in the salivary glands (Table 3.1) were used to infect lizards in their natural habitat at Rusinga Island, none of the lizards picked up the infection by day 30. There were no indicative changes in the packed cell volume (PCV) of the lizards or rectal temperature. Rat inoculation and xenodiagnosis was negative using blood from these lizards on day 30.

3.3.2 Lizard infection with *T. congolense*

Glossina m. centralis though containing mature riverine and savannah *T. congolense* (Table 3.1) parasites was not able to transmit infections to the lizards. The lizards remained aparasitaemic for 30 days, and showed no indicative signs of fever or anaemia. Blood from these lizards on day 30 was not infective to laboratory rats or *G. m. centralis.*

Mature infections of *T. congolense* were not detected in the riverine flies (*G. fuscipes*, *G. tachinoides* and *G. p. gambiensis*) and hence few flies were used for the infection of lizards (Table3.1). The lizards remained aparasitaemic throughout the infection period in all experiments and showed no indicative changes in packed cell volume (PCV) or rectal temperatures. Blood from these lizards, when inoculated into rats failed to produce an infection and was not infective to *G. m. centralis*.

3.4 Discussion

The present study has shown that lizards can be infected experimentally with *T. brucei* but not with *T. congolense*. The prepatent period in the lizards varied depending on the species of fly used for infection. Infections using *G. m. centralis* took the shortest time (8 days) whereas infections with *G. tachinoides* took the longest (14 days). These results indicate that *G. m. centralis* is a more efficient vector of trypanosomiasis than flies from the *palpalis* group (*G. fuscipes* and *G. tachinoides)*. Infections in the lizards persisted for a prolonged period without causing any severe clinical signs of disease. The clinical symptoms of trypanosomiasis include anaemia, wasting, fever, abortions and disorders of the nervous system in terminal stages. *Trypanosoma brucei* survived in the monitor lizard and retained its infectivity to laboratory rodents and *Glossina*. Several workers have previously attempted to infect lizards with trypanosomes and obtained varied results. Woo and Soltys (1969) showed that American caymans (*Caiman sclerops* and *Pseudemys scripta elegans*) living in a temperate environment could be infected experimentally with *T. brucei*. Caymans remained infected for 12 weeks and the organisms were infective to mice throughout this time. On the other hand Bruce *et al.,* (1911) and Fraser and Duke (1912) in an attempt to investigate the

ability of reptiles to maintain *T. brucei* were unable to establish infections in crocodiles and monitor lizards, but, Watson (1960) inoculated a virulent laboratory strain of *T. b. gambiense* to a young crocodile and obtained a transient parasitaemia. This infection was not transmissible to *G. palpalis* nor could rats be infected by parasitaemic blood. In addition, Molyneux (1973) attempted to infect various species of lizards with *T. b. brucei* and *T. b. gambiense*. *Varanus niloticus* and *V. exanthematicus* would only support brief infections of *T. b. brucei* and *T. b. gambiense*. The infections lasted at most four days and were induced by high doses of organisms. However, *Agama agama* and *Testudo sulcata* could maintain infections of *T. b. brucei* for 55 and 66 days respectively. Molyneux (1973) also attempted to passage a laboratory strain of *T. b. gambiense* through *Agama* lizards. After nine passages the parasites became adapted to the reptilian host, a higher parasitaemia and a greater virulence being reached at each passage.

Radiotelemetry has been used to measure the deep body temperatures of free-ranging animals. Lizards are able to thermoregulate up to a certain point depending on air and substrate temperatures. During the day, lizards exhibit classical patterns of behavioural thermoregulation such as basking on rocks, periodic shuttling between sunny and shady microenvironments, pressing the venter on the sand and lying in water. During the night, lizards like the monitor lizards spend most of their time in water (Christian and Weavers, 1996). In this study, lizards were infected in Nairobi at temperatures ranging from 16°C - 26^{0}C. These are cooler conditions than are found in their natural habitat, and yet they were able to retain *T. brucei* infection. In their natural habitat at the lakeshore temperatures are constant and high (28°C- 33°C). Lizards presumably maintain body temperatures close to mammalian body temperature (37°C)

and thus may be capable of retaining *T. brucei* infection for prolonged periods. Attempts to infect lizards at Rusinga Island with a *T. brucei* parasite isolated from lizards at Sio Port, Busia were unsuccesful. This could be due to the poor maturation rates reached with this parasite in the riverine flies.

A "good" reservoir should ideally maintain a chronic infection without adverse effects. In this study, lizards appeared to self-cure after a few weeks. Parasites could no longer be detected by the buffy coat technique nor was the blood infective to both rats and tsetse. This is often the case with wildlife (Mihok *et al.,* 1992a,b; Olubayo *et al.,* 1994).

The results of this study indicate that monitor lizards serve as cryptic reservoirs for *T. brucei*. Since *G. fuscipes* derives most of its feeds from lizards, these flies act as vectors between lizards, man and livestock. It is therefore also possible that the lizards may have acted as an unnoticed pool of *T. brucei* infection since the early epidemics of sleeping sickness in the 1900's. If this is the case, this may create problems in the eradication of endemic foci of sleeping sickness. Control strategies in the past have mainly involved tsetse eradication and suppression using a combination of methods. Trypanosomiasis control efforts should consider the possible role of lizards and other reptiles which are abundant in many tropical parts of Africa where sleeping sickness is a recurrent problem.

CHAPTER FOUR

4.0 EFFECT OF LIZARD BLOOD ON SURVIVAL OF *GLOSSINA* SPECIES

4.1 Introduction

Glossina species are obligate blood feeders. Both sexes feed on a wide variety of vertebrate hosts (Moloo, 1993). Although some are opportunistic utilising available hosts, others show preferences for particular host species. Even so, in certain circumstances, many tsetse will feed on the available hosts (Moloo, 1980). Hence, there are many local variations in the feeding habits. Preferences are not based on the nutritional value of the blood. They are a reflection of behavioural selection for specific hosts (Glasgow, 1963). Behavioural selection is related to coincidence in the habits of the tsetse and their favoured hosts, and the complacency of the host species (Ford, 1971). The host range and the feeding behaviour of tsetse is important in the epidemiology of human and animal trypanosomiasis.

Bloodmeals taken at intervals of 2-3 days provide the sole source of energy for adult *Glossina*. During post-emergence development of the fly, the first few meals contribute to the development of flight thoracic muscles in both sexes (Langley, 1977; Bursell *et al.,* 1974). Thereafter, male flies spend the net gain from each bloodmeal in basal metabolism, flight activity, spermatophore production or for storage as fat or amino acids. Female flies invest considerable energy resources in larval production. Tsetse take large amounts of blood sometimes up to two to three times their own body weight (Moloo & Kutuza, 1970). The rapid ingestion of the bloodmeal creates certain problems. The speed of flight is reduced and the fly becomes extremely susceptible to

predation. The haematophagous habit is therefore characterised by rapid concentration of the meal and excretion of copious quantities of water. Water loss is controlled by a diuretic hormone that regulates the activity of the malphigian tubules. This results in concentration of the bloodmeal and thus facilitates digestion. The excretion of water is extremely rapid: it commences during feeding and is virtually complete 30 min later (Bursell, 1960). The meal and its ionic composition play an important role in determining the rate of water loss. The rate at which water elimination is achieved will limit the rate at which the meal is subsequently digested and utilised. Flies which do not undergo diuresis cannot fully utilise their bloodmeals.

Glossina species have been shown, by use of synthetic substrates and spectrophotometric methods (Cheeseman & Gooding, 1985), to possess several proteolytic enzymes in their midguts. These digestive enzymes include: carboxypeptidases, aminopeptidases, trypsin, chymotrypsin, trypsin-like and chymotrypsin-like enzymes (Cheeseman & Gooding, 1985). The enzymes vary in the level of activity with fly species and age and also with the meal utilised. Digestive proteases induced by the serum component of the bloodmeal in *G. m. morsitans* also induce the transformation of trypanosomes (Imbuga *et al.,* 1992 a, b).

Gooding (1973) reviewed aspects of proteolytic enzyme release and their control in response to bloodmeal intake in haematophagus arthropods. Subsequently, many approaches have been employed in establishing the characteristics of proteolytic enzymes in tsetse (Gooding & Rolseth, 1976; Cheeseman & Gooding, 1985). However, these studies have not included the influence of bloodmeals from natural/preferred, occasional or non-preferred hosts of tsetse. Reptiles are preferred hosts for the *palpalis* flies and non-preferred hosts for the *morsitans* group. In this study, experiments were

carried out to determine the effects of crocodile, lizard and goat blood (preferred and non-preferred hosts) on survival of *palpalis* and *morsitans* tsetse. The effects of the different host bloods on protease and trypsin levels in the different tsetse were also studied.

4.2 MATERIALS AND METHODS

4.2.1 Tsetse flies used for survival experiments

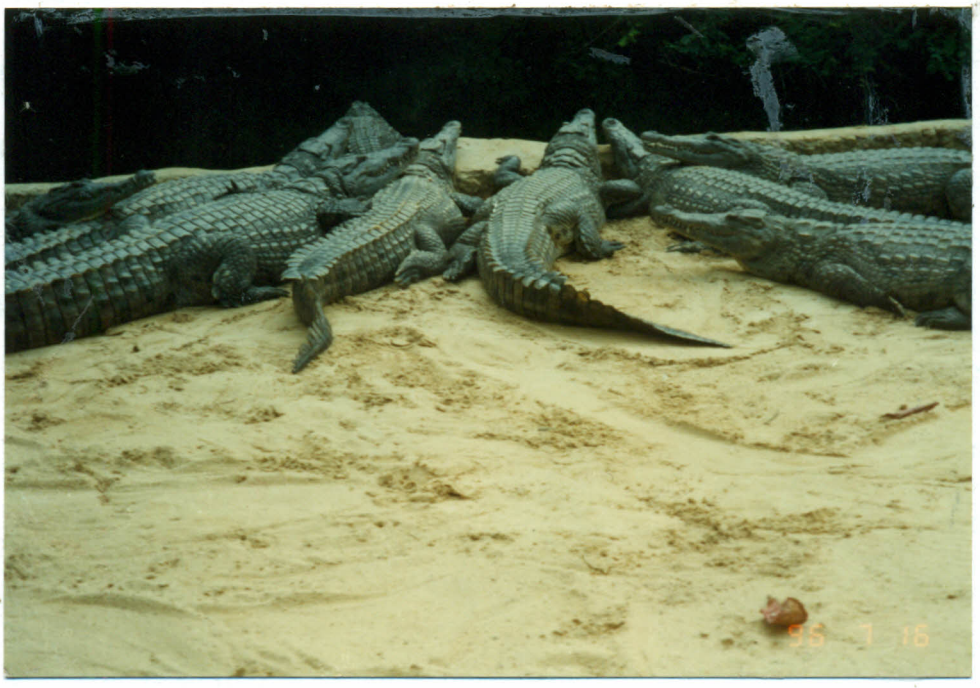
The effect of lizard, crocodile and goat blood on survival, water loss and protease release was studied using teneral *G. m. centralis, G. m. morsitans,* (*morsitans* group), *G. fuscipes, G. tachinoides* and *G. p. gambiensis* (*palpalis* group) tsetse.

4.2.2 Bloodmeal source and collection

Goats, lizards and crocodiles were used as bloodmeal sources. Blood from goats was obtained by jugular venipuncture into heparinised tubes whereas blood from lizards was obtained from the ventral tail vein as described by Esra *et al.,* (1975).

Crocodiles used in this study were obtained from Mamba village crocodile farm at Mombasa, Kenya (Plate 4.1). Blood samples were obtained from the supra vertebral vessel located caudal to the occipit and immediately dorsal to the spinal cord (Olson *et al.,* 1975). The skin behind the occipit was cleaned in 70% alcohol and a 22G needle inserted through the skin in the midline directly behind the occipit. Blood collected was frozen at -20 °C in the freezer until required for use.

Plate 4.1



Crocodiles at Mamba Village (Mombasa) used as blood source

4.2.3 Effect of hosts' blood on survival of flies

Experiments were carried out to determine the effect of goat, lizard and crocodile blood on survival of five tsetse species. In total, 750 male flies were used with 50 flies for each experiment. Teneral male tsetse were fed *in vitro* on goat, lizard and crocodile blood through silicone membranes (Mews *et al.*, 1977). The flies were subsequently maintained on the same host blood for 30 days. Counts of flies were done every day for 30 days and expressed as proportion surviving.

4.2.4 Determination of bloodmeal size

Experiments were carried out to determine bloodmeal size in five species of tsetse fed on goat, lizard and crocodile blood. In total, 150 flies were used. An average meal size was obtained from 10 male flies of each species for five experiments. Flies were placed in 4 x 2 cm specimen tubes (one fly per tube). Bloodmeal size was estimated by weighing individual flies in specimen tubes before and after feeding. Flies were immobilised by brief chilling at 4° C for 3 min and then weighed individually using an electronic balance.

4.2.5 Determination of water loss in the flies

Experiments were carried out to determine water loss in five tsetse species fed on goat, lizard and crocodile blood. A total 150 male teneral flies were used with 10 tsetse for each experiment. Five experiments were carried out. The rate of water loss from the meal was determined by weighing fed flies individually in tared specimen tubes immediately after feeding, and then transferring them to fresh clean tubes for

re-weighing at intervals of 5 min, 10 min, 15 min, 30 min, 1 h, 2 h, 6 h, and 24 h. The flies were maintained at about 25^0 C throughout the study.

4.2.6 General protease assays

Experiments were carried out using 75 tsetse flies to determine general protease activity in five species of tsetse fed on goat, lizard and crocodile blood. Dissections of the posterior gut were conducted 48 h post feeding and assays carried out on five pooled guts of male flies of each species. The general protease assay was carried out as described by Stiles *et al.*, (1991) using a commercial kit (Bio-Rad Watford). Test samples were placed in one of the nine 0.3 cm wide wells cut in a casein substrate gel in 8.5 x 6.5 cm diffusion plates. The plates were covered with plastic lids and incubated at 27^0 C for 16 h. The resultant lytic zones around the wells were enhanced by flooding the gels with 3% (v/v) acetic acid (which also stopped the reaction) and the diameter was measured with Vernier callipers. Buffer blanks were run as controls. Protease levels were reported for each fly as International Units (IU) of trypsin-like activity, based on a standard curve for commercial trypsin (Sigma, St. Louis, MO).

4.2.7 Trypsin assays

Studies were carried out to determine the time course production of trypsin in *G. m. centralis* and *G. fuscipes* fed on goat, lizard and crocodile blood. A total of 600 tsetse were used with 20 pooled midguts each for *G. m. centralis* and *G. fuscipes*. Teneral male flies were fed on goat, lizard and crocodile blood 24 h post eclosion. Only fully engorged flies were used for trypsin determination. Tsetse midguts were dissected promptly in phosphate buffered saline (pH 8.4) at 0, 24, 48, 72, and 96 h following the

ingestion of the bloodmeal. Midguts were pooled for each group of twenty flies and stored in the freezer at -20^0 C until analysis was done. At a later date, midguts were thawed, homogenised and centrifuged in microfuge tubes in 200 µl 50 mM Tris/HCl at 1200 g for 5 min. Supernatants were transferred to fresh microfuge tubes and used in the trypsin assays.

Trypsin activity was assayed using a chromogenic substrate, carbobenzoxy-val-gly-arg-4-nitranilide acetate (Chromozym-TRY; Boehringer-Mannheim, FRG) as described by Imbuga *et al.,* (1992 a). The reaction mixture consisted of Chromozym-TRY (40 µmol) and crude midguts of 20 pooled guts. The reactions were initiated by the addition of Chromozym-TRY and the change in absorbance at 410 nm monitored for 10 min using a GBC 916 spectrophotometer linked to a TS computer system (GBC, Victoria, Australia). The change in molar extinction coefficient at 410 nm (ε_{410} = 8800; (Erlanger *et al.,* 1961) was used to determine the amount of substrate hydrolysed; 1 unit trypsin is the amount of enzyme required to hydrolyse 1 µmol Chromozym-TRY min^{-1} at 30^0 C. The enzyme activity was determined using the formula:

activity= ((change in A_{410nm}/min)/ε_{410})) *(V/v) * dilution factor

where v= volume of Chromozym-TRY used in assay

V= total volume of the assay

* = multiplied

4.3 Results

4.3.1 Bloodmeal size

All the tsetse species fed readily on blood from goat, lizard and crocodile *in vitro*. *G. m. centralis, G. m. morsitans* and *G. p. gambiensis* took larger meals (26.4 - 31.6 mg) than *G. fuscipes* and *G. tachinoides* (9.6-17.4 mg). *G. tachinoides* the smallest fly took the smallest meal (9.6-11.2 mg) (Table 4.1). Analysis of variance (ANOVA) of the meal sizes considering effects of meal type, tsetse species and their interaction detected significant effects of tsetse species (F= 119.7, df=4, P<0.001), whereas bloodmeal type (F= 8.4, df=2, P=0.07) and the interaction (meal x tsetse) (F= 6.02, df=8, P=0.29) effects were not significant.

4.3.2 The effect of host blood on the survival of different tsetse species

Monitor lizard blood killed all the *morsitans* species of flies by day 13 in *G. m. centralis* and day 10 in *G. m. morsitans.* It supported survival of *G. fuscipes* (43%), *G. p. gambiensis* (67%) and *G. tachinoides* (70%) by day 30. In contrast, the effects of crocodile blood on *morsitans* species of flies were less dramatic with 60% survival of *G. m. centralis* and 52% survival of *G. m. morsitans* by day 30. Maintaining p*alpalis* flies on crocodile blood supported survival of *G. fuscipes* (59%), *G. tachinoides (68%)* and *G. p. gambiensis* (56 %). Goat blood supported survival of both the *morsitans* and *palpalis* flies (Figs. 4.1a - 4.5 a).

4.3.3 The effect of host blood on water loss in tsetse flies

Flies started excreting water immediately after feeding. *Glossina m. morsitans* and *G. m. centralis* flies fed on goat bloodmeal excreted 43 % and 45% of the water

Table 4.1 Mean bloodmeal sizes (mg) (±SE) in five tsetse species fed on goat, lizard and crocodile blood

Fly species	goat blood	lizard blood	crocodile blood
Gmc[a]	30.1 ± 1.14	26.9 ± 0.9	28.2 ± 0.7
Gmm[a]	31.4 ± 0.61	30.6 ± 1.42	26.4 ± 1.86
Gpgamb[a]	29.4 ± 0.48	31.6 ± 0.45	28.4 ± 0.54
Gff[b]	17.4 ± 0.52	16.8 ± 0.25	15.6 ± 0.57
Gtach[c]	9.6 ± 0.44	10.1 ± 0.38	11.2 ± 0.33

Meal sizes in flies with the same letter are not significantly different (SNK test)

Gmc = *Glossina m. centralis*
Gmm = *G. m. morsitans*
Gpgamb = *G. p. gambiensis*
Gff = *G. f. fuscipes*
Gtach = *G. tachinoides*

Fig 4.1a Survival of *G. m.centralis* maintained on goat, lizard and crocodile blood for 30 days

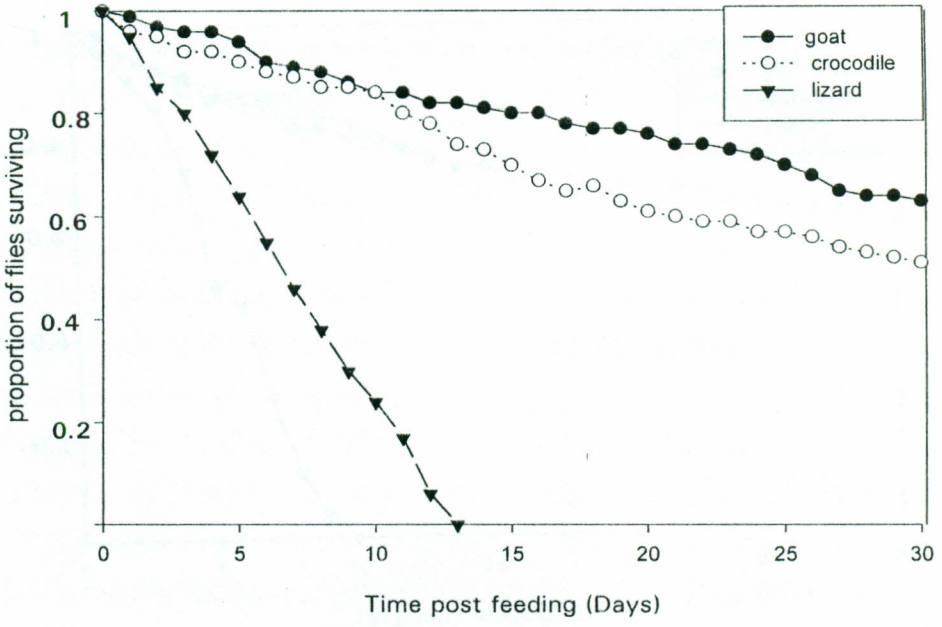


Fig. 4.1b Water loss in *G. m. centralis* post-feeding on goat, crocodile and lizard blood

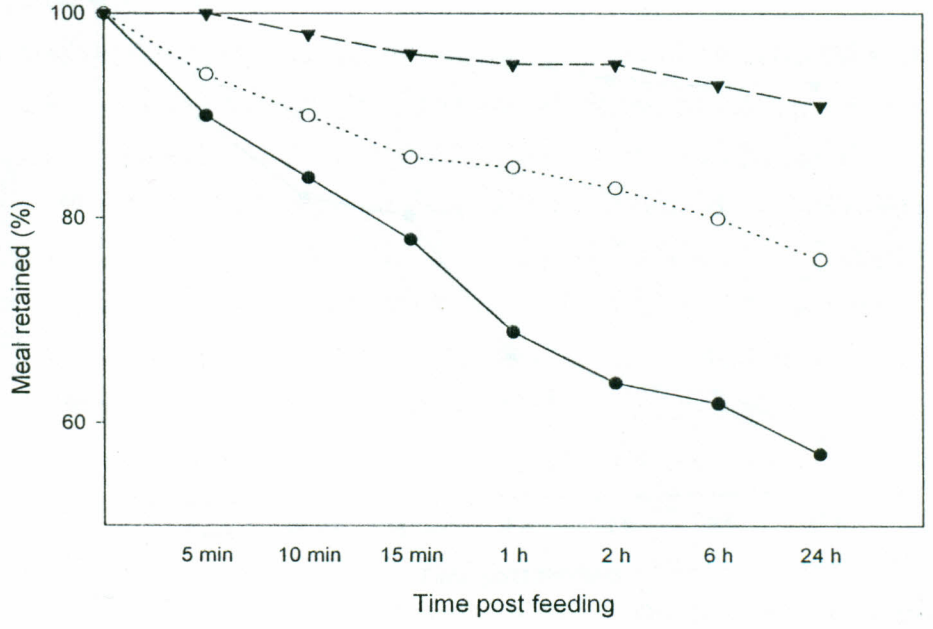


Fig. 4.2a Survival of *G. m. morsitans* maintained on goat, lizard and crocodile blood for 30 days

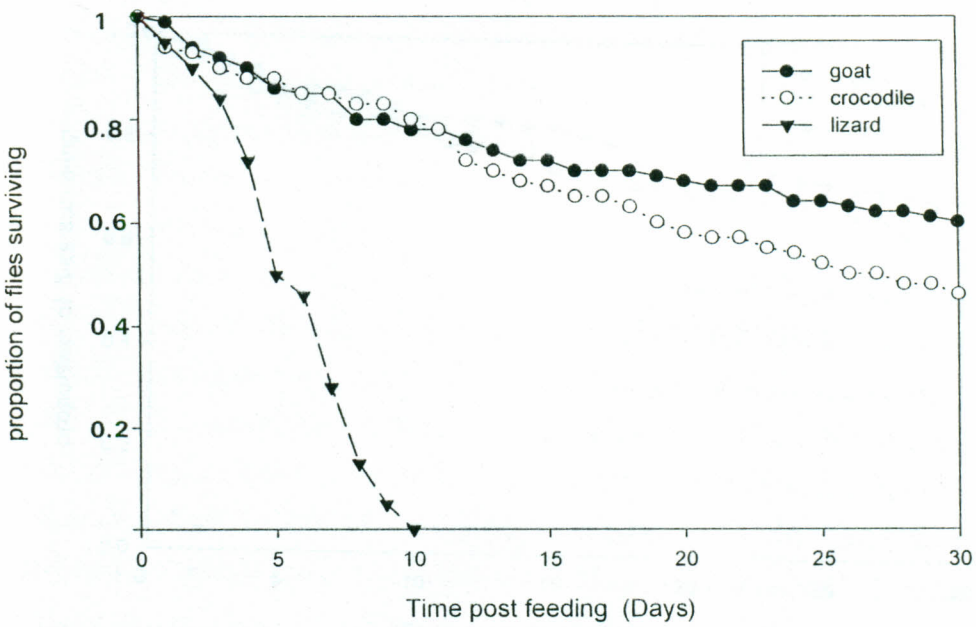


Fig. 4.2b Water loss in *G. m. morsitans* post-feeding on goat, crocodile and lizard blood

