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# Culverts and Trypanosome Transmission in the Serengeti National Park (Tanzania)\*

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## Abstract

A survey of the 116 culverts along the 76 km of the Mwanza Road between the Banagi Junction and Kirawira, Serengeti National Park, was undertaken in July 1975. The commonest vertebrates seen were bats (16 culverts), spotted hyaenas (11 culverts), warthogs (5 culverts) and porcupines (3 culverts). All the vertebrates appear to use the culverts as temporary shelters rather than breeding sites. Six arthropods of medical interest were found in the culverts, pupae of *Glossina swynnertoni*, larvae, pupae and adults of *Auchmeromyia luteola* and *A. bequaerti*, *Ornithodoros moubata* sp., *Ixodid* ticks and *Xenopsylla* sp. Seven hyaenas (*Crocuta crocuta*) associated with culverts along the road where it leads through plains were immobilized with Immobilon or succinylcholine. From 6 of these animals three strains of *T. (T.) brucei* and one of *T. congolense* could be isolated by inoculating white laboratory rats, in the field, with venous blood.

Along the same road 733 *G. swynnertoni* were caught, triturated in saline and injected into 49 rats without revealing a single mature trypanosome infection.

*A. luteola* and *A. bequaerti* were found in close association. Blood ingested by the larvae under laboratory conditions takes 4–5 days to digest. The main host of *Auchmeromyia* sp. larvae in this area was the warthog although occasional feeds were taken on hyaenas. Trypanosomes of *T. (T.) brucei* remain viable in the gut of *Auchmeromyia* sp. larvae for about 21 hours but attempts to transmit the infections to another host after interruption of the meal proved to be unsuccessful. 502 blood fed *Auchmeromyia* sp. collected in the field were triturated in saline and injected into 32 rats but no strains of trypanosomes could be isolated.

It is concluded that tsetse flies probably only occasionally may be involved in the cyclical transmission of trypanosomes to and from hyaenas and that another possible way is the oral route. On the other hand mechanical transmission by *Auchmeromyia* larvae seems unlikely.

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\* This study was supported by the Swiss National Fund, Grant Nr. 3'2360'74. We wish to thank the authorities of the Tanzanian National Parks and the Serengeti National Park for the permission to carry out this survey and for their assistance.

## General introduction

R. GEIGY <sup>1</sup>

During earlier studies into the occurrence of Sleeping Sickness in the Serengeti National Park it was recognized that a number of wild animals acted as reservoirs of *Trypanosoma rhodesiense*. Strains of *T. (T.) brucei* <sup>2</sup>, isolated from hartebeest, lion and hyaena were shown to be positive in the 'Blood Incubation Infectivity Test' (RICKMAN & ROBSON, 1970) and to infect human volunteers and thus were *T. rhodesiense* (GEIGY et al., 1973 and 1975).

The Mwanza Road between Banagi and Kirawira appeared to be a suitable location to study in greater detail the transmission of trypanosomes from game reservoirs to man, by *Glossina* or possibly other haematophagous arthropods. In 1971 no less than 15 strains of *T. (T.) brucei* were isolated from 25 hyaenas and 8 lions examined in that area. One of these isolates was proven to be *T. rhodesiense*. This road leads through woodland, thorn thickets and open plains, some of which contain scattered trees. This area of the Serengeti is known as the corridor and the famous annual game migration passes through this region. At other times of the year this area is well populated with game, including topi, hartebeest, gazelle, eland, impala, waterbuck, dik-dik, buffalo, giraffe, wildebeest, zebra, warthog, elephant, lion and hyaena.

The road leads through areas which are flooded during the wet season and has therefore been raised in places above the surrounding countryside. In order to allow water to drain freely, 116 culverts have been constructed along the 76 km of the road between Banagi and Kirawira. These culverts consist of between one and six metal tubes of 60 or 90 cm diameter built into the earth road.

On examining the culverts it was found that the bottom of the tubes were often covered with earth, sand or gravel, deposited there by the water flooding through them. The depth of this deposit in some cases was several centimeters. The deposits were suitable habitats for the larvae of *Auchmeromyia* sp. while the adult flies were often found on the roof of the tubes. *Ornithodoros* and *Ixodid* ticks were also to be found among the culvert deposits. During the dry season a number of animals, particularly hyaena, warthog and porcupine use the tubes as shelters and provide a ready supply of food for the haematophagous arthropods.

The culverts are used by animals probably as temporary shady resting places, complementary to the large trees on the plain. In addition

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<sup>1</sup> Swiss Tropical Institute, Basle.

<sup>2</sup> Former *T. brucei* subgroup.

to using the culverts hyaenas have their own dens on the plains, often not far from the road, where they live in family groups and rear their young. Warthogs in addition to the culverts use abandoned burrows of aardwarks or hyaenas as resting places. Porcupines were not very common in the culverts but they too probably only use them as temporary resting sites.

Much of the region through which the Mwanza Road passes is tsetse habitat and many *Glossina swynnertoni* are to be found. From earlier studies it is known that *G. swynnertoni* is the main vector of trypanosomes in this region and will feed on a variety of game particularly buffalo, warthog and giraffe (MOLOO et al. 1971).

Since infected game is found in close association with tsetse and other bloodsucking arthropods, both inside and outside the culverts, on a road used by tourists, it seemed possible that the areas around the culverts were important sites of trypanosome transmission to man. A survey was, therefore, carried out between 8th and 30th July 1975 to investigate this further.

A laboratory and room for keeping rats <sup>3</sup> were kindly provided at the Serengeti Research Institute, Seronera. In addition, the Director of S.R.I. showed us even more hospitality by accommodating us in his guest houses. We wish to express to both Dr. and Mrs. Mcharo our thanks for all the kindness they showed us during the survey.

The party consisted of 5 scientists: Prof. R. Geigy, Swiss Tropical Institute, Basle, as leader of the team; Dr. R. Beglinger, Veterinary School, Zurich, who, with his colleague Dr. med. R. Müller, was in charge of the darting and subsequent examination of the vertebrates; Dr. P. F. L. Boreham, Imperial College of Science and Technology, University of London, an expert in arthropod bloodmeal identifications and Miss M. Kauffmann, from the Swiss Tropical Institute, Basle, who has much experience of field work in Africa. In addition we had the help of two experienced African field assistants, Mr. Oswald Adam and Mr. Pius Hermes from the Swiss Tropical Institute Field Laboratory, Ifakara (Tanzania), and a driver, Mr. Amidu Ranadhan. The Ifakara Field Laboratory kindly put at our disposal two landrovers and the necessary scientific equipment.

