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DISTRIBUTION, DENSITY & INFECTION RATES OF TESTSE FLIES IN SELECTED SITES OF SOUTHERN RIFT VALLEY OF ETHIOPIA

SHANDALA MSANGI

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ADDIS ABABA UNIVERSITY
Faculty of Veterinary Medicine

FREIE UNIVERSITÄT BERLIN
Fachbereich Veterinärmedizin

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A thesis submitted in partial fulfilment for the degree of Master of Science in Tropical Veterinary Epidemiology at the Freie Universität Berlin and Addis Ababa University

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SHANDALA MSANGI

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DISTRIBUTION, DENSITY AND INFECTION RATES OF TSETSE FLIES IN SELECTED SITES OF SOUTHERN RIFT VALLEY OF ETHIOPIA

By Shandala Msangi

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Dr. Susanne Münstermann .....

Dr. Getachew Tilahun .....

DEDICATION SHEET

This thesis is dedicated to my wife **Ester** and my daughter **Jane**.

TABLE OF CONTENT

Page

LIST OF FIGURES iii

LIST OF TABLES iii

LIST OF ANNEXES iv

LIST OF ABBREVIATIONS v

ACKNOWLEDGEMENTS vi

ABSTRACT vii

1. INTRODUCTION 1

2. OBJECTIVES 3

2.1 General Objective 3
2.2 Specific Objectives 3

3. LITERATURE REVIEW 4

3.1 General consideration 4
3.2 The genus Glossina 4
3.2.1 Biology 4
3.2.2 Systematics 5
3.2.3 Tsetse species and distribution in Africa 6
3.3 Tsetse fly control 7
3.3.1 Estimation of population size 7
3.3.1.1 Sampling techniques 8
3.3.1.2 Sampling biases 9
3.3.2 Tsetse population reduction and eradication 11
3.3.2.1 The use of baits 11
3.3.2.1.1 Traps and targets 12
3.3.2.1.2 Insecticide treated livestock 14
3.3.2.2 The Sterile Insect Technique (SIT) 15
3.3.3 Constraints in control efforts 15
3.4 The role of tsetse in the epidemiology of trypanosomosis 17
3.4.1 Tsetse flies as vectors of trypanosomes 17
3.4.2 Trypanosome infection rates in tsetse 18
3.4.2.1 Determination techniques 18
3.4.2.2 Factors affecting infection rates 19
3.4.3 Tsetse age determination 21
3.4.3.1 Ovarian dissection 21
3.4.3.2 Wing fray 22
3.4.4 Tsetse host preferences 23

4. MATERIALS AND METHODS 25
4.1 THE PROJECT AND STUDY AREA 25
4.1.1 The SIT project area 25
4.1.2 The study area 25
4.2 STUDY DESIGN 28
4.3 SAMPLING 28
4.5. DISSECTION OF FLIES 29
4.5.1 Infection rate 29
4.5.2 Bloodmeal collection and identification 30
4.6 FLY AGE DETERMINATION 31
4.7 DATA RECORDING AND ANALYSIS 31
5. OUTPUT 32
6. RESULTS 33
6.1 TSETSE POPULATIONS 33
6.1.1 Tsetse species and distributions 33
6.1.2 Tsetse relative densities 33
6.2 TRYPANOSOME INFECTION RATES AND SPECIES 36
6.2.1 Glossina pallidipes 38
6.2.3 Glossina fuscipes 38
6.2.3 Comparisons between G. pallidipes and G. fuscipes 39
6.2.4 Associations between trypanosome infection rates and age of tsetse: 39
6.2.5 Immature infections 41
6.3 AGE AND SEX COMPOSITION OF THE TSETSE POPULATIONS 42
6.4 THE RELATIONSHIP BETWEEN WING FRAY CATEGORIES AND OVARIAN AGE 43
6.5 TSETSE FLY HOSTS 44
6.5.1 Blood meal prevalence 44
6.5.2 Identification rate 45
6.5.3 Feeding pattern 47
6.6 TSETSE CHALLENGE 48
6.7 SUMMARY RESULTS 49
7. DISCUSSION 50
7.1 TSETSE POPULATIONS 50
7.1.1 Tsetse species and distributions 50
7.1.2 Tsetse relative density 50
7.1.3 Effects of odour attractants on the relative density 51
7.1.4 Sex ratios and age composition of the caught flies 51
7.2 TSETSE INFECTION RATES 52
7.3 RELATIONSHIP BETWEEN AGE IN WING-FRAY AND OVARIAN CATEGORIES 54
7.4 TSETSE FLY FEEDING PREFERENCE 55
7.4.1 Prevalence of bloodmeals 55
7.4.2 Feeding pattern 56
7.5 RELATIONSHIP BETWEEN TSETSE CHALLENGE AND TRYPANOSOME PREVALENCE IN CATTLE 57
7.6 LIMITATIONS AND POSSIBLE SOURCES OF ERRORS IN THE STUDY 57
7.6.1 Sampling techniques 57
7.6.2 Estimation of infection rate 58
7.6.3 Tsetse age estimation 58

8. CONCLUSIONS AND RECOMMENDATIONS 59
9. REFERENCES 61
10. ANNEXES 72
11. CURRICULUM VITAE 79
12. THE SIGNED DECLARATION SHEET 82

LIST OF FIGURES

Page

Fig 1: Tsetse relative density in North Omo at different times of the year 34
Fig.2: Tsetse relative densities in North Omo at different altitudes in the wet spell. 35
Fig. 3: Tsetse relative densities in North Omo at different altitudes in the dry spell. 35
Fig 4: Infection rates of different infection types. 39
Fig 5a.Relationship between age, indexed by wing fray, and infection rate in *G. pallidipes* 40
Fig 5b.Relationship between age, indexed by wing fray, and infection rate in *G fuscipes* 40
Fig 6: Percentage number of flies in each wingfray for *Glossina* spp 42
Fig 7: Percentage number of flies in each sex for the two tsetse species 42
Fig. 8: Feeding pattern of *G. pallidipes* during dry and wet spell in North Omo. 46
Fig. 9: Feeding pattern of *G. fuscipes* during dry and wet spell in North Omo. 46

LIST OF TABLES

Table 1a: Tsetse relative densities in North Omo in different vegetation types. 34
Table 1b: Males and females relative densities in North Omo. 35
Table 2: Tsetse relative densities in North Omo from traps baited either with Cow Urine (CU) alone or Cow Urine with Acetone (CU+A) during the dry spell. 36

Table 3a: Mature infection rates for the wet and dry spells and two sexes of *Glossina* spp. in North Omo with 95% CI 37

Table. 3b. Mature infection type and rate in *Glossina* species in North Omo with 95%CI 37

Table 3c: Immature infections in *Glossina* spp. in North Omo with 95% CI. 37

Table 4: Linear regression analysis of age, indexed by wingfray, on log-transformed infection rates for different infection types in tsetse flies 41

Table 5: Relationship between wing-fray categories and ovarian age in female tsetse dissected in North Omo 43

Table 6: Prevalence of blood meals by sex. 44

Table 7: Prevalence of blood meals during the first (dry) and second (wet) survey. 45

Table 8: Ruminants tsetse hosts as identified by ELISA. 47

Table 9a: Estimates of tsetse challenge during the dry spell as a product of relative density, infection rate and percentage of bloodmeal taken from cattle. 48

Table 9b: Estimates of tsetse challenge during the wet spell a product of relative density, infection rate and percentage of bloodmeal taken from cattle. 48

LIST OF ANNEXES

Annex10.1a-h: Estimated curves for relationship between tsetse infection rates and age in wingfray in non-logarithm scale 69

Annex.10.2. a-h: Population structure of tsetse. 71

Annex 10.3. Classification of tsetse flies. 73

Annex 10.4: Classification of trypanosomes of economic and epidemiological importance. 74

Annex 10.5. The rainfall pattern in the Southern Rift Valley of Ethiopia 78

LIST OF ABBREVIATIONS

ABTS	Azino-di-ethyl-benzithiozolinsulphonate
BgVV	Bundesinstitut fur gesundheitlichen Verbraucherschutz und Veterinärmedizin
BUL	Bushland
CFT	Complement Fixation Test
CUL	Cultivated land
DNA	Deoxy-ribose Nucleic Acid
ELISA	Enzyme Linked Immuno-Sorbent Assay
FAO	The Food and Agricultural Organization of the United Nations
FFF	Forest
GAR	Goat Anti Rabbit
G.f	*Glossina fuscipe.*
G.p	*Glossina pallidipes*
GPS	Global Positioning System
GGL	Grassland
IgG	Immunoglobulin G
Km	Kilometer
Km^2	Square kilometer.
m	Meters
m-asl	Meters above sea level
OD	Optical Density
PCR	Polymerase Chain Reaction
r	Correlation coefficient
RD	Relative Density
RFF	Riverine forest
s.d.	Standard deviation
s.e.	Standard error
SIT	Sterile Insect Technique
SRVETEP	Southern Rift Valley of Ethiopia Tsetse Eradication Project
WGL	Wooded Grass land

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ABSTRACT

A tsetse survey to assess the risk of trypanosomosis to livestock was conducted in the South Western Rift Valley of Ethiopia. The challenge was estimated as the product of tsetse relative density, infection rate and the proportion of bloodmeal taken by tsetse flies from livestock.

A total of 2900 km^2 of the study area was divided into 100 km^2 monitoring grids, and stratified in three strata according to altitude as lowland (0-1600 m altitude), mid-altitude (1601-2000 m altitude) and highland (above 2000 m altitude.) The survey was done twice according to this stratification, first during the dry spell (April-mid May) and second in late June-July 1999 (wet).

Twentyfive NG2U-traps were deployed in each grid. Few biconical traps were deployed along the rivers to monitor riverine species. Traps were removed after three days, and the flies were counted, identified and sexed. Relative density was calculated as the number of flies caught per trap per day. Tsetse infection rates were determined by dissection method. Aging of flies was done mainly by wingfray method. Bloodmeal identification was based on enzyme-linked immunosorbent assay (ELISA) using host specific antisera.

Two tsetse species were detected, *Glossina pallidipes* as the predominant species which was detected up to 1931 m above sea level and *Glossina fuscipes*, detected up to 1710 m altitude. During the dry spell, the relative density of *G. pallidipes* was 0.6 flies/trap/day and 0.06 flies/trap/day for *G. fuscipes*. During the wet spell, these figures rose to 2.4 and 0.1 for *G. pallidipes* and *G. fuscipes* respectively. The lowland areas recorded the highest density for both species. There were no flies caught in the highland area.

About 915 *G. pallidipes* and 652 *G. fuscipes* were dissected for infection rate estimation. All flies were from lowland areas. For *G. pallidipes*, the overall infection rate was found to be 7.9%. There was a significantly higher infection rate in females (9.4%) than in males (4.1%) ($\chi^2 = 6.42$, $P < 0.05$). The infection rate was also significantly higher in the wet (9.5%) than it was in the dry months (5.6%), ($\chi^2 = 4.57$, $P < 0.05$). All the three important pathogenic trypanosome types were detected, with an infection rate of 5.4%, 2.2% and 0.2% for *Vivax*, *Congolense* and *Brucei* types respectively. As regard to *G. fuscipes*, the overall infection rate was found to be 7.6%. Only *Vivax* and *Congolense* infection types were detected. Females also showed significantly higher infection rates (9.4%) than males (5.8%). ($\chi^2 = 4.68$, $P < 0.05$). There was no significant

difference in the infection rates in the wet (7.8%) and dry period (7.7%) ($\chi^2 = 0$, $P > 0.05$). The infection rate was 7.5% and 0.03% for *Vivax* and *Congolense* type infections respectively. For both *G. pallidipes* and *G. fuscipes* there was a strong relationship between infection rate and age, indexed by wingfray, with a correlation coefficient (r) of 0.98 and 0.99 respectively.
During the dry spell, cattle were identified as the main source of bloodmeals for both *G. pallidipes* (45%) and *G. fuscipes* (40%). In the wet spell, cattle provided 66.7% of the bloodmeals for *G. pallidipes* while *G. fuscipes* changed their main host to man (69.2%) with cattle providing only 7.7% of their meals. Implications of this change are discussed.

The trypanosomosis risk index (challenge) for individual tsetse species in dry months was estimated to be 152.5 for *G. pallidipes* and 18.6 for *G. fuscipes*, while in the wet spell, these figures were 1521 for *G. pallidipes* and only 6 for *G. fuscipes*. The overall trypanosomosis risk index in dry months was 171 and in the wet months it was estimated to be 1527.

It is concluded that the risk is higher in the wet than in the drier months of the year, this finding corresponded well with trypanosome prevalence in animals. According to the stratification of the altitude in this particular study, the lowland was categorised as having medium to high tsetse challenge, mid-altitude as having low to medium challenge, while there was no challenge in the highlands. *G. fuscipes* contributed very low to the overall challenge in the study area as compared to *G. pallidipes*.

1. INTRODUCTION

African animal trypanosomosis constrains the production of milk, meat and animal traction across much of Sub-Saharan Africa. The tsetse-transmitted disease is particularly important in Ethiopia where at least six million cattle are exposed to the disease (Swallow and Woudyalew, 1994). Ethiopia, one of the largest African countries, has a land area of about 1.3 million square km (Tikubet, 1993). Pergman *et al* (1981) noted that Ethiopia has the largest domestic animal population in Africa, and trypanosomosis is widespread in domestic livestock in the western, south-western lowland regions and the associated river systems. According to FAO (1993), Ethiopia has a population of 31 million cattle, 23.2 million sheep, 18.1 million goats, 2.7 million horses, 0.63 million mules, 5.2 million donkeys and 1.07 million camels. Unpublished data indicate that tsetse transmitted trypanosomosis is the major cause for the large land resources in the Southern Rift Valley of Ethiopia to remain underutilized. About 20,000 km^2 of agricultural suitable land of the Southern Rift Valley is estimated to be infested by the tsetse fly *Glossina pallidipes*. Almost all the livestock populations in and adjacent to this valley are at risk of acquiring the disease at any one time. It is estimated that mortality due to trypanosomosis is more than 16% as compared to the national average of 8%. An estimated US$ 0.8 million is expended annually in drug supply to the area by the Government.

There are several intervention methods used currently in the fight against bovine trypanosomosis in Africa. These include the use of drugs against trypanosomes in cattle, either as prophylactics or as therapeutics. However, the occurrence of drug resistance, the high recurrence of costs of the drugs and the logistics of delivery, do not make this strategy for disease control an attractive one. Others are the use of various methods aimed at controlling the vector *Glossina* and the introduction of trypanotolerant cattle such as the N'dama of west and central Africa into areas infested by tsetse flies. However, vector elimination or reduction remains the permanent solution of controlling trypanosomosis. Langley (1994) noted that it is generally recognized that the control of trypanosomosis is best achieved by removing tsetse flies from the environment, though few African countries have ever practiced tsetse control on a wide scale. Despite the known limitations, the use of trypanocidal drugs, generally administered by the livestock owners themselves, is the major method by which animal trypanosomosis is today controlled in most African countries (Leak, 1999). However, Jordan (1986) warned that killing trypanosomes in

man and domestic animals by trypanocidal drugs is not a permanent solution and that zero disease can only realistically be achieved by elimination of the vectors. Likewise, Leak (1999) noted that complete eradication of the vector is rarely achieved, yet in the absence of a vaccine for trypanosomosis and as drug resistance to available trypanocidal drugs increases, control of tsetse fly remains the most desirable means of controlling the disease.

However, methods of tsetse control require, among other things, the knowledge of tsetse species distribution and abundance in the area. Katondo (1984) advised that outbreaks of trypanosomosis could be contained by implementation of plans based on sound knowledge of *Glossina* distribution. Unfortunately, for many countries such knowledge is fragmentary. One of the most important outcomes from the results of species distribution surveys is the estimate of the actual tsetse infested areas within a given region with an option for tsetse control (Rawlings *et al.*, 1993).

Tsetse infection rate is an important parameter in the assessment of trypanosomosis risk. Knowledge of the dynamics of trypanosome infection rates in tsetse is fundamental to our understanding of the epidemiology of tsetse transmitted trypanosomosis (Leak and Rowlands 1997). Application of such knowledge can be important, for example, in planning of tsetse control activities using sterile male insect techniques (Leak and Rowlands, 1997). The information on trypanosomes and their relative infection rate in tsetse flies is needed also for the assessment of the actual trypanosomosis risk to the people and their livestock and drug resistance studies (Mohamed-Ahmed *et al.*, 1989).

Different tsetse species have different host preferences. In some instances tsetse flies take most of their bloodmeals from animals other than cattle, hence posing less risk to cattle. Moloo *et al.* (1980) found that species such as *G. brevipalpis* take bloodmeals mostly from unusual hosts like hippopotamus. Also, there is little success in such control activities like use of pour-ons which depend solely on flies feeding on treated animals if the flies feed on animals other than cattle. The knowledge of tsetse host preferences is, therefore, of vital importance in estimating the risk of trypanosomosis in a particular area.

In view of the above, this study was designed to address these aspects (tsetse species distribution and density, infection rate and tsetse host preferences in the area) as an important part in estimating the trypanosomosis risk in the area. This will form an important baseline information for the control of trypanosomosis in the Southern Rift Valley of Ethiopia.

2. OBJECTIVES

2.1 General Objective

To establish the entomological component of the risk of trypanosomosis to livestock in the Southern Rift Valley of Ethiopia.

2.2 Specific Objectives

To conduct a tsetse fly survey in the North Omo, Southern Rift Valley of Ethiopia during the dry and wet spells and establish the following:

- Distribution and relative abundance of the vectors.
- Habitat preferences of different tsetse species.
- Variations in the infection rate of different trypanosome species in tsetse.
- Effect of age on the trypanosome infection rate in tsetse.
- Hosts preferences of the different tsetse species.

3. LITERATURE REVIEW

3.1 General consideration

Tsetse transmitted animal trypanosomosis is one of the major constraints to social economic development in Africa. In a review on the current situation of animal trypanosomosis in Africa, Murray and Gray (1984) reported that tsetse flies infest approximately 10 million km^2 of the continent, affecting 38 countries. About 30% of the 147 million cattle in the countries affected by tsetse flies are exposed to the disease. The annual losses in livestock production due to trypanosomosis are estimated at US $5 billion and there is evidence that the overall situation is deteriorating.

3.2 The genus Glossina

3.2.1 Biology

Glossina are large insects, rather dull in appearance, varying in colour from light yellowish-brown to dark-brown. Some species have alternating darker and lighter bands on their abdomen. Tsetse flies reproduce by adenotropic viviparity. According to the description of the biology of tsetse flies given by Jordan (1986), females usually mate within a day or so after emerging from their pupal case when the female is about to or is actually in the process of taking her first bloodmeal. Active and viable sperms can remain in the female spermathecae throughout the life of the female, which is about 3-4 months. Mating takes place once, and multiple mating is probably a relatively rare occurrence. Male flies are fully potent when they are few days old and can mate on many occasions, but in the field they are unlikely to pair more than few times. In females, eggs are ovulated sequentially in the four ovarioles and are ovulated into the uterus at intervals of about 9-10 days with the first ovulation occurring when the fly is about 9 days (Jordan, 1986). After fertilization, the first instar larva develops within the egg and hatches after three and a half days, feeding on uterine gland secretions. It then moults twice and larviposition

of the third instar larva occurs. This then burrows rapidly into soil, contracts rapidly to form a barrel-shaped puparium, which darkens later. After four days, ecdysis results in the true pupae. After 30 days, at a temperature of about 25°C, a single fly emerges from each puparium with a sex ratio of 1:1.

