FEEDING BEHAVIOUR, SURVIVAL AND REPRODUCTIVE PERFORMANCE OF *GLOSSINA PALPALIS GAMBIENSIS* INFECTED WITH *TRYPANOSOMA (DUTTONELLA) VIVAX*

BY

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DECLARATIONS

I, JOSEPH NJUGUNA MAKUMI declare that this thesis is my original work and has not been presented for a degree in any other university.

Signed .....

CANDIDATE

Date ....10/6/89....04/08/1988

I declare that this thesis has been submitted for examination with my approval as university supervisor.

Signed Prof. C. P. M. Khamala

.....

Date 1988

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Lastly but not least, I wish to thank Miss Catherine Barua who typed this manuscript.

DEDICATION

Dedicated to my parents

Makumi and Nduta

and

to my dear wife Jane Wangu

for all you have done.

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A study was carried out to determine the effect of Trypanosoma (Duttonella) vivax Ziemann infection in Glossina palpalis gambiensis Vanderplank on the feeding behaviour, survival and reproductive performance. A colony of this subspecies was maintained in the laboratory: results showed that the performance of the flies in terms of female survival and fecundity and weight of puparia produced was consistently good. Teneral male and female flies infected with T. vivax stock ILRAD 3145 were compared with uninfected controls by measuring the following parameters: (1) mean number of feeds in 20 consecutive days; (2) mean rate of bloodmeal intake ug/sec. of the total days fed; (3) mean weight of bloodmeal intake/day of the total days fed; (4) mean total bloodmeal engorgement in 20 days; (5) mean number of probes/day of the days fed; (6) mean frequency of the multiple probe feeds, and (7) mean number of pre-feeding probes of the multiple probe feeds. There were no significant differences in feeding between the infected and the uninfected controls. The results suggested that the presence of T. vivax epimastigotes in the labrum had no deleterious effect on the feeding behaviour of the tsetse vectors.

The frequency of probing during feeding by hungry tsetse flies in relation to transmission of T. vivax was also studied. The infected tsetse flies were allowed to

probe consecutively without feeding on two goats and then allowed to complete feeding on a third one, and the rate of transmission calculated. Evidence was found to suggest that infected flies transmitted trypanosomes both during probing and when they completed feeding on the susceptible hosts. The survival and reproductive performance of the infected and the uninfected flies were compared by recording daily mortality and pupal production of a group of 100 infected females and 88 infected males and an equal number of uninfected controls. Results suggested that the presence of trypanosomes in the mouthpart had no adverse effect on the survival of the flies. The weights of pupae produced were also not affected suggesting that the reproductive physiology of the female and the nutrition of the pregnant females in relation to nourishment of the intra- uterine larvae was therefore not affected by infection of the flies with T. vivax.

CHAPTER ONE

INTRODUCTION

1:1 General Introduction

Tsetse flies are of health and economic importance because they transmit pathogenic trypanosomes which in man give rise to human trypanosomiasis (African sleeping sickness) and in livestock they cause animal trypanosomiasis (Nagana).

An accurate account of the geographical distribution of tsetse and the prevalence of trypanosome infections in man and animals is difficult. The relevant baseline data of such studies would be unreliable because of shortage of technically skilled personnel in the field, inadequate methods of the detection of infection and the failure on the reporting system. A total of 31 species and subspecies of tsetse flies have been described (Table 1) which infest 37 countries in tropical Africa in a 'fly-belt' that covers an area approximately 10.5 million Km^2 between 15°N and 29°S which is about half of the arable land of Africa (F.A.O./W.H.O./O.I.E., 1982). In this area, trypanosomiasis is essentially a zoonosis i.e. wild game and domestic animals act as reservoirs of infection from which trypanosomes are transmitted by tsetse flies to man and also to livestock (Ashcroft, 1959).

Table 1: The genus Glossina Wieldemann 1830

I The fusca group

1. G. fusca fusca Walker 1849
2. G. fusca congolensis Newstead and Evans 1921
3. G. tabaniformis Westwood 1850
4. G. longipennis Corti 1895
5. G. brevipalpis Newstead 1910
6. G. nigrofusca nigrofusca Newstead 1910
7. G. nigrofusca hopkinsi Van Emden 1944
8. G. fuscipleuris Austen 1911
9. G. medicorum Austen 1911
10. G. frezili Gouteux 1987
11. G. severini Newstead 1913
12. G. schwetzi Newstead and Evans 1921
13. G. haningtoni Newstead and Evans 1922
14. G. vanhoofi Henrard 1952
15. G. nashi Potts 1955

II palpalis group

16. G. palpalis palpalis Robineau-Desvoidy 1930
17. G. palpalis gambiensis Vanderplank 1949
18. G. tachinoides Westwood 1850
19. G. pallicera pallicera Bigot 1891
20. G. pallicera newsteadi Austen 1929
21. G. fuscipes fuscipes Newstead 1910
22. G. fuscipes martinii Zumpt 1933
23. G. fuscipes quanzensis Pires 1948

24. G. caliginea Austen 1911

III morsitans group

25. G. longipalpis Wiedemann 1930

26. G. morsitans morsitans Westwood 1850

27. G. morsitans submorsitans Newstead 1910

28. G. morsitans centralis Machado 1970

29. G. pallidipes Austen 1903

30. G. austeni Newstead 1912

31. G. swynnertoni Austen 1923

Trypanosomiases in Africa are still one of the most serious threats to the health of man and an obstacle to the development of livestock industry. It is estimated that some 35 million people (W.H.O., 1979) and 30% of the 147 million cattle in countries affected by tsetse flies (Murray and Gray, 1984) are exposed to the risk of infection. Animal trypanosomiasis is in many areas the major veterinary problem to livestock production in Africa. In vast areas of the continent, breeding of domestic livestock is difficult due to the high incidence of the disease thus becoming one of the principal constraint to rural development. It impedes economic development which in Africa depends largely on the agricultural sector. The presence of tsetse flies not only excludes domestic livestock from a considerable area of Africa but also causes severe losses to livestock production due to poor growth, weight loss, low milk yield, reduced capacity for work, infertility and abortion. The annual loss in meat production alone due to trypanosomiasis is valued at US $ 5 billion (F.A.O./W.H.O./O.I.E., 1963; Trail et al, 1985).

The tsetse fly is an obligatory haematophagus insect that make it an efficient vector of trypanosomiasis. The vector/trypanosome/host interaction maintains endemic and/or enzootic trypanosomiasis in a variety of ecological zones.

Trypanosomes are transmitted cyclically i.e. they undergo development in tsetse flies with a few species being transmitted directly (non-cyclically) by other biting dipteran flies (Wells, 1972). Cyclical transmission of infection, however, represents the most important problem, because once the tsetse fly becomes infected it remains infective for a long period, in contrast to the ephemeral nature of non-cyclical transmission.

There are seven important species and subspecies of Salivarian trypanosomes and tsetse flies transmit six of these which are of socio-economic importance, namely, T. b. gambiense Dutton, to man in western and central Africa, T. b. rhodesiense Stephens & Fantham in eastern and southern Africa, T. brucei brucei Plimmer & Bradford, T. congolense Broden, T. simiae Bruce, Hamerton, Bateman and Mackie and T. vivax Ziemann to livestock.

Trypanosomes are transmitted through the bite of an infected tsetse fly when it feeds on its host. The epidemiological factors involved in the transmission of African trypanosomiasis have been described (Scott, 1970; Apted, 1970; Maclennan, 1970; Willet 1970). The most important can be listed as the density and infection rates in tsetse flies , reservoir hosts, host-fly contact, species and stock variations of pathogenic trypanosomes and climatic factors. Trypanosomes are transmitted through the bite of an

infected tsetse and the feeding process of the vectors has been described by several workers including Jobling (1933), Buxton (1956), Gordon et al (1956) and Bursell (1970).

The frequent occurrence of trypanosomiasis epidemics in many parts of tropical Africa in recent years, despite control efforts over the last 60 years, have necessitated a review of some of the factors involved in the transmission of pathogenic trypanosomes to man and livestock. Trypanosomiases have been reported in areas where tsetse fly populations are difficult to detect, and epidemics occur in areas of very low tsetse challenge (Nash, 1944a). Rates of infection of trypanosomes in tsetse flies have been found to be low whereas studies on mammalian hosts have shown infection rates to be comparatively higher (England and Baldry, 1972; Robson et al, 1972; Moloo et al, 1973). Thus infection rate by itself cannot satisfactorily explain the frequent occurrence of epidemics in endemic areas. The risk to African trypanosomiasis should therefore be re-examined in the light of these observations, and knowledge of feeding behaviour of infected tsetse flies is one of the important factors in the understanding of the epidemiology of trypanosomiasis

Studies on the trypanosome-vector relationship have revealed a specific association between parasites and

mechanoreceptors responsible for detecting rate of the blood flow in the labrum (Rice et al, 1973). Jenni et al (1980) proposed that this close association of the parasites and mechanoreceptive sensilla in the labrum may affect the feeding behaviour of infected tsetse flies resulting in frequent probing. The chances of transmission of pathogenic trypanosomes would thus be increased. Such behaviour would indicate an advantageous adaptation by parasites that may have a profound epidemiological and epizootiological implications (Jenni et al, 1980), and would seem to be an important measure of the risk to trypanosomiasis (Livesey et al, 1980).

However, studies by other workers (Moloo, 1983 and Moloo and Dar, 1985) have shown that feeding behaviour of infected tsetse flies was not significantly different from that of uninfected ones. These workers found no differences in feeding behaviour between tsetse flies infected with T. vivax, T. congolense or T. b. brucei and the uninfected ones.

1:2 Literature Review

The literature concerning the feeding process of tsetse flies is enormous and covers mainly host-trypanosome interactions, the role of labral-cibarial sensilla, probing and transmission of trypanosomes. Detailed studies have been conducted into the mechanisms by which a tsetse fly obtains its bloodmeal and the function of the mouthparts (Gordon et al, 1956).

1:2:1 Host-trypanosome interaction in the vector

When T. vivax trypanosomes are ingested with blood some attach to the walls of the food canal of the tsetse fly. Here they undergo development in the course of which the blood stream trypomastigotes are transformed first into elongated trypomastigotes with the kinetoplast not far behind the nucleus and then into epimastigote forms with kinetoplast anteriorly to the nucleus. These eventually invade the hypopharynx and transform to the infective coated metacyclics (Lloyd & Johnson, 1924; Gardiner et al, 1986). The attachment of T. vivax epimastigotes in the labrum of infected G. fuscipes was studied by Vickerman (1973) who demonstrated that the epimastigotes are attached to the

cuticular lining of the labrum by flagellar hemidesmosomes. The parasites are aligned in such a way that the flagellar axonemes run parallel to the length of the labrum. The parasites are swept posteriorly as blood rushes past during feeding. The attachment mechanisms of trypanosomes is usually regarded as a prerequisite to maintain position in a flowing aqueous environment (Livesey et al, 1980; Jefferies et al, 1986) and which appears to be important for the establishment of infection. Thevenaz and Hecker (1980) studied the distribution and attachment of T. (N). congolense in the proximal part of the proboscis in G. m. morsitans. These authors presented evidence to show the interactions within the trypanosome populations and between the parasites and the vector. They suggested that these relationships may influence the feeding behaviour of the tsetse fly in that trypanosomes may impair the function of the labral-cibarial sensilla.

1:2:2 Labral-cibarial sensilla and their role in feeding behaviour

Rice et al (1973) described the sensilla (LC1) in the labrum of G. austeni previously identified by Newstead (1906) as "stalked" hairs. These authors classified the labral-cibarial sensilla (LC1 and LC2) as "mechanoreceptors' whose function is to monitor the

movement of saliva in the food canal and provide a means of ensuring that the lumen of the labrum is not clogged with coagulated blood. These workers proposed a relationship between the labral-cibarial sensilla and the feeding behaviour of G. austeni. They stated that "Quite often, flies can be observed to break off from sucking and recommence their probing and sampling activities at another site; the stimulus for this is presumably the absence of a sufficient quantity of blood as detected by the LC1 and LC2 mechanoreceptive sensilla, acting as fluid flow meters".

Killick-Kendrick et al (1977) suggested that in Leishmania-infected sandflies, mechanical blockage by a plug of parasites may interfere with the function of sensilla in the labrum. These authors proposed that, because parasites were attached to the cuticle lining in the pharynx, the chemosensilla would be rendered inoperative. Full engorgement would not occur, although external sense organs would allow continuation of probing. Molyneux (1977) reported that Leishmania infected sandflies have difficulty in obtaining a bloodmeal, and so probe for longer periods than uninfected ones.

Molyneux and Lavin (1979) examined the labra of G. morsitans infected with I. (N). congolense using the stereoscan electron microscope (SEM). These authors

presented evidence to show heavy colonization of the labrum occurring in the region bearing LCI sensilla. They proposed that this may impair the function of the sense organs. Rice et al. (1973) suggested that the tsetse fly would as a result, probe more frequently than if uninfected. These workers related rate of transmission of parasites to the frequency of probing by the infected tsetse flies and suggested that the chances of transmission would be increased because the parasites in the hypopharynx (and salivary glands in Trypanozoon), would be more likely to be inoculated into the mammalian host. Molyneux and Lavin (1979) related the observed low T. brucei infection rates in flies, but high rates in mammals in some situations, to the probing frequency of infected tsetse. Similarly, they proposed that the high incidence of trypanosomiasis in cattle in areas where Glossina populations were difficult to detect could be explained by a higher probing rate in the infected flies.

Working with T. b. brucei, Jenni et al (1980) described a specific association between trypanosomes and mechanoreceptors responsible for detecting rate of blood flow in the proboscis of G. m. morsitans and G. austeni. These authors reported that infected flies probed significantly more frequently on the belly of mice than uninfected controls. They suggested that the observed differences in feeding behaviour resulted from

impaired function of the labral mechano-receptors in the infected Glossina. They concluded that to obtain the same bloodmeal size, an infected tsetse fly took longer to engorge, or if it did not take sufficient blood, had to feed more frequently.

Livesey et al (1980) reported details of fluid mechanics of blood flow in the proboscis of infected and uninfected G. m. morsitans and discussed the epidemiological significance of their observations. They reported a reduction in the rate of passage of blood in the labra of infected tsetse flies and suggested that the important factors in measuring risk of trypanosomes are the increased frequency of probing and the increased voracity of infected flies, and not just the infection rates.

Moloo (1983) and Moloo and Dar (1985) studied the feeding behaviour of G. m. morsitans and G. m. centralis infected with T. vivax, T. congolense or T. brucei and presented evidence which could not be reconciled with the above suggestion. The results differed from those of a previous study, (Jenni et al. 1980) for Glossina infected with T. brucei. These authors reported that the rate of blood flow through the food canal of the proboscis was not significantly affected by the presence of the trypanosomes. Hence, the close association between the parasites in the

labrum and LC1 mechano-receptors had no deleterious effect on the flow rate.

Moloo (1983) disputes the contention by Jenni et al. (1980) that Trypanozoon infection rates were high in the mammalian hosts but low in Glossina in the field because the infected tsetse flies probed more frequently and fed more voraciously than the uninfected ones. Moloo (1983) agreed with the observed high Trypanozoon infection rates in the mammalian hosts in some situations than in the vectors, but suggested this may be due to the actual numbers of the mammalian hosts under continuous challenge in relation to the numbers of Trypanozoon-infected tsetse flies. It is probable that the number of Trypanozoon-infected tsetse flies outnumber their hosts in enzootic/endemic regions. Infected tsetse flies feed and hence probably transmit trypanosome infection more than once in their lifespan. This author argued that if the specific relationship between the epimastigote colonies and the mechano-receptors in the labrum had deleterious effects on the feeding behaviour of the latter, then in terms of evolution of the parasite/ vector relationship, one would expect selection to operate against the infected tsetse flies and hence cyclical transmission. But this does not seem to be true since trypanosomiasis transmitted largely by Glossina affects man and his livestock over enormous territory of tropical Africa.