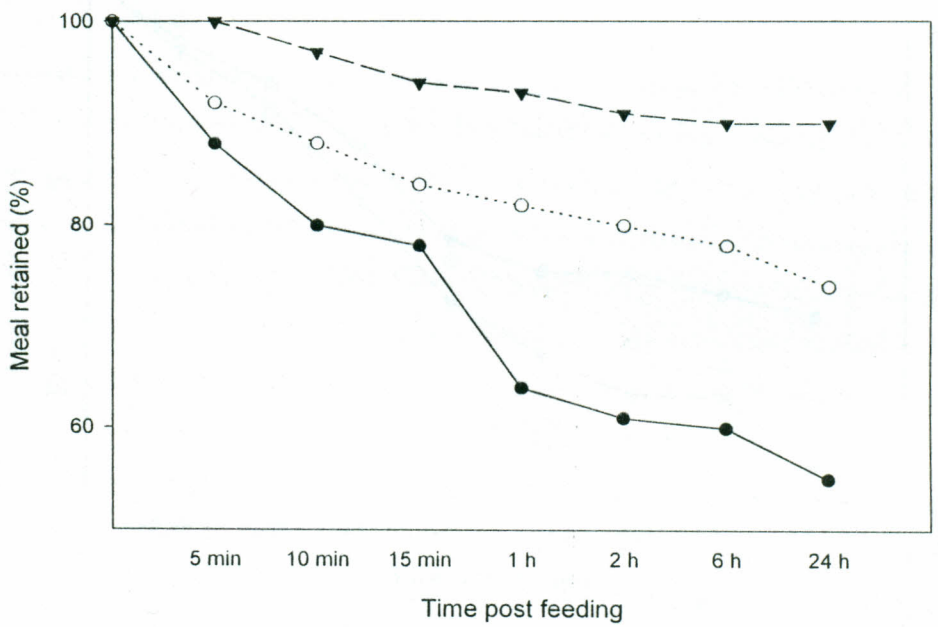


Fig. 4.3a Survival of *G. tachinoides* maintained on goat, crocodile and lizard blood for 30 days

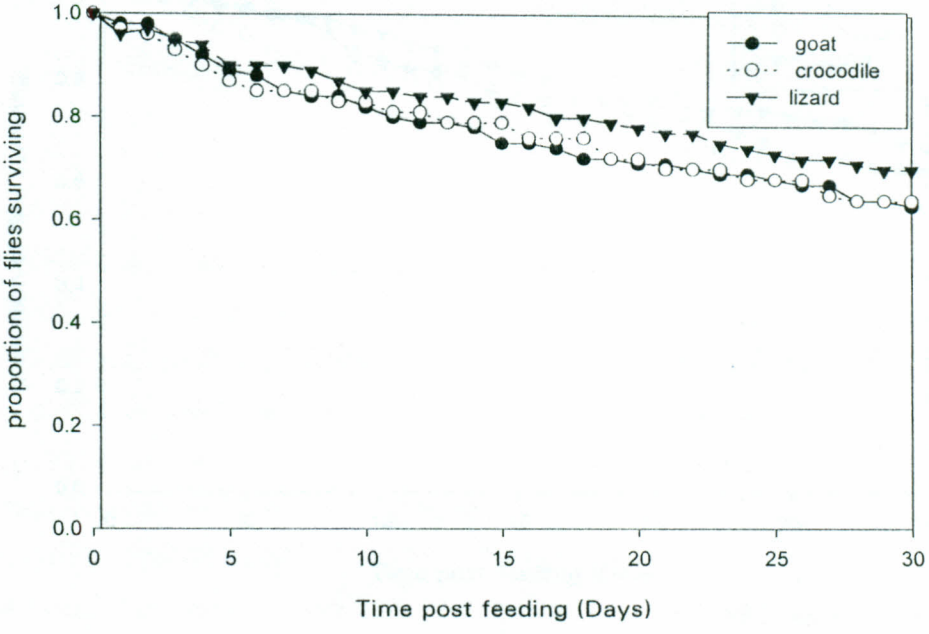


Fig. 4.3b Water loss in *G. tachinoides* post-feeding on goat, crocodile and lizard blood

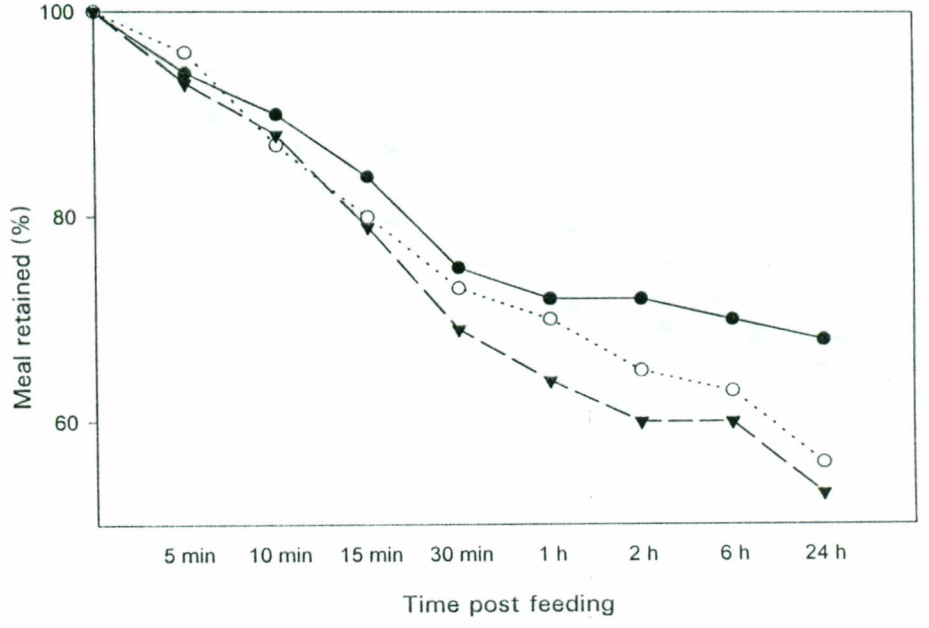


Fig. 4.4a Survival of *G. p. gambiensis* maintained on goat, lizard and crocodile blood for 30 days

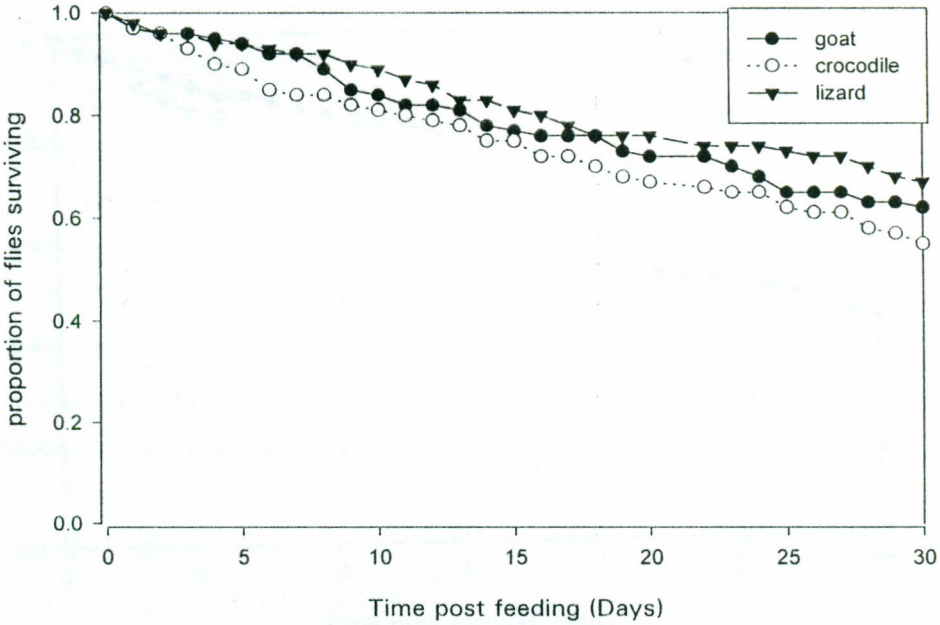


Fig. 4.4b Water loss in *G.p.gambiensis* post-feeding on goat, crocodile and lizard blood

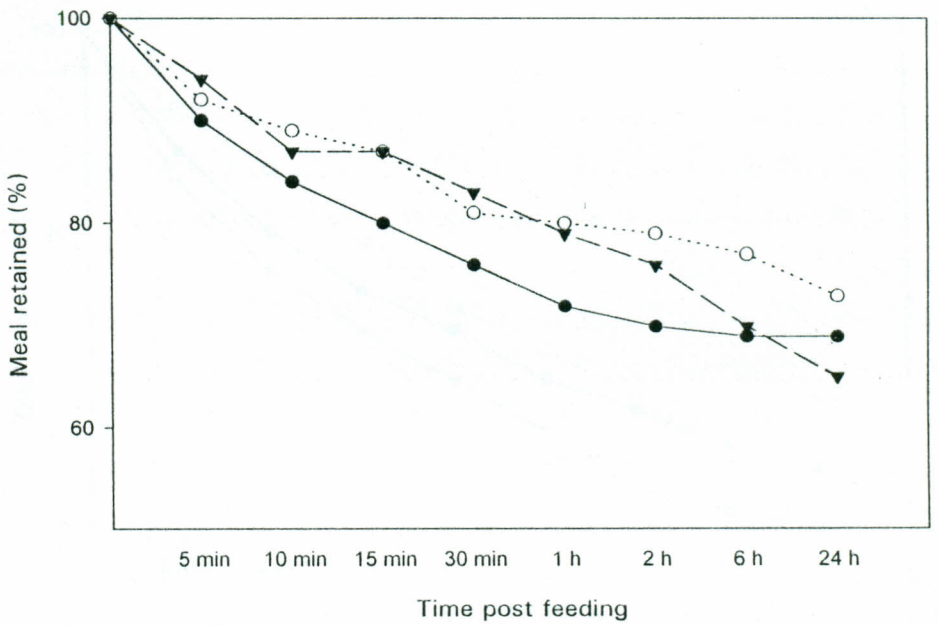


Fig 4.5a Survival of *G. fuscipes* maintained on goat, lizard and crocodile blood for 30 days

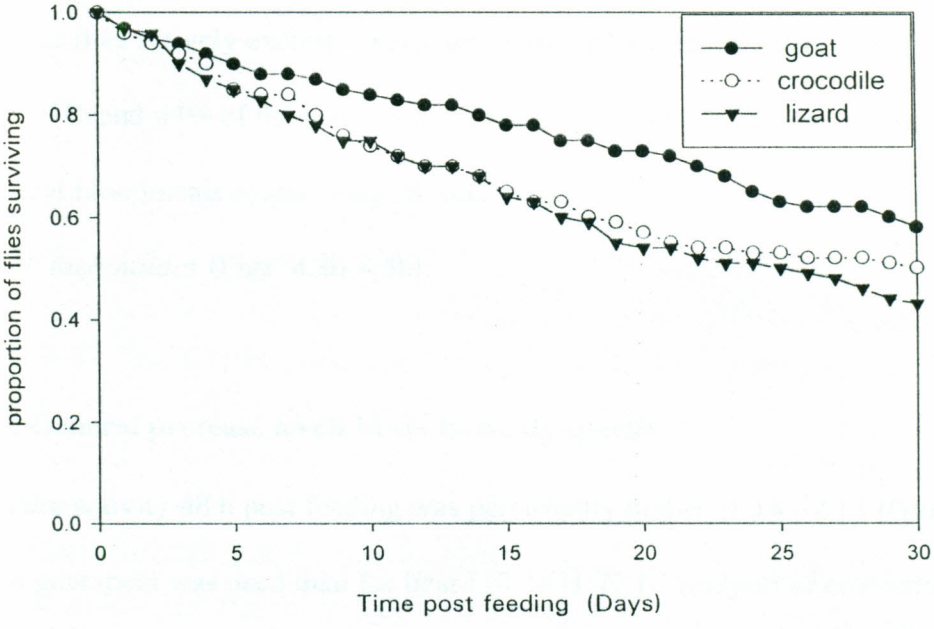
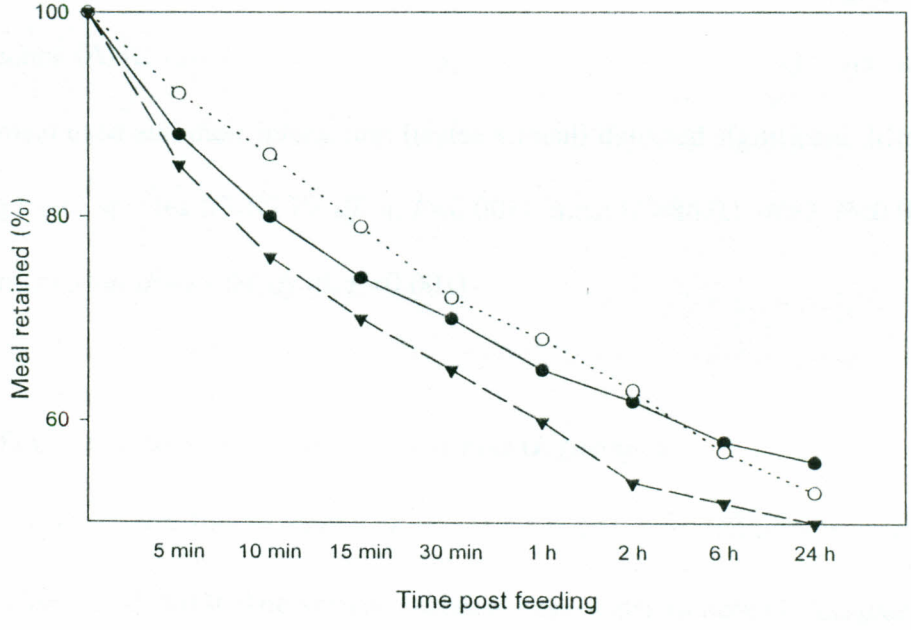


Fig. 4.5b Water loss in *G. fuscipes* post- feeding on goat, crocodile and lizard blood



respectively in the first 24 h post feeding. However, water loss in these *morsitans* flies was inhibited when lizard blood was used. By 24 h only 9% and 10% of the water had been excreted in *G. m. morsitans* and *G. m. centralis* respectively (Figs. 4.1b-4.2b). *Palpalis* flies actively excreted water when all the bloodmeals were used. In *G. fuscipes* 50%, 47% and 44% of the water was excreted by 24 h post feeding on lizard, crocodile and goat bloodmeals respectively. Similar trends were followed by *G. p. gambiensis* and *G. tachinoides* (Figs. 4.3b-4.5b).

4.3.4 General protease levels in six tsetse fly species

Protease activity 48 h post feeding was persistently higher (1.14 - 2.12 IU/midgut) when goat meal was used than for lizard (0.24 -1.72 IU/midgut) or crocodile (1.32-1.86 IU/midgut) blood. The riverine group flies maintained higher protease levels than the *morsitans* group. Low protease levels were found in *G. m. centralis* (0.24 IU/midgut) and *G. m. morsitans* (0.47 IU/midgut) 48 h after a lizard bloodmeal (Fig. 4.6). Analysis of variance (ANOVA) of protease activity based on two factors: tsetse species and bloodmeal used and their interaction (tsetse x meal) detected significant differences among tsetse species (F=85.79, df=4, P<0.001), meal (F=86.03, df=2, P<0.001) and their interaction (F=79.84, df=8, P<0.001).

4.3.5 Trypsin activity in *G. m. centralis* and *G. fuscipes*

Trypsin activity was low in teneral *G. m. centralis* (0.003-0.012 μ moles/min/ml) and *G. fuscipes* (0.01-0.03). The activity increased cyclically in both *G. fuscipes* and *G. m. centralis* following ingestion of goat, lizard and crocodile blood and peak activity was attained between 48-72 h post feeding. Trypsin activity then decreased in *G. fuscipes*

Fig. 4.6 Protease activity (48 h) in five tsetse species fed on goat, lizard and crocodile blood.

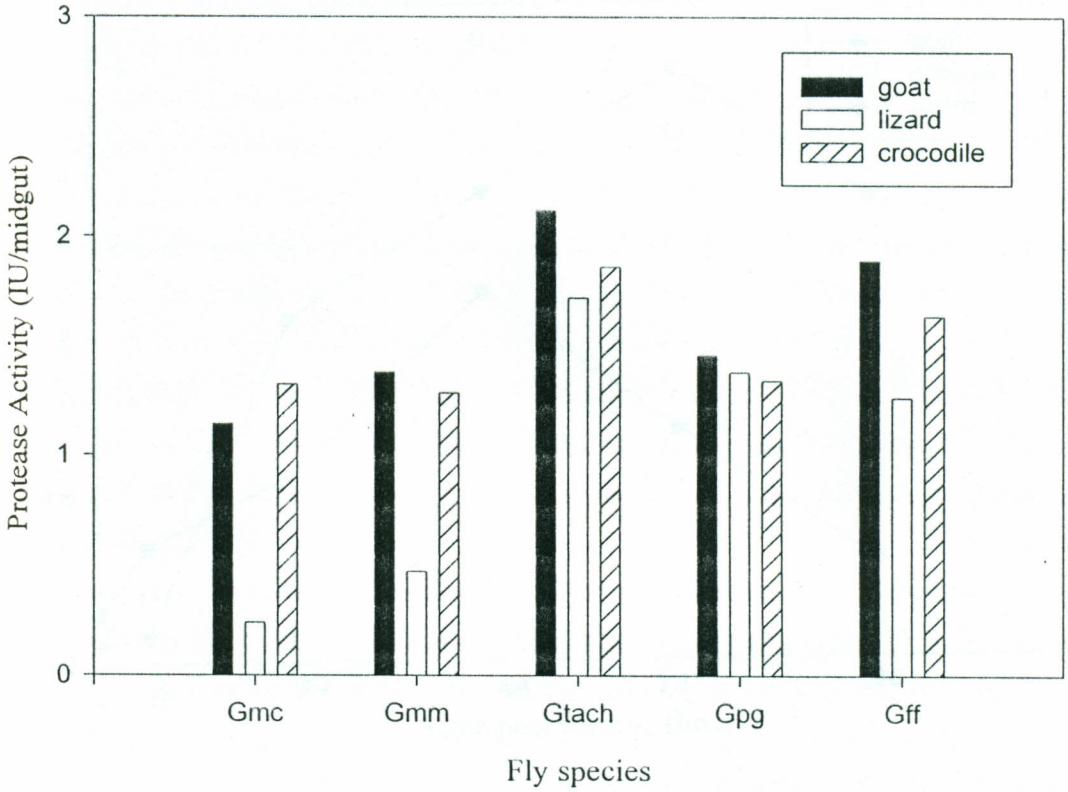


Fig. 4.7 Time course of trypsin activity in *G. fuscipes* fed on goat, lizard and crocodile blood

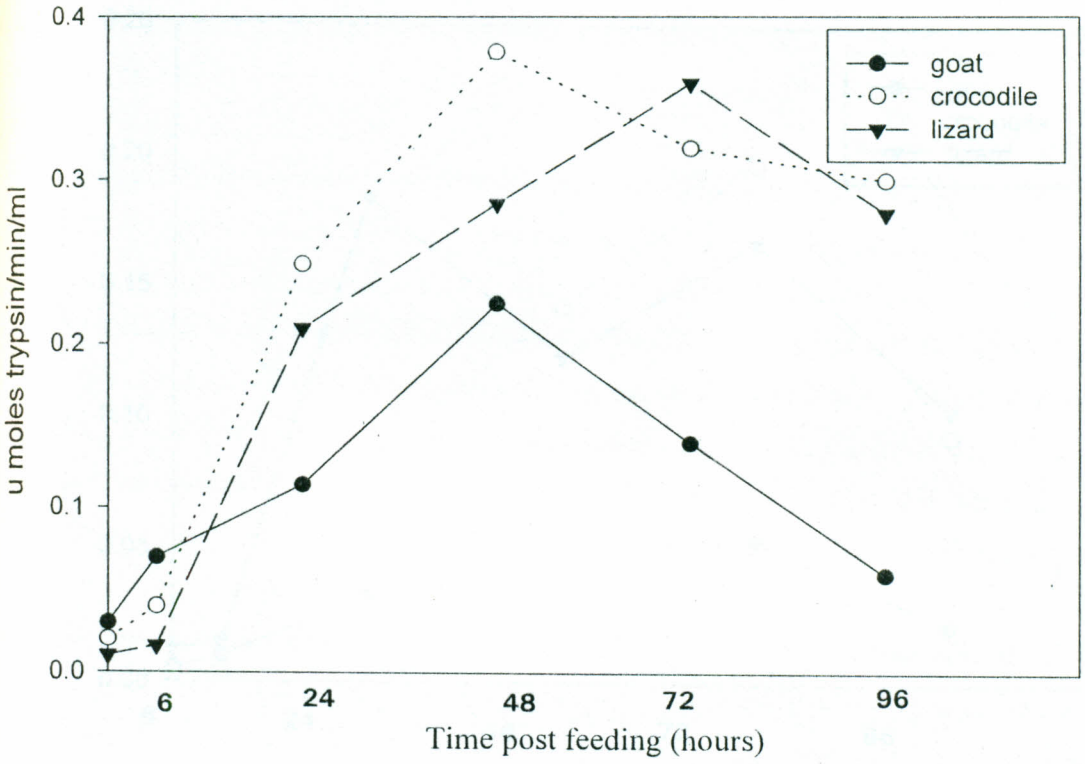
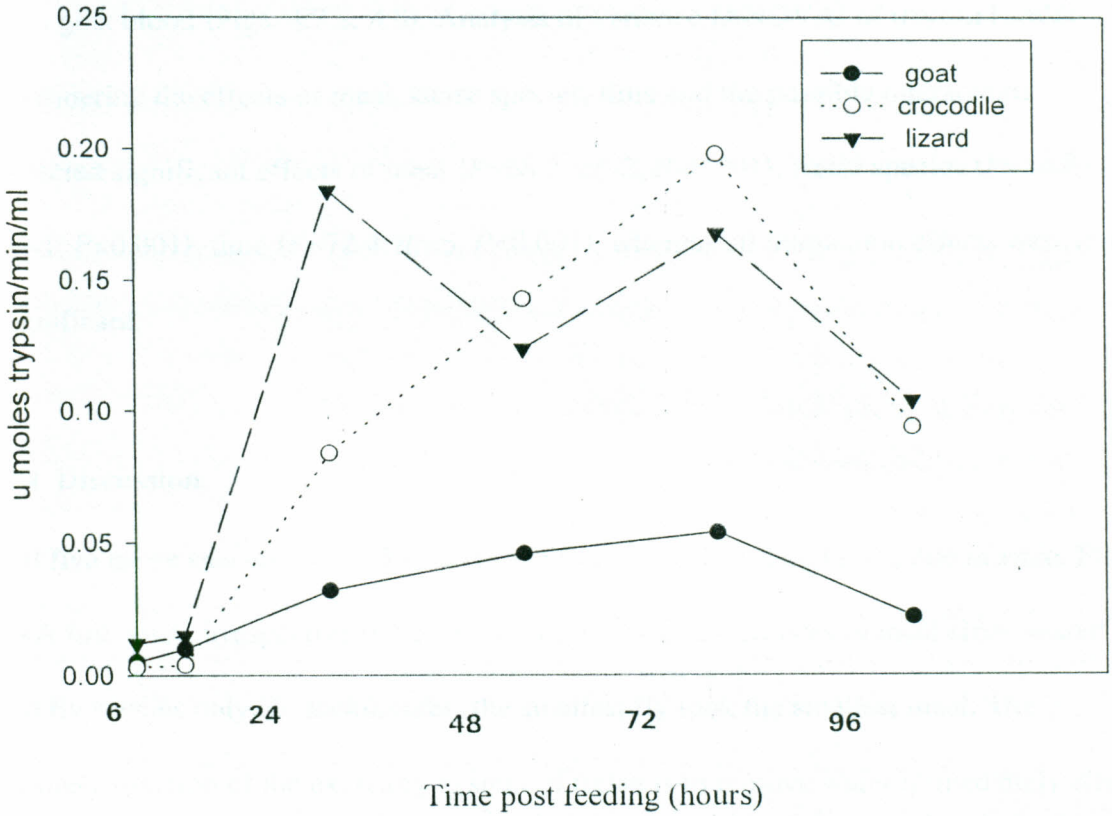


Fig.4.8 Time course of trypsin activity in *G. m. centralis* fed on goat, lizard and crocodile blood



(0.225-0.06μ moles/min/ml) (Fig. 4.7) and *G. m. centralis* (0.054-0.022 μmoles/min/ml) (Fig. 4.8) when goat blood was used and by 96 h had almost returned to the level of unfed, teneral flies. However, when lizard and crocodile blood were used, enzyme activity fell after the peak but did not return to the levels of activity found in the teneral fly. Trypsin activity was persistently higher in *G. fuscipes* than in *G. m. centralis*. Lizard and crocodile bloods induced higher activity in both tsetse species than goat blood (Figs. 4.7 & 4.8). Analysis of variance (ANOVA) of trypsin levels considering the effects of meal, tsetse species, time and the possible interactions detected significant effects of meal (F=68.7, df=2, P<0.001), tsetse species (F=46.8, df=1, P<0.001), time (F=72.4, df=5, P<0.001), whereas all interaction effects were not significant.