It is known that females live longer than males, both in the laboratory and in the field. The female must live for at least 36 days to produce more than two offsprings if the species is to survive. Leak (1999) noted that tsetse flies are k-strategists, having a low rate of reproduction; they produce a single egg at a time and one egg every 9-10 days. The eggs and larvae produced are retained in the female uterus until just before the third stage larvae is ready to pupate, and this high level of parental "care" ensures a high probability of each individual surviving. This differs from most insects, which are r-strategists, producing large numbers of eggs each having a low chance of surviving to adulthood. Jordan (1986) noted that in most insects, the immature stages are abundant, but are particularly vulnerable to natural and man induced mortality. However, in *Glossina*, few offsprings are produced but eggs and larva are well protected within the female parent where little mortality occurs. The pupae are also protected by a hard case burrowed in the soil.

Both males and females feed only on blood. Females feed more frequently than males to nourish the larva, and at 25°C, females need to feed, on the average, about every other day and males at rather longer intervals. Females feed up to 2-3 times their body weight (Jordan, 1986).

3.2.2 Systematics

Tsetse flies are classified into the genus *Glossina* of the family Glossinidae, order Diptera-the two winged flies (Buxton, 1955). Two features clearly distinguish the genus *Glossina* from other diptera. One is the discal cell of the wing, shaped like a cleaver and referred to as "hatchet cell" lying between longitudinal wing vein IV and V. The second feature is the presence of secondary branches on the hairs of the arista of the antennae. The genus is further subdivided into well-marked species groups (Subgenera). These are *fusca* (Austenina), *palpalis* (Nemorhina) and *morsitans* (Glossina) groups. The groups can readily be distinguished on the basis of the differences of male and female genital armatures. Within the subgenera, males of the *palpalis* group are identified mainly from the morphology of the inferior claspers, males of the *morsitans*

group from the superior claspers and *fusca* group females most easily from the signum. Standard conventional keys for identification of tsetse flies identify individual tsetse species. There are 31 species and subspecies identified at present, as shown in annex 3.

3.2.3 Tsetse species and distribution in Africa

Katondo (1984) noted that outbreaks of trypanosomosis could be contained only by implementation of sound knowledge of *Glossina* species and their distribution. However, for many African countries, such knowledge is fragmentary. According to Jordan (1986) the Genus *Glossina* occurs only in Africa, covering a total area of 11 million km^2. Its northern limit corresponds with the southern edges of the Sahara and Somali deserts. The southern limit is less well defined, where in south-west, it varies between 10^0 and 20^0 S corresponding with the northern edges of the Kalahari and Namibia deserts where as in south-east it is generally at about 20^0-29^0S. These limits are determined by climate, often through its effect on vegetation and temperature. In Southern Africa, where rainfall is high, the limits of Glossina are related to seasonal low temperature and tsetse fly do not occur above 1800m, but the upper limit decreases with distance from the equator.

The different tsetse species have different ecological preferences in terms of habitat and hosts. Leak *et al.* (1987) reported that *morsitans* group tsetse inhabit savanna areas of Africa, which, in addition to being extensive, are more likely to be the habitat of domestic livestock and of game animals which serve as reservoirs of trypanosome infection. *Fusca* group tsetse species, on the other hand, inhabit the forest areas which are less suited for livestock production, whilst *palpalis* group tsetse flies are largely confined to gallery forest and forest relics, where the degree of contact with livestock is limited. They are, therefore, likely to present a low trypanosomosis risk especially during the rain season.

Moloo (1985) provided tables on the distribution of *Glossina* species in Africa. According to the tables, most of the *fusca* and *palpalis* group of tsetse are concentrated in west and central Africa, while most of *morsitans* group of tsetse species are found in Eastern and Southern Africa. *Glossina brevipalpis* and *G. longipennis* seem to be the only *fusca* group species found in Eastern Africa. The tables show that all three groups of tsetse species are present in Ethiopia. These

include *G. brevipalpis* and *G. longipennis (fusca)*, *G. tachinoides* and *G. f. fuscipes (palpalis)*, and *G.m.submorsitans* and *G. pallidipes (morsitans)*

3.3 Tsetse fly control

The earlier and old tsetse control techniques, including vegetation clearing, ground and aerial insecticide spraying and selective game destruction have been widely phased out due to both high cost involved and environmental side effects caused by these methods (FAO, 1992; Leak, 1999). Aerial spraying also requires sophisticated equipment and expertise (FAO, 1992). These methods therefore, need not to be described in details here. Leak (1999) noted that traps and targets are more acceptable means of controlling tsetse than either ground or aerial spraying of insecticides in terms of the direct ecological and environmental impact they might have. The improved and advanced methods in tsetse control strategies involve the use of odour baited traps (Dransfield *et al.*, 1990), the use of the insecticide-impregnated targets (Vale *et al.*, 1988) and insecticide treated cattle (Pour-Ons) (Shereni, 1990). Tsetse control using impregnated targets or traps, together with odour attractants where appropriate appears to be a very successful method. It is technically fairly simple yet efficient and relatively cost-effective. The techniques are also attractive because of the minimal environmental pollution (Leak 1999).

3.3.1 Estimation of population size

The information required in a study of the epidemiology of an insect-borne disease is not so much the proportion, but the number of vectors biting the hosts. Therefore, in order to estimate the exposure of an animal to the disease, such analysis like age composition, should be combined with a study of the numbers of flies biting or an experiment to estimate the size of the population (Saunders, 1962). Commonly employed methods in estimating the population density of flies in the field are briefly described.

3.3.1.1 Sampling techniques

Fly rounds: These are marked paths through the bush along which men walk to catch flies, usually with hand nets, stopping at intervals to do so (FAO, 1982). It is an old method, but still used by various workers in sampling including Saunders (1962), Allsopp (1985), Mohamed Ahmed *et al.* (1989). In a review on tsetse ecology, Leak (1999) noted that one of the major problems with flyround sampling was the repellence of humans to many species of tsetse, e.g. *G. m. morsitans* and *G. pallidipes*. Catches of some important species of the *morsitans* group consisted mainly of males and very hungry flies, and consequently gave biased information. Also, flyrounds do not take into account the diurnal changes in activity of tsetse, as it is a measure taken only once. These observations led to development of other sampling techniques such as traps.

Trapping: Various trap designs have been developed over the years for both sampling and control purposes. They include F3, NGU and Epsilon for Savannah species and biconical, monoconical and pyramidal traps for riverine species. Traps provide a standardized system of sampling that is not dependent on varying ability to catch flies and they catch a more representative sample of the two sexes. Traps can be used for species that are sometimes repelled by odour of men e.g. *G. pallidipes* and *G. morsitans*. However, traps have to be well constructed and maintained and stationary traps are very dependent for efficiency on the site chosen (FAO, 1992). Traps have been used widely, probably more than any other sampling methods in Africa and have been described by various authors (e.g. Saunders, 1962; Hendrickx *et al.*, 1993 in Togo; Woolhouse *et al.*, 1993 in Zambezi valley Zimbabwe; Mohamed-Ahmed and Dairri., 1987 in Sudan; Vale, 1974).

Electric nets: This is a battery powered grid of parallel wires. Flies colliding with the grid are electrocuted and fall into a collecting tray from which they are later removed. This method gives a sample that is less biased than most other methods (FAO, 1982). Electric nets are used to sample populations of flies in flight, for instance when they are approaching or are near to real or artificial hosts. Parker and Brady (1990) noted that the use of electric nets has revolutionized our understanding of tsetse behaviour and ecology, and it is now being used increasingly elsewhere in entomology. Hargrove (1991) obtained the largest daily catch of tsetse from vehicle mounted electric nets as compared to other sampling methods including stationary bait ox, odour baited

traps and mobile fly-rounds. Electric nets could be used stationary (Parker and Brady, 1990) or vehicle mounted (Hargrove, 1991).

Ox-surveys: This is a less common method, but used occasionally. A team, consisting of an ox and 2-4 men catching flies coming to feed on the ox, usually with hand nets. This could be done as mobile surveys (Vale *et al.*, 1988) or stationary bait ox (Hargrove, 1991).

Puparial searches: For some species of tsetse, searching for puparia can be a useful and complementary way of determining their presence and breeding sites, although it is time consuming, and may not be very productive for certain species. This technique may be of particular use for species that do not readily come to traps such as *G. austeni*. (Leak, 1999).

3.3.1.2 Sampling biases

In principle, any sampling system should be unbiased, so that individuals of different species, sex, age and nutritional status are sampled in proportion to their representation in the population or alternatively, any bias should be known and allowed for (Parker and Brady, 1990). The size and composition of samples obtained from populations of *Glossina* are a function of both the density of the population being sampled and of its activity, which is itself dependent on variable factors such as the prevailing meteorological conditions and the element of the population attracted by the particular catching method in use (Jordan, 1974). Certain categories of the populations, e.g. age group, hunger stage, pregnancy stage, are more active than others and respond differently to the sampling method, therefore samples tend to be biased (FAO, 1992). Robinson (1995) observed that there are considerable biases in the fraction of the tsetse population that is sampled by different methods. For instance, in an area where *G. morsitans* and *G. pallidipes* are both present, traps tend to catch more *G. pallidipes* and are biased towards older females, while mobile techniques tend to catch more *G. morsitans* and are particularly efficient for male flies. Any information on population variables such as age, distribution, mortality rates or sex ratios must be treated therefore with extreme caution. He noted that mortality in the collecting devices adds another bias to sampling since certain flies of different species, sex, age and stages in reproductive cycles may be more susceptible to death through exhaustion or desiccation and will therefore not be dissected.

Sex ratio: Saunders (1962), reviewing Jack (1939), explained that in the field female tsetse flies are much less active than males and therefore usually form a very low proportion in samples caught on fly-rounds. This is especially so with *G. morsitans*, *G. pallidipes* Aust. and *G. swynnertoni* in which the female proportion is often under 10%. It is known that female tsetse flies live longer than males (Jordan, 1974). Since in nature males and females hatch in equal numbers, tsetse populations are expected to consist of more females. However, a review by Vale (1993) showed that hand net catches from human baits or from other baits accompanied by hand net catches consist mainly of males of a few tsetse species, such as *G. m. morsitans* with other species such as *G. pallidipes* being poorly represented. The only host-like bait that gives fair representation of the relative abundance of each sex was traps. Likewise Saunders (1962) reported that few female flies are attracted to a party of men on a flyround but this proportion of females may be raised by the use of screens, bait animals and traps. Murray *et al.* (1983) noted that flyround sampling and vehicles usually produce a grossly distorted sex ratio in favour of males because sexually appetitive as well as hungry males are highly responsive to moving objects, whereas only very hungry females are attracted. Also the presence of man is known to repel *G. pallidipes* and *G. morsitans*. Stationary traps normally produce a more representative sex ratio. *G. f. fuscipes* caught with pyramidal traps by Katunguka-Rwakishaya and Kabagambe (1996) showed higher percentage of females than males and ranged between 51-64.6% in Uganda. Therefore most traps catch a relative higher proportion of females than other sampling methods especially flyrounds. Results obtained by Vale and Phelps (1978) from studies on *G. m. morsitans* Westwood. and *G. pallidipes* Aust. captured by various sampling methods showed that odour baited traps were not only convenient but were also able to attract large samples with representative sex and species composition and indicated their value for ecological studies. However, different trap designs vary in their sex ratio bias, for instance for *G. pallidipes*, NGU and F3 traps catch a higher proportion of females than biconical traps do, (FAO, 1992).

Age structure: Saunders (1962) showed that the oldest females and those most advanced in pregnancy are caught on flyrounds. Murray *et al.* (1983) noted that in general young flies (tenerals) of most species are greatly over-represented in human bait samples and mostly under-represented in trap samples. Sample bias, as regard to age structure is especially serious since certain analytical methods depend on a representative age distribution. Since trypanosome infections in the fly are age dependent, infection rate data also suffer limitations imposed by sampling bias. However, experiments have shown that biconical and NGU traps efficiency for teneral *G. pallidipes* is the same for other age categories (FAO, 1992).

Species: Sampling methods differ in their effectiveness in sampling different species. A single method is, therefore, likely to give a biased picture of the species composition in an area. The biconical traps, for instance, are effective in Kenya for *G. pallidipes* but not for co-existing *G. austeni* and *G. longipennis*. These are more effectively sampled using sticky traps or bait animals and moving vehicles respectively. Because species composition is dependent on the sampling method, it is difficult to determine the relative abundance of different species in a given area (Murray *et al.*, 1983).

Hunger state: Murray *et al.* (1983) noted that bait animals and stationary traps catch mainly hungry flies, but trap samples often include a small proportion of engorged flies of both sexes. This is because engorged flies regard the trap as a resting site. When a variety of sampling methods were compared, refuge traps were found to catch *G. morsitans* in the widest range of hunger states, giving the most representative picture of the nutritional states in the population at large.

3.3.2 Tsetse population reduction and eradication

Various efforts have been employed in the control of tsetse flies and the disease they transmit across Africa. Murray and Gray (1984) noted that governments, bilateral and international agencies have undertaken new initiatives including training in evaluating the impact of trypanosomosis on productivity, development of better diagnostic techniques, improving tsetse trapping techniques, control campaigns and in research. In Ethiopia however, tsetse and trypanosomosis control measures are restricted mainly to monitoring the tsetse population and trypanosomosis incidence in cattle and the use of trypanocidal drugs to control the disease (Tikubet, 1993). This is largely due to the lack of appropriate resources to undertake systematic vector and diseases control programs.

3.3.2.1 The use of baits

Analysis of host-oriented behaviour of tsetse flies *G. m. morsitans* West. and *G. pallidipes* Aust. led to 10-1000-fold improvement in the cost effectiveness of baits for surveying and control.

Baits are now used widely to replace air and ground broadcasting of insecticides (Vale, 1993). Baits can be stationary (traps and targets) or mobile (cattle).

3.3.2.1.1 Traps and targets

Different trap designs have been used in various countries in the effort to control tsetse flies. Dransfield and Brightwell (FAO, 1992) reported that NGU traps baited with acetone, cow urine and octenol have been used to control *G. pallidipes* and *G. longipennis* in Kenya while biconical traps without odours were used to control *G. m. submorsitans* in Bukina Faso. Noninsecticidal pyramidal traps have been used to control peridomestic populations of *G. fuscipes* and *G. palpalis* in Congo. In Uganda, *G. f. fuscipes* were controlled by the use of insecticidal pyramidal traps. However, Vale (1993) warned that traps must be serviced about once a month if they are to remain in good order and that a trap that has gone topsided or that has torn netting can be completely ineffective.

Vale (1993) advised that, if the intention is simply to kill tsetse flies, rather than to retain them for surveys, it is more economical to replace the traps by targets that need less regular repair and that can be coated with a long-lasting insecticide. Targets are usually more effective because they do not require the flies to enter. In addition to insecticide treatment of visual components, the targets are provided with treated netting to ensure that tsetse in fight collide with the insecticide (Vale 1993). Insecticide-treated targets have been used in various countries including Zimbabwe, Kenya, Ethiopia, Rwanda and Bukina Faso for control of both *G. pallidipes* and *G. morsitans* (FAO, 1992; Vale *et al.*, 1988). In Kenya, Dransfield *et al.* (1990) obtained 99.9% reduction of *G. pallidipes* and *G. longipennis* by using insecticide-impregnated targets. By using targets consisting of black cloth and netting, baited with 1-Octen-3-ol and acetone or butanone and coated with deltamethrin Vale *et al.* (1988) were able to reduce a population of *G. m .morsitans* West. and *G. pallidipes* Aust. by 99.99% in Zimbabwe and concluded that targets offer a simple and ecologically clean method of controlling tsetse and preventing invasion.

However, Williams *et al.* (1992) noted that the control of tsetse fly populations, using either traps or targets depends on the movement patterns of the flies, which determine how many flies find the trap and the efficiency of the trap, which determine the proportion of those flies that are killed. It is also noted (FAO, 1992) that stationary traps are very much dependent for efficiency

on site chosen. For instance, Vale (1997) obtained an 8-fold better catch in a sunny-cleared area (7m radius) as compared to an area under large-shady trees surrounded by leafy bushes. It is also noted by Leak (1999) that the use of traps or targets require a considerable amount of costly maintenance. Unless good supervision is maintained, traps or targets may become ineffective after a few months; reimpregnation may not be carried out adequately or at necessary intervals and targets or cloth materials can quickly rot, particularly in the hot and humid regions. In addition their sustained use for tsetse control in many cases, has been severely compromised by theft of targets or trap material. Consequently, attempts are being made to develop easily maintained or disposable targets, which could be partially managed by the beneficiary communities.

Odour attractants: Odours are used together with sampling or control techniques to increase their efficiency in catching flies. A number of odour attractants have been identified. In the review on tsetse ecology, Leak (1999) classified odour attractants in three groups, those associated with animal breath such as acetone, octenol and carbon dioxide; those associated with urine such as phenols and those associated with skin secretions such as sebum. The latter have proved less promising than breath and urine. Phenols, acetone and octenol have been shown to increase the tsetse catch in traps by several times in *G. pallidipes* (Hassanali *et al.*, 1986) and in *G. longipennis* (Jaenson *et al.*, 1991). Octenol has also shown to increase catches of tabanids up to three fold (Jaenson *et al.*, 1991). Urine from cattle and buffalo is also known to be a good attractant for tsetse flies especially *G. pallidipes* and it has been shown by Vale *et al.* (1986) that it increases the trap entering response of this species. However, very high release rates may become repellent. The effectiveness increases if it is allowed to "age" for about ten days. The active components are phenols (FAO, 1992). Carbon dioxide was the first attractant to be identified in host odour (Vale, 1993). All tsetse species, including members of the *palpalis* group are attracted by carbon dioxide given off in animal breath and it can substantially increase the catch. However, its use in the field is impractical (FAO, 1992). At present, attractants are most effective for *morsitans* tsetse group, whilst further improvements are needed for attractants of *palpalis* group flies. The control of *fusca* group tsetse species is difficult since synthetic phenols or other attractants less attracts them. However, generally they occur in areas without cattle and are therefore economically less important vectors of trypanosomosis to cattle (Leak, 1999).

Community participation in tsetse control: Leak (1999) suggested that while the technical means of controlling tsetse flies are available, the main difficulty lies in sustaining control over a

long period. This is especially the case when using traps and targets. This is because targets and trapping techniques are long term operations, requiring some initial organisation and direction followed by a long maintenance phase. It is widely felt that successful control using traps and targets should involve the local community. It is believed that if the communities, which are the intended beneficiaries of the control are involved in the exercise, the operation will be much more economical and sustainable. In theory, the operation should also stand more chance of success if the beneficiaries, having vested interest in the success of the operation, are involved in carrying it out. This could also be the case with tsetse control by insecticide treatment of livestock.