The survey was undertaken as follows: on most days the Mwanza Road was visited, the party being divided into a veterinary and an entomological team. Each culvert was visited in turn and, if a hyaena was present, an attempt was made to dart it. The veterinary group followed the animal until it laid down, blood was taken and immediately injected into rats. Blood was also used for preparing thick and thin films and for serum. The entomological group searched the de-

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<sup>3</sup> All laboratory rats used were kindly provided by EATRO, Tororo, Uganda.



posits in the culverts and collected *Auchmeromyia* larvae and ticks. Tsetse flies were also caught along the Mwanza Road. The material collected was taken back to the laboratory. The *Auchmeromyia* larvae and tsetse flies were triturated in saline and injected into rats to try to isolate trypanosome strains. All rats were examined for the presence of trypanosomes and then as appropriate, stabulates were prepared in liquid nitrogen. At the end of the survey Miss Kauffmann remained at S.R.I. for a further three weeks in order to continue observations on the rats.

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# I. Survey of the Culverts

R. GEIGY <sup>4</sup> and P. F. L. BOREHAM <sup>5</sup>

## Introduction

In order to understand the role of the culverts in the transmission of trypanosomes it was important to examine them and to determine what vertebrates and haematophagous invertebrates were present. During November 1974 a brief visit was made to the area by two of us (R. Geigy and M. Kauffmann) in order to locate the culverts. A total of 95 culverts were found on the Mwanza Road and labelled. When the survey commenced in July 1975 it was immediately apparent that, during road work a number of new culverts had been installed in the intervening period and a few had been missed at the preliminary survey. These additional culverts have been given the suffix a, b, or c as appropriate, with the exception of 4 extra culverts (01 to 04) positioned before the first culvert found in November 1974. (+) means very few present, + = present, ++ = greater number present, +(+) = in between + and ++.

## Survey methods

Each culvert along the Mwanza Road (Fig. 1) was examined between one and four times and a note made of any vertebrate present in the tubes. Samples of the deposits in the tube were taken, passed through a coarse mesh sieve and examined for the presence of haematophagous arthropods. In addition the tubes were searched for adult Dipteran flies and signs of occupation by vertebrates. A note was also made of the type of habitat and fauna surrounding the culvert and the presence or absence of tsetse flies.

## Results

The results of the data collected from the culvert survey are shown in Table 1. 22 of the 116 culverts were unsuitable for examination since they were wet or contained water except one (culvert 28), which had been damaged by a bulldozer. However, in these culverts it was still possible to look for vertebrates and adult *Auchmeromyia*.

## Discussion

Four main types of habitat were noted along the road: woodland, showing much evidence of elephant damage, thorn thicket with pre-

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dominant gall acacias, mixed woodland and thorn thicket showing variable amounts of two types of vegetation and open plains.

#### a) Vertebrates

Seven different vertebrates were observed inside the culverts during this survey: The spotted hyaena (*Crocuta crocuta*), the warthog (*Phaenocoerus aethiopicus*), the porcupine (*Hystrix galeata*), bats (probably the yellow winged bat *Lavia frons*), the bat eared fox (*Otocyon megalotis*), francolins (*Francolinus* sp.) and small lizards. Bats were the commonest vertebrate seen, being found in 16 culverts with between one and four individuals in a single tube. All the bats were hanging from the roofs of the tubes and were using them as resting sites.

Eleven culverts had hyaenas in them and eleven hyaenas were seen in one culvert (culvert 31 which consisted of three 60 cm tubes).

As regards the use of these culverts by hyaenas as resting places, the situation had changed since November-December 1971. At that time, 25 hyaenas were darted inside the culverts between No. 34 and 66. Probably due to the improvement of the Mwanza Road and continual roadwork going on, traffic had increased considerably since 1971. The hyaenas abandoned the use of culverts, wherever other shady places were available. In 1975 in every case the culverts containing hyaenas were located in open plains or in areas close to a plain (e.g. culverts 45 and 49). On occasions hyaenas were observed lying in half flooded culverts cooling themselves in the mud and water. It was apparent that the culverts were used by the hyaenas as cool shady resting places.

Similarly warthogs used the culverts as shelters and the 5 culverts in which warthogs were present contained much more earth with dry grass scattered inside. It appeared that the same resting site was used continually by the warthog. The culverts containing warthogs were located both in the plains and areas covered with woodland and thorn-bush.

It is interesting to note that in culvert 43 one tube contained a hyaena while 3 warthogs were in the next tube. Porcupines were seen in 3 culverts associated with vegetation. Two of the culverts contained single individuals while the third contained an adult female and a juvenile. Porcupines, unlike other vertebrates, were very difficult to chase out of their tubes. A single bat eared fox was observed in one culvert (64), while small lizards were seen to run out of culvert 35 and 59 as they were approached. A pair of francolins were seen to leave culvert 32, whether they had been resting inside or accidentally wandered in, remains uncertain. During this survey no mongooses were observed inside culverts although in the preliminary survey, dwarf mon-

