HOST RANGE DETERMINATION OF TSETSE FLY *GLOSSINA MORSITANS SUBMORSITANS* BY BLOODMEAL ANALYSIS IN THE UPPER DIDESSA RIVER VALLEY (WESTERN ETHIOPIA)

Habtamu Belete [1], Getachew Tikubet [2], Beyene Petros [3] and E.K. Kang'ethe [4]

[1] Department of Microbiology and Parasitology, Faculty of Medicine
Addis Ababa University, PO Box 22442 code 1000, Addis Ababa, Ethiopia
E-mail: Habtamubel@yahoo.com

[2] ICIPE-Ethiopia Collaborative Project, PO Box 3853, Addis Ababa, Ethiopia

[3] Department of Biology, Faculty of Science, Addis Ababa University
PO Box 1176, Addis Ababa, Ethiopia

[4] Department of Public Health, Pharmacology and Toxicology
University of Nairobi, PO Box 29053, Nairobi, Kenya

ABSTRACT: Enzyme linked immunosorbent assay (ELISA) was employed to determine the rate of digestion of blood proteins ingested by teneral and non-teneral laboratory reared *Glossina morsitans morsitans* at different time intervals after feeding. This test showed that non-teneral flies digested the species distinguishing bloodmeal components faster than tenerals. At 48 hr post-feeding, the bloodmeal donor was identifiable in 87.5% of the teneral tsetse and 55.5% of non-teneral tsetse flies. Among 160 bloodmeals of *G. m. submorsitans* collected from the upper Didessa river valley 54.4% (87/160) were with identifiable hosts, of which warthog accounted for about 52.9 (46/87) of meals, whereas human and buffalo blood accounted for about 21.8% (19/87) and 12.6% (11/87) of the meals, respectively. Others like giraffe, goat, cattle and elephant accounted for few bloodmeals. Thus warthog appeared to be the major host for *G. m. submorsitans* in the study area.

Key words/phrases: Bloodmeal, ELISA, Ethiopia, *Glossina*, Traypanosomosis

INTRODUCTION

The ability to identify natural hosts of blood-sucking insects is an integral part of many ecological investigations, and is of paramount importance in epidemiological studies of insects of medical or veterinary importance (Rurangirwa *et al.*, 1986). Accurate identification of bloodmeals in haematophagous arthropods such as the tsetse fly is, therefore, an important aspect of the epidemiology of vector borne diseases and host-vector interactions (WHO, 1987).

It is important to know the kind of animals on which different species of tsetse depend for food. This enables to characterize and understand the epidemiological as well as epizoological picture of trypanosomosis in a given locality. Several difficulties might be encountered with bloodmeal identification in tsetse flies. Tests may be performed on bloodmeals that have deteriorated to some extent due to digestion processes and storage before analysis. Also, the wide range of hosts used by different species of tsetse flies as source of food require several types of antiserum for the identification of bloodmeals.

The most detailed review of the feeding habit of *Glossina spp.* was reported by Weitz (1963) and Moloo (1993) based on the identification of over 22,600 bloodmeals. Although further bloodmeals have been collected and identified since that time, the general conclusions reached have not been affected. After identifying the source of several bloodmeal samples of different species of tsetse flies Clausen *et al.* (1998) have also categorized host preference of *Glossina* as follows: *Glossina austeni* and *G. fscipleuris* seemed to have a distinct feeding preference for Suidae (mainly bushpig), while *G. morsitans* ssp. fed mainly on Suidae (mainly warthog) although local variations were observed and in some areas hippopotamus or ruminants replaced the warthog as the main host. The principal food source for *G. longipalpis* and *G. fusca* were found to be bushbucks. *G. pallidipes* fed mainly on ruminants and *G. brevipalpis* on hippopotamus. Nevertheless, it has been shown that changes in environment, fauna and host availability may result in modification of tsetse feeding pattern.