3.3.2.1.2 Insecticide treated livestock

This is a natural extension of the artificial bait system. The principle is simply that tsetse coming to feed on cattle or other treated domestic livestock will be killed by picking up a lethal deposit of insecticide on the ventral tarsal spines whilst feeding. Alternatively, they will be repelled by the insecticide and will therefore not attempt to feed. Whilst repellent effect may protect treated livestock, control of the tsetse population depends upon a relatively large proportion of feeds being taken from domestic rather than wild animals, a sufficient proportion of the livestock population being treated, their movement pattern and a sufficiently low level of reinvasion. The treated livestock will then be equivalent to moving insecticide-impregnated targets, complete with build-in odour attractants. As cattle tend to aggregate in herds, a higher number of treated livestock may be required than the number of cloth targets that would be required in an area. None of these parameters has yet been adequately defined and there are, therefore, no clear guidelines to determine how many cattle need to be treated in a given area (Leak, 1999). The method has demonstrated the potential to replace artificial attractive devices in areas where sufficient livestock are available (FAO, 1992). This is because pour-on treatments provide an extra benefit, as other nuisance flies and ticks may also be controlled. The technique may be less susceptible to disruption than targets or trap method, as there are no traps or targets to be stolen. Additionally, the livestock owners are likely to have a greater interest in ensuring that their livestock are treated (Leak, 1999).

3.3.2.2 The Sterile Insect Technique (SIT)

The method involves the release of sterilized males in the field which then mate with wild females, which are then unable to reproduce. This is usually done after suppression of the tsetse population by other methods. Leak (1999) noted that the SIT requires large numbers of tsetse of the target species to be reared in laboratory colonies. The puparia produced by these flies are sterilized with radiation and the sterile males emerging from these puparia are released in larger numbers than the wild males in the natural population, over a long period into the area from which tsetse are to be eradicated. Commonly, the sterile males are given an initial bloodmeal treated with trypanocides before release so as to reduce the risk of them subsequently transmitting trypanosomes. The success of the technique depends upon a high probability of wild females mating with a sterile male rather than the wild ones. Jordan (1974) noted that the principle advantage of the method is that it becomes more economical when the natural population is low. This is the reverse of the situation with other methods of control when it is often relatively easy to drastically reduce a population but difficult and costly to remove the survivors of the control campaign. However, the method is very expensive, sophisticated and it is species specific. The technique has been used successfully in some parts of Africa e.g. eradication of three species of flies *G.palpalis gambiensis*, *G. tachinoides* and *G. m. morsitans* in Burkina Faso (Clair *et al.*, 1990), eradication of *G. austeni* in Zanzibar, Tanzania (IAEA, 1997) and eradication of *G. p. palpalis* in central Nigeria (Oladumande *et al.*, 1990).

3.3.3 Constraints in control efforts

Despite various efforts emloyed in the control of tsetse fly, control efforts have not overall been a success due to various factors. These are briefly described bellow.

Reinvasion: One of the factors responsible for the lack of success in sustaining control of tsetse flies is their high mobility, resulting in continuous invasion pressure into cleared areas. Brightwell *et al.* (1992) noted that the high mobility of Savannah species means that re-invasion into control areas is a constant problem, and that such re-invasion is frequently seasonal, especially for the species of the *morsitans* group. Dransfield *et al.* (1990) using traps, showed that the population of *G. longipennis* was reduced by 90% during the dry season but was not reduced at all during the rains due to re-invasion. Likewise Jordan (1986) noted that it is relatively easy to

kill a lot or even all flies in an area by various methods, but much more difficult to ensure that the area remains fly-free. Unless there are natural barriers, artificial barriers are a costly and labor intensive option.

Wild hosts: Tsetse flies are known to have a wide range of hosts including wild animals, which maintain them in the field. Allsopp *et al.* (1972) advised that because of the importance of the relationship between tsetse flies and game animals in the transmission of pathogenic trypanosomes, it is evident that much more research is required in this field. Likewise Jordan (1986) indicated that there is close association between game animals, Savannah species of *Glossina* and trypanosomosis. The natural events such as the rinderpest epizootic at the end of the nineteenth century, which resulted into death of many animals, could also lead to the disappearance of tsetse flies and trypanosomosis. However, host destruction is not deliberately employed today. Murray *et al.* (1983) noted that wild animals, particularly wild bovidae, do not suffer from severe clinical disease but become carriers and constitute an important reservoir of infection.

Management, funding and training: Murray and Gray (1984) cited some of the constraints in the effort to control tsetse flies as lack of funds to implement control programs and lack of extension services with personnel trained in how to plan and implement the measures currently available. Likewise, Leak (1999) noted that management or supervision of any tsetse control scheme remains one of the main constraints to their wide spread successful use. Despite community participation schemes, in which affected communities are educated about the target or trap schemes and their beneficial effects, sometimes with degree of success, Leak cited theft as an aspect which has proved to be a wide spread and serious obstacle to successful and sustainable use of traps and targets.

3.4 The role of tsetse in the epidemiology of trypanosomosis

3.4.1 Tsetse flies as vectors of trypanosomes

The causative organisms of trypanosomosis are various species of the genus *Trypanosoma*. These are presented in annex 4. In Africa, certain species of *Trypanosoma* are transmitted from one vertebrate host to another by the blood sucking tsetse flies of the genus *Glossina*. Jordan (1986) noted that the normal hosts of these trypanosome species are wild large mammals of Africa, which, unless stressed, generally do not suffer any pathogenic effect of trypanosomes and that some of these animals can harbour trypanosome infections for long periods of time, perhaps indefinitely. These animals then act as reservoir hosts from which tsetse flies can acquire infections. Once infected, tsetse flies themselves become reservoirs of the parasites, as trypanosomes undergo continuous cycles of development within the fly. In the insect gut only the trypomastigote forms occur and these transform into epimastigote forms in the mouthparts or salivary glands prior to their further transformation into metacyclic forms, the only form which is infective to mammalian hosts. This cycle of development varies in duration according to the species of trypanosome, tsetse species and temperature. In the subgenus *Duttonella* development occurs only in the proboscis, in *Nannomonas* in the midgut and proboscis and in *Trypanozoon* in the midgut and salivary glands. This varying degree of complexity of the cycles of development can, in turn, be related to the rates of infections found in wild populations of *Glossina*. Thus highest infection rates are found with trypanosomes of the subgenus *Duttonella* and the lowest with those of the subgenus *Trypanozoon*. Once forms of trypanosomes infective to the vertebrate hosts have developed, any one fly has the potential to infect any animal from which it subsequently takes a bloodmeal. Transmission of trypanosomes after they have undergone a cycle of development culminating in the production of infective metacyclic trypanosomes is referred to as cyclical transmission, and this is by far the commonest mode of transmission. Leak (1999) noted that only tsetse are known to be capable of transmitting African trypanosomes cyclically, whilst biting insects may transmit them mechanically and are responsible for mechanical transmission of some trypanosome species in Southern America and Asia. Jordan (1986) noted that this circulation of trypanosomes between wild animals and tsetse flies is of no practical significance until man or his domestic animals, which are usually susceptible to the pathogenic effects of the trypanosomes, are introduced into the cycle and become hosts of the parasites, causing sleeping sickness in man or nagana in domestic animals.

3.4.2 Trypanosome infection rates in tsetse

Manifestation of trypanosomes in the flies is determined and expressed as infection rate and is an important factor in the risk assessment.

3.4.2.1 Determination techniques

Dissection Method: Several techniques have been used to examine trypanosome infections in tsetse flies but the fly dissection is probably the most widely used (Otieno, 1983). The method, first introduced by Lloyd and Johnson (1924) is used in routine epidemiological surveys to determine trypanosome infection rates in *Glossina*, despite introduction of other methods such as recombinant DNA technique. This is partly because DNA technique is not simple enough to be performed in most laboratories (Basompen *et al.*, 1996). In the dissection method the identification of the trypanosome species is based on the location of the parasites in the infected fly. The mouth parts (hypopharynx and labrum), the mid gut and salivary glands are dissected and examined separately. Parasites detected in both mouth parts are considered mature *Vivax*, those found in both midgut and mouth parts are identified as *Congolense* while those recovered from mouth parts, midgut and salivary glands are identified as *Brucei* type infections. Some of the inadequacies of the dissection method is that in practice, the procedure is cumbersome and that infected salivary glands are rarely found by this method (Otieno, 1983, Jordan, 1974). Also this method does not allow the identification of mixed infections or distinction of *Trypanosoma* species of the same sub-genera as it is based on location of trypanosomes in internal organs of the tsetse fly (Leak and Rowlands, 1997). For instance, it is not possible to distinguish *Trypanosoma simiae* infections, which are not pathogenic to cattle, from *T. congolense* infections. However, Leak (1999) admits that for determining overall trypanosome infection rates, the technique is reasonably reliable, particularly when data obtained are used for comparison purposes.

Dot-ELISA: This is a monoclonal antibody based test, introduced by Basompen *et al.* (1996) for the diagnosis of the natural trypanosome infection in vectors. The method is capable of detecting and identifying infecting trypanosome species in naturally infected flies when the assay is performed under field conditions. This method is more sensitive than the fly dissection method (Basompen *et al.*, 1996). However, this method has not been widely used in the field. Leak (1999) noted that the method is also less sensitive when used for detecting infections in tsetse

mouth parts. It would therefore be less useful for determining mature trypanosome infection rates, the parameter most important for epidemiological studies. A major drawback to the test is its inability to test each proboscis more than once.

DNA-probes: The technique was introduced by Kukla *et al.* (1987). It is species and subspecies specific involving trypanosome DNA hybridization. The authors indicate that it was possible to detect trypanosomes in organs where parasite development is known to characteristically occur. The technique detects not only the infection in the tsetse fly, but can also identify the strain of the parasite after hybridization with DNA probes specific for each strain. However, the disadvantages of this method, as explained by Leak (1999) include difficulties in distinguishing mature and immature infections. The method is complicated and costly.

Polymerase chain reaction (PCR): Masiga *et al.* (1992) describing this method, explain that amplification of DNA sequences by PCR can be a simple and rapid technique for detecting trypanosomes from the proboscis and salivary glands of tsetse. The method is highly sensitive. However, midgut samples require more complicated purification of DNA. In addition to this complication and high costs involved, Leak (1999) noted that with this method it is also difficult to distinguish between mature and immature infections.

Probing: Infected flies may be examined for trypanosomes, by causing them to salivate whilst probing on to warmed glass slides. This is particularly useful for experimental work with laboratory colonies of flies rather than for determining infection rates in wild flies (Leak, 1999).

3.4.2.2 Factors affecting infection rates

Jordan (1974) reviewing data from various sources came out with a number of possible factors affecting infection rates in tsetse flies.

Age structure: Jordan (1974) had noted that because individual tsetse flies can become infected with *vivax* group and *Congolense* group of trypanosomes throughout their life, older flies are more likely to be infected than younger flies. The overall infection rate of a population of *Glossina* will, therefore, be highest when longevity is greatest. Mohamed-Ahmed and Dairri (1987) in Somalia also reported this correlation. In their study the trypanosome infection rate of

female *G. pallidipes* was directly proportional to the age of the flies. Likewise, Harley (1967) found that 80% of infections were found in flies more than 40-50 days old. Leak (1999) observed that in natural field situations, female tsetse usually have higher infection rates than males; however, this is to some extent because females live longer than males, and therefore have greater likelihood of feeding on an infected host and picking up and maturing infection. When age is taken into account, infection rates of males and females are similar.

Sampling Method: Jordan (1974) noted that different values for the extent to which the same tsetse population is infected with trypanosomes can be obtained from samples of flies collected by different sampling methods. A sampling method which catches particular age groups out of a flies can lead to erroneous conclusions. Harley (1967) found marked differences in infection rate, when he tested different sampling methods simultaneously.

Hosts: A major factor in determining the trypanosome infection rate in a natural population of tsetse flies is the source of food of the tsetse flies. Whether or not the most widely used hosts are good trypanosome reservoirs is of great importance (Jordan, 1974). It is noted by Leak (1999) that as the proportion of feeds taken from suids increases, the population of *Trypanasoma vivax* infection in the tsetse declines. This is because suids are refractory to infection with *T. vivax*.

Species of tsetse flies: Fly species differ in their capacity to transmit trypanosomes. Jordan (1986) noted that although all *Glossina* species that have been examined can habour trypanosomes, not all species could be infected with all species of trypanosomes. The data reviewed by Jordan (1974) suggest that species of *Glossina* that feed primarily on bovidae could be more heavily infected than those species feeding on less satisfactory trypanosome reservoirs. Riordan (1977) obtained possibly the highest trypanosome infection rate ever reported in *G.m.morsitans* caught close to a route along which herds of heavily infected trade cattle in Nigeria passed regularly. In some months of his study, an infection rate of up to 90% was detected. Leak (1999) observed that *morsitans* group flies, except *G. austeni*, are good vectors of all trypanosome species. *Palpalis* group species appear to be poor vectors of most trypanosome species and forest species of the *fusca* group appear to be good vectors of *Trypanosoma Congolense* and *T. vivax* but poor vectors of *trypanozoon* trypanosomes.

Temperature: The temperature experienced by the pupae and young adults has an effect on the infection rates. It has been found in laboratory studies that pupae and young adults of *G.*

morsitans, kept at high temperatures gave rise to mature flies that can develop unusually high infection rates (FAO, 1982, Leak, 1999).

3.4.3 Tsetse age determination

Determination of the age of tsetse flies is crucial for studies of population dynamics. Techniques for aging vector populations such as tsetse flies have been developed for epidemiological and population dynamic studies, and for monitoring control operations. The age structure of a population enables survival or mortality rates to be calculated, and these are essential for estimating the rates of change in population size. These can then be related to seasonal climatic fluctuations or to additional mortality imposed on a population as a result of vector control. For monitoring trap or target control schemes, the declining age of a population will enable the prediction of the time to extinction, or adequate control, of the population (Leak, 1999). Saunders (1962) noted that the age composition of the sample is a guide to their importance as vectors of trypanosomes. This is because many flies that become infected with trypanosomes acquire them at late feeds and having acquired them, retain the infection for life. The epidemiological importance of the vectors therefore increases with age and the highest infection rate will, theoretically, occur within the oldest age group. There have been various studies on the prevalence of trypanosome infections in *Glossina* from the field (Leak and Rowlands, 1997; Woolhouse *et al.*, 1993; Mohamed-Ahmed *et al.*, 1989; Riordan, 1977). Most of these studies have shown the relationship between infection rate and tsetse age categories. Mostly two techniques have been employed in the tsetse age determination in various studies. These are ovarian dissection developed by Saunders (1962) and wing fray by Jackson (1946).

3.4.3.1 Ovarian dissection

Saunders (1962) describing the method, explained that the "ovarian" method of age determination is based upon the changes occurring in the ovaries during gonotrophic cycles. Each ovary of *Glossina* contains two polytrophic ovarioles, and single eggs are produced alternatively from right and left ovaries. Eggs are ovulated sequentially into the uterus at intervals of about 9-10 days. The method depends primarily upon evidence that an ovariole has ovulated. This evidence is either in the form of an open expanded sac in the follicular tube which appears

immediately after ovulation or a small follicular relic (corpus luteum), which remains on the posterior end of the follicular tube after the regression of the open sac. The number and position of these structures combined with the relative sizes of the four egg follicles and the state of the egg or larva in the uterus are then used for the age determination of the fly. Application of this method is straightforward only up to the end of the fourth ovulation, when each ovariole has ovulated once; after this point it is less certain. This work was first done for *G. morsitans* and then applied to *G. pallidipes*, *G. palpalis* and *G. brevipalpis*.

3.4.3.2 Wing fray

The method was first described by Jackson (1946) for aging *G. morsitans*, Westwood. The progressive fraying of the trailing edge of the wing with age gives a reasonable indication of the mean population age. The degree of wear and tear on the trailing edge of the wing is compared with a standard and the age estimated under six categories.

The author provided tables of root mean fray values with which the numbers of flies in each category is multiplied. The products are added and the totals divided by the number of flies in the sample give the Mean Wing Fray Value (MWFV). The corresponding average age is then read from tables.

Harley (1965) advised that the method of determining the physiological age of females of *Glossina* by dissection of the reproductive organs gives a much more precise estimate of the age of flies, at least up to 40-50 days old, than earlier methods based on wing fray and wing colour. Some of the inadequacies of this method is that it can only be applied to females. Also when a fly has ovulated four or more times, the exact number can not be determined, though it can be recognized as having ovulated at least four times. In wing fray technique, though the age of individuals can not be told with any confidence, the mean age of the samples can be estimated (Jackson, 1946). Inadequacies of this method are far more. It suffers possible artificial damage of the wings inside the cage before collection. Trypanosome infections can alter fly behaviour and thus the rate of wing fray in infected flies. The rate of wing fray can vary seasonally and between sexes and species of tsetse (Leak and Rowlands, 1997).

In his studies on wing fray and ovarian age in females of *G. morsitans centralis* in Botswana, Allsopp (1985) confirmed that although the relationship between the two was strong, it varied

from month to month. The rate of wing fraying with age was greatest in the hot dry season and in both very young and very old females, it was affected by humidity. He noted that obviously both age and activity manifest themselves as degree of wing fraying. The more the wings move, either with time (age) or as a result of specific behaviour (activity), the more eroded they become, but it is considered unlikely that wingfray could be used as a reliable measure of either. Nevertheless, Allsopp (1985) admitted that despite limitations of the wing fray method, it is still used as a convenient, if crude, field aging technique and for males, there are few practical alternatives. However, in a most recent study on six species of tsetse flies, Leak and Rowlands (1997) demonstrated a highly significant association between wing fray and ovarian age, with correlation coefficients of up to 0.86-0.90 at two sites in Ethiopia. From this analyses, the authors concluded that wing fray category could be used instead of ovarian age in analysis of the association between trypanosome infection rate and age in tsetse flies.

3.4.4 Tsetse host preferences

Out of a variety of different animals, tsetse flies prefer certain species for their bloodmeals. The knowledge regarding host preference is required for vector control strategies and for detection of *Trypanosoma* reservoirs (Staak *et al.*, 1986). Lamprey *et al.* (1962) showed that in an area of Tanzania, although the mammalian fauna comprised of only 3%, 0.2% and 0.02% of warthog, rhinoceros and buffalo respectively, these species provided 77%, 2% and 14% of *G. swynnertoni* bloodmeals. Animal such as impala, comprising 70% of the fauna, provided only 1% of the bloodmeal. Leak (1999) observed that impala, gazelle, zebra, waterbuck and wildebeest, very common in many tsetse infested areas of Africa, are rarely fed upon by tsetse. Due to these preferences, the knowledge of host preferences of tsetse flies is important in estimating the risk of trypanosomosis in animals. Moloo *et al.* (1980) found that a species such as *G. brevipalpis* takes bloodmeals mostly from unusual hosts e.g. hippopotamus. Therefore, they are of little risk to animals. Because of this, Mihok *et al.* (1992) commented that some of the parasites might not be relevant to livestock transmission cycles. Similarly, Gates and Williams (1984), in their studies on *G. m. morsitans* and *G. pallidipes* feeding preferences, showed that although 12,000 heads of cattle represented 75% of the animal biomass in the surveyed area, they provided only 5.6% of the total bloodmeals, while 74.8% were from warthogs and bushpigs. In such a situation, knowledge on tsetse host preference is even more important in planning appropriate control programs. For instance, Bauer *et al.* (1995) noted that the sole use of deltamethrin pour-ons or

spray on cattle would be very likely to fail in situations where most of the flies feed on hosts other than cattle. Likewise Clausen *et al.* (1998) advised that knowledge of tsetse feeding behaviour is also vital in monitoring the success of tsetse control programs and that the proportion of feeds from wild or untreated animals is likely to affect the success of control programs based on insecticide sprays or pour-ons. However, while it is known that tsetse flies prefer certain species of vertebrate for their bloodmeals, host availability could change this situation. Jordan (1986) noted that there is abundant circumstantial evidence that tsetse species can, in absence of wildlife, support themselves on cattle and perhaps on man. However, this is particularly so with *palpalis* tsetse group.

