Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 28 (1971)
Heft: 3

Artikel: Sleeping sickness survey in Musoma District, Tanzania
Autor: Onyango, R.J. / Woo, P.T.K. / Moloo, S.K.
DOI: https://doi.org/10.5169/seals-311726

East African Trypanosomiasis Research Organization, Tororo, and
Swiss Tropical Institute, Basle

Sleeping Sickness Survey in Musoma District, Tanzania

I. Investigation of the Incidence of Sleeping Sickness in the Human Population

R. J. ONYANGO[1] and P. T. K. WOO[2]

Abstract

In a survey of sleeping sickness in the Ikoma-Serengeti area, carried out in October and November 1970, about 3,000 people living in the area were examined and none was found infected.

Introduction

Rhodesian sleeping sickness was introduced into the Musoma District mainly in the Ikoma area in the 1920's. It is believed to have been an extension of an epidemic in Maswa, Mwanza District, which lies to the south. The outbreak of Rhodesian sleeping sickness in Maswa probably began in 1919–1921 during a period of famine but early patients were first diagnosed in 1922 (DAVEY 1924). *Glossina swynnertoni* was incriminated as the main vector of the outbreak. The spread of the disease was thought to be due to infected persons and the maintenance of intensive man-fly contact as the game population was quite small and scattered (SWYNNERTON 1923, 1925). Davey and McClean, however, having travelled in the affected area during an investigation of the same outbreak, found ample evidence that game was quite abundant in the area (DAVEY 1924). From 1925 the yearly incidence of sleeping sickness in Ikoma ranged between 12 and 265 (FAIRBAIRN 1948). The endemic situation continued until 1954 when the last 3 cases of sleeping sickness were notified from the area. It is reported that a large number of cases of sleeping sickness was diagnosed during the endemic period

[1] E.A.T.R.O., Tororo, Uganda.
[2] University of Guelph, Guelph, Canada.

largely among goldmine workers in the Kilimafedha and associated mines in the district. One possible reason for the disappearance of the disease in the mid-fifties is that a number of mines had been closed because of their unprofitability; consequently the population moved away (YOUNG 1968).

In 1964, 2 patients suffering from sleeping sickness were notified from the Musoma District, one of them a resident of Seronera, the administrative centre of Serengeti National Park. From 1964 to date the following cases have been notified: 1 case in 1965; 4 in 1966; 6 in 1967; 14 in 1968 and 6 in 1969. Of these, 2 were tourists. The reappearance of sleeping sickness into this heavily tsetse-infested area caused some concern particularly as this area had been developed as a tourist centre. For this reason a survey of human trypanosomiasis (sleeping sickness) in the Musoma District was undertaken mainly in the Serengeti National Park, Ikoma Game Reserve and populated areas surrounding the game sanctuary particularly those to the north and north west about 10 miles from the borders of the Park. The aims of the survey were to find out the mechanism and the extent of transmission of sleeping sickness in this part of Tanzania. To achieve this objective it was necessary to examine people living in the area for evidence of parasitaemia; to examine the tsetse species so as to ascertain the rates and types of their infections and also their feeding habits; and to determine the infection rates in a limited number of game species and cattle with pathogenic trypanosomes particularly those of the *Trypanosoma brucei* subgroup. Full accounts of the investigations and their results are reported in this paper in four parts. In the first part an account is given of the investigations undertaken among the human population living in the survey area.

Subjects

A preliminary census of human population was not taken before the survey. It was assumed that because an excellent co-operation between the administration and the people existed, the majority of the population would respond to written and verbal notices and come willingly for examination at the preselected centres. In fact with regard to the people living in the Serengeti National Park, nearly a 100 % attendance was achieved. Although a good number of people in the Serengeti National Park are immigrant labourers, many of them had lived and worked within the park for over 2 years. The period of residence for the female population and children may have been shorter, temporary and renewed frequently after visits to the original homeland miles away from the Park. Contact of this latter group with the vector and the infective agent was therefore irregular. Population sampled in the homesteads and in the villages surrounding the Park represented the resident local population and therefore were more permanent. The number of young adult males in the villages tended to be fewer than expected because members of this group left home in search of lucrative employment elsewhere. An attempt was made to examine people of all ages and both sexes. During the survey, inquiries were made about ill persons who could not come to the centres. Those reported to be ill were either

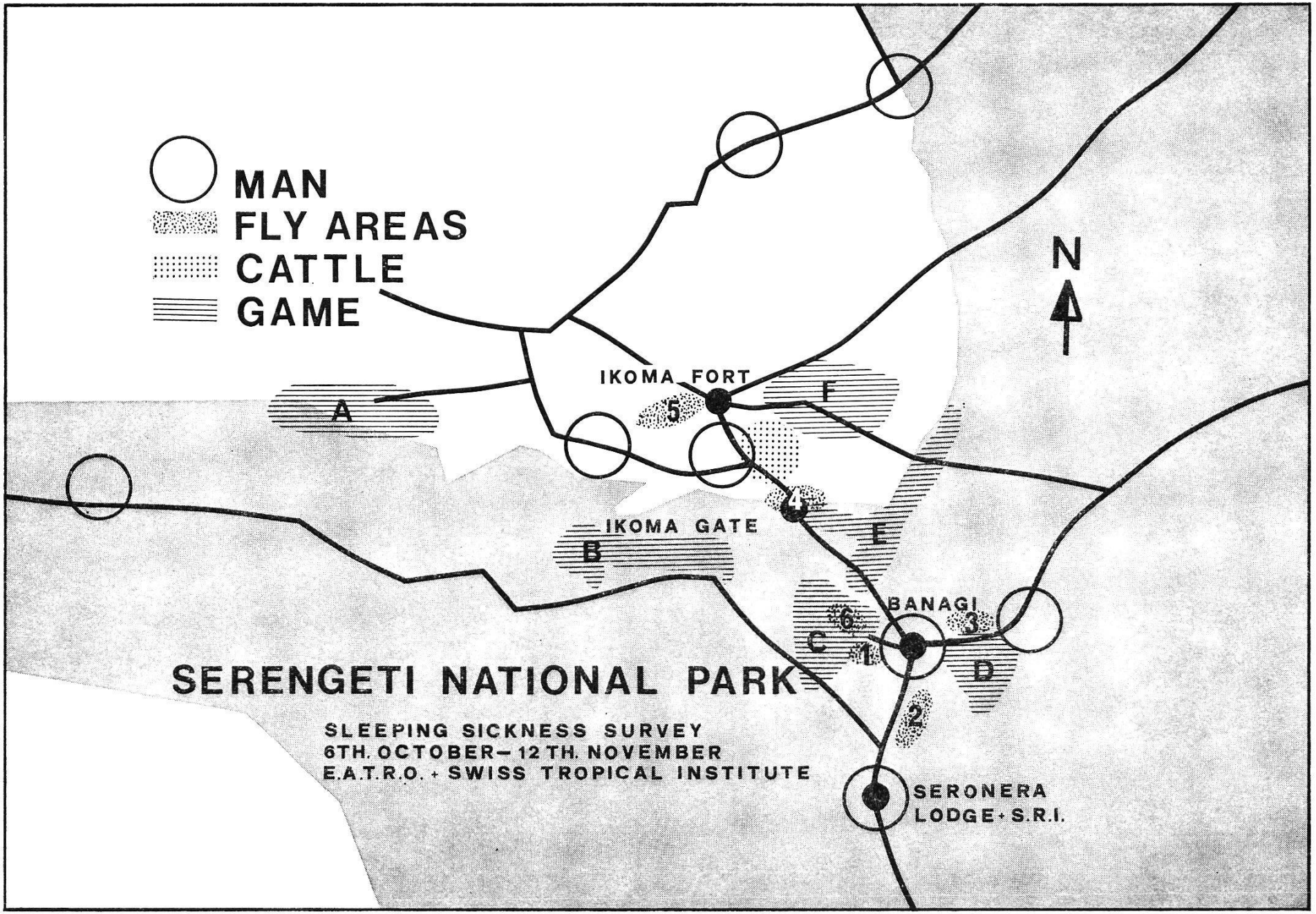

Fig. 1. Map of part of the Musoma District showing locations of the main survey areas

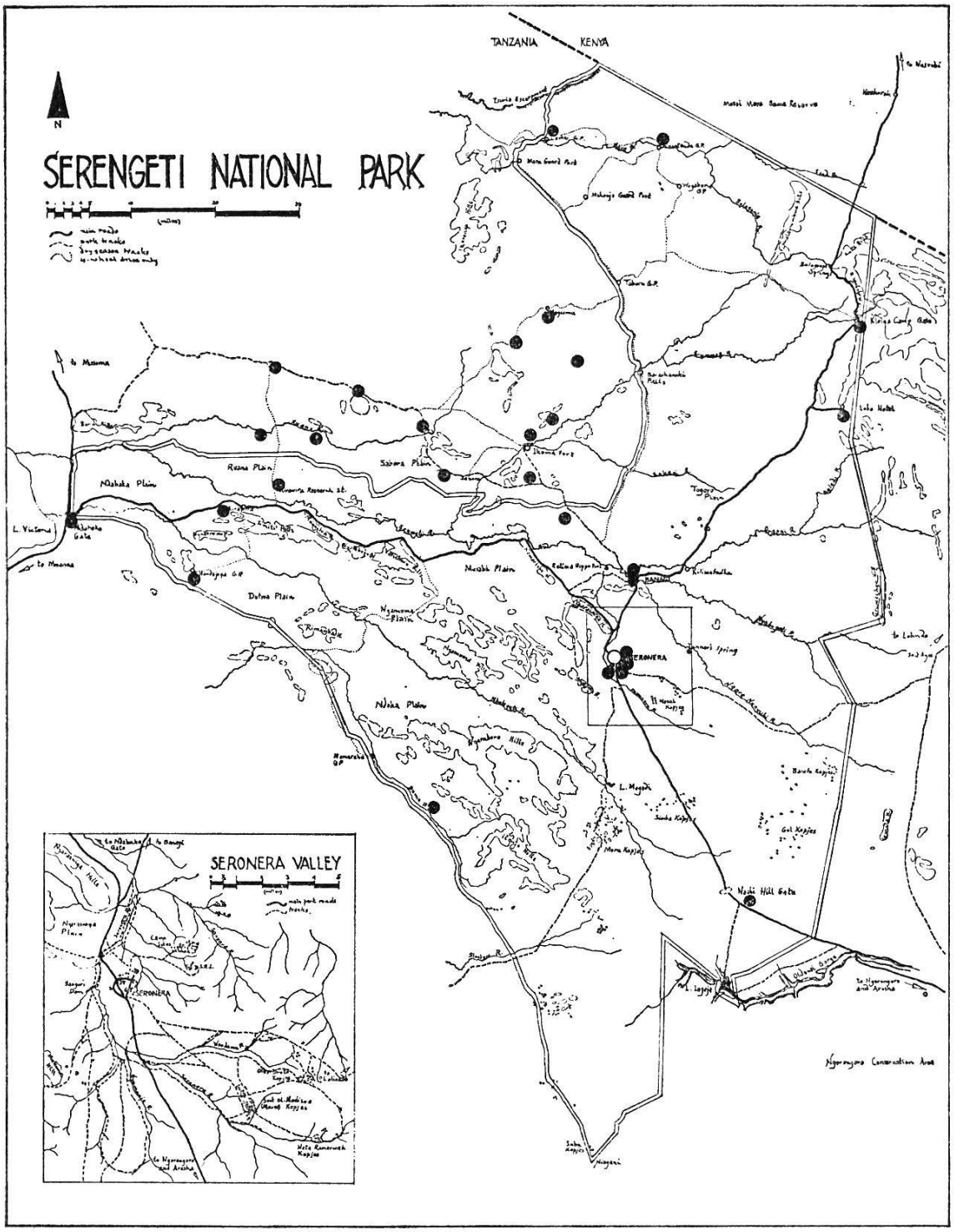

Fig. 2. Map of the area covered by the survey team examining the human population.

visited at their homes or brought in a vehicle for examination at the relevant centres.

All the resident population in the various establishments within the Serengeti National Park and villages in the Ikoma area and a few tourists at Serengeti Lodge were examined. Examinations of the people were carried out at 29 centres (see maps Fig. 1 and 2). The histogram (Fig. 3) shows the age and sex distribution of all the people examined at these centres. In all there were a total of 2,941 people.

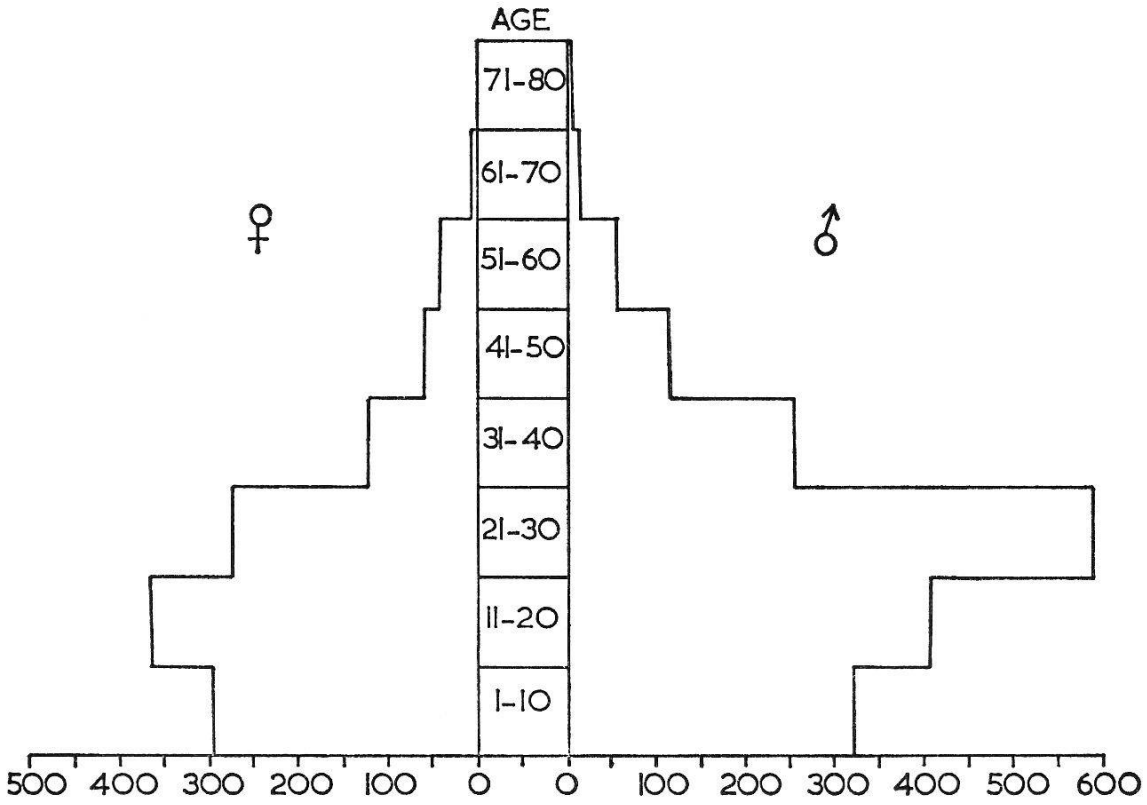

Fig. 3. Histogram showing age and sex distribution of the population surveyed in the Serengeti National Park and Ikoma area.