1:2:3 Probing and Rate of transmission of trypanosomes by infected tsetse flies

Corson (1932) studied the transmission of T. rhodesiense to rats by G. morsitans. He reported that a single G. morsitans infected with T. rhodesiense transmitted the infection to 12 different rats when allowed to feed on them. In a study on the infectivity to man of a strain of Trypanosoma rhodesiense transmitted cyclically by Glossina morsitans, Fairbairn and Burtt (1946) stated that "of 247 rats merely probed into by T. b. rhodesiense infected flies, 22 were not infected". Thus 93.7% were infected by probing alone. They reported that of 35 men who were rebitten by the same fly on different days which had failed to infect them originally, 12 were infected after an interval of some few weeks.

Moloo (1981) studied the transmission of a West African stock of Trypanosoma vivax by G. m. centralis and G. m. morsitans amongst rabbits, mice, rats and goats. He reported that the infected tsetse flies were able to transmit the infection throughout life to susceptible hosts but not at every feed.

Roberts (1981) compared rates and frequency of transmissions by G. m. morsitans infected with T.

congolense into mice when allowed to probe consecutively without feeding and when allowed to feed to repletion. He reported that rates and frequency of transmission were lower when the flies were allowed to feed on them than when allowed to probe without feeding. In the consecutive probings on mice, the overall transmission rates for the first trial was 54%. The overall rate dropped to 37.3% 3-7 days after the initial probings.

1:2:4 Longevity of infected Glossina

Duke (1928) studied the mortality of G. palpalis used in transmission experiments. He compared the longevity of flies infected with various strains of trypanosomes with that of uninfected ones. The author reported that T. rhodesiense was not pathogenic to the flies but rather appeared to increase their survival. He concluded that the presence in its intestine of developing forms of the polymorphic trypanosome was not to any noticeable extent injurious to the fly.

In a study of the survival of infected flies, Buxton (1955) reported that there was no evidence that the multiplication of trypanosomes shortened the life of the fly. He added that infected flies may live a

long time and there was no evidence that the infection was harmful to them. Baker and Robertson (1957) studied the relative susceptibility of male and female G. morsitans to infection with trypanosomes, and the effect of such infections on the longevity of the flies. They showed that the average longevity for all flies with T. rhodesiense or T. b. brucei infections was 25.9 days compared to the uninfected with an average length of 25.8 days. Female flies with salivary gland infections outlived those with only gut infections. The authors concluded that development of trypanosomes had no detectable pathogenic effect on the flies.

In his breeding experiments, Vanderplank (1948b) recorded the mean length of life of female tsetse flies and found it to be about 2-3 months. Nash (1936a) reported that in captivity females lived slightly longer than males, but in nature, they live at least twice as long probably because males lead a more hazardous life (Jackson, 1937).

1:2:5 Mechanical transmission of trypanosomes

The importance of direct transmission in the epidemiology of trypanosomes has been recognized for a long time. Duke (1919) was the first to suggest that animal trypanosomes could be found in the absence of tsetse flies. There are historical reports of nagana in

Africa in the apparent absence of tsetse flies (Chambers, 1917; Barnett, 1947; Buxton, 1955), but these may have been caused by inefficient catching techniques and not their absence in such areas.

Successful transmission of various species of trypanosomes directly by Glossina and other species of biting flies have been shown by Dutton et al (1907), Taylor (1930) and Bailey (1966). The important conclusion from these reports is that mechanical transmission is most easily contrived from animals with relatively high parasitaemia. The only study that included both T. rhodesiense and G. morsitans was conducted by Bailey (1965) who reported that tsetse flies could mediate mechanical transmission from trypanosome-infected patients and mice to uninfected mice at rates of 22.8% and 4.9% respectively. Gingrich et al (1983) studied mechanical transmission of T. rhodesiense by G. morsitans fed in vitro and in vivo and reported transmission rates of 4.9 and 40.9% respectively. These authors concluded that mechanical transmission by Glossina play a more important role in the epidemiology and epizootiology of trypanosomiases than presently acknowledged.

Glossina palpalis gambiensis is mainly confined to the riverine vegetation over vast areas in West Africa (Katondo, 1984) where it is closely associated with T. gambiense infection (LRU, 1984). The ecology and

behaviour of this species and the close man-fly contact in some circumstances make it an efficient vector of human and animal trypanosomiases. Man takes cattle to watering areas in the dry season where they come into contact with tsetse flies which may be infected with various trypanosomes. It is known that tsetse flies can transmit mixed infections to susceptible hosts (Moloo et al., 1982) and the species has been incriminated as a vector of T. congolense and T. vivax to livestock (Kaminsky, 1986).

1:3 Objectives of the Study

From the literature review, it is clear that few studies have been done to investigate the feeding behaviour of tsetse flies infected with pathogenic Trypanosoma species. The available literature on the subject is conflicting and therefore inconclusive. It is also evident that comparative studies between infected and uninfected tsetse flies have not been carried out on any of the palpalis group, which are important vectors of human trypanosomiasis, and in certain circumstances, also of animal trypanosomiasis. This study was therefore designed to investigate the feeding behaviour of Glossina palpalis gambiensis, the transmission of T. vivax to susceptible goats and the effect of infection, if any, on the survival and

reproductive performance ofreproductive performance ofthe flies. the two main factors that would influence the presence and abundance of the vectors.

The specific objectives were five, viz:

- To maintain a colony of G. p. gambiensis to provide surplus of flies to carry out the study.
- To compare feeding by infected and uninfected flies.
- To study the frequency of probing by infected tsetse flies and its significance in the transmission of T. vivax to susceptible hosts.
- To compare the survival and reproductive performance of infected and uninfected tsetse flies.
- To determine whether G. p. gambiensis can also transmit T. vivax directly (non-cyclically) to susceptible hosts.

CHAPTER TWO

MAINTENANCE OF A COLONY OF *GLOSSINA PALPALIS GAMBIENSIS* AND THE INFECTION OF TENERAL FLIES WITH *TRYPANOSOMA VIVAX*

2:1 Introduction

A colony of *Glossina palpalis gambiensis* was maintained in the laboratory to provide a surplus of adults to be used for feeding behavioural studies. The colony was maintained under controlled laboratory conditions and its performance monitored by recording daily the female stock, mortality, pupae production and weight and teneral (young unfed flies) emergences. Geigy (1948) described the conditions and techniques for breeding *Glossina palpalis* for laboratory investigations, but it was not until later that Nash *et al* (1960) successfully reared this species for mass production of adults. These authors maintained the flies at 25-26°C and 80% relative humidity and allowed them to feed on goats. Since then, successful laboratory colonisation of a number of *Glossina* species has been achieved using living animal host as a source of food (Nash *et al*, 1968 and 1971; Mews *et al*, 1972). However, only limited success has been achieved with *in vitro* feeding using diets of defibrinated or heparinised

blood through a membrane (Mews, 1976). Since Nash et al (1960) showed that the highest insemination rate occurred when 3-day old females were mated with about 7-day old males, the females in this study were therefore mated with 8-day old males and left for 24 hours.

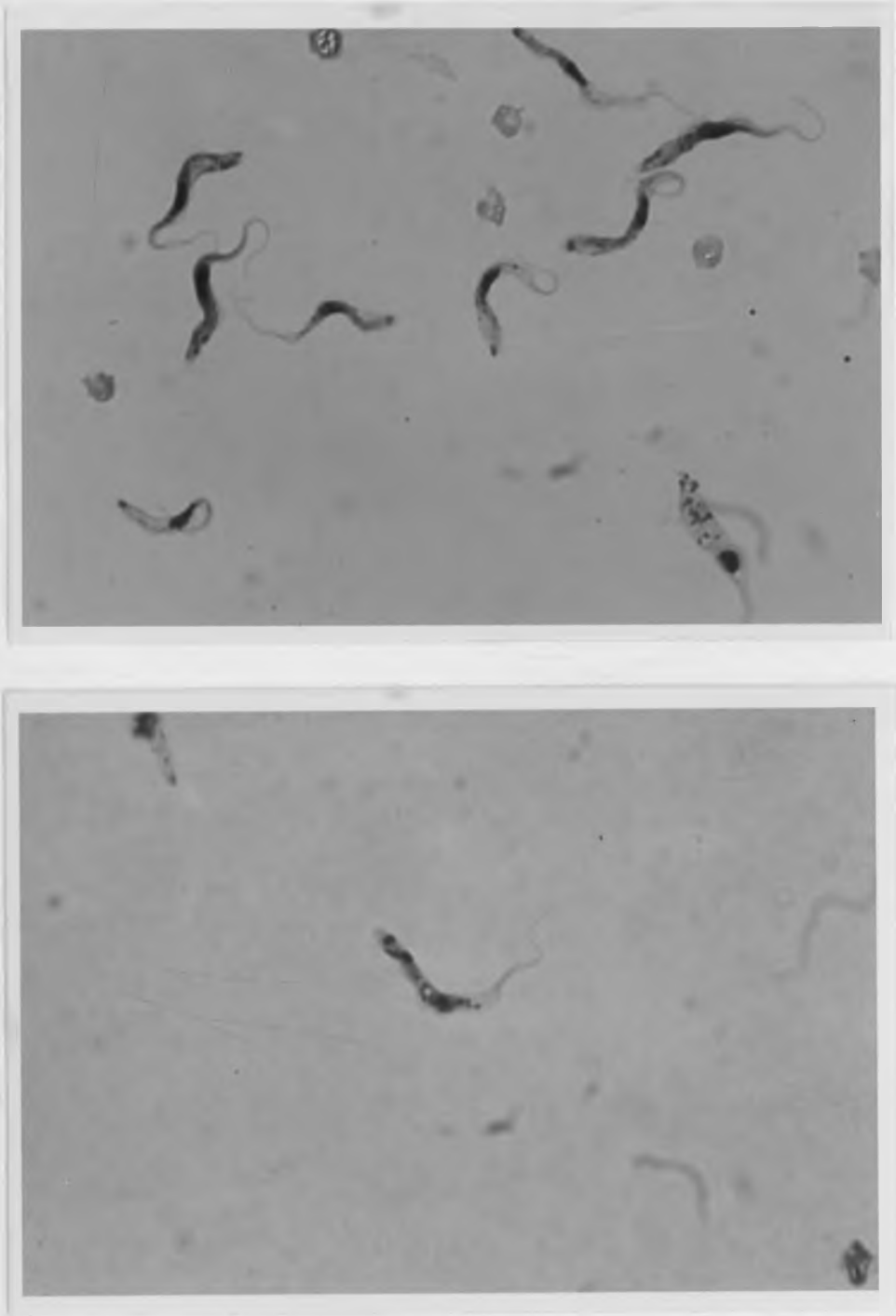
2:2 Materials and Methods

Glossina palpalis gambiensis from the ILRAD colony was used in this study. The pupae to start the colony originated from the Centre of Research on Animal Trypanosomes (CRTA) in Bobo-Dioulasso, Burkina Faso in 1983. Half-lop rabbits were used to feed the colony tsetse flies and for studies on the feeding behaviour and survival of infected flies. The goats used were adult males, (cross-breeds between East African Maasai and Galla goats) weighing 20 - 25 Kg purchased from an area of Kenya free from trypanosomiasis. They were housed in maximum insect proof isolation units and allowed free access to water and hay with concentrated rations.

Trypanosoma (Duttonella) vivax Ziemann was used in the study. The stock ILRAD 3145 (Plate 1), was a derivative of Zaria Y486 which was isolated from a cow in Nigeria in 1976 (Leeflang , Buys and Blotkamp, 1976).

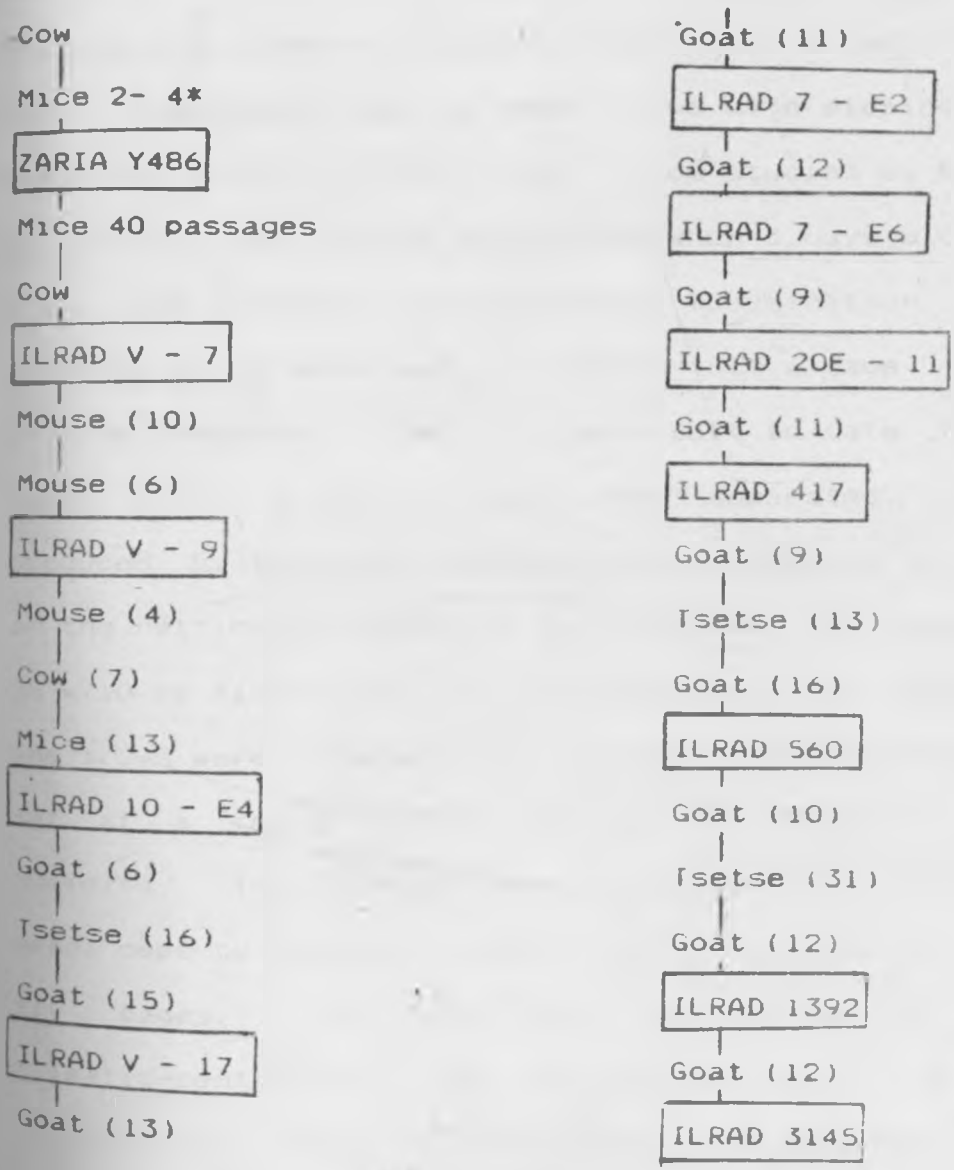
PLATE 1.

T.vivax ILRAD 3145 bloodstream forms from a goat (1280X)



The history of this stock is given as a flow diagram below.