The preference of tsetse for certain host is detected by serological species identification of the blood recovered from the abdomen of the fly (Staak *et al.*, 1986). Host specific antisera are used to identify the source of vertebrate blood from the gut of tsetse. The concept of serological diagnostic tests is that each vertebrate species possesses one or more plasma proteins with antigenic determinants unique to the species and so identification depends on the ability of antiserum to recognize only the unique proteins of the host's blood (Leak, 1999). A number of such studies have been reported (Weitz, 1963; Clausen *et al.*, 1998; Staak *et al.*, 1986).

4. MATERIALS AND METHODS

4.1 The project and study area

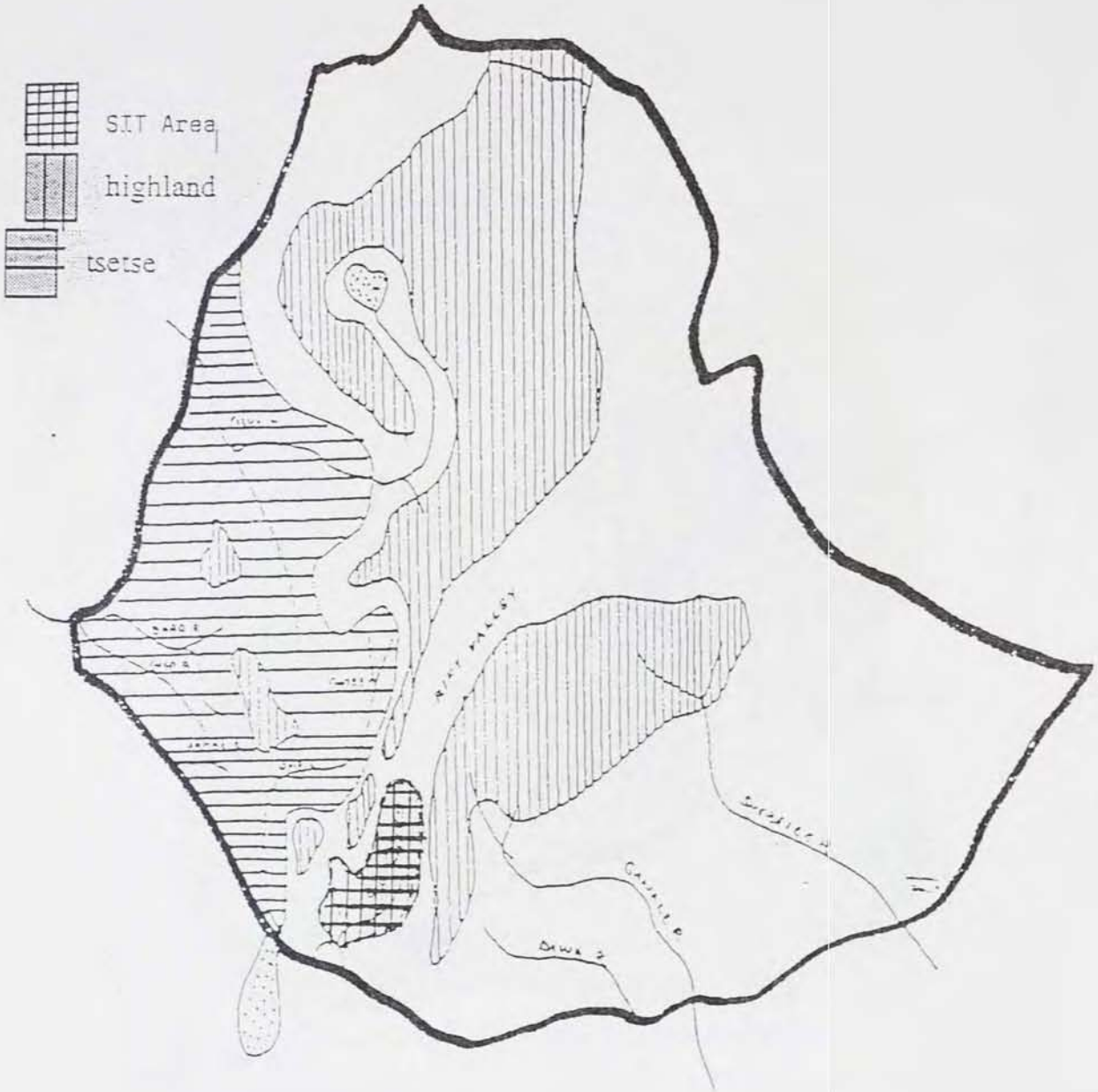
4.1.1 The SIT project area

The study was done between April and September 1999 in the Southern Rift Valley of Ethiopia. The SIT project, which is undertaken by the Southern Rift Valley of Ethiopia Tsetse Eradication Project (SRVETEP) lies between latitude 4° 45" and 7° 15"N and longitude 36° 40" and 38° 20" E (Map 1). It lies between the Eastern and Western walls of the Southern Rift Valley. The area comprises four agro-ecological zones, namely, humid to sub-humid, dry sub-humid, semiarid and arid zones. The project area is divided into three blocks of 6,000km^2, 5000km^2 and 13,000 km^2 (Map 2). Each block is further subdivided into 100km^2 grids. The blocks are then subdivided into five small working project areas, comprising about 24 grids each. There were five working teams, one for each small area, headed by a veterinarian.

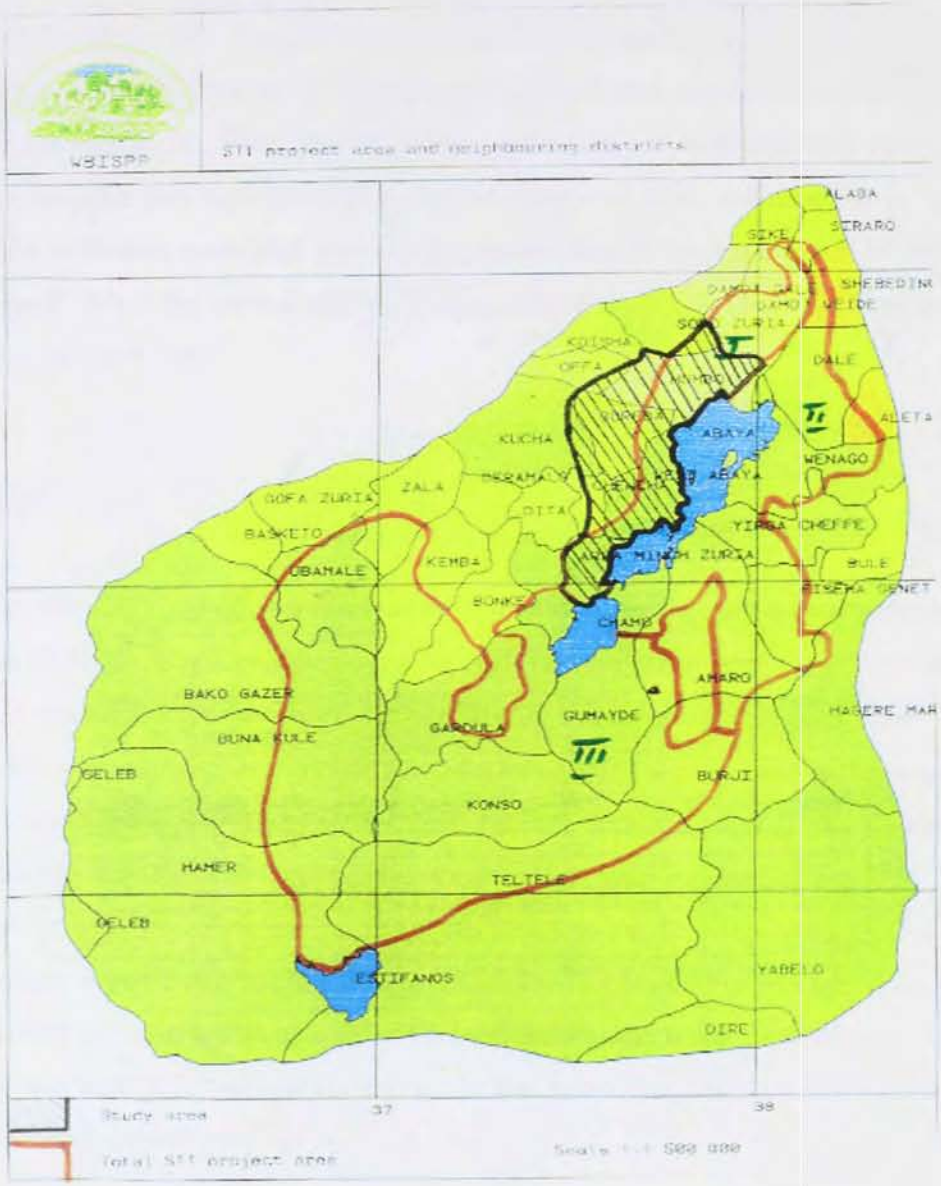
4.1.2 The study area

The study was conducted in the north-western block of the SIT project area, covering a total of 29 grids (2900 km^2), areas in and outside the first control block for the project (Map.2). Investigations in the area outside the control area were meant to assess the potential re-invasion points. Tsetse surveys by the SRVETEP in the area had already started since September 1998. Six different vegetation types were identified in the study area, namely, Forest (FFF), Grassland (GGL), Wooded Grassland (WGL), Cultivated Land (CUL), Bushland (BUL) and Riverine Forest (RFF). The majority of the SIT project area has a bimodal rainfall pattern with short rains occurring in March-May and a major rainfall in July-September (Details see Annex 5). However, during this study period, there was a delay, and the short rains started in mid May joined by major rains in late June. Agropastoralism is the main animal production system in the area. Quite few wild animals were present in the study area including warthogs, impala, leopards, crocodiles and monkeys.

Map 1: A map of Ethiopia showing tsetse distribution and the entire SIT project area.



Map. 2


WBISPP
STI project area and neighbouring districts
ALABA
SIRARO
SEKE
SHEBEDIN
DALE
ALETA
WENAGO
KUCHA
ABAYA
ZALA
GOFA ZURIA
DITA
YIRGA CHEFFE
BASKETO
UBAMALE
KEMBA
BONKE
CHAMO
AMARO
BAKO GAZER
GUMAYDE
GARDULA
BUNA KULE
GELEB
BURJI
KONSO
HAMER
TELTELE
DERC
YABELO
Study area
37
38

4.2 Study design

An epidemiological cross-sectional sampling was used in this study. An average of 25 NG2U-traps (Brightwell *et al.*, 1991) were deployed in such a manner as to cover all the six vegetation types found in the study area. All traps were given an identity and their location was geo-referenced using Global Positioning System (GPS). The area was stratified into three altitude levels, areas between 0-1600 meters above sea level (m asl), areas between 1601-2000 m asl, and areas above 2000 m asl. For convenience in data analysis, in this particular study, this stratification will be referred to as *lowland, mid altitude* and *highland* respectively. Data were collected according to this stratification. Tsetse dissection for both infection rate determination and bloodmeal collection was done twice, first in April-mid-May and repeated in late June-July. The first data collection coincided with the dry season and in this text, it will be will be referred to as "dry-spell" while the second data collection, which also coincided with the rain season will be referred to as "wet-spell".

4.3 Sampling

Transects for the collection of a representative sample of each vegetation type were designed along which 25 NG2U traps were deployed for each 100 km^2 grid. Along the rivers hand-net catching and biconical traps were used additionally for the monitoring of *G. fuscipes*. The trap sites were cleared for sufficient visibility and all trap supports were coated with car-grease to protect fly catches from ants. Traps were checked before deploying and during fly catch collection for any damage. Torn and damaged traps were either replaced or repaired. Observations of wild animals or their activity were also noted. The traps were left for 72 hours after which they were transferred to a different site. Two surveys were conducted during dry spell. In the first survey traps were baited with cow urine alone while a combination of cow urine and acetone was used in the second survey. In the wet period, only one survey was conducted and traps were baited with cow urine alone.

Flies for the estimation of density were collected after every 72 hours, flies for dissection were collected twice a day. Because of the large area covered, this was not at all times possible and some flies therefore remained for more than 24 hours in the traps. These flies were treated

separately when dissected for age determination but were also included in the density estimation. Flies from hand-net catches were not considered for density estimation.

Fly cages from the field were transported to a temporary laboratory made in the field. Here flies were mechanically immobilised, identified, counted and sorted into their respective species, sexes and into teneral and non-teneral flies.

4.5. Dissection of flies

4.5.1 Infection rate

The number of flies (N) (sample size) for dissection to give 95% confidence in determining the infection rate was given by the formula.

$$N = 1.96^2 * Pexp^* (1-Pexp)/d^2$$

Pexp = Expected tsetse infection rate.

$d.^2$ = Allowed error.

The expected tsetse infection rate was determined by dissecting about a hundred flies collected from different sites of the study area, which gave a rough infection rate of 9%. A 4% error was allowed and this gave a sample size of about 200 flies for each comparison made.

The mouth parts (hypopharynx and labrum), midguts and salivary glands were dissected by the method described by Lloyd and Johnson (1924). The trypanosomes were identified according to their site in the fly's body and differentiated into mature and immature to give unbiased infection rates. Trypanosomes recovered in both mouth parts were identified as mature *Vivax* (*Duttonella*) infection, and if only the labrum was infected, this was identified as immature *Vivax*. If both hypopharynx and midgut were infected, then the trypanosomes were identified as mature *Congolense* (*Nannomonas*) group. Infection of mid-gut only was identified as immature *Congolense*. In case all the three parts were infected, then these were identified as mature *Brucei* (*Trypanozoon*) group. Both non-teneral male and female flies were dissected.

4.5.2 Bloodmeal collection and identification

During the sorting and dissections, squash smear was made from all flies found with fresh undigested blood in their gut, on Whatmann filter paper for host identification. The filter papers were first soaked in 0.1% sodium azide solution and air dried to kill bacteria and prevent degradation of the blood during storage. These were then stored in a desiccator. A record sheet for identification with dates and details of collection area, possible hosts and tsetse species were filled and together with the filter papers were packet-sealed in envelops and taken for identification to The National Veterinary Institute (NVI), in Debre-Zeit, Ethiopia.

The bloodmeal identification was based on enzyme-linked immunosorbent assay (ELISA) using host specific antisera as described by Clausen *et al.* (1998) with some modifications. The species specific antisera were obtained from BgVV-Berlin, prepared by repeated immunization of rabbits. Cross-reactivity was either minimized or removed by repeated absorption. The final working dilution was set to be 1:500. A commercially available goat antirabbit IgG-peroxidase (GAR IgG-PO) (Nordic Immunology, Tilburg- The Netherlands) was used as a conjugate-enzyme system at a dilution of 1:1000. The control antigens were pre-diluted at 1:300 in 0.05M carbonate buffer, pH 9.6, while the test samples were eluted from filter papers with 1-3ml in the same carbonate buffer depending on the size and colour of the sample. This elution was assumed to be at a dilution of 1:300. The final working dilution for both was set to be 1:600. Incubation was done for 1 hour at 37 ^{0}C. All washings and the rest of dilutions was done with phosphate buffered saline at pH 7.2 mixed with Tween 20. Azino-di-3-ethyl-benzthiazolinsulfonate (ABTS) was used as a chromogen. 50µl/well was used as a working volume for all reagents. A parallel control plate was run for all tests done. Results were read after 5 minutes with an ELISA reader by comparing the colour and OD reading of the test and control plates.

The bloodmeal identification was done in a 3-series test procedure in which specific antisera for human, ruminant, monitor lizard, suids (domestic pig, warthog and bushpig), horses, camel and avian (series 1) were used. Series 2 involved re-testing the ruminant reactors from series 1 with cross-absorbed antisera specific for bovine, sheep, goat, duiker and impala. Test series 3 utilised specific antisera against dog, elephant, cat, fowl, hippopotamus, crocodile, rat and lion.

4.6 Fly age determination

Before dissecting flies for various studies, fly wings were carefully removed from all flies. Average aging of both males and females was done by categorizing the degree of wear of wings on scales of 1-6, using wing fray method described by Jackson (1946). The first hundred flies dissected for determination of the sample size were aged by both wing fray and ovarian dissection, as described by Saunders (1962). The relationship between wing fray and ovarian categories were determined by regression analysis. The rest of the flies were aged by wing-fray method only.

4.7 Data recording and analysis

The following information were recorded from the dissection results:

- Species of *Glossina* dissected.
- Place and habitat from which the dissected fly was caught.
- Site or location of trypanosome in the fly body (positive dissection).
- Identification of trypanosomes according to their sites.
- Age category according to wing fray analysis.

The total numbers of flies caught for each trap for each day was recorded. The arithmetic mean catches per day per trap for different vegetation type or habitat and for the whole sampling season was calculated to give the apparent densities for different vegetation type, altitude and season. The total counts for different tsetse species were used in estimating the distribution of different tsetse species in the study area. Efforts were also made to get the mean monthly temperature and rainfall. Tsetse challenge (or trypanosomosis risk) was calculated by the method described by Leak *et al.* (1993) as the product of relative density (RD), trypanosome infection rate (IR), and the proportion of feed taken from livestock. Tsetse challenge was used as an index of the numbers of infected tsetse feeding on cattle per unit of time (Leak, 1999). The overall infection rates among the different fly species, infection rate of different trypanosome species, infection rates in different seasons (temporal variation) was subjected to Chi-square (χ^2) test comparisons. Significance was considered at $P< 0.05$.

After $\log_{10}(x+1)$ transformation of data, linear regression analysis and the correlation coefficient (r) was used to determine the relationship between the following parameters:

- Infection rates and age categories
- Age in wing-fray and ovarian categories.

Excel, statgraphics and *EpiInfo* analysis software were used throughout in the data analysis.
The results of bloodmeal analysis for possible hosts for the flies were used to estimate the proportion of bloodmeal provided by different hosts. The results were presented as percentages in tables and histograms.

5. OUTPUT

1. The tsetse challenge (distribution and relative density, tsetse infection rate and feeding patterns) in selected sites of the Southern Rift Valley of Ethiopia has been established.
2. This information will contribute to the assessment of the trypanosomosis risk in the respective areas and give a baseline data for the forthcoming control program.

6. RESULTS

This study had a general objective of estimating the tsetse challenge (Relatve density, infection rate and percentage of bloodmeal taken by tsetse flies from cattle) in the Southern Rift Valley of Ethiopia, and the results are described in detail below.