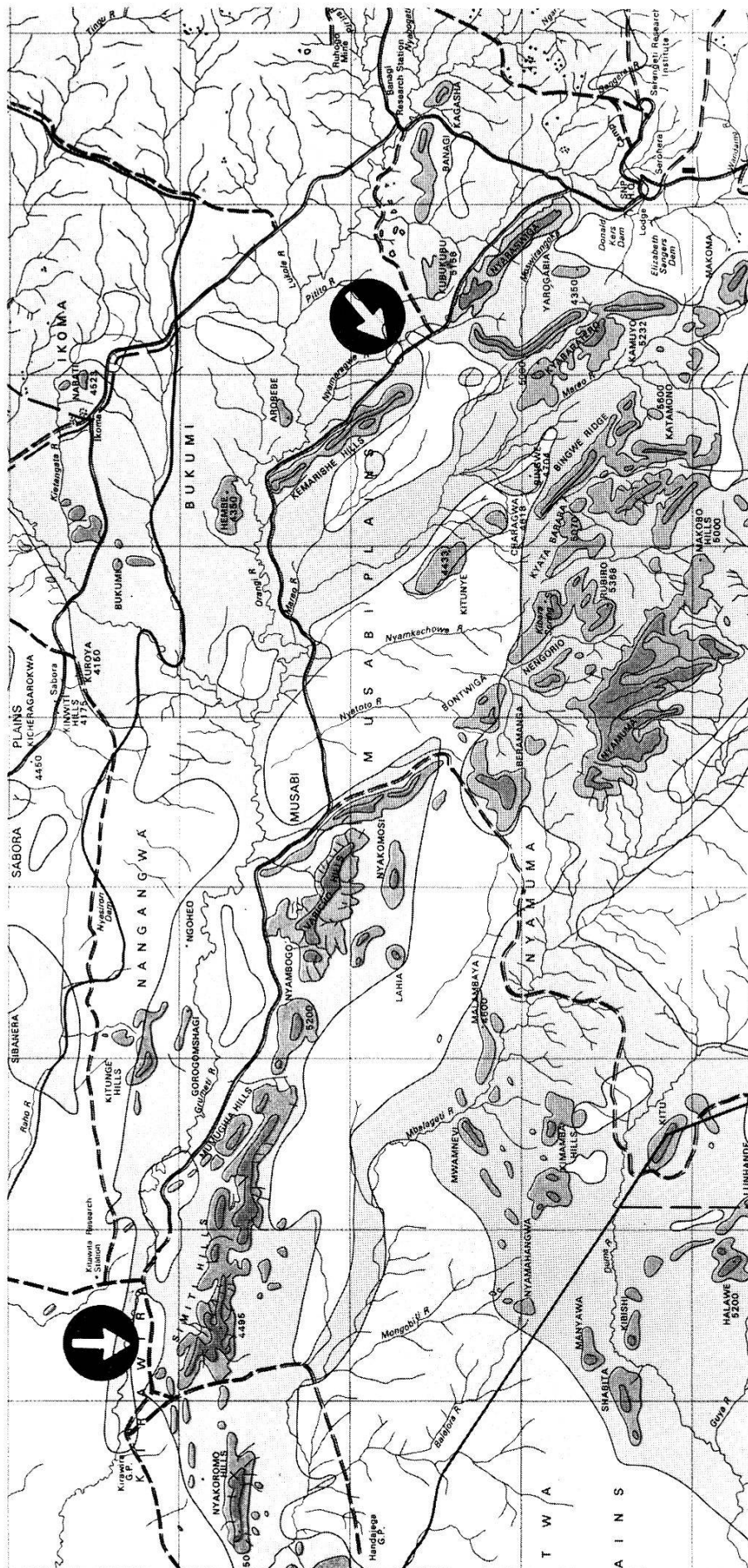


Table 1. Results of the Culvert Survey

Culvert	Habitat	Vertebrates	<i>Auchmeromyia</i> adult	larvae	<i>Orni-</i> <i>thodorus</i>	Ticks <i>Ixodids</i>	<i>Glossina</i> <i>swynner-</i> <i>toni</i> (along road)
01	woodland + thornbush	—	—	—	—	—	+
02	thornbush	—	flooded	—	—	—	+
03	thornbush	4 warthogs	+	+	—	—	—
04	thornbush	—	+	+	—	—	—
1	thornbush	—	+	+	+	—	+
2	thornbush	warthog	+	+	fed	—	+
3	woodland + thorn	porcupine quills	—	+	fed	—	+
3a	woodland + thorn	porcupine/warthog traces	+	+	+	—	+
4	woodland	porcupine	+	+	+	—	+
5	woodland + thorn	—	—	—	—	—	1 pupa
6	woodland + thorn	—	—	+	+	—	+
7	woodland + thorn	—	+	+	+	—	+
8	woodland + thorn	—	—	+	+	—	+
9	open woodland	—	—	+	+	—	+
10	open woodland	—	—	+	(+)	—	+
11	open woodland and few thorn	hyaena	—	—	—	—	+
12	woodland + thorn	—	—	+	+	—	—
13	woodland + thorn	7 warthogs	+	+	—	—	—
14	woodland + thorn	—	+	+	+	—	+
15	woodland + thorn	porcupine quills	—	+	fed	—	+
15a	woodland + thorn	—	—	—	—	—	—
16	woodland + thorn	—	—	—	+	—	+
17	woodland + thorn	—	—	—	+	—	—
17a	woodland + thorn	bats, butterflies	—	wet	+	—	pupae inside

18	woodland + thorn	bat	—	water	—	—	—
18a	woodland + thorn	bat/lions around	+	wet	—	+	+
19	woodland + thorn	—	—	water	—	+	+
20	woodland + thorn	smell hyaena	—	wet	—	+	+
20a	woodland + thorn	6 hyaenas outside	+	+	—	+	+
21	woodland + thorn	used by warthogs	—	—	—	+	+
22	smaller plain	used by warthogs	—	+	—	+	—
23	smaller plain	1 warthog	+	pupae	+	fed	+
24	woodland + thorn	warthogs & hyaena around	+	+	+	fed	—
25	plain	1 hyaena	+	+	+	fed	—
26	plain	—	water	—	—	—	—
27	thornthicket	4 hyaenas	wet or water	—	—	—	—
28	woodland gallery	bat, used by hyaena	—	—	+	—	—
29	plain	—	tube to high from ground. Bulldozer damage	+	+	+	—
30	plain	bat	—	+	+	+	—
31	plain	11 hyaenas	—	+	+	—	—
32	thornthicket	1 hyaena/francolins	+	+	+	fed	—
33	thornthicket	—	+	+	+	fed	—
34	thornthicket	used by warthogs	pupae	—	+	fed	—
35	plain	— lizard	+	+	+	+	—
36	plain	—	—	+	+	+	—
37	plain	—	—	+	+	+	—
38	plain	hyaena	—	+	+	—	—
39	plain	—	—	+	+	deads	—
40	plain	hyaena/skin rests of topi	—	+	+	fed	—



Table 1. Results of the Culvert Survey

Culvert	Habitat	Vertebrates	<i>Auchmeromyia</i> adult	larvae	<i>Orni-</i> <i>thodorus</i>	Ticks <i>Ixodids</i>	<i>Glossina</i> <i>swynner-</i> <i>toni</i> (along road)
41	plain	—	—	+	+ fed	—	—
42	plain	—	gravel	+	+	—	—
43	plain	1 hyaena/3 warthogs	—	+	+	—	—
44	woodland	—	+	—	—	—	+
45	woodland	1 hyaena	—	—	—	—	+
46	woodland	bat	little earth	—	—	—	+ + pupae inside
47	open woodland	—	little earth	pupal cases	+	—	+
48	woodland	—	little earth	—	+	—	+ + pupae inside
49	woodland	hyaena in plain 5 hyaenas, bat, porcupine quills	little earth, partly wet	—	+	—	(+)
50	woodland	2 bats	—	+	+ + fed	—	+
50a	woodland	bat	little earth, damp	—	+	—	—
51	woodland	—	gravel	—	+	—	(+) pupae inside
52	woodland	—	gravel	—	—	—	(+)
			pupal cases outside hard, damp earth				

52a	woodland	—	—	—	+	—	+	+	pupae inside (+)
53	woodland	bat	—	—	+	—	+	+	+
54	woodland	— bat eared fox / lion excrements near culvert	pupal cases	—	+	—	+	+	+
55	woodland + thorn	—	little earth	—	+	—	+	+	+
56	woodland + thorn	—	pupal cases	—	+	—	+	+	+
57	woodland + thorn	African hare nearby	little earth	—	+	—	+	+	+
58	woodland near plain	4 hyaenas	wet	—	—	—	—	—	—
59	plain – thornbush nearby	— lizard	water	—	—	—	—	—	—
60	woodland	zebras and hyaenas in plain	—	—	+	—	+	+	+
61	woodland + thornbush	—	—	—	+	—	+	+	+
62	plain	bats	—	—	+	—	+	+	+
63	plain	—	+	+	+	—	+	+	+
64	plain	game in plain	—	—	+	—	+	+	+
65	plain	bat eared fox	pupal cases	+	+	—	+	+	+
66	plain (warthog, hyaena)	used by warthog	—	+	+	—	+	+	+
67	woodland + thornbush	—	pupal cases	—	—	—	—	—	—
68	woodland near plain	—	+	+	+	—	+	+	+
69	woodland near plain	used by warthog	pupae	+	+	—	+	+	+
		used by warthog	—	—	—	—	—	—	—

