Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 43 (1986)
Heft: 4

Artikel: Production and evaluation of specific antisera against sera of various vertebrate species for identification of bloodmeals of "Glossina morsitans centralis"
Autor: Rurangirwa, F.R. / Minja, S.H. / Musoke, A.J.
DOI: https://doi.org/10.5169/seals-313649

Acta Tropica *43*, 379–389 (1986)

[1] International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya
[2] Small Ruminant Collaborative Research Support Program, P.O. Box 58137, Nairobi, Kenya
[3] Veterinary Research Laboratories, P.O. Kabete, Kenya

Production and evaluation of specific antisera against sera of various vertebrate species for identification of bloodmeals of *Glossina morsitans centralis*

F. R. Rurangirwa[2], S. H. Minja[1], A. J. Musoke[1], V. M. Nantulya[1], J. Grootenhuis[3], S. K. Moloo[1]

Summary

Specific antisera against sera of 46 species of vertebrates were prepared. The antisera to 21 Bovidae species were raised in goats except the antiserum to goat serum which was raised in sheep. The antisera to 3 Suidae species were produced either in domestic pigs or warthogs, while antisera to most of the other vertebrate species were raised in rabbits. The antisera were used in an enzyme-linked immunosorbent assay (ELISA) to identify the source of bloodmeals ingested by teneral and non-teneral tsetse at different time intervals after feeding. The bloodmeal donors were identifiable in 100% of the teneral tsetse up to 40 h post-feeding and in 87.5% in those tested up to 74 h post-feeding. Non-teneral tsetse digested the species-distinguishing bloodmeal components faster than the tenerals. Bloodmeals could be identified in 100% non-tenerals at 20 h post-feeding but only 67.5% and 50% of the bloodmeals could be identified 40 h and 74 h post-feeding, respectively. The antisera were also able to identify mixed bloodmeals from closely related species.

Key words: *Glossina;* bloodmeal; host identification; ELISA.

Introduction

Accurate identification of the source of bloodmeals of haematophagous insects is important for the investigation of host-vector interactions in vector-borne diseases. In recent years the serological approach has been preferred to

Correspondence: Dr. A. J. Musoke, ILRAD, P.O. Box 30709, Nairobi, Kenya

other methods of identification of the source of bloodmeals (Weitz, 1956), since such methods have been found to be accurate and consistent. The serological approach is based on the concept that each vertebrate species possesses one or more plasma proteins with antigenic determinants unique to the species. Thus, identification of the source of tsetse bloodmeals will depend on the ability of the antisera to recognise only the unique proteins of the host's blood.

A good serological test for bloodmeal identification should be sensitive enough to detect minute quantities of residual bloodmeals and specific enough to differentiate closely related host species. The need to achieve the two attributes has led to the development of many serological methods (Tempelis, 1975). The main constraint to the various tests in use has been the specificity of the antisera. Whereas sensitivity will be found in a number of established serological tests such as haemagglutination inhibition, complement fixation and enzyme-linked immunosorbent assay (ELISA), the reliability of the test largely depends on the specificity of the antisera.

This paper describes the preparation of specific antisera against sera of diverse species of vertebrates, and demonstrates that with this level of specificity it is possible to identify mixed bloodmeals from closely related species.

Materials and Methods

Preparation of antisera

Sera from various animal species (Table 1) were used to raise species-specific antisera. The antisera were raised by repeated immunization of the animals with 1 ml of whole serum initially mixed with 1 ml of Freund's complete adjuvant (FCA) and subsequently in Freund's incomplete adjuvant (FIA) at intervals of 2 weeks. Re-immunization in FIA was repeated until potent antiserum was obtained. The animals were test-bled 8 weeks after the first immunization, and, thereafter, every two weeks. The potency of the antisera was determined by agar gel immunodiffusion.