Methods

Four methods of examinations were used during the investigation:

1. Blood film (thin and thick on glass slides) examination.
2. The haematocrit centrifuge technique (HCT).
3. The serum IgM (immunoglobulin M_2) level determination.
4. The immunofluorescent antibody test (IFT).

Blood samples for all the tests were obtained from a skin puncture on the tip of a finger in the case of every adult and juvenile and on the tip of the big toe of every child and infant. One skin puncture produced enough blood for samples required for all the four tests. The first drops were used to make both thin and thick blood films, a subsequent squeeze of the skin puncture produced enough blood for capillary tubes used in the HCT and additional pressure produced blood samples to be absorbed onto a filter paper and left to dry for use in both the serum IgM level estimations and the IFT.

All the thin blood films were fixed in ethanol at the time of taking the samples. Both these and the thick films were brought back to the laboratory at the Serengeti Research Institute (S.R.I.) and stained with Giemsa stain. Some were examined under the microscope at S.R.I. and others were brought back to the laboratories of the East African Try-

panosomiasis Research Organization, Tororo, for examination. Each blood film was examined under oil immersion lens and discarded after 200 microscope fields had been scanned.

Blood samples were examined immediately by the HCT described by Woo (1970). A portable Honda (1 kilowatt) generator was available to supply electric current for the centrifuge. It was carried at the back of a landrover to the examination centres at which surveys were carried out. Only at a few inaccessible places that were reached only by a four-seater aeroplane which was not big enough to carry all the equipment was the generator not taken and hence the HCT was not used to test the few samples obtained. The tests for the estimation of serum IgM level and for the immunofluorescent antibodies were carried out on blood spots on absorbent filter paper at E.A.T.R.O. The methods of the tests used are similar to those described by CUNNINGHAM et al. (1967) and BAILEY et al. (1967), respectively.

Results

Blood films

Out of a total of 2,941 people sampled, blood films taken from 102 were lost. Examination of the rest showed no trypanosomes. About 47 blood films were found to be positive for malaria parasites *(P. falciparum)* and one for microfilaria *(D. perstans)*.

Haematocrit centrifuge technique

A total of 2,761 freshly obtained blood specimens in capillary tubes were examined by this method; none showed the presence of trypanosomes. Thirteen specimens were positive for microfilaria.

Serum IgM levels

Of the specimens collected 47 were lost and so only 2,894 were tested. Those that showed a significant rise in IgM (immunoglobulin M_2) levels are shown in the histogram (Fig. 4) by their ages and sexes. They numbered 909 and formed 31.4 % of the population.

Indirect immunofluorescent tests (IFT)

The first 1,691 people had the test performed on their dried blood sample irrespective of whether they had raised IgM serum levels or not. Only 11 of these were positive by the test; all except one were males. Of the remaining 1,203, only those that had raised IgM serum levels

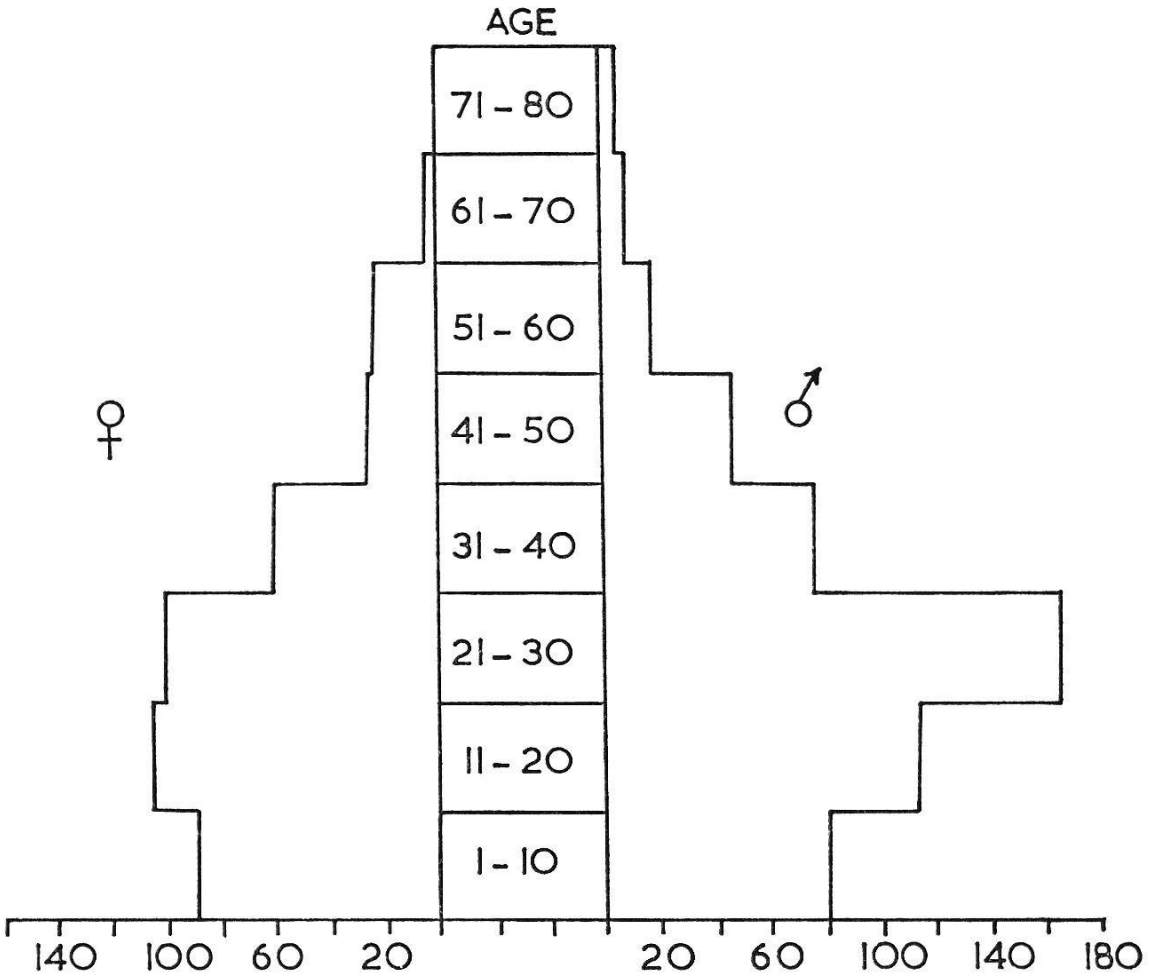

Fig. 4. Histogram showing the age and sex distribution of people with significant raised IgM serum levels.

had their samples tested for immunofluorescent antibodies. In this group those with raised serum IgM levels numbered 484; among them 25 showed evidence of immunofluorescent antibodies. Fourteen were males and 11 females. The degree of fluorescence achieved was only 3, none showed maximum fluorescence of degree 4. People positive by the IFT formed 1.2 % of the whole population examined.

Lost specimens

One hundred and two blood films and only 47 dried blood samples on filter paper were lost. Many whose blood films were lost had their blood samples examined for raised IgM serum levels as well as for immunofluorescent antibodies. Some of those whose dried samples were lost had their blood slides examined for the presence of parasites. Thus the people whose both blood films and dried blood samples on filter paper were lost numbered only 15 and they came from 6 centres only. They consisted of 4 females and 11 males. The largest number came from Ikoma Robanda where 7 were lost. In other places the number lost was as follows: Mugumu Primary Court: 3 lost out of 205 samples;

Mugumu Lungahule: 2 out of 237; Nata Sibora: 1 out of 114; Lobo: 1 out of 165 and Serengeti National Park: 1 out of 526 samples.

Discussion

The results of this investigation showed that at the time of the survey (i.e. between 6th October and 16th November, 1970) no overt trypanosome infection was discovered among the human population examined. Even the HCT, which is said to be more sensitive than blood film examination, gave negative results. The screening tests used together (i.e. IgM estimation and IFT) also gave negative results. The interpretation of this is that a few months before and including the duration of the survey of that year there was no transmission of pathogenic trypanosome strains capable of producing parasitaemia in man. Nor did the survey team find an evidence of a healthy carrier among that population. Of a total of 17 cases notified between 1964 to 1968 the highest monthly incidence occurred in the month of November (i.e. 5 cases altogether). From monthly incidence of cases of sleeping sickness at Ikoma between the years 1934 and 1946 there appeared to be no increase in incidence during any particular month or season (FAIRBAIRN 1948). The negative results together with those found by other survey teams whose reports appear in parts II, III and IV will be discussed together in part V where an attempt will then be made to explain the epidemiological factors which are at play in this part of Tanzania.

References

BAILEY, N. M. et al. (1967). The indirect fluorescent antibody technique applied to dried blood, for use as a screening test in the diagnosis of human trypanosomiasis in Africa. – Trans. roy. Soc. trop. Med. Hyg. *61,* 696–700.

CUNNINGHAM, M. P. et al. (1967). The estimation of IgM immunoglobulin in dried blood, for the use as a screening test in the diagnosis of human trypanosomiasis in Africa. – Trans. roy. Soc. trop. Med. Hyg. *61,* 688–695.

DAVEY, J. B. (1924). The outbreak of human trypanosomiasis (*Trypanosoma rhodesiense* infection) in Mwanza District, Tanganyika Territory. – Trans. roy. Soc. trop. Med. Hyg. *17,* 474–481.

FAIRBAIRN, H. (1948). Sleeping sickness in Tanganyika Territory, 1922–1946. – Trop. Dis. Bull. *45,* 1–17.

SWYNNERTON, C. F. M. (1923). The entomological aspects of an outbreak of sleeping sickness near Mwanza, Tanganyika Territory. – Bull. ent. Res. *13,* 317–370.

SWYNNERTON, C. F. M. (1925). The evidence as regards the game in the outbreak of human trypanosomiasis near Mwanza. – Trans. roy. Soc. trop. Med. Hyg. *19,* 70–80.

WOO, P. T. K. (1970). The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. – Acta trop. *27,* 384–386.

YOUNG, A. S. (1968). SRI report, unpublished.

Sleeping Sickness Survey in Musoma District, Tanzania

II. The Role of *Glossina* in the Transmission of Sleeping Sickness

S. K. MOLOO[1], R. F. STEIGER[2], R. BRUN[2] and P. F. L. BOREHAM[3]

Abstract

A survey to determine the role of local *Glossina* species on the transmission of human sleeping sickness has been carried out in the Ikoma/Seronera/Kilimafedha triangle. Three different tsetse species were encountered; they were *G. swynnertoni, G. pallidipes* and *G. brevipalpis.* Out of 6,348 *G. swynnertoni* and 623 *G. pallidipes* examined not a single carried salivary gland infection. *G. swynnertoni* had a wide range of vertebrate hosts including bovids, suids, elephant, hippopotamus, primates, carnivores, aardvark and avians, of which buffalo, warthog and giraffe were the hosts most generally favoured. Comparison of the feeding patterns of this species showed that *G. swynnertoni* is readily adaptable. The local tsetse species are primarily zoophilic and, since game was abundant, they attacked man only through chance meeting.

Introduction

Transmission of the human sleeping sickness was studied from the point of view of the local *Glossina* species acting as vectors of *T. rhodesiense.* Since the time at our disposal was limited, it was not possible to survey a very large area of the District. Consequently, six study sites were selected within the Ikoma/Seronera/Kilimafedha triangle which is the centre of human activity in the Serengeti National Park and Ikoma, and where game and tsetse were abundant. To evaluate the problem of human trypanosomiasis, the following investigations were undertaken: (i) distribution of the local *Glossina species;* (ii) the incidence of salivarian trypanosome infections; (iii) feeding habits of the vector; (iv) the nature of man-fly contact.

Survey Area, Materials and Methods

The six sites examined are shown on the map (Fig. 5). The numbers refer to the order in which the areas were surveyed, that is Area 1 was studied in the first week of the 6-week survey period and Area 6 in the last.

Vegetation

The habitat is for the most part gently undulating, though hilly in places. It has good drainage. In many places beneath the surface of the

[1] E.A.T.R.O., Tororo, Uganda.
[2] Swiss Tropical Institute, Basle, Switzerland.
[3] Imperial College Field Station, Ascot Berks, U.K.