6.1 Tsetse populations

6.1.1 Tsetse species and distributions

Two tsetse species were detected in North Omo, Southern Rift Valley of Ethiopia, *Glossina pallidipes* and *G. fuscipes*. *G. pallidipes* was the predominant species and it was found in almost all lowland areas under 1600m above sea level. However, this species was not detected in highland areas over 2000m above sea level. It was detected up to 1931m above sea level where two female flies were captured at this point. The species is wide spread and it was detected in all types of vegetation found in the study area including forest, grassland, wooded grassland, cultivated land, bush land and riverine forests. *G. fuscipes* was found confined to river galleries, especially the Deme river and catchment basin, the Gogera-Mancha , Gasa and Woyo rivers. It was also confined to riverine type of vegetation, although it was detected in wooded grassland close to the rivers. This species was detected up to 1710m above sea level.

6.1.2 Tsetse relative densities

Table 1a and b, Fig. 1, 2 and 3, show the mean relative densities of the two species in different vegetation types, altitudes and for the two sexes. There was a marked difference in the relative densities of the two species during the wet and dry spells (Fig.1). During the dry spell, the relative density for *G. pallidipes* was 0.6 flies/trap/day and 0.06 flies/trap/day for *G. fuscipes*. These figures rose to 2.4 and 0.1 for *G. pallidipes* and *G. fuscipes* respectively during the wet spell. There was also a marked preference for vegetation type (Table 1a). Although *G. pallidipes*

was found in almost all types of vegetation, the highest relative density was detected in the bush land vegetation where an average of 44.25 flies/trap/day was recorded. The lowest relative density for *G. pallidipes* was in grassland type of vegetation where only 0.05 flies/trap/day were recorded. This preference was also marked in *G. fuscipes*, which was almost always found in riverine vegetation with a relative density of 0.38 flies/trap/day. It was also detected in wooded grassland closer to the rivers at a density of 0.075 flies/trap/day.

Fig 1: Tsetse relative density in North Omo during wet and dry spells.

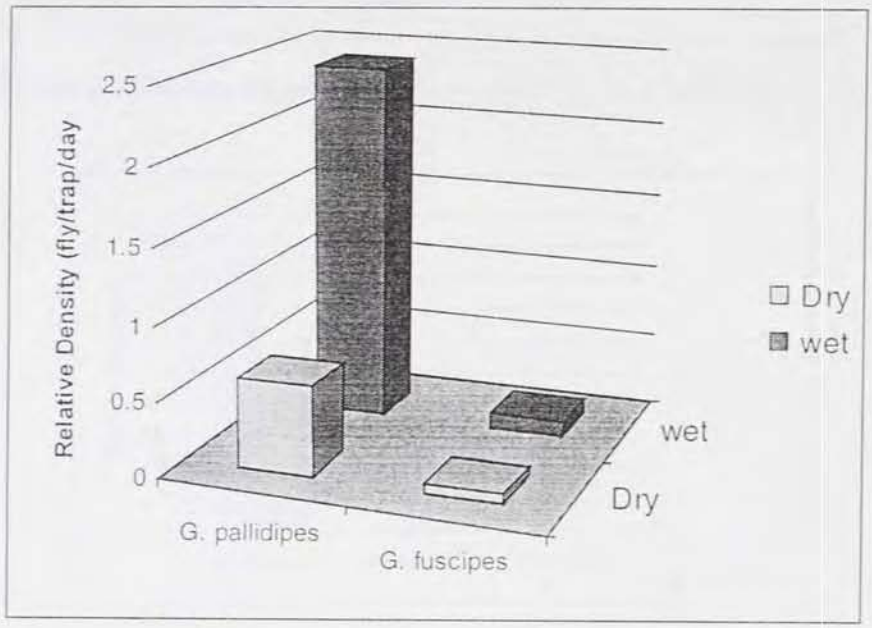


Table 1a: Tsetse relative densities in North Omo in different vegetation types.

Vegetation type	*Glossina pallidipes*		*Glossina fuscipes*	
	3-days catch	Relative density (Flies/trap/day)	3-days catch	Relative density (Flies/trap/day)
Forest	12	0.57	0	0
Grassland	1	0.05	0	0
W. Grassland	1721	1.9	69	0.075
Cultivated land	57	1.43	0	0
Bushland	531	44.25	0	0
Riverine forest	376	3.13	46	0.38

Fig.2: Tsetse relative densities in North Omo at different altitudes in the wet spell.

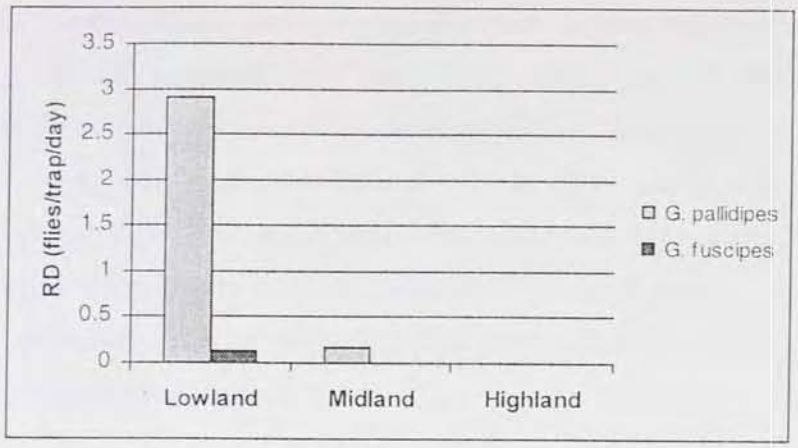


Fig. 3: Tsetse relative densities in North Omo at different altitudes in the dry spell.

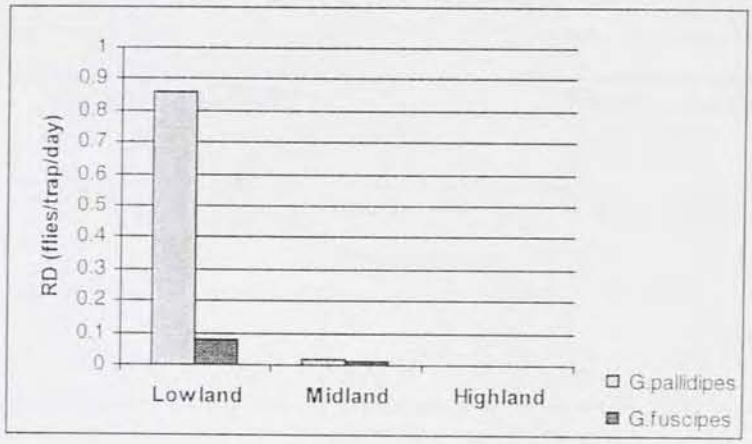


Table 1b: Males and females Relative Densities in North Omo.

Sex	*Glossina pallidipes*		*Glossina fuscipes*	
	3-days catch	Relative density (flies/trap/day)	3-days catch	Relative density (flies/trap/day)
Males	788	0.7	39	0.03
Females	1909	1.7	76	0.07

Altitude has a significant effect on the tsetse distribution and abundance (Fig 2 and 3). During the wet spell, the lowland areas between 0-1600m above sea level recorded the highest relative densities for both *G. pallidipes* (2.9 flies/trap/day) and *G. fuscipes* (0.13 flies/trap/day). At the

midland altitude areas between 1601-2000m above sea level, this figure dropped to 0.17 and 0.01 for *G. pallidipes* and *G. fuscipes* respectively. In the highland areas over 2000 m above sea level no flies were detected during both surveys. For both species, females had higher densities (1.7 flies/trap/day for *G. pallidipes* and 0.07 flies/trap/day for *G. fuscipes*) than males (0.7 flies/trap/day for *G. pallidipes* and 0.03 flies/trap/day for *G. fuscipes*).

Table 2 shows the relative densities from the results of two surveys carried out during the dry spell (January-mid May 1999) at different times in the same area, using NG2U-traps baited with either cow urine alone or cow urine and acetone. The combination of the two odour attractants increased *G. pallidipes* catch by almost five fold but its effect on the *G. fuscipes* was very little as the increase was less than one fold.

Table 2: Tsetse relative densities in North Omo from traps baited either with Cow Urine (CU) alone or Cow Urine with Acetone (CU+A) during the dry spell.

Odour attractant	*Glossina pallidipes*		*Glossina fuscipes*	
	3-days catch	Relative density (flies/trap/day)	3-days catch	Relative density (flies/trap/day)
CU	984	0.6	93	0.06
CU + A	5211	3.13	142	0.08

6.2 Trypanosome infection rates and species

A total of 1567 flies were dissected during this study. About 915 flies were *G. pallidipes* while 652 were *G. fuscipes*. All of these flies were collected from the lowland areas. It was impossible to get flies for dissection from midland and highland areas. Table 3a-c show mature and immature infection rate for the two sexes, times of the year and the infection types.

Table 3a: Mature infection rates for the wet and dry spells and two sexes of Glossina spp. in North Omo with 95% CI.

	Glossina pallidipes				*Glossina fuscipes*			
	N	Infected	%	95%CI	N	Infected	%	95%CI
Dry	389	22	5.6	3.6-8.6	322	25	7.7	5.1-11.2
Wet	526	50	9.5	7.1-12.3	330	26	7.8	5.2-11.3
Male	269	11	4.1	2.1-7.2	375	22	5.8	3.7-8.7
Female	646	61	9.4	7.3-11.9	277	28	9.4	6.8-14.3

Table. 3b. Mature infection type and rate (%) in *Glossina* species in North Omo with 95%CI

Tsetse species	Dissected flies	Mature *Vivax*	Mature *Congolense*	Mature *Brucei*	Overall infections	95% CI
G. pallidipes		50	20	2	72	6.2-9.8
	915	(5.5%)	(2.2%)	(0.2%)	(7.8%)	
G. fuscipes		49	2	0	51	5.8-10.1
	652	(7.5%)	(0.3%)	(0%)	(7.8%)	

Table 3c: Immature infection-types in *Glossina* spp. in North Omo with 95% CI.

Tsetse	Dissected	Immature infections		Overall	95%CI
species	Flies (N)	Vivax	*Congolense*	infections	
G.pallidipes	915	5	23	28	
		(0.5%)	(2.5%)	(3%)	2-4.4
G.fuscipes	652	1	20	21	
		(0.1%)	(3%)	(3.2%)	2-4.9

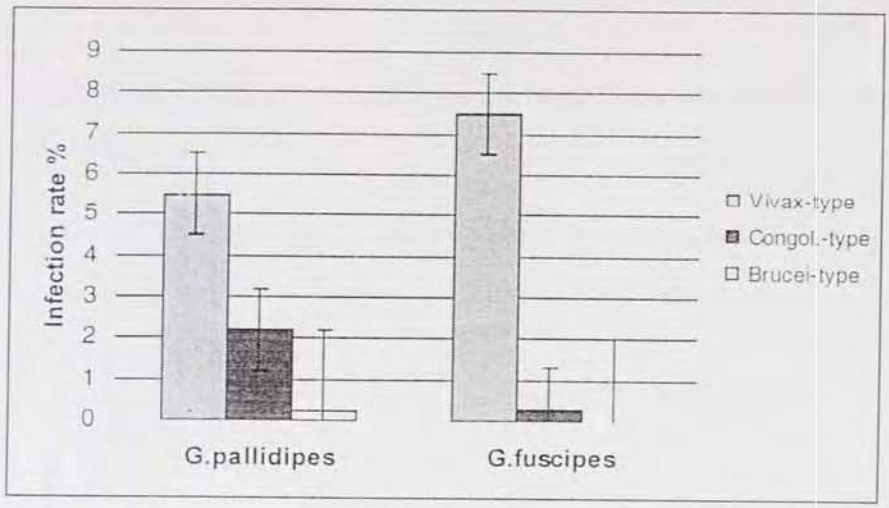
6.2.1 *Glossina pallidipes*

All the three important pathogenic trypanosome species were detected in this fly. The overall infection rate for this tsetse species was found to be 7.9 %. There was a significantly higher infection rate in females (9.4 %) than in males (4.1 %) (χ^2 =6.42, P< 0.05). The infection rate was also significantly higher during the wet (9.5%) than during the dry spell (5.6 %) (χ^2 = 4.57, P< 0.05), (Table 3a). From the overall infection rate, *Vivax* type infection made up 69.4% of all the infected flies, while *Congolense* type made 27.7% and *Brucei* type making only 2.7%. There was a significant difference among the three infection rate types (χ^2 = 50, P < 0.001). The percentage infection rates in all dissected flies were 5.46, 2.18 and 0.22 for *Vivax*, *Congolense* and *Brucei* types respectively (Table 3b).

6.2.3 *Glossina fuscipes*

Unlike in *G. pallidipes*, only *Vivax* and *Congolense* infection types were detected in this tsetse species. *Congolense* type was quite rare and only two flies were found infected with this type. There were no *Brucei* type infections. The overall infection rate was 7.6%. Table 3a shows the infection rates of the two sexes, time of the year and infection types for the species. Females showed a significantly higher infection rate (9.4%) than males (5.8%) (χ^2 = 4.68, P <0.05). However, there was no significant difference between infection rate during the wet (7.8%) and during the dry spells (7.7%), (χ^2 = 0, P > 0.05). *Vivax* type infection constituted 96% of all infected flies while *Congolense* type made only the remaining 4%. The three infection rates showed a highly significant difference among them (χ^2 = 92.89, P < 0.001). *Vivax* type infection was 7.5% of all dissected flies, *Congolense* was 0.03% and *Brucei* type was 0 (Table 3b, Fig 4).

Fig 4: Infection rates of different infection types.



6.2.3 Comparisons between *G. pallidipes* and *G. fuscipes*

The two tsetse species appear to have no significant difference between their overall mature infection rates (χ^2 = 0, P >0.05). However, there was a significant difference between their *Congolense* type infection rate (χ^2 = 9.71, P < 0.05). While in *G. pallidipes* this rate was 2.2% of all dissected flies, it was only 0.03% for *G. fuscipes*. There was no detected significant difference for the *Vivax* type infection between the two species (χ^2 = 2.71, P > 0.05). The difference in the two species for the *Brucei* type infection was also not significant (χ^2 = 1.43, P > 0.05).

6.2.4 Associations between trypanosome infection rates and age of tsetse:

The overall infection rate ranged between 2.7% in wingfray category 1 to 14.5% in wingfray category 6 for *G. pallidipes* and from 2.2% in wingfray category 1 to 32.43% in wingfray category 6 for *G. fuscipes*.

A regression coefficient for a linear term for wing-fray in log-linear analysis of overall trypanosome infection rates for the two tsetse species showed a significant increase in the infection rate with age (Fig 5a and b). In *G. pallidipes*, the correlation showed a significantly strong relationship between the two variables (r = 0.98, P<0.01). *G. fuscipes* had the same trend

with a correlation coefficient (r) of 0.99 ($P < 0.01$). A linear regression analysis for wing fray in log-transformed infection rate for different infection types is shown in table 4.

Further illustrations of this relationship on non-logarithmic scale is shown in various curves in Annexes 10.1a-h

Fig 5a. Relationship between age, indexed by wing fray, and infection rate in *G pallidipes*.

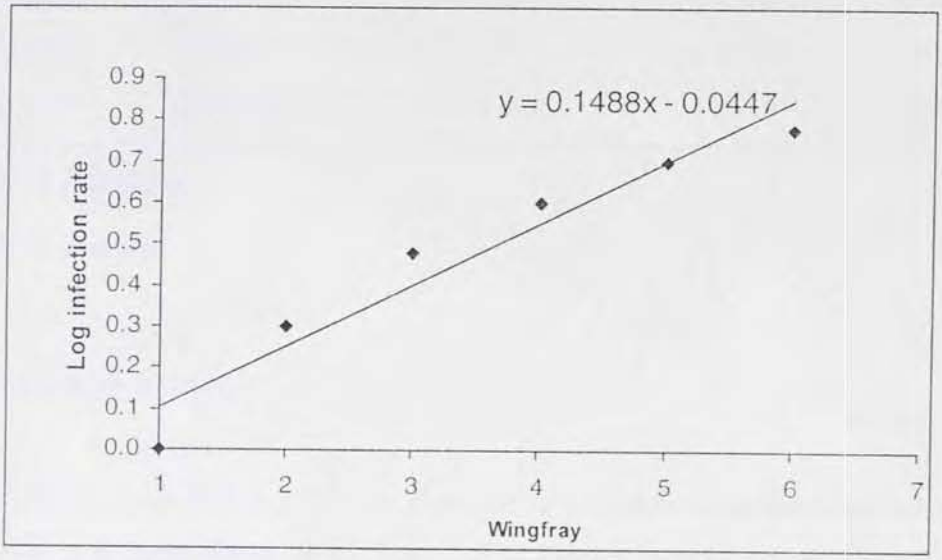


Fig 5b. Relationship between age, indexed by wing fray, and infection rate in *G. fuscipes*

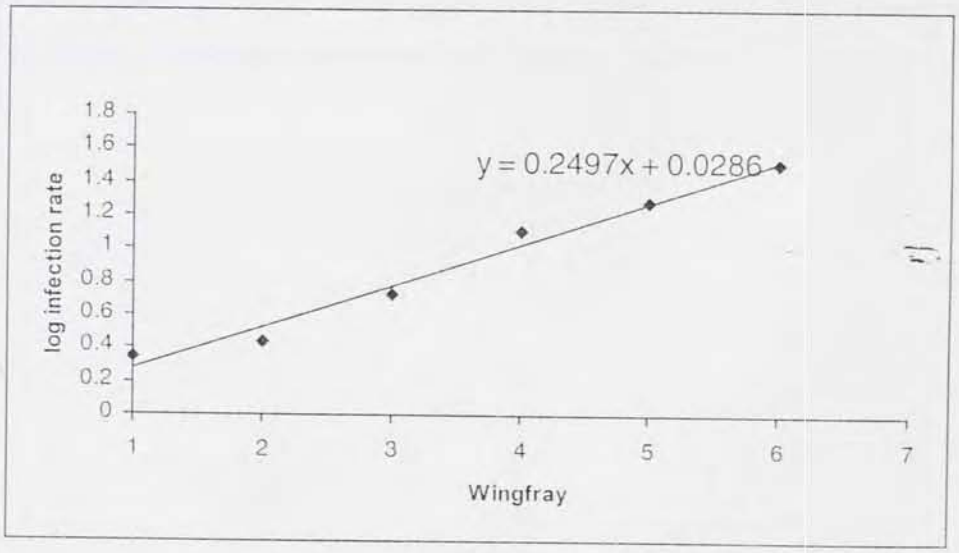


Table 4: Linear regression analysis of age, indexed by wingfray, on log transformed infection rates for different infection types in tsetse flies.

Trypanosome-type	Correlation coefficient (r) ± s.e.	Intercept ± s.e.	Regression coefficient ± s.e.	P-Value
Vivax-type [1]	0.92 ± 0.1	0.22 ± 0.1	0.12 ± 0.02	0.0083
Congo.-type [1]	0.91 ± 0.1	-0.14 ± 0.1	0.12 ± 0.02	0.01
Vivax-type [2]	0.99 ± 0.06	0.03 ± 0.05	0.24 ± 0.01	0.0001

[1] = For *G. pallidipes*.