4.4 Discussion

All five tsetse species fed readily on blood from goat, lizard and crocodile *in vitro*. Flies took full meals irrespective of the blood source, with differences in meal sizes related to the fly species only. *G. tachinoides*, the smallest fly took the smallest meal. The primary function of the excretory system of tsetse is to remove water immediately after feeding. Results showed that the ingestion of blood was normally accompanied by rapid loss of water in the first 24 h. This loss was drastically inhibited in *morsitans* flies fed on lizard blood. The low protease levels in *morsitans* flies at 48 h post a lizard bloodmeal indicated that these flies were not able to fully utilise the lizard blood, this may have been responsible for the high mortality seen in *G. morsitans* subspecies post feeding.

The movement of water across the gut of tsetse is controlled by an active transport mechanism involving movement of Na^+ ions (Langley, & Pimley, 1973). The movement is assisted by a favourable osmotic gradient between the gut contents and the haemolymph. Two models have been proposed to explain water loss in tsetse. Water passes directly down the gut, or water passes through the gut wall to the malphigian tubules and there is no transfer to the tsetse haemolymph (Langley, 1972). Metabolic inhibitors ouabain and acetazolamide reduce the rate of water loss by *G. m. morsitans.* Ouabain interferes with the Na^+/K^+ active transport system across membranes. Acetazolamide is a specific inhibitor of carbonic anhydrase and also interferes with ion and water transport across membranes (Tobe, 1974). Ouabain-sensitive transport mechanisms in insects have also been reported in *Periplaneta americana* and *Drosophila hydei*. The lack of active water loss in *morsitans* flies after feeding on lizard blood and to a lesser extent, crocodile blood in this study is puzzling and could be partly due to similar toxins. The ionic composition of blood may be critical in determining water loss in tsetse. Blood of animals which possess a very high erythrocyte potassium concentration depress the rate of water excretion probably due to pathological leakage of potassium from intact erythrocytes or haemolysed blood (Langley & Pimley, 1973). The water transport system is assisted by a favourable osmotic gradient between the food medium and the gut contents of the fly. The system is incapable of working efficiently if the osmotic pressure of the gut medium exceeds that of vertebrate blood (Langley & Pimley, 1973). The nature of the blood cells may also play a role in influencing digestion. The erythrocytes of circulating reptile blood are nucleated oval cells with a high DNA content. The high DNA content may influence digestion in *morsitans* flies. By contrast, higher vertebrates eg. goats have

small enucleated erythrocytes. The inhibition of water loss in *morsitans* flies fed on lizard blood in this study could be due to a combined effect of toxins, viscosity of blood, ionic composition and osmotic pressure, upon the normal rate and extent of food uptake and manipulation of the meal prior to digestion. Results of Moloo (1993) have shown that reptiles are not a major food source of *morsitans* flies. Hence, these flies may simply have not evolved mechanisms of dealing with the unique physiological characteristics of reptilian blood, particularly its high DNA content. Duke (1935) showed the presence of large cylindrical masses of light green crystals in the intestines of *Glossina palpalis* fed on monitor lizard and crocodile blood. The crystals were associated with later stages of digestion. No crystals of this type were found in *morsitans* flies after feeding on lizard blood in this study.

Gels containing protease substrate have been used to measure proteolytic activity in microlitre quantities in crude tsetse gut extracts (Stiles *et al.,* 1991). The maximum enzyme activity in tsetse is found in the posterior gut 48-72 h post feeding (Gooding, 1985). In this study, although goat, lizard and crocodile bloodmeals had no significant effects on meal sizes, the type of blood ingested by the fly elicited different levels of proteolytic enzymes. General protease activity was higher in tsetse fed on goat than on crocodile and lizard blood. There were striking low protease levels in *morsitans* flies fed on lizard blood. This could be due to the presence of unique serum protease inhibitors in lizard blood. The low protease levels may have interfered with digestion in these flies. *Morsitans* flies rarely feed on lizards, but the physiological basis for this has never been studied. The protease levels were higher in riverine than in *morsitans* tsetse ($P<0.001$). Cheeseman and Gooding (1985) showed interspecific variation in the quantities and types of proteases; different species of tsetse produce different quantities

and types of proteases to digest a given bloodmeal. Inconsistencies in levels of general proteases in tsetse have also been reported by Stiles *et al.,* (1991), who showed that the quantity of casein-substrate-specific proteases in the posterior midgut lysates of *G. p. palpalis* were 1.56 fold higher than in *G. p. gambiensis.* Mihok *et al.,* (1995) found no differences in protease activity between infected and un-infected flies. Similarly, Mihok *et al.,* (1995) found no difference in protease levels between *G. m. centralis* and *G. m. morsitans* despite large differences in susceptibility to infection. Protease levels were, however weakly correlated with infection in *G. m. morsitans*, the tsetse responding most dramatically to lectin inhibitors (Mihok *et al.,* 1993). The levels of these proteases may affect trypanosome survival in the guts of flies. Protease secretion is controlled by a secretogogue mechanism with one or more serum proteins providing the normal stimulus to the midgut secretory cells. It is also possible that the products of digestion act directly on the secretory cells, inhibiting their activity. Stiles *et al.,* (1990) observed reduction in midgut proteases in *G. palpalis* following irradiation. Stiles *et al.,* (1989) reported that the midgut epithelial cells were significantly damaged following irradiation.

Trypsin production in two species (*G. fuscipes* and *G. m. centralis*) showed that it is a time-dependent process peaking between 48-72 h after a bloodmeal. Varying quantities of trypsin were produced by each fly species in response to a given bloodmeal. Trypsin levels were significantly higher in *G. fuscipes* than *G. m. centralis* ($P<0.001$). Lizard and crocodile blood resulted in higher trypsin levels than goat blood in both species. It is possible to relate trypsin activity at 48-72 h post bloodmeal to infection rates. For example, large differences in the susceptibility of *G. m. centralis* and *G. fuscipes* to various trypanosomes reported in this work are reflected in parallel

differences in trypsin activity. Similarly, a host blood that repeatedly reduced infection in tsetse (lizard) induced higher trypsin levels than a host blood that repeatedly increased infection (goat). Imbuga *et al.,* (1992 a, b) showed that tsetse midgut trypsins play an important role in the transformation of bloodstream trypanosomes. Transformation is only possible in the presence of optimal enzyme concentrations and of whole blood. It is likely that a certain trypsin level is required for this transformation above which it has a lytic effect. Host bloods which do not support high infection rates may induce high outputs of midgut trypsin; similarly, refractory tsetse may have higher trypsin levels post feeding than susceptible tsetse.

CHAPTER FIVE

5.0 EFFECT OF HOSTS' BLOOD ON TRYPANOSOME INFECTIONS IN TSETSE FLIES

5.1 Introduction

The developmental cycle of trypanosomes involves a vertebrate host and a tsetse vector. The transition from vertebrate to tsetse is critical for the trypanosome must adapt quickly to a hostile environment inside the tsetse gut to survive. The life cycle of *T. brucei* and *T. congolense* can be divided into two phases in the fly: establishment and maturation. To establish in the midgut, trypanosomes must change from bloodstream forms to procyclics and then move to the ectoperitrophic space to form an actively-dividing population, losing the glycoprotein coat (Vickerman, 1985). The procyclics then mature to infective forms, move to the mouthparts (*T. congolense*) or salivary glands (*T. brucei)*, and transform to dividing epimastigotes and finally metacyclics.

Gut establishment involves a complex interaction between the fly, endosymbionts and the trypanosome itself (Maudlin & Ellis, 1985; Maudlin & Welburn, 1988). Croft *et al.,* (1982) were the first to discover factors in tsetse that kill trypanosomes *in vitro*. Since then, numerous studies have revealed the presence of proven or potential trypanocidal factors in the tsetse gut and haemolymph for the various species of African trypanosomes (Maudlin, 1991). These include.(1) midgut lectins (Maudlin & Welburn, 1987, 1988; Welburn & Maudlin, 1989); (2) trypanoagglutinins and trypanolysins (Ibrahim *et al.,* 1984; Ingram & Molyneux, 1991) and (3) digestive proteases (Stiles *et al.,* 1991; Imbuga *et al.,* 1992a, b ; Osir *et al.,* 1993). These substances interact with the biology of the fly, its symbiotic organisms

and the genetic type of the trypanosome to determine the fate of an incipient infection. In both susceptible and refractory tsetse, parasite mortality appears to be dependent on the type and quantity of these factors as well as on the trypanosome species/strain taken up in the bloodmeal. Since the midgut factors are bloodmeal-induced, infection rates should be influenced by the type of host blood at the time of an infective meal (Moloo, 1981; Mihok *et al.,* 1993). These findings are not surprising as midgut factors involved in lysis and transformation are themselves induced by bloodmeals (Maudlin, 1991). Finally, most host blood contains cytolytic factors that act on trypanosomes directly. For example, fresh buffalo and waterbuck sera cause lysis of the bloodstream trypanosomes (Reduth *et al.,* 1994). Hosts are therefore major determinants in the epidemiology of trypanosomiasis.

The *palpalis* tsetse obtain significant proportions of their bloodmeals from reptiles, e.g monitor lizards and crocodiles (Moloo, 1993). For example, Mohammed-Ahmed & Odulaja (1997) reported that 78-93% of all bloodmeals taken by *Glossina fuscipes* near Lake Victoria region in Kenya were from lizards. Other hosts present in the area included goats, hippopotami, snakes and cattle. *G. fuscipes* has been responsible for epidemics of sleeping sickness near Lake Victoria both in Kenya and Uganda (Okoth & Kapaata, 1988). The history of sleeping sickness in this region has provoked controversy about the origins, spread of the disease and the identity of the causative organisms. Genetic exchange may be important in generating diversity in trypanosome strains but its' role in the generation of epidemics of human sleeping sickness is not clear (Hide *et al.,* 1996).

A close contact exists between man, wild animals, lizards and *G. fuscipes* at watering points along the Lake Victoria shores. Infected flies, though feeding mainly

on lizards, feed occasionally on man and his livestock. The outcome of such feeds is not known. Although work has been carried out on the vectorial capacity of *palpalis* flies (Moloo & Kutuza, 1988 a, b; Moloo *et al.,* 1992), no studies have been carried out to determine the outcome of trypanosome infections in riverine tsetse depending primarily on reptilian bloodmeals. For instance, it is not known whether reptile blood supports survival and viability of trypanosomes and for how long. The effects of reptile blood on the riverine fly's ability to acquire early midgut infections, as well as maturation of trypanosomes are also not known. Mihok *et al.,* (1993) using *G. m. centralis* and *G. m. morsitans* showed that the bloodmeal used by the fly is important in determining the outcome of the infection. In this study, investigations were carried out to determine the effect of reptilian blood (lizard and crocodile) on trypanosome viability, early establishment and maturation in representative economically-important tsetse. These studies may be important in explaining the natural transmission cycle of trypanosomes between reptiles, man and livestock.

5.2 Blood incubation infectivity test (BIIT)

Trypanosomes of the subgenus *Trypanozoon* (*T. b. gambiense*, *T. b. rhodesiense* and *T. b. brucei)* are indistinguishable morphologically. The only practical distinguishing feature is the ability of two of the parasites, *T. b. gambiense* and *T. b. rhodesiense* to cause disease in humans. *T. b. brucei* is a parasite of wildlife and occasionally livestock and is not infective to humans. Human sera is trypanolytic for *T. b. brucei*. This lytic activity has been used in a simple assay, the blood incubation infectivity test (BIIT) to distinguish between the human-infective and the non-human infective trypanosomes (Rickman and Robson, 1970). The test has been used widely to analyse bloodstream

populations isolated from animals for human serum resistance and also to demonstrate trypanotolerance characteristics of African wildlife (Mehlitz *et al.,* 1982; Rickman and Kolala 1982; Mulla and Rickman, 1988). BIIT consists of a standardised *in vitro* incubation of trypanosomes in human serum or blood with subsequent inoculation into susceptible laboratory rodents. If no infection develops, it is concluded that all the trypanosomes were killed by the serum and were most likely not *T. b. rhodesiense* or *T. b. gambiense.* This test is the only practical alternative to the actual infection of humans.

Monitor lizards come in close contact with man in areas where sleeping sickness is endemic. The circulation of the human disease between man, livestock and monitor lizards should not be ignored. Studies of serum resistance were therefore carried out using human, lizard, goat, and crocodile serum in order to determine the potential role of these hosts in the transmission of trypanosomes.

5.3 MATERIALS AND METHODS

5.3.1 Tsetse flies used in the infectivity studies

G. m. centralis, G. m. morsitans, G. pallidipes, (*morsitans* group) and *G. fuscipes*, *G. p. gambiensis* and *G. tachinoides* (*palpalis* group) were used for the infection experiments.The flies were laboratory reared at 25°C, relative humidity 85%, 12 h period of light and 12 h period of darkness.

5.3.2 Trypanosomes

Trypanosoma brucei (Ng3), savannah *T. congolense* (Ng5) and riverine *T. congolense* (ANR3) were used. The parasites were expanded in individual goats under controlled conditions in order to obtain standardised material. *G. m. centralis* with mature infections were used to initiate infections through cyclical challenge. At peak parasitaemia (1x 10^5 parasites) goats were bled via the jugular vein and 100 ml of blood cryopreserved for infectivity studies.

5.3.3 Blood incubation infectivity test (BIIT)

Experiments were carried out to test the lytic action of goat, lizard, crocodile and human sera on 15 stocks of trypanosomes (Table 5.1) in a blood incubation infectivity test. The test was carried out as described in the original version used by Rickman and Robson (1970). The trypanosomes were raised in Wistar rats. At peak parasitaemia (10^7 parasites/ml) the rats were bled by cardiac puncture using heparin as an anticoagulant. The standard BIIT was carried out by mixing one volume of heparinised rat blood containing trypanosomes with three volumes of serum. Incubations were carried out at 37° C for 2 h followed by inoculation of 0.3 ml of the mixture into five rats. The rats were monitored daily for parasitaemia.

5.3.4 The early pattern of tsetse midgut infections with trypanosomes

The pattern of infection of three trypanosome species in six species of tsetse was studied on days 3 and 6 using goat, lizard or crocodile blood.

Cryopreserved parasites were diluted in fresh (occasionally frozen) goat blood to a final concentration of 1x 10^6 parasites/ml. Dilutions were then made 1:100 in goat, lizard

Table 5.1 Stocks of trypanosomes used in the blood incubation infectivity test (BIIT)

stabilate	clone/stock	source	reference
Iltat1.1	*T. brucei* DNA clone	cow	Masake *et al.*, 1991
Wr 3	*T. brucei* stock	white rhino	Mihok *et al.*, 1990
Wr 5	*T. brucei* stock	white rhino	Mihok *et al.*, 1991
Sere 1	*T. brucei* stock	lion	Mihok *et al.*, 1993
Sere 2	*T. brucei* stock	buffalo	Mihok *et al.*, 1991
Mara 7	*T. brucei* stock	white rhino	Mihok *et al.*, 1991
Wr 18	*T. brucei* stock	white rhino	Mihok *et al.* 1991
Wr 19	*T. brucei* stock	white rhino	Mihok *et al.* 1991
Ng 10	*T. brucei* DNA clone	buffalo	Mihok *et al.*, 1992
Ng 15	*T. brucei* DNA clone	buffalo	Mihok *et al.*, 1992
Ng3	*T. brucei* DNA clone	buffalo	Mihok *et al.*, 1992
K 60	*T. congolense* DNA clone	dog	Masake *et al.*, 1991
Ng 5	*T. congolense* DNA clone	buffalo	Mihok *et al.*, 1993
Anr 3	*T. congolense* DNA clone	pig	Gibson *et al.*, 1988
Tsw 103	*T. congolense* DNA clone	pig	McNamara *et al.*, 1994

and crocodile blood for a final concentration of 10^4 parasites/ml. Parasite-blood mixtures were incubated at 38.5° C for 1 h prior to tsetse feeding to duplicate conditions used in tests designed to detect the presence of trypanotoxins in wildlife sera (Rickman & Kolala, 1982). The solutions were fed to teneral 1-day-old flies through a silicone membrane (Plate 5.1) (Mews *et al.*, 1977) after confirmation of the parasite viability and motility. Dissections were carried out on midguts of an average 37 flies (range 34-50) for each experiment on day 3 to detect procyclic forms. Dissections were performed in phosphate saline glucose (PSG) buffer. Additional flies were fed on the same blood without parasites on day 3. Blood was held at 4° C in the refrigerator between feeds. Dissections were then carried out on an average of 40 flies (range 35-47) for each experiment on day 6-7 to detect midgut procyclic forms.

5.3.5 The overall pattern of tsetse midgut infections with trypanosomes

Experiments were conducted to study the pattern of midgut infections of three trypanosome species in six species of tsetse on days 3, 6, 10 and 21 using goat and lizard blood. The overall pattern of infection with lizard blood was only studied in riverine tsetse. Flies given an infective goat or lizard meal on day 0 were fed subsequently on the same uninfected blood on days 3, 6, 9, 12, 15 and 18 respectively. Midgut dissections were carried out on an average 31 flies (range 28-35) of each tsetse species on each of days 3, 6, 10, and 21 in order to establish the pattern of infection.

Plate 5.1

Feeding flies *in vitro* through silicone membranes

5.3.6 The effect of goat blood on maturation of trypanosomes in tsetse

Experiments were carried out to study the maturation of *T. brucei* in six tsetse species using goat blood. Male and female tsetse (*morsitans* and *palpalis*) were given an infective goat meal containing $1x10^4$ parasites/ml (Ng3, Anr3 and Ng5) on day 0. The flies were subsequently maintained on goat blood for 30-33 days. On average, 36 flies (range 31-42) were dissected in each experiment between days 35 to 37 after infection following 2 days of starvation. Midguts, salivary glands and proboscides were dissected in PSG (pH 8.0) and then examined by phase-contrast microscopy (x400) to detect parasites. Infections were scored as immature if they were only found in the gut, and mature if they were found in the gut and hypopharynx (*T. congolense*) or gut and salivary glands (*T. brucei)*.

5.3.7 The effect of prior blood feeding on early infection rates in *palpalis* (group) flies

Experiments were carried out to investigate the effect of pre-feeding *palpalis* flies on goat, lizard or crocodile meals on the pattern of *T. brucei* infection. In total, 654 flies were used with an average 34 flies (range 28-39) dissected for each experiment on day 6 of infection. Teneral flies obtained 24 h post eclosion and flies fed once after emergence on goat, lizard or crocodile blood were used. Flies were given an infective meal on day 0 and subsequently fed on the same blood without parasites on day 3. Alternatively, flies were given a clean meal on day 0 and then the same blood with parasites on day 3. Dissections were performed on the tsetse midguts between day six and seven post infection to reveal midgut procyclics.

5.3.8 The effect of different bloodmeals on early infections in *G. fuscipes*

Nine experiments were carried out using all possible combinations of goat, lizard and crocodile blood on days 0 and day 3 to study the effect of reptilian blood on the early pattern of *T. brucei* (Ng3), and *T. congolense* (Ng5, Anr3) infections in *G. fuscipes*. In total, 265 tsetse were used with an average of 31 flies (range 28-36) dissected for each experiment. All nine possible bloodmeal combinations used on day 0 and 3 and were as follows: goat:goat, goat:lizard, goat:crocodile, lizard:lizard, lizard:goat, lizard:crocodile, crocodile:crocodile, crocodile:goat and crocodile:lizard. Dissections were performed on day 6 and 7 post feeding to reveal midgut infections. A further nine experiments were carried out to determine the effect of varied meals on days 6 and 8 on the probable outcome of infections on day 10. In total 273 tsetse were used with an average of 32 flies (range 30-34) dissected for each experiment. Dissections were performed on day 10 post feeding to show midgut infections in these additional groups.

5.3.9 The effect of different feeding patterns on maturation of *T. brucei* in *palpalis* (group) flies

Three experiments were carried out to investigate the effect of goat, lizard or interrupted goat and lizard meals on maturation of *T. brucei* (Ng3) in both male and female *palpalis* tsetse. Altogether 604 tsetse were used with an average 32 flies (range 29-35) dissected between days 35 and 37 post infection. *G. fuscipes, G. tachinoides* and *G. p. gambiensis* were given an infective meal containing 1 x 10^4 trypanosomes/ml. (*T brucei* only) in goat or lizard blood. The flies were maintained subsequently either wholly on goat blood or wholly on lizard blood or alternating goat and lizard

bloodmeals. Dissections were carried out between days 35 and 37 post-feeding on the midgut, proboscis and salivary glands to determine maturation rates.

5.3.10 Preparation of bloodstream and procyclic *T. brucei* forms for agglutination assays

Male Wistar rats were infected with *T. brucei*. At peak parasitaemia (10^7 parasites/ml) the rats were bled by cardiac puncture using heparin as an anticoagulant. Bloodstream forms were separated from rat blood using a DEAE column as previously described (Lanham and Godfrey, 1970). Procyclic culture forms of the parasite were obtained by transferring the bloodstream forms into an SDM-79 cultivation media (Brun and Jenni, 1985) that contained 10% foetal calf serum without the use of antibiotics. The trypanosomes were cultivated at 27^0 C with twice-weekly passages. The parasites were pelleted from the medium by centrifugation (1,000 g, 10 min, 4° C) and counted using an improved Neubauer haemocytometer.

5.3.11 Agglutination assays

Teneral *G. fuscipes* were either left unfed or were fed on goat or lizard blood (24 h after emergence), and then starved for 72 h. The flies were immobilised by brief chilling at 4° C, after which their midguts were carefully removed. The midguts were then dipped several times in phosphate-buffered saline (PBS) (0.1M sodium diphosphate (pH 8.0) containing 0.15M NaCl) to remove any haemolymph, and then resuspended in 1.0 ml ice-cold PBS. Each pool of 20 midguts was then homogenised using a Virtis homogenizer, followed by centrifugation (12,000 g, 5 min, 4° C) in a Heraeus 2 Minifuge. The supernatant was stored at -20°C in the refrigerator until required for use.

The pooled samples of crude midgut homogenate from unfed teneral and fed flies were used to prepare doubling serial dilutions in phosphate buffered saline (PBS), containing $MgCl_2$ (0.1 mg/ml). The samples (2 μl) were placed into wells of a flat-bottomed microtitre plate, and an equal volume of the trypanosomes (bloodstream or procyclic forms) containing $5x10^6$ *T. brucei* parasites/ml was added to each dilution. After mixing, the plate was incubated at 27° C for 2 h and the agglutination scored using an inverted microscope. All the tests were carried out in duplicate, with the controls consisting of only parasites and phosphate buffered saline (PBS). The reciprocal of the highest dilution giving agglutination was considered as the end point.