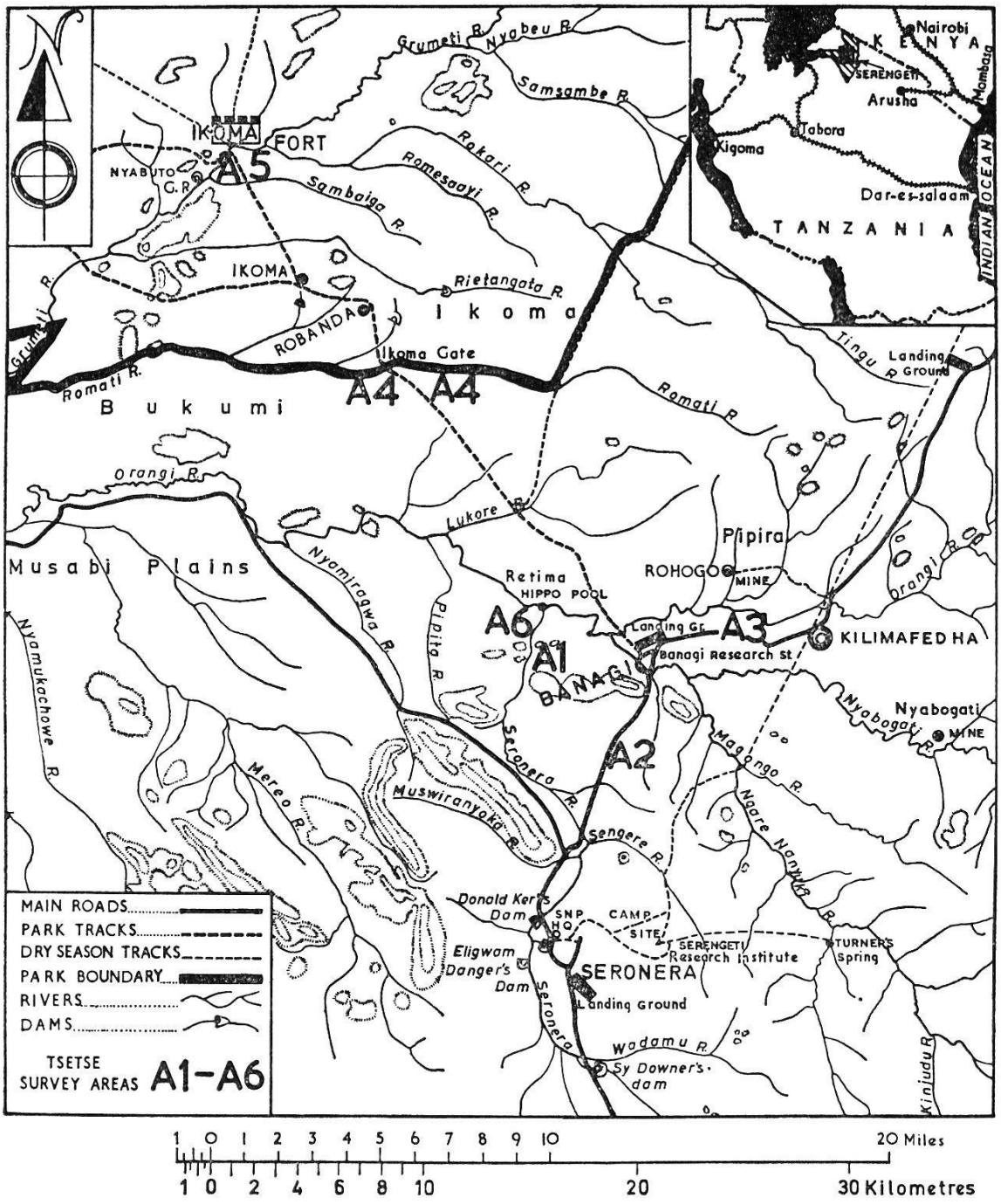

Fig. 5. A map of the part of Musoma District showing locations of tsetse survey areas.

soil there is a cement-like material which binds soil and decomposed rock into a hard pan. Rocky granite kopjes are scattered here and there.

Dry thorn-bush or nyika of one type or another occupies much of the area. The trees and shrubs belong to a great variety of genera but among them Acacia, Euphorbia, Sansevieria and Balanites communities often predominate. The common characteristic of the genera which dominate the nyika is thorniness, with a few important exceptions such as the tree-Combretums and the sparsely scattered baobab (Adansonia). In the greater part of the thorn-bush there is no general canopy. Shrubs

which often form thickets are in the main small and evenly scattered throughout the area of grass. Shrubs fringing the banks of rivers and streams form continuous and in places, particularly in Ikoma (Area 5), dense thickets. Areas of open grassland with black cotton soil and patches of hard pan are also features of the habitat. It is characteristic that, except in extreme conditions of dryness, grasses are usually closer and taller than that which commonly grows under "miombo" (see MULLIGAN 1970, p. 294).

Distribution of game

The approximate frequency distribution of different game species for Areas 1–4 and 6 for October are given in Table 1 (JARMAN 1970). The data for Area 5 were not available. It should be mentioned that the frequency of some species such as wildebeest, zebra, gazelles and eland can fluctuate from day to day.

Contents of Table 1 and the observations made during the survey are summarized below. Although as complete as practicable, these data

Table 1. Frequency distribution of game animals in the survey areas in October 1970

Hosts	Areas				
	1	2	3	4	6
Impala	190–200	115–125	235–245	155–165	190–200
Thomson's gazelle	175–185	260–270	5–15	5–10	175–185
Zebra	55–65	–	1–5	305–315	55–65
Wildebeest	–	–	–	280–290	–
Topi	40–50	15–25	25–35	25–35	40–50
Buffalo	20–30	5–10	105–115	5–10	20–30
Hartebeest	15–25	80–90	20–30	5–10	15–25
Giraffe	20–30	10–20	5–15	5–15	20–30
Warthog	20–30	5–15	5–15	5–15	20–30
Dik-dik	20–30	1–5	5–15	5–10	20–30
Grant's gazelle	15–25	–	1–5	10–20	15–25
Hyaena	5–15	5–10	5–10	5–10	5–15
Lion	1–5	1–5	1–5	5–10	1–5
Reedbuck	1–5	–	1–5	5–10	1–5
Leopard	1–5	1–5	1–5	–	1–5
Bushbuck	1–5	–	5–15	–	1–5
Waterbuck	1–5	1–5	1–5	–	1–5
Steinbok	–	1–5	1–5	5–10	–
Duiker	1–5	1–5	–	–	1–5
Elephant	1–5	–	1–5	–	1–5
Rhinoceros	1–5	–	1–5	1–5	1–5
Eland	–	–	–	5–10	5–10

on game possibly do not include some of the rarer animals which were not seen.

Area 1. – This area contained a variety of game. Among the most common animals and in the order of apparent frequency were: impala, Thomson's gazelle, zebra, topi, buffalo/giraffe/warthog/dikdik, hartebeest (kongoni), Grant's gazelle and hyaena. Other animals present included elephant, rhinoceros, waterbuck, reedbuck, bushbuck, duiker, lion and leopard. Hippopotami were seen in restricted places.

Area 2. – The order of apparent frequency of common animals was Thomson's gazelle, impala, hartebeest, topi, giraffe and warthog. Buffalo, hyaena, waterbuck, duiker, dikdik, steinbok, lion, leopard and ostrich were also present.

Area 3. – Impala, buffalo, topi, hartebeest and Thomson's gazelle/giraffe/warthog/dikdik/bushbuck was the order of apparent frequency of common animals present. The fauna also included zebra, Grant's gazelle, lion, reedbuck, leopard, waterbuck, steinbok, elephant and rhinoceros, but these were less frequent.

Area 4. – In this area game was abundant but restricted in variety compared to the other areas. The following was the order of apparent frequency of common game animals: zebra, wildebeest, impala, topi, Grant's gazelle and giraffe/warthog. Other less frequent animals included hyaena, Thomson's gazelle, buffalo, hartebeest, dikdik, lion, reedbuck, steinbok, eland, aardvark and ostrich.

Area 5. – The animals seen or their presence suspected were giraffe, buffalo, zebra, lion, hyaena, impala, topi, bushbuck, waterbuck, hartebeest, dikdik, warthog, wildebeest, Thomson's gazelle, leopard, duiker, aardvark and ostrich.

Area 6. – In view of their close proximity, this area contained a variety of game species similar to that in Area 1. The order of apparent frequency of common animals was impala, Thomson's gazelle, zebra, topi, warthog/giraffe/buffalo/dikdik, hartebeest/Grant's gazelle, hyaena and, in restricted places, hippopotamus and crocodile. The less common animals present included rhinoceros, elephant, lion, reedbuck, leopard, bushbuck, waterbuck, eland and duiker.

Methods of catching Glossina

The following sampling methods were used with a view to determine the distribution of *Glossina* species. The flies caught by these methods were dissected to find trypanosome infections.

(a) Trapping. – Two Langridge traps (modification of single-screen Swynnerton trap, SWYNNERTON (1933)) were used. Each was suspended from a pole between two trees with wires in order to allow free move-

ment in breeze and so enhance its conspicuousness. The flies were trapped for 3–5 days in each area and the traps emptied at 08.00, 10.00, 14.00 and 18.00 hours.

(b) Random catch. – Two groups of three field assistants each using hand-nets caught flies which were attracted to them. Catches were made between 08.00 and 14.00 hours. The catching parties covered about 8 km² in each of the Areas 1, 2, and 4–6.

(c) Landrover catch. – A landrover was driven through the open woodland and beside thickets, stopping from time to time to catch flies which had entered the vehicle and those that alighted on the body.

(d) Fly-round. – This method was employed in Area 3 alone. A transect footpath of 500 × 200 yd. was cut and divided into 50 yd. sections. The transect traversed thorn-bush woodland and thicket patches. A group of 6 field assistants caught flies from 08.00 to 14.00 hours for a period of four days, six rounds per day.

(e) Bait-animal catch. – Two field assistants caught flies that had alighted on a black cow which was tethered to a tree in Area 2. Catches were made between 06.00 and 18.00 hours over a period of three days.

Trypanosome infections in Glossina

Proboscis, midgut and salivary glands of non-teneral *G. swynnertoni* and *G. pallidipes* were examined under phase-contrast optics. In some cases, the organs containing infective trypanosomes were preserved in liquid nitrogen by the method of DAR & WILSON (1970).

Blood meals

Two catching parties of 2 field assistants each covered about 8 km² in each of the six areas. Flies in Hunger Stage I (JACKSON 1933) were caught from their resting sites and their gut contents expressed on to filter papers. These feeds were dried in a dessicator over calcium chloride and then stored at –20°C. The main groups of hosts were identified by precipitin tests (WEITZ 1952) and the individual species of each group was determined subsequently by the inhibition test (WEITZ 1956). Weak feeds derived respectively from the family *Bovidae* and *Suidae,* which failed to react in the tests specifically, were classed as unidentified species of the groups concerned. "Cat" feeds could be derived from any member of the *Felidae* family including lion and leopard. Feeds which were assigned as "Carnivore" were members of the order *Carnivora* excluding the cat, dog, hyaena and mongoose families. It is possible that these feeds were derived from zorilla and/or honey badger.

Man-fly contact

Observations were made on the reaction of tsetse to the presence of field assistants in all six areas as well as to the men working in the Park.

Results

Distribution of Glossina

The results of sampling methods given in Table 2 are intended to show the relative distribution of the three tsetse species encountered. The local distribution of *G. swynnertoni, G. pallidipes* and *G. brevipalpis* is described below.

Area 1. – *G. swynnertoni* were numerous and were caught in open woodland as well as along thickets close to drainage. *G. pallidipes* were also present but in low numbers confined to riverine thickets.

Area 2. – This area of open woodland with widely scattered small thickets supported a large number of *G. swynnertoni. G. pallidipes* was not encountered.

Area 3. – *G. swynnertoni* were numerous throughout this area of open woodland with riverine thickets. The latter also supported small populations of *G. pallidipes.*

Table 2. Average numbers of tsetse caught per day by four methods

Area	Species	Flies caught per day							
		Random catch		Trap		Bait catch		Fly-round	
		♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
1	*G. swynnertoni*	174	3	46	45	–	–	–	–
	G. pallidipes	1 *	0	2	3	–	–	–	–
2	*G. swynnertoni*	181	22	45	37	122	31	–	–
	G. pallidipes	0	0	0	0	0	0	–	–
3	*G. swynnertoni*	–	–	51	35	–	–	285	4
	G. pallidipes	–	–	2	4	–	–	0	0
4	*G. swynnertoni*	168	10	43	36	–	–	–	–
	G. pallidipes	0	0	4	4	–	–	–	–
5	*G. swynnertoni*	231	16	23	24	–	–	–	–
	G. pallidipes	27	13	57	52	–	–	–	–
	G. brevipalpis	0	0	0	1 *	–	–	–	–
6	*G. swynnertoni*	194	12	48	50	–	–	–	–
	G. pallidipes	1	2	2	4	–	–	–	–

* Caught in five days.
– Catching method not used.

Area 4. – Large numbers of *G. swynnertoni* were encountered in this area of open woodland with thicket patches. Langridge traps caught a few *G. pallidipes* close to large thickets of *Euphorbia candelabra.*

Area 5. – *G. swynnertoni* and *G. pallidipes* were both numerous. The latter were encountered mainly in and around thickets which were numerous and close. One *G. brevipalpis* female was trapped adjacent to dense thicket.

Area 6. – Numerous *G. swynnertoni* were encountered throughout the area while a few *G. pallidipes* found were confined mainly to continuous thickets fringing the river and the Retima Hippo Pool.

Infection rates

The incidence of trypanosome infections in *G. swynnertoni* and *G. pallidipes* are given in Table 3. Although the infection rates in flies from all the six areas were high, not a single salivary gland infection was found. All the mature infections belonged to the *vivax* and *congolense* groups.