Table 1. Results of the Culvert Survey

Culvert	Habitat	Vertebrates	<i>Auchmeromyia</i> adult	larvae	Ticks <i>Orni- thodorus</i>	<i>Ixodids</i>	<i>Glossina</i> <i>swynner- toni</i> (along road)
70	woodland near plain	—	— wet	—	—	—	—
71	thornbush + trees	—	too wet for sampling				+
72	thornbush + trees	—	no earth	no sampling			+
73	thornbush more open	—	++ pupal cases	++ fed	++ fed	+	++ (+)
74	plain	—	++	+ fed	+	—	+ some in culvert
75	thornthicket	—	—	—	++ +	—	— pupal case
75a	thornthicket	—	—	—	+	—	—
75b	thornthicket	—	++ (+)	++ (+) fed	++ fed	—	+
76	thornthicket	bat used by warthog	++	++	+	+	++ (+)
77	thornthicket	—	(+)	some fed ++ (+)	++	—	++ (+) pupae inside
78	thornthicket	used by warthog porcupine quill	++	starving ++ (+) all	++	—	++ (+)
79	thornthicket	—	dead by starvation				
80	plain	—	flooded no sampling too wet for sampling				—
81	plain	warthog traces – hyaena nearby	—	—	+	—	—
82	plain	—	—	too wet for sampling		—	—
83	plain	—	—	too wet for sampling		—	—
83a	plain	—	—	water, no sampling		—	—

83b	plain	—	— water, no sampling	—
84	end of plain	2 porcupines	— no earth, no sampling	—
85	entering woodland	African hare nearby	— too wet for sampling	—
86	thornbush	quills of porcupine	+ + too wet for sampling	—
86a	thornbush	quills of porcupine	— too wet for sampling	—
87	thornbush	bat	+ (+) too wet for sampling	+
87a	thornbush	wildebeest bones nearby	— too wet for sampling	+ + caught
87b	thornbush near plain	—	+ too wet for sampling	+
87c	smaller plain	—	+ (+) + + fed —	—
88	smaller plain	—	pupae	+ flea
89	smaller plain	bats	+ + + + some —	—
90	smaller plain	warthog footprints	pupae fed	—
91	smaller plain	inside hoof of young warthog	+ + + some + + fed	—
92	smaller plain	wildebeest bones outside	fed	—
93	smaller plain	warthog footprints	+ + + some —	—
93a	smaller plain	used by warthog	fed	—
94	smaller plain	warthog footprints	+ + + +	—
94a	smaller plain	warthog footprints	+	—
95	woodland near Kirawira junction	warthog footprints	+ + + +	—
		—	+ flooded no sampling	—
		—	— too wet for sampling	—
		—	— flooded no sampling	—

gooses (*Helogale undulata*) were seen. It would appear that all the animals inside the culverts only used them as resting sites and not as breeding places. The hyaenas and warthogs probably use the same culverts over a period of time, as they were observed in or around the culverts on a number of occasions.

#### b) Invertebrates

In this survey we were only concerned with those bloodsucking invertebrates which might be possibly involved in trypanosome transmission. Six such arthropods were found: *Glossina swynnertoni* Austen, *Auchmeromyia luteola* and *bequaerti*, *Ornithodoros moubata* sp., Ixodid ticks (*Amblyomma cohaerens*, *Hyalomma* sp. and *marginatum* and *Rhipicephalus kochi*)<sup>6</sup> and fleas (*Xenopsylla* sp.). Pupae and empty pupal cases of *G. swynnertoni* were found quite commonly in the deposits of culverts but on no occasion were adult tsetse seen inside. Many *G. swynnertoni* were present outside the culverts, especially in the early part of the road (up to culvert 10) and just after the Musabi plains (culvert 44–48). The typical habitat for this *Glossina* species was open woodland sometimes interspersed by thorn bush. This tsetse likes to deposit its larvae under fallen wood which is common in this type of habitat. The population of tsetse flies varied considerably during the course of this study. During cold, overcast or windy days few were present, but on warm days swarms of hungry flies followed the car and could be caught along different sections of the road. The presence of tsetse pupae inside the culverts suggested an interesting possibility. When the adult emerge they may well rest inside the tubes for the first 24 hours before looking for a bloodmeal. If the culvert happens to be inhabited, this bloodmeal may well be taken from the occupant of the culvert. Trypanosome infections in hyaenas and warthogs are common in the Serengeti (BAKER, 1968, GEIGY, MWAMBU & KAUFFMANN, 1971, GEIGY & KAUFFMANN, 1973) and since the first bloodmeal is the most important for a tsetse to acquire an infection (WILLETT, 1966) it is quite possible for the flies to pick up infections within the culverts. Adult *Auchmeromyia* flies were found in 39 culverts resting on the side or top of the tubes, while larvae were found in the soil of 52 culverts. Once again populations were seen to change rapidly. The larvae would not survive in wet or flooded culverts and the absence of suitable food supply meant starvation and death after several days. On occasions dead larvae were found (culverts 39 and 79).

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<sup>6</sup> We thank Dr. R. Immler, CIBA-GEIGY Ltd., Basle, Biotechnical Products, for identifying the ticks.

It was also noticeable that after rain adult flies could be found in tubes where they had not previously been. Thus adult *Auchmeromyia* might rapidly repopulate culverts. Full details of the findings on *Auchmeromyia* are given in Part 3 of this publication.

*Ornithodoros moubata* sp. was found in about half of the culverts. Bloodfed and unfed nymphs and adults were present, but no attempt was made to distinguish the different stages. *Ornithodoros moubata* sp. was often present in very large numbers within the culverts and could be found in all types of habitat. It seemed to be more resistant to rough soil and gravel and to flooding than *Auchmeromyia*.

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## II. Immobilization of Animals and Isolation of Trypanosomes

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### Introduction

On a preliminary visit to the Mwanza Road between Banagi and Kirawira in the Western Corridor of the Serengeti National Park during November 1974, Professor Geigy discovered that 11 of the 95 culverts were occupied by spotted hyaenas (*Crocuta crocuta*). This observation was confirmed in July 1975, when 11 out of 116 culverts examined were found to shelter hyaenas. Most of the culverts were located in the Musabi Plains (Fig. 1).

During the period of the survey three clans of hyaenas were identified living in the plains, each consisting of about 15 to 20 animals. No hyaenas were found in the thornbush or in the riverine fringing forests. During the heat of the day some animals used the culverts as a cool and often muddy resting place while others dozed under single trees or bushes. The natural shelters of hyaenas are burrows which they dig in the ground. Several such dens were present in each of the clan areas. These dens are primarily used by mothers and their young cubs for a period of a few weeks. We observed only once a single adult animal disappearing into a den. On cool or rainy days hyaenas were rarely found in the culverts.