The antisera to sera of the Bovidae species were produced in goats while antiserum to goat serum was produced in sheep. Antisera against the sera of most other species which did not belong to the Bovidae or Suidae were produced in rabbits. Antiserum against the serum of the domestic pig was raised in warthog while the antisera against warthog and bushpig were raised in domestic pigs. Each antiserum was tested against the sera of closely related species by agar gel immunodiffusion to detect cross-reactivity. The serum of the species that cross-reacted most was subsequently used to absorb the antiserum. The most cross-reactive heterologous serum was coupled onto cyanogen bromide-activated Sepharose 4B following described methods (Pharmacia Fine Chemicals, AB Uppsala, Sweden), and used to absorb the antiserum. Absorptions were carried out repeatedly until each antiserum reacted with homologous serum only.

Preparation of enzyme-coupled species-specific antisera

Each species-specific antiserum was precipitated with 35% ammonium sulphate, dialysed against phosphate-buffered saline (PBS) pH 7.2 and coupled to horse radish peroxidase enzyme (Sigma Chemical Company) using the method of Wilson and Nakane (1978) as modified by Lindqvist et al. (1982). The concentration of the conjugate for use in the tests was selected from a block titration of the conjugate against the homologous serum.

Selection of filter paper for collection of bloodmeals

Whatman filter papers Nos. 1, 2 and 4 (Whatman Limited, England) were soaked in a 2% solution of sodium azide and dried at 37 °C. Twenty and 40 μl of bovine blood in ethylenediamine

Table 1. Common and generic names of vertebrate species to which specific antisera were made

Common name	Generic name	Common name	Generic name
Baboon	*Papio papio*	Jackal	*Canis* spp.
Bongo	*Boocercus euryceros*	Kongoni	*Alcepaphus bucelaphus cokii*
Buffalo	*Syncerus caffer*	Lechwe	*Kobus leche*
Bushbuck	*Tragelaphus scriptus*	Lion	*Panthera leo*
Camel	*Camelus ferus bacterianus*	Marabou (Stork)	*Leptoptilos crumeniferus*
Cat (serval)	*Felis serval*	Mouse	*Mus musculus*
Cattle	*Bos indicus* and *Bos taurus*	Oryx	*Oryx callotis*
Dik-dik	*Rhynchotragus* and *Madoqua* spp.	Ostrich	*Struthio camelus*
		Pig (Bush)	*Potamochoerus porcus*
Dog	*Canis lupus familiaris*	Pig (Domestic)	*Sus scrofa domestica*
Donkey	*Equus asinus*	Rabbit	Sub-family-*Dipodinae*
Duiker	*Cechalophus* and *Silvicapra*	Rat (Black)	*Rattus rattus*
Eland	*Taurotragus oryx*	Rat (Brown)	*Rattus norvegicus*
Elephant	*Loxodonta africana*	Reedbuck	*Cervicapra arundineum*
Flamingo	*Phoenicopteridae* spp.	Rhinoceros	*Diceros bicornis* and *Ceratotherium simum*
Fowl	*Galla domestica*		
Gazelle (Grant's)	*Gazella granti*	Sheep	*Ovis aries*
Gazelle (Thomson's)	*Gazella Thomsonii*	Sitatunga	*Tragelaphus spekei*
Giraffe	*Giraffa camelopardalis*	Suni	*Nesotragus moschatus*
Goat	*Capra hireous*	Topi	*Damaliscus korrigum*
Hare	Sub-family-*Dipodinae*	Warthog	*Phacochoerus aethiopicus*
Human	*Homo sapiens*	Waterbuck	*Kobus defassa* and *Kobus ellipsiprymnus*
Hyaena	*Crocuta crocuta* and *Hyaena hyaena*		
		Wildebeest	*Conochaetus tauricus*
Horse	*Equus cabalus*	Zebra	*Equus* spp.
Impala	*Aepyceros melampus*		

tetracetic acid (EDTA) were spotted on each filter paper and dried at room temperature before storing at –20°C. Each spot on the filter paper was later eluted into 1 ml of PBS, ph 7.2, at 4°C overnight. The eluates were subjected to immunoelectrophoresis alongside equal volumes of bovine serum and developed with rabbit anti-bovine serum. Patterns of precipitating lines were observed and comparison made of each filter paper. Results showed that filter paper No. 1 was the most suitable for sample collection and was used in all subsequent experiments.