Figure 1. History of *T. vivax* ILRAD 3145



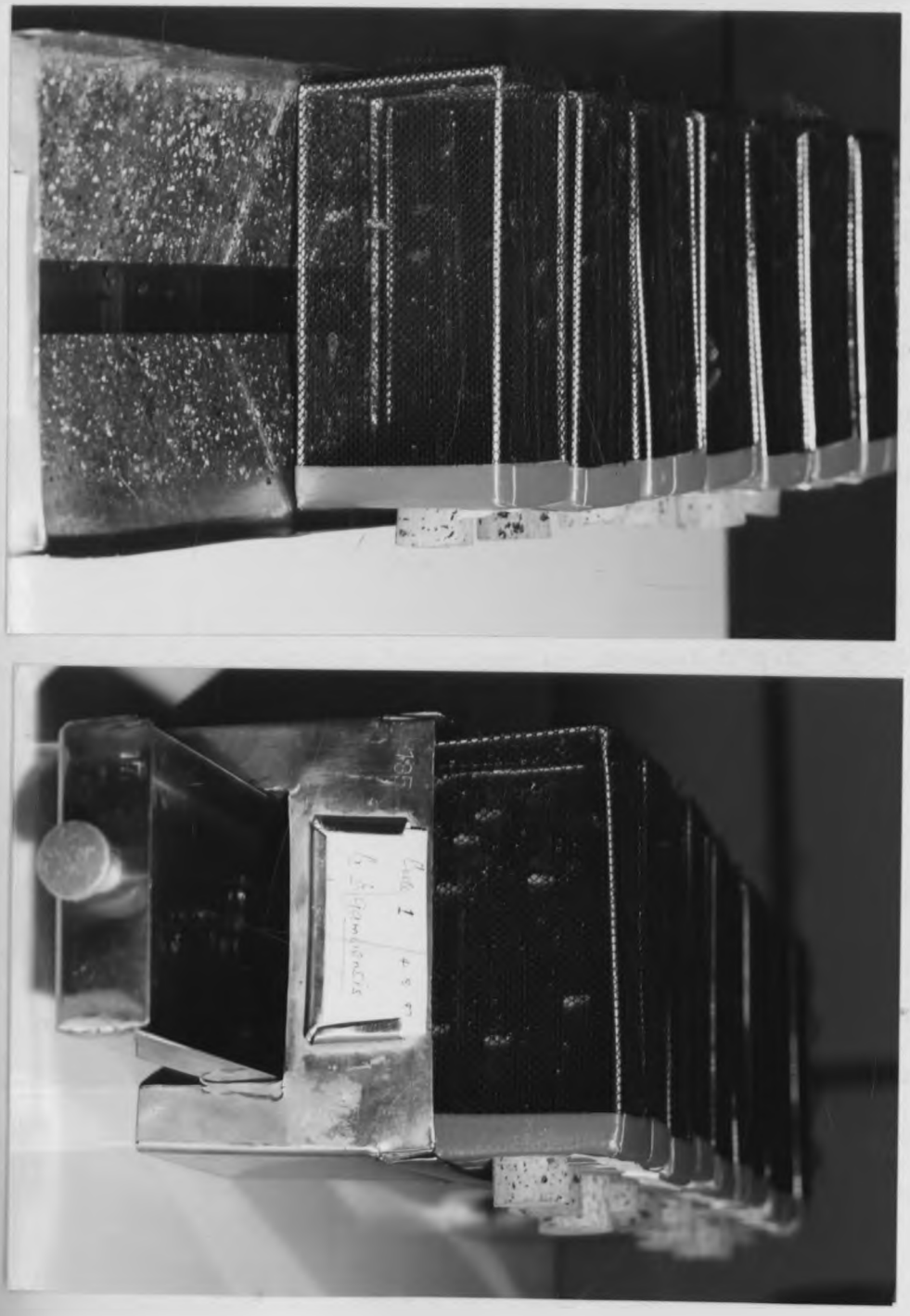
* Number of days after challenge

2:2:1 A colony of *Glossina palpalis gambiensis*

The colony, which I maintained during the period of the study, comprised about 1800 mated females. The flies were housed in Geigy-20 cages, 20 flies per cage (Geigy, 1948). The cages measuring 14 x 18.5 x 11 cm. were made of stainless steel frame covered with a black netting and a one-inch diameter hole with a cork at one end. These were kept on metal trays with sloping sides measuring 55.5 x 15.5 x 11 cm. From studies by Nash *et al* (1960), the females were mated when 3 days old with 8-day old males to achieve highest insemination rates. A group of 20 males was introduced into a cage holding 20 fed females. The flies were left to mate for 24 hours after which the sexes were separated. Larvae produced by the mated females crawled through the mesh of the netting to pupate in a 2.5 cm deep tray measuring 55 x 10 cm fixed below this to hold the pupae (Plate 2). The trays were arranged on a trolley in the holding room set at a temperature of 25±1°C and 80±2% relative humidity. The trolley legs had glycerol in attached metal cups to prevent predatory ants from getting to the fly cages. The flies were maintained in these climatic-controlled rooms when not feeding. The room was kept at 12 hours of subdued indirect electric light during the day and 12 hours of darkness at night.

PLATE 2.

Adult female Glossina palpalis gambiensis in Geigy- 20 cages arranged on a tray showing pupae in a collection tray.



The flies were fed by strapping the cages on cleaned ears of half-lop rabbits held in wooden boxes (Plate 3). The ears were cleaned with tissue soaked with 70% alcohol and dried with cotton wool. The cages were covered with a black cotton cloth to reduce disturbance to the flies and allowed to feed for 10 minutes. The trays were checked daily for pupae which were collected into plastic tubes with netting at one end. The pupae were counted, weighed and the number and mean pupal weights recorded in monthly record sheets. The pupae were then transferred into emergences cages (Plate 4) in the holding room. The cages, made of an aluminium frame with netting, measure 40 cm high x 30 cm broad x 10 cm deep with a removable aluminium tray to hold the pupae. The newly emerged flies (tenerals) crawled up the sides of the cage to expand their wings and harden. To remove the flies, the pupal tray was replaced by an aluminium sheet and the whole cage placed in the cooling chiller at 2 - 3°C which thus immobilized the flies. The flies were removed and the pupal tray replaced. The sexes were separated using a soft brush based on the external genitalia, counted and recorded. These were put in Geigy cages in groups of 20 flies of either sex and fed and maintained as described above. The dead females were removed from the cages and the total number of deaths recorded.

PLATE 3.

Tsetse flies in Geigy - 20 cages strapped on ears of half lop rabbits

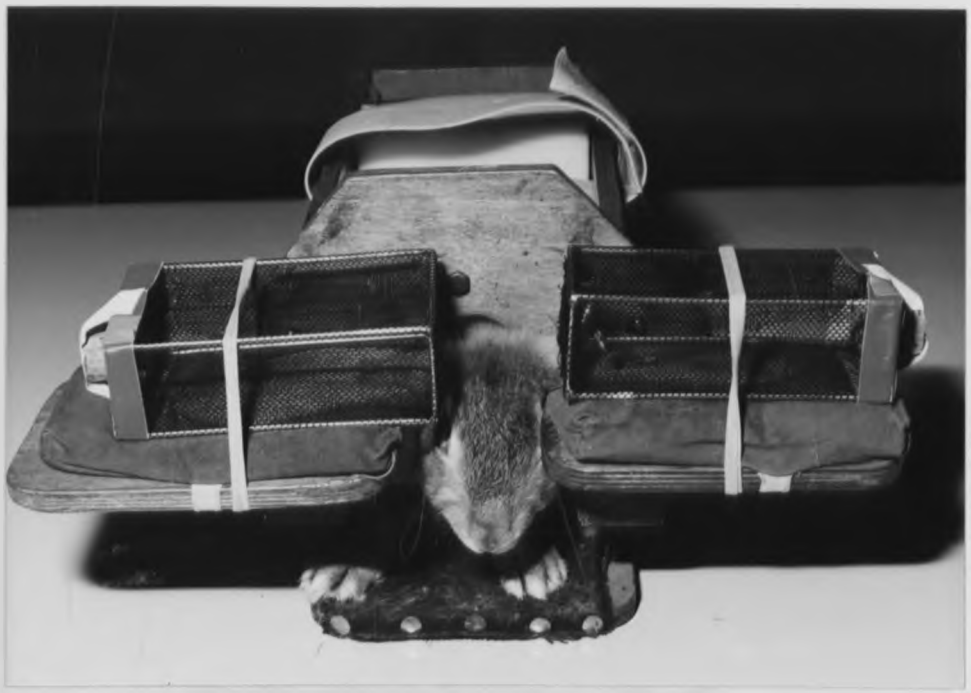
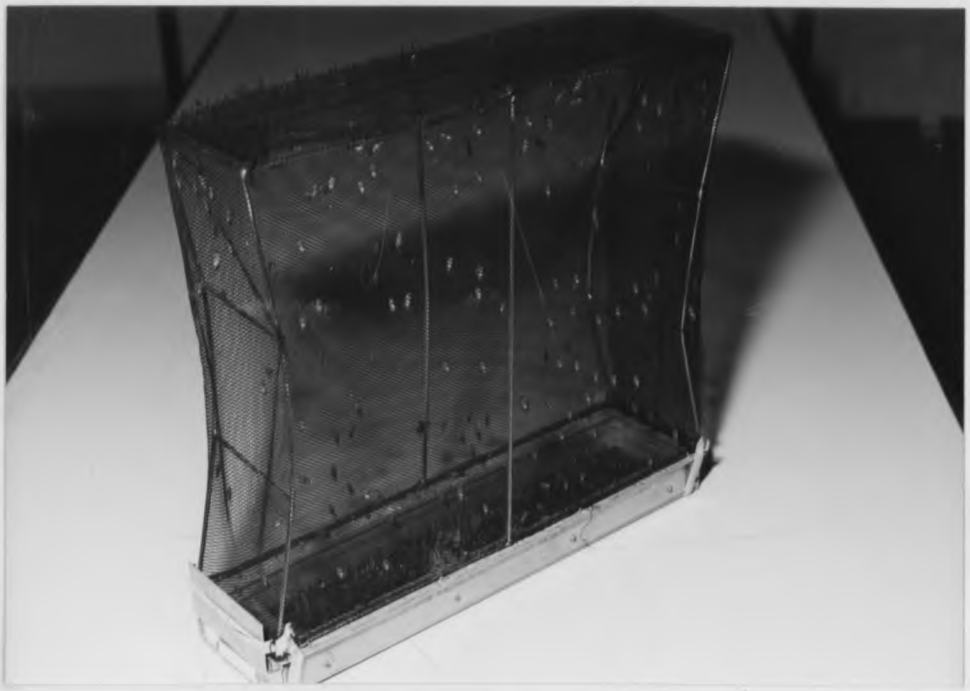


PLATE 4.

Emergence cage with pupae in removable tray beneath and emerged flies on the sides where they expand and harden the wings.



In order to learn all the techniques involved in tsetse fly maintenance, a group of 200 females were used. These were put in Geigy-20 cages and maintained as described above. The flies were fed, mated and pupae collected as previously described. The group was maintained for 90 days and its performance compared with the main colony of G. p. gambiensis.

2:2:2 Experimental methods

Preparation of stabilate

A goat was injected intramuscularly with T. vivax stock ILRAD 1392 for the preparation of its derivative. The stabilate ILRAD 1392 was removed from the trypanosome bank and thawed at room temperature. Three capillaries of the stabilate were diluted with 3 ml phosphate saline glucose (PSG), pH 8, in a 5 ml syringe. The buffer was prepared by the method of Lanham and Godfrey (1970) by dissolving the following salts in 1000 ml of water:-

anhydrous di-sodium Hydrogen orthophosphate (Na_2HPO_4) 2.392g

Sodium dihydrogen orthophosphate (NaH_2PO_4) 0.239g

Sodium chloride (NaCl) 1.7g

Glucose 10g

A drop of the diluted material was examined under the microscope and the number of live parasites counted. The material, containing 5 parasites/microscope field at 400X and transported under ice in a bucket, was injected intramuscularly (i.m.) using 23G x 2.5cm needle into the thigh muscle of a goat free from infection.

Parasitaemia: The goat was checked daily for trypanosomes in the peripheral blood by unstained thin wet blood film and buffy coat examination (Woo, 1969) as follows. Blood samples were drawn from the ear by puncturing a peripheral vein using 23G x 2.5 needle. The blood was collected into sodium-heparinised haematocrit capillary tubes. A drop of blood was applied on a clean microscope slide and covered with a cover slip. The remaining blood in a capillary tube was sealed at one end with plasticine and spun at 10,000 r p m for 5 minutes in a centrifuge (Combifuge - Haraeus Christ). The unstained thin wet blood film was examined for trypanosomes by phase-contrast microscopy at 400X magnification using a combination of Phaco 2 NPL FLUOTAR 40/0.7 and Periplan Gw 10X eye pieces (E. Leitz Wetzlar, Giessen, Germany). The packed red blood cell volume (PCV) of haematocrit was measured using a haematocrit chart (Haeraeus Christ). The buffy coat i.e. layer between red blood cells and plasma was examined for the parasites at 320X magnification under phase- contrast microscopy.

At the first rising parasitaemia, (25 parasites/field) a stabilate was prepared from the blood of the infected host. Blood was collected by puncturing the jugular vein of the goat using 20G x 2.5cm needle, into sodium heparinised, evacuated "Venoject" blood collecting tubes. The tubes were kept under ice in a bucket. About 5 ml of the blood was diluted with an equal volume of 20% glycerol in ice-cold phosphate saline glucose, pH 8, and a drop of the material applied on a clean microscope slide. The thin wet blood film was examined at 400X magnification under phase-contrast microscopy and the number of live parasites per field recorded.

The diluted blood containing 5 parasites/field was pipetted using 150 mm pasteur pipettes into a hundred 75ml haematocrit capillary tubes filled 2/3 with blood. The tubes were then sealed at one end with plasticine. and packed in groups of 25 each in plastic tubes under liquid nitrogen. The tubes were labelled. capped and transferred into an insulating jacket which was suspended in the vapour phase of liquid nitrogen in a canister for 24 hours and then cryopreserved in liquid nitrogen at -196°C. The stabilate was numbered ILRAD 3145 and was used in all the experiments.