[2] = For *G. fuscipes*.

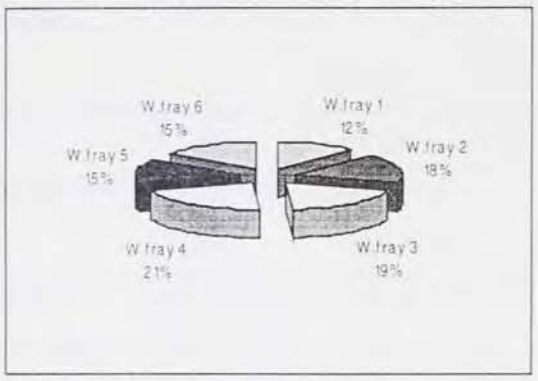
6.2.5 Immature infections

There was no detectable significant difference in immature infection rates between sexes, tsetse species or at different times of the year, and it appears to have no specific relationship with the fly age. While in *G. fuscipes* there was an up and down trend, in *G. pallidipes* the infection rate had almost a constant rate over the fly age (Table 3c and Annex 10h). However, there was significantly higher *Congolense* type immature infections than the *Vivax* type for both species, Table 3c.

6.3 Age and sex composition of the tsetse populations

The trapped flies for dissection showed a significant difference in their age composition. In *G. pallidipes*, the fraction of flies in wingfray 6 was significantly higher in female than in male flies, ($\chi^2 = 7.57$, $P < 0.05$). This fraction was also significantly higher in the wet than in the dry spell in this species. Fig 6 shows the distribution and number of flies in each wing fray category for both species. These results also show that in *G. pallidipes* almost 15% of all the flies were in wing fray 6, while in *G. fuscipes* wing fray 6 had only 6.5% of all the caught flies. Regarding sex ratios, while in *G. pallidipes* the females made a total of 71% of all flies, in *G. fuscipes*, which were caught by hand nets, the females made only 43% (Fig 7). Female *G. fuscipes* caught by traps from the same area, made 66%. Annexes (10.2a-h) show the population structure of tsetse flies.

Fig 6: Percentage number of flies in each wingfray for Glossina spp.



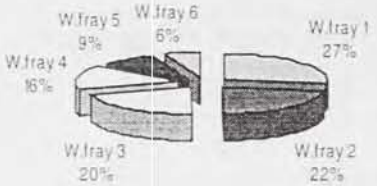
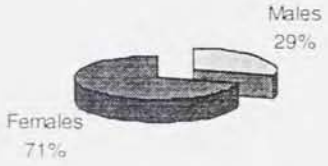
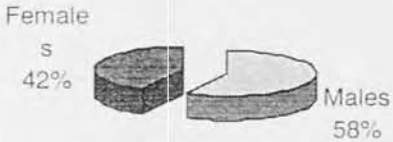


Fig. 7: Percentage number of flies in each sex for the two tsetse species.





6.4 The relationship between wing fray categories and ovarian age

Table 5 shows the relationship between the two variables in *G. pallidipes*. The linear regression analysis of the wing fray categories on the ovarian age indicated a highly significant association between the two ($r = 0.81$, $P < 0.001$). The regression gave an increase of about 0.8 units of wing fray for every unit increase of ovarian age. Although this association was also significant for *G. fuscipes* ($P < 0.001$), the regression analysis gave a lower correlation coefficient (r) of about 0.63. The result of association between the two variables from *G. pallidipes* collected from traps after 48-72 hours gave a much lower correlation coefficient (r) of about 0.59, indicating a weaker association. Most of these particular flies, which were left longer in the traps, had high wing fray categories, had their two wings differing in the extent of fraying, and were therefore difficult to score.

Table 5: Relationship between wing-fray categories and ovarian age in female tsetse dissected in North Omo.

Tsetse species	Mean Wingfray ± s.d	Mean Ovarian Age ± s.d	Correlation Coefficient (r)	Intercept ± s.e	Regression Coefficient ± s.e
G. pallidipes [1]	3.5 ± 1.60	2.85 ± 1.58	0.81	1.16 ± 0.2	0.83 ± 0.06
G. pallidipes [2]	4.3 ± 1.64	1.80 ± 1.20	0.59	2.90 ± 0.4	0.78 ± 0.19
G. fuscipes	2.82 ± 1.60	3.0 ± 1.59	0.63	0.91 ± 0.3	0.63 ± 0.10

1 = Flies collected twice a day.

2 = Flies collected after 48-72 hours.

6.5 Tsetse fly hosts

6.5.1 Blood meal prevalence

G. fuscipes had a higher percentage (12.3%) of fresh bloodmeals than *G. pallidipes* (7.4%). While in *G. pallidipes* males had higher prevalence of bloodmeals (8.5%) than females (7%), in *G. fuscipes* females had higher bloodmeal prevalence (14.4%) than the males (10.7%). Both *G. pallidipes* and *G. fuscipes* had higher percentage of fresh blood during the wet than during the dry spell (Table 6 and 7).

Table 6: Prevalence of bloodmeals by sex.

Tsetse species.	Total flies dissected		Male		Female	
	N	Total Bloodmeals (%)	N	Total Bloodmeals (%)	N	Total Bloodmeals (%)
G. pallidipes	915	68 (7.4%)	269	23 (8.5%)	646	45 (7%)
G. fuscipes	652	80 (12.3%)	375	40 (10.7%)	277	40 (14.4%)

Table 7: Prevalence of blood meals during the first (dry) and second (wet) surveys.

	Total flies dissected		Dry spell		Wet spell	
Tsetse species	Total Bloodmeals		Total Bloodmeals		Total Bloodmeals	
	N	(%)	N	(%)	N	(%)
G. pallidipes	915	68 (7.4%)	389	27 (7%)	526	41 (8%)
G. fuscipes	652	80 (12.3%)	322	25 (7.7%)	330	55 (16.7%)

6.5.2 Identification rate

During the dry spell, a total of 52 bloodmeal samples (27 from *G. pallidipes* and 25 from *G. fuscipes*), were collected and analysed. Twenty-five samples (48%) were identified to either their groupings (e.g. ruminants), families (e.g. bovidae) or species (e.g. cattle). Twenty samples (74%) from *G. pallidipes* and only 5 samples (20%) from *G. fuscipes* were identified. During the wet spell survey, 50 bloodmeal samples, (22 from *G. pallidipes* and 28 from *G. fuscipes*) were analysed. Again, twenty-five samples (50%) were identified. Twelve samples (54.5%) from *G. pallidipes* and thirteen samples (46.5%) from *G. fuscipes* were identified (Table 8).

Fig. 8. Feeding pattern of *G. pallidipes* during dry and wet spells in North Omo.

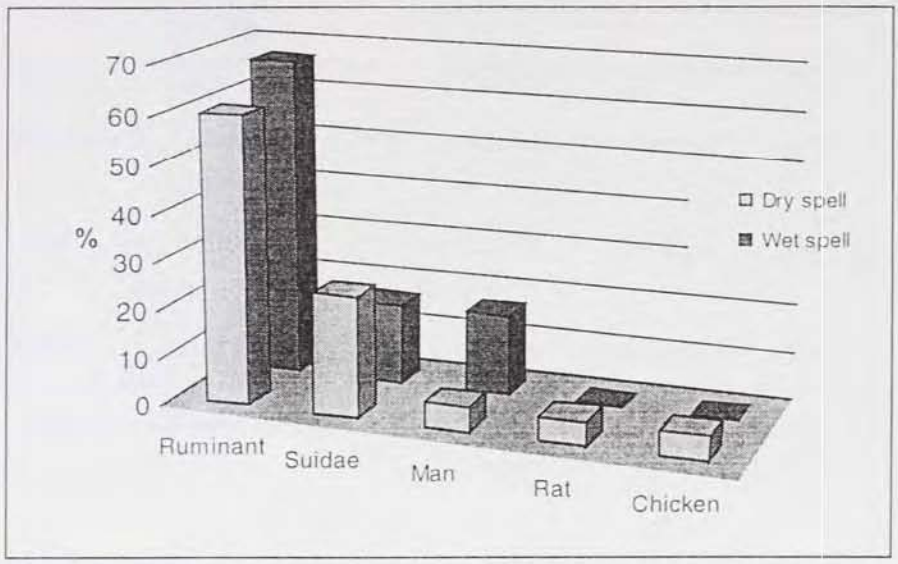


Fig. 9 Feeding pattern of *G. fuscipes* during dry and wet spell in North Omo.

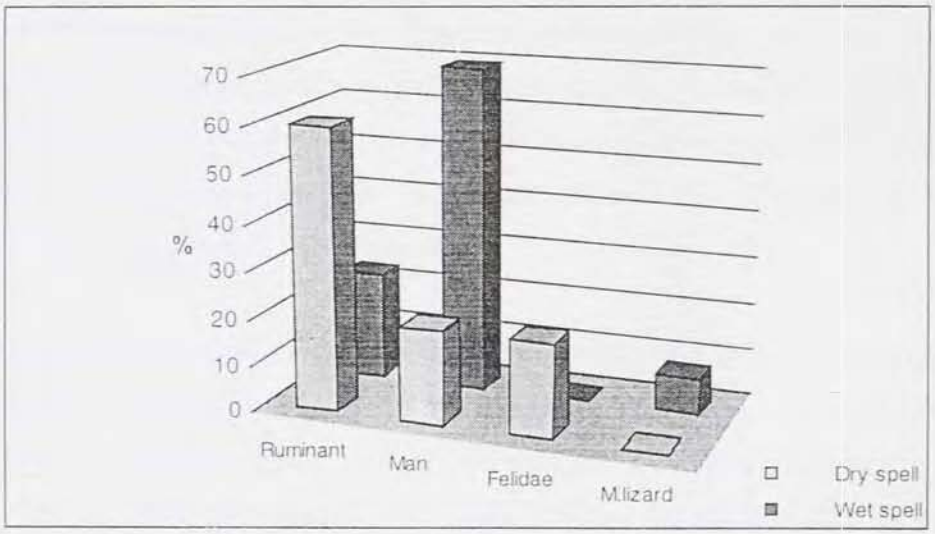


Table 8: Ruminant tsetse hosts as identified by ELISA.

	Dry spell				Wet spell			
	All identified	All ruminant	Cattle	Other ruminant	All identified	All ruminant	Cattle	Other ruminant
G.p.	20 (74%)	12 (60%)	9 (45%)	3 (15%)	12 (54.5%)	8 (66.7%)	8 (66.7%)	0 (0%)
G.f.	5 (20%)	3 (60%)	2 (40%)	1 (20%)	13 (46.4%)	3 (23%)	1 (7.7%)	2 (15.4%)

Other ruminant = specific antisera not available.

6.5.3 Feeding pattern

During the dry spell, the main source of bloodmeal for *G. pallidipes* was identified as ruminants (60%) of which 75% were cattle and 25% from other wild ruminants. Cattle alone made 45% of all identified animals. *Suidae* were identified as the next most important host for this species, giving 25% of the identified bloodmeals. Other minor hosts include man, rat and chicken. In the wet spell, ruminants continued to be the main source of bloodmeal for *G. pallidipes* (66.7%) of which all (100%) were from cattle, making 66.7% of all identified hosts. *Suidae* and man were identified as minor hosts (Fig 7, Table 8).

Although the identification rate for *G. fuscipes* was very low during the dry spell (20%), 60% of the identified bloodmeals originated from ruminants, 66% being cattle and 34% from other wild ruminants. Cattle made 40% of all identified animals. During the wet spell, *G. fuscipes* changed their main host from ruminants (23%) to man (69.2%). Of the identified ruminants, 33.4% were cattle and 66.6% being other unidentified wild ruminants. Cattle made up only 7.7% of all identified hosts. Monitor lizard was identified as a minor host (7.7%) (Fig 8,Table 8).

6.6 Tsetse challenge

The estimates of tsetse challenge, adjusted to include the proportion of bloodmeal taken from cattle and summed up for the two species during the dry and wet periods is shown in Table 9a and 9b

Table 9a: Estimates of tsetse challenge during the dry spell, as a product of relative density, infection rate and percentage of bloodmeal taken from cattle.

Tsetse species	Relative density (flies/trap/day)	Infection rate %	Bloodmeal from cattle %	Tsetse challenge (Index) 95%CI
G pallidipes	0.6	5.65	45	152.5 (132-166)
G.fuscipes	0.06	7.76	40	18.62 (12-25)
Total Challenge				171 (166.5-175.5)

Table 9b: Estimates of tsetse challenge during the wet spell, as a product of relative density, infection rate and percentage of bloodmeal taken from cattle.

Tsetse species	Relative density (flies/trap/day)	Infection rate %	Bloodmeal from cattle %	Tsetse challenge (Index) 95%CI
G. pallidipes	2.4	9.50	66.7	1521 (1473-1569)
G. fuscipes	0.1	7.88	7.7	6 (4.5-7.5)
Total challenge				1527 (1520-1534)

6.7 Summary results

Two tsetse species were detected in the study area, *G. pallidipes* as the predominant species and *G. fuscipes*. During the dry spell the relative density of *G. pallidipes* was 0.6 flies/trap/day and 0.06 flies/trap/day for *G. fuscipes*. During the wet period these figures rose to 2.4 and 0.1 for *G. pallidipes* and *G. fuscipes* respectively.

Of the 915 *G. pallidipes* dissected, 7.9% were infected. The infection was significantly higher in female flies (9.4%) than in males (4.1%). The infection was also higher in the second (wet spell) survey (9.5%) than in the first (dry spell) survey (5.6). All the three important pathogenic trypanosomes were detected, *Vivax* type (5.46%), *Congolense* type (2.2%) and *Brucei* type (0.22%). 652 *G. fuscipes* were dissected and 7.6% were positive. Females had a higher infection rate (9.4%) than males (5.8%). There was no significant difference in the infection rate between the first survey (dry, 7.8%) and the second survey (wet, 7.7%). Only *Vivax* (7.5%) and *Congolense* type (0.03%) infections were detected.

During the first survey (dry), cattle were identified as the main source of bloodmeal for both *G. pallidipes* (45%) and *G. fuscipes* (40%). In the second survey (wet), cattle remained the main source of blood for *G. pallidipes* while *G. fuscipes* changed their main host to man (69.2%) with cattle providing 7.7% of their meals.

The challenge index for the two tsetse species during the dry spell was estimated to be 152.5 for *G. pallidipes* and 18.6 for *G. fuscipes*. These figures rose to 1521 for *G. pallidipes* and dropped to 6 for *G. fuscipes* during the wet period. The overall challenge index during the dry spell was 171 and 1527 for the wet spell.

7. DISCUSSION

7.1 Tsetse populations

7.1.1 Tsetse species and distributions

This study, like the earlier study by Leak *et al.* (1993) in South West Ethiopia, has proved *G. pallidipes* to be the most important vector of pathogenic trypanosomes to cattle in the North Omo, South West Rift Valley of Ethiopia. Most of the flies were caught in the lowland areas and the density decreased as the altitude increased. This supports earlier reports (Langridge, 1976; Tikubet and Gematchu, 1984) that climate, which is largely influenced by altitude, has an impact on tsetse populations. Observations in Southern Africa, where the rainfall is high, the limits of Glossina are related to seasonal low temperature and tsetse flies do not occur above about 1800m, but the upper limit decreases with distance from the equator. Langridge (1976) reported 1600 meters above sea level to be the possible rough upper altitudinal limit to tsetse distribution in Ethiopia. However, later Tikubet and Gematchu (1984), caught *G. pallidipes* at 1700 m altitude. In this study, this species was caught up to 1930 m altitude. This is an indication of further advancing of *G. pallidipes* to higher altitude, posing trypanosomosis risk to areas previously free from the disease. *G. fuscipes* was caught up to 1710 m asl, and was confined to rivers or areas near to the rivers. Leak (1999) had noted that *G. fuscipes* has poor water-proofing and inadequate water reserves and is therefore confined to hygrophitic habitat and that it is rarely found far from open water in lucustrine or riverine habitat.

7.1.2 Tsetse relative density

Observations showed that the relative density of *G.pallidipes* increased during the wet period. Mohamed-Ahmed and Dairri (1987) and Leak *et al.* (1987) reported similar results. This could suggest an absolute increase in the number of tsetse flies due to favorable environment, enough moisture, vegetation growth and more suitable habitat or spread of the flies from the rivers and

thickets, where they usually inhabit during the dry season, to more open areas during the rain (Brightwell *et al.*, 1992) and therefore increasing the density in the open areas. Leak *et al.* (1993) also cited the latter as the possible reason for the high densities of *G. pallidipes* obtained during dry season when the traps were deployed in the Ghibe river valley, South Western Ethiopia.

7.1.3 Effects of odour attractants on the relative density

Various studies, (Hassanali *et al.*, 1986; Vale *et al.*, 1988) reported that addition of phenols to acetone increase *G. pallidipes* catches by several times. This study reflected similar results, when cow urine alone or a combination of acetone and cow urine was used for *G. pallidipes*. Similarly, an unpublished report by Leak (ILRI, 1995) showed a 2-3 fold increase in the catch of *G. pallidipes* when a combination of cow urine and acetone was used in Ethiopia. This study supported the suggestion by Leak (1999) reviewing tsetse ecology, that at present attractants are most effective for *morsitans* group tsetse, whilst further improvements are needed for attractants of *palpalis* tsetse group. In this study, the combination of the two odour attractants increased the catch for *G. pallidipes* by about 5x while the increase for *G. fuscipes* was less than 1x.

7.1.4 Sex ratios and age composition of the caught flies

The results showed higher percentage of females than males in the catches. 71% of *G. pallidipes* and 66% of *G. fuscipes* caught by NG2U-traps were females. Mohamed-Ahmed and Dairri (1987) and Allsopp *et al.* (1972) obtained similar results. Leak (1999) reported that the sex ratio of tsetse flies emerging from puparia are close to unity, but as females live longer than males, more females are generally found in a representative adult population sample. He noted that in an unbiased sample, females would comprise between 70-80% of an average population. However, female *G. fuscipes* caught by hand-net in this study made only 42%, with the males making the majority. This is again similar to other earlier observations made by Vale (1993) and Okiwelu (1977). Vale (1993) reported that hand-net catches from human or other baits accompanied by hand-net catchers, consisted mainly of males. He showed that the only host-like bait that gave fair representation of the relative abundance of each sex were traps. A sample of *G. pallidipes* caught by vehicular trapping by Gates and Williamson (1984) gave only 47% females. The sex ratio is therefore largely influenced by the sampling technique. Since in an unbiased sample females

comprises of 70-80% (Leak, 1999), then it can be concluded that stationary trapping is a relatively unbiased sampling technique.

Generally, in this study, *G.pallidipes* showed an even distribution of number of flies in all age categories. *G. fuscipes* showed a different age distribution of the flies in which about 50% of all the flies appeared to be young with wing fray category of one and two. Katunguka-Rwakishaya and Kabagambe (1996) and Mohamed-Ahmed and Dairri (1987) made similar observations for this species. This could reflect the behaviour of *G. fuscipes* being confined to the rivers with short distance movements and therefore the rate of fraying of the wings is very low resulting into low wing fray categories. In contrast to results obtained by Mohamed-Ahmed and Dairri in which females were not included, this study indicated much more older flies in wet than in the dry spell. This can be expected to occur biologically, as flies are likely to live longer during the rains as a result of enough moisture and more suitable habitat. Females also had higher proportion of older flies than males. This could explain higher infection rate in females observed in *G. pallidipes*.