5.3.12 Induction of agglutination by bloodmeal in *G. fuscipes*

Studies were carried out to determine the time course of agglutination activity in *G. fuscipes* post feeding. *G. fuscipes* were fed on goat or lizard blood 24 h post emergence. Afterwards twenty flies were killed at 24 h intervals until 120 h. Midgut homogenates were prepared from these flies and assayed as above for their ability to agglutinate bloodstream parasites.

5.3.13 Effects of sugars on midgut infections of *T. brucei* in *palpalis* flies

Three experiments were carried out to study the effects of adding D-glucosamine, D-glucose, D-galactose, mannose and melibiose to the infective meal on midgut infections of *T. brucei* in three tsetse species of the *palpalis* group. In total, 846 tsetse were used with an average 32 (range 29-34) flies dissected for each experiment. The sugars were prepared to a concentration of 0.06 M in goat or lizard blood and used in the infective

meal on day 0. A subsequent meal was given on day 3 without the sugars. Dissections of midguts were carried out on days 6-7 post infection to reveal procyclics.

A chi-square test was performed on the data.

5.4 Results

5.4.1 Blood incubation infectivity test (BIIT)

In a blood incubation infectivity test to study the effects of goat, lizard, crocodile and human sera on 15 stocks of trypanosomes, human serum lysed all the stocks of trypanosomes preventing infection in rats except for *T. brucei* (Ng15), which was resistant in all trials (N=5). The rats were parasitaemic 5 days post infection with this parasite (Table 5.2). This *T. brucei* parasite, which was used in many previous experiments (Mihok *et al.,* 1994), was therefore not used again because of its potential for human infectivity. Otherwise, there was only minimal evidence for trypanotoxins in reptilian blood, consisting of a slightly longer prepatent period in rats relative to goat and human sera (Table 5.2). Also both reptile sera lysed two stocks of *T. brucei* obtained from white rhinoceros (WR18, WR19), preventing infection in rats.

5.4.2 Early pattern of midgut infections with *Trypanosoma brucei*

The patterns of infection were quite different for each trypanosome species. Infections of *T. brucei* were high in all the flies on day 3 (39.4 - 81.7%) and low by day 6 (21.6 - 56.3 %). Overall, goat blood supported higher infections on day 3 (72.5%) and day 6 (48.9%) than lizard and crocodile blood. A log linear analysis of infection rates based on tsetse species, day of infection, meal taken on day 0, and all interaction effects showed that the main effects were all highly significant (Table 5.3), 2-way interaction

Table 5.2 Blood incubation infectivity test (BIIT) in 15 stocks of trypanosomes using goat, lizard, crocodile and human sera

Stock	goat	lizard	crocodile	human
	Average prepatent period in 5 rats			
Iltat1.1	2	9	7	-
Wr3	3	9	6	-
Sere1	5	8	9	-
Wr18	8	-	-	-
Wr19	9	-	-	-
Ng5	11	15	14	-
Ng3	5	9	9	-
Anr3	9	12	11	-
Tsw 103	10	12	14	
WR 5	8	13	13	-
Sere2	7	12	10	-
Ng10	4	11	8	
Ng15	6	10	9	5
K60	11	16	14	-
Mara7	3	11	7	-

Table 5.3 Log linear analysis of infection rates on day 3 and day 6 in 1440 tsetse flies of six species infected with *T. brucei* (Ng3) in goat, lizard or crocodile blood.

Factor	*df*	χ^2	*P*
Days	1	120.4	< 0.001
Tsetse	5	74.2	< 0.001
Meal	2	26.1	< 0.001
Days x Tsetse	5	10.2	0.069
Days x Meal	2	6.2	0.044
Tsetse x Meal	10	4.9	0.900

effects were also either significant (day x meal, P=0.04) or nearly so (day x tsetse P=0.07). Hence the data were re-analysed for each day. On day 3, the interaction effect between tsetse and meals was not significant (χ^2=4.9, df=10, P=0.89); the effect of tsetse species was highly significant (χ^2=56.7, df=5, P<0.001) and the effect of meal was marginally not significant (χ^2=5.40, df=2, P=0.07). The *morsitans* group flies were more susceptible to *T. brucei* than the *palpalis* group tsetse, with *G. m. centralis* being the most susceptible and *G. fuscipes* the least susceptible species (Fig. 5.1).

On day 6, the interaction effect between tsetse and different bloodmeals was not significant (χ^2=5.02, df=10, P=0.88). In contrast to day 3, both the effect of tsetse species (χ^2=32.55, df=5, P<0.001) and the effect of meal (χ^2=6.29, df=2, P<0.001) were highly significant. Whereas infection rates were high and fairly uniform among meals on day 3, lizard and crocodile meals had a strongly depressive effect on the outcome of infection on day 6 (Fig. 5.2). Species trends were similar on days 3 and 6 with the *morsitans* group flies being more susceptible than the *palpalis* group. *G. m. centralis* supported the highest infections whereas *G. fuscipes* had the lowest infection rates (Fig. 5.1).

5.4.3 Early pattern of midgut infections with *T. congolense* (savannah) parasites.

Infections of *T. congolense* (savannah) were high in all the flies on day 3 (45.3-83.9%). *G. m. centralis* had the highest midgut infections 83.9% while *G. fuscipes* had the lowest 45.3%. Following a second bloodmeal on day 3 there were reductions in midgut infections in all the flies by day 6 (40.2-4.5%) (Fig. 5.3). Goat, lizard and crocodile

Fig. 5.1 *T. brucei* (Ng3) infections on days 3 and 6 in six species of tsetse

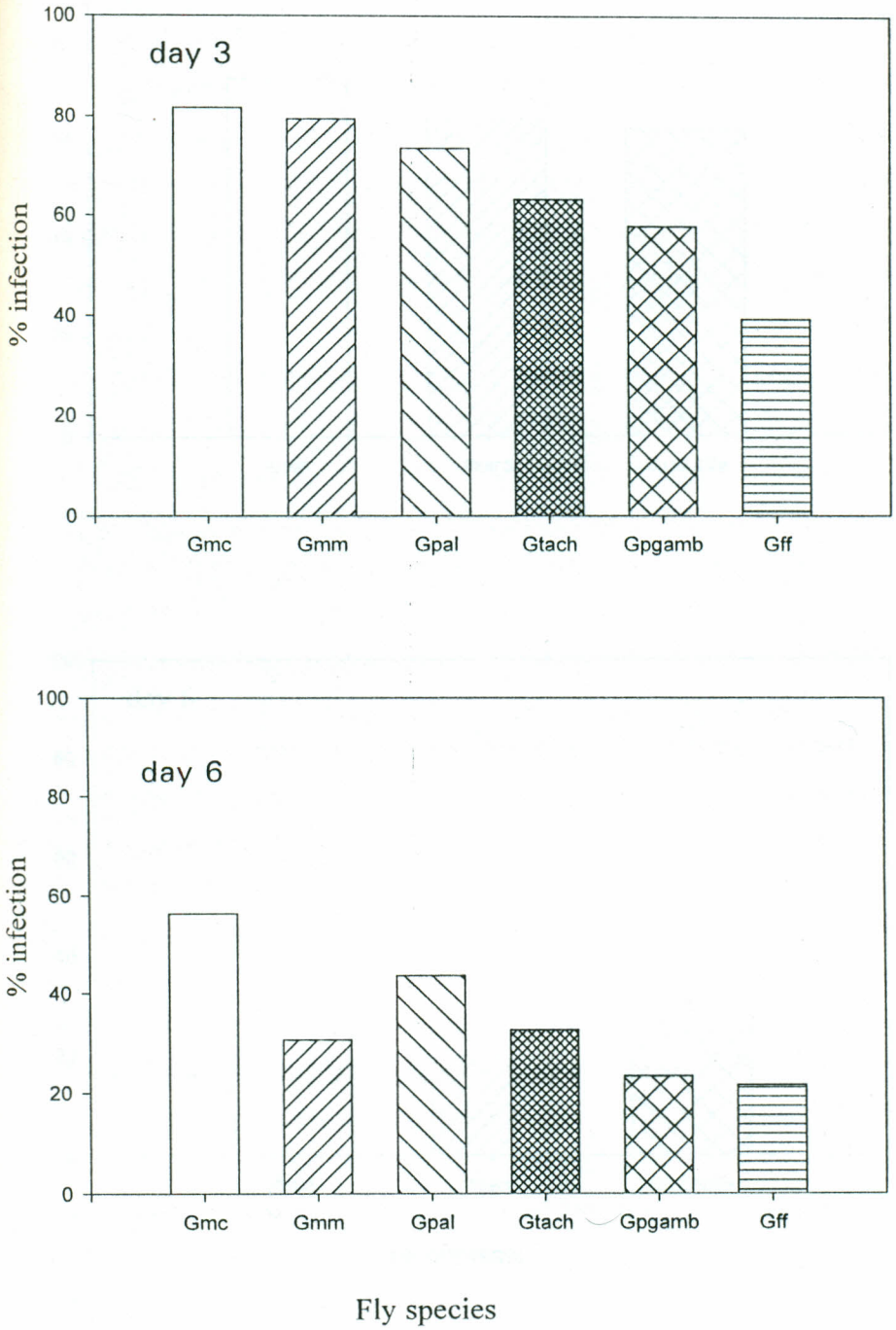


Fig. 5.2 *T. brucei* (Ng3) infections on days 3 and 6 using goat, lizard or crocodile blood.

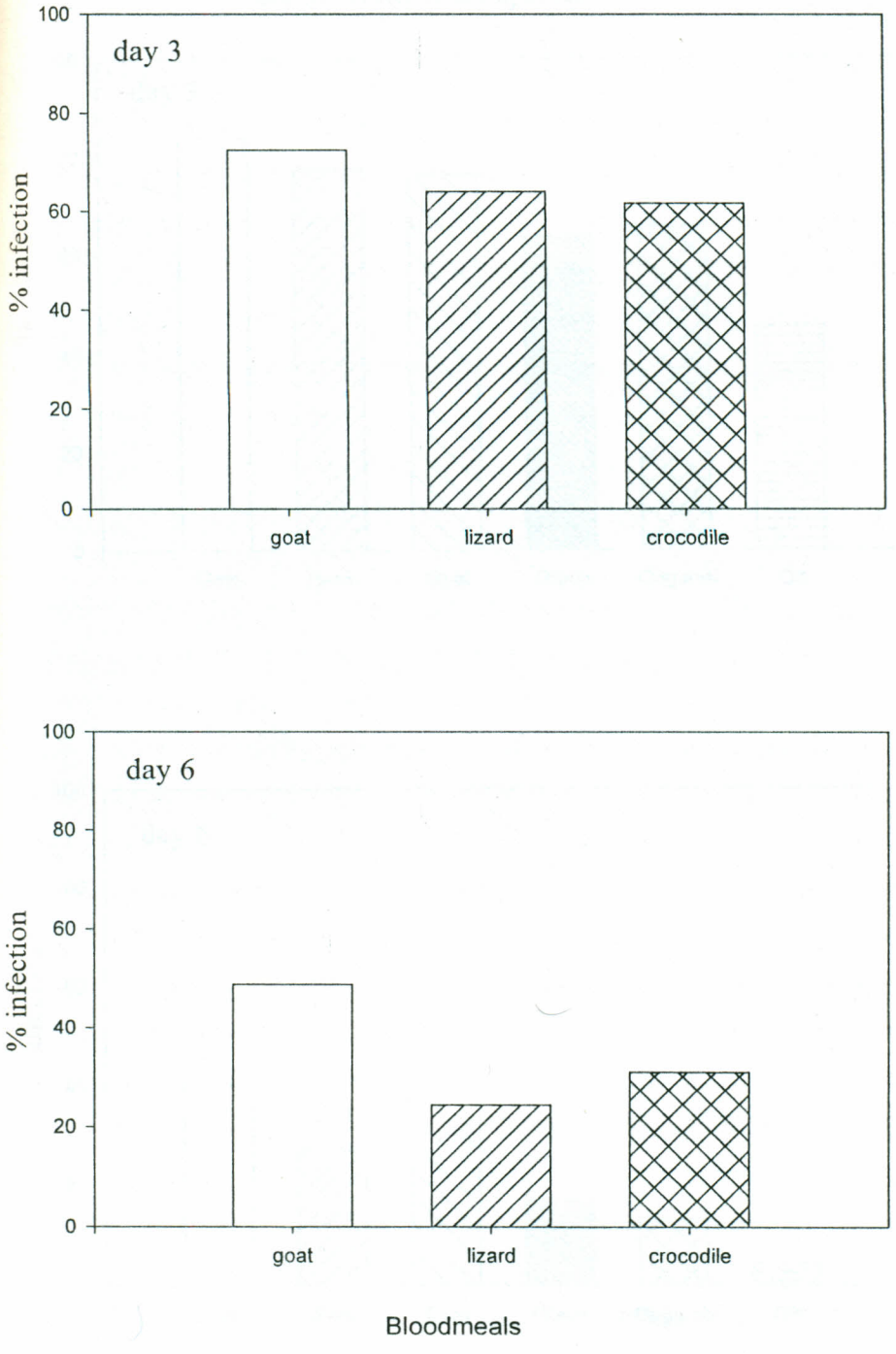


Fig. 5.3 *T. congolense* (Ng5) infections on days 3 and 6 in six different tsetse species

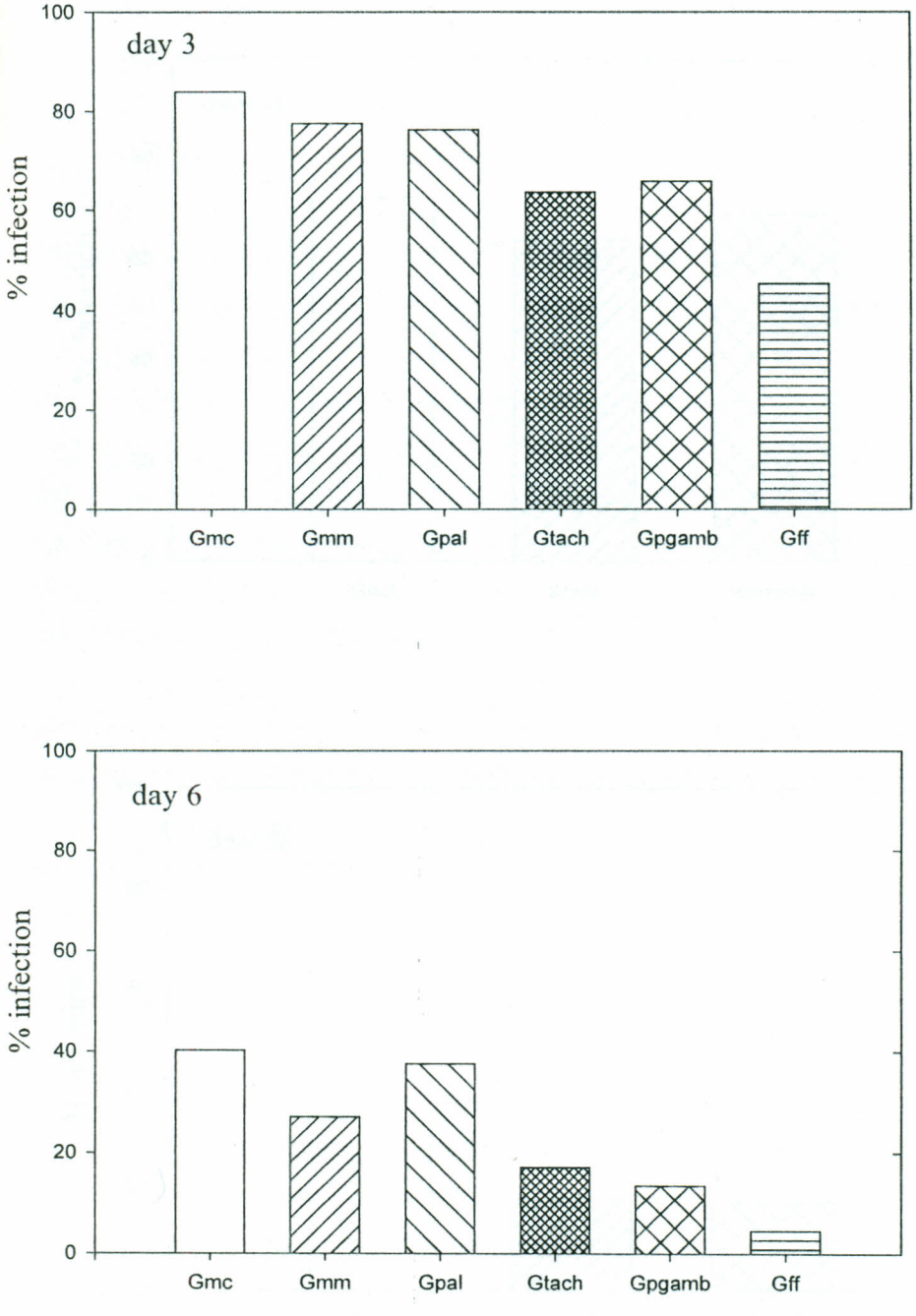
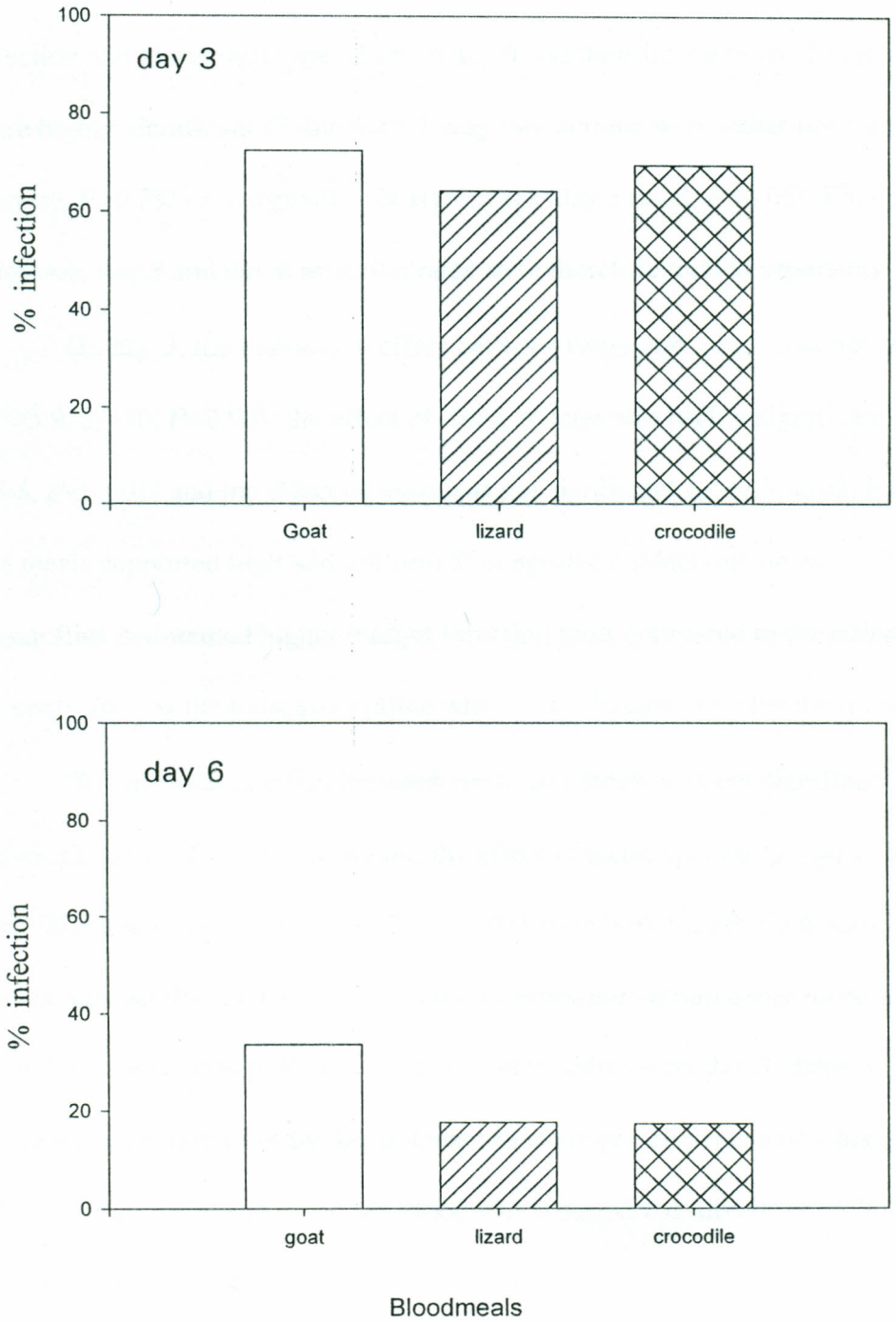


Fig. 5.4 *T. congolense* (Ng5) infections on days 3 and 6 using goat, lizard or crocodile blood



blood supported high infections of *T. congolense* in all flies on day 3 (64.2-72.4%). By day 6, midgut infections were reduced (33.6-17.6%) (Fig 5.4). The data on infections were analysed using log linear analysis based on three factors; tsetse species, day of infection and bloodmeal type taken on day 0 and their interactions. The main effects were highly significant (Table 5.4); 2-way interactions were either not significant (day x tsetse, P=0.29) or marginally not significant (day x meal, P=0.06). For comparative purposes, day 3 and day 6 infection rates were therefore treated separately.

On day 3, the interaction effect between tsetse and meals was not significant (χ^2=5.9, df=10, P=0.98); the effect of tsetse species was highly significant (χ^2=41.30, df=5, P<0.001) and the effect of meal was not significant (χ^2=7.9, df=2, P=0.16). All the meals supported high and uniform *T. congolense* infections on day 3. The *morsitans* group flies maintained higher midgut infection rates compared to the *palpalis* group. *G. m. centralis* was the most susceptible whereas *G. fuscipes* was the most resistant.

The interaction effect between tsetse and meals was not significant on day 6 (χ^2=5.33, df=10, P= 0.97), however, the effect of tsetse species (χ^2=44.3, df=5, P<0.001) and meal (χ^2=15.9, df=2, P<0.001) were both highly significant. Species trends were similar on day 3 and 6 with the *morsitans* group being more susceptible than the *palpalis* group. Following a second bloodmeal on day 3, there was a large decline in infection rates by day 6. Goat blood supported persistently higher infections (38.5%) than lizard and crocodile blood which supported infections of 21.8% and 20% respectively (Fig. 5.4).

Table 5.4 Log linear analysis of infection rates on day 3 and day 6 in 1205 tsetse flies of six species infected with *T. congolense* (Ng5) in goat, lizard or crocodile blood.

Factor	*df*	χ^2	*P*
Days	1	219.8	< 0.001
Tsetse	5	77.9	< 0.001
Meal	2	18.6	< 0.001
Days x Tsetse	5	6.1	0.297
Days x Meal	2	5.6	0.060
Tsetse x Meal	10	4.3	0.941

5.4.4 Early pattern of midgut infections with *T. congolense* (riverine)

Trypanosoma congolense (riverine) infections were higher in all the flies on day 3 (39.3-79.5%) than on day 6 (13.5-43%) (Fig. 5.5). Bloodmeals obtained from goat, lizard and crocodile supported high infections on day 3 (62.4-72.1%). Midgut infections were reduced by day 6 (25.9-34.1%). The data were analysed using log linear analysis based on tsetse species, day of infection, meal taken on day 0 and their interactions. The main effects, tsetse species, day of infection and infective meal were all highly significant; 2-way interaction effects (day x tsetse) and (day x meal) were not significant (Table 5.5).

On day 3, the interaction between tsetse and meals was not significant (χ^2=8.7, *df*=10, *P*=0.98); the effect of tsetse was highly significant (χ^2=47.2, *df*=5, *P*<0.001) and the effect of meal was not significant (χ^2=6.4, *df*=2, *P*=0.49). There were significant differences in infection rates on day 3 among tsetse. The *morsitans* group maintained persistently higher midgut infections than the *palpalis* group (Fig. 5.5). All the meals however supported high infections on day 3 (Fig. 5.6).

Following a second meal on day 3, the interaction effect between tsetse and meals on day 6 was not significant (χ^2=6.2, *df*=10, *P*=0.79). In contrast to day 3, the effect of meal was significant (χ^2=8.23, *df*=2, *P*=0.01). The effect of tsetse was also highly significant (χ^2=26.5, *df*=5, *P*<0.001). The depressive effect of reptilian meals on infection rates was evident only on day 6. Goat meal supported higher midgut infection rates than crocodile and lizard meals (Fig. 5.6).