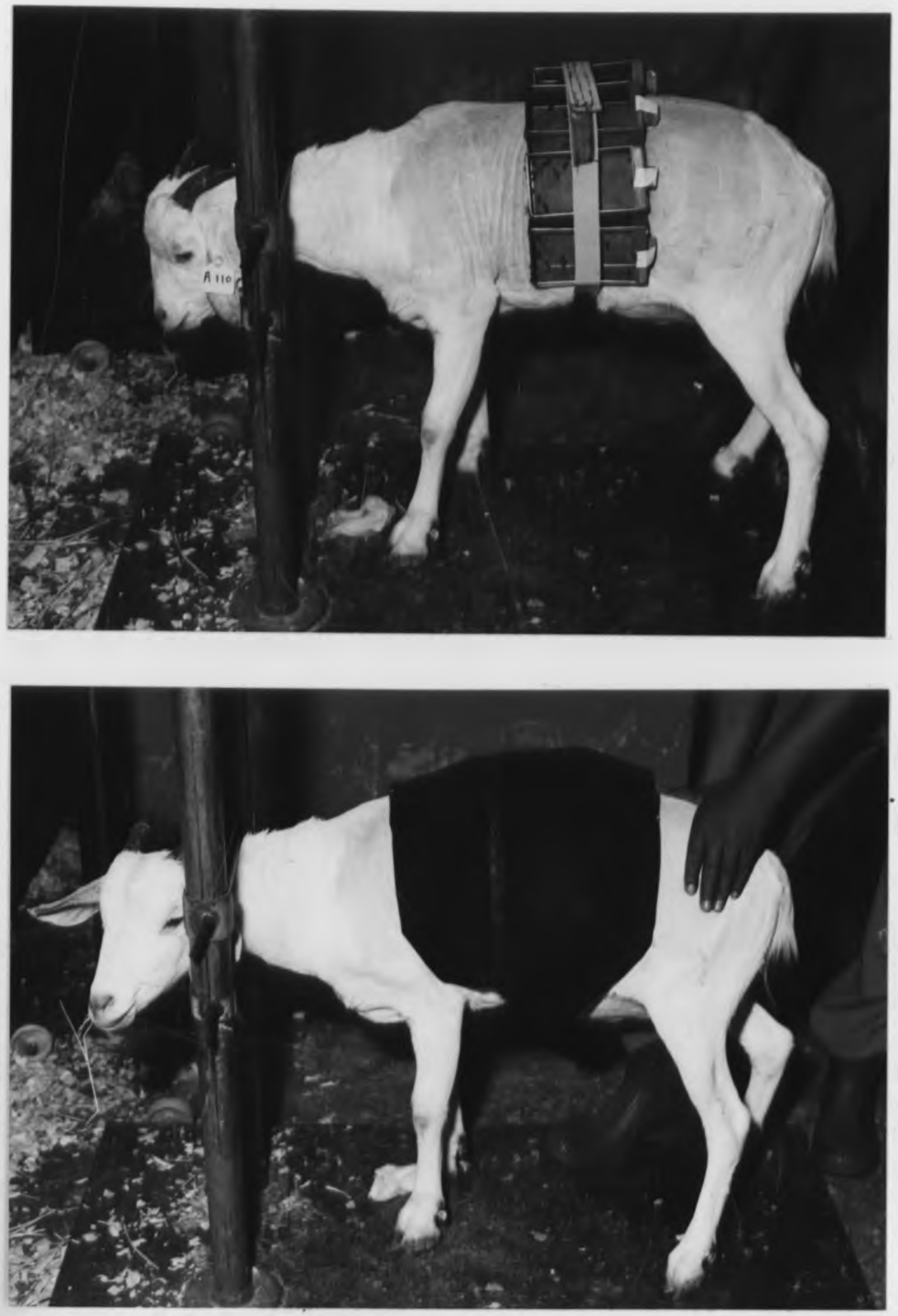
Tsetse fly infection with *T. vivax*

A total of 438 male and 430 female flies divided into three groups were infected with *T. vivax* as follows. A goat was infected with the stabilate ILRAD 3145 by injecting intramuscularly (i.m.) material containing 5 parasites/field as described above. The parasitaemia of the goat was monitored daily as previously described, and at the first rising parasitaemia, (15 parasites/ field) the goat's flanks were shaved with a clipper and cleaned with cotton wool soaked with 70% alcohol. The flanks were then dried with cotton wool and the cages holding 20 teneral flies each were arranged on the flanks and strapped with plastic webbing (Plate 5). The cages were covered with a black cotton cloth to avoid disturbance to the flies and the flies left to feed for 10 minutes after which the cages were removed and packed.

A control group comprising the same number of male and female flies was similarly allowed to feed on an uninfected goat. The females were mated when 3 days old with 8-day old males as described previously. As the developmental cycle of *T. vivax* in most tsetse vector is completed by day 25, flies were fed daily, on the respective goats for 22 days.

PLATE 5.

Tsetse flies strapped on shaved flanks of a goat.



Isolation of infected tsetse flies

The tsetse were not fed for 2 days and on day 25 post-emergence, these hungry flies were induced to extrude saliva on warmed slides (Burtt, 1946) as follows: Flies were removed from the cages using a long glass tubing with netting at one end and put singly in individual glass tubes which were corked. The flies in the tubes were placed singly on the warmed Wellcome slides and the tube moved to induce the fly to probe on a single clear disc (Plate 6). The slides were warmed an a glass plate at 37°C. The flies in glass tubes were labelled to correspond with the wells on the slides on which an individual fly extruded saliva.

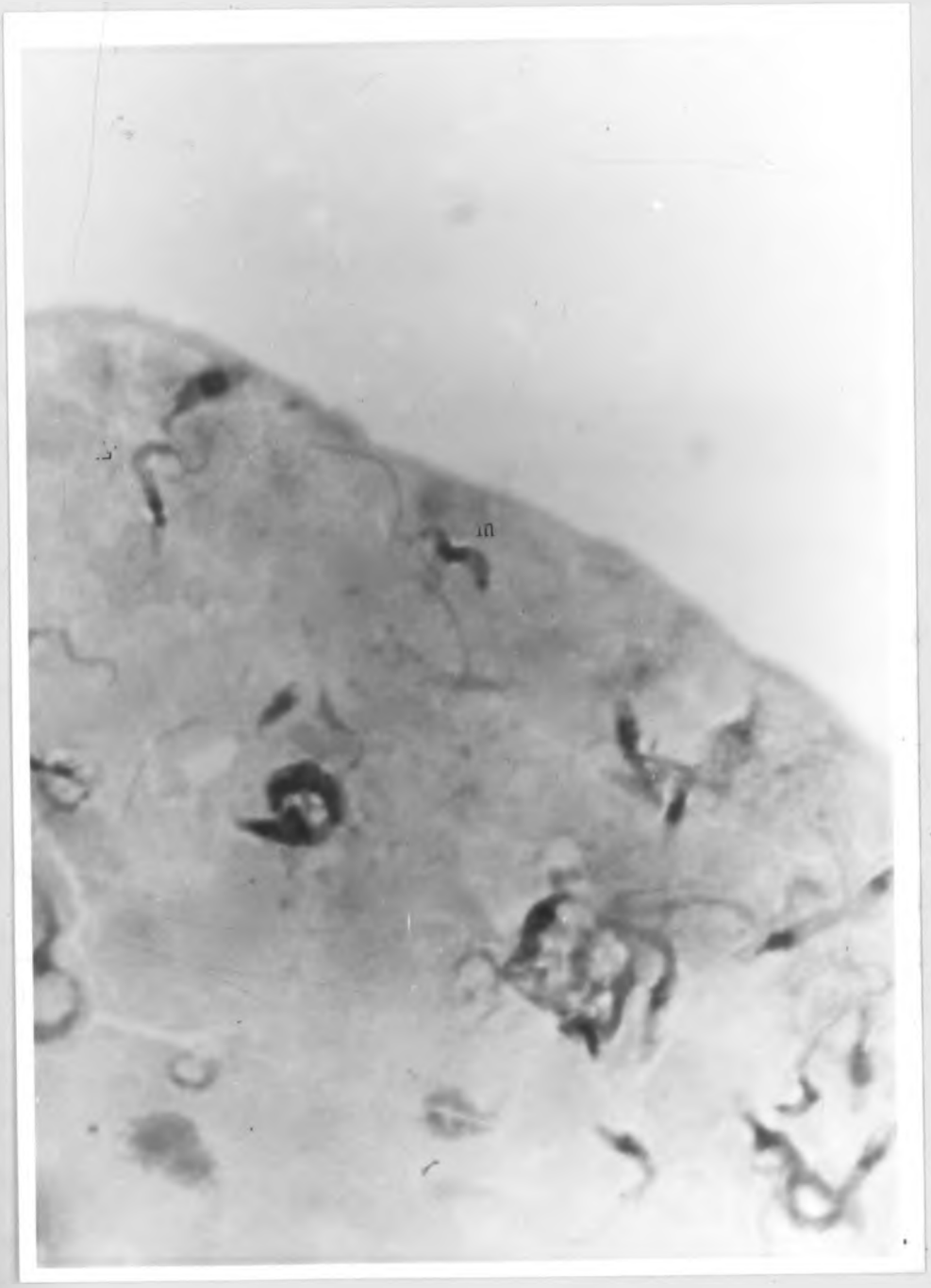
The saliva extruded by the flies into the wells was examined for parasites at 320X magnification under phase-contrast microscopy. The parasites were classified as 'metacyclic'-like and/or "longforms" based on their morphology (Plate 7). Flies with mature infection (i.e. 'metacyclic'-like) were identified and labelled accordingly. The control flies were similarly handled and subjected to the above treatment.

PLATE 6.

Isolation of infected tsetse flies by inducing them to extrude saliva on warmed Wellcome slide.

PLATE 7.

Extruded saliva with coated metacyclics (m) and uncoated long forms (L) from infected tsetse flies (614X)



Infection Rates

Flies that did not probe on the warmed slides and those that did not extrude any parasites were dissected to determine the actual infection rate. The proboscis was removed with sharp dissecting pins and placed on a clean microscope slide with a drop of phosphate-buffered saline (PBS), pH 7.4. The labrum and hypopharynx were separated using sharpened dissecting pins under a dissecting microscope (Wild-Heerbrugg). These were covered with a cover slip and examined for trypanosomes at 320X magnification. The tsetse flies were recorded to be infected if trypanosmes were observed in the labrum and/or hypopharynx (Plate 8 and 9).

2:3 RESULTS

2:3:1 A colony of Glossina palpalis gambiensis

Figure 2 shows the performance of the main colony with mortality of the mated females about 20 per week. The stock was maintained between 1500 and 1700 females between week 3 and 11 but was increased to 1800 females in week 12 when the demand for experimental adult flies increased. The production i.e. pupae produced was good with an average 4900 pupae per month. The mean monthly teneral emergences was 4500 which is 93.7% of the total

PLATE 8.

Infected labrum of G.p. gambiensis with trypansomes (T) attached on the lining of the labrum (614X)

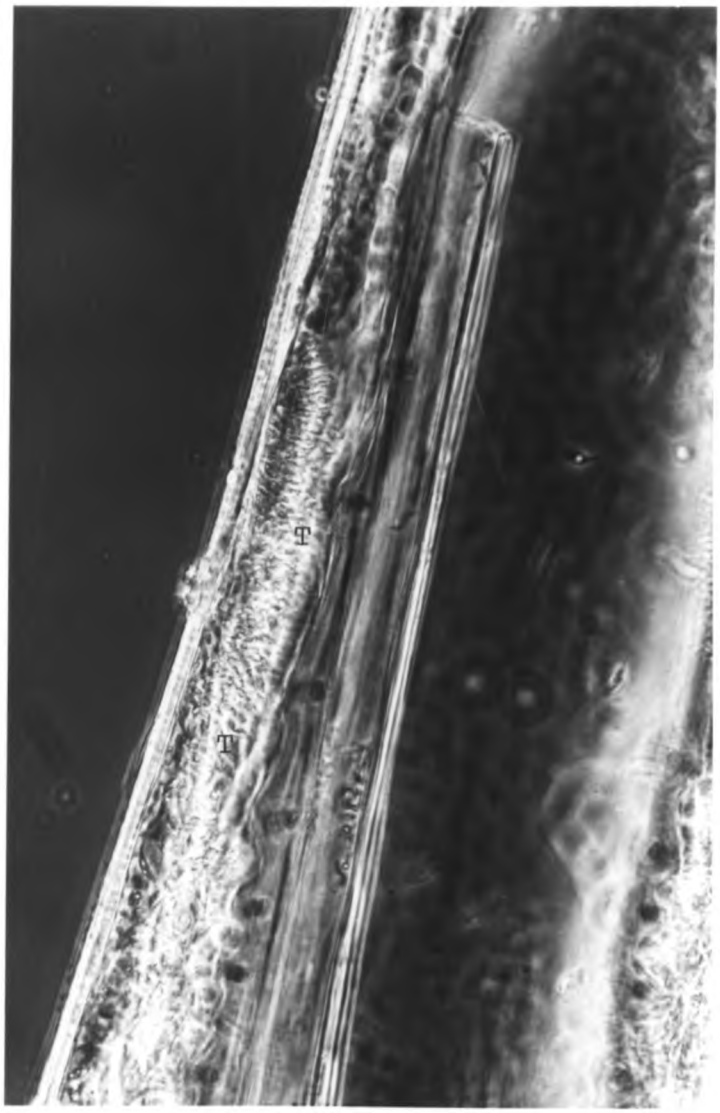


PLATE 9.

Infected hypopharynx showing metacyclic trypanosomes (614X)

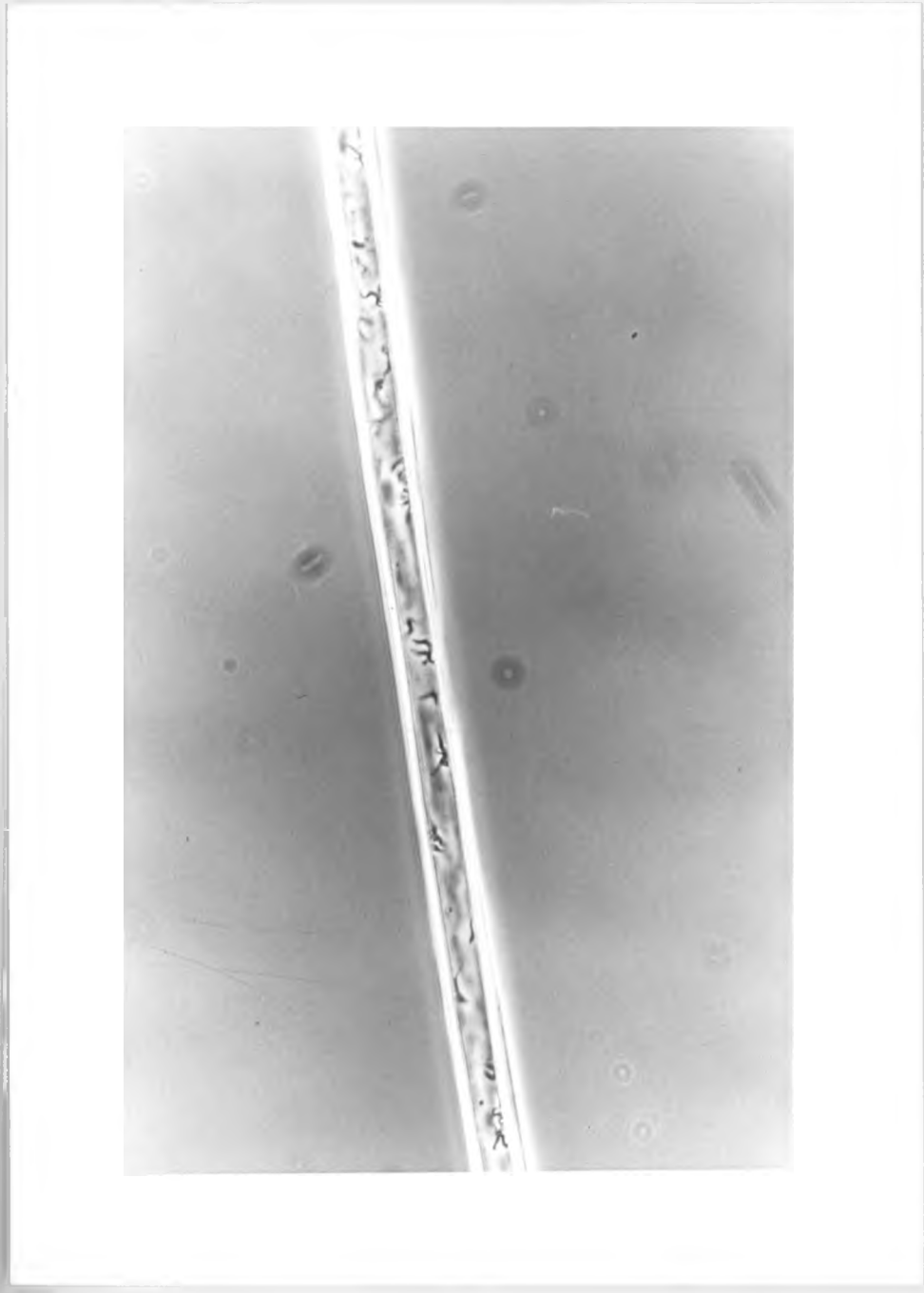
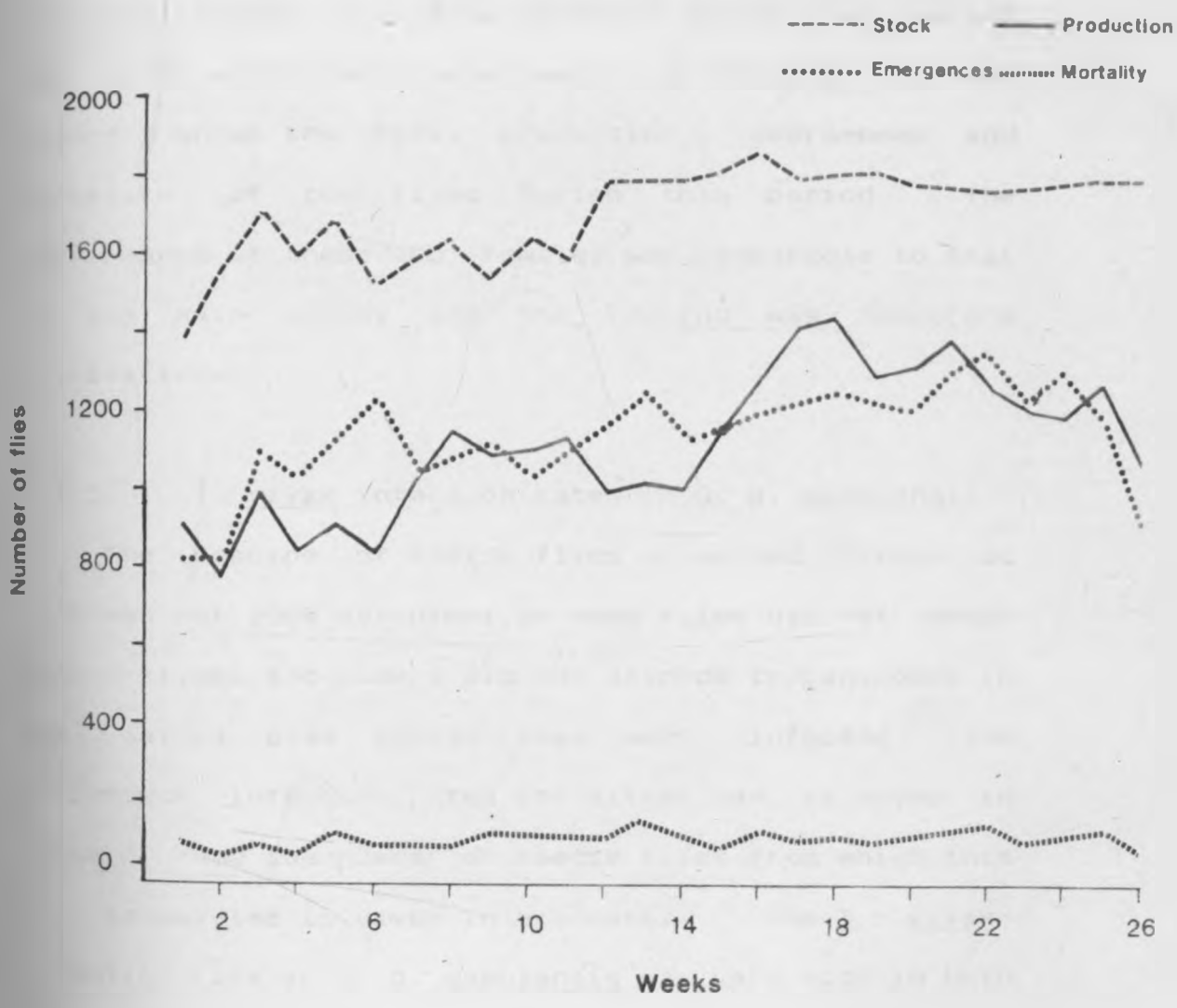