Table 3. Rates of trypanosome infection in *G. swynnertoni* and *G. pallidipes*

Area	Species	Number examined ♂♂	♀♀	Overall infections % ♂♂	♀♀	♂♂+♀♀	Mature infections %
1	*G. swynnertoni*	939	134	17.2	17.9	17.3	13.7
2	*G. swynnertoni*	777	237	15.2	15.6	15.3	12.3
3	*G. swynnertoni*	789	277	15.6	18.8	16.4	14.8
4	*G. swynnertoni*	893	123	15.3	14.6	15.3	12.2
5	*G. swynnertoni*	1,217	223	11.5	17.5	12.4	10.2
	G. pallidipes	359	264	10.6	10.2	10.4	8.7
6	*G. swynnertoni*	580	159	15.3	19.5	16.2	12.9

Hosts of Glossina

The sample of *G. pallidipes* blood meals is too small – eight squashes in all – to draw valid conclusions from it. Of these, 2 were from warthog, 2 from buffalo, 1 from elephant and 3 from unidentified suids.

The results of identification tests of blood meals of *G. swynnertoni* from all the six areas from which they were collected are shown in Tables 4/5 and Fig. 6. These results are summarized as follows:

Area 1. – Hippopotamus, giraffe, buffalo and elephant each contributed 19.8 % of all feeds. Only 7.9 % were from warthog. Con-

Hosts	Sex	Areas 1	2	3	4	5	6	Total
Man	♂						1	1
	♀						**1** 0.5%	**1** 0.1%
Baboon	♂		1					1
	♀		**1** 0.8%					**1** 0.1%
Carnivore	♂				3	1		3
	♀				**3** 2.3%	**1** 0.4%		**4** 0.5%
Hyaena	♂		2		1			3
	♀		**2** 1.6%		**1** 0.8%			3 0.3%
Cat	♂	2	2			1	1	5
	♀	**2** 2.0%	**2** 1.6%			**1** 0.4%	**1** 0.5%	**6** 0.7%
Aardvark	♂		3		2	1		6
	♀		**3** 2.5%		**2** 1.6%	**1** 0.4%		**6** 0.7%
Elephant	♂	20					27	47
	♀	**20** 19.8%					5 **32** 17.6%	5 **52** 6.0%
Elephant and unidentified bovids	♂						2	2
	♀						**2** 1.1%	**2** 0.2%
Rhinoceros	♂				2		5	7
	♀				**2** 1.6%		2 **7** 3.9%	2 **9** 1.0%
Unidentified suids	♂		12	1	4	20		37
	♀		**12** 9.8%	**1** 1.0%	**4** 3.1%	12 **32** 14.0%		12 **49** 5.7%
Warthog	♂	7	33		26	118	2	186
	♀	**7** 6.9%	1 **34** 27.9%		8 **34** 26.6%	26 **144** 63.2%	**2** 1.1%	35 **221** 25.6%
Warthog and unidentified bovids	♂	1	2			1		4
	♀	**1** 1.0%	**2** 1.6%			**1** 0.4%		**4** 0.5%

Hippopotamus	♂	20	**20**	19.8 %												25	**28**	15.4 %	45	**48**	5.6 %
	♀	—														3			3		
Unidentified bovids	♂	10	**10**	9.9 %	15	**18**	14.8 %	5	**11**	10.9 %	6	**8**	6.3 %	5	**6**	2.6 %	6	**12**	6.6 %	47	**65**	7.5 %
	♀	—			3			6			2			1			6			18		
Giraffe	♂	20	**20**	19.8 %	24	**24**	19.7 %	1	**2**	2.0 %	19	**19**	14.8 %	17	**17**	7.5 %	19	**23**	12.7 %	100	**105**	12.2 %
	♀	—			—			1			—			—			4			5		
Giraffe and buffalo	♂	1	**1**	1.0 %				1	**1**	1.0 %	3	**3**	2.3 %				1	**2**	1.1 %	6	**7**	0.8 %
	♀	—						—			—						1			1		
Buffalo	♂	20	**20**	19.8 %	5	**5**	4.1 %	72	**86**	85.1 %	34	**47**	36.8 %	14	**16**	7.0 %	45	**55**	30.2 %	190	**229**	26.6 %
	♀	—			—			14			13			2			10			39		
Waterbuck	♂																1	**1**	0.5 %	1	**1**	0.1 %
	♀																—			—		
Eland	♂																7	**9**	4.9 %	7	**9**	1.0 %
	♀																2			2		
Bushbuck or eland	♂																3	**5**	2.7 %	3	**5**	0.6 %
	♀																2			2		
Topi	♂										—	**1**	0.8 %							—	**1**	0.1 %
	♀										1									1		
Topi or hartebeest	♂										2	**2**	1.6 %	—	**1**	0.4 %				2	**3**	0.3 %
	♀										—			1						1		
Impala	♂										1	**1**	0.8 %	1	**1**	0.4 %	2	**2**	1.1 %	—	**4**	0.5 %
	♀										—			—			—			4		
Avian	♂				19	**19**	15.6 %				—	**1**	0.8 %	7	**7**	3.1 %				26	**27**	3.1 %
	♀				—						1			—						1		
Total meals	♂	101	**101**		118	**122**		80	**101**		103	**128**		184	**228**		147	**182**		733	**862**	
	♀	—			4			21			25			44			35			129		

Fig. 6. Feeding patterns of *G. swynnertoni* in the six survey areas.

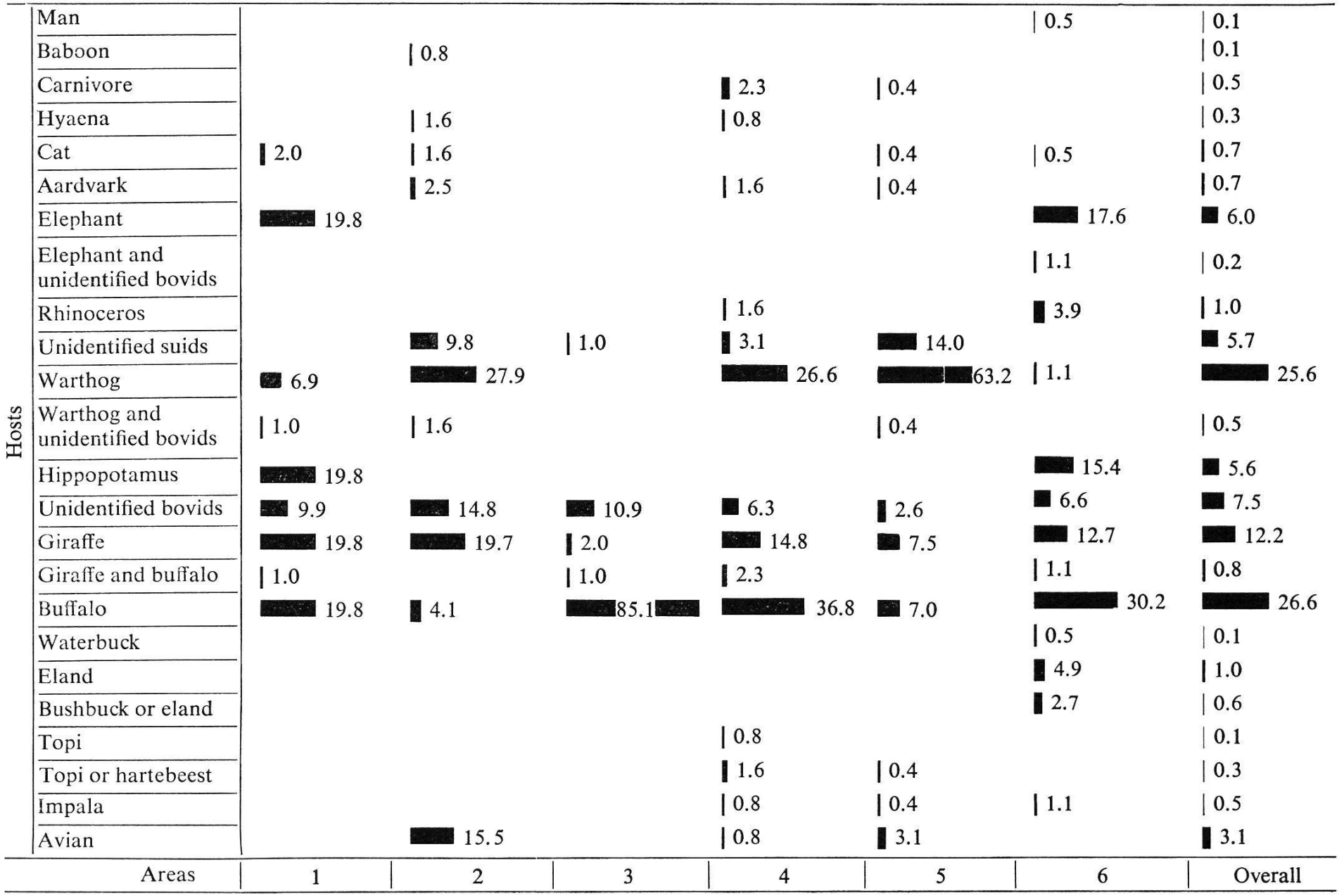

Table 5. Hosts of *G. swynnertoni* in the six survey areas

Hosts	Areas					
	1	2	3	4	5	6
	%	%	%	%	%	%
Primates	–	0.8	–	–	–	0.5
Bovids	51.5	39.5	99.0	64.1	18.3	60.8
Suids	7.8	38.7	1.0	29.0	77.3	1.1
Carnivores	1.9	3.2	–	3.1	0.9	0.5
Elephant	19.4	–	–	–	–	18.3
Rhinoceros	–	–	–	1.5	–	3.8
Hippopotamus	19.4	–	–	–	–	15.1
Aardvark	–	2.4	–	1.5	0.4	–
Avian	–	15.3	–	0.8	3.1	–

tribution from cat was 2 % while of the two double feeds, one came from giraffe/buffalo and the other from warthog/unidentified bovid.

Area 2. – The order of preferred hosts was warthog (27.9 %), giraffe (19.7 %) and birds (15.6 %), most probably ostrich which were present in the area. The less favoured hosts included buffalo (4.1 %), aardvark (2.5 %), hyaena (1.6 %), cat (1.6 %) and baboon (0.8 %). The two double feeds were from warthog/unidentified bovids.

Area 3. – The feeding pattern of the tsetse in this locality showed very strikingly that buffalo was the host most commonly fed on. Including the giraffe/buffalo double feed, 86.1 % of all feeds were from this host. Giraffe contributed 2 % of feeds and suids only 1 %.

Area 4. – The order of favoured hosts was buffalo (39.1 %), warthog (26.6 %) and giraffe (17.1 %). Carnivore (2.3 %), rhinoceros (1.6 %), aardvark (1.6 %), topi or hartebeest (1.6 %), topi (0.8 %), impala (0.8 %) and avian (0.8 %) contributed altogether 10.3 % of all feeds. Three double feeds found were from giraffe/buffalo.

Area 5. – Warthog, which contributed 63.6 % of all feeds, was the most favoured host in this area. Giraffe and buffalo provided only 7.5 % and 7.0 % of all feeds, respectively. Birds, possibly ostrich, contributed 3.1 % of the total feeds. Single feeds were from cat, topi or hartebeest, impala and aardvark. One double feed was from warthog/unidentified bovid.

Area 6. – In view of the proximity of Areas 6 and 1, and hence their similarity in fauna, the feeding pattern of the tsetse in this locality was similar to that in Area 1. The order of favoured hosts was buffalo (31.3 %), elephant (18.7 %), hippopotamus (15.4 %) and giraffe (13.8 %). Eland contributed 4.9 %, rhinoceros 3.9 %, eland or bushbuck 2.7 %, warthog 1.1 %, impala 1.1 %, cat 0.5 % and waterbuck

0.5 % of all feeds. Two double feeds were from elephant/unidentified bovids while a third from giraffe/buffalo. One feed was derived from man.

The overall results for the entire survey area are given in the last column of Table 4. Of 875 identified meals (double feeds included), 442 (50.5 %) came from bovids and nearly a third (31.3 %) from suids, possibly all warthog. Of the bovid feeds, 53.4 % were from buffalo and 25.3 % from giraffe. The other important hosts were elephant, hippopotamus and avians, which provided 6.2 %, 5.5 % and 3.1 % of all feeds, respectively. Contributions from carnivores were 1.5 %, rhinoceros 1.0 %, aardvark 0.7 % and primates 0.2 % of all feeds.

Man-fly contact

The local tsetse species were mostly dependent on game for food and little on man. Contact between man and fly was therefore "impersonal". However, of the three species encountered, *G. swynnertoni* was attracted most to man and attacked the fly-catching parties as well as other human beings in the area quite freely; in some cases even managing to draw blood undetected by their human hosts. Hence, the probability of man-fly contact for this species could be high. In Ikoma (Area 5) where *G. swynnertoni* and *G. pallidipes* were both present in equally high numbers, the latter seldom attacked the catching parties, thus man-*G. pallidipes* contact was negligible.

Discussion

Three different tsetse species were present within the Ikoma/Seronera/Kilimafedha triangle; they were – *G. swynnertoni,* Aust., *G. pallidipes,* Aust. and *G. brevipalpis.* Newst. *G. swynnertoni* was the most widely distributed species and occurred in high numbers in all the six areas surveyed. *G. pallidipes,* on the other hand, had restricted distribution; apart from Area 5 in Ikoma where they were quite numerous, the populations of this species encountered were small and confined largely to riverine vegetation. *G. brevipalpis* was restricted to a small localised area in Ikoma. The distribution of these three tsetse species was closely related to their vegetational requirements. The dry thorn-bush or nyika forms the main vegetation type in the area and is one to which *G. swynnertoni* is well adapted; hence its wide distribution. *G. pallidipes* too is adapted to life in the savannah but requires dense thickets. This latter type of vegetation was encountered mainly close to drainage, while in Ikoma (Area 5), continuous thickets

favoured by *G. pallidipes* were quite extensive. The localised distribution of *G. brevipalpis* was due to its requirement of dense thickets providing heavy shade (SWYNNERTON 1936). Of the areas surveyed, Area 5 (Ikoma) provided vegetational requirement for all the three tsetse species while Area 2, having open woodland with sparsely scattered small thickets, supported only *G. swynnertoni*. The remaining four areas afforded in the main the vegetational requirements for *G. swynnertoni* and, in restricted places, for *G. pallidipes* also.