Identification of single bloodmeals

Tsetse (*Glossina morsitans centralis*) were allowed to feed in vitro on heparinized whole blood of buffalo, cattle, eland, oryx or warthog. At different time intervals post-feeding, groups of tsetse were dissected. The gut and its contents from individual flies were squashed onto different sectors of the filter papers, dried at room temperature and stored at –20°C. The bloodmeals were eluted in PBS as described above and used for identification of the hosts.

Bloodmeals from teneral and non-teneral tsetse

In order to assess the rate of digestion of bloodmeals, teneral (young unfed tsetse) and non-teneral tsetse were fed in vitro (Mews et al., 1977) on fresh defibrinated blood of a steer and their guts dissected out at different time intervals after feeding and the contents squashed onto different sites on the filter papers. The gut contents were analysed for the presence of the distinguishing serum components of the bloodmeals.

Mixed bloodmeals

Blood obtained from two or three different animal species was mixed and fed in vitro to non-teneral tsetse which had been "starved" for 48 h. The guts of these tsetse were dissected 24 h later and analysed to identify the host animals.

In another experiment, tsetse were fed on blood from one animal species and 24 h later the same tsetse were fed on blood of another species and, 1 or 24 h after the last feed, the guts were dissected out and squashed on filter papers. The eluates from the filter papers were later used for identification of the two animal species.

Analysis of the bloodmeals by enzyme immunoassay

Twenty-five microlitres of each eluate and a similar volume of corresponding serum (positive control) were each added to 75 μl of carbonate buffer pH 9.0 in flat bottomed wells of microtitre plates (Titre Tek-Flow Laboratories, McLean, Virginia, USA) in duplicate. The plates were incubated overnight at room temperature to allow coating of bloodmeal components to the plates. The plates were washed by flicking off the buffer followed by three washes with phosphate-NaCl-Tween 20 (0.05 M phosphate, 0.5 M NaCl, 0.5% Tween 20 buffer, pH 0.8) at 5-min intervals. One hundred microlitres of selected concentrations of the conjugate were added and left for 45 min after which the above washing procedure was repeated. One hundred microlitres of substrate (0.1% ortho-phenylene diamine, Sigma) were added to each well and left at room temperature for 1 h in the dark. Colour development was read visually or with a micro-ELISA auto reader (Model MR-580, Dynatech, Plochingen, West Germany) at 492 nm. Any O.D. reading that was 0.100 and above was considered positive.

Results

Specificity of the antisera raised against host sera

Table 2 shows the extent of cross-reactivity of the antisera produced in goats and/or sheep among the various species of the Bovidae as determined by agar gel immunodiffusion. Most cross-reactivity was removed by absorption of the antisera with serum of the most cross-reacting species. For example, antiserum to buffalo serum was absorbed with bongo serum to remove the cross-reactivity with most species of Bovidae. Absorption with bongo serum, however, did not remove the cross-reactivity with cattle serum. Further absorption using cattle serum was needed to make the antiserum buffalo-specific. Antiserum to each of the 20 species of Bovidae was absorbed similarly until rendered species-specific. After the cross-reactivity within the Bovidae had been totally absorbed, the antiserum was tested for cross-reactivity with the non-Bovidae species and absorbed accordingly. Tables 3a, b, c and d show the specificity of some of the absorbed antisera as detected by ELISA.