Fig. 5.5 Riverine Anr3 (*T. congolense*) infections on days 3 and 6 in six different tsetse species

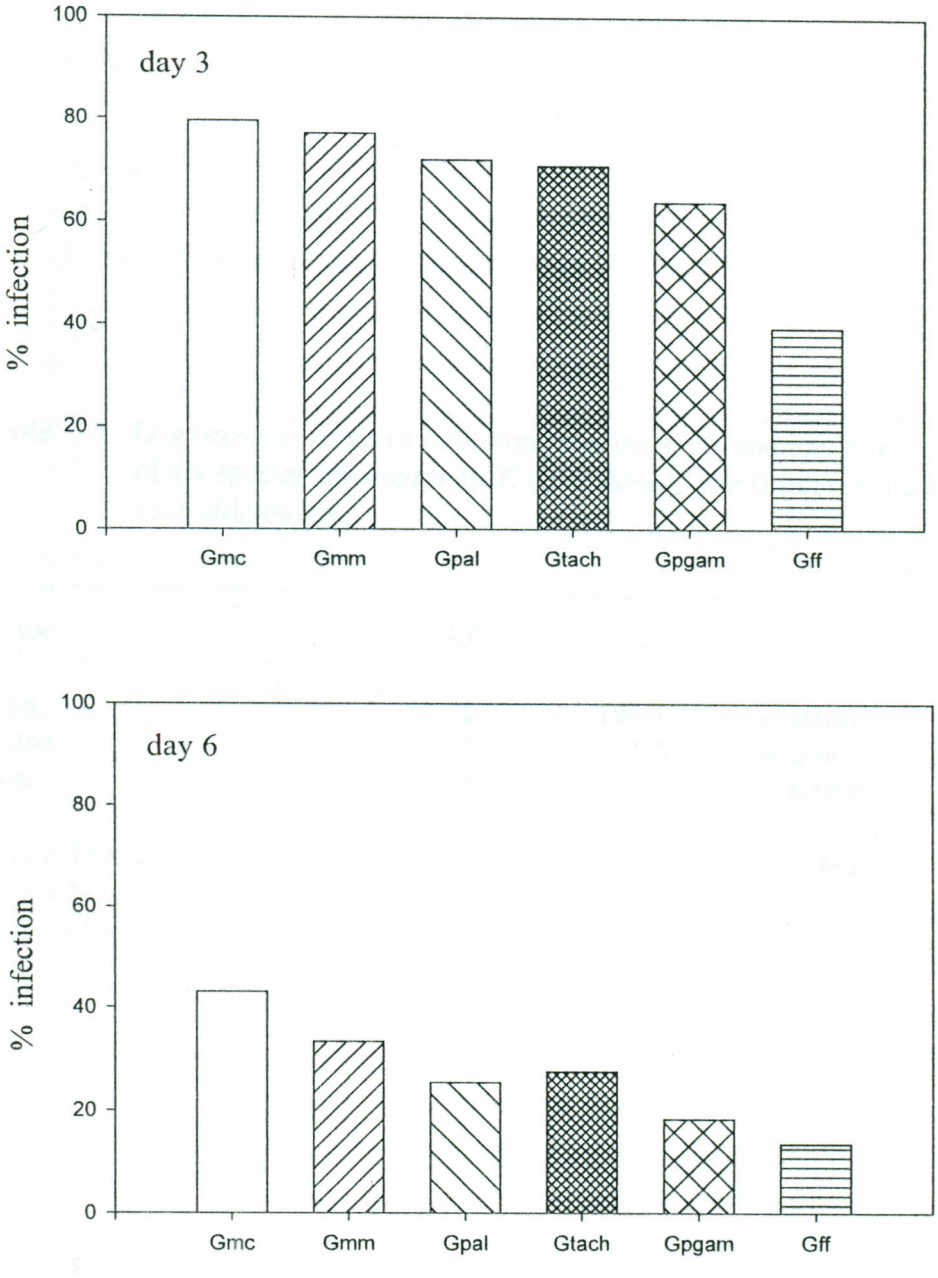
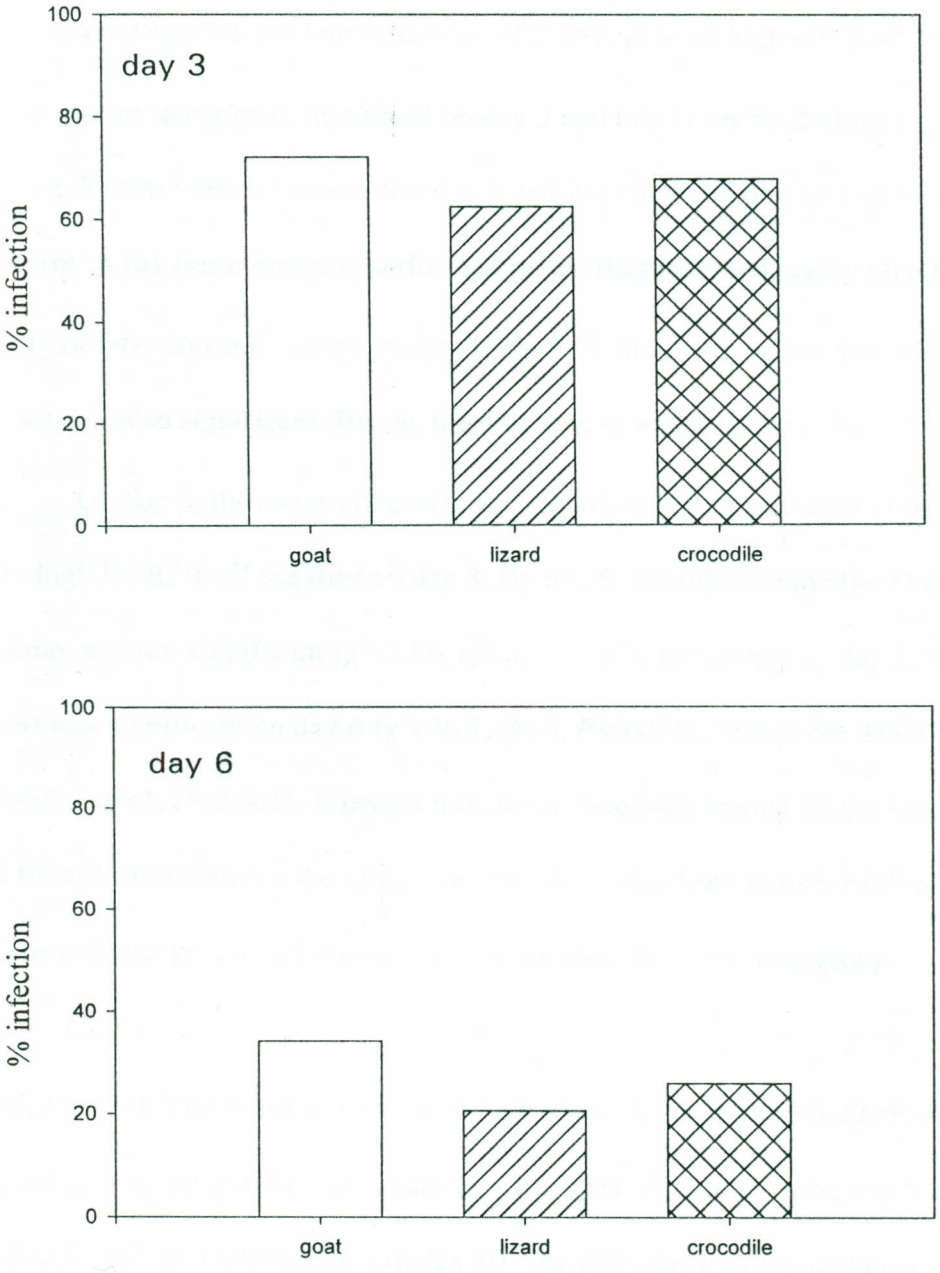


Table 5.5 Log linear analysis of infection rates on day 3 and day 6 in 1188 tsetse flies of six species infected with *T. congolense* (Anr3) in goat, lizard or crocodile blood.

Factor	*df*	χ^2	*P*
Days	1	196.1	< 0.001
Tsetse	5	61.5	< 0.001
Meal	2	9.2	0.010
Days x Tsetse	5	4.7	0.462
Days x Meal	2	8.6	0.433
Tsetse x Meal	10	5.9	0.978

Fig. 5.6 *T. congolense* (Anr 3) infections on days 3 and 6 using goat, lizard or crocodile blood



5.4. 5 The late pattern of midgut infections with *T. brucei*

The pattern of midgut infections on days 3, 6, 10, and 21 were quite different for each trypanosome species. Midgut infections of *T. brucei* were high (39.8-80%) in all the tsetse species using goat bloodmeal on day 3 and low (13.6-56.2%) by day 6. Infections did not decrease much further after day 6 and infections on day 10 and 21 were fairly uniform. A log linear analysis performed on the data revealed highly significant effects of day of infection and tsetse species (Table 5.6); the 2-way interaction effect (day x tsetse) was also significant. Hence, the data was re-analysed for each of the days.

On day 3, the tsetse effect was not significant. *T. brucei* infections persisted at very high levels in all the flies on day 3. By day 6, the interaction effect between tsetse and day was not significant (χ^2=13.6, *df*=5, *P*=0.93). In contrast to day 3, the effect of tsetse was significant on day 6 (χ^2=46.1, *df*=5, *P*<0.001), so was the day effect (χ^2=35.3, *df*=1, *P*<0.001). Whereas infections were high among all the flies on day 3, the flies cleared many of the infections after day 3 resulting to lower infections on day 6. The *palpalis* group cleared more infections than the *morsitans* group.

On day 10, the interaction between tsetse and day was not significant (χ^2=32.2, *df*=5, *P*=0.98). The effect of tsetse was highly significant (χ^2=43.0, *df*=5, *P*<0.001); the day effect was marginally significant (χ^2=5.8, *df*=1, *P*=0.04). There was less dramatic decline in infections from day 6 to day 10. The *morsitans* group, however, supported infections better than the *palpalis* group. On day 21, the interaction effect (tsetse x day) was not significant. The day and tsetse effects were either not significant (χ^2=35.6, *df*=1, *P*=0.82) or highly significant (χ^2=48.8, *df*=5, *P*<0.001). *T. brucei* infections stabilised after day 10 in all the tsetse. Infections on day 21 were not different from

Table 5.6 Log linear analysis of infection rates on days 3,6, 10 and 21 in 673 tsetse flies of six species infected with *T. brucei* (Ng3) in goat blood.

Factor	*df*	χ^2	*P*
Days	3	90.5	< 0.001
Tsetse	5	61.5	< 0.001
Tsetse x days	15	25.6	0.03

those on day 10. However, *G. fuscipes* was still the most resistant and *G. m. centralis* the most susceptible (Fig. 5.7).

5.4.6 The late pattern of midgut infections with *T. congolense* (riverine and savannah)

The patterns of midgut infections on days 3, 6, 10 and 21 were similar for the two stocks of *T. congolense.* Infections were fairly high (42.3-77.2%) on day 3 in all the tsetse species but decreased (17.6-57.4%) by day 6. The *palpalis* group cleared more infections than the *morsitans* group. Infections reached a constant level by day 10 and no further decline was observed by day 21. The *morsitans* group were more susceptible than the *palpalis* group (Fig. 5.8.). A log linear analysis based on day of infection, tsetse species and their interaction performed on the data showed that the main effects were significant whereas the interaction effect (day x tsetse) was not significant (Table 5.7).

5.4.7 The late pattern of midgut infections with *T. brucei* using lizard blood

In the preliminary investigation, there was heavy mortality of the *morsitans* (group) flies when maintained on the lizard blood. Experiments to study the late pattern of infection using lizard blood were conducted using the *palpalis* flies. Infections on day 3 were uniform and fairly low (38.2-71.8%) among the tsetse. Following a second meal, there was a decline in infections to as low as 13.6-36.5% by day 6. Though differences among flies were not significant, *G. tachinoides* supported the highest infections whereas *G. fuscipes* had the lowest. The overall infection rates throughout the 21 days were much lower in these *palpalis* group of flies using lizard blood (Fig. 5.9) than

Fig 5.7 *T. brucei* (Ng3) infections on days 3,6, 10 and 21 in flies fed on goat blood

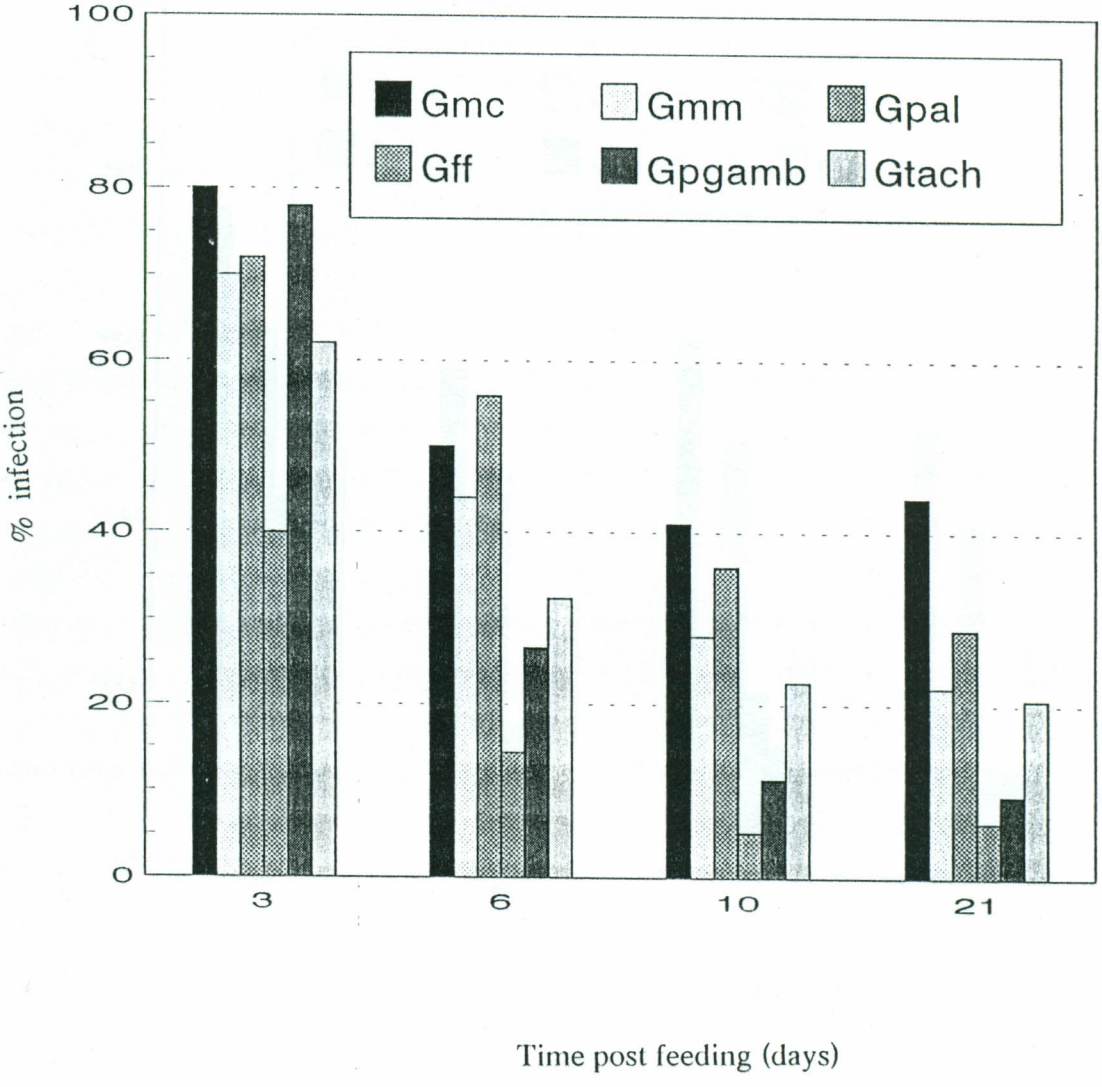


Fig 5.8 *T. congolense* infections on days 3,6, 10 and 21 in flies fed on goat blood

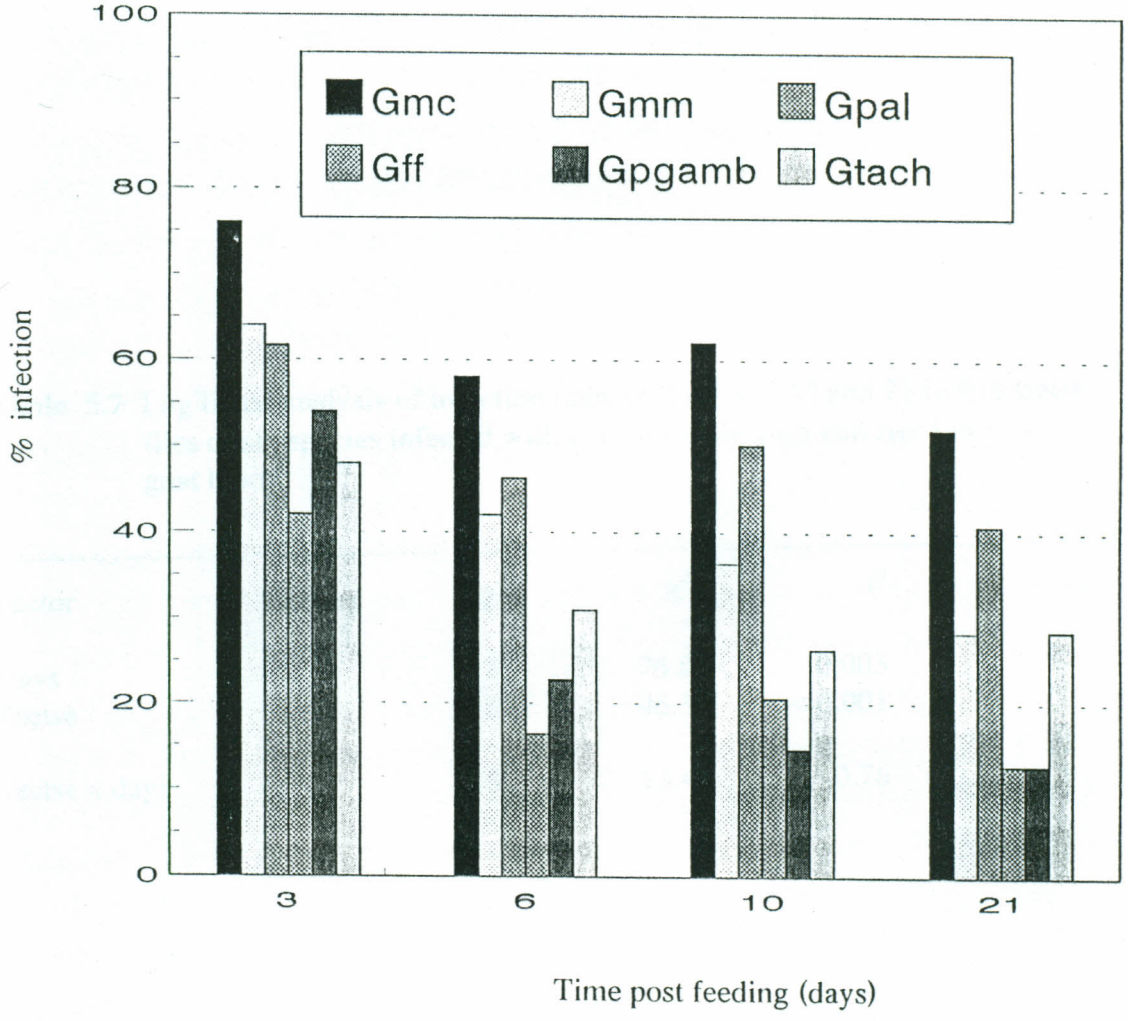
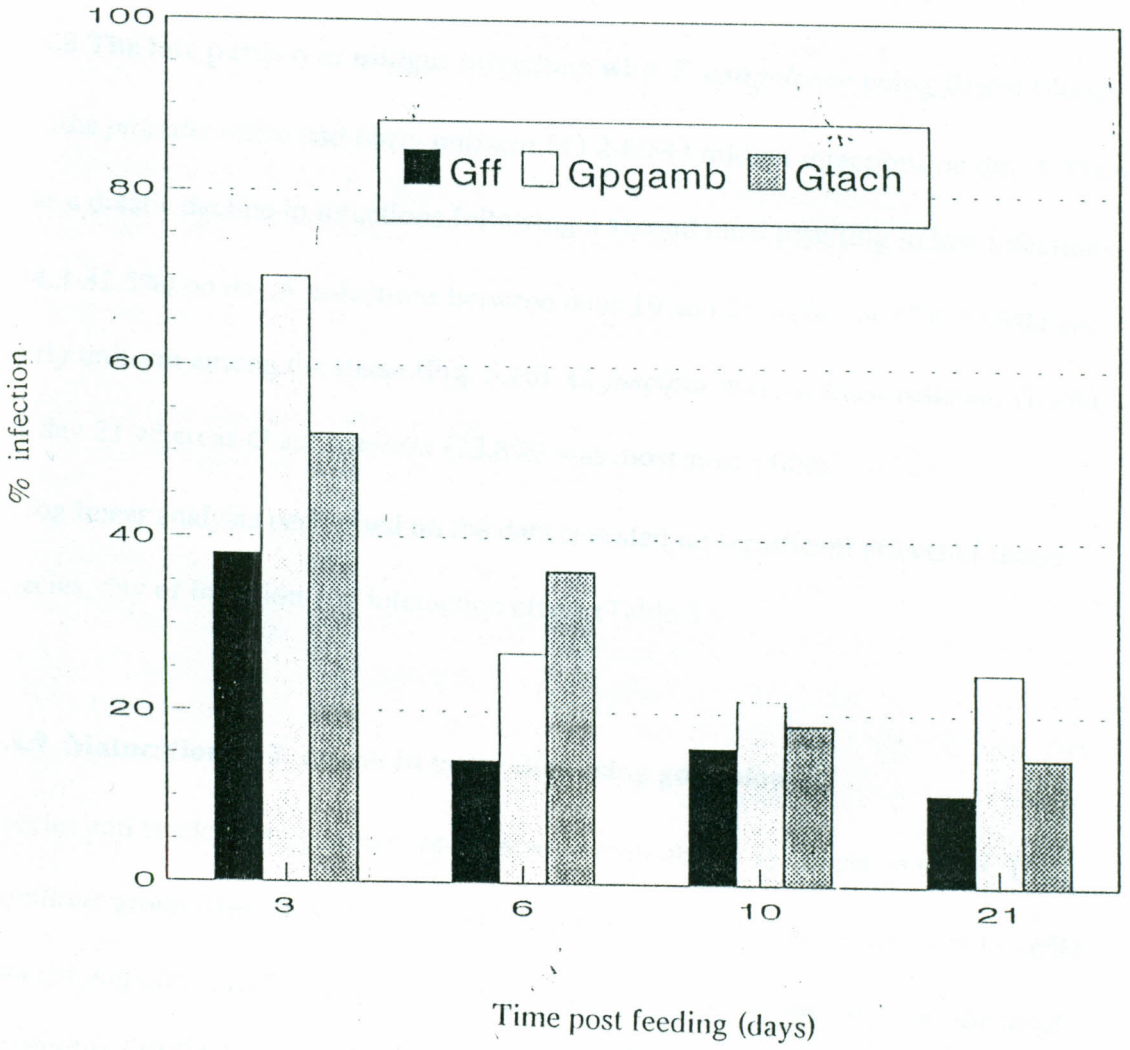


Table 5.7 Log linear analysis of infection rates on days 3,6, 10 and 21 in 958 tsetse flies of six species infected with *T. congolense* Ng5 and Anr3 in goat blood.

Factor	*df*	χ^2	*P*
Days	3	76.8	0.003
Tsetse	5	46.3	< 0.001
Tsetse x days	15	13.4	0.76

Fig. 5.9 *T. brucei* (Ng3) infections on days 3,6, 10 and 21 in flies fed on lizard blood



when goat meal was used. A log linear analysis of infection rates revealed significant effect of the day of infection whereas tsetse species and their interaction (tsetse x day) effects were not significant (Table 5.8).

5.4.8 The late pattern of midgut infections with *T. congolense* using lizard blood

All the *palpalis* tsetse had fairly uniform (43.2-60%) midgut infections on day 3. There was a drastic decline in infections following a second meal resulting in low infections (14.3-32.5%) on day 6. Infections between days 10 and 21 were low (7.6-27.8%) and fairly uniform among the tsetse (Fig. 5.10). *G. fuscipes* was the most resistant (7.5%) by day 21 whereas *G. tachinoides* (23.8%) was most susceptible.