Out of 6,384 *G. swynnertoni* dissected, 796 (12.5 %) contained mature trypanosome infections, as against 54 (8.7 %) of the 623 *G. pallidipes* examined. It is noteworthy that not a single fly showed salivary gland infection, indicating that the incidence of mature *brucei* type infection in the survey area must be very low indeed. It is known that in nature the incidence of positive salivary gland infections is generally very low. For example, in a Rhodesian sleeping sickness area in Tanzania, VANDERPLANK (1947) dissected 35,112 *G. swynnertoni* and *G. pallidipes*; infections attributed to *T. brucei* were under 0.1 %. In an endemic area in South Busoga, Uganda, the corresponding figures for the dissections made by WILSON and his colleagues (1971) were 0.09 % in 13,240 *G. pallidipes*. The same team, working in Mara Region, Kenya, from April, 1969 to October, 1970 found this type of infection in 0.07 % each of 10,375 *G. pallidipes* and 5,928 *G. swynnertoni* examined. It is noteworthy that of the total of four *G. swynnertoni* found carrying *brucei* infections, three were from a single batch caught in Talek area (WILSON et al. 1971). During an outbreak due to *T. rhodesiense* in the Lake Province, Tanzania, DUKE (1923) dissected a total of 2,206 *G. swynnertoni* caught from three different areas and obtained the following rates of *T. brucei* infection: (i) 0.24 % of 819 flies from the locality where the inhabitants were heavily infected; (ii) 0.1 % of 772 flies from villages where fly-man transmission was relatively low; and (iii) 0 % of 665 flies from uninhabited localities.

G. swynnertoni had a wide range of vertebrate hosts including bovids, suids, elephant, hippopotamus, primates, carnivores, aardvark and avians, of which buffalo, warthog and giraffe were the hosts most generally favoured. WEITZ (1963) has shown that various species of *Glossina* exhibit selective feeding patterns. The work on this subject carried out by WEITZ & GLASGOW (1956), GLASGOW et al. (1958) and WEITZ et al. (1958) has shown that the main host of *G. swynnertoni* is warthog. The present study indicates that the feeding habits of this tsetse species varied according to locality. For example, while warthog was the favoured host in Area 2 and 5, buffalo provided the bulk of the feeds in Area 3. In Area 4 and 6, buffalo was again found to have contributed more feeds than warthog. It is noteworthy that hippopotamus, elephant, buffalo and giraffe each provided 19.8 % of the

feeds in Area 1 and, even in Area 6 the number of feeds from hippopotamus and elephant were large. A high proportion of bird feeds, possibly ostrich, in Area 2 and 5 is also notable. Clearly, *G. swynnertoni* is adaptable in its feeding habits.

The blood of zebra, gazelles, wildebeest or dikdik was not found in any meal although these animals were present in large numbers in at least some of the areas surveyed. Reedbuck, steinbok and duiker were also present, though in small numbers, but provided none of the feeds. Impala, topi and hartebeest were common in many areas, but contributed only 0.9 % of the total feeds. In some areas gazelle and impala were present practically throughout the day and yet ignored by tsetse. It is probable that these animals which are highly active can avoid tsetse attacks very efficiently. A proportion of feeds from topi is of interest. Although WEITZ et al. (1958) found some *G. swynnertoni* meals derived from hartebeest, topi or wildebeest, a specific topi feed has never before been identified from any *Glossina* species.

It should be noted that this is the first time a study of the tsetse feeding habits has been carried out in six areas within a given locality and within a few days of each other. The results show striking differences between areas and this seems to correspond very well to the availability of hosts. For example, in Areas 2 and 5 with low buffalo populations (5–10) the number of feeds on suids were 37.7 % and 77.2 %, respectively, whereas in Area 4 with a high frequency of buffalo (105–115), 85.1 % of the feeds were from this host. Again, a large number of feeds from warthog (77.2 % of all feeds) in Area 5 corresponds to high warthog and low buffalo populations. Another example of *G. swynnertoni* feeding on a host readily available is given by the giraffe feeds. Giraffe were present in large numbers in Area 1, 2 and 6, and the number of feeds on this host were likewise high in these areas: 19.8, 19.7 and 12.7 %, respectively. The high percentage of giraffe feeds in Area 4 (17.8 %) are probably related to small numbers of buffalo present in the area. Furthermore, large herds of giraffe were observed in this area during the period of the survey. The high percentage of Avian feeds in Area 2 is also noteworthy. This is almost certainly due to the greater number of ostrich present in this area compared with other areas.

Weak feeds derived respectively from the family *Bovidae* and *Suidae,* which failed to react in the tests for the species, were classed as unidentified species of the groups concerned. "Cat" feeds were tested for members of the *Felidae* family including lion and leopard. Feeds which were assigned as "Carnivore" were members of the older Carnivore excluding the cat, dog, hyaena and mongoose families. It is possible that these feeds were derived from zorilla or honey badger.

In addition to furnishing the bulk of the blood meals for tsetse,

game provides a reservoir for the causative agent of Rhodesian sleeping sickness (WILLETT & FAIRBAIRN 1955; HEISCH et al. 1958). The correlation of the animals on which *G. swynnertoni* feeds with the incidence of *T. brucei* infections in such hosts (ASHCROFT 1959; GARNHAM 1960; GUILBRIDE et al. 1962; BAKER 1968; GEIGY et al. 1971) and with the degree of their susceptibility to experimental infection with these trypanosomes (ASHCROFT et al. 1959) indicates quite strikingly that the favourite hosts are not efficient reservoirs of *T. rhodesiense* and thus confirm a similar suggestion made by ASHCROFT et al. (1959). The data compiled by ASHCROFT (1959) show that only one of the 26 buffaloes examined harboured *brucei* trypanosomes while, in the 150 warthogs, 68 giraffes and 11 elephants, the corresponding rates were 2 %, 1.5 % and 0 %, respectively. GARNHAM (1960) and GUILBRIDE et al. (1962) examined altogether 424 hippopotami, none of which showed *T. brucei* infection. In the less favoured waterbuck, hartebeest, eland, bushbuck, and impala, the incidence of this type of infection was as high as 24 %, 9 %, 5 %, 5 % and 1.3 %, respectively (ASHCROFT 1959). Studies on the reservoir potential of the game animals have shown that warthog and some ruminants, possibly buffalo and giraffe, usually become infected with *T. rhodesiense* and *T. brucei* but are resistent, the parsitaemia being scanty and not persisting for long. On the other hand, bushbuck, eland, hyaena and impala, which were rarely fed on by *G. swynnertoni,* are noted for their tolerance to such infections, nearly always become infected and show a blood positive period of considerable duration (ASHCROFT et al. 1959). Aardvark, which provided a small proportion of *G. swynnertoni* feeds (0.7 % of total feeds), is highly susceptible to *T. rhodesiense* and usually killed by it (VANDERPLANK 1941; BURTT 1946b). It is conceivable that such animals might in certain circumstances function, though, temporarily, as reservoirs of the parasite.

It is known that under the condition of game scarcity, *G. pallidipes* will attack man but not as readily as *G. swynnertoni,* which shows remarkable attraction to man. Owing to its confinement to dense thickets, *G. brevipalpis* seldom comes into contact with man and does not readily attack him (SWYNNERTON 1923b). This would explain the predominance of *G. swynnertoni* in the random and fly-round catches. It has been pointed out by SWYNNERTON (1923a) that even when cattle are present, this species attacks man quite freely. Study on the reaction of flies to the simultaneous presence of bait cow and man in Area 2 has confirmed his observation. In order or readiness to attack man, the three species of tsetse encountered may be ranged as follows: (1) *G. swynnertoni,* (2) *G. pallidipes* and (3) *G. brevipalpis. G. swynnertoni* is a clandestine tsetse and gentle "biter" (SWYNNERTON 1923a)

as indicated by their several successes in drawing blood off the catching parties undetected.

The results of the random and fly-round catches show that, although in nature the two sexes occur in approximately equal numbers, the proportions of females caught in all the survey areas were exceedingly low. This indicates a "non-hungry" picture (FISKE 1920; NASH 1948). The local tsetse species are primarily zoophilic and, since game was abundant, the flies attacked man only through chance meeting.

References

ASHCROFT, M. T. (1959). The importance of African wild animals as reservoirs of trypanosomiasis. – E. Afr. med. J. *36,* 289–297.

ASHCROFT, M. T., BURTT, T. & FAIRBAIRN, H. (1959). The experimental infection of some African wild animals with *Trypanosoma rhodesiense, T. brucei* and *T. congolense.* – Ann. trop. Med. Parasit. *53,* 147–161.

BAKER, J. R. (1968). Trypanosomes of wild mammals in the neighbourhood of the Serengeti National Park. – Symp. zool. Soc. Lond. No. *24,* 147–158.

BURTT, E. (1946). Observations on an antbear *(Crycteropus afer)* in relation to infection with *Trypanosoma rhodesiense.* – Trans. roy. Soc. trop. Med. Hyg. *39,* 529–532.

DAR, F. K. & WILSON, A. J. (1970). The freeze preservation of insect proboscis forms of *T. vivax* and *T. congolense,* and blood forms of *T. vivax.* – E. Afr. Trypanosomiasis Res. Org. Rept. 1969, 23–24.

DUKE, H. L. (1923). An inquiry into an outbreak of human trypanosomiasis in a *"Glossina morsitans"* belt to the east of Mwamza, Tanganyika Territory. – Proc. roy. Soc. (B), *94,* 250–265.

FISKE, W. F. (1920). Investigations into the bionomics of *Glossina palpalis.* – Bull. ent. Res. *10,* 347–463.

GARNHAM, P. C. C. (1960). Blood parasites of hippopotamus in Uganda. – E. Afr. med. J. *37,* 495.

GLASGOW, J. P., ISHERWOOD, F., LEE-JONES, F. & WEITZ, B. (1958). Factors influencing the staple food of tsetse flies. – J. anim. Ecol. *27,* 59–69.

GUILBRIDE, P. P. L., COYLE, T. J., MCANULTY, E. G., BAKER, L. & LOMAX, G. D. (1962). Some pathogenic agents found in hippopotamus in Uganda. – J. comp. Path. *72,* 137–141.

HEISCH, R. B., MCMAHON, J. & MANSON-BAHR, P. E. C. (1958). The isolation of *Trypanosoma rhodesiense* from a bushbuck. – Brit. med. J., Nov. 15, 1203–1204.

JACKSON, C. H. N. (1933). The causes and implications of hunger in tsetse flies. – Bull. ent. Res. *24,* 443–482.

JARMAN (1970). Personal communication.

MULLIGAN, H. W. (1970). The African Trypanosomiases. 950 pp. ill. London: George Allen and Unwin Ltd.

NASH, T. A. M. (1948). Tsetse flies in British West Africa. – H. M. Stationery Office, London, 77 pp.

SWYNNERTON, C. F. M. (1923 a). The entomological aspects of an outbreak of sleeping sickness near Mwanza, Tanganyika Territory. – Bull. ent. Res. *13,* 317–370.

SWYNNERTON, C. F. M. (1923 b). The relation of some East African tsetse flies to the flora and the fauna. – Trans. roy. Soc. trop. Med. Hyg. *17,* 128–141.

SWYNNERTON, C. F. M. (1933). Some traps for the tsetse flies. – Bull. ent. Res. *24,* 69–102.

SWYNNERTON, C. F. M. (1936). The tsetse flies of East Africa. – Trans. roy. ent. Soc. London, *84,* 1–579.

VANDERPLANK, F. L. (1941). A note on the relation between the virulence of *Trypanosoma rhodesiense* towards rats and the normal blood temperature of the previous mammalian host. – Trans. roy. Soc. trop. Med. Hyg. *35,* 43–46.

VANDERPLANK, F. L. (1947). Seasonal and annual variation in the incidence of trypanosomiasis in game. –Ann. trop. Med. Parasit. *41,* 365–374.

WEITZ, B. (1952). The antigenicity of sera of man and animals in relation to the preparation of specific precipitating antisera. – J. Hyg. *50,* 275–294.

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

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

WEITZ, B. & GLASGOW, J. P. (1956). The natural hosts of some species of *Glossina* in East Africa. – Trans. roy. Soc. trop. Med. Hyg. *50,* 593–612.

WEITZ, B., LANGRIDGE, W. P., NAPIER BAX, P. & LEE-JONES, F. (1958). The natural hosts of *Glossina longipennis* Corti and some of other tsetse flies in Kenya. – International Scientific Committee for Trypanosomiasis Research, 7th Meeting. Publication No. 41, 303–312.

WILLETT, K. C. & FAIRBAIRN, H. (1955). The Tinde experiment: a study of *Trypanosoma rhodesiense* during eighteen years of cyclical transmission. – Ann. trop. Med. Parasit. *49,* 278–292.

WILSON et al. (1971). Personal communication.

Sleeping Sickness Survey in Musoma District, Tanzania

III. Survey of Cattle for the Evidence of *T. rhodesiense* Infections

P. M. MWAMBU[1] & J. S. P. MAYENDE[1]

Abstract

During a sleeping sickness survey of the Musoma District, particularly the Serengeti National Park and its environs, 798 head of cattle in the Ikoma area (just outside the park) were examined for evidence of *T. rhodesiense* infections. Four of the isolations of *T. brucei* subgroup organisms gave an equivocal result with the BIIT.