Most species of *Glossina* have host preference for feeding predominantly on only one available host species while others show a great deal of adaptability in their feeding habit. Staak *et al.* (1986) reported that the preference of tsetse flies for certain host species differed according to the sampling area. Such reports together with the fact that there is much local variation in the fauna of Africa indicate the necessity for bloodmeal analysis for host range determination in a specific area and situation.

Glossina morsitans submorsitans Newstead 1924 has an east to west distribution from Ethiopia to Senegal and it has been shown to be the most important vector of animal trypanosomosis in Ethiopia (Jordan, 1986). The present study is, therefore, aimed at determining the host range of *Glossina morsitans submorsitans* in the upper Didessa river valley where this tsetse species is dominant and at the same time responsible for transmitting animal trypanosomosis (Nagana) in the area.

MATERIALS AND METHODS

Study area

The study was conducted in the Upper Didessa River Valley, in Western Ethiopia, situated at 8° 40'N and 36° 20'E (Fig. 1) where the problems of animal trypanosomosis is high in the escarpment (MOA, 1989). The area is infested with both *Glossina morsitans submorsitans* and *G. tachinoides* (Westw.) where the latter is riverine, restricted only to the course of the Didessa river (Langridge, 1976).
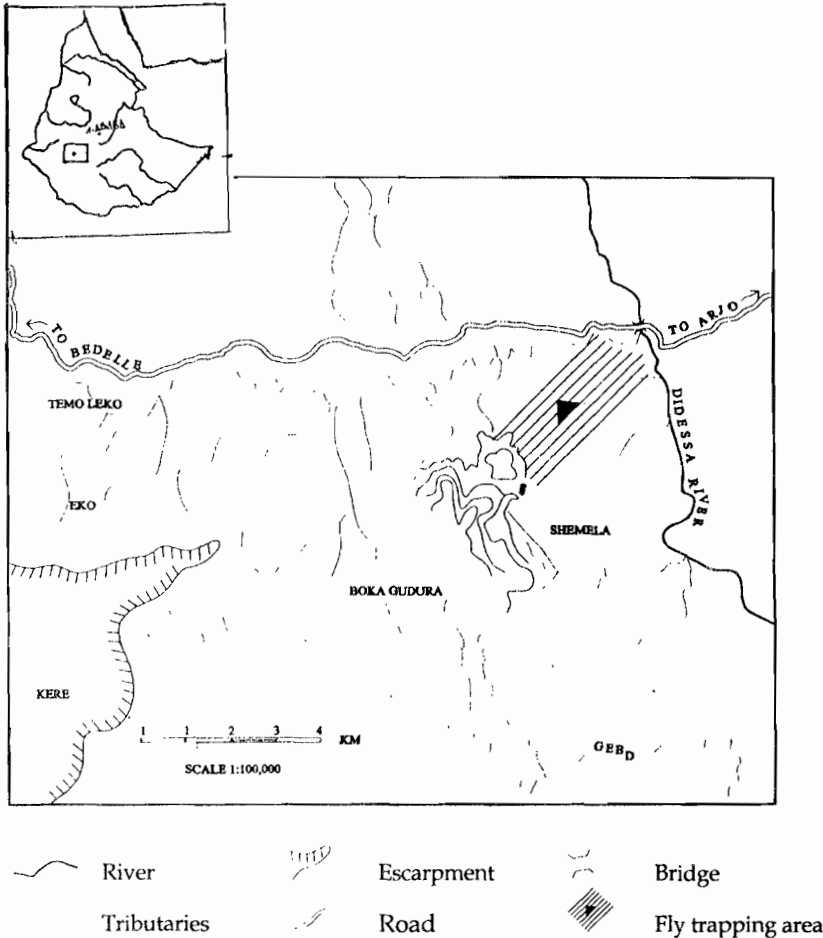

◯◯ River	Escarpment	Bridge
Tributaries	Road	Fly trapping area

Fig. 1.   Map of the study area in the upper Didessa River valley.