### Methods

At the commencement of this survey, animals were immobilized with Immobilon <sup>9</sup> (a mixture of etorphine and acepromazine), using a powder gun or pistol <sup>10</sup>. The doses of Immobilon per animal varied between 0.5 and 1 ml, each ml containing 2.45 mg etorphine hydrochloride and 10 mg acepromazine maleate. Immobilon was chosen because of its fast action and short recovery time, after injection of the antidote Revivon <sup>9</sup> (diprenorphine hydrochloride). Immobilon is widely used in veterinary medicine for both domestic and wild animals.

Since the response of Immobilon in hyaenas turned out to be so unpredictable, succinylcholine 22–24 mg per animal was used (KRUUK, 1972, BERTRAM, 1973).

It was possible at the beginning of the survey to dart hyaenas in the culverts as they ran out. However, as the survey continued, the animals became much shyer, leaving the culverts as soon as they heard a car approaching. Furthermore, the flight distance increased rapidly. Initially it was 30 to 40 metres but, towards the end of the survey, it was at least 100 metres. Thus darting became progressively more difficult as the accuracy of the dartgun is limited to 50–60 metres.

The flight distance was further influenced by weather conditions. On cool

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<sup>9</sup> Reckitt & Colman, Hull, U.K.

<sup>10</sup> Dist-Inject Mod. 30 and 60, Peter Ott AG., Basle, Switzerland.

Table 2

	Hyaena No.	Age/sex	Dosis	Response	Recovery
Immobilon	1	old female	1.00 ml	dead after 4 min.	—
	2	young male	0.50 ml	down after 3 min.	20 min. after Revivon i.v.
	3	adult female	0.50 ml	dead after 6 min.	—
	4	young male	0.75 ml	weakly immobilized after 3 min.	2 min. after Revivon i.v.
succinyl-choline	5	old female sick	24 mg	down after 3 min.	killed
	6	young male	24 mg	down after 6 min.	1 hour
	7	adult female	24 mg	down after 5 min.	45 min.

days the hyaenas were more active and alert. A chase by car was not possible due to the nature of the ground.

After immobilization, the hyaenas were tied and blood collected from the jugular vein. 30 ml citrated blood (1:4) was removed and 5 ml injected into each of 5 rats intraperitoneally.

A further 20 ml was taken without anti-coagulant and used for thin and thick films and the preparation of serum. Serum was stored in liquid nitrogen.

Since little is known about electrocardiograms (ECG) of hyaenas, ECG measurements<sup>11</sup> were taken from two animals, immobilized with succinylecholine.

*Glossina* were caught on and along the Mwanza road. They were kept in batches in Geigy cages and brought back to the laboratory in thermos flasks (temperature about 4–7 °C). Batches of 7 to 57 flies were triturated in saline and portions of 5 ml supernatant injected into white rats. Each rat was pretreated with antibiotics, 500 I.U. Penicillin plus 500 mcg Streptomycin i.p.

## Results

### 1. Immobilization of animals

In the fourteen days spent hunting in the Musabi Plains, 7 hyaenas from the three clans were immobilized, 4 with Immobilon and 3 with succinylcholine; details are shown in Table 2.

In addition, 3 hyaenas were darted with 0.5 ml Immobilon which had no apparent effect. The dose was then raised to 0.75 ml and, even in this range, 2 more hyaenas did not show any signs of immobilization. These 5 animals were followed over a period of 30 to 45 minutes.

A total of five hyaenas were darted with doses of 22 to 24 mg succinylcholine per animal. Three collapsed after 3–6 minutes, one disappeared in a den five minutes after being hit and one animal fled without any reaction.

<sup>11</sup> Cardioline, Polymed, Glattbrugg, Switzerland.

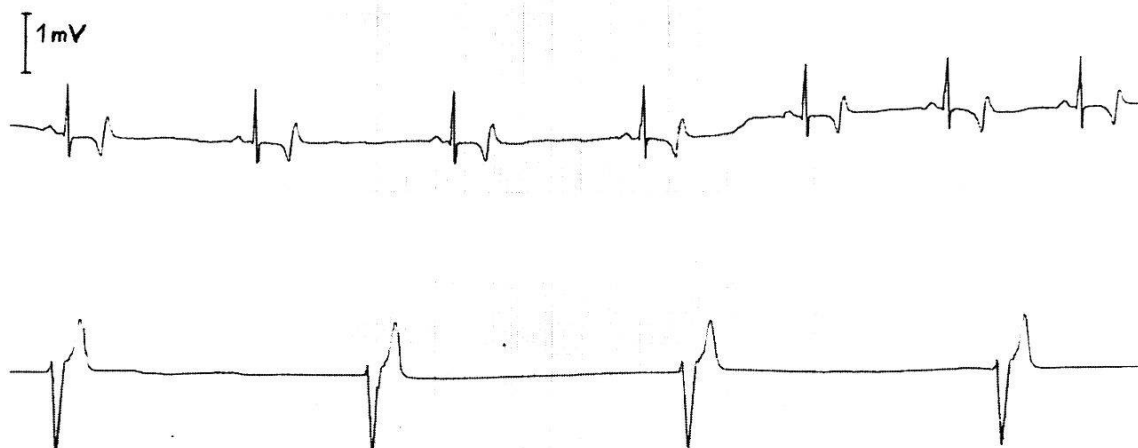


Fig. 2. Electrocardiograms of hyaenas. Top: healthy adult male, hyaena No. 6, bottom: old sick female, hyaena No. 5.

One of the immobilized hyaenas (No. 5) was subsequently killed as it showed strange behaviour patterns and appeared to be sick. It was suffering from several old infected bite wounds. The autopsy revealed a severe septicaemia and peritonitis. Hyaenas Nos. 2, 6 and 7 had to be artificially respired.

Blood from three further animal species was collected:

- 1 Thomson's gazelle (*Gazella thomsoni*), young male, close to culvert 20,
- 1 Porcupine (*Hystrix galeata*), young female, between culvert 3 and 4,
- 1 Rock hyrax (*Heterohyrax brucei*), old male, near the Serengeti Research Institute.

All these animals had recently been hit by cars.

Electrocardiograms. Both strips (Fig. 2) show the aVR-lead at a paper speed of 25 mm/sec. The upper trace originates from a healthy adult male hyaena (No. 6), immobilized with succinylcholine. The heart frequency averaged 53 beats per minute; P-Q interval is 0.128, QRS complex 0.064 and Q-T interval 0.36 sec.

The lower trace (hyaena No. 5) shows an ECG of a dying heart. No atrial activity was seen, an ectopic pacemaker appeared in the ventricular musculature. The heart frequency was 27 beats per minute.

## 2. Isolation of trypanosomes from mammals

Fresh, citrated blood from 7 immobilized hyaenas as well as from the porcupine, Thomson's gazelle and rock hyrax was injected into white

rats. All the rats inoculated with blood from hyaena No. 5, where autopsy revealed severe septicaemia and peritonitis, died overnight. Rats inoculated with blood from hyaena 1, 3 and 7 developed *T. (T.) brucei* infections.