The results have revealed a 3.5% infection in cattle with *T. brucei* subgroup organisms, a group which includes *T. rhodesiense*. The BIIT for differentiating *T. brucei* (sensu stricto) from *T. rhodesiense* has, however, shown majority of the isolates to be *T. brucei* with others giving inconclusive BIIT results.

Introduction

The area where the examination of cattle was carried out lies immediately outside and north of the western extension of the Serengeti National Park (see map in Fig. 1, part I). This area is inhabited by Waikoma people who keep only the East African short-horned zebu cattle. These cattle are grazed on communal basis, and modern methods of livestock husbandry are not practised. The cattle while grazing come into frequent contact with tsetse flies and game animals from the Serengeti National Park and Game Reserve. Other times they are in close contact with the people in the homesteads (Bomas) where they are tethered for the nights and part of the mornings.

Previous records of cattle trypanosomiasis in Ikoma are difficult to trace and the infecting *Trypanosoma* species are not clearly known. The Tanzania Veterinary Department, however, is aware of the existence of the disease and mention is made in the departmental Annual Reports of treatment of cattle against trypanosomiasis.

Material and Methods

Blood samples

All cattle examined were resident and bred in the area. It had been arranged to collect blood samples from the animals at a public crush, but the latter was found unsuitable for the work and so sampling was performed at the cattle owners' homesteads.

Each of 798 cattle presented was bled from a vein in the ear and thick and thin blood films were made. Drops of blood were also collected from 765 cattle and absorbed on Whatman No. 4 filter paper.

[1] East African Trypanosomiasis Research Organization, Tororo, Uganda.

Among the herd 260 cattle randomly selected were aseptically bled from the jugular vein for immediate, intraperitoneal inoculation into mice. Two Swiss white mice were inoculated with blood from each animal.

The thick and thin blood films were allowed to dry and then carried back to the laboratory in slide boxes. Blood samples on filter paper were dried in the sun, after which they were bundled together, wrapped in envelopes and preserved in a deep freeze (–20°C).

Examination of the blood samples

Thick and thin blood films were stained with Giemsa's stain. Stained thick blood films were examined under an oil immersion objective (×100) with ×8 oculars until parasites were found, or until at least 200 microscope fields had been scanned. Where parasites were present, the respective thin blood films were examined for the identification of the trypanosome species.

The dried blood samples on filter paper were processed and examined as outlined in previous work by the indirect fluorescent antibody test (IFT) (CUNNINGHAM et al. 1967). Three antigens (i.e. *T. brucei, T. vivax* and *T. congolense*) were used.

Wet films made from the tails of the inoculated mice were examined on every working day starting on the fourth day after inoculation, for four weeks. Thereafter the mice which were aparasitaemic were examined once weekly up to 60 days after inoculation. Mice that were positive were left to attain good parasitaemias, then thin blood films were made, stained and examined for the identity of the infecting trypanosomes. Where the trypanosomes were found to be of the *T. brucei* subgroup, the mice were bled from the heart and the infected blood preserved in liquid nitrogen.

Blood Incubation Infectivity Test (BIIT)

Ten *T. brucei* subgroup stabilates isolated in mice and preserved in liquid nitrogen were tested to differentiate those that were *T. brucei* sensu stricto from those that were likely to be *T. rhodesiense* using BIIT (RICKMAN & ROBSON 1970). *T. brucei* and *T. rhodesiense* are morphologically indistinguishable members of the *T. brucei* subgroup and yet the former is non pathogenic to man. Stored human whole blood obtained from Tororo Hospital blood bank was used in the test. The experimental and control samples were incubated at 37°C for 5 hours before the inoculation of rats. All the white rats used in this test were mature. Apart from the initial test, two additional tests were performed for each trypanosome stabilate. BIIT negative and positive

controls (a man-tested *T. brucei,* E.A.T.R.O. 999, and two proven *T. rhodesiense,* one man-tested E.A.T.R.O. 1135 and the other isolated from man-E.A.T.R.O. 1084) were included during the test. The rats used in the test were examined daily for 40 days.

Results

A summary of the results of the tests is given in tables 6 and 7. Results in Table 6 show an overall infection in 28 cattle (3.5 %) with

Table 6. The number of cattle found infected with *T. brucei* subgroup organisms by thick blood films and mouse inoculation

Method of examination	No. of cattle examined	Positive results No.	Positive results % of 798
Thick films (a)	798	22 *	2.8
Mouse inoculation (b)	260	12 **	1.5
Combined (a) and (b)	798	28	3.5
IFT	765	251	31.5

* 5 of these were mixed (with *T. vivax*) infections.
** 2 of these were mixed (with *T. congolense*) infections.

Table 7. Results of the BIIT on the 10 isolated *T. brucei* subgroup organisms and on BIIT positive and negative controls

Stabilate No.	Results			Remarks
	Test	Retest 1	Retest 2	
E.A.T.R.O. 1801	0	0	0	BIIT negative
E.A.T.R.O. 1815	+	0	0	Equivocal result
E.A.T.R.O. 1817	0	0	0	BIIT negative
E.A.T.R.O. 1818	+	0	0	Equivocal result
E.A.T.R.O. 1820	0	0	0	BIIT negative
E.A.T.R.O. 1825	+	+	0	Equivocal result
E.A.T.R.O. 1828	0	0	0	BIIT negative
E.A.T.R.O. 1835	0	+	+	Equivocal result
E.A.T.R.O. 1843	0	0	0	BIIT negative
E.A.T.R.O. 1845	0	0	0	BIIT negative
E.A.T.R.O. 999 (Neg. control)	0	0	0	BIIT negative
E.A.T.R.O. 1135 (Pos. control)	+	+	+	BIIT positive
E.A.T.R.O. 1084 (Pos. control)	+	+	+	BIIT positive

T. brucei subgroup organisms, as diagnosed by the standard diagnostic techniques (SDT). The immunofluorescent test (IFT) which detects the common antibodies to any trypanosome infection, however, showed more positive cases. In addition to *T. brucei* subgroup infection *T. congolense* and *T. vivax* group infections were diagnosed by the SDT. The *T. vivax* and *T. congolense* infections will be discussed in a later publication.

Of the ten stabilates tested and retested (see Table 7), by the BIIT, six gave negative results. Of the remaining four, two (E.A.T.R.O. 1815 and 1818) were positive on initial test, but negative on the two retests. The positive result in each case was obtained in only one of the two rats used. The third (E.A.T.R.O. 1825) was positive on initial test and one retest but negative on a second retest. The positive result in this case was obtained in only one of the two rats. The fourth (E.A.T.R.O. 1835) was negative on initial test but positive on the two retests. Again as in the above cases, only one of the two rats used in each retest was parasitaemic.

The BIIT positive (E.A.T.R.O. 1084 & 1135) and negative (E.A.T.R.O. 999) control stabilates used gave the expected results, both rats in the case of the positive controls showing parasitaemia throughout the period they were alive.

Discussion

The results of the examination of blood samples obtained from cattle clearly indicate a high incidence of cattle trypanosomiasis in the Ikoma area. *T. brucei* subgroup, *T. congolense* and *T. vivax* groups are all involved. The results of mouse inoculation alone indicate that if this technique had been used in the examination of the entire 798 cattle, a higher number of *T. brucei* infections might have been diagnosed. Many of them could have given unequivocal results with the BIIT and therefore could have been supposed to be *T. rhodesiense*. Results of the investigation of sleeping sickness in the human population in the Ikoma area (Part I) and dissections of tsetse flies caught in areas contiguous to where cattle were examined (Moloo et al., Fig. 1, this paper, part II) revealed neither *T. rhodesiense* in particular nor *T. brucei* subgroup infections in general. The epidemiological significance of these results will be discussed in full in Part V in this issue.

Conclusion

Cattle in Ikoma, an area immediately bordering the National Park, were found to have a high infection rate of *T. brucei* subgroup,

organisms, and a few of these infections may have been *T. rhodesiense,* a pathogenic trypanosome to man. The four strains which gave inconclusive results by the BIIT need further retesting to ascertain their infectivity for man.

References

CUNNINGHAM, M. P., WILSON, A. J. & KIMBER, C. D. (1967). Modification of the indirect fluorescent antibody test as applied to bovine trypanosomiasis. – E.A.T.R.O. annual rep. for 1966, p. 29.

RICKMAN, L. R. & ROBSON, J. (1970). The blood infectivity test: a simple test which may serve to distinguish *Trypanosoma brucei* from *T. rhodesiense.* – Bull. Wld Hlth Org. *42,* 650–651.

East African Trypanosomiasis Research Organization, Tororo, and
Swiss Tropical Institute, Basle

Sleeping Sickness Survey in Musoma District, Tanzania

IV. Examination of Wild Mammals as a Potential Reservoir for *T. rhodesiense*

R. GEIGY [1], P. M. MWAMBU [2] and M. KAUFFMANN [1]

Abstract

The incidence of trypanosomiasis was investigated in 115 mammals belonging to 13 species. Twelve strains of *T. brucei* subgroup were isolated; 2 from hyaena, 5 from lion, 1 from warthog, 1 from waterbuck and 3 from hartebeest. Five strains of these showed positive reaction with the BIIT and are suggestive of *T. rhodesiense*. Further investigations are necessary to confirm the identity of the trypanosomes.

Introduction

During the survey 115 wild mammals, belonging to 13 species were examined in 6 different areas; 4 within the Serengeti National Park, 1 just along the Park boundary near Kirawira and 1 in the Ikoma game reserve (cf. map and Table 8; for vegetation and game distribution cf. part II, Table 8 and Fig. 5, p. 190). Most animals were shot, but 8 lions and 3 hyaenas were darted with tranquillizers (Sernylan or Succinylcholin), 1 lion cub was caught alive and later released.

Material and Methods

Isolation of trypanosomes

From the animals that were shot blood was collected as soon as possible after death, whenever possible by cardiac puncture, otherwise from the severed neck, or in the case of the darted animals, by venous puncture. Rats not being available in sufficient numbers, between 7 to 10 mice were inoculated intraperitoneally. Two methods were then used for the first 70 mammals. Two mice were inoculated with 0.5 to 1 ml of whole blood, the rest with blood diluted 4 : 1 with sodium citrate 3.8 % (1 to 1.5 ml per mouse). For the last 45 mammals only citrate blood was used for inoculation. In the whole, about 940 mice were used. Surviving mice were examined repeatedly by wet preparation up to 4 to 8 weeks after inoculation. As soon as a mouse became heavily

---

[1] Swiss Tropical Institute, Basle, Switzerland.
[2] E.A.T.R.O., Tororo, Uganda.

infected with either *T. brucei* subgroup or *T. congolense* stabilates were made using the E.A.T.R.O. standard technique and preserved in liquid nitrogen; sometimes a second stabilate was made after a further passage in mice.

All *T. brucei* subgroup strains were later submitted to the "Blood Incubation Infectivity Test" (BIIT) (RICKMAN & ROBSON 1970) for the differentiation between *T. brucei* and *T. rhodesiense* strains. For additional examination, several thick and thin films were made direct from the game animals and sometimes also with the rest of the citrated blood. A few trials with 1 hyaena, 1 lion, and 3 topi were made with the haematocrit centrifuge technique (HCT) (WOO 1970). About half the inoculated mice were also examined in this way before being destroyed at the end of the experiment (WOO & KAUFFMANN 1971).

An autopsy was performed on most of the killed animals by Dr. Lossos (E.A.T.R.O.) and/or Mr. Burton Gwamaka (S.R.I.) and specimens preserved for later histopathological examination.

Results

The overall infection rate for trypanosomes including all findings proved to be 38.2 %.

Table 8. Description of the 6 * different areas in central and western Serengeti National Park where 115 animals belonging to 13 species have been darted or shot (cf. map p. 190)

Area	Description	Approx. distance of area centre from Banagi
A	along Park boundary N/E Kirawira Research Station	38 miles
B	Mwanza Road, north Orangi River Junction	18 miles
C	Triangle between Track Banagi-Mwanza Road and Banagi-Ikoma Road (around Retima Hippo Pool)	6 miles
D	Triangle between Banagi-Kilimafedha south of Mangi River	3.5 miles
E	Banagi-Ikoma Gate Road, mainly eastwards along Park boundary	10 miles
F	Ikoma controlled shooting area	8 miles east Ikoma Fort

* A single lion cub was caught alive and returned south of Seronera (SS).

Six strains of *T. brucei* subgroup, 12 of *T. congolense* and 6 mixed *T. brucei-congolense* came up in mice. Thirteen other infections of *T. vivax* (4 mixed with *T. brucei* subgroup or *T. congolense)* and 11 (T. unidentified) were found in thick and thin films. A further T.? was detected by the haematocrit centrifuge technique in one of the 3 topi

Table 9. Distribution of examined and infected wild animals

Area	Animals examined	Isolated in mice			Found in blood films only			Animals infected
		brucei	congol.	b + c	vivax	v+b	or c?	
A	9	0	0	0	0	0	3	3
B	11	0	1	0	2	0	0	3
C	42	1	3	0	2	0	3	9
D	14	0	0	0	2	0	2	4
E	24	4	4	6	3	4	2 *	19
F	13	1	4	0	0	0	1	6
SS	1	0	0	0	0	0	0	0
Total	115	6	12	6	9	4	11	44 = 38.2%

* In one case HCT only.

Table 10. Incidence of Trypanosomiasis found in wild mammals Serengeti National Park and Ikoma Fort Controlled shooting Area

Species	No examined	No infected	*T. brucei* group	*T. congolense*	*T. vivax*	T.?
Crocuta crocuta	5	5	2	4	0	0
Panthera leo	9	8	5	6	4	1
Equus burchelli	10	1	0	0	1	0
Phacochoerus aethiopicus	13	5	1	1	0	3
Tragelaphus scriptus	2	1	0	1	0	0
Kobus defassa	10	6	1	1	2	2
Redunca redunca	10	2	0	0	2	0
Alcelaphus buselaphus	11	8	3	2	3	2
Connochaetes taurinus	10	1	0	0	0	1
Damaliscus korrigum	11	1	0	0	0	1
Aepyceros melampus	11	2	0	0	0	2
Gazella granti	2	1	0	1	0	0
Gazella thomsonii	11	3	0	2	1	0
13 species	115	44	12	18	13	12

All strains of *T. brucei* group as well as *T. congolense* came up in mice. T.?: only very few badly preserved trypanosomes were found.