The climate is marked with wet and dry seasons; most of the rain falling between June and September. It has an average total annual rainfall of 1950 mm and mean monthly maximum and minimum temperatures of 25° C and

12.5° C, respectively. The altitude of the valley ranges between 1250–1900 m above sea level. The vegetation of the area is characterized by wooded savanna where different grass species of *Hyperrhenia* grow to a height of up to two meters. The dominant tree species are *Terminalia spp.* and *Combretum* spp. During the dry season most of the trees and the grass, except those found along the water course of the river, become leafless as a result of annual burning practice by pastoralists. This forces tsetse to seek refuge in the unburned strips of vegetation along watercourses or isolated evergreen tickets.

From our observation, the species of wild animals present in the area are *Phacochoerus aethiopicus* (warthog), *Hippotragus equinus* (roan antelope), *Patamochoerus porcus* (bushpig), *Colobus polykomos* (monkey), *Papio anubis* (baboon), *Crocuta crocuta* (Hyena), *Dipodina spp.* (rabbit) and *Hippopotamus amphibuis* (hippopotamus). Ungulates such as *Syncerus caffer* (buffalo) and *Tragelaphus scriptus* (bushbuck) are rare. There are virtually no cattle or other domestic animals in the valley.

Collection of wild blood-fed flies

The widely used Biconical (Charllier *et al.*, 1977) and the recently introduced Ngu (NG2G) traps (Bightwell *et al.*, 1987) baited with odor attractants were used in order to capture as many blood-fed tsetse flies as possible from the study area. Of the flies captured, those containing visible fresh blood or partially digested blood in their stomach were squashed on Whatman No. 1 filter paper protected with 0.1% sodium azide. The filter paper was then air dried, labeled and kept in a desiccater over silica jell at 20° C until analysis.

Determination of bloodmeal detection time following feeding of laboratory reared and fed flies

About 2000 pupae of *G. m. morsitans* were obtained from ICIPE (Nairobi, Kenya). The pupae were then kept in the insectary at the Ethiopian Health and Nutrition Research Institute, Addis Ababa. Flies were maintained at 25°C and 60–70% RH. Samples of newly emerged flies (teneral) as well as previously fed flies (non-teneral) which had been starved for 72 h were taken and allowed to feed on rabbits for 10 minutes. Finally, fly guts were squashed onto azide-coated Whatman filter paper No 1 at 1, 6, 24, 48, 72, 96 and 120 h, post-feeding. The filter papers were then labeled and put in a desiccater until analysis.

Bloodmeal analysis

The direct ELISA procedure described by Beier *et al.* (1988) was used in this study after it was modified for detecting bloodmeal sources of tsetse flies. The dried blood spots in the filter paper were cut out and placed in wells of microtiter plate. Elution of the spots was done with PBS (Phosphate buffered saline, NaCl, 8.0 gl^{-1}; KCl, 0.020 gl^{-1}; Na_2HPO_4, 1.15g^{-1}; KH_2PO_4, 0.20 gl^{-1}) pH, 7.2

in 500 μl per elution. After dilution of the sample (1:1000) in coating buffer (Na$_2$ CO$_3$ 1.59 gl^{-1}; NaHCO$_3$, 2.93 gl^{-1} in 100 ml distilled water), pH.9.6 100 μl per well was added to flat-bottomed wells of microtiter plate in duplicates. Wells without blood were used as conjugate and substrate controls. To allow coating of bloodmeal component, the plates were then incubated overnight at room temperature. Washing of the plates was made by flicking off the buffer followed by three washes with PBS-Tween 20 (0.05 M phosphate, 0.5 M NaCl, 0.5% Tween 20, pH 8) at 5 min intervals. One hundred microliters of 1:1000 concentration of peroxidase conjugate (labeled anti-species IgG) were added and left for 45 min after which the above washing procedure was repeated.