Stabilates of these three strains were prepared as soon as a suitable parasitaemia was reached (i.e. day 6 or 7 after inoculation). Stabilates were stored in liquid nitrogen.

Four rats inoculated with blood from hyaena No. 6 died overnight, the fifth developed a *T. congolense* infection. A stabilate of this strain was made on day 10. One out of 5 rats inoculated from hyaena No. 2 showed a few trypanosomes with the haematocrit-technique on day 16, but became negative again until day 33 and 34. No trypanosomes could be found on day 35. Thereafter no more controls were made as the laboratory was closed down.

### 3. Other parasites

Microscopical examination of thick and thin films from hyaena blood confirmed that all hyaenas harbour *Babesia*, *Hepatozoon* and unsheathed microfilariae (GEIGY & KAUFFMANN, 1973). *Hepatozoon* was found again only in neutrophils.

No blood parasites were seen in any of the other three animal species.

In the back of the nasal passage of hyaena No. 3 we found a Pentastomid worm which was identified as *Linguatula multiannulata* (HAFFNER, SACHS & RACK, 1967)<sup>12</sup>.

### 4. Examination of *Glossina* for trypanosome infections

A total of 733 *G. swynnertoni*, collected between culvert 1 and 60, were triturated and injected into 49 white rats. In most cases, 5 ml supernatant were inoculated into each of two rats. Tail blood was examined for the presence of trypanosomes for three weeks or more. All rats remained negative.

## Discussion

We could not find any literature about the use of etorphine or Immobilon in hyaenas. There are some records about successful immobilization of other carnivores like bears, lions, wolves and hunting dogs

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<sup>12</sup> We thank Dr. John Riley, University of Dundee, for identifying the Pentastomid worm.

(BEEMAN et al., 1974; JONES, 1972; WALLACH et al., 1967). Therefore it was decided to test Immobilon in hyaenas. But, as the results show, the drug-combination did not work well in this species. Two animals died of dyspnoea and cessation of respiration. It was also impossible to determine an adequate dosage. With a dose of 0.5 ml one animal went down, one died and three did not show any reaction.

The lack of success with Immobilon was not due to the failure of the capture gun. In several cases the hyaenas tore out the syringes and it was seen that all of these had been discharged. The possibility of using a bad batch of drug can also be excluded since the same batch was tested on pigs in Zurich and it worked satisfactorily.

It is therefore concluded that it was the etorphine (a morphine-like compound) which is not very effective in hyaenas, because the other component of Immobilon, acepromazine, reacts much slower (dogs i.m. about 15–20 minutes).

There are several complicating factors which influence the effect of a drug in wild animals. A free-living wild animal needs a higher dose than a captured one. A darted animal is looking for cover and must therefore be followed. It is almost certain that the running animal, under stress from confrontation with man, discharges adrenaline, becomes hyperthermic and produces a metabolic acidosis which may be intensified fatally by a drug induced depression of respiration. The response to a drug is further influenced by nutritional factors, individual reaction and state of health (e.g. parasites). These factors could not be controlled. These missing standard conditions make a prediction of the drug action in a wild animal very difficult.

The ECG of the healthy hyaena appears to be similar to that of a dog. The heart frequency (53 beats/min) seems to be quite low. It is known that succinylcholine produces tachycardia in animals (WEST-HUES & FRITSCH, 1961). SPECTOR, quoted in GRAUWILER, 1965, found in striped hyaenas (*Hyaena hyaena*) heart frequencies between 55 and 58/min. But he does not state if this records were made under medication.

The incidence of *T. (T.) brucei* in hyaenas compares well with the results of the former surveys, carried out in the same area in 1970 and 1971, when 15 strains were isolated from 36 hyaenas (GEIGY et al. 1973). As in the former two surveys, no mature trypanosome infections (*T. (T.) brucei* or *T. congolense*) could be found in *G. swynnertoni* collected in the same area, even though this time the flies were examined by trituration and injection into white rats and not by dissection and microscopical examination (MOLOO & KUTUZA, 1974). These results suggest again that probably hyaenas are mainly infected through eating infected game (MOLOO et al., 1973) and only occasionally may play a role in the cyclical transmission by tsetse flies.

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### III. Studies on the Genus *Auchmeromyia* Brauer and Bergenstamm (Diptera: Calliphoridae)

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#### Introduction

The genus *Auchmeromyia* is comprised of five species, all of which are to be found only in the Ethiopian region of Africa. The larvae are of particular interest since they are haematophagous unlike the adult flies which feed on faeces, fallen fruit and fermenting vegetables (ZUMPT, 1965). Although they have not been proved to transmit any pathogen, this possibility cannot be ruled out. The taxonomy of the adults of this group is reviewed by ZUMPT (1956; 1965) whilst a key to the larvae of the two most widespread species is given by WETZEL (1970).

#### *Auchmeromyia luteola* (Fabricius)<sup>15</sup>

The Congo Floor Maggot is the most extensively studied member of this genus (ROUBAUD, 1913; PATON, 1935; GARRETT-JONES, 1950). It is found in many parts of Africa south of the Sahara but its range does not extend south of Durban (GARRETT-JONES, 1950). Larvae are found in wet and dry areas, mainly inside houses, and especially where primitive housing conditions exist. They live in the dusty or sandy floor of such houses and feed on man, usually at night, while he is asleep on the ground.

Although primarily associated with man, *A. luteola* has been described in association with other animals such as domestic pigs, warthogs (*Phacochoerus aethiopicus*) and aardvarks (*Orycteropus afer*) (GEIGY & HERBIG, 1954). It is interesting to note that ZUMPT (1965) states 'warthogs and antbears may not be true hosts of *A. luteola*, because only a few specimens, among great numbers of the usual *Auchmeromyia* species associated with these animals, have so far been found near the burrows. I suspect that the original host may be a burrowing animal not yet traced by the entomologist.'

An extensive laboratory study into the biology of *A. luteola* was undertaken by GARRETT-JONES (1950). The larvae take about 20 minutes to feed to completion on their host, before dropping off onto the ground. They will feed daily if given the opportunity, except the day before a moult. There are three larval stages and the minimum number of feeds for complete development is six, two in each stage, although up to 20 may be taken. The duration of the life cycle under natural conditions was estimated at approximately 10 weeks so that there would be about five generations per year.

#### *Auchmeromyia bequaerti* Roubaud

The distribution of *A. bequaerti* is reported from Zaire, the former Belgian Congo, across Eastern Africa and south to Zululand. It is found in and near warthog and aardvark burrows (ZUMPT, 1965).

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<sup>15</sup> PONT, A. (in press). Catalogue of the Calliphoridae of Africa, asserts that the name *Auchmeromyia senegalensis* Macquaert has priority over the species hitherto known as *Auchmeromyia luteola* (Fabricius).