Table 11. Data of individuals found infected

Common name and scientific name	Indiv. number (field number) sex/area	Strains isolated B	Strains isolated C	Mice infected over total inoculated	E.A.T.R.O. stabilate	Thick films B	Thick films C	Thick films V	Thick films ?	Thin films B	Thin films C	Thin films V	Thin films ?	HCT
Spotted hyaena	1 * (71) ♀ E	+	0	3/10	1857	0	0	0	0	0	0	0	0	0
Crocuta crocuta	2 (98) ♀ F	0	+	9/9	1823	0	0	0	0	0	0	0	0	–
	3 (99) ♂ F	0	+	3/10	1876	0	0	0	0	0	+	0	0	–
	4 * (103) ♀ E	0	+	4/7	–	0	0	0	0	0	0	0	0	–
	5 * (109) ♂ E	+	+	8/8	1809/1851	0	0	0	0	0	0	0	0	–
Lion	1 * (72) ♂ E	+		1/10	1858	0	0	0	+	0	+	0	0	+
Panthera leo			+	1/10	1844									
	2 * (73) ♂ E	0	+	2/10	1859	0	+	0	0	0	0	+	0	–
	3 * (74) ♂ E	+	0	10/10	1860	0	0	0	0	+	0	0	0	–
	4 * (104) ♂ E	+		2/10	1811	0	0	+	0	0	0	+	0	–
			+	4/10	–									
	5 * (105) ♂ E	+		1/10	1822	0	0	0	0	0	0	0	0	–
			+	5/10	–									
	6 * (106) ♀ E	0	+	8/8	–	–	–	–	–	0	+	+	0	–
	7 * (107) ♂ E	0	+	8/8	1808	0	–	–	–	0	+	+	0	–
	8 * (108) ♂ E	+	0	8/8	1804	–	+	0	0	0	0	0	+	–
Zebra	10 (70) ♀ C	0	0	0/8	–	0	0	+	0	0	0	0	0	–
Equus burchelli														
Warthog	1 (1) ♀ A	0	0	0/8	–	0	0	0	+	0	0	0	0	–
Phacochoerus	4 (4) ♀ A	0	0	0/8	–	0	0	0	+	0	0	0	0	–
aethiopicus	8 (8) ♀ A	0	0	0/8	–	0	0	0	+	0	0	0	0	–
	12 (97) ♀ F	+	0	3/5	1803	0	0	0	0	0	0	0	0	–
	13 (100) ♂ F	0	+	9/10	1838	0	0	0	0	0	0	0	0	–
Bushbuck	1 (84) ♀ B	0	+	2/10	1837	–	–	–	–	0	0	0	0	–
Tragelaphus scriptus														
Defassa waterbuck	2 (76) ♀ D	0	0	0/10	–	0	0	0	0	0	0	+	0	–
Kobus defassa	3 (77) ♀ D	0	0	0/10	–	0	0	+	0	0	0	+	0	–

Defassa waterbuck	4	(78)	♂	C	0	+	9/10	1807	0	+	0	0	0	0	0	0	–
Kobus defassa	5	(79)	♀	C	0	0	0/10	–	0	0	0	+	0	0	0	0	–
	6	(80)	♂	C	0	0	0/10	–	0	0	0	0	0	0	0	+	–
	8	(82)	♂	C	+	0	1/10	1836	0	0	0	0	0	0	0	0	–
Bohor reedbuck	4	(88)	♀	B	0	0	0/10	–	–	–	–	–	0	0	+	0	–
Redunca redunca	6	(90)	♂	B	0	0	0/10	–	–	–	–	–	0	0	+	0	–
Coke's hartebeest	1	(41)	♀	E	0	0	0/8	–	0	0	+	0	0	0	0	0	–
Alcelaphus	2	(42)	♂	E	+	+	8/8	1852	0	+	0	0	0	0	0	0	–
bucelaphus	3	(43)	♀	E	0	0	0/8	–	0	0	+	0	0	0	0	0	–
	4	(44)	♀	E	0	0	0/8	–	0	0	+	0	0	0	0	0	–
	6	(46)	♀	E	0	0	0/8	–	0	0	0	+	0	0	0	0	–
	7	(47)	♀	E	+	+	5/8	1810/1873	0	0	0	0	0	0	0	0	–
	9	(49)	♂	E	+	0	5/8	1856	0	0	0	0	0	0	0	0	–
	11	(101)	♂	F	0	0	0/10	–	0	0	0	+	0	0	0	0	–
Wildebeest	1	(31)	♀	C	0	0	0/8	–	0	0	0	+	0	0	0	0	–
Connochaetes taurinus																	
Topi	10	(60)	♂	E	0	0	0/8	–	0	0	0	0	0	0	0	0	+ *
Damaliscus korrigum																	
Impala	3	(12)	♀	D	0	0	0/8	–	0	0	0	+	0	0	0	0	–
Aepyceros melampus	7	(16)	♀	D	0	0	0/2	–	0	0	0	+	0	0	0	0	–
Grant's G.	2	(93)	♂	F	0	+	4/5	1841	0	0	0	0	0	0	0	0	–
Gazella granti																	
Thomson's G.	4	(24)	♂	C	0	+	7/8	–	0	+	0	0	0	0	0	0	–
Gazella thomsonii	5	(25)	♂	C	0	+	3/7	1855	0	+	0	0	0	0	0	0	–
	6	(26)	♀	C	0	0	0/8	–	0	0	+	0	0	0	0	0	–
Total	44				12	18			0	6	7	10	1	4	8	2	2

examined. Where the diagnosis is put down as T.?, only one or two badly preserved forms were seen.

In Table 9 the results are classified according to their distribution over the 6 areas examined. *T. brucei* subgroup was found in area C, E and F, *T. congolense* in B, C, E and F and *T. vivax* in B, C, D and E. In area A only three unidentified infections were detected. The highest infection rate was found in area E, where 19 out of 24 animals showed a detectable parasitaemia, i.e. 75 % comprising 10 of the 12 *T. brucei* subgroup infections.

Table 10 shows the distribution of the trypanosome strains found in the 13 species of mammals examined.

Table 11 contains the detailed data of each animal showing trypanosomes, such as strains isolated and preserved in liquid nitrogen as well as all the findings from thick and thin films and HCT. The most interesting result is the high incidence of *T. brucei* subgroup and *T. congolense* in lion and spotted hyaena; 7 of the 12 strains of the former were isolated from these two species of mammals, of which all but one animal harboured either one or the other or both parasites. The negative lion was a young cub, about three months old and probably to young to develop a patent infection.

The 12 *T. brucei* subgroup strains were examined by the BIIT, using fresh human blood for each test and incubating for 5 hours. Eleven strains were available as stabilates from the first passage in mice, the last one (hartebeest 42) from passage two only. The results are given in Table 12. One hyaena, 2 lions, 1 waterbuck and 1 hartebeest gave positive results, i.e. reacted like *T. rhodesiense*. Up to now the strains were tested over 1 to 10 mice passages (the work is still going on). The strain isolated from waterbuck 82 (stabilate E.A.T.R.O. 1836) gave consistently positive results over mouse-passage 2 to 9, reacting exactly as the *T. rhodesiense* strains isolated from man and used as controls. Eight of the 9 known *T. rhodesiense* strains were isolated from patients at E.A.T.R.O., the last one in the Serengeti National Park (stabilate of third passage made by E.A.T.R.O.). In 1 case we started with a stabilate E.A.T.R.O. 931, made from metacyclic forms after glossina-passage. As regards the other four strains, BIIT frequently gave positive, sometimes negative results. Three times an early test was negative, while a later test carried out with mice from the same passage became positive. In the case of hyaena 71 (E.A.T.R.O. 1857) another series of tests was carried out over 9 passages, starting from a second capillary tube. The BIIT was then negative throughout. These results are difficult to interpret, as RICKMAN & ROBSON (1970) found that each strain they tested was always negative or always positive. But on the other hand, in their study never more than 6 tests were carried out on any one strain, whereas in the present study be-

Table 12. Strains of *T. brucei*-subgroup isolated 1970/Serengeti: Results of BIIT

Host	Hy 71	Hy 109	Lion 72	Lion 74	Lion 104	Lion 105	Lion 108	Warthog 97	Water-buck 82	Harte-beest 42	Harte-beest 47	Harte-beest 49
Species	B	B+C	B+(C)	B	B+(C)	B+(C)	B	B	B	B+C	B+C	B
Stabilate	1857	1809	1858	1846 1860 ×	1811	1822	1804	1803	1836	1852	1810 × 1873	1854 × 1856 × 1866 ×
1. passage												
2 passage	0 0 0 0	0	0 0 0 0	0 0	+ +	0 0	0 0 0 0	0	+ +		+	0 0
3. passage	0 0 + 0		0 0	0	+	0 0	0 0		+	0 0	+ ++	0 0 0
4. passage	0 0		0 0		+ +	0 +	0 0		+ ++		0	0
5. passage	+ 0 ++ 0		0 0		++ ++	+ +	0 0		+		00 ++	
6. passage	0 0 + 0		0 0		++	0 0	0 0		+		0 +	
7. passage	0 + 0		0 0		+	0 0	0 0		++		+	
8. passage	0 + 0		0 0		0	0 0	0 0		++		0 0	
9. passage	0 0		0 0			0 0	0 0		+		0	
10. passage	0		0 0			0 0	0 0					

Table 13. Other parasites found in thick and thin films

Host	Number examined	Babesia nuttallia theileria	Hepatozoon	Borrelia	Micro-filaria
Spotted hyaena	5	5	5	0	5
Lion	9	8	9	0	0
Zebra	10	10	0	1	2 *
Warthog	13	1	0	0	1
Bushbuck	2	2	0	0	0
Waterbuck	10	10	0	0	3 *
Reedbuck	10	2	0	3	0
Wildebeest	10	5	0	0	0
Hartebeest	11	10	0	0	1
Topi	11	7	0	0	0
Impala	11	2	0	1	0
Grant's gazelle	2	0	0	0	0
Thomson's gazelle	11	4	0	0	2
Total	115	66	14	5	14

* With sheath.

Table 14. Mice control sheet lion 6 (106)

106 Lion ♀ Mouse adult	Days after inoculation											
	wet preparation										HCT	
	6	7	8	9	10	12	14	15	20	24	24	33–75
729	0	0	0	0	0	0	0	0	0	0	(+)	0
730	+	0	(+)	0	0	0	0	0	0	0	(+)	(+)
731	0	0	0	0	0	0	0	0	0	0	(+)	(+)
732	0	(+)	(+)	0	0	0	0	0	0	0	(+)	(+)
733	0	(+)	0	0	0	0	0	0	0	0	0	(+)
734	0	0	0	0	0	0	0	0	0	0	(+)	(+)
735	0	(+)	(+)	0	0	0	0	0	0	0	(+)	(+)
736	(+)	(+)	(+)	0	0	0	0	0	0	0	?	(+)

? = haematocrit tube broken.
(+) = 1–3 trypanosomes seen in whole preparation or tube.

tween 8 and 18 tests were undertaken over 6 to 8 passages on the positive-negative strains. One explanation might be that in these cases we had a mixed population of *T. brucei-T. rhodesiense*. The strains giving positive results with BIIT will be tested on volunteers for pathogenicity for man.

In contrast to the findings of BAKER et al. (1967), no *T. brucei* subgroup or *T. congolense* were found in the 10 wildebeest examined. This may be due to the fact that our wildebeest belonged to migrating herds, whereas in the earlier survey resident herds had been examined.

While searching for trypanosomes in thick and thin films, a number of other parasites were found. A preliminary list is given in Table 13.

Discussion

In contrast to other authors, we never found a case of *T. congolense* in thick or thin films which did not appear in mice as well. Two factors offer themselves to explain this discrepancy. By inoculating 6 to 10 mice instead of two only, the chances of isolating *T. congolense* increase considerably, since frequently only 1 to 3 out of up to 10 mice inoculated became positive (cf. Table 11). Secondly, *T. congolense* may produce only a very feeble and fleeting infection in laboratory animals as happened on this occasion in the case of lion 6 (106) and hyaena 3 (99) and 4 (103). Eight mice were inoculated from lion 6 (106), 2 of them showed very few trypanosomes on day 6, another 3 came up on day 7, 4 were still positive on day 8, but on days 10, 12, 14, 15, 20 and 24 not a single trypanosome was spotted in wet preparations. After that HCT was used on the whole series and a very few forms were found in 6 of the 8 mice (cf. Table 7).

From hyaena 3 (99) 10 mice were inoculated, 1 of them showed a few trypanosomes on day 10 only, then all controls remained negative up to day 21. On day 29 the series was tested by HCT and 2 mice found to be positive, one even showing a high parasitaemia; the strain having been identified as *T. congolense* was preserved in liquid nitrogen. Only in one of these three cases (lion 106) *T. congolense* was found in a thin film (cf. Table 11).

Two mice out of ten and one out of ten inoculated with the blood of lion 104 and 105, respectively, developed *T. brucei* subgroup infections after 6 to 9 days. All the other mice remained negative by wet preparation examination. On day 26, these mice were examined by HCT: 3 mice of the former and 5 of the latter showed a feeble infection with *T. congolense* (WOO & KAUFFMANN 1971).

It seems essential that enough mice are inoculated, as it happened frequently that even out of 10 mice only 1 or 2 produced a detectable

parasitaemia (cf. Table 11). In addition, one can sometimes separate mixed infections by using a sufficient number of mice.