One hundred microliters of substrate (0.15 ortho-phenylene diamine, Sigma) in substrate diluent (citric acid buffer pH 4.0 (9 gl^{-1}), 0.05% (v/v) Tween-20 in PBS 500 ml, 5ml, 30% H$_2$O$_2$ in 1 litre distilled water) were added to each well and left at room temperature for 1 h in the dark. Color development was read with a micro- ELISA auto-reader at 492 nm. Any optical density (O.D.) reading that was 3x the average negative control readings and above was considered positive.

RESULTS

The capacity of ELISA to identify the bloodmeals taken from a known host (rabbit) at different times post-feeding, *i.e.* from 1 h up to 120 h after ingestion, showed that the degradation of the species-specific blood components was slower in teneral than in non- teneral tsetse (Fig. 2).
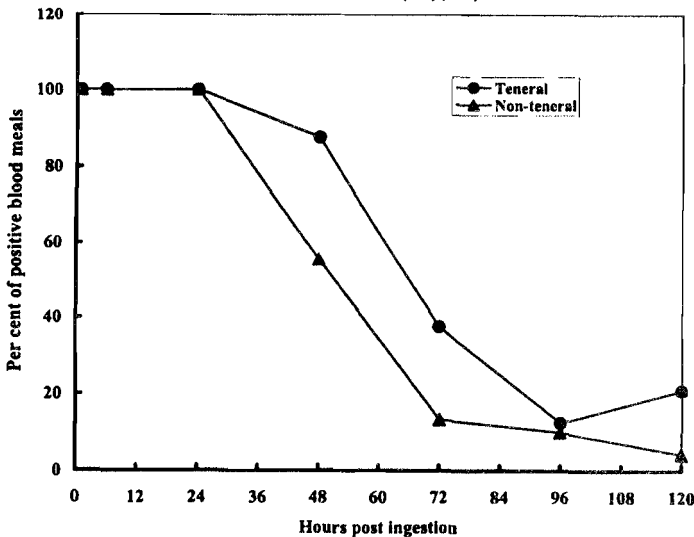

Fig. 2. ELISA results for bloodmeals of teneral and nonterneral *G. m. submorsitans* at successive time after feeding on rabbit.

As assessed by ELISA at 48 post-ingestion, the bloodmeals were identifiable in 87.5% (43/49) of fed teneral tsetse as compared to 55.5% (41/74) in fed non-teneral tsetse. At 72 h post-feeding, the bloodmeal was identifiable in 37.5% (15/40) of fed teneral and 13.3% (9/68) of non-teneral flies (Fig. 2). At 96 h the donor were identifiable in 12.5% of tenerals compared to 10% of non-teneral tsetse. High rate of digestion of blood proteins started about 24 h post-ingestion in non-teneral flies whereas in teneral tsetse, it occurred after 48 h (Fig. 2). At 48 h post feeding 92% (23/25) and 83.3% (20/24) of bloodmeals of teneral male and female flies, respectively were identifiable as compared to nonteneral male and female flies which were only 57.5% (23/40) and 52.9% (18/34) of the bloodmeals, respectively. Thus, there were no differences in the rate of bloodmeal digestion between male and female teneral (P> 0.3702) and non-teneral (P> 0.4329) flies.