Fig. 2: Performance of the main colony of G. p. gambiensis



production.The results indicate that the performance of the flies in terms of female survival and fecundity and weight of puparia produced has been consistently good.

Table 2 shows the performance of a group of 200 mated female G. p. gambiensis maintained for 90 days. The total number of puparia produced during this period was 1,550 with a mean pupal weight of 29.19 ± 0.2 mg. Figure 3 shows the stock, production , emergences and mortality of the flies during this period. The performance of these 200 females was comparable to that of the main colony and the rearing was therefore satisfactory.

2:3:2 T. vivax infection rates in G. p. gambiensis

The probing of tsetse flies on warmed slides at 37°C was not 100% efficient as some flies did not probe on the slides and others did not extrude trypanosomes in the saliva even though they were infected. The percentage infection rates for either sex is given in Table 3, and the number of tsetse flies from which this was calculated is given in brackets. The T. vivax-infection rate in G. p. gambiensis was very high in both male and female flies. The infection rates in males were 87.6% in group I, 95% in group II and 87.9% in group III. The corresponding infection rates in females were 92.8, 90.9 and 90.8% respectively. The mean infection rate was 90.2% in males and 91.5% in

Table 2: Performance of 200 mated female G. p. gambiensis for 90 days

Initial number of females	Potential no.* of reproductive cycles/ initial female	Mean no. of puparia/ initial female	Mean no. of puparia/ reproductive cycles	Mean puparial weights (mg±S.E)
200	7.1	7.8	1.0	29.19±0.2

* The mean number of 9 day reproductive cycles for which each female was alive between day 16 and 90 (maximal number of cycles assuming 100% survival is 8.3 for 9 age groups).

Fig. 3 : Performance of 200 female G. p. gambiensis

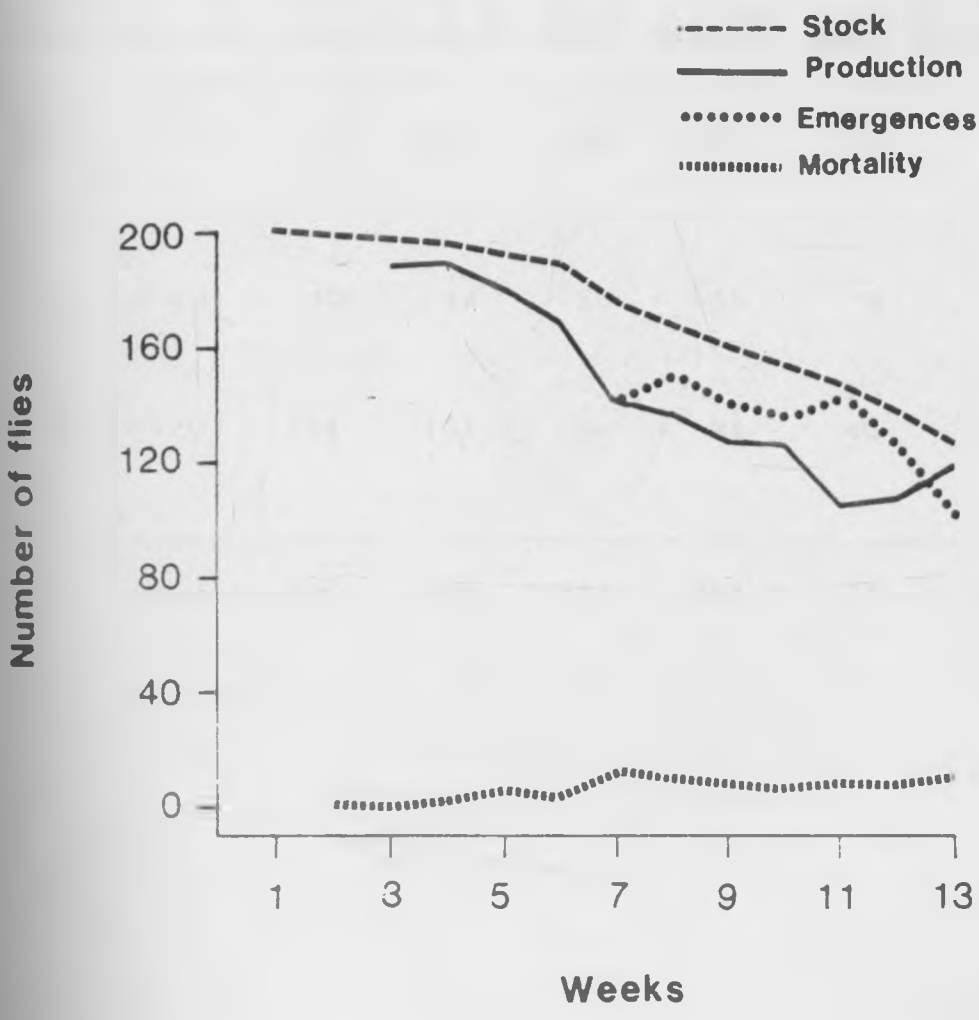


Table 3: *P. vivax* infection rates in *G. p. gambiensis*

Group	Number Infected		Number Surviving		Number infected as determined by Warm Slide		Probe		Dissection		Infection rate (%)	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
I	200	200	169	181	120	138	28	30	87.6	92.8		
II	60	60	40	44	33	35	5	5	95.0	90.9		
III	178	170	141	131	84	91	40	28	87.9	90.8		
Total	438	430	350	356	237	264	73	63	90.2	91		

females. This compares with earlier results of 91.9% (Moloo et al. 1987) for this I. vivax stock . It is therefore evident that this species is highly susceptible to this stock of I. vivax. Moloo et al (1987) suggested that successful establishment of I. vivax-infection in a particular tsetse species depended on the biochemical characteristics of its attachment sites in the food canal and the efficiency of bloodstream trypomastigotes of a particular stock to attach and undergo complete development to metatrypanosomes in the hypopharynx of the vector. These characteristics of the tsetse vector and the I. vivax parasites used could be genetically determined. Such characteristics could have contributed to the high I. vivax-infection rates in this species.

2:4 Discussion

The mass rearing of Glossina species is important when large number of adults are required for experimental purposes. The sterile insect release (SIR) for control of tsetse flies also requires mass production of sterile males. The in vitro feeding technique (Mews et al.. 1977) is easier and allows a large number of flies to be fed at a time. The major problem with this method is contamination of blood during preparation. The blood has to be prepared under

sterile conditions, and the plate and membrane sterilised before the flies are allowed to feed. The *in vivo* feeding technique for mass production of flies (Nash *et al.*, 1958) involves strapping the fly cages on a living host. Only a few cages can be strapped at a time and therefore a large number of hosts must be maintained which can be expensive in terms of personnel and maintenance of the animals.

The technique used in the current study is adequate for mass production of tsetse flies. The performance of the colony in terms of female survival and fecundity and weight of puparia was consistently good. These factors reflect the nutritional state of the pregnant females and are a measure of the efficiency of the rearing technique.

The major problem in attempts to rear tsetse is insecticidal contamination, which can be lethal even in minute quantities. To overcome postulated contamination, staff working in the laboratory had to wash and wear special clothing before entry into the holding rooms. Predatory ants are another threat to colony maintenance. Metal cups with glycerol were therefore attached to trolley legs to prevent predatory ants from getting to the fly cages. Disturbance of tsetse during feeding was minimised by covering the cages with a black cotton cloth.

The factors influencing development of Salivarian

trypanosomes in Glossina have been discussed by Molyneux (1977) who listed them as:- (1) Endogenous factors associated with the fly, (2) Ecological factors and (3) parasite and host. Rickettsia-like organisms (RLOs) have been associated with susceptiblity of Glossina to infections with various trypanosomes (Maudlin and Ellis, 1985) but this was not observed for T. vivax (Moloo et al., 1987). It has been suggested that the infection rate is determined by, among other factors, the characteristics of the tsetse vectors and parasites which are genetically inherited (Moloo et al., 1987). Lectin-like molecules have been suggested to play an important role in parasite attachment and differentiation (Pereira, Adrade and Ribeiro, 1981; Ibrahim, Ingram and Molyneux, 1984) which is a prerequisite for trypanosome development in the vector.

CHAPTER THREE

EFFECT OF TRYPANOSOMA (DUTTONELLA) VIVAX ON THE FEEDING BEHAVIOUR, SURVIVAL AND REPRODUCTIVE PERFORMANCE OF GLOSSINA PALPALIS GAMBIENSIS

3:1 Introduction

The development of T. vivax in Glossina species is confined to the proboscis whereas T. congolense and T. b. brucei initially develop in the midgut and then invade respectively, the mouthparts and the salivary glands where they complete development to the infective forms (Bruce et al, 1910, 1911; Lloyd and Johnson, 1924; Hoare, 1972). The T. vivax bloodstream trypomastigotes are ingested with blood and some attach to the walls of the labrum where they divide to form rosettes of short epimastigotes. Some of these invade the hypopharynx where they transform to metacyclics and acquire a surface coat (Gardiner et al, 1986). These are the infective forms and are introduced with saliva into the skin when a tsetse fly feeds on a host.

Rice et al (1973) proposed a relationship between labral-mechanoreceptive sensilla and the developing trypanosomes in Glossina austeni and suggested that they may influence the feeding behaviour of the infected flies. This association of trypanosomes and the

sensilla was also described by Molyneux and Lavin (1979) in G. morsitans infected with T. congolense. Similar associations were shown by Jenni et al (1980) in G. m. morsitans infected with T. brucei who suggested that infection may impair the function of the sensilla. Thus the infected flies fed more voraciously and took longer to engorge than the uninfected ones. This contention was however disputed by other workers (Moloo, 1983; Moloo and Dar, 1985) who presented evidence to show no differences in feeding behaviour between G. m. morsitans infected with T. b. brucei, T. congolense or T. vivax and uninfected controls.

The feeding process of tsetse flies begins with the eversion of the haustellum on contact with a suitable host which then pierces the skin. It is known that "the fly never feeds after the first piercing, but partly withdraws the haustellum, moves its head and thrusts it deeply in a fresh direction" (Gordon et al, 1948). The insect may withdraw the haustellum, walk a short distance and again pierce the skin (Gordon et al, 1948a) which may be repeated several times before the fly begins to ingest blood. This is termed "probing" and Dethier (1954) observed that flies could be induced to probe on quite unsuitable surfaces. The important requirement seemed to be temperature which must be above ambient. Roberts (1981) compared the rate of transmission when G. morsitans infected with

T. congolense probed consecutively and when they completed feeding on mice. He reported that transmission occurred in both situations but the rate was higher during consecutive probings than when they completed feeding. Fairbairn and Burtt (1946) also showed that T. rhodesiense could be transmitted when infected flies probed on man.

In his transmission experiments, Duke (1928a) investigated the effect of T. rhodesiense and T. b. brucei on the infected flies. He concluded that the presence in its intestine of developing forms of polymorphic trypanosomes was not to any noticeable extent injurious to the fly. These findings were supported by Baker and Robertson (1957) who showed that G. morsitans infected with T. rhodesiense or T. b. brucei lived slightly longer than the uninfected flies. They suggested that the trypanosomes exerted some favourable influence to the flies.

Mechanical transmission of T. b. gambiensis by G. palpalis in the laboratory was demonstrated by Dutton et al., (1907). Bailey (1966) showed that T. b. rhodesiense could be mechanically transmitted by G. morsitans. Wells (1972) reviewed literature on mechanical transmission of trypanosomes by Glossina and other biting flies. He concluded that mechanical transmmision is more widespread than presently acknowledged.