A log linear analysis performed on the data revealed no significant effects of tsetse species, day of infection and interaction effect (Table 5.9)

5.4.9 Maturation of *T. brucei* in tsetse flies using goat blood

Species and stocks of trypanosomes differed in their ability to mature in tsetse. The *morsitans* group flies (Gmc, Gmm, Gpal) had higher overall maturation rates (33.6%) than the *palpalis* (17.5%) group (Gff, Gpgamb, Gtach). *G. m. centralis* was the most susceptible (30.5% in males, 19.4% in females) while *G. fuscipes* was the most resistant (5.4% in males, 0% in females). Males had consistently higher overall maturation rates (17.3%) than females (8.51%) in all the tsetse species (Fig. 5.11). A log linear analysis based on tsetse species, sex of the tsetse and their interaction was performed. Although the main effects, tsetse species (χ^2=12.89, *df*=5, *P*=0.02) and sex (χ^2=7.12, *df*=1, *P*<0.05) were significant; the interaction effect (tsetse x sex) was not significant (χ^2=11.8, *df*=5, *P=0.04*).

Table 5.8 Log linear analysis of infection rates on days 3,6, 10 and 21 in 344 *palpalis* tsetse of three species infected with *T. brucei* (Ng3) in lizard blood.

Factor	*df*	χ^2	*P*
Days	3	48.4	0.03
Tsetse	2	24.8	0.07
Tsetse x days	6	10.8	0.48

Table 5.9 Log linear analysis of infection rates on days 3,6, 10 and 21 in 673 *palpalis* tsetse of three species infected with *T. congolense* in lizard blood

Factor	*df*	χ^2	*P*
Days	3	76.4	0.06
Tsetse	2	44.8	0.08
Tsetse x days	6	23.4	0.68

Fig. 5.10 *T. congolense* infections on days 3,6, 10 and 21 in flies fed on lizard blood

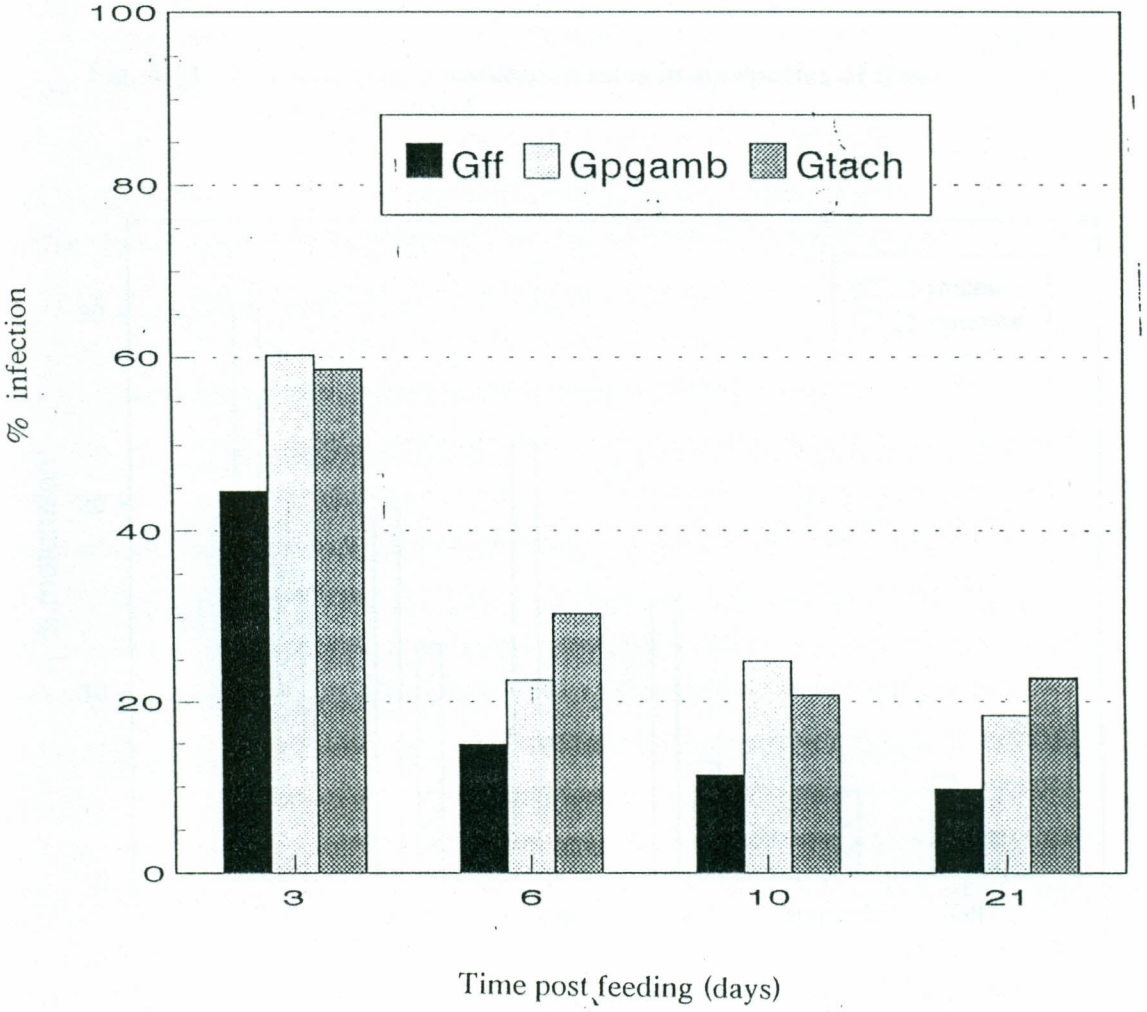
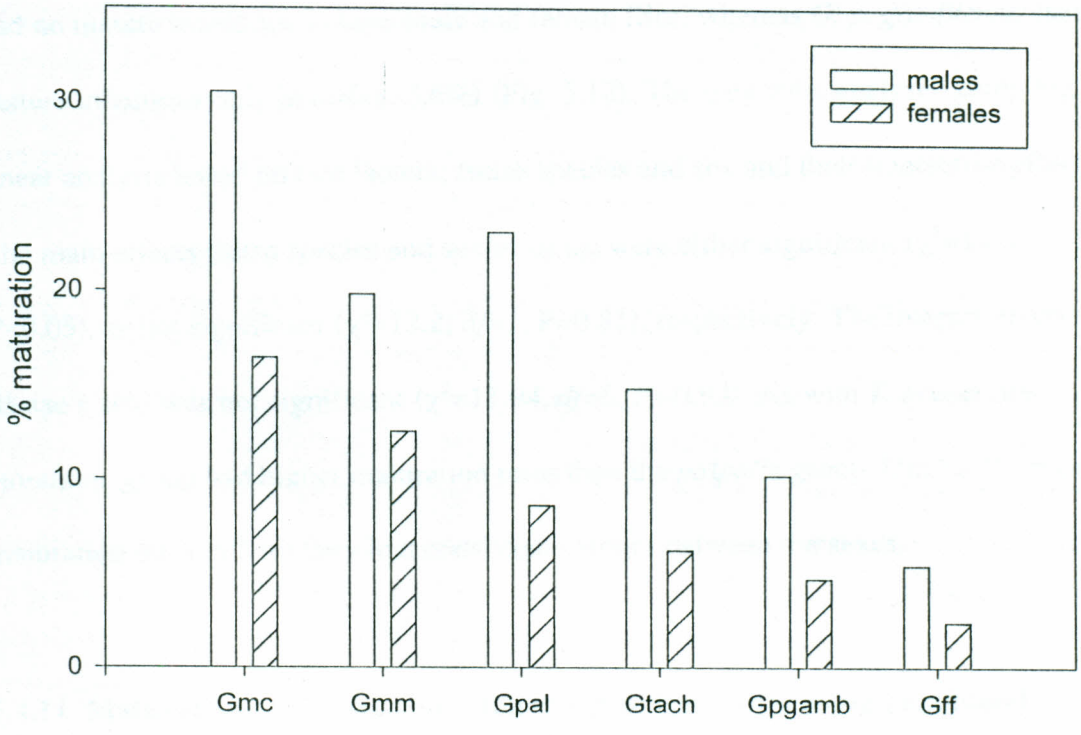


Fig. 5.11 *T. brucei* (Ng3) maturation rates in six species of flies using goat blood



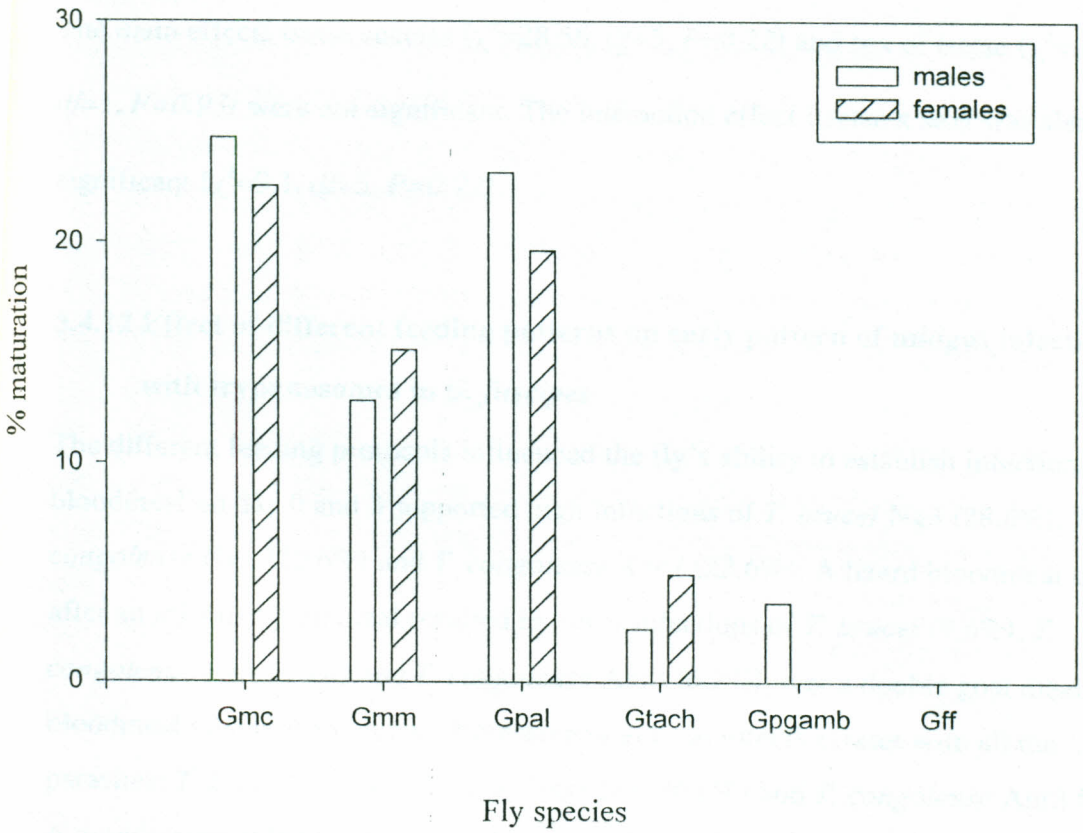
5.4.10 Maturation of *T. congolense* (savannah) in tsetse flies using goat blood

There were differences in the maturation pattern of *T. congolense* between different species of flies. *G. m. centralis* had mature infections in 24.7 % males and 22.5% females. This compared closely with *G. pallidipes* (23.1% males, 19.6% females) and *G. m. morsitans* (19.8% males, 12.5 females). Among the *palpalis* flies *G. tachinoides* had the highest maturation rates (2.4% males, 4.9% females). *G. fuscipes* had no mature infections in both male and female flies, whereas *G. p. gambiensis* had mature infections only in males (3.6%) (Fig. 5.12). The data were analysed using log linear analysis based on two factors; tsetse species and sex and their interaction effect. The main effects tsetse species and sex of tsetse were either significant (χ^2=14.6, *df*=5, *P*<0.05), or not significant (χ^2=12.2, *df*=1, P=0.81), respectively. The interaction effect (tsetse x sex) was not significant (χ^2=11.04, *df*=5, *P=0.93)*. As with *T. brucei*, the *morsitans* group had higher maturation rates than the *palpalis* group. Unlike *T. brucei,* maturation rates did not vary in a consistent manner between the sexes.

5.4.11 Maturation of *T. congolense* (riverine) in tsetse flies using goat blood

The *morsitans* group of flies had lower maturation rates with riverine *T. congolense* than those observed for savannah *T. congolense. G. m. centralis* had mature infections in 14.7% males and 12.5% females, *G. m. morsitans* (6.8 % males, 5.1% females), and 13.1% in males and 9.6% in females of *G. pallidipes*. Similarly, there were very low maturation rates in all *palpalis* group of flies. *G. p. gambiensis* and *G. tachinoides* however, showed higher maturation rates with the riverine than the savannah

Fig. 5.12 *T. congolense* (Ng5) maturation rates in six species of flies using goat blood



T. congolense. Differences in maturation rates between male and female tsetse were inconsistent. *G. tachinoides* the most efficient of the *palpalis* flies used had mature infections in 3.2% males and 4.6 % females whereas *G. p. gambiensis* had mature infections in 6.8% males and 5.2% females. *G. fuscipes* was however not able to mature this parasite in both males and females (Fig. 5.13). A log linear analysis of maturation rates based on two factors; tsetse species and sex and their interaction was performed. The main effects tsetse species (χ^2=28.56, *df*=5, *P*=0.22) and sex of tsetse (χ^2=5.8, *df*=1, *P=0.93)* were not significant. The interaction effect (tsetse x sex) was also not significant (χ^2=9.1, *df*=5, *P=0.72)*.

5.4.12 Effect of different feeding patterns on early pattern of midgut infections with trypanosomes in *G. fuscipes*

The different feeding protocols influenced the fly's ability to establish infections. Goat bloodmeal on day 0 and 3 supported high infections of *T. brucei* Ng3 (28.6%), *T. congolense* Ng5 (20.6%) and *T. congolense Anr3* (22.6%). A lizard bloodmeal on day 3 after an infective goat meal resulted in lower infections of *T. brucei* (9.6%), *T. congolense* Ng5 (8.8%) and *T. congolense* Anr3 (6.2%) over a double goat meal. Lizard bloodmeal on day 0 and 3 had depressive effects on infection rates with all the parasites; *T. brucei* (5.6 %), *T. congolense* Ng5 (6.6%) and *T. congolense* Anr3 (3.8%). A double crocodile bloodmeal on day 0 and 3 had similar depressive effects. However, a goat bloodmeal taken on day 3 after an infective reptilian meal resulted in partial reversal of the depressive effects of a double reptilian meal (Fig. 5.14). A log linear analysis of infection rates was performed considering two factors; meal combination, parasite and their interaction effect. Meal combination (χ^2=69.9, *df*=8, *P*< 0.001) and parasite (χ^2= 22.2, *df*=2, *P*=0.004) effects were significant; the interaction effect (meal combination x parasite) was not significant (χ^2= 10.7, *df*=16 , *P*=0.71).

Fig. 5.13 Riverine *T. congolense* (Anr3) maturation rates in six species of tsetse using goat blood

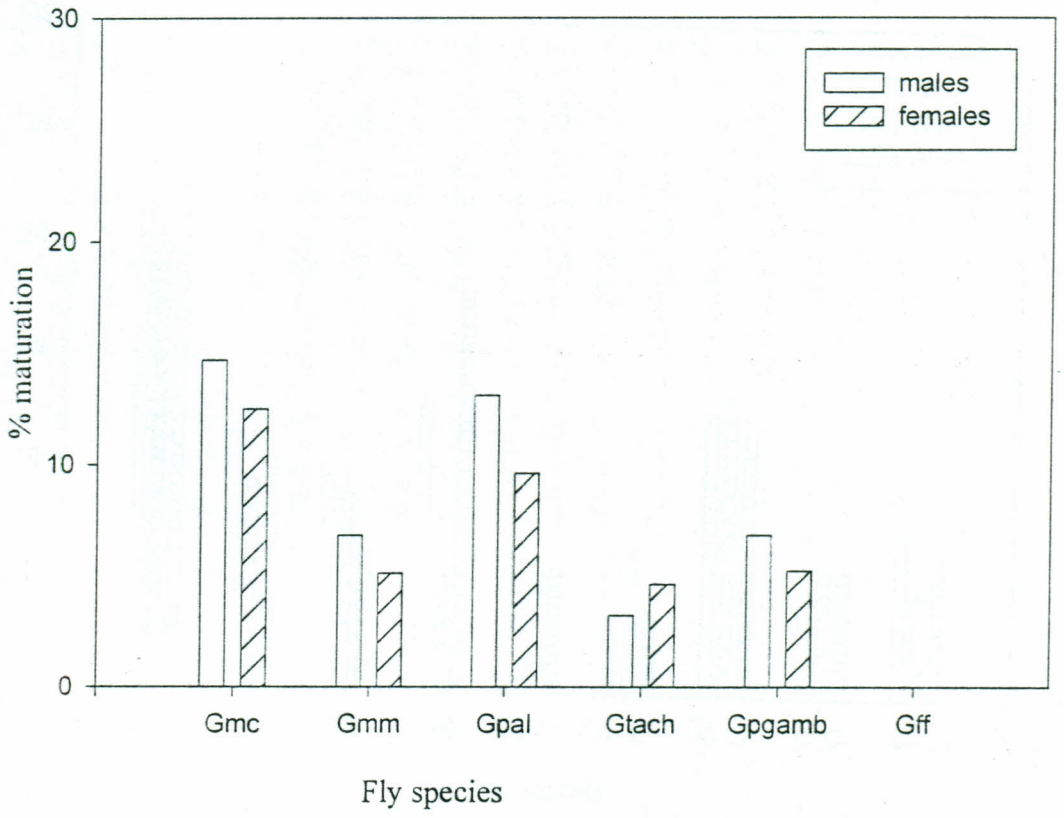
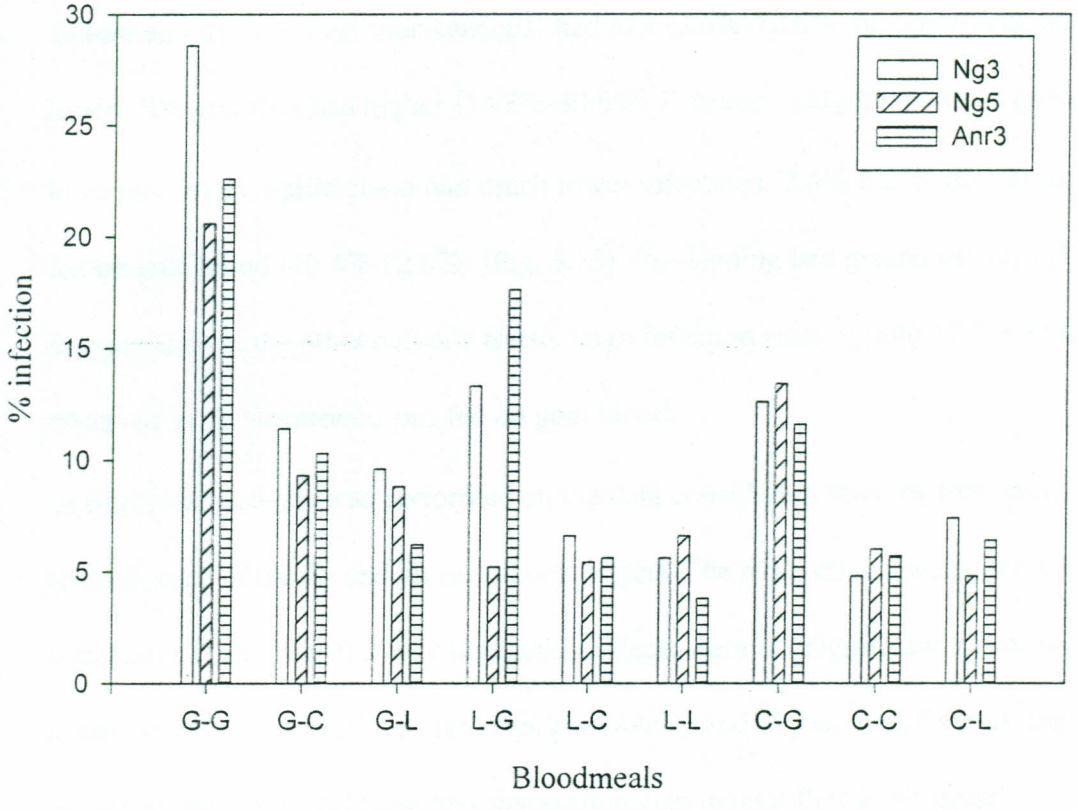


Fig. 5.14 Effect of different feeding patterns on early infections of trypanosomes in *G. fuscipes*



G-G goat : goat
G-C goat : crocodile
G-L goat : lizard
L-G lizard : goat
L-C lizard : crocodile
L-L lizard : lizard
C-G crocodile : goat
C-C crocodile : crocodile
C-L crocodile : lizard

Additional meals on day 6 and 8 did not have any effects on infections (Table 5.10). The meal utilised by the fly in the first two days (day 0 &3) was critical in determining the outcome of the infection on day 10.

5.4.13 The effect of pre-feeding on early infections with *T. brucei* in *palpalis* flies

G. tachinoides, G. p. gambiensis and *G. fuscipes* fed on goat, lizard and crocodile blood before an infective meal (non-tenerals) had low (3.6%-12.6%) midgut infections of *T. brucei.* Teneral flies had higher (14.8%-40.6%) *T. brucei* midgut infections on day 6. Flies pre-fed on reptile blood had much lower infections (3.6%-8.2%) than those pre-fed on goat blood (10.4%-12.6%) (Fig. 5.15). Pre-feeding had greater effects in *G. fuscipes* than in the other *palpalis* tsetse. High infection rates of upto 17.7% were observed in *G. tachinoides* pre-fed on goat blood.

A log linear analysis was performed on the data considering three factors; meal, tsetse species, state of the fly and all interaction effects. The main effects were all highly significant (Table 5.11); 2-way interaction effects were not significant. *G. tachinoides* maintained higher infections than *G. p. gambiensis* and *G. fuscipes.* Overall, flies fed before an infective meal were less susceptible than teneral flies in all tsetse.

5.4.14 Effects of different feeding patterns on maturation of *T. brucei* in *palpalis* flies

G. tachinoides, G. p. gambiensis and G. fuscipes maintained wholly on goat blood had mature infections with *T. brucei* on day 35 post infection in both male and female flies. Infections were higher in male (11.8%) than female (3.9%) flies in all the three tsetse species. *G. tachinoides* was the most susceptible with 12.5% mature infections

Table 5.10 Effect of different feeding patterns on infections with trypanosomes on day 10 in *G. fuscipes*

Feeding protocol				% infections		
Day 0	Day 3	Day 6	Day 8	Ng3	Ng5	Anr3
goat	goat	goat	goat	24.4	18.6	16.4
goat	goat	lizard	lizard	16.8	12.6	13.8
goat	crocodile	goat	goat	8.6	7.8	9.4
goat	crocodile	crocodile	crocodile	6.8	5.4	8.6
goat	lizard	lizard	lizard	5.6	5.6	7.4
goat	lizard	goat	goat	8.6	9.4	6.4
crocodile	crocodile	crocodile	crocodile	4.8	5.8	6.1
lizard	lizard	goat	goat	4.2	3.5	4.8
lizard	lizard	lizard	lizard	3.8	2.8	4.2

Fig. 5.15 Effect of pre-feeding *palpalis* tsetse on goat, lizard or crocodile blood on infections with *T. brucei* on day 6.