Table 3. Climatic data collected at Seronera, Serengeti National Park, Tanzania, 12th July to 31st July 1975 \*

Date	% Humidity	Temp. °C		Rainfall (mm)	Sunshine (h)
		Maximum	Minimum		
12	60	27.5	13.5	0.0	10.0
13	67	28.0	12.6	1.0	9.0
14	56	26.0	10.5	0.0	7.5
15	78	28.0	12.5	7.0	6.9
16	68	25.7	10.5	0.0	10.5
17	67	27.5	10.0	0.0	6.5
18	64	27.0	11.5	0.0	7.0
19	73	26.9	12.0	0.0	6.0
20	84	26.0	14.5	2.1	0.8
21	87	24.0	12.1	0.6	6.0
22	79	27.9	14.7	10.3	5.5
23	91	26.5	15.3	5.3	0.1
24	83	20.2	14.3	0.0	5.0
25	91	25.0	15.4	3.3	3.4
26	96	24.0	15.1	4.8	0.1
27	86	18.5	13.2	0.0	7.0
28	77	25.6	11.0	0.0	11.0
29	52	27.5	18.0	0.0	8.0
30	55	27.0	14.0	0.0	8.0
31	64	26.0	11.0	0.0	10.0

\* Data kindly provided by the Serengeti Research Institute.

#### *Auchmeromyia reidi* Zumpt

This species has only been described from one area of Sudan where it was found in a warthog burrow (ZUMPT, 1959).

#### *Auchmeromyia choerophaga* (Roubaud)

*A. choerophaga* is a West African species associated with warthog and aardvark burrows. It is suggested that the larvae may require three months to reach maturity since the chance to feed only occurs at irregular intervals. Experimentally, *A. choerophaga* can be maintained on both the blood of man and domestic pigs (ROUBAUD, 1914; ZUMPT, 1965).

#### *Auchmeromyia boueti* (Roubaud)

*A. boueti* is also only found in West Africa but with a more restricted distribution than *A. choerophaga*. Again it appears to be associated with warthogs and aardvarks.

There are many gaps in our knowledge of this genus and especially their relationship to wild animals. *Auchmeromyia* sp. were commonly found in the culverts along the Mwanza Road in the Serengeti National Park, thus presenting a unique opportunity to study some aspects of their biology and, in particular, the possibility of *Auchmeromyia* larvae being involved in trypanosome transmission.

Details of the survey methods are given in part I of this paper. *Auchmeromyia* sp. were collected from the culverts between 12th and 31st July 1975.

Climatic data collected at Seronera for the period of the study is shown in Table 3. As has already been reported (part I) *Auchmeromyia* sp. larvae and adults were common in the culverts: larvae being found in 52 of the 116 culverts and the adults in 39 culverts.

### *Taxonomic Note*

No attempt was made to identify the species of *Auchmeromyia* in the field since it was anticipated that only one species would be present and suitable facilities were not available. Some adults, larvae and pupae, however, were brought to London and were kindly identified by Mr. James Dear at the British Museum (Natural History). Of the 17 adult flies collected inside culvert number 7, 15 were male *A. luteola* while the other two were female *A. bequaerti*. Seven larvae collected in culvert 24 pupated in the laboratory and were kept until emergence. The adult flies were identified as two male and one female *A. luteola* and four male *A. bequaerti*. The larvae proved very difficult to identify even using the characters described by WETZEL (1970). We must, therefore, assume that we are dealing with a mixture of two species and references throughout will be made to *Auchmeromyia* sp.

The presence of two species of *Auchmeromyia* living in the same habitat is of interest and the finding of *A. luteola* in the complete absence of man, i.e. in the middle of a game park where the nearest human habitation was many miles away, confirms the observation of GEIGY & HERBIG (1954) that this species is not entirely dependent upon man.

### *Anatomy of the Digestive Tract of Auchmeromyia sp.*

The first description of the gross internal anatomy of *A. luteola* was given by DUTTON et al. (1904). These authors noted that, after a bloodmeal, the dorsal diverticulum (crop) expands tremendously extending from the head to about the fifth segment. It can clearly be seen through the transparent body wall. The functional anatomy of the larval intestinal tract can be described as follows:

When a 3rd stage larva commences to suck blood it attaches itself to the host with its oral lips and proceeds to incise the skin by means of the anterior chitinous biting parts – the cephaloskeleton. The oral cavity behind these biting parts leads directly into the foregut which is joined dorsally to the muscular and extendible crop. The sucking action during the uptake of a bloodmeal is caused by contraction of the crop in which the blood is first stored. The blood is gradually transferred from the crop into the foregut, the diameter of which progressively widens until it passes into the midgut. The midgut is the largest part of the gut and is convoluted. The midgut is lined by a peritrophic membrane and most of the blood digestion takes place

Table 4. Rate of digestion of human blood by *Auchmeromyia* sp. collected in culverts in the Serengeti National Park, as determined by the precipitin test

No. of fed larvae	Digestion period (h)	Description of contents	% positive
6	36	No apparent digestion	100
10	48	Foregut bright red, little digestion	100
10	60	Foregut still contained blood except one small larva	100
10	72	About half the larvae contained undigested blood in crop	90
10	96	No blood in crop, blood in hind gut black and thick	70
11	102	Most larvae had black liquid in the gut, almost complete digestion	36

here. At the junction between the midgut and the rectum two lateral branches of Malpighian tubules lead into the gut. In the rectum blood digestion is completed and the black liquid is periodically ejected through the terminal anal opening.

#### *Digestion of the bloodmeal*

GARRETT-JONES (1950) has suggested that, under laboratory conditions, *A. luteola* will feed daily implying a rapid digestion of blood. It seemed unlikely to us that a host would be regularly available to *Auchmeromyia* sp. in the culverts and thus it was of interest to know exactly how long it took for a blood meal to be digested.

Second and third stage larvae were collected in the field and kept in dry earth without food, until the gut was empty. Groups of larvae were allowed to feed on the human arm until fully engorged. The larvae were then kept in dry soil at ambient temperatures for various periods of time. After killing the larvae, the gut contents were smeared onto filter paper and subsequently tested by the precipitin test to determine whether human blood proteins could be detected (WEITZ, 1956; BOREHAM, 1972; 1975). The results are shown in Table 4.

No digestion occurred in the crop and some blood remained here for 2–3 days before passing into the midgut. The precipitin test showed that, in 36% of the fed larvae, the bloodmeal could still be detected after five days. The digestion rate of blood will obviously vary with temperature and although facilities were not available to control this, nevertheless this experiment does indicate that daily feeding by *Auchmeromyia* sp. is not necessary for survival.

*Natural hosts of Auchmeromyia sp.*

While the culverts were being surveyed for mammals, any blood fed larvae found were kept in dry earth and returned to the laboratory within 2–6 h. Blood smears were prepared on filter papers as described by WEITZ (1956). In some cases, where freshly fed third stage larvae were collected, only part of the bloodmeal was used and the remainder used in attempts to isolate trypanosome strains. The filter papers were interleaved with non-absorbent paper and stored at  $-20^{\circ}\text{C}$  until brought back to Imperial College. Bloodmeal identifications were performed as described by WEITZ (1960) and BOREHAM (1972). Every feed was tested for both suid and hyaena blood and any meal which failed to react in one or both of these tests was subsequently examined to determine whether mammalian, avian or reptilian serum proteins were present. Feeds which did not react were all very well-digested bloodmeals and a negative result indicates a bloodmeal unsuitable for testing rather than a host not included in the test system, such as amphibia.