Table 2. Reactivity of unabsorbed antisera against sera of some Bovidae species as revealed by agar gel immunodiffusion

Serum of	Antiserum to sera of																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1 Suni	+++	–	–	+	+	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–
2 Buffalo	–	+++	+	–	++	++	–	+	–	+	–	+	–	++	+	–	+++	–	+	–
3 Topi	+	–	+++	+	–	–	–	+	+	–	–	–	++	–	–	–	–	–	–	–
4 Oryx	–	–	+	+++	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
5 Bushbuck	+	+	+	–	+++	–	–	+	–	++	+	+	–	+++	+	+	+++	+	–	–
6 Reedbuck	+	–	+	–	+++	++++	+	+	–	–	–	–	–	+	–	+	–	–	+++	–
7 Lechwe	+	–	+	+	–	++	++++	+	–	–	–	–	–	+	–	+	–	–	++	–
8 Duiker	–	+	++	+	–	–	–	+++	–	–	–	+	–	–	–	+	–	–	+	–
9 Wildebeest	–	–	+	+	–	–	–	+	+++	–	–	–	++	–	–	–	–	+	–	–
10 Dik-dik	+	–	–	+	–	–	–	+	–	+++	–	–	–	–	–	+	–	–	–	–
11 Eland	++	+	+	–	++	–	–	–	–	++	++++	–	–	+	–	+	+++	+	+	–
12 Grant's gazelle	+	–	–	+	–	–	–	+	–	++	–	++++	–	–	–	+	–	+	+	++
13 Kongoni	–	–	++	+	–	–	–	+	+	–	–	–	++++	–	–	–	–	–	–	–
14 Cow	–	+	+	–	++	–	–	+	–	–	–	–	–	++++	–	–	+++	–	++	–
15 Goat	–	–	–	–	–	–	–	–	–	–	–	–	–	–	++++	–	–	–	–	–
16 Impala	+	–	+	+	–	–	–	+	–	–	–	–	–	+	–	++++	–	–	+	–
17 Bongo	+	+	+	–	++	–	–	+	–	++	+	–	–	+	–	–	++++	–	–	–
18 Sheep	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	++++	–	–
19 Waterbuck	–	–	–	–	–	++	++	–	–	–	–	–	–	–	–	–	–	–	++++	–
28 Thomson's gazelle	–	–	–	–	–	–	–	+	–	–	–	+++	–	–	–	+	–	–	+	++++

+ denotes reactivity; ++++ being maximum; – denotes no reactivity.

Table 3a. The specificity of absorbed antisera to some Bovidae species as detected by ELISA

Antisera to serum of	Sera of						
	Buffalo	Cow	Eland	Oryx	Bushbuck	Waterbuck	Kongoni
Buffalo	*0.638**	0.020	0.007	0.010	0.021	0.021	0.015
Cow	0.011	*0.664*	0.026	0.004	0.019	0.023	0.022
Eland	0.020	0.040	*0.648*	0.064	0.030	0.036	0.042
Oryx	0.007	0.005	0.000	*1.051*	0.012	0.015	0.015
Bushbuck	0.021	0.029	0.049	0.025	*0.635*	0.025	0.017
Waterbuck	0.038	0.024	0.014	0.048	0.001	*0.538*	0.007
Kongoni	0.039	0.031	0.027	0.037	0.026	0.048	*0.409*

* Any reading less than 0.100 was considered negative.

Table 3b. The specificity of absorbed antisera to some Bovidae species as detected by ELISA

Antisera to serum of	Sera of						
	Dik-dik	Suni	Impala	Duiker	Sheep	Goat	Thomson's gazelle
Dik-dik	*0.436**	0.039	0.021	0.026	0.000	0.028	0.000
Suni	0.016	*0.851*	0.018	0.014	0.020	0.014	0.024
Impala	0.021	0.029	*0.607*	0.004	0.000	0.001	0.033
Duiker	0.002	0.003	0.008	*1.227*	0.000	0.003	0.000
Sheep	0.040	0.024	0.005	0.016	*0.492*	0.008	0.006
Goat	0.015	0.018	0.013	0.029	0.002	*0.395*	0.000
Thomson's gazelle	0.060	0.044	0.022	0.020	0.029	0.070	*0.477*

* Any reading less than 0.100 was considered negative.