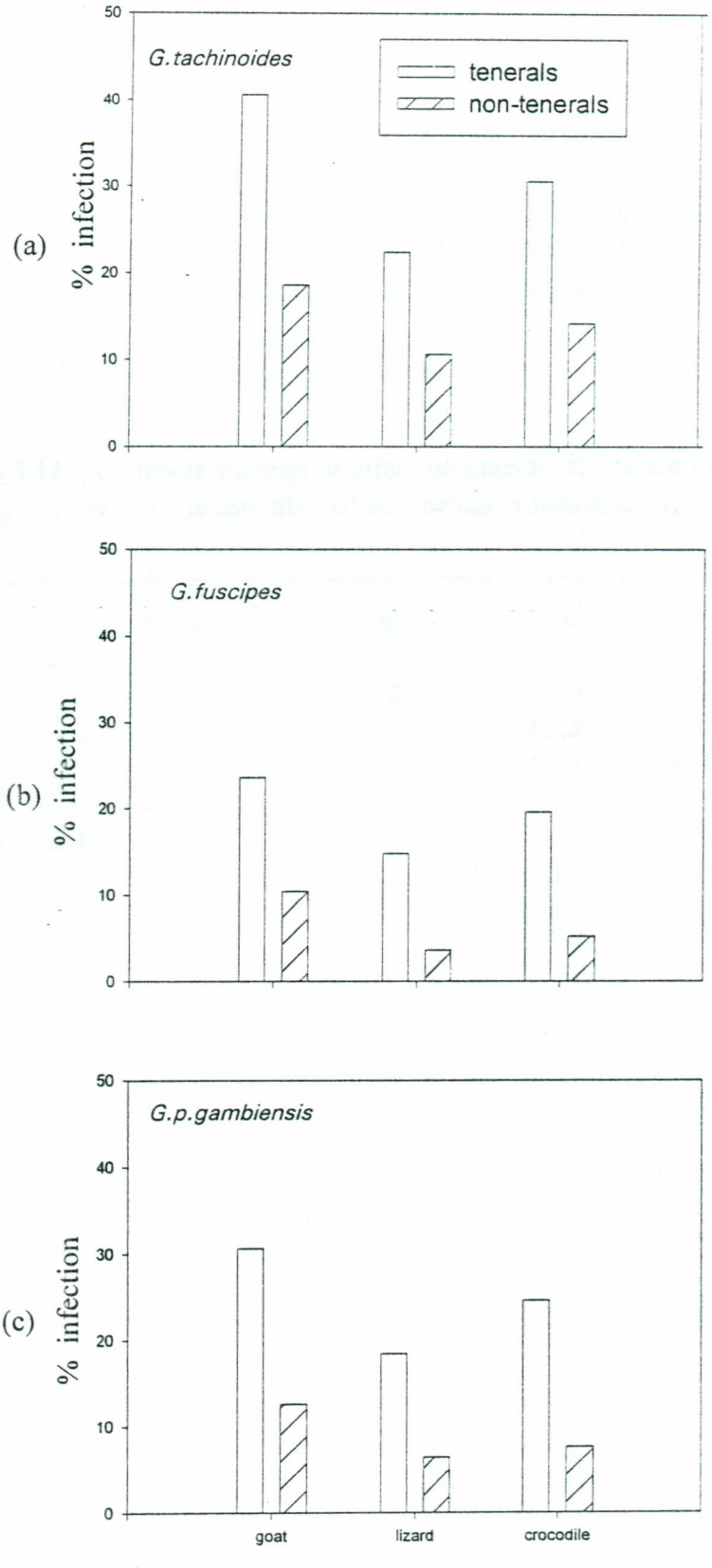


Table 5.11 Log linear analysis of infection rates in 654 tsetse flies of three species of *palpalis* tsetse infected as tenerals or non-tenerals with *T. brucei*.

Factor	*df*	χ^2	*P*
Tsetse	2	9.9	0.007
Meal	2	13.5	0.001
State	1	14.3	< 0.001
Tsetse x meal	4	8.7	0.09
Tsetse x state	2	5.6	0.08
Meal x state	2	10.6	0.91

in males and 5.6% in females whereas *G. fuscipes* was the most resistant with mature infections in 4.6% males and 1.9% females. Flies maintained wholly on lizard blood for 35 days had few or no mature infections. *G. fuscipes* in particular, maintained wholly on lizard blood had no mature *T. brucei* infections in both male and female flies. *G. tachinoides* had mature infections in males (2.4%) only whereas *G. p. gambiensis* maintained wholly on lizard blood had low maturation rates in both male (1.9%) and female (4.2%) flies. Maintaining flies on interrupted goat and lizard meals for 35 days resulted in low but improved maturation rates of *T. brucei* over feeding flies wholly on lizard bloodmeal.

G. fuscipes had mature infections in males (1.8%) only whereas *G. tachinoides* had mature infections in 7.6% males and 2.9% females (Fig. 5.16). A log linear analysis performed on maturation rates based on tsetse species, meal utilised, sex of fly, and the interaction effects showed that the meal ($P=0.001$)and sex ($P<0.001$) effects were significant whereas the tsetse species ($P=0.07$) and the interaction effects were not significant (Table 5.12). Though the tsetse species effect was not significant, *G. tachinoides* was the most susceptible whereas *G. fuscipes* was the most resistant. Flies maintained wholly on goat blood had the highest maturation rates with *T. brucei* (Fig. 5.16)

5.4.15 Induction of agglutination activity by bloodmeals

The gross pattern of agglutination activity followed in *G. fuscipes* for 120 h post feeding on goat and lizard meals was similar for goat and lizard blood types and followed a cyclical pattern. Agglutination titres were low before ingestion of a

Fig. 5.16 Effect of different feeding patterns on maturation rates of *T. brucei* in riverine tsetse

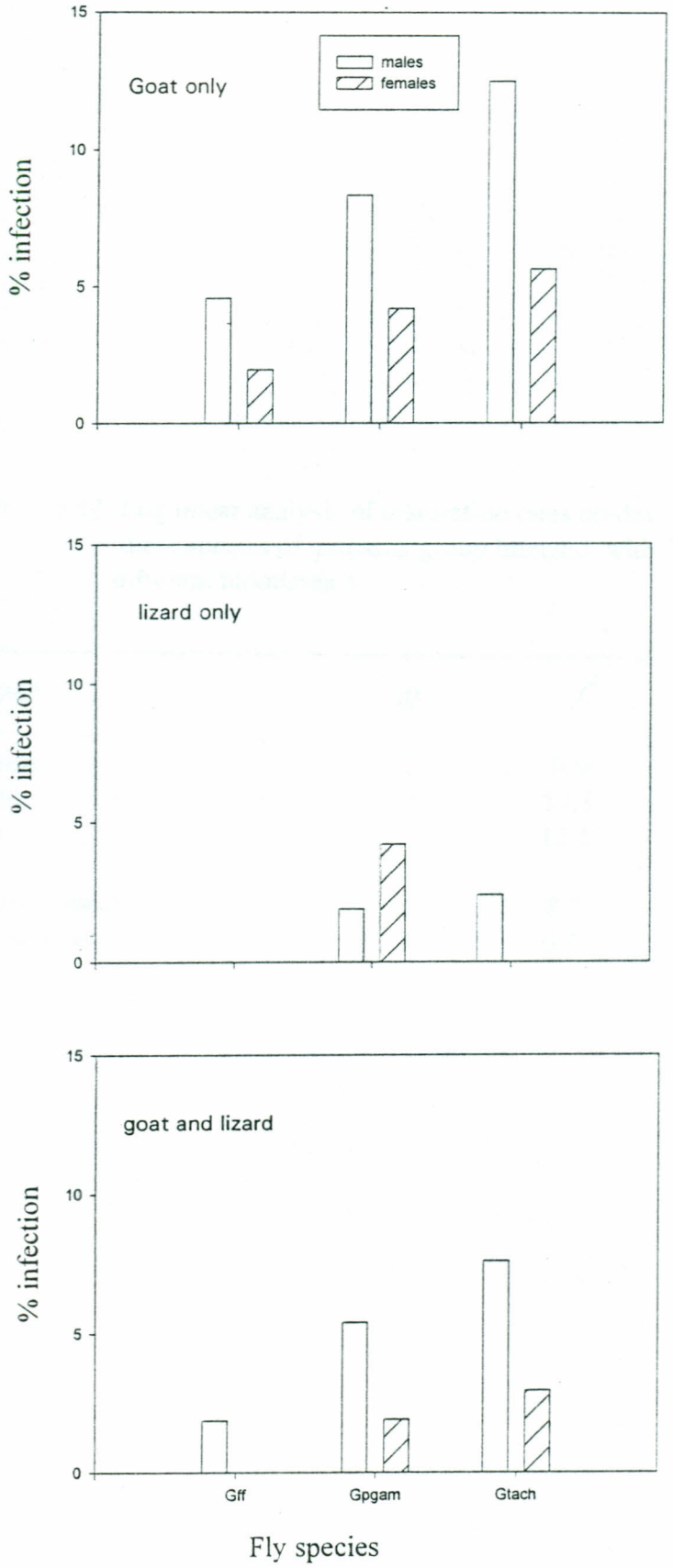
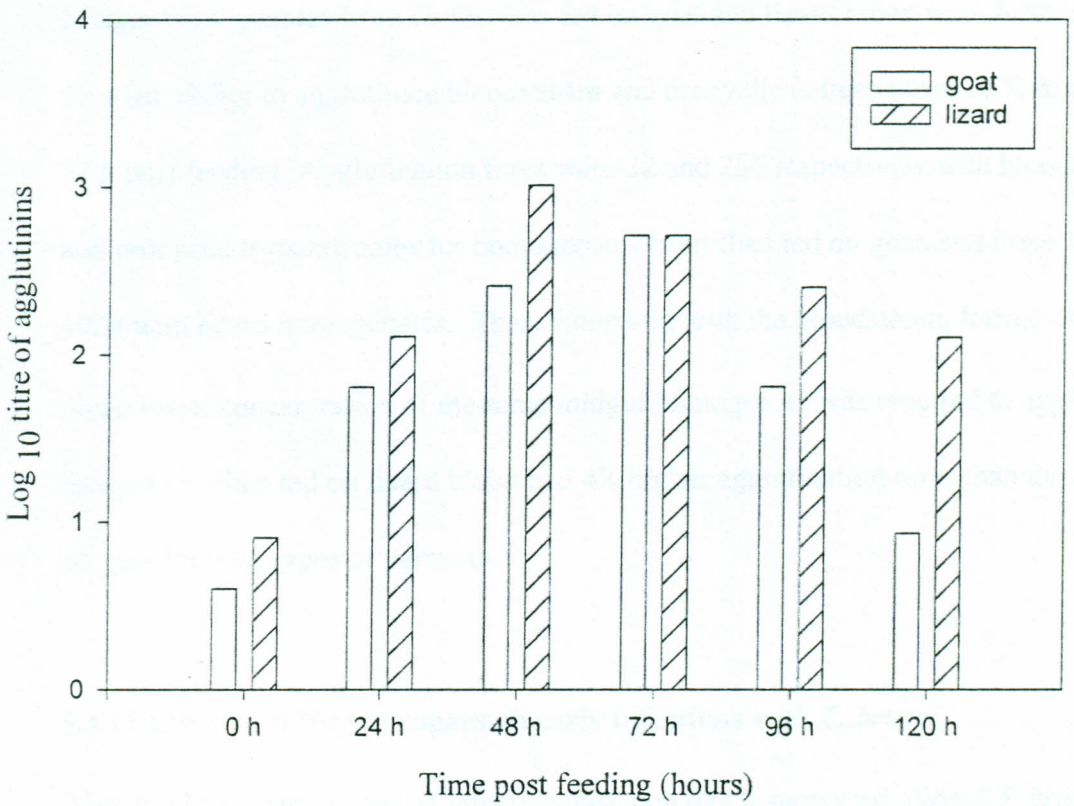


Table 5.12 Log linear analysis of maturation rates on day 35 in 604 tsetse flies of three species of *palpalis* group infected with *T. brucei* and maintained on different bloodmeals.

Factor	*df*	χ^2	*P*
Tsetse	2	9.9	0. 07
Meal	2	14.5	0.001
Sex	1	12.6	< 0.001
Tsetse x meal	4	8.7	0.68
Tsetse x sex	2	6.6	0.74

Fig. 5.17 Time course agglutinin activity towards bloodstream *T. brucei* in *G. fuscipes* fed on goat and lizard blood



bloodmeal (0 h). They increased following ingestion of both meals, reaching a peak at 48-72 h. Titres decreased afterwards (Fig. 5.17). Flies fed on lizard blood had higher agglutination titres at all times; titres also remained quite high after 72 hours at a time when titres with goat blood were dropping 100-fold.

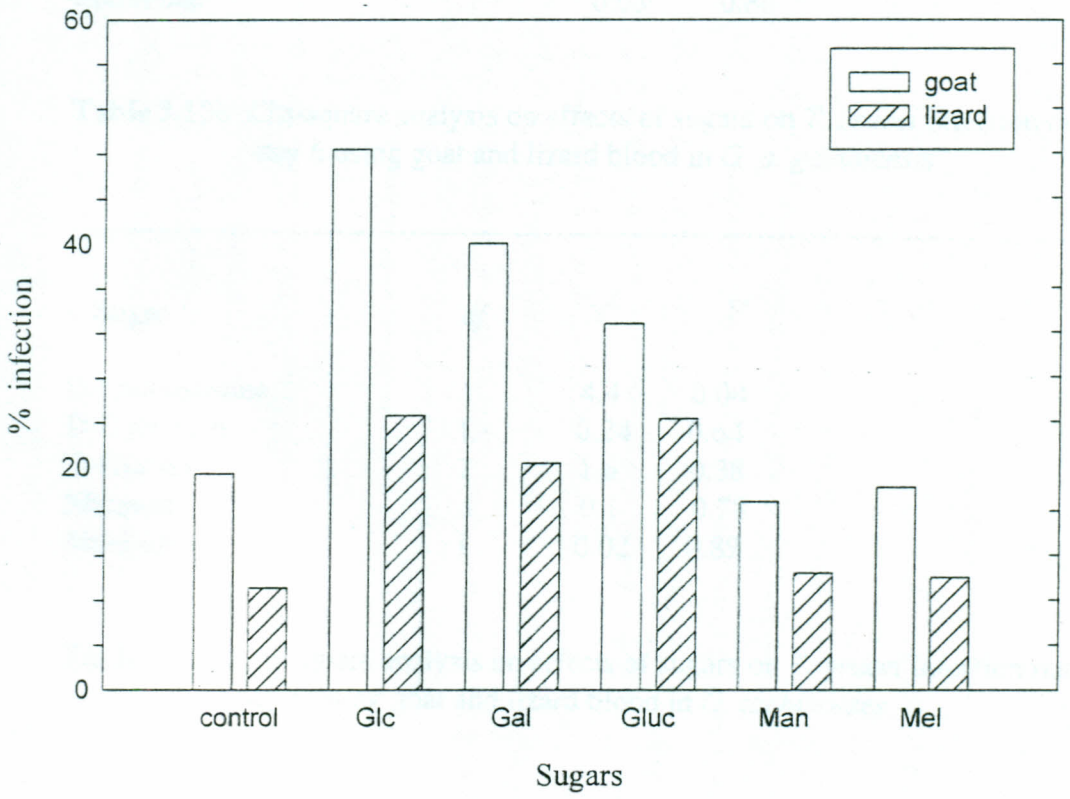
5.4.16 Agglutination of bloodstream and procyclic form trypanosomes

Midgut homogenates from *G. fuscipes* fed on goat and lizard blood were further tested for their ability to agglutinate bloodstream and procyclic culture forms of *T. brucei* at 72 h post feeding. Agglutination titres were 32 and 256 respectively with bloodstream and procyclic trypanosomes for homogenates from flies fed on goat, and titres 128 and 1024 with lizard homogenates. Thus, compared with the bloodstream form parasites, a much lower concentration of the same midgut homogenate was required to agglutinate procyclics. Flies fed on lizard blood had 4X higher agglutination titres than those fed on goat for both types of parasites.

5.4.17 Effect of different sugars on early infections with *T. brucei*

Goat and lizard blood used as infective meals on day 0 supported midgut *T. brucei* infections in 19.4% and 9.1% *G. fuscipes* respectively on day 6. This increased significantly (P=0.01) to 48.4 % and 24.6% respectively when 0.06M D-glucosamine was added to the infective meal. Addition of D-galactose resulted in a significant increase of the midgut infections (P=0.04) to 40.0% using goat bloodmeal and 20.% using lizard bloodmeal. Other sugars, D-glucose (32.8%), mannose (16.8%) and

Fig 5.18 Effect of sugars on early infection rates of *T. brucei* in *G. fuscipes*



Glc = D-Glucose Gal = D-Galactose Gluc = D-Glucosamine
Man = Mannose Mel = Melibiose

Table 5.13a Chi-square analysis on effects of sugars on *T. brucei* infection rates on day 6 using goat and lizard blood in *G. fuscipes*

Sugar	*df*	χ^2	*P*
D-Glucosamine	1	5.7	0.01
D-Galactose	1	4.1	0.04
D-Glucose	1	1.1	0.29
Mannose	1	0.1	0.78
Melibiose	1	0.03	0.86

Table 5.13b Chi-square analysis on effects of sugars on *T. brucei* infection rates on day 6 using goat and lizard blood in *G. p. gambiensis*

Sugar	*df*	χ^2	*P*
D-Glucosamine	1	4.4	0.04
D-Galactose	1	0.24	0.64
D-Glucose	1	1.6	0.38
Mannose	1	0.1	0.78
Melibiose	1	0.02	0.89

Table 5.13c Chi-square analysis on effects of sugars on *T. brucei* infection rates on day 6 using goat and lizard blood in *G. tachinoides*

Sugar	*df*	χ^2	*P*
D-Glucosamine	1	0.05	0.82
D-Galactose	1	6.8	0.03
D-Glucose	1	0.03	0.86
Mannose	1	5.4	0.01
Melibiose	1	0.04	0.87

Fig. 5.19 Effect of sugars on early infection rates of *T. brucei in G. p. gambiensis*

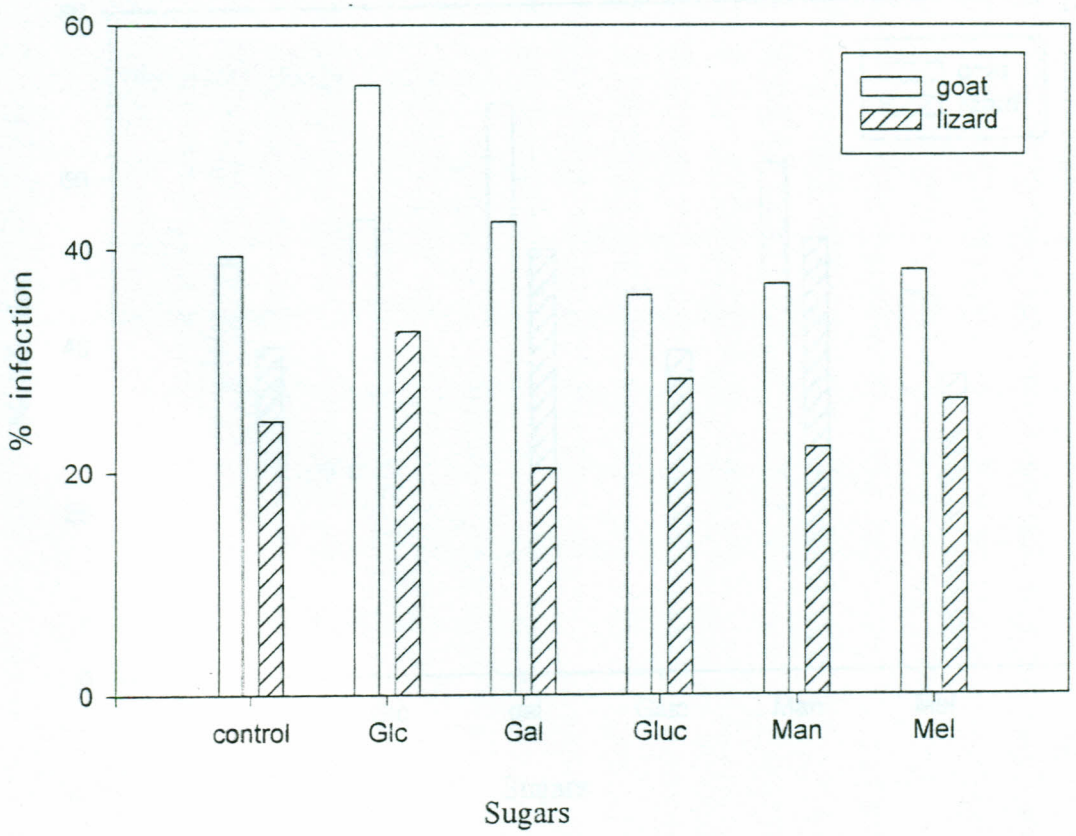
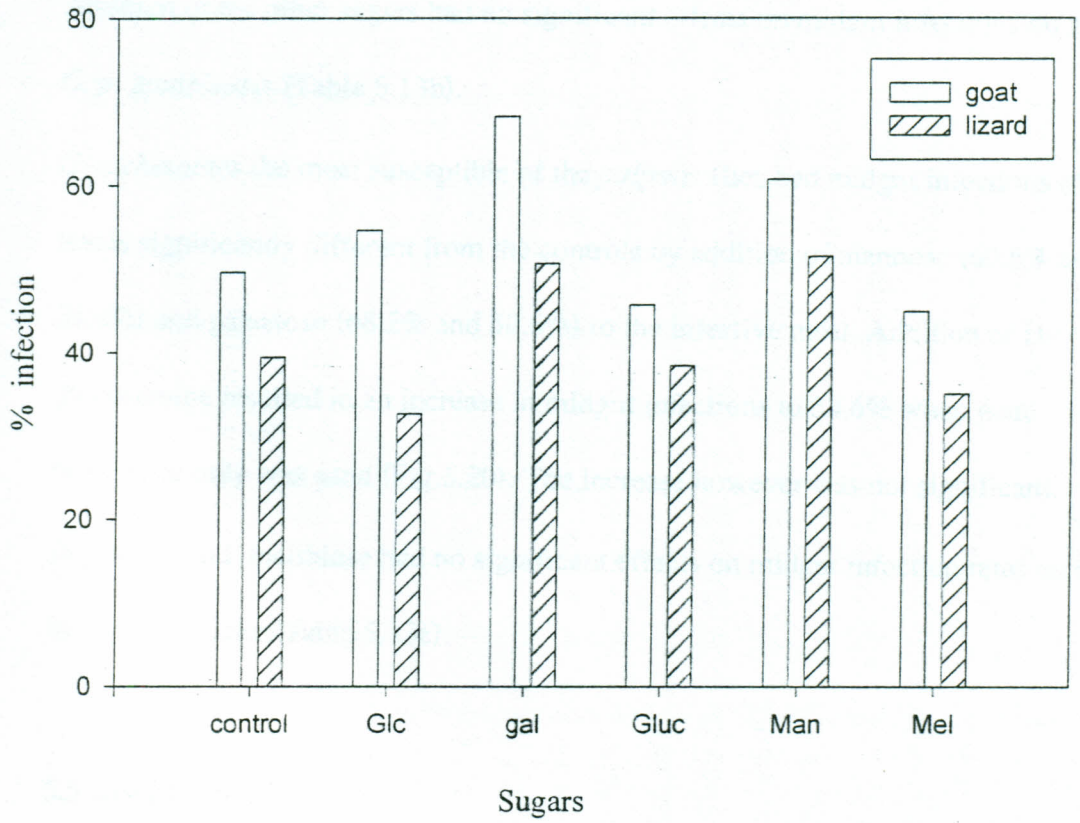


Fig.5.20 Effect of sugars on infection rates of *T. brucei* in *G. tachinoides*



melibiose (18.1%) (Fig.5.18) had no significant effects on midgut infections in *G. fuscipes* (Table 5.13a)

The effect of addition of sugars to the infective meal on midgut *T. brucei* infections in *G. p. gambiensis* is shown in Fig.5.19. Midgut infections were significantly increased from 39.4% and 24.6% when goat and lizard blood were used alone to 54.6% and 32.6% respectively (P=0.01) when D-glucosamine was added to the infective meal. Addition of the other sugars had no significant effects on midgut infections on day 6 in *G. p. gambiensis* (Table 5.13b).

G. tachinoides the most susceptible of the *palpalis* flies had midgut infections of 49.6% levels significantly different from the controls by addition of mannose (60.6% and 51.4%) and galactose (68.2% and 50.6%) to the infective meal. Addition of D-glucosamine resulted in an increase in midgut infections to 54.6% when goat bloodmeal only was used (Fig.5.20). The increase however was not significant.

D-glucose and melibiose had no significant effects on midgut infection rates on day 6 in *G. tachinoides* (Table 5.13c).