3:2 Materials and Methods

3:2:1 Feeding behaviour of *Glossina palpalis gambiensis*

Male and female flies infected with *T. vivax*, in batches of 12 each, and the same number of uninfected ones, were placed into individual small glass tubes with netting at one end. The tubes were capped and numbered. Every morning the flies were allowed to feed on cleaned ears of half-lop rabbits (Plate 10). The ears were cleaned with Kleenex paper tissue soaked with 70% alcohol and then dried with dry cotton wool. The flies were fed every day, the feeding being alternated between two rabbits to subject them to the same treatment. The following parameters were recorded for each tsetse fly:

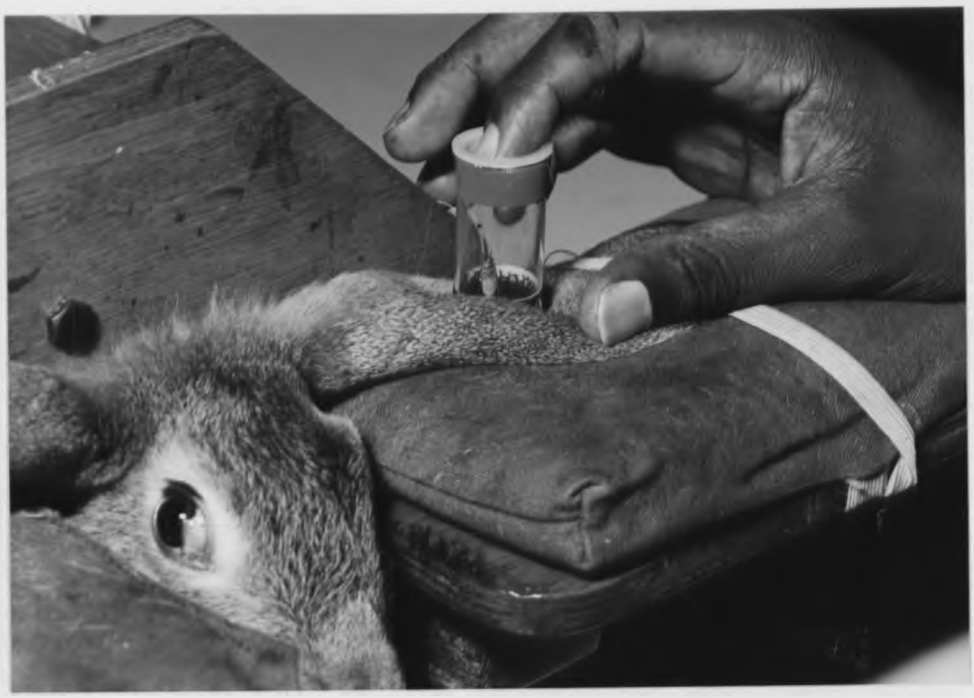
1 Number of probes: The number of times the fly inserted the proboscis and penetrated the skin of host were counted and recorded.

2 Weight (in mg) of bloodmeal ingested by weighing each fly before and immediately after engorgement.

3 Duration of bloodmeal engorgement: The time taken (in seconds) by individual flies to engorge was recorded using a stop watch. This was taken as the time the fly started to feed to the time it withdrew the proboscis from the skin of the host.

PLATE 10.

Tsetse fly in a small glass tube being induced to probe on an ear half lop rabbit



Hunger Cycle

This was defined as the period between feeds. In order to determine any differences in feeding behaviour when flies fed every day, T. vivax-infected tsetse in batches of 12 of either sex were allowed to feed at three-day intervals on ears of rabbits and the same parameters as described above recorded.

Probing by infected male and female tsetse flies in relation to transmission of infection

Tsetse flies infected with T. vivax were placed into individual clear plastic tubes and numbered. Each fly was placed on a flank of a goat and left to feed. The fly was removed after a single probe and transferred onto a second goat where it was again left to feed and interrupted after a single probe. The fly was then allowed to probe and feed to repletion on the third goat. The experiment was similarly repeated with female tsetse flies and the goats on which they probed or fed marked. The goats were bled daily and the unstained thin wet blood film and buffy coat examined for parasites as previously described.

3:2:2 Mechanical transmission of *T. vivax* by *G. p. gambiensis*

A goat was infected intramuscularly (i.m.) with *T. vivax* as previously described. A wet blood film and PCV of the goat were examined daily. When trypanosomes were first detected in the buffy coat, (i.e. HCT positive) a male fly in a glass tube was allowed to feed on the shaved flank of the infected goat. The feeding was interrupted and the fly transferred on to the shaved flank of an uninfected goat where it was left to feed to repletion.

The experiment was repeated with other flies when the number of parasites observed in a thin wet blood film under a microscope field at 400X was:-

i) 1 parasite/20 fields

ii) 1 parasite/field

iii) 15 parasites/field and

iv) 30 parasites/field.

In a repeat experiment male and female flies were allowed to feed on a goat when the parasitaemia was, i) 5 ii) 10 and iii) 30 parasites/field, and the feeding interrupted as described above. Each fly was transferred on to an individual uninfected goat. The flies were left to feed on the new hosts to repletion. The goats were examined daily for parasites as described above for 20 consecutive days. The goats were thereafter

examined every five days, if not infected up to day 40. The prepatent period of the infection in goats were recorded.

3:2:3 Survival and reproductive performance of infected tsetse flies

Male and female G. p. gambiensis with mature infections were put separately in Gelgy-20 cages. An equal number of uninfected flies of each sex were similarly kept as controls. The test and control flies were kept in separate trays in the holding room and fed daily on rabbits. The rabbits were bled daily from a peripheral ear vein and the blood examined for trypanosomes as previously described. The rabbits were changed if found to be infected to prevent infection of the control flies. The cages were examined daily for dead flies which were removed from the cages, counted and recorded. The dead flies were dissected and the mouthparts examined for parasites. The number of infected and uninfected flies of each sex were recorded every day till all died.

The pupae produced in each group were weighed individually. The mean pupal weights were calculated from the total number of pupae produced.

3:3 Results

3:3:1 Feeding behaviour of G. p. gambiensis infected with T. vivax

The data recorded for 20 days for each tsetse fly (See Appendix I) were used to calculate the means of the following parameters:-

1 Mean number of feeds taken during 20 consecutive days by the infected and the uninfected tsetse flies.

2 Mean rate of bloodmeal intake (in ug/sec.) of the total days fed.

3 Mean weight of bloodmeal intake (mg)/day of the total days fed.

4 Mean total bloodmeal engorgement during 20 days.

5 Mean number of probes/day during the days fed.

6 Mean frequency of the multiple probe feeds.

7 Mean number of pre-feeding probes of the multiple probe feeds.

Means of the means of the experimental and control groups were compared for differences using two-tailed Students' t-test (Snedecor and Cochran, 1967). The results are given in Table 4.

Table 4

Feeding behaviour of G. p. gambiensis infected with T. vivax in 20 consecutive days

	1*		2		3		4		5		6		7	
	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected	Unifected	Infected	Uninfected	Infected	Uninfected
Male														
$\bar{x}$±SE	14.9±0.9	15.4±0.7	340.0±23	344.0±20	10.8±0.7	11.6±1.0	157.3±9.6	173.8±13	1.3±0.1	1.3±0.04	5.2±1.5	5.1±1.2	2.0±0.3	1.8±0.3
DF	22		22		22		22		22		22		9	
t	0.433		0.133		0.615		1.034		0.377		0.133		0.517	
P	>0.05		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05	
Female														
$\bar{x}$±SE	13.2±0.9	13.7±0.7	547.2±33	513.6±27	33.3±2.1	42.2±1.3	437.1±38	596.0±23	1.3±0.04	1.3±0.1	4.0±0.9	5.3±0.2	1.8±0.2	1.8±0.2
DF	21		21		22		21		21		21		9	
t	0.436		0.794		3.622		3.037		0.141		0.726		0.186	
P	>0.05		>0.05		<0.05		<0.05		>0.05		>0.05		>0.05	

* The numbers 1-7 in the table are the parameters measured as listed below

1. Mean number of feeds taken during 20 consecutive days by infected and uninfected tsetse.
2. Mean rate of bloodmeal intake (in µg/sec) of the total days fed.
3. Mean weight of bloodmeal intake (mg)/day of total days fed.
4. Mean total bloodmeal engorgement during 20 days.
5. Mean number of probes/day during the days fed.
6. Mean frequency of the multiple probe feeds.
7. Mean number of pre-feeding probes of the multiple probe feeds.

The total blood meal engorgement during the days fed by the control flies was higher (569.8±23) than for the infected flies (437.1±38.2 mg). The mean weight of blood meal intake by the uninfected female flies was therefore higher (42.2±1.3 mg) than for the infected flies (33.3±2.1 mg). All the other parameters i.e. mean number of feeds, rate of bloodmeal intake, frequency of the multiple probe feeds and number of pre-feeding probes were not significantly different for the infected and uninfected females at the 5% level ($P<0.05$) (Table 4).

There were no significant differences for the mean weight and total bloodmeal ingested by infected and uninfected male flies. Therefore the differences for the mean and the total weight of blood ingested by the females are not in themselves alone conclusive. There were no significant differences in the mean number of feeds, rate of bloodmeal intake, number of probes per day, frequency of multiple probe feeds and number of non-feeding probes between the infected and uninfected male flies. Thus the observed differences in the females could be due to chance. These results suggest that there were no significant differences in feeding behaviour when flies were fed for 20 consecutive days. *P. vivax* infection did not therefore affect the feeding behaviour of this species in terms of number of feeds, mean weight and total bloodmeal engorged, rate of

bloodmeal intake, total number of probes, number of multiple probes and non-feeding probes.

Female flies ingested about 30.2% more blood of that taken by the males and the rate of bloodmeal intake by the female flies was also higher than in males. The mean number of feeds, mean number of probes/day and the frequency of the multiple probe feeds were not significantly different between the two sexes.

Hunger cycle of tsetse flies

In the previous experiment the flies were fed for 20 consecutive days. It was observed that not all flies fed every day and therefore the maximum number of days a tsetse fly took without a blood meal, i.e. period from feed to feed were calculated from the above results. This was termed the "hunger cycle" and was found to be three days. The means of the parameters listed above were compared for differences and the results are given in Table 5. There were no significant differences between the infected and the uninfected flies in all the parameters studied. The results tally with earlier ones when flies were fed for 20 consecutive days. As there were no differences in any of the parameters when the hungry flies were fed at three-day intervals. the observed differences in the females in the mean weight and total bloodmeal engorgement when they were fed for

Table 5

Feeding behaviour of Glossina palpalis gambiensis infected with T. vivax when allowed to feed at three-day intervals

	1*		2		3		4		5		6		7	
	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected	Infected	Uninfect
Male														
$\bar{x}$±SE	7.7±0.2	7.5±0.2	497.2±21.1	468.4±21.1	19.1±1.1	19.6±1.0	146.3±10.1	145.6±8.0	1.3±0.1	1.3±0.1	4.1±0.6	4.3±0.5	1.2±0.1	1.0±0.03
DF	21		22		22		22		22		19		19	
t	0.787		0.954		0.409		0.056		0.294		0.220		1.297	
P	>0.05		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05	
	N.S.		N.S.		N.S.		N.S.		N.S.		N.S.		N.S.	
Female														
$\bar{x}$±SE	7.6±0.2	7.7±0.2	638.6±52.8	689.9±50.6	47.1±3.7	50.4±1.6	356.3±29.7	385.5±15.1	1.4±0.1	1.4±0.1	5.3±1.0	5.9±1.4	1.5±0.2	1.1±0.1
DF	16		16		16		16		16		13		13	
t	0.326		0.702		0.821		0.877		0.281		0.391		1.287	
P	>0.05		>0.05		>0.05		>0.05		>0.05		>0.05		>0.05	
	N.S.		N.S.		N.S.		N.S.		N.S.		N.S.		N.S.	

* Parameters 1 - 7 listed below N.S. - Not significantly different

1. Mean number of feeds taken during 20 consecutive days by infected and uninfected tsetse.
2. Mean rate of bloodmeal intake in µg/sec of the total days fed.
3. Mean weight of bloodmeal intake (mg)/day of total days fed.
4. Mean total bloodmeal engorgement during 20 days.
5. Mean number of probes/day during the days fed.
6. Mean frequency of the multiple probe feeds.
7. Mean number of pre-feeding probes of the multiple probe feeds.

20 consecutive days could be due to chance. The observed differences are not supported by any other parameter when flies were fed at three-day intervals. This suggests that the presence of T. vivax in the labrum and hypopharynx of G. p. gambiensis did not in any way affect the feeding behaviour of this species when allowed to feed for 20 consecutive days and also at three-day intervals.

Probing by infected male and female tsetse flies in realation to transmission of infection

Table 6 shows the number of goats challenged with each tsetse fly, transmission of infection and the prepatent period of infection in goats. Two male flies probed consecutively on four goats all of which became infected. Two male flies were allowed to probe and complete feeding on two goats one of which became infected. The mean prepatent period was 9.6 days. Four female flies probed consecutively on four goats while two others completed feeding on two different goats. All the goats became infected and the mean prepatent period was 10.2 days.

It is clear from these results that probing by infected tsetse during feeding can result in transmission of infection to different goats.

Table 6: Transmission of T. vivax to goats by infected male and female tsetse

Sex of tsetse	Total No. of goats challenged	Transmission 1	2	3	Prepatent period (days) 1	2	3
Male:							
1.	3	+	+	+	10	9	9
2.	3	+	+	-	10	10	-
Female:							
1.	3	+	+	+	8	10	10
2.	3	+	+	+	11	12	11

Key:

1 - 1st Probe

2 - 2nd Probe

3 - Feed

Transmission rates were similar when flies probed consecutively and when they ingested blood. One out of the two goats on which the male flies completed feeding was not infected. However, all the female flies transmitted infection to goats by consecutive probing and also when they completed feeding on the hosts. One goat on which male tsetse completed feeding did not become infected. It could be that the fly had extruded more or all mature metacyclics from the hypopharynx into the skin during previous probing or the extruded metacyclics were too few or uncoated to initiate the infection in the host. This is supported by the observation of fewer metacyclics in the hypopharynx of different tsetse flies dissected immediately after than in those examined before feeding. It is therefore evident that a large proportion of the metacyclics in the hypopharynx are inoculated into the host when infected tsetse flies feed.

3:3:2 Mechanical Transmission of *T. vivax*

Table 7 gives the parasitaemia of the infected goat at the time when the flies used to challenge uninfected ones were allowed to feed, the number and the prepatent period of the infection in goats. The results show that none of the flies which was interrupted when feeding on a goat at low parasitaemia transmitted the infection.

Table 7: Goats infected when 'challenged' with tsetse interrupted while feeding on an infected goat at different levels of parasitaemia

Sex of tsetse	Number of parasites/F at time of feed by tsetse	No. of goats used	Infection	Prepatent period (days)
M	5/F	2	-	-
F	5/F	2	-	-
M	10/F	2	-	-
F	10/F	2	-	-
M	30/F	2	+	14
F	30/F	2	-	-

One fly interrupted when feeding on a goat with a high parasitaemia, (30/F), transmitted the infection to another goat. The prepatent period was 14 days. The flies were interrupted when they had ingested a small amount of blood and it is therefore possible that trypanosomes were picked up with blood and inoculated directly to the next host. Thus G. p. gambiensis transmitted T. vivax directly to goats when the feeding was interrupted at high parasitaemia.

3:3:3 Survival and Reproductive performance of infected tsetse flies

Table 8 shows the average length of life for the infected and uninfected flies and the range of length of life in days. The average length of life for the infected male and female flies were 85.3 and 98.4 days respectively. The corresponding values for the uninfected flies were 68.7 and 106.7 days. The mean survival in days for the infected males is significantly greater than for the uninfected ones.