Table 3c. The specificity of absorbed antisera to Suidae species as detected by ELISA

Antisera to serum of	Sera of		
	Warthog	Domestic pig	Bush pig
Warthog	*0.605**	0.002	0.001
Domestic pig	0.001	*0.362*	0.000
Bush pig	0.001	0.002	*0.279*

* Any reading less than 0.100 was considered negative.

Table 3d. The specificity of absorbed antisera to animal species belonging to diverse families

Antisera to serum of	Sera of							
	Rhinoceros	Rabbit	Camel	Human	Giraffe	Warthog	Horse	Elephant
Rhinoceros	*0.683**	0.008	0.039	0.000	0.062	0.043	0.000	0.046
Rabbit	0.012	*0.707*	0.009	0.000	0.017	0.009	0.041	0.033
Camel	0.019	0.000	*0.932*	0.047	0.008	0.037	0.000	0.050
Human	0.019	0.055	0.001	*0.580*	0.028	0.001	0.014	0.042
Giraffe	0.000	0.000	0.000	0.059	*0.774*	0.000	0.011	0.000
Warthog	0.000	0.002	0.007	0.012	0.017	*0.443*	0.005	0.007
Horse	0.039	0.004	0.060	0.052	0.034	0.030	*1.007*	0.036
Elephant	0.005	0.016	0.000	0.020	0.044	0.021	0.047	*0.685*

* Any reading less than 0.100 was considered negative.

Identification of bloodmeals in teneral and non-teneral tsetse

The degradation of the species-specific serum components in defibrinated steer blood was slower when fed to teneral than to non-teneral tsetse (Fig. 1) as assessed by ELISA. At 40 h post-ingestion, the bloodmeals were identifiable in 100% of teneral tsetse fed as compared to 67.5% in non-teneral tsetse. At 64 h post-ingestion, the bloodmeal donor was identifiable in 87.5% tsetse fed as teneral, while in the non-teneral the percentage remained at 67.5%. At 74 h the donor was identifiable in 87.5% of the tsetse fed as teneral compared to 50% of non-teneral tsetse.

Bloodmeal identification in mixed feeds

Identification of host species in tsetse fed on a mixture of blood from 2 or 3 different animal species is shown in Table 4. In 40 tsetse fed on a mixture of blood from two different species, both host species were identified in 39 of them (97.5%). In those fed on a mixture of blood from 3 different species, all 3 host species were identified in 87.5% (21/24). In tsetse given two different bloodmeals 24 h apart and dissected 1 or 24 h after the second meal (Table 5), the host species of the second meal was identified in most tsetse (85%), while the host species of the first meal was identifiable in 60%. The donor species of the first bloodmeal was most consistently identifiable only in tsetse that had ingested eland blood as the first meal (Table 5).