5.5 Discussion.

During the life cycle of gut adapted trypanosomes (*Trypanozoon, Nannomonas*) a critical time is the initial establishment of a gut infection in the fly. Trypanosomes taken up in the infective bloodmeal by tsetse are subjected to severe biochemical, physiological and physical environmental changes which are harsh to the survival of the parasite in the midgut. Most bloodstream forms die shortly after entering the vector, before transforming into gut adapted procyclic forms (Turner *et al.*, 1988). During the next few days, many gut infections are lost as the few surviving procyclics

often fail to establish (Welburn *et al.*, 1989). Progression from establishment to maturation is not automatic. Many incipient gut infections fail to mature.

Certain host's blood sera have lytic effects on trypanosomes thus hindering their successful establishment (Rickman & Kolala, 1982). In this study, there was only minimal evidence of *T. congolense* and *T. brucei* lysis by lizard and crocodile blood. This was reflected in the longer prepatent periods taken by the parasites to grow in laboratory rats after incubations in reptilian sera compared to goat sera.

Goat blood supported persistently higher infections of *T. brucei* and *T. congolense* in all the tsetse species used on day 3 and 6 and at the time of maturation compared to lizard and crocodile blood. The effect of the initial meal was generally expressed in parasite mortality following a second bloodmeal on day 3. Feeds on lizard and crocodile blood resulted in the elimination of more infections than feeds on goat blood. In many experiments, the first two meals on day 0 and 3 were critical in determining the outcome of trypanosome infections in tsetse. Goat blood on day 0 and 3 supported higher infections than reptile blood. Goat blood on day 3, after an infective lizard and crocodile meal, also partially reversed the depressive effects of reptile blood. *Palpalis* flies maintained wholly on lizard bloodmeal matured few or no *T. brucei* infections. This probably reflects normal situation where lizards harbour cryptic infections that rarely mature in tsetse depending solely on reptiles as a food source. Flies maintained either on goat blood or interrupted goat and lizard blood had higher maturation rates with *T. brucei* than those maintained wholly on lizard blood. This may represent the start of an epidemic where goats (or humans) transmit *T. brucei* to flies which normally feed solely on lizards. These overall patterns suggest that transmission indices in situations where flies feed mainly on reptiles are much lower than would be

expected if they fed on livestock. Goats with their ability to facilitate transmission, may therefore act as particularly effective disease reservoirs.

It may be possible that reptile blood contains some factor/s deleterious to the trypanosomes and hence only a small proportion of the trypanosomes manage to become established in the vector. Agglutinin and trypsin levels were higher in flies fed on lizard than goat blood. This was in conformity with differences in infection rates in flies fed on the two meals. Regular feeding of tsetse on hosts which induce high trypsin and agglutinin levels may reduce the probability of trypanosome infection. The unique ability of goat blood enhancing midgut infection rates has previously been reported by Mihok *et al.,* (1993) and Olubayo *et al.,* (1994) who showed that infection rates can be modified dramatically by host species-specific blood factors ingested with the infective meal. Blood from goats in particular increased greatly midgut infection rates of *T. congolense* savannah-type and *T. brucei* subspecies in *G. m. morsitans* and *G. m. centralis*. Blood from wildlife such as eland and buffalo substantially reduced infection rates. Critical events in the fly occurred shortly after a second bloodmeal, between day 3 and 6 (Olubayo *et al.,* 1994). The mechanism by which goat blood facilitates midgut infection rates in tsetse remains unknown. However, useful clues may lie in the carbohydrate molecules on the red blood cell (Olubayo *et al.,* 1994; Nguu *et al.,* 1996). Mihok *et al.,* (1995) observed a significant increase in infection rates after feeding *G. m. morsitans* on a diet containing a high proportion of red blood cells (RBC's). A similar observation was made by Maudlin & Welburn (1994) in *G. m. morsitans* using cow RBC's suspended in saline. This showed that the factors responsible for enhancing the infection were lodged on the RBC's. Mihok *et al.,* (1995) found that this enhancement was probably not due to the small size of goat RBC's; instead the

carbohydrate molecules on the cells may play an important role. Ingram and Molyneux (1990) reported carbohydrate molecules on the human RBC's that specifically bind to tsetse midgut or haemolymph proteins. It is possible therefore, that the exposed carbohydrate moieties on goat RBC's result in a better fit with midgut lectins thus inhibiting their trypanolytic effects more efficiently. This would then increase survival chances of trypanosomes in the midgut.

Results of this study showed that the *morsitans* group flies were more efficient vectors of both *Nannomonas* and *Trypanozoon* organisms than the *palpalis* flies when goat and other blood were used. *G. m. centralis* was the most susceptible whereas *G. fuscipes* was the most resistant. The poor vectorial capacity of the *palpalis* group in this study is consistent with the findings of Moloo and Kutuza (1988 a, b) who showed that *G. p. palpalis, G. fuscipes* and *G. tachinoides* were poor vectors of *T. congolense* and *T. brucei* when compared with *G. m. centralis*. Moloo *et al.,* (1994) while studying susceptibility to *T. simiae* also showed that *G. p. gambiensis* was not susceptible whilst *G. brevipalpis, G. m. centralis,* and *G. pallidipes* were susceptible. Reifenberg *et al.,* (1997) also showed higher mature infection rates in *morsitans*-group tsetse than in the *palpalis* group when infected with Savannah-type *T. congolense*. This may suggest that factors responsible for hindering establishment may be at higher levels and more active in the *palpalis* group compared to the *morsitans* group. In this study, the differences in susceptibility of *G. m. centralis* and *G. fuscipes* were reflected in parallel differences in trypsin and agglutinin activity. Similarly, a host blood which repeatedly reduced infection (lizard) induced higher trypsin and agglutinin levels than a host blood that repeatedly increased infection (goat). Imbuga *et al.,* (1992 a, b) showed that trypsins induced by bloodmeal sera play a role in transformation of bloodstream trypanosomes

and that transformation is only possible in the presence of optimal enzyme concentrations.

There were higher maturation rates of *T. brucei* infections in male than female tsetse in *morsitans* and *palpalis* flies. This bias in maturation of *T. brucei* favouring higher rates in male tsetse has previously been reported (Maudlin *et al.,* 1991; Mihok *et al.,* 1992a,b). The phenomenon appears to be specific to *T. brucei* which is hypothesised to be more sensitive to haemolymph lectin than *T. congolense* (Maudlin *et al.,* 1991). Milligan *et al.,* (1995) suggested that a sex-linked factor may be killing or preventing migrating parasites from maturing. Maturation is affected by trypanosome species and stock. In this study, there was poor maturation of *T. congolense in* the *palpalis* flies especially *G. fuscipes* even when the meal used was favourable. Studies of the kinetics of maturation of trypanosomes in tsetse have shown that the process of maturation is triggered during a specific time period following establishment in the midgut which varies amongst trypanosome species and stocks (Dale *et al.,* 1995). Higher rates of maturation are achieved in stocks which mature earlier in tsetse, the longer a trypanosome strain takes to establish the first mature infection, the lower the final transmission rate will be (Milligan *et al.,* 1995).

In *Glossina* species, a bloodmeal stimulates the release of different molecules, including lectins (Molyneux & Stiles, 1991). Results of this study showed that midgut agglutinins (lectins) in *G. fuscipes* varied depending on the meal utilised. Lizard meal resulted in a high output of agglutinins in *G. fuscipes.* The meal with the highest output of agglutinins was most successful at preventing trypanosome establishment in the midgut and accordingly produces lower infection rates. The bloodmeal-induced agglutination activity in *G. fuscipes* followed a pattern similar to that of trypsins.

Starting immediately after the bloodmeal, the activity increased and reached a peak 48-72 h later. Similar findings have been reported in *G. palpalis,* where peak agglutination activity occurs at 48 h post bloodmeal and in *G. m. morsitans* where peak activity occurs 48-72 h post feeding (Stiles *et al.,* 1990; Abubakar *et al.,* 1995). An interesting observation was that, compared with the bloodstream-form parasites, a much lower concentration of the lectin was required to agglutinate the procyclis. Similar observations were made by Abubakar *et al.,* (1995). A possible explanation for this observation may be that the lectin-binding sites on the surface of procyclics are more accessible such that both the interaction with the lectin and agglutination are facilitated. Initial work by Ibrahim *et al.,* (1984) showed that *G. austeni* midgut extract was capable of agglutinating *T. brucei* and that this activity was specifically inhibited by carbohydrates. This finding indicated the involvement of lectins or lectin-like molecules. It is now generally accepted that lectins mediate both lysis and differentiation of trypanosomes (Maudlin & Welburn 1987; 1988). According to a model proposed to explain the lectin-mediated establishment of trypanosomes, the action of an endochitinase produced by large numbers of micro-organisms degrades chitin and leads to an accumulation of glucosamine within the midguts of susceptible flies (Maudlin, 1991). The glucosamine, in turn, blocks the lectin-mediated trypanocidal activity. On the other hand, refractory flies with few micro-organisms produce relatively less glucosamine and the parasites entering such flies are lysed by the lectins.

A comparison of teneral and non-teneral *palpalis* flies showed that a single bloodmeal prior to infection had a great effect on susceptibility. Teneral flies also had lower agglutinin and trypsin levels than non-tenerals. Maudlin and Welburn (1987)

showed that teneral flies possessed lower lectin titres than non-tenerals and concluded that the secretion of midgut lectin was stimulated by bloodmeal. They noted that a single bloodmeal greatly increases the rate of trypanosome killing in the midgut. Thus the higher susceptibility to trypanosome infection observed in categories of flies was attributed to low midgut lectins.

The host-related and tsetse-related differences in infection rates can be reduced by addition of D(+) glucosamine to the infective meal (Mihok *et al.*, 1992c; 1993). Glucosamine probably acts as a specific inhibitor to midgut lectins. Mihok *et al.*, (1992c) showed that D-glucosamine promotes midgut *T. brucei* infection rates in *G. m. centralis* and *G. pallidipes*. In this study midgut infection rates in *G. fuscipes* were enhanced by D-glucosamine, and D-galactose with glucosamine as the most efficient. Midgut infections in *G. tachinoides* were enhanced by addition of D-galactose and mannose to the infective meal whereas in *G. p. gambiensis* only addition of D-glucosamine was effective. However, midgut infection rates in the *palpalis* group did not reach the high infection levels observed in *G. m. morsitans* or *G. m. centralis* (Maudlin and Welburn, 1987, 1988; Mihok *et al.*, 1992c). The effects of galactose and mannose shown in this study have not been previously reported in the *morsitans* flies. It may be that midgut lectins of the *palpalis* flies differ from those of the *morsitans* group, or the *palpalis* group may possess other trypanocidal factors of differing specificity and activity. Welburn *et al.*, (1994) showed that midgut infections in *G. p. palpalis* are increased by D-galactose in addition to D-glucosamine. Super infection was obtained by a combination of D-glucosamine and D-galactose suggesting a second trypanocidal molecule in these flies. Grubhoffer *et al.*, (1994) showed the presence of two lectin systems in the midgut of *G. tachinoides* with different functional and

structural characteristics. Van den Abbeele *et al.,* (1996) showed evidence of two trypanosome binding proteins in *G. p. gambiensis*. There is sufficient evidence to suggest the presence of more than one trypanocidal molecule in the *palpalis* flies. This may explain their innate refractoriness to trypanosome infections. It seems probable that variation in the infectivity of tsetse species with trypanosomes is likely to be a factor comparable in importance to food source in determining the overall infection rates in tsetse populations.

CHAPTER SIX

6.0 GENERAL DISCUSSION, CONCLUSIONS AND SUGGESTIONS FOR FURTHER RESEARCH

6.1 General discussion

The life cycle of trypanosomes (*Trypanozoon*, *Nannomonas*) involves a vertebrate host and a tsetse vector. While some vertebrate hosts are susceptible to infection with trypanosomes others are refractory, especially wild animals and some livestock, that act as reservoirs of the parasites. The mechanisms of trypanotolerance are however not well understood. In the past, studies to determine the reservoir role of wild hosts have been hampered by an inability to obtain sufficient samples of blood from hosts for diagnosis and also limitations of available diagnostic techniques. The standard parasitological techniques routinely applied in the field are not sufficiently sensitive to detect low trypanosome levels. Diagnosis is an essential requirement in the management of disease at the epidemiological level for evaluating the performance of disease control strategies. The centrality of diagnosis in trypanosomiasis management has led to introduction of highly sensitive diagnostic techniques. The new technologies are likely to revolutionalise diagnosis of trypanosomiasis in animals, man and tsetse flies.

The present study has demonstrated the occurrence of *T. brucei* in wild monitor lizards. Though a wide range of parasitological techniques was used, the parasites were detected using rat inoculation and xenodiagnosis only. This confirms the difficulty of using simple parasitological techniques for detection of trypanosomes especially when they are present at low levels. The finding of *T. brucei* at an endemic focus of sleeping

sickness (Busia, Kenya) suggests that lizards may serve as reservoirs of human disease in addition to cattle and other wild animals. This finding is of particular relevance to the unexplained resurgence of sleeping sickness in specific areas around the Lake Victoria shores after many years of absence.

In this study, the ability of *morsitans* and *palpalis* flies to develop infections from lizards was also demonstrated. It was also possible to infect lizards experimentally with *T. brucei* but not *T. congolense* by tsetse challenge. The *T. brucei* survived for prolonged periods without showing clinical signs of the disease. These results suggest that monitor lizard blood exerts no immediate lytic effects on *T. brucei* organisms. These results were further confirmed by the blood incubation infectivity test (BIIT) which demonstrated minimal evidence for trypanotoxins in monitor lizard blood. Certain wild animal sera e.g. hippopotami and hyena, have trypanolytic effects on trypanosomes hindering their successful establishment (Rickman & Kolala, 1982). Thus, lizards are able to support the entire developmental cycle of trypanosomes. These findings suggest that monitor lizards could act as reservoirs of *T. brucei,* maintaining viable and infective parasites. These findings are consistent with those of Woo and Soltys (1969) and Molyneux (1973) who showed that it was possible to experimentally infect lizards with *T. brucei*. The finding of *T. brucei* in lizards suggests that the epidemiology of trypanosomiasis is complex. In the past, lizards have not been recognised as reservoirs of trypanosomiasis and hence have not been given consideration when formulating control strategies. It is however clear from the results of this study that lizards play a role in the trypanosome life cycle and could be important 'pools' of disease.

Digestion in tsetse flies is mainly proteolytic. *Glossina* spp. possess several proteolytic enzymes in their midguts. In this study, the general protease levels were higher in flies fed on goat than lizard blood. Low protease levels were found in *morsitans* flies fed on lizard blood. This may have interfered with digestion in these flies resulting in heavy mortalities. *Morsitans* flies may have problems digesting lizard blood due to its high DNA content. Protease levels were higher in *palpalis* than *morsitans* flies. Inconsistencies in protease levels among flies have been previously reported by Stiles *et al.,* (1991) in the *palpalis* flies. The levels of these proteases may affect trypanosomes in the gut of flies.

Trypsin levels were low in teneral *G. m. centralis and G. fuscipes*. Trypsin production was induced by bloodmeal. This was a time dependent process peaking at 48-72 h. The trypsin peak coincided with peak levels of agglutinins (lectins). Both molecules may be involved in trypanosome differentiation. Maudlin and Welburn (1988) showed that lectins mediate lysis and differentiation of trypanosomes whereas Imbuga *et al.,* (1992 a, b) provided evidence for involvement of trypsin. Trypsins and lectins are closely related (Osir *et al.,* 1993). Trypsin levels were higher in *G. fuscipes* than in *G. m. centralis.* These differences may be partly responsible for the differences in susceptibility with trypanosomes between the two fly species.

In the development of trypanosomes a critical time is the initial establishment of a gut infection in the fly. Within hours of entering the vector, most bloodstream forms die before transforming into gut adapted procyclic forms (Turner *et al.,* 1988). During the next few days, many gut infections are lost as the few surviving procyclics often fail to establish (Welburn *et al.,* 1989). Progression from establishment to maturation is not automatic. Many incipient gut infections fail to mature. The present study has

demonstrated that the blood used as an infective meal can have significant influence on the vectors infection rate with pathogenic trypanosomes. Lizard and crocodile blood eliminated many more infections than goat blood and substantially reduced early infections with *T. brucei* and *T. congolense* in all the tsetse species used. There were significant differences between mature *T. brucei* infections in male and female tsetse when lizard and goat blood were used in all species. This bias in maturation of *T. brucei* favouring higher rates in male tsetse has previously been reported (Maudlin *et al.*, 1991; Mihok *et al.*, 1992). The phenomenon appears to be specific to *T. brucei* which is hypothesised to be more sensitive to haemolymph lectin than *T. congolense* (Maudlin *et al.*, 1991). Maudlin *et al.*, (1991) suggested that a sex-linked factor may be controlling maturation. The meal used by *palpalis* flies had significant effects on maturation rates with *T. brucei.* Flies maintained totally on lizard blood had lower maturation rates than flies maintained on interrupted meals of goat and lizard. This may represent the natural situation in the wild where lizards harbour parasites that never get to maturation sites in flies utilising them solely as food source. In the event of an occasional feed on a favourable host e.g goat, or man the parasites undergo maturation and can be transmitted to other hosts marking the beginning of an epidemic. The overall patterns suggest that though lizards harbour trypanosomes, transmission indices in situations where flies feed mainly on reptiles are much lower than would be expected if they fed on goat. It may be possible that reptile blood contains some factors deleterious to the trypanosomes and hence only a small proportion of the trypanosomes manage to become established in the vector. The unique ability of goat blood enhancing midgut infection rates has been reported previously by Mihok *et al.*, (1993) and Olubayo *et al.*, (1994). The mechanism by which goat blood facilitates midgut infection rates in tsetse remains unknown. However, useful clues may lie in the carbohydrate molecules on the red blood cell (Olubayo *et al.*, 1994; Nguu *et al.*, 1996).

Agglutinin production in *G. fuscipes* followed a cyclical pattern, starting immediately after the bloodmeal, the activity increased and reached a peak at 48-72 h. Agglutination titres were higher in flies fed on lizard than goat blood. These differences accorded with differences between infection rates in flies utilising the two meals. Regular feeding of tsetse on monitor lizards may reduce the probability of trypanosome

infection. These results conform with results of previous workers (Maudlin and Welburn, 1988; Imbuga *et al.,* 1992 a, b) and suggest that lectins and trypsins influence establishment of trypanosomes in tsetse.

Tsetse flies can be susceptible or refractory to infection with trypanosomes. In this study, the *morsitans* group tsetse showed higher establishment and maturation levels with trypanosomes than the *palpalis* group. The poor vectorial capacity of the *palpalis* group in this study is consistent with the findings of Moloo and Kutuza (1988 a, b) who showed that *G. p. palpalis, G. fuscipes* and *G. tachinoides* were poor vectors of *T. congolense* and T. *brucei* when compared with *G. m. centralis*. This may suggest that the factors responsible for hindering establishment may be at higher levels and more active in the *palpalis* flies than in the *morsitans* flies. In fact, lectin and trypsin levels in this study were higher in the *palpalis* than the *morsitans* flies.

Maturation is affected by trypanosome species and stock. In this study, there was poor maturation rates of *T. congolense* in the *palpalis* flies even when goat blood was used. Maudlin and Welburn (1988) obtained similar findings when they noted variation in maturation between stocks of *T. congolense* in the same fly species. Maturation is dependent on a signal from tsetse midgut lectin. Whereas midgut lectin promotes cell death, it is essential for trypanosome maturation.

Refractoriness in tsetse is mediated by midgut lectin(s) which induce procyclic trypanosome death. Results of this study show that midgut agglutinins varied depending on the meal utilised. Flies fed on lizard blood had higher titres of agglutinin than those fed on lizard blood. The meal with the highest output of agglutinins is most successful at preventing trypanosome establishment in the midgut and accordingly

produces lower infection rates. A comparison of teneral and non-teneral flies in this study showed that a single bloodmeal prior to infection has a great effect on susceptibility. Maudlin and Welburn (1987) showed that teneral flies possessed lower lectin titres than non-teneral ones and concluded that the secretion of midgut lectin was stimulated by bloodmeal. They noted that a single bloodmeal greatly increased the rate of trypanosome killing in the midgut. Thus, the higher susceptibility to trypanosome infection observed in categories of flies may be attributable to low midgut lectins. It seems probable that variation in the infectivity of tsetse with trypanosomes is likely to be a factor comparable in importance to food source in determining the overall infection rates in tsetse populations.

The host-related and tsetse-related differences in infection rates were reduced by addition of D(+) glucosamine to the infective meal. Glucosamine probably acts as a specific inhibitor to midgut lectins. Mihok *et al.,* (1992c) showed that D-glucosamine enhances midgut *T. brucei* infections in *G. m. centralis* and *G. pallidipes*. In this study, midgut infections in *G. fuscipes* were enhanced by D-glucosamine and D-galactose with glucosamine as the most efficient. Midgut infections in *G. tachinoides* were enhanced by addition of D-galactose and mannose while in *G. p. gambiensis* only glucosamine was effective. However, midgut infection rates in the *palpalis* group did not reach the high levels observed in *G. m. morsitans* or *G. m. centralis* (Maudlin and Welburn, 1988; Mihok *et al.,* 1992c). The effects of galactose and mannose observed in this study have not been previously reported in the *morsitans* flies. It may be that midgut lectins of the *palpalis* flies differ from those of the *morsitans* group, or, the *palpalis* group possess other trypanocidal factors of differing specificity and activity. Welburn *et al.*, (1994) showed super infection in midgut infections in *G. p. palpalis*

when a combination of D-glucosamine and D-galactose were added to the infective meal suggesting a second trypanocidal molecule in these flies. Grubhoffer *et al.,* (1994) showed the presence of two lectin systems in the midgut of *G. tachinoides* with different functional and structural characteristics. There is therefore sufficient evidence to suggest presence of more than one trypanocidal molecule in the *palpalis* flies. This may explain their innate refractoriness to trypanosome infections.

A long period was chosen to allow maturation of slow-growing forms. The timing of maturation is closely correlated with the success of maturation. The longer the time taken for maturation of the midgut infections the poorer the transmission index (Dale *et al.,* 1995).

6.2 Conclusions

1. Diagnosis of wild monitor lizards for trypanosomes showed that lizards naturally harbour *T. brucei.* This *T. brucei* parasite was infective to tsetse and laboratory rats. Monitor lizards could therefore act as reservoirs of *T. brucei.*

2. Experimental infection of lizards with trypanosomes by tsetse challenge showed that it was possible to infect lizards with *T. brucei* (not *T. congolense)* parasites for prolonged periods without showing clinical symptoms of disease. The *T. brucei* parasites retained their infectivity for laboratory rats and tsetse. Thus, cyclical transmission of *T. brucei* between monitor lizards and tsetse is possible.

3. Monitor lizard blood supported survival of *palpalis* flies and had lethal effects on the *morsitans* flies. Lizards are important as maintenance hosts of *palpalis* flies. Trypsin levels induced by bloodmeals were higher in *palpalis* than *morsitans* flies. Lizard bloodmeal resulted in higher levels of trypsin than goat bloodmeal in all the flies.

4. There was minimal evidence for the presence of trypanotoxins in reptilian blood consisting of a slightly longer prepatent period in rats inoculated with trypanosomes compared with goat blood. Reptiles are important in the developmental cycle of pathogenic trypanosomes.

5. The *morsitans* flies were more efficient vectors of trypanosomes than the *palpalis* flies. *G. m. centralis* was the most susceptible whereas *G. fuscipes* was the most resistant. Goat blood facilitated higher infections of *T. brucei* and *T. congolense* than reptile blood in all the tsetse species. Maintaining *palpalis* flies totally on lizard blood resulted in lower maturation rates of *T. brucei* than interrupted feeds of goat and lizard. This suggests that transmision indices in areas dominated by lizards are much lower than in areas where goats are predominant.

6.3 Suggestions for further research

1. Research should be carried out in areas of differing epidemiology on many more lizards to determine the actual infection rates with trypanosomes.

2. Studies should be carried out to determine factors in monitor lizard blood responsible for the death of mice and *morsitans* flies. These factors may be important in the control of flies. Mechanisms responsible for inhibition of water loss in *morsitans* flies after lizard bloodmeals should also be investigated.

3. Experiments to infect lizards with trypanosomes using mainly riverine tsetse should be studied in more detail. This would be best done in the natural habitat of the lizards. This study would explain the natural transmission of trypanosomes between lizards and these flies.

4. Studies should be carried out to determine the effect of reptilian bloodmeals on infections with human infective trypanosomes (*T. brucei gambiense* and *T. b. rhodesiense)* in economically important tsetse vectors.

6.4 References

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