As in the case of other similar surveys, the animal species found to be the favourite hosts for the glossina species present (here mainly *G. swynnertoni*) are not at all the same as the ones serving as hosts for the glossina-transmitted trypanosomes (cf. part II). Furthermore, the "fly" and the "game" areas in general do not coincide with the exception of "fly area" 6, which lies in the middle of "game area" C, and areas 3 and D, which overlap (cf. map, part I, fig. 1). For obvious reasons, the areas where hunting took place were many times the size of the ones where flies were caught. Wherever lions were immobilized with tranquillizers, many tsetse flies could be observed near and around the resting groups or prides of lions, all the favourite hosts of tsetse flies being kept at a considerable distance. Examination of the darted animals, specially the older ones, revealed the presence of many hippoboscids, a bloodsucking species of diptera which has been suspected as a means of mechanical transmission of trypanosomes (BAKER 1967). The specimens collected were identified by Dr. Oldroyed[3] as *Hippobosca longipennis,* Fabricius 1805, a species recorded in Africa from lion, leopard, cheetah, hyaena, jackal and other carnivores. About 20 hippoboscids found on lions were dissected in the laboratory, but no trypanosomes were found in the mouthparts or in the gut.

References

BAKER, J. R. (1967). A review of the role played by the hippoboscidae (Diptera) as vectors of endoparasites. – J. Parasit. *53,* 412–418.

BAKER, J. R. et al. (1967). Trypanosomes of wild mammals in an area northwest of the Serengeti National Park, Tanzania. – Z. Tropenmed. Parasit. *18,* 280–284.

RICKMAN, L. R. & ROBSON, J. (1970). The testing of proven *Trypanosoma brucei* and *T. rhodesiense* strains by the blood incubation infectivity test. – Bull. Wld Hlth Org. *42,* 911–916.

SACHS, R., SCHAUER, G. B. & BAKER, J. R. (1967). Isolation of trypanosomes of the *T. brucei*-group from lion. – Acta trop. *24,* 109–112.

WOO, P. T. K. (1970). The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. – Acta trop. *27,* 384–386.

WOO, P. T. K. & KAUFFMANN, M. (1971). The haematocrit centrifuge technique for the detection of low virulent strains of trypanosomes of the *Trypanosoma congolense* sub-group. – Acta trop. *28,* in press.

YOUNG, A. S. (1968). SRI report, unpublished.

[3] British Museum, London.

Sleeping Sickness Survey in Musoma District, Tanzania

V. The Endemicity of Rhodesian Sleeping Sickness in Ikoma-Serengeti Area – Final Discussion

R. J. ONYANGO[1], R. GEIGY[2], P. M. MWAMBU[1] and S. K. MOLOO[1]

Since 1922 when Rhodesian sleeping sickness was first introduced into the Ikoma area the disease must have been maintained as a zoonotic infection, the parasite being continuously transmitted between fly and game. People living in the area have been infected whenever they encroached onto the tsetse bush and encountered infected flies. The incidence of the disease in man has thus depended on the degree of man-fly contact which might have been brought about by the various human activities such as hunting, poaching, game watching, etc. The reduction in the number of human infections and eventual disappearance of the disease in man in the early 1950's might have been due to the decrease in human activity in the fly belts and thus the reduction of man-fly contact. The recrudescence of human trypanosomiasis in 1964 in the Ikoma-Serengeti area could be attributed to the increase in human population and their activities which enhanced man-fly contact. Population movements in the area in the form of (I) tourism, which is estimated to bring about 40,000 visitors into the park; (II) game protection carried out by game rangers and scouts who travel around within the park to prevent poaching; (III) road construction work within the park; (IV) game poaching by local people; (V) travels by employees and their families to and from the many scattered establishments within the park, etc. promotes a certain amount of man-fly contact during which an infection with *T. rhodesiense* may be contracted by susceptible persons. It is possible that the increase of the disease transmission from 4 in the first half to 37 in the second half of the ten year period, 1961 to 1970 inclusive, was due to the increase in the frequency of man-fly contact in the area.

ONYANGO & WOO (cf. part I) have found no evidence of infection in about 3,000 people who were living in the Ikoma-Serengeti area. Yet during the 4 months previous to the survey 4 patients were diagnosed all of whom were employees within the Park. In December 1970, a month following the survey, one patient living in the Ikoma area outside the park was also found infected. These isolated cases are

[1] E.A.T.R.O., Tororo, Uganda.
[2] S.T.I., Basle, Switzerland.

an example of the complicated nature of the epidemiology of the Rhodesian sleeping sickness that baffles many investigators and has been the subject of a number of reviews (FAIRBAIRN 1948, ASHCROFT 1959, NASH 1960, FORD 1965 and ONYANGO 1966). Such sporadic cases confirm the belief that infection in this area occurs by chance.

The results of the entomological investigation (cf. part II in this issue) are indeed intriguing. Low infection rates in tsetse with mature *T. brucei* in the order of 0.1 % to 0.6 % is a known fact; despite these low rates intense transmission may still occur in endemic areas. The total absence of salivary gland infection in flies in an endemic area is an unexpected finding. Yet this finding in the area does not necessarily imply that transmission of *T. brucei* subgroup organisms will not be effected by flies which have no salivary gland infection. Recently it has been experimentally shown that "when flies from cages with known infected flies were individually fed on normal mice, the transmission rate was approximately 5 times higher than revealed by salivary gland infections" (WARD & BELL 1971). It may be that the proventricular forms of *T. brucei* and *T. rhodesiense* are as infective as the metacyclic forms in the salivary glands.

In all the 862 blood meals analysed only two were derived from primates, one from man and one from baboons. This finding together with the fact that no *brucei* subgroup trypanosomes were isolated from tsetse flies suggest that transmission of human trypanosomiasis is likely to be restricted.

The finding during the survey that *G. swynnertoni* readily attacks man when the opportunity arises shows that this tsetse species may be an efficient vector of *T. rhodesiense* if man-fly contact is established. Judging from the activities of the population in and around the Serengeti National Park and those of the visitors, particularly tourists, the circumstances that can bring about "personal" man-fly contact seem to come about rarely.

MWAMBU & MAYENDE (cf. part II) found a few *T. brucei* subgroup infections in cattle. Out of 28 strains isolated 10 were tested by BIIT and of these 4 behaved suspiciously like T. rhodesiense. Cattle have been proved to harbour *T. rhodesiense* in another part of East Africa (ONYANGO et al. 1966), hence the probability that cattle in Ikoma may be acting as reservoirs is not surprising. Since these cattle come in contact with tsetse in the Game Reserve only and these tsetse populations have little contact with the human population, chances of transmission of *T. rhodesiense* between cattle, tsetse and man appear remote.

GEIGY et al. (cf. part IV) in a survey of a limited number of game species isolated 12 strains of *T. brucei* subgroup, 5 of which behaved like *T. rhodesiense* by the BIIT. These suspicious strains were isolated from lions, hyaena, waterbuck and hartebeest. Their presence in these

mammalian species and their scarcity in the tsetse vector possibly indicate that the mammalian host must have been fed on only occasionally. These suspicious isolates will be tested in volunteers for their pathogenicity to man. Suffice to say, the role of game reservoirs of *T. rhodesiense* has been established. The species usually associated with the disease is bushbuck. Other ungulates have also been shown to harbour the parasite without ill effects on their health (ASHCROFT 1959). *T. brucei* has been isolated in the past from lions (SACHS et al. 1967) but not from hyaena. Isolation from hyaena is therefore interesting.

Conclusion

The reappearance of cases of Rhodesian sleeping sickness in the Ikoma-Serengeti area since 1964 confirms the endemicity of the disease in the area. From the results of the investigations described above it is difficult to pinpoint any focus responsible for the disease in man. It would appear that game-fly transmission occurs in a haphazard manner. The transmission to man is a chance occurrence which depends on many factors, the major one being man-fly contact, which in this locality appears restricted. To offer maximum protection to employees and resident population, adequate clearing of vegetation round the settlements and villages is advisable. This should be coupled with systematic case finding. For the individuals who may come in contact with the fly while sightseeing or during the course of duty, it is essential that they report to competent medical authorities any febrile episodes occurring a few days to a few weeks after a visit to the area. If this is done, the few infections that will be contracted will be quickly treated; hence these visits to the area could be made with impunity.

Acknowledgement

We wish to record our sincere thanks to the Ministries of Health and Social Welfare and of Agriculture Food and Cooperatives for placing before us all the assistance we needed in the way of transport and personel; to the Director of Tanzania National Parks for his encouragement; to the Director and the staff of the Serengeti Research Institute for valuable discussions and laboratory space; and to many other people (that we are unable to mention here individually by name) who gave us technical and other kinds of assistance for which we are indeed grateful. We also wish to thank the Secretary General of the East African Community for permission to publish this paper.

References

ASHCROFT, M. T. (1959). A critical review of the epidemiology of human trypanosomiasis in Africa. – Trop. Dis. Bull. *56*, 1073–1093.

FAIRBAIRN, H. (1948). Sleeping sickness in Tanganyika Territory, 1922–1946. – Trop. Dis. Bull. *45*, 1–17.

FORD, J. (1965). Distribution of *Glossina* and epidemiological patterns in the African trypanosomiases. – J. trop. Med. Hyg. *68,* 211–225.

NASH, T. A. M. (1960). A review of the African trypanosomiasis problem. – Trop. Dis. Bull. *57,* 973–1003.

ONYANGO, R. J. (1969). New concepts in the epidemiology of Rhodesian Sleeping Sickness. – Bull. Wld Hlth Org. *41,* 815–823.

SACHS, R., SCHALLER, G. B. & BAKER, J. R. (1967). Isolation of trypanosomes of the *T. brucei* group from lion. – Acta trop. *24,* 109–112.

WARD, R. A. & BELL, L. H. (1971). Transmission of *Trypanosoma brucei* by colonized *Glossina austeni* and *G. morsitans.* – Trans. roy. Soc. trop. Med. Hyg. *65,* 236–237.

Zusammenfassung

Im Oktober und November 1970 sind im Serengeti-Ikoma-Gebiet epidemiologische Erhebungen über das Vorkommen von *T. rhodesiense*-Infektionen durchgeführt worden. Diese vom Gesundheitsministerium von Tanzania veranlaßten Untersuchungen sind ausgelöst worden durch das periodische Wiederaufflackern von Herden östlicher Schlafkrankheit in jenen Gegenden vom Jahre 1964 an nach 10jährigem Stillstand. Die mit diesem Unternehmen beauftragte Forschergruppe setzte sich zusammen aus Medizinern, Veterinären, Entomologen und Protistologen, die von der E.A.T.R.O., Tororo (Uganda), und vom Schweizerischen Tropeninstitut in Basel gestellt wurden.

Es ergaben sich folgende Resultate: Von 3000 untersuchten Personen wies keine eine Trypanosomen-Infektion auf. Bei 8000 sezierten Tsetsefliegen sind wohl eine Anzahl Organinfektionen mit Trypanosomen festgestellt worden, jedoch keine für den Brucei-Typus charakteristischen Speicheldrüseninfektionen. Untersuchungen an 798 Rindern im Ikoma-Gebiet ergaben 3,5 % Infektionen vom Brucei-Typ, während an 115 Wildtieren 12 Brucei-Stämme von Hyäne, Löwe, Warzenschwein, Wasserbock und Kuhantilope isoliert werden konnten. Der in diesen Fällen durchgeführte Blut-Inkubationstest nach RICKMAN & ROBSON ergab zum Teil inkonstante Resultate, wies aber in einigen Fällen deutlich auf *T. rhodesiense* hin. Diese Frage wird weiter verfolgt. Zusammenfassend läßt sich auf Grund der negativen Resultate bei Mensch und Tsetsefliege, und der relativ niedrigen Infektionsraten bei Rind und Wildtieren heute feststellen, daß im Serengeti-Ikoma-Gebiet die Übertragungsmöglichkeiten offenbar sehr gering und dem Zufall überlassen sind und daß daher das Risiko für Schlafkrankheitsbefall gering ist.

Résumé

Au cours des mois d'octobre et de novembre 1970, une enquête épidémiologique sur l'incidence des infections à *T. rhodesiense* dans la région de Serengeti-Ikoma a été réalisée par un groupe de chercheurs comprenant des équipes de médecins, vétérinaires, entomologistes et protozoologistes de l'E.A.T.R.O., Tororo (Uganda), et de l'Institut Tropical Suisse à Bâle. Cette enquête, réclamée par le Ministère de la Santé de la République de la Tanzanie, a été déclanchée par la réapparition dans cette région de foyers de maladie du sommeil du type est-africain en 1964, après dix années d'absence. Les résultats peuvent se résumer ainsi: Sur 3.000 personnes examinées aucune infection n'a pu être mise en évidence. L'analyse de près de 8.000 mouches Tsé-Tsé a bien permis de constater dans un nombre de cas la présence de trypanosomes dans les organes, mais aucune infection de glandes salivaires, caractéristique du type Brucei, n'a pu être démontrée. L'examen de 798 têtes de bétail de la région d'Ikoma a révélé un taux d'infection à type

Brucei de 3,5 %, alors que sur 115 animaux sauvages 12 souches de Brucei ont pu être isolées de hyène, lion, phacochère, le cobe Defassa et la bubale. Le « Blood Inoculation Infectivity Test » d'après Rickman et Robson pratiqué sur ces souches a donné des résultat inconstants, mais cependant dans quelques cas nettement en faveur de *T. rhodesiense*. L'étude de ce problème sera poursuivi ultérieurement.

Etant donné d'une part la négativité des investigations menées chez l'homme et chez la mouche Tsé-Tsé et d'autre part vu le taux d'infection relativement bas du bétail et des animaux sauvages, les auteurs arrivent à la conclusion, que dans la région Serengeti-Ikoma la transmission est basse, épisodique et le fait du hazard et que la probabilité d'y contracter une infection à *T. rhodesiense* est faible.