7.2 Tsetse infection rates

G. pallidipes showed significantly higher infection rates in the wet than in the dry spell. Although it was not significant, Mohamed-Ahmed and Dairri (1987) reported similar results. Similarly, Harley (1965) obtained the highest infection rate in *G. pallidipes* during the wet season with warm temperature. This could reflect either an increase in mean age of the flies during the rain as a result of more suitable environment or availability of infective vertebrate hosts as a source of bloodmeal during the rains. Woolhouse *et al.* (1994) reported significant fluctuations of infection rate with time even after age effect was excluded and therefore ruled out the changes in age as the only cause of infection rate fluctuations. Increase in temperature could also have a significant effect on the fluctuations in tsetse infection rate. Laboratory studies have shown that increase in temperature during the pupal stage could result into higher infection rates in the adult emerging from these pupae (FAO, 1982). However, other studies showed no significant relations with the rains. Woolhouse *et al.* (1993) obtained a significant temporal fluctuation in infection rate that correlated negatively with temperature and rain fall. In this study, *G. fuscipes* showed no significant difference in the infection rates between the two seasons. This could indicate that unless there is extremes of long rain or dry weather, season has no significant effect on the life

span or infection rate of *G. fuscipes*. This can be expected to occur naturally as this species is always confined to the rivers with enough moisture and suitable vegetation cover. Leak (1999) noted that many studies on the effect of climate on *palpalis*-tsetse group showed that cumulative effects of a series of long rain seasons or long dry seasons are thought to have been of importance in influencing the population of this tsetse species.

The infection rates were higher in females than in males for both *G.pallidipes* and *G.fuscipes*. Leak and Rowlands (1997) reported significantly higher *Vivax*-infections in females than in males. Leak (1999) suggested that in the field conditions, infection rates are usually higher in females than males from the same resting sites since the average life-span of males (one month) is shorter than that of females (2-3 months). Moreover, females usually take bigger meals and at shorter intervals. Other studies (Mohamed-Ahmed *et al* 1989, Woolhouse *et al.*, 1993) found no significant difference between the two sexes. However, in this study females showed higher percentage of old flies (flies in wingfray 6) than in males. This could explain the difference observed in the infection rates.

The infection rate in *G. fuscipes* reported in this study is higher than that reported by Leak and Rowlands (1997) in Ghibe Valley. However, both the animal trypanosome prevalence and vector-host contact, reflected by high bloodmeal prevalence for this species, reported in this study are all high. These two factors could explain the high infection rate observed in this species.

In this study, there was a significant variation in the overall infection rates with age, indexed by wingfray. This was also reported by Woolhouse *et al.* (1994). For both *G.pallidipes* and *G. fuscipes*, there was an increase of *Vivax* type infection with age. *Congolense* type infection showed a similar trend in *G. pallidipes*. Several studies (Mohamed-Ahmed and Dairri, 1987; Leak and Rowlands, 1997; Woolhouse *et al.*, 1993, 1994) reflected similar results, supporting earlier suggestions (Jordan, 1974) that flies could continuously be infected at any time of their age. This is however in contrast to laboratory results by Welburn and Maudlin (1992) which suggest that tsetse flies acquire infection of *Trypanozoon* and *Nannomonas*-type in the first bloodmeal and that the later feeds do not contribute much to the trypanosome infection rate in tsetse populations. They concluded that tsetse flies, once fed, remain relatively (although not absolutely) refractory to infections. Other laboratory results by Wendy and Venessa (1992) showed that about 15% of the infected flies had established an infection from trypanosomes picked on the second or third feed.

In this study, *Vivax* type infections were the most common, followed by *Congolense* type. *Brucei* type were the least common and it was completely absent in *G. fuscipes*. This is in agreement with several other earlier studies (Harley, 1965; Leak *et al.*, 1993; Woolhouse *et al.*, 1994). Some workers (Leak and Rowlands, 1997) attributed this phenomena with the long developmental route in an unfavorable tsetse gut environment, with anti-trypanosome factors, such as lectins, for both *Congolense* type and *Brucei* type and also other barriers for *Brucei* type during its movement to the salivary glands. Others (Baldry, 1969; Otieno, 1983; review by Jordan, 1974) attributed this to the inadequacy of the dissection method and the difficulties in dissecting the salivary glands intact. Indeed, Otieno (1983) obtained three *T. brucei* trypanosomes from supposedly *T. Congolense* after mice inoculation of triturated mouth parts which showed mature *Congolense* type infection, and questioned the adequacy of the dissection method in estimating tsetse infection rates. However, the former reason could also hold since Welburn and Maudlin (1992) laboratory results showed inhibition of tsetse gut colonization by *Congolense* infections after taking the first uninfected bloodmeal. Other studies also showed *Congolense* type infection to be more common than *Vivax* type during the rainy season (Mohamed-Ahmed and Dairri, 1987) but flies were reported to have been feeding on warthogs, which are said to be refractory to *Vivax* infections (Jordan 1965). Flies in this study fed mostly on ruminants.

Glossina fuscipes has been reported to be a poor vector or refractory to *Congolense* type infections (Leak and Rowlands, 1997). Leak *et al.* (1987) reported 92% of the trypanosome infections to be from *T vivax* in a site where only *palpalis* group tsetse flies were found. In this study 96% of trypanosome infections were from *Vivax* type, with *Congolense* type making only 4%. In *G. pallidipes* on the other hand, *Congolense* type infection made about 28% of the infections. If this is the case, this could be an important finding in the epidemiology of bovine trypanosomosis.

7.3 Relationship between age in wing-fray and ovarian categories

The result demonstrated a highly significant association between age in wingfray and ovarian categories for *G. pallidipes* with regression coefficient (r) of 0.81. Leak and Rowlands (1997) reported correlation coefficient of 0.86-0.90 for six species in Ethiopia, including *G. pallidipes*. In this study, *G. fuscipes* showed a lower relationship (r) of 0.63 despite catching by hand net and

immediate dissection and therefore expected low damage of wings by the traps contrary to *G.pallidipes* which were caught by traps. The possible explanation for this observation is that *G. fuscipes*, being confined to the rivers, has very short distance movements, consequently a low rate of wear and tear of their wings, and therefore low wing-fray categories. A fly may therefore have high ovarian category, but very low wing-fray category as a result of limited movements. *G. pallidipes* collected from traps after 48-72 hours, showed much lower correlation of 0.59. This result proved earlier suggestions by Leak and Rowlands (1997) that wings could be artificially damaged in the cages before collection. It can therefore be concluded here that wing-fray can be used in estimating the mean tsetse population age instead of ovarian dissection, provided the flies are collected frequently from the traps and are not left for more than 24 hours. The wingfray method is more reliable in savanna species than in riverine species.

7.4 Tsetse fly feeding preference

7.4.1 Prevalence of bloodmeals

Generally, the results suggest that on average *G. fuscipes* had a higher prevalence (12.3%) of fresh bloodmeals than *G. pallidipes* (7.4%). This could reflect the high vector-host contact in the strategic sites of *G. fuscipes* in rivers where animals and humans are present most of the time. These findings could also explain the high infection rate observed in this riverine species. In *G. pallidipes*, although traps caught more females than males, males had higher frequencies (8.5%) of fresh bloodmeals than the females (7.4%). This was not expected, as females are known to feed more frequently than males due to their greater energy needs. Gates and Williams (1984) reported higher incidence of bloodmeals in females than males in a sample of *G. pallidipes* caught by vehicular trapping. With mobile traps, Vale (1974) also obtained higher percentage of recent fed females than males. The finding in this study may indicate, however, that fed males are more active than females. For both *G.pallidipes* and *G. fuscipes*, there were higher fresh bloodmeal frequencies in the wet than it was in the dry spell, possibly due to a better availability of hosts in the rains. Higher infection rates in the flies were also detected in the rains. This is in agreement with earlier finding by Katunguka-Rwakishaya and Kabagambe (1996) who suggested that high prevalence of bloodmeals in the warm humid months during their study could suggest

high transmission to mammalian hosts during this period if trypanosome infection rates in tsetse are high.

7.4.2 Feeding pattern

The present study identified ruminants as the most preferred host for *G. pallidipes* providing 60% and 67% of the feed to the fly during dry and rain season respectively. *Suidae* and man were identified as the next important hosts. Leak et *al.* (1993) reported similar results in Ghibe Valley, Ethiopia. As regard to *G. fuscipes*, cattle appeared to be preferred most during the dry period. Interestingly, this species shifted from cattle to human during the rains. The possible explanation for this observation could be that during the dry spell cattle were frequently seen grazing along the rivers, which were the only places they could get green pastures. After the rains, the green pastures were available in many open places; therefore cattle were no longer available for this river fly. This shift of *G. fuscipes* to humans coming to the rivers as their only source of water could suggest high risk of human sleeping sickness during the rains. Tikubet (1993) had already warned that the presence of *T. brucei gambiense* is strongly suspected in the nearby Gambelle regions of South Western Ethiopia. This behaviour of *G. fuscipes*, together with the low identification rate observed in this species (despite a better quality blood and higher bloodmeal prevalence than in *G. pallidipes*) could indicate a wide range and uncommon hosts of this species. Clausen *et al.* (1998) also reported this opportunistic feeding behaviour of *G. fuscipes*. Allsopp *et al.* (1972) reported that in the presence of many game animals, bushbuck is the most preferred host of *G. pallidipes* among the wild animals. Indeed, it appears that in the presence of game animals, *G. pallidipes* will go for them rather than the livestock. Clausen *et al.* (1998) and Staak *et al.* (1993) reported the hosts for most of *morsitans* group Glossina, including *G. pallidipes* to be wild ruminants (bushbuck and buffalo) rather than livestock (cattle). Similarly, Gates and Williams (1994) reported that although cattle provided 75% of the animal biomass in the surveyed area, bushbuck provided about 14% while cattle provided only 8% of the bloodmeals. In this study, very few wild animals were observed in the study area, eg warthogs, antelopes and monkeys. This could give an explanation for the absence of wild ruminants in the identified hosts of the flies in this study. This supports the earlier report that host preference is influenced also by their availability. Jordan (1986) noted that there is abundant circumstantial evidence that tsetse species can, in absence of wildlife, support themselves on cattle and perhaps on man.

The information on tsetse feeding behaviour is not only important in trypanosomosis risk assessments and control programs in livestock, but it gives also an indication of the potential risk of human sleeping sickness and the part played by various hosts in the transmission cycle.

7.5 Relationship between tsetse challenge and trypanosome prevalence in cattle

Although there were few points for the statistical inference to be made on this relationship, the results of a parallel study conducted in the same area showed high trypanosome prevalence during period of high tsetse challenge. Trypanosome prevalence in cattle in the study area was 22.7% during the dry spell and 29.9% during the wet spell (Karanja, pers com).

7.6 Limitations and possible sources of errors in the study

7.6.1 Sampling techniques

Certain categories of the populations, e.g. age group, hunger stage, pregnancy stage, are more active than others and respond differently to the sampling method, therefore samples tend to be biased (FAO, 1992). Robinson (1995) also had observed that there are considerable biases in the fraction of the tsetse population that is sampled by different methods. For instance, in an area where *G. morsitans* and *G. pallidipes* are both present, traps tend to catch more *G. pallidipes*, biased towards older females, while mobile techniques tend to catch more *G. morsitans* and are particularly efficient for male flies. He noted that mortality in the collecting devices adds another bias to sampling since certain flies of different species, sex, age and stages in reproductive cycles may be more susceptible to death through exhaustion or desiccation and will therefore tend not to be dissected. However, some of the factors which could affect infection rate in tsetse are the sex and age composition. Older flies and females, which live longer than males are expected to have higher infection rates. To minimize biases, NG2U traps were used in the sampling. Traps are known to be the most unbiased sampling method for the relative abundance of both sex (Vale, 1993) and age (FAO, 1992). NG2U traps are also efficient for savanna species. Efforts were also

made to collect flies for dissection from the traps as frequently as possible to avoid mortalities of certain categories of the population.

Hand nets were used for catching *G. fuscipes* for dissection in determining the infection rate after traps failed to get enough flies for this species. The method proved to be very biased towards males. Murray *et al.* (1983) had noted that fly round sampling usually produces a grossly distorted sex ratio in favour of males because sexually appetitive as well as hungry males are highly responsive to moving objects. This study confirmed infection rates in males to be lower than in females and as catches of males were higher due to the sampling technique used, this could result in low overall infection rate in this species and therefore be a possible source of error.

7.6.2 Estimation of infection rate

The dissection method was used for this purpose. With this method, it is not possible to identify mature mixed infections since the identification is based on the location of the parasites in the body of the tsetse fly. In case of a mixed infection of *Vivax* and *Congolense* type for example, this will be identified as *Congolense*, and therefore underestimate *Vivax* type infections. Also the technique can not distinguish mixed mature and immature infections of different subgenera. For instance, a mixed infection of mature *Vivax* and immature *Congolense* will be identified as *Congolense* type infections and therefore over estimating *Congolense* infection. It is also not possible to distinguish trypanosomes of the same subgenera. For instance, it is not possible to distinguish *Trypanosoma simiae* infections, which are not pathogenic to cattle, from *T. Congolense*, since they all appear in the same site. This could over estimate the risk to cattle.

7.6.3 Tsetse age estimation

The results from the wing fray method could be severely affected if the flies are not collected frequently from the traps. This is because fly wings tend to be artificially dammaged as flies struggle inside the cages. However, to minimize this, *G.pallidipes* flies were collected twice a day, while *G. fuscipes* were collected by hand net and dissected immediately.

8. CONCLUSIONS AND RECOMMENDATIONS

1. From the tsetse species distribution, abundance, infection rate and bloodmeal analysis the tsetse challenge in the different altitude strata defined for this study can be categorised as follows:

Highlands (areas above 2000 m)	No challenge
Midlands (1601 - 2000 m)	Low to medium challenge
Lowlands (0 - 1600 m)	Medium to high challenge

The challenge was higher during the wet than during the dry period and it corresponded well with the trypanosome prevalence in animals. *G.pallidipes* was identified as the main vector of the pathogenic trypanosomes and it had advanced as high as 1930 m, thus posing a risk also to areas considered tsetse free in earlier studies. As in general "the highlands of Ethiopia" are considered to be altitudes above 1700 m, a low challenge has to be assumed there as well.

2. From the results it can be concluded that the population of *G.pallidipes* and *G.fuscipes* in the Southern Rift Valley of Ethiopia do not differ much from other populations of the same species in Africa in terms of infection rate, infection type, relationship between infection rate and age, population structure and the host preference.

3. The knowledge of tsetse host preference is not only important in trypanosomosis risk assessments and control programs in livestock, but it gives also an indication of the potential risk of human sleeping sickness or the part played by various hosts in the transmission cycle. Since this study was not exhaustive, further studies are therefore recommended in this field. Identification of tsetse bloodmeals requires a well established laboratory. Since at present there is no laboratory carrying out bloodmeal analysis routinely in the region, transfer of this technology to Ethiopia is strongly recommended.

4. According to these assessments, *G.fuscipes* appears to pose a low risk, due to its low preference for cattle and possibly the low ability of *Congolense* infections, an important parasite in cattle, to mature in it. However, *G.fuscipes* needs to be controlled also as it is

still a risk to human populations living along and depending on the rivers for their daily water needs.

5. It is recommended to carry out further studies on the vectorial capacity of *G.fuscipes* for *T.congolense* in the project area to determine its importance in the epidemiology of bovine and human trypanosomosis.

6. It can be concluded from the study that suppression and monitoring of *G.pallipides* can be done using the NG2U trap. For *G.fuscipes*, however, both NG2U and biconical traps produced only low catches which were increased in this study by handnet catching, a biased and cumbersome method. It is recommended to investigate into a more effective trap for this species.

7. The tsetse survey results as well as those of the other study on trypanosome prevalence (Karanja, 1999, pers. comm.) underpin the assumptions made by the Ethiopian Government which led to the establishment of SRVETEP. High tsetse densities, wide distribution of flies in the project area, high infection rates which correspond well with high trypanosome prevalence in animals in addition to increasing drug resistance in South Western Ethiopia (Codja *et al.*, 1993) depict a situation which makes vector control the most promising approach to trypanosomosis control.

8. The project has opted for SIT as the ultimate control method. It appears that the *G.fuscipes* population in the north-western corner of the project area justifies the production of sterile males of *G.pallidipes* as well as of *G.fuscipes*.

9. The study identifies a potential re-invasion point for *G.fuscipes* along the river Gogera. This finding needs to be considered when planning the control strategy for the project.

10. Although this study was not conducted for the whole year, the results presented here give an indication of the variations in trypanosomosis risk between the dry and rainy season in the project area.

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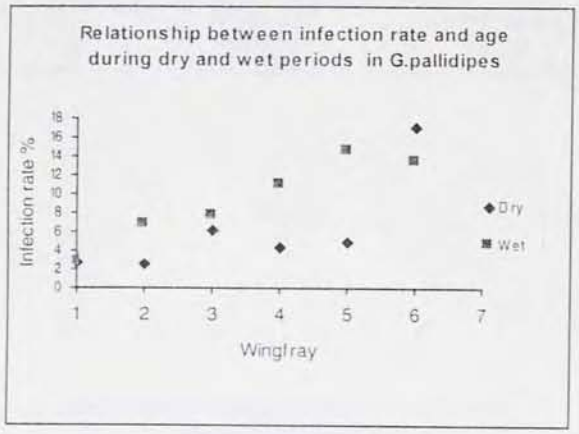
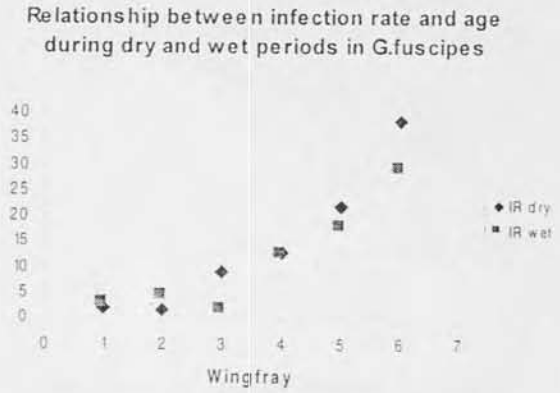
10. ANNEXES

Annex10.1a-h: Estimated curves for relationship between tsetse infection rates and age in wingfray in non-logarithm scale.

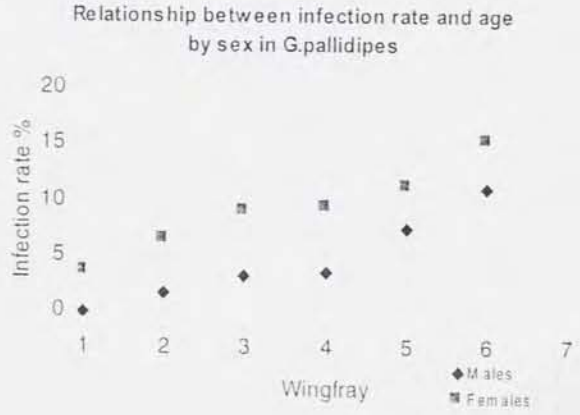
a.