Of the 160 bloodmeal samples collected in the field, hosts could be identified from 87 (54.4%) of the samples (Fig. 3). About 53% (46/87) of the flies had fed on warthog. Humans were the next most common host at about 22% (19/87). Buffalo made about 13% (11/87) of the bloodmeal. Giraffe and elephant accounted 8% (7/87) and 1.1% (1/87), respectively. Cattle meals were very few, *i.e.*, 3.3% (3/87). Mixed feeding was identified in four bloodmeals comprising two meals of human/warthog, one of cattle/ elephant and one of buffalo/.giraffe.
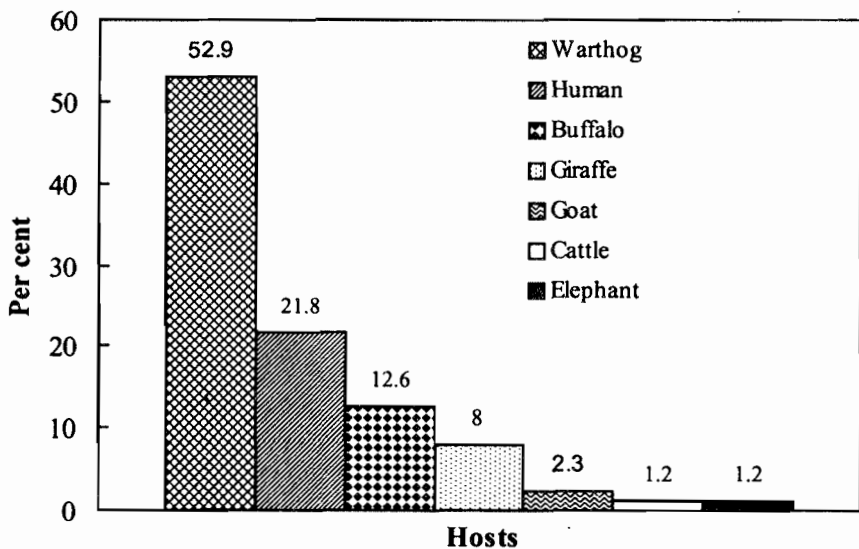

Fig. 3. Frequency distribution of the bloodmeal source of *G. m. submorsitans* in the upper Didessa River valley.

DISCUSSION

The time after ingestion of a bloodmeal at which blood proteins can be detected by immunological methods, such as ELISA, will depend on both the size of the bloodmeal and the rate of its breakdown due to digestion (Service *et al.,* 1986). Furthermore, most workers have recorded the detection time after feeding as a guide to the sensitivity of their technique. The ELISA test was chosen for the present study as it is more sensitive than other immunological tests such as precipitin and haemagglutination inhibition test (Rurangirwa *et al.,* 1986).

In the bloodmeal identification by using the rabbit-fly model system, 87.5% of the teneral flies were positive for rabbit blood 48 h after ingestion. This is in close agreement with that reported by Rurangirwa *et al.* (1986) where at 40 h post-feeding in *G. m. centralis,* the bloodmeal donor could still be identified in 100% of the teneral and 67.5% of the non-teneral flies. The fact that at 72 h post-ingestion only 37.5% of the meals in teneral and 13.3% in non-teneral *G. m. submorsitans* was identified indicates that the highest rate of digestion of blood components takes place after 48 h and 72 h post feeding in non-teneral and teneral tsetse, respectively.

Differences observed in the rate of digestion of plasma proteins between teneral and non-teneral tsetse *(G. m. submorsitans)* are comparable to those reported by Weitz and Buxton (1953) and show that the distinguishing blood components of cattle are degraded faster in non-teneral than teneral tsetse given bloodmeal. This suggests that the proteolytic enzymes are released at faster rate in non-teneral than fed teneral tsetse. As Rurangirwa *et al.* (1986) suggested, it is possible that after digestion of the first feed, the release of the proteolytic enzymes (in non-teneral) is increased by stages at subsequent feeds.

Obviously detection time will depend not only on the size of the bloodmeal but also on temperature, which is the most important environmental factor determining digestion rates (Service *et al.,* 1986). The speed of digestion also varies between species of *Glossina* (Langley and Stafford, 1990), with their age (Weitz and Buxton, 1953), with physiological condition amongst individuals of the same species (Moloo, 1976) and type of blood ingested (Rurangirwa *et al.,* 1986). Significant differences between male and female *G. m. submorsitans* were not observed in the rate of digestion, suggesting that males and females of *G. m. submorsitans* digest their bloodmeal at the same rate hence sex factor does not have any influence.