Figures 4 and 5 show the survival of the infected and the uninfected flies against time in days. The survival curves for all the flies show that rate of mortality was generally increasing with age. In both the infected and the uninfected male flies, 50% died within the first 65 days (Fig. 4). This was different

Table 8: Survival of infected and uninfected male and female G. p. gambiensis

	Number of flies		Average length of life in days		Range of length of life in days	
	Male	Female	Male	Female	Male	Female
Infected	88	100	85.3	98.4	29-186	26-205
Uninfected	88	100	68.7	106.7	27-160	28-196

Fig. 4 : The survival curves for infected and uninfected male G. p. gambiensis

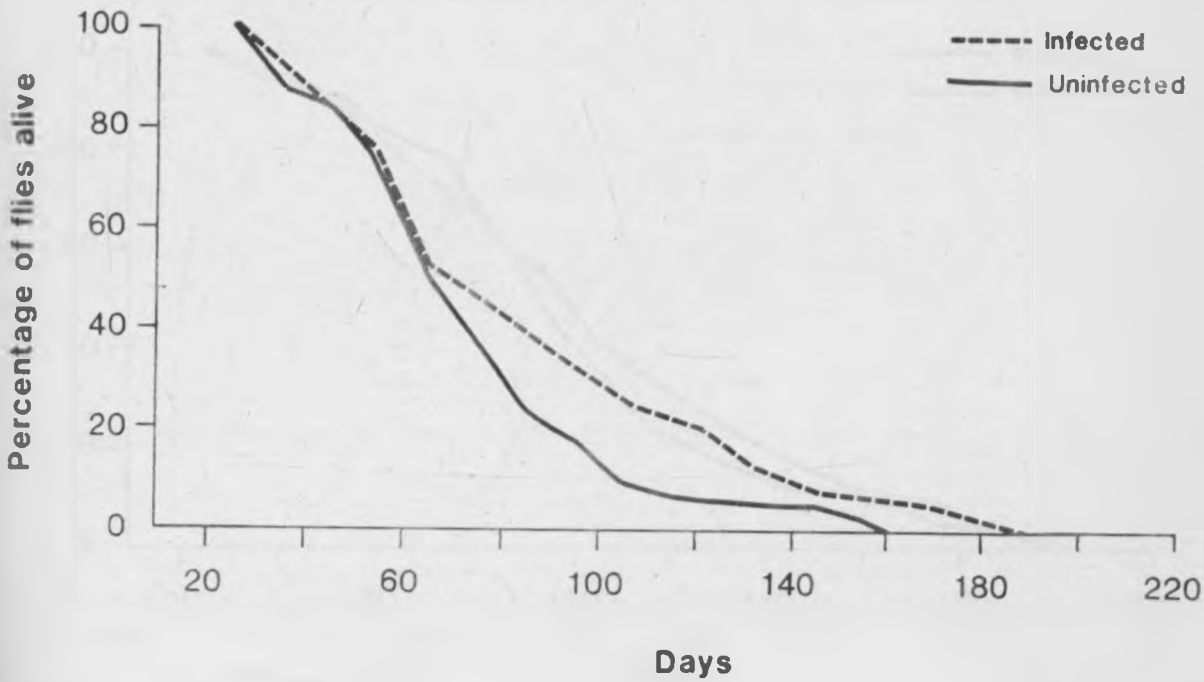
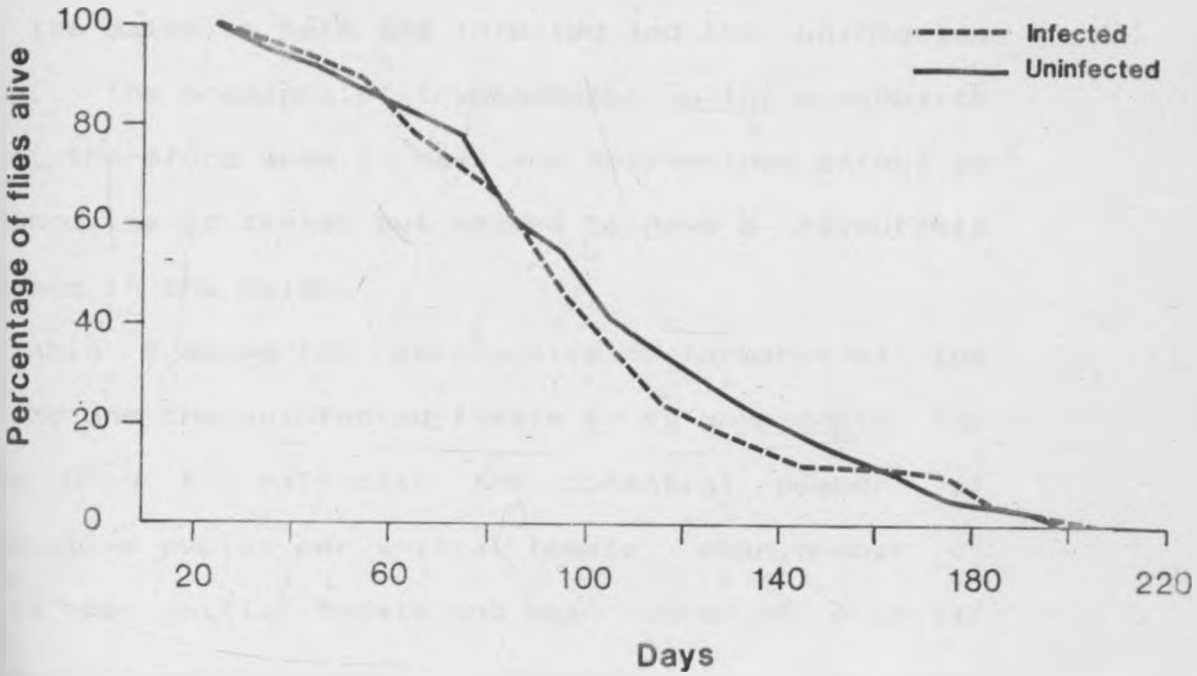


Fig. 5: The survival curves for infected and uninfected female G. p. gambiensis



in females where 50% of the infected and the uninfected died within the first 90 and 100 days respectively, i.e. the females had a longer mean survival than the males (Fig. 5). A few of the infected and the uninfected male and female flies in both cases survived for a long time.

Figure 6 shows the large variations in the length of life of individual flies. Infected male flies lived longer than the uninfected ones, but this was not apparent in the females. The female flies lived longer than the males in both the infected and the uninfected groups. The presence of trypanosomes in the mouthparts did not therefore seem to have any deleterious effect on the longevity of tsetse but seemed to have a favourable influence in the males.

Table 9 shows the reproductive performance of the infected and the uninfected female G. p. gambiensis. The method used to calculate the potential number of reproductive cycles per initial female, mean number of puparia per initial female and mean number of puparia/ reproductive cycle is given in Appendix II. The mean pupal weights were calculated from the weights of all pupae produced during the study on the longevity of the flies. The mean pupal weights for the infected and the uninfected tsetse flies were not significantly different at 5% level. Results show that the potential number of reproductive cycles per initial female, the mean number of puparia/initial female and mean number of

Fig. 6 Days on which individual flies died during the period of the study

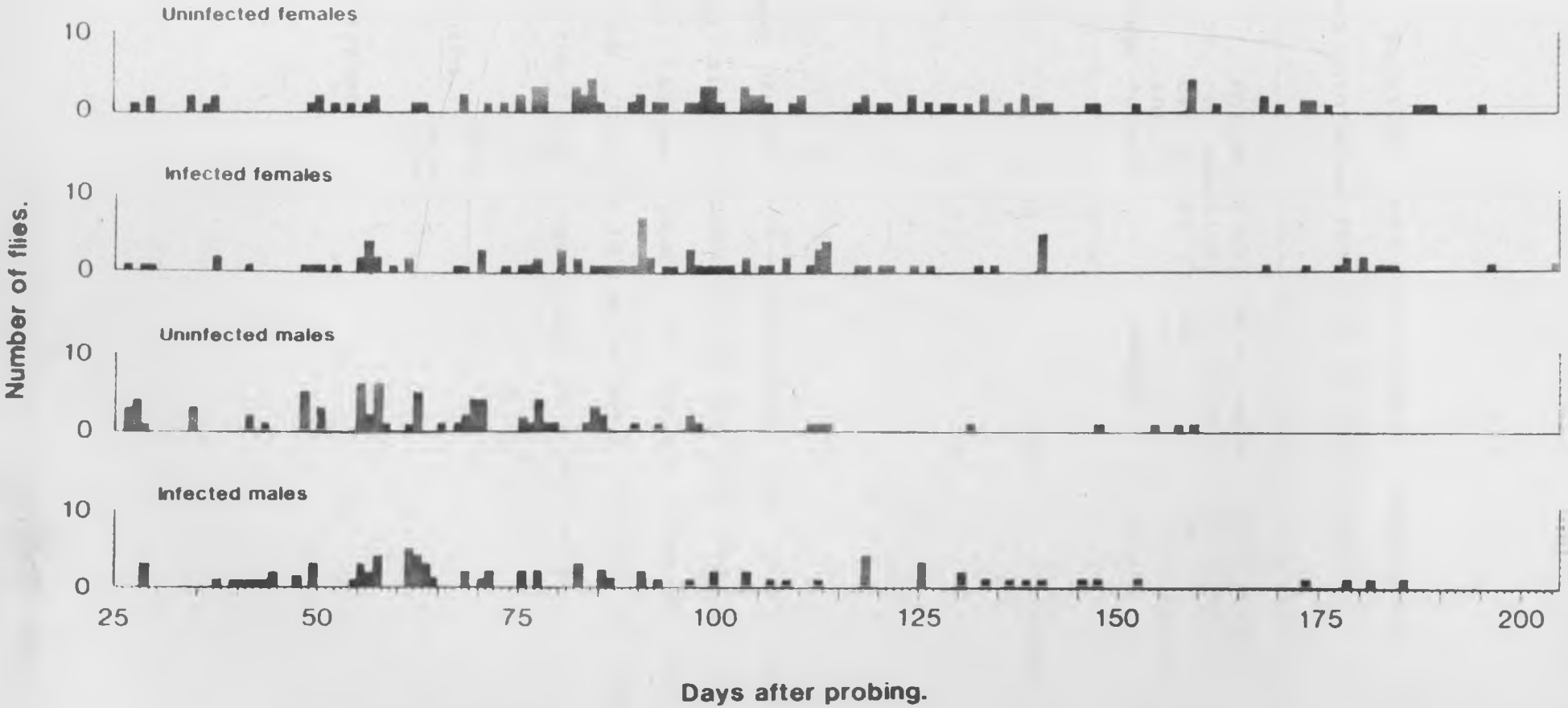


Table 9: Survival and reproductive performance of infected and uninfected female G. p. gambiensis

	Initial Number of females	Number of age groups completed	Potential No. reproductive cycles per initial female*	Mean number of puparia per initial female	Mean Number of puparia per reproductive cycle	Mean puparial weight (mg±S.E)
1	100	18	7.03	6.35	0.90	24.65±0.5
2	100	18	7.57	6.28	0.83	25.33±0.3

Key:

* the mean number of 9 - day cycles for which each female was alive between day 16 and the end of the experiments (maximal number of cycles assuming 100% survival are 18.0 for 18 age groups i.e.flies of the same age. The first 3 age groups were excluded).

1 - Infected

2 - Uninfected

puparia per reproductive cycle were not different between the infected and the uninfected females. The weight of pupae produced by the infected and the uninfected flies was however lower than that of tsetse in the colony (29.93±0.3 mg). Infection with T. vivax did not therefore affect the reproductive performance of the species as reflected by the mean pupal weight, mean number of puparia/female and mean number of puparia/ reproductive cycle.

3:4 Discussion

The present study has shown that T. vivax did not affect the feeding behaviour of Glossina palpalis gambiensis as measured by the number of feeds, mean weight and total bloodmeal engorgement, total number of probes and the non-feeding probes. Tsetse flies search for food in the wild when they are hungry (Ford, 1969) and Nash (1969) stated that the feeding frequency in the bush was normally three days for G. pallidipes. Tsetse flies ingest more than their own weight of blood (Bursell, 1970) and the act of feeding is itself quite rapid. These results show that the presence of trypanosomes in the labrum and hypopharynx did not affect the amount of bloodmeal, the rate of intake or the time taken by the fly to ingest blood. Thus the voracity of the flies was not affected by the presence

of trypanosomes in the labrum, i.e. the trypanosomes did not affect the feeding behaviour of this palpalis group of tsetse.

The results also demonstrate that probing behaviour by infected tsetse during feeding could result in transmission of infection. The failure to infect one goat on which an infected tsetse fed could have been caused by lack of metacyclics in the hypopharynx or they might have been too few to initiate infection. Very few mature metatrypanosomes were observed in the hypopharynx when flies were dissected immediately after a bloodmeal compared to those dissected before feeding. It was evident that most of the trypanosomes were injected with saliva during feeding. It was observed that all the goats were infected when female flies probed consecutively and when they completed feeding on others. These results suggest that transmission can occur both during probing and also when the infected tsetse flies feed on the host which is similar to observations by Roberts (1981) that T. congolense was transmitted by G. m. morsitans during consecutive probing.

The study also demonstrated that G. p. gambiensis can transmit this T. vivax stock directly (non-cyclically) to susceptible goats. One goat was infected when a male tsetse fly interrupted while feeding on a host with a high parasitaemia completed feeding on another goat. Thus the rate of direct transmission

would seem to be dependent on the parasitaemia of the animal at the time of the interrupted feed and also on the time interval before the next meal. This supports findings by Taylor (1930) who observed that G. morsitans directly transmitted T. b. gambiense and T. b. brucei. Gingrich et al. (1983) also showed that T. b. gambiense could be directly transmitted by G. morsitans and observed that mechanical transmission could be more widespread in the field. and suggested more investigation of the fly feeding behaviour and direct transmission.

Infection of the flies with T. vivax did not affect their survival. The vectorial efficiency of a vector is determined by the infection rate and the survival. However this may be influenced by its behaviour e.g. host-preference and daily activity. The longer the survival of infected tsetse, the more the possibility of transmitting the infection to susceptible hosts. As the infection does not shorten or lengthen the lifespan of the flies, it can be concluded that the possibility of transmission to new hosts with time is not affected by infection.

The reproductive performance of flies determines the density of tsetse which is an important factor in the epidemiology/epizootiology of trypanosomiasis. In the field the higher the fly density the greater the

possibility of picking up an infection and transmitting it to new hosts. Transmission of trypanosomes can be decreased by reducing the survival of infected flies. Transmission of trypanosomes has been interrupted by reducing fly density (ICIPE, 1987). Infection with trypanosomes did not affect the development of the intra-uterine larvae and therefore the fly density is not affected by infection. This may be the situation in the field where transmission is density-dependent.

CHAPTER FOUR

GENERAL DISCUSSION AND CONCLUSIONS

4:1 Discussion

In the present study, an adequate supply of adult tsetse flies was necessary; this was fulfilled by successful maintenance of a colony of G. p. gambiensis which provided surplus material for this study. The stock colony was maintained at approximately 1800 mated females with good overall performance. The performance of the 200 mated females kept for 90 days compared favourably with the main colony. The mean pupal weight (29.19±0.2) was similar to the weight (29.93±0.3) of the pupae in the main colony.

Infection rates of trypanosomes in tsetse flies is an important factor in the epidemiology/epizootiology of trypanosomiasis because the higher the infection rate the greater the possibility of transmitting the infection to new hosts. In these studies the high infection rate of T. vivax in G. p. gambiensis tallied with previous similar studies. This was attributed to the simpler mode of development in this vector. Bruce et al. (1910) showed that T. vivax development in tsetse flies is confined to the proboscis, and Vickerman (1973) demonstrated that the blood stream trypomastigotes

attached to the wall of the food canal and transformed to epimastigotes which multiplied at the foci of attachment to form rosettes of epimastigotes. Some of these invaded the hypopharynx and transformed into the infective coated metatrypanosomes (Lloyd & Johnson, 1924; Gardiner et al, 1986). The high infection rates implied that G. p. gambiensis could be an important vector of T. vivax. In view of the fact that this species is ecologically riverine, it is well positioned to afflict cattle brought in for watering and, if infected with T. vivax, transmission can take place. The rate of transmission would depend on the infection rate in the tsetse flies and the frequency of such cattle-fly contact.