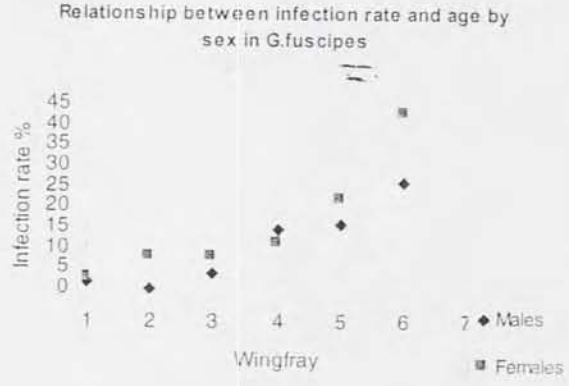
b.



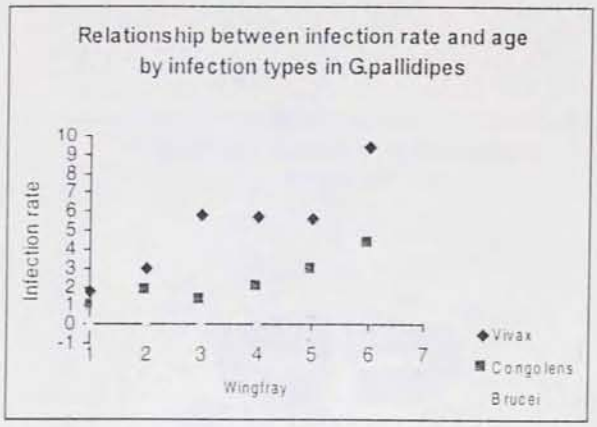
c.



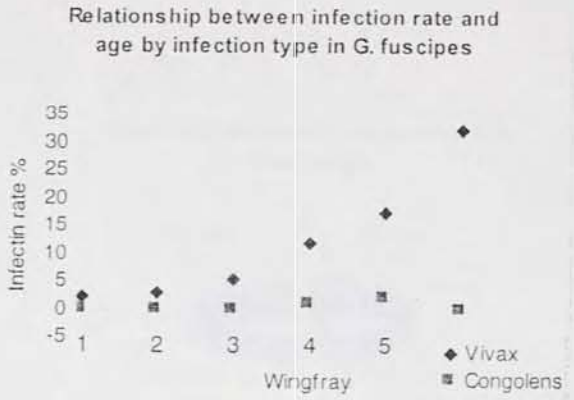
d.



e.


Relationship between infection rate and age by infection types in G.pallidipes
Infection rate
Wingfray
Vivax
Congolens
Brucei

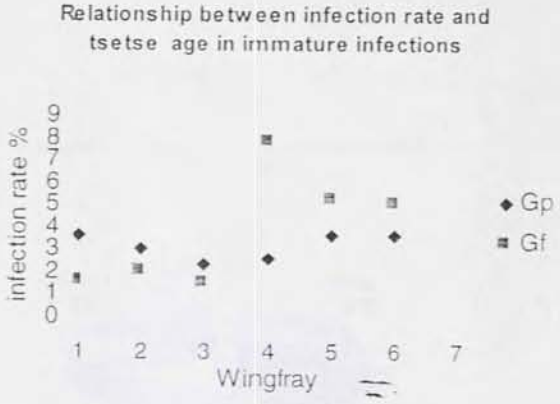
f.


Relationship between infection rate and age by infection type in G. fuscipes
Infectin rate %
Wingfray
Vivax
Congolens

g.

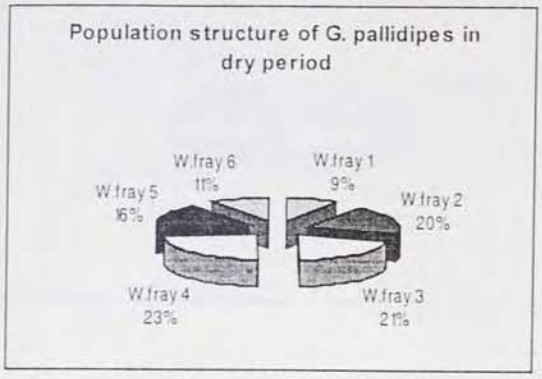

Relationship between age and infection rate in Glossina spp.
Infection rates %
Wingfray categories
G.p
G.f

h.

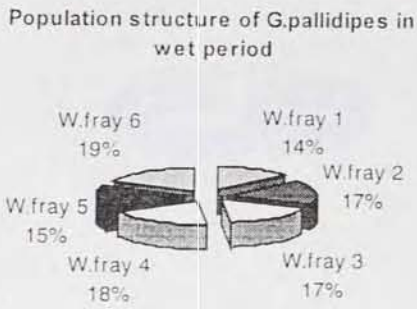

Relationship between infection rate and tsetse age in immature infections
infection rate %
Wingfray
Gp
Gf

Annex.10.2. a-h: **Population structure of tsetse**

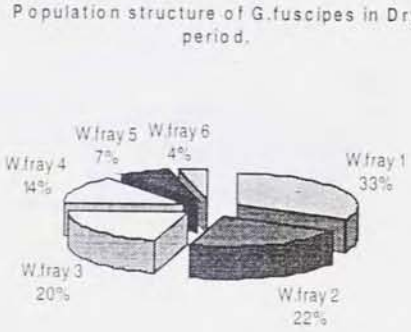
a.



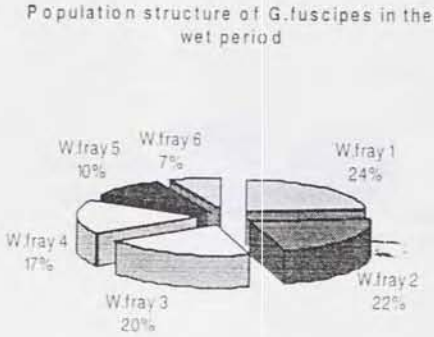
b.



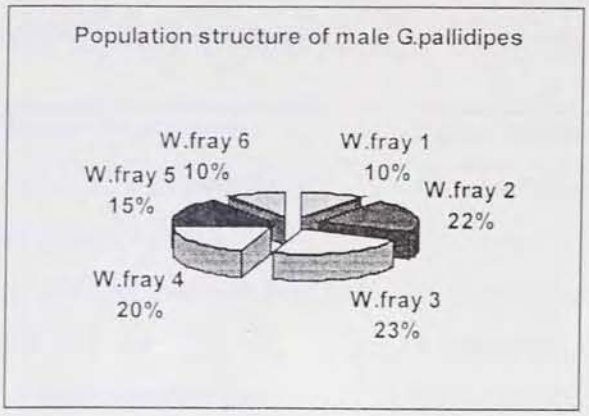
c.



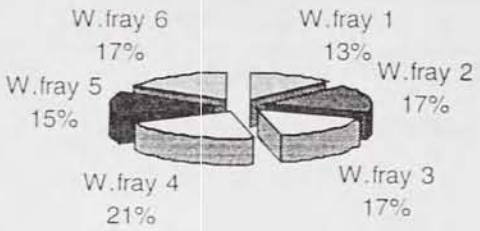
d.



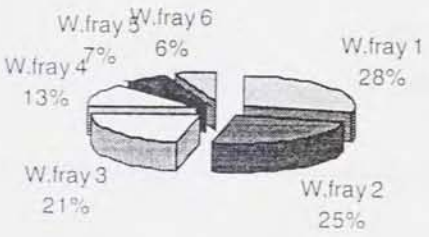
e.


Population structure of male G.pallidipes
W.fray 6
10%
W.fray 1
10%
W.fray 5
15%
W.fray 2
22%
W.fray 4
20%
W.fray 3
23%

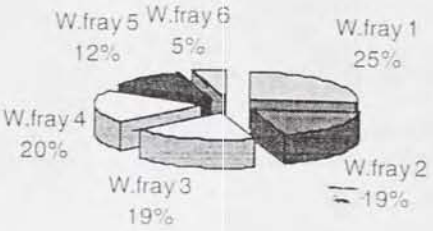
f.


Population structure of female G.pallidipes.
W.fray 6
17%
W.fray 1
13%
W.fray 5
15%
W.fray 2
17%
W.fray 4
21%
W.fray 3
17%

g.


Population structure of male G.fuscipes
W.fray 5
7%
W.fray 6
6%
W.fray 4
13%
W.fray 1
28%
W.fray 3
21%
W.fray 2
25%

h.


Population structure of female G.fuscipes
W.fray 5
12%
W.fray 6
5%
W.fray 1
25%
W.fray 4
20%
W.fray 2
19%
W.fray 3
19%

Annex 10.3. Classification of tsetse flies

Tsetse species and Subspecies (by group or subgenera).

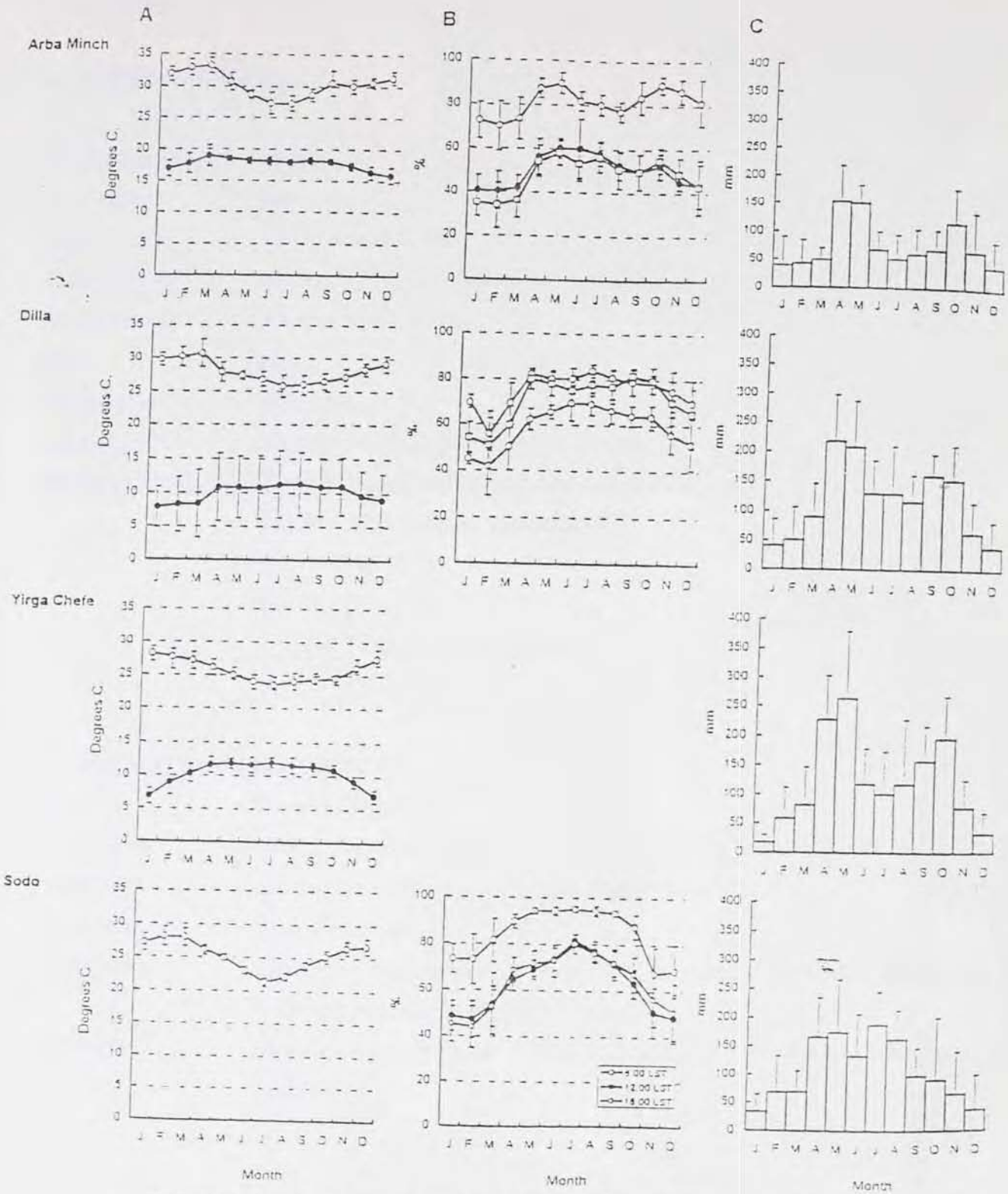
Morsitans (Glossina)	*Palpalis* (Nemorhina)	*Fusca* (Austenina)
G. morsitans submorsitans Newstead 1910	*G.palpalis palpalis* Rob-Desvoidy 1830	*G. fusca fusca* Walker 1849
G. morsitans centralis Machado 1970	*G. palpalis gambiensis* Vanderplank 1949	*G. nigrofusca nigrofusca* Newstead 1910
G. morsitans morsitans Westwood 1850	*G. fuscipes fuscipes* Newstead 1910	*G. nigrofusca hopkinsi* van Emden 1944
G. austenni Newstead 1912	*G. fuscipes quanzensis* Pires 1948	*G. medicorum* Austen 1911
G. pallidipes Austen 1903	*G. fuscipes martinii* Zumpt 1935	*G. tabaniformis* Westwood 1850
G. swynnertoni Austen 1923	*G. tachinoides* Westwood 1850	*G. brevipalpis* Newstead 1910
G. longipalpis Wiedemann 1830	*G. pallicera pallicera* Bigot 1891	*G. longipenis* Corti 1895
	G. pallicera Newstead Austen 1929	*G. frezili* Gouteux 1987
	G. caliginea Austen 1911	*G. severini* Newstead 1913
		G. hamingtoni Newstead & Evans 1922
		G. fuscipleuris Austen 1911
		G. vanhoofi Henrard 1952

Annex 10.4 Classification of trypanosomes of economic and epidemiological importance

Genus Trypanosoma Gruby, 1843

- Section Salivaria.
  - Subgenus Duttonella Chalmers, 1918
    - *Trypanosome vivax* Ziemann, 1905
  - Subgenus Nannomonas Hoare, 1964
    - *Trypanosoma Congolense* Broden, 1904
    - *Trypanosoma simiae* Bruce *et al*, 1911
  - Subgenus Trypanozoon, Luhe, 1906
    - *Trypanosoma brucei brucei* Plimmer & Bradford, 1899
    - *Trypanosoma brucei rhodesiense* Stehhen & Fanthan, 1910
    - *Trypanosoma brucei gambiense* Dutton, 1902
    - *Trypanosoma brucei evansi* (Steel, 1885)
    - *Trypanosoma brucei equiperdum* Doflein, 1901
  - Subgenus Pycnomonas Hoare, 1964
    - *Trypanosoma suis* Ochmann, 1905
- Section Stecoraria.
  - Subgenus Megatrypanum Hoare, 1964
    - *Trypanosoma theileri* Laveran, 1902
  - Subgenus Herpetosoma Defloein, 1901
    - *Trypanosoma lewis* (Kent, 1880)
    - *Trypanasoma drayi* Hoare, 1931
  - Subgenus Schizotrypanum Chagas, 1909
    - *Trypanasoma cruzi* Chagas, 1909

Annex 10.5



The mean monthly minimum and maximum temperature (A), the mean monthly relative humidity at 6.00 h, 12.00 h and 18.00 h LST (B) and the mean monthly rainfall (C) as recorded in Arba Minch, Dilla, Yirga Chefe and Sodo from 1987 to 1997. Error bars indicate Standard Deviation

11. CURRICULUM VITAE

1. PERSONAL DATA.

Family name:	Msangi.
First name	Shandala
Date of birth	19th September 1960
Place of birth	Mwanga, Kilimanjaro Region, Tanzania.
Marital status.	Married with one child.
Sex.	Male.
Nationality.	Tanzanian.
Languages.	English and Swahili, written and spoken.
Mailing address.	Tropical Pesticides Research Institute (TPRI).
	P.O.Box 3024, Arusha, Tanzania.
	Tel. (057) 8813-15.
	Fax: 8217
	E.mail:1 TPRI@Marie.gn.apc.org.
	E:mail:2:msangis@hotmail.com

2. EDUCATION BACKGROUND.

1969-1975	Primary Education
1976-1979.	Ordinary level Secondary Education (Tarime Secondary School)
1980-1982	Advanced level Education (Moshi High School)
1982-1983	National Service.
1986-1989.	University Undergraduate. Bachlor of Veterinary Medicine (BVM) at Sokoine University of Agriculture (SUA).
1998-1999.	Msc. Course in Tropical Veterinary Epidemiology, FUB and AAU joint programme.

3. APPOINTMENTS AND WORKING EXPERIENCE.

PERIOD.	TITLE & RESPONSIBILITIES.
1984-1985.	Field Officer in TPRI, assisting in loboratory and field research work.
1990-1993.	District Veterinary Officer (DVO) in Kibaha District, Tanzania.

Arranging and overseeing treatment and control measures against all animal diseases in the district.

Animal husbandry extension services.

1993-1997 ASSISTANCE RESEARCH OFFICER.
At the TPRI under ministry of Agriculture.

- Planning and writing research proposals on livestock disease vector control programs (Tsetse flies and ticks).
- Management of these projects through laboratory and field trials, collection of research data, treatment of animals under the projects and preparing & presenting project implementation reports.

4. MEMBERSHIP TO SCIENTIFIC SOCIETIES.

- Tanzania Veterinary Association (TVA)
- Tanzania Society for Animal Production (TSAP).
- Tanzania Entomological Association (TEA).
- East African Society for Parasitologist (EASP-TZ).

5 PUBLICATIONS.

Msangi, S.J., Ngomuo A J, Kasuku, A.A. (1990):

The efficacy of oxfendazole against strain of *Haemonhus contortus* resistant to fenbendazole and thiophanate. *Tanzania Veterinary Journal (The Tropical Veterinarian)*, **10**, 1-7.

Kimaro, E.E., Kasege P.K., Msangi S.J., Matechi, H.T., Sikay S.M., and Doriye R., (1995):

The control of ticks and tsetse flies by application of a Pour-On formulation containing alphamethrin and cypermethrin (Pouricide ®) to cattle in Makuyuni, Northern Tanzania. *Proc. Of the 2nd EASP Symposium*, Arusha.

Kimaro, E.E., Muangirwa C.J, Msangi S.J., Sikay M. and Doriye, R., (1996):

The response of tsetse flies *Glossina swynnertoni* to traps, hosts and associated odour attractants. *Proc. Of Tanzania Entomological Association Second Annual Scientific conference*, Arusha.

12. THE SIGNED DECLARATION SHEET

I, the under signed, declare that this thesis is my original work and has not been presented for a degree in any University.

Name: SHANDALA MSANGI Signature: .....

Date of submission: 15th NOVEMBER 1999

This thesis has been submitted for examination with our approval as University advisors.

Dr. Susanne Münstermann .....

Dr. Getachew Tilahun. .....

C-1

bution, Density & Infection Rates
tse Flies in selected sites of
rn rift valley of Ethiopia

Shandala Msangi