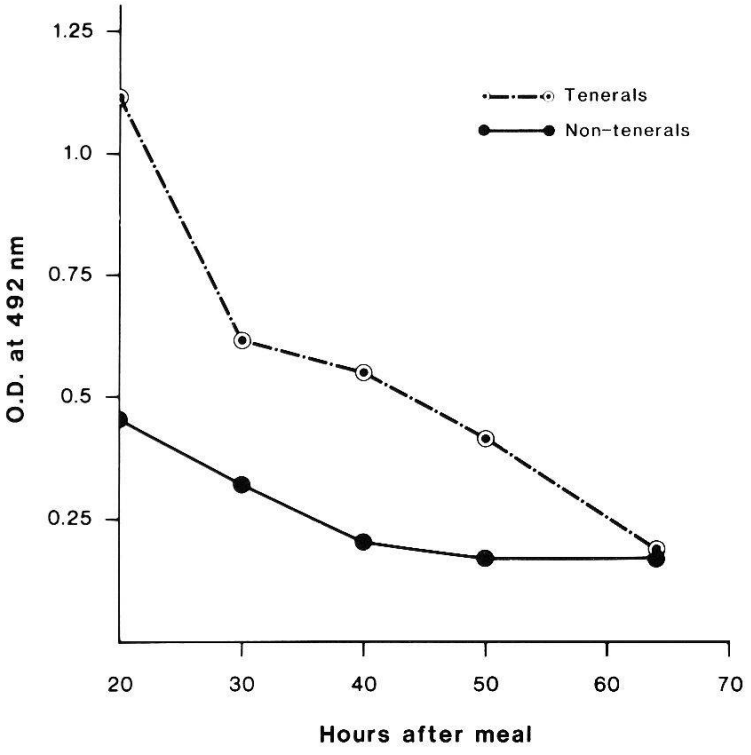

Fig. 1. The degradation of species-specific serum components of a steer in teneral and non-teneral tsetse as determined by ELISA. Each point represents the mean value of analyses of the bloodmeal from 16 different tsetse flies.

Table 4. Number of animal species identified in bloodmeals from tsetse fed on mixed blood from two or three species when the bloodmeals were analyzed 24 h after feeding

Species mixed	Number of species mixed	Number of tsetse in which all species were identified
B + E or B + O	2	7/8*
B + C or B + W	2	8/8
E + O or E + C	2	8/8
E + W or O + C	2	8/8
O + W or C + W	2	8/8
C + B + W or C + O + E	3	8/8
W + E + O or W + E + C	3	6/8**
W + E + O or W + E + C	3	7/8**

B = Buffalo; C = Cow; E = Eland; O = Oryx; W = Warthog
* one species only identified in one tsetse
** two species only identified in the remaining tsetse

Table 5. Identification of the host species of blood in tsetse given two different bloodmeals, 24 h apart, then dissected and analysed 1 or 24 h after the second meal

Tsetse group number	Host of first meal	Host of second meal	Number of tsetse in which the host was identified	
			First host	Second host
I*	B	C, E, O or W	2/8	7/8
	C	B, E, O or W	2/8	7/8
	E	B, C, O or W	8/8	5/8
	O	B, C, E or W	7/8	7/8
	W	B, C, E or O	7/8	7/8
II*	B	C, E, O or W	1/8	7/8
	C	B, E, O or W	7/8	7/8
	E	B, C, O or W	7/8	8/8
	O	B, C, E or W	3/8	7/8
	W	B, C, E or O	4/8	6/8

B = Buffalo; C = Cow; E = Eland; O = Oryx; W = Warthog
* In Group I the second meal was ingested 24 h after the first and the tsetse were dissected 1 h after the second bloodmeal while in Group II the second bloodmeal was ingested 24 h after the first, and the tsetse were dissected 24 h after the second bloodmeal.

Discussion

Specific antisera to sera of 46 species of vertebrates were prepared and successfully used to identify bloodmeals of tsetse fed on different host blood using the ELISA test. By using closely related animals in production of antisera,

the extent of cross-reactions was minimized, thus making absorption of the cross-reactivity less laborious. The absorbed antisera were capable of differentiating very closely related animal species without resorting to differences in titres (Boreham, 1975; Staak, 1983) or a combination of two tests such as ELISA and complement fixation test (Staak, 1983) in order to arrive at a definite identification. The specificity was such that the colour change in the presence of heterologous serum proteins was the same as that in the presence of buffer alone.

The importance of using closely related animal species in raising species-specific antisera was recognized some years back. Tempelis (1975) found that the fowl, besides being a good producer of precipitating antibody, was an appropriate host in which specific antisera to other birds could be raised.

The ELISA test was chosen for this study as it is more sensitive than the agglutination test, quantitative precipitation, haemagglutination and immunofluorescent assays (Buxton and Rissing, 1980). A test which uses minute quantities of samples is suited for tsetse bloodmeal analysis. ELISA detected as little as 58 μg of residual blood components (Burkot et al., 1981) or dilutions of blood or serum beyond a dilution of 10^{-6} (Lindqvist et al., 1982). Also, ELISA is easy to perform and is amenable to large scale use since many samples can be tested simultaneously.