The study revealed that high infection rates did not affect the feeding behaviour of the flies. However, similar studies by Jenni et al (1980) using G. m. centralis infected with T. b. brucei revealed a low vectorial efficiency. The difference could either be inherent in the species used or since flies fed on the belly of anaesthetised mice. It is possible that the tsetse flies inserted the proboscis into the peritoneum which caused difficulty in obtaining a bloodmeal resulting in higher probes. Earlier work by Moloo et al.

(1985) did not show any difference in probing frequency in mice between infected and uninfected G. m. centralis. The results were however higher for the infected and uninfected tsetse when fed on mice than when the flies probed on rabbits. It has also been shown that with T. b. brucei the epimastigotes were in the salivary glands (Lloyd and Johnson, 1924) and not in the labrum where LC1 mechanoreceptors were located.

The present study supports earlier work by Moloo (1983) working with G. m. morsitans who found no differences in feeding behaviour of tsetse flies infected with T. b. brucei, T. vivax or T. congolense. Thus the suggestion by Jenni et al. (1980) that infected flies fed more voraciously than uninfected is not supported in the present study for the palpalis group of tsetse flies.

Jenni et al. (1980) also suggested that the observed differences in rate of blood flow in the proboscis was caused by the close association of parasites and LC1 mechanoreceptors in the labrum and LC2 sensilla in the pharynx. These authors further urged that such an association may have a deleterious effect on the function of the sensilla resulting in impaired function of the sense organs. Vickerman (1973) showed that the epimastigotes were attached to the cuticular

lining of the labrum by flagellar hemidesmosomes and suggested that the parasites were swept posteriorly as blood rushes past during feeding. These observations confirmed that most of the trypanosomes were dislodged from the labrum of the infected tsetse flies and swept posteriorly into the midgut during feeding. The close association of trypanosomes and mechanoreceptors in the labrum was therefore possibly broken during feeding.

The present study has shown that consecutive probing by infected tsetse could result in transmission of trypanosomes. This suggests that a single probe by an infected tsetse fly could result in transmission of T. vivax. In feeding behaviour studies, infected male flies made a total of 92 different non-feeding probes while females probed 69 times. Therefore an equal number of transmissions would have been made by these flies if the feeding was interrupted and they completed feeding on new susceptible hosts. However, this would have been dependent on the presence of mature metacyclics in the hypopharynx. This was in agreement with Robert's (1981) suggestion that probing by infected flies could be an important factor in the spread of salivarian trypanosomes in endemic areas. He asserted that infected flies may transmit trypanosomes through consecutive probing if the feeding is interrupted as long as metacyclics are inoculated with the saliva during probing. However, his other

suggestion that the rate of transmission of T. congolense was greater when the infected flies probed consecutively than when they completed feeding was not supported by this study for T. vivax.

Feeding behaviour of tsetse flies is an important factor in the epidemiology/epizootiology of trypanosomiasis because it may influence transmission of trypanosomes. Since trypanosomes are transmitted when an infected tsetse feeds on a host, frequent feeding could result in more transmissions. Although the feeding behaviour was not affected by infection with T. vivax, trypanosomes were transmitted during consecutive probing by the infected tsetse. In the field where large herds of cattle are kept, sometimes in areas frequented by wild animals, a single infected tsetse may transmit infection to several hosts when its feeding is interrupted. This could explain the occurrence of epidemics in areas where tsetse populations are apparently low, and also the observed high infection rates of trypanosomes in mammalian hosts compared to infection rates in tsetse. If infected tsetse fed more voraciously and probed more frequently than uninfected, then in terms of evolution, this would reveal an advantageous adaptation in the transmission of trypanosomes.

It has been shown that G. p. gambiensis can transmit T. vivax directly (non-cyclically) if the feeding is interrupted when the donor host is highly parasitaemic. No transmission occurred at low parasitaemia. The feeding was interrupted when the fly had ingested a small amount of blood. It is therefore possible that a few or no bloodstream forms were taken up with the blood when the tsetse fly fed at low parasitaemia to contaminate the mouthparts. But this may have been possible at high parasitaemia. It is therefore possible that G. p. gambiensis could be involved in direct transmission of trypanosomes in the field. The close intermingling of heavily infected and non-infected hosts and frequently interrupted fly feeds in the field, may result in high mechanical transmission of trypanosomes. As few wild flies are infected with polymorphic trypanosomes even in the presence of trypanosomiasis, it may be that, as suggested by Duke (1923a), direct transmission may be responsible in part at least, for the very high prevalence of the disease encountered in some areas in the past. The importance of biting flies other than Glossina in direct transmission of trypanosomes is well documented. However, the importance of Glossina species in the mechanical transmission has not been evaluated in the field and may play some greater role than presently acknowledged and need to be studied further.

The observation that infected males lived significantly longer than uninfected controls agrees with earlier results by other workers (Duke, 1928a; Buxton, 1955; Baker and Robertson, 1957) who observed that infection seemed to exert some favourable influence on the life of tsetse. The longevity of tsetse is important in the field because once infected it normally remains so for its entire life. The chances of transmission of trypanosomes to susceptible hosts increases with increase in longevity of infected flies. Tsetse feed and hence transmit trypanosomes more than once in its life. It is known that tsetse can transmit infection throughout life to susceptible hosts but not at every feed (Molo, 1981). Increased longevity by infected tsetse would increase the risk of trypanosomiases. However, in the field tsetse are exposed to several hazards and may not therefore live as long as laboratory reared ones. The most important cause of death is predation and inability to find a suitable host.

One of the important factors in the epidemiology/ epizootiology of trypanosomiases is the tsetse-density. The greater the tsetse density the higher the risk of trypanosomiases as there is a higher possibility of a large proportion of tsetse picking up and subsequently transmitting the infection. Tsetse flies are viviparous in nature, i.e. produce larva at an advanced stage of

development which therefore increases its chance of survival. As infection did not affect the reproductive physiology in terms of nourishment to the intra-uterine larva, this suggested that trypanosomes did not have any effect on fly density in terms of fecundity and mortality. These results differed from studies by Townson (1971) and Kershaw et al (1954) who observed higher mortality in Brugia pahangi-infected Aedes aegypti and Loa loa-infected Chrysops species.

4:2 CONCLUSIONS

1. T. vivax did not affect the feeding behaviour of G. p. gambiensis. There were no significant differences between the infected and the uninfected flies in number of feeds, mean weight and total bloodmeal ingested, rate of bloodmeal intake and total number of probes.
2. Consecutive probing by infected tsetse flies during feeding resulted in transmission of T. vivax to susceptible goats. The rate of transmission was not different when the flies completed feeding on other goats.
3. G. p. gambiensis transmitted T. vivax directly (non-cyclically) to susceptible hosts when the feeding was interrupted at high parasitaemia.
4. Infection with this stock of T. vivax did not

affect the survival and reproductive performance of *G. p. gambiensis*.

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APPENDIX I

Mean number of feeds taken during 20 consecutive days by infected and uninfected *G. p. gambiensis*

Males		Females	
Infected	Uninfected	Infected	Uninfected
13	17	14	17
14	16	15	13
19	15	11	18
12	18	17	11
16	17	11	13
10	14	13	14
11	11	11	14
16	17	15	11
16	18	7	15
18	14	16	11
20	11	15	16
14	17		11
Mean ± SE 14.92±0.9	15.42±0.7	13.18±0.9	13.67±0.7

Mean rate of bloodmeal intake (in ug/sec.) during days fed by infected and uninfected G. p. gambiensis

	Males		Females	
	Infected	Uninfected	Infected	Uninfected
	364	423	745	506
	457	268	508	467
	351	299	558	585
	312	318	407	640
	227	313	562	453
	309	415	399	507
	312	472	663	498
	408	355	636	405
	280	350	540	614
	480	274	491	666
	236	384	601	439
	341	257		382
Mean ± SE	340±23.0	344±20.0	547±32.9	514±26.9

Mean weight of bloodmeal, mg/day of total days fed, ingested by infected and uninfected G. p. gambiensis in 20 consecutive days

Males		Females	
Infected	Uninfected	Infected	Uninfected
15.53	16.55	45.35	42.64
11.75	8.17	30.02	39.14
9.36	10.75	37.50	32.77
10.67	6.87	25.30	50.96
8.02	8.32	32.79	41.62
10.85	16.50	20.42	39.67
13.15	15.07	38.75	41.59
10.79	10.93	37.85	41.39
8.80	9.69	30.97	40.47
12.03	10.52	29.48	47.49
6.24	15.23	38.13	43.90
12.78	10.34		44.41
Mean ± SE 10.83±0.7	11.5±1.0	33.32±2.1	42.17±1.3

Total bloodmeal engorgement (in mg.) during 20 days by infected and uninfected G. p. gambiensis

Males		Females	
Infected	Uninfected	Infected	Uninfected
201.9	281.4	634.9	724.9
164.5	130.8	450.4	508.8
177.8	161.2	412.5	589.9
128.1	123.6	429.7	560.6
128.4	141.4	360.8	541.1
108.5	231.0	265.5	555.4
144.7	165.7	426.3	579.4
172.6	185.9	567.3	607.1
140.7	174.4	216.8	522.4
216.5	147.3	471.7	702.5
124.7	167.5	572.0	457.5
179.0	175.9		488.5
Mean ± SE 157.3±9.6	173.8±12.7	437.1±38.2	569.8±23.0

Mean number of probes/day, including the feeding ones, by infected and uninfected G. p. gambiensis in 20 consecutive days

Males		Females	
Infected	Uninfected	Infected	Uninfected
1.46	1.41	1.43	1.75
1.21	1.25	1.40	1.39
1.05	1.38	1.36	1.28
1.17	1.21	1.12	1.46
1.53	1.06	1.18	1.50
1.18	1.25	1.43	1.27
1.27	1.07	1.46	1.13
1.56	1.44	1.60	1.17
1.31	1.50	1.29	1.13
1.39	1.36	1.25	1.15
1.20	1.23	1.20	1.50
1.40	1.29		1.27
Mean ± SE 1.31±0.1	1.29±0.04	1.34±0.04	1.33±0.1

Frequency of the multiple probe feeds by infected and uninfected G. p. gambiensis in 20 consecutive days

Males		Females	
Infected	Uninfected	Infected	Uninfected
11	12	7	24
6	8	12	7
2	9	7	10
4	8	4	10
18	2	4	12
4	7	8	7
5	2	9	4
16	14	13	4
6	15	4	4
13	10	7	4
8	6	5	15
11	8		4
Mean ± SE 8.67±1.5	8.42±1.2	7.27±0.9	8.75±1.8

Mean number of pre-feeding probes by infected and uninfected G. p. gambiensis in 20 consecutive days

	Males		Females	
	Infected	Uninfected	Infected	Uninfected
	3.0	3.0	2.0	2.0
	1.7	1.7	1.0	2.0
	1.5	2.5	2.0	1.0
	2.0	1.3	2.0	2.0
	2.0	2.0	2.0	1.5
		1.0		2.0
Mean ± SE	2.03±0.3	1.81±0.3	1.8±0.2	1.75±0.2

Mean number of feeds taken by infected and uninfected *G. p. gambiensis* when allowed to feed at three day intervals

Males		Females	
Infected	Uninfected	Infected	Uninfected
8	8	8	7
8	7	8	8
8	8	6	8
8	8	8	6
8	8	8	8
8	7	8	8
8	7	8	8
8	6	7	8
7	8	7	8
6	7		
7	8		
8			
Mean ± SE 7.7±0.2	7.5±0.2	7.6±0.2	7.7±0.2

Each tsetse was allowed to feed on 8 different occasions in 22 days

Mean rate of bloodmeal intake (in ug/sec.) during days fed by infected and uninfected G. p. gambiensis when allowed to feed at three day intervals

Males		Females	
Infected	Uninfected	Infected	Uninfected
497	518	570	567
462	531	628	557
551	436	468	590
550	481	426	673
371	540	610	977
602	534	582	696
455	322	892	589
435	392	715	653
558	414	856	907
424	424		
457	463		
604	566		
Mean ± SE	497.2±21.6	468.4±21.1	638.6±52.8	689.9±50.6

Mean weight of bloodmeal (mg)/day of total days fed, ingested by infected and uninfected G. p. gambiensis when allowed to feed at three day intervals

Males		Females	
Infected	Uninfected	Infected	Uninfected
14.2	21.9	38.5	50.1
20.0	18.1	54.4	43.7
21.1	16.7	35.6	49.2
23.8	17.5	32.3	54.2
15.3	20.2	55.1	59.5
16.5	22.8	37.4	50.5
16.2	14.2	55.4	50.1
21.0	15.1	53.0	44.8
18.2	23.9	62.2	51.6
15.9	20.1		
19.0	20.5		
27.1	24.6		
Mean ± SE 19.0±1.1	19.6±1.0	47.1±3.7	50.4±1.6

Total bloodmeal engorgement (in mg) by infected and uninfected G. p. gambiensis when allowed to feed at three day intervals

Males		Females	
Infected	Uninfected	Infected	Uninfected
113.2	175.0	307.7	350.0
160.0	126.5	435.2	349.2
168.4	133.8	213.5	393.4
190.5	140.1	258.1	325.4
122.4	161.7	443.5	476.3
132.2	159.7	299.5	403.7
129.2	99.6	442.9	400.5
167.9	106.0	371.2	358.6
127.2	143.5	435.2	412.5
95.1	161.1		
133.3	143.8		
216.6	196.5		
Mean ± SE	146.3±10.1	145.6±8.0	356.3±29.7	385.5±15.1

Mean number of probes/day, including feeding ones, by infected and uninfected G. p. gambiensis when allowed to feed at three day intervals

Males		Females	
Infected	Uninfected	Infected	Uninfected
1.3	1.1	1.5	1.4
1.0	1.0	1.6	1.3
1.5	1.3	1.5	1.8
1.1	1.3	1.4	1.5
1.4	1.4	1.3	1.0
1.3	1.6	1.5	1.1
1.5	1.4	1.3	1.3
1.3	1.1	1.4	1.8
1.0	1.3	1.0	1.0
1.2	1.3		
1.3	1.3		
1.1	1.3		
Mean ± SE	1.3±0.1	1.28±0.1	1.39±0.1	1.36±0.1

Frequency of the multiple probe feeds by infected and uninfected G. p. gambiensis when allowed to feed at three day intervals

Males		Females	
Infected	Uninfected	Infected	Uninfected
4	2	7	4
7	4	9	4
2	4	6	11
6	6	4	5
4	7	7	2
7	6	3	4
3	2	4	11
2	4	2	
4	4		
2	4		
	4		
Mean ± SE	4.1±0.6	4.27±0.5	5.25±0.8	5.86±1.4

Mean number of pre-feeding probes by infected and uninfected G. p. gambiensis when allowed to feed at three day intervals

Males		Females	
Infected	Uninfected	Infected	Uninfected
1.0	1.0	1.3	1.0
1.3	1.0	1.3	1.0
1.0	1.0	1.0	1.2
1.0	1.0	1.0	1.5
1.0	1.3	1.0	1.0
1.3	1.0	1.3	1.0
2.0	1.0	2.0	1.2
1.0	1.0	3.0	
1.0	1.0		
1.0	1.0		
	1.0		
Mean ± SE	1.16±0.1	1.03±0.03	1.48±0.2	1.13±0.1

APPENDIX II

Number of females alive	Number of pupae produced
Number of females alive / Original number of females	Number of pupae produced / Number of females alive
Mean pupal weight	

Total number of reproductive cycles	Reproductive cycles per original female	Total pupae produced
Pupae per original females	Pupae per reproductive cycle	
Mean pupal weight		