In the collection of bloodmeal samples traps usually catch abiased hungry samples of the flies, which are seeking hosts with only little residual

bloodmeal in their gut. Thus, it is of great practical significance that at up to 48 h post feeding 87.55 and 55.5% of host bloodmeal from fed teneral and non-teneral *G. m. submorsitans*, respectively, could still be identified.

It is known that the distribution and abundance of at least some *Glossina spp.* are closely related to the abundance and habits of wild animals. Warthog was found to be an apparently favored or available host of *G. m. submorsitans* in the upper Didessa river valley. Similarly, 45% feeds on warthog have been noted in a previous report (Weitz, 1963). According to the report of Snow and Boreham (1979), 90% feeds taken by *G. m. submorsitans* in the west Kiang of Gambia where cattle are very restricted in their contact with woodland areas which is the major areas of *G. m submorsitans* habitat, were also from warthog. In Northern Nigeria, where wild bovidae were relatively scarce, warthog feeds constituted greater proportion of *G. m. submorsitans* feeds than in East Africa, where bovids were abundant (Jordan *et. al.*, 1962).

Results of the present study agree with the most recent work of Clausen *et al.* (1998) who reported that the bloodmeals of *G. m. morsitans* and *G. m. submorsitans* were derived from suidae (57.1%) mainly from warthog which accounted about 37% followed by hippopotamus 17%. Warthog is one of the reliable hosts of tsetse (Nash, 1969) having a relatively restricted home range in favored tsetse habitats and being most active in the early morning and late afternoon when flies are usually most actively searching for food (Torr, 1994).

Although the position of warthog in the epidemiology of Nagana in Ethiopia has not been investigated, their abundance may be important for two reasons. First, the result of bloodmeal determination shows that they are the major hosts of *G. m. submorsitans* and possibly support the large population of this species throughout the upper Didessa valley from which domestic animals are normally absent. Secondly, they may be important reservoirs for trypanosomes since *Trypanosoma congolese, T. vivax T. brucei* and *T. suis* have all been isolated from them (Hoare, 1972), and a 10% infection with the trypanosome parasites has been reported (Jordan, 1986; Claxton *et al.*, 1992).

Humans were found to be the second most favored host in the present study. The presence of small irrigation schemes along the Didessa River valley where people work, including others who are going to the valley for several purposes such as wood and charcoal collection, honey gathering, as well as the presence of an all weather road crossing the valley account for the relatively large number of bloodmeals identified from humans. Cattle meals were very few, as the livestock are normally absent in the area due to the disease.

Furthermore, the finding that a mixture of blood from more than one host can be identified is of epizoological interest since such double feeds may facilitate the transmission of the disease from the reservoir host to the cattle or vise-versa. Knowledge of feeding habit is vital in planning tsetse control programs. The success of control programs based on insecticide sprays or pour-on on cattle would very likely fail in situations where most of the flies feed on hosts other than cattle (Bauer *et al.*, 1995). Further work on the infection rate of warthog for various types of trypanosomes is needed in order to determine the role of this wild animal in the epizoology of the animal trypanosomosis in the area.

ACKNOWLEDGEMENTS

REFERENCES

1. Bauer, B., Amsler-Delafosse, S., Clausen, P.-H., Kabore, I. And Petrich-Bauer, J. (1995). Successful application of deltamethrin pour-on to cattle in campaign against tsetse flies (*Glossina* spp.) in the pastoral zone of samorogouan, Burkina Faso. *Tropical Medicine and Parasitology* **46**:183–189.

2. Beier, J.C., Perkins, P.V. and Wirtz, R.A. (1988). Bloodmeal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. *J. Med. Entmol.* **25**:9–16.

3. Bightwell, R., Dransfield, R.D., Kyorku, C., Golder, T.K., Tammo, S.A. and Mungai, D. (1987). A new trap to *Glossina pallidipes*. *Trop. Pest Managemen*, **33**:151–159.