Using the antisera prepared, it was possible to identify the host species of the bloodmeal in 100% of the teneral tsetse given first meal and 67.5% of the non-teneral flies 40 h after they had ingested blood of a steer. At 74 h post-feeding, the bloodmeal donor could still be identified in 87.5% of the fed teneral and 50% of the non-teneral tsetse, respectively. These results are comparable to those reported by Weitz and Buxton (1953) and show that the distinguishing serum components of cattle are degraded faster in non-teneral than in teneral tsetse given first meal. This suggests that the proteolytic enzymes are released at a faster rate in non-teneral than in fed teneral tsetse. It is possible that after digestion of the first feed the release of the proteolytic enzymes (in non-tenerals) is escalated at subsequent feeds.

In the field situation where tsetse density is very low, it is difficult to encounter recently engorged flies in their resting places. One is more likely to catch or trap flies with only little residual bloodmeal. It is thus encouraging to note that up to 74 h post-feeding 87.5% and 50% bovine bloodmeal from the fed teneral and non-teneral tsetse, respectively, could still be identified. Furthermore, in tsetse fed on a mixture of blood from 2 or 3 different animal species, simultaneously or sequentially, the host species were identified in most cases. This is of epidemiological interest since double feeds have been reported for tsetse caught in the field (Moloo et al., 1971).

Acknowledgments

We thank Mrs. Anita Kaushal, Mr. S. B. Kutuza, Mrs. Catherine Nkonge and Mr. Dismus Lugo for excellent technical assistance and Ms. Doris Churi for typing the manuscript.

This is ILRAD Publication No. 397.

Boreham P. F. L.: Some applications of bloodmeal identifications in relation to the epidemiology of vector-borne tropical diseases. J. trop. Med. Hyg. *78*, 83–91 (1975).

Burkot T. R., Goodman W. G., DeFoliart G. R.: Idéntification of mosquito bloodmeals by enzyme-linked immunosorbent assay. Amer. J. trop. Med. Hyg. *30*, 1336–1341 (1981).

Buxton T. B., Rissing J. P.: The antibody and antigen ELISA: future applications. Qualityline *2*, 4–6 (1980).

Lindqvist K. J., Gathuma J. M., Kaburia H. F. A.: Analysis of bloodmeals of haematophagous insects by haemoagglutination inhibition and enzyme immunoassay. In: Proceedings of the Third Annual Medical Scientific Conference, Nairobi, Kenya, ed. by P. M. Tukei and A. R. Njogu, p. 122–133 (1982).

Mews A. R., Langley P. A., Pimley R. W., Flood M. E. T.: Large-scale rearing of tsetse flies (*Glossina* spp.) in the absence of living host. Bull. ent. Res. *67*, 119–128 (1977).

Moloo S. K., Steiger R. F., Brun R., Boreham P. F. L.: Sleeping sickness survey in Musoma District, Tanzania. II. The role of *Glossina* in the transmission of sleeping sickness. Acta trop. (Basel) *28*, 189–205 (1971).

Staak C.: Identification of bloodmeals from tsetse flies. In: Proceedings of the Third International Symposium, World Association of Veterinary Laboratory Diagnosticians, June 13–15, 1983, Ames, Iowa, USA, Vol. 2, p. 659–664 (1983).

Tempelis C. H.: Host-feeding patterns of mosquitoes, with a review of advances in analysis of bloodmeals by serology. J. med. Ent. *11*, 635–653 (1975).

Weitz B.: Identification of bloodmeals of blood-sucking arthropods. Bull. Wld Hlth Org. *15*, 473–490 (1956).

Weitz B., Buxton P. A.: The rate of digestion of bloodmeals of various haematophagous arthropods as determined by the precipitin test. Bull. ent. Res. *44*, 445–450 (1953).

Wilson M. B., Nakane P. K.: Recent developments in the periodate method of conjugating horesradish peroxidase (HRPO) to antibodies. In: Immunofluorescence and Related Staining Techniques, ed. by W. Knapp, K. Holubar and G. Wick, p. 215–224. Elsevier/Biomedical Press, Amsterdam 1978.