4. Challier, A., Eyraud, M., Lafaye, A. and Laveissiere, C. (1977). Amelioration due rendement du piege biconique pour glossines (Diptera, Gelossinidae) par l'emploi d'un cone inferieur blue. *Ser. Med. et. Parasitol. Cah* OSTROM **XV**:283–286.

5. Clausen P.H., Adeyemi, I., Bauer, B., Breloeer, M., Salchow, F. and Staak, C. (1998). Host preferences of tsetse based on bloodmeal identfications. *Medical and Veterinary Entomology* **12**:169–180.

6. Claxton, J.R., Faye, J.A. and Rawlings, P. (1992). Trypanosome infection in warthogs (*Phacochoerus aethiopicus*) in Gambia. *Vet. Parsitol.* **41**:179–187.

7. Hoare, C.A. (1972). *The Trypanosomes of Mammals.* Blackwell Scientific Publications. Oxford 749 pp.

8. Jordan, A.M., Jones, F.L. and Weitz, B. (1962). The natural hosts of tsetse flies in northern Nigeria. *Ann. Trop. Med. Parasitol.* 56:430–442.

9. Jordan, A.M. (1986). *Trypanosomiasis Control and African Rural Development.* 1st ed. Longman Group Ltd., New York, p. 344.

10. Langley, P.A. and Stafford, K. (1990). Feeding frequency in relation to reproduction in *Glossina morsitans morsitans* and *G. pallidipes. Physiol. Entomol.* 15:415–421.

11. Langridge, W.P. (1976). *A tsetse and Trypanosomiasis Survey of Ethiopian.* Ministry of Overseas Development, p. 98.

12. MOA (1989). Annual report of national tsetse and trypanosomiasis investigation centre Bedelle, Ethiopia, p. 41.

13. Moloo, S.K. (1976). Aspects of the nutrition of adult female *Glossina morsitans* during pregnancy. *J. Insect Physiol.* 22:563–567.

14. Moloo, S.K. (1993) The distribution of *Glossina spp.* in Africa and their natural hosts. *Ins. Sci. and Appl.* 14:511–527.

15. Nash, T.A.M. (1969*). The tsetse fly, African Bane.* Collins Clear Type Press, London, p. 215.

16. Rurangirwa, F.R., Minja, S.H., Musoke, A.R., Nantulay, V.M., Grootenhuis, J. and Moloo, S.K. (1986). Production and evaluation of specific antisera against sera of various vertebrate species for identification of bloodmeals of *Glossina morsitans centralis. Acta Trop.* 43:379–389.

17. Service, M.W., Voller, A. and Bidwell, D.E. (1986). The enzyme linked immunosorbent assay (ELISA) test for identification of bloodmeals of heamatophagus insect *Bull. Ent. Res.* 76:321–330.

18. Snow, R.K. and Borhame, P.F.L. (1979). The feeding and ecology of tsetse fly *Glossina m. submorsitans* in relation to nagana transmission in Gambia. *Acta Trop.* 36:47–51.

19. Staak, C., Kampe, U. and Korkowski, C. (1986). Species identification of bloodmeals from tsetse (Glossinidae). *Trop. Med. Parasitol.* 37:59–60.

20. Torr, S.J. (1994). Responses of tsetse flies (Diptera: *Glossindae*) to warthog (*Phacochoerus aethiopicus pallas*). *Bull. Ent. Res.* 66:713–729.

21. WHO (1987). Bloodmeal Identification in vectors. *Parasit. Today* 3:324–326.

22. Weitz, B. (1963). The feeding habits of *Glossina. Bull. Wld. Hlth. Org.* 28:711–729.

23. Weitz, B. and Buxton, P.A. (1953). The rate of digestion of various haematophagus arthropods as determined by precipitin test. *Bull. Wld. Hlth. Org.* 44:445–450.