The results of the bloodmeal analyses are shown in Table 5.

Although the precipitin test will not distinguish between domestic and wild pigs, it can be assumed that all the pig feeds were, in fact, derived from warthogs. Domestic pigs are not present inside the Park and bushpigs have not been reported in the locality where the survey was performed. In order to confirm this, 128 bloodmeals of *Auchmeromyia* sp., representative of each culvert, were tested by the haemagglutination inhibition test (BOREHAM, 1972) to identify the species of suid. 95 bloodmeals gave positive results all of which were from warthogs. The remaining 33 meals were too weak to identify further. Only in culvert 68, were feeds other than warthog found: three out of the 32 meals contained blood of both warthogs and hyaenas.

A total of 15 blood fed larvae were collected from a burrow into which a hyaena was seen to flee. On analysis, all the bloodmeals were found to have been derived from warthogs, suggesting that the hyaena was only using the burrow as a temporary shelter rather than as a permanent den.

A single larva was found in the hair of a hyaena which had been immobilized (No. 4). This larva contained blood in the crop, but was not attached to the skin when discovered. Analysis of the blood revealed that it had fed on a hyaena.

The results show that the most important host of *Auchmeromyia* sp. along the Mwanza Road was the warthog (98% of all positive feeds). Three meals contained hyaena blood as well as warthog blood and the single larva collected on a hyaena had also fed on this host. This is the first record of *Auchmeromyia* sp. being associated with



Table 5. Feeding patterns of *Auchmeromyia* sp. larvae collected in culverts on the Mwanza Road, Serengeti National Park, Tanzania, 1975

Culvert	Date	No. tested	Result
1	14/7	38	37 suid, 1 negative
	18/7	5	5 suid
2	14/7	6	6 negative
	18/7	4	1 suid, 3 negative
14	18/7	33	33 suid
23 right tube	21/7	70	70 suid
23 left tube	21/7	44	44 suid
24	12/7	48	48 suid
	21/7	16	16 suid
	31/7	13	13 suid
29	21/7	16	16 suid
32 right tube	12/7	36	36 suid
32 left tube	12/7	57	56 suid, 1 negative
32	21/7	57	57 suid
33	17/7	17	17 suid
	21/7	1	1 suid
35	17/7	18	18 suid
36	17/7	14	14 suid
40	17/7	1	1 negative
65	24/7	11	10 suid, 1 negative
68	24/7	32	29 suid; 3 suid and hyaena
69	24/7	25	25 suid
73	24/7	14	14 suid
74	24/7	11	11 suid
75 B	25/7	12	12 suid
76	25/7	12	12 suid
87 C	25/7	24	24 suid
89	25/7	16	16 suid
90	25/7	11	11 suid
91	25/7	27	27 suid
92	25/7	21	21 suid
Total 23		710	

hyaenas. ROUBAUD (1914; 1916) states that *Auchmeromyia* sp. are parasitic only on hairless mammals such as man, warthog and aardvark and subsequent literature gives no suggestion that other hosts are involved. We have now been able to show that at least occasionally hairy mammals such as the hyaena can act as hosts.

#### *Feeding patterns of ticks along the Mwanza Road*

In order to obtain additional information on the vertebrate animals utilising the culverts, bloodmeal analyses on a few fed *Ornithodoros*



Table 6. Feeding patterns of *Ornithodoros* and *Ixodid* ticks collected in culverts on the Mwanza Road, Serengeti National Park

Culvert	Date	No. tested	Result
<i>Ornithodoros</i>			
1	18/7	2	2 suid
2	18/7	1	1 suid
14	18/7	13	13 suid
23	21/7	2	2 suid
24	12/7	1	1 suid
	21/7	1	1 suid
29	21/7	6	6 suid
32	12/7	6	6 suid
	21/7	3	3 suid
33	17/7	4	4 suid
	21/7	1	1 suid
34	17/7	1	1 suid
35	17/7	2	2 suid
36	17/7	2	2 suid
40	17/7	1	1 suid
42	17/7	7	7 suid
50	24/7	8	8 suid
60	24/7	3	3 suid
66	24/7	1	1 suid
68	24/7	1	1 suid
69	24/7	2	2 suid
73	24/7	8	8 suid
75 B	25/7	9	9 suid
76	25/7	1	1 suid
90	25/7	17	17 suid
Total 22		103	103 suid
<i>Ixodid</i>			
23	21/7	1	1 suid
90	25/7	10	10 suid
Total 2		11	11 suid

*moubata* sp. and *Ixodid* ticks were undertaken. Because of the risk of African Swine Fever Virus, all filter papers containing tick bloodmeals were immersed in ether for 30 min before precipitin testing. The results are shown in Table 6.

All meals were derived from warthogs and none from other hosts. These results indicate that warthogs must use the culverts extensively as temporary resting sites even though they were only actually seen in

five culverts during this survey, although their presence was suspected in several others.

*Possible role of Auchmeromyia sp. in trypanosome transmission*

a) Trypanosomes in *Auchmeromyia* sp. larvae

It is known that both hyaenas and warthogs in the Serengeti National Park are infected with trypanosomes including *T. (T.) brucei* (GEIGY et al. 1971 and Part II p. 71). Since the blood sucking larvae of *Auchmeromyia* sp. were found in close association with these animals the possibility was considered that the larvae might be involved in trypanosome transmission either cyclically or, more probably, non-cyclically. In order to investigate this, 502 blood fed *Auchmeromyia* sp. larvae were triturated with saline and injected into 32 rats. Each rat was also injected with a mixture of penicillin (500 IU) and streptomycin (500 mcg) to eliminate the risk of infection. When blood smears were prepared from larvae, the remainder (often with part of the blood meal) was included in the pool. Thus, should trypanosomes be isolated in the rats, the probable host source would also be identified. In the event, after examination of the rats for at least three weeks, no trypanosomes were isolated. It is therefore concluded that none of the *Auchmeromyia* sp. larvae examined contained infective *T. (T.) brucei* or *T. congolense* organisms.

b) Survival of trypanosomes in *Auchmeromyia* sp.

In order to determine for what period of time trypanosomes remained infective in *Auchmeromyia* sp., larvae were fed on heparinised whole rat blood taken from an animal showing a heavy *T. (T.) brucei* parasitaemia. The strain used was the *T. (T.) brucei* strain isolated from hyaena number 1 which produced a virulent infection in rats, being visible 3–4 days after inoculation. Groups of 5 larvae were killed at intervals, triturated in saline and injected into clean rats. Rats were examined for at least three weeks to determine whether they developed a parasitaemia. The results are shown in Table 7.

A number of larvae were dissected and examined for the presence of trypanosomes. In one case, a single larva was shown to have trypanosomes mainly in the hind gut two days after ingesting infected blood. In this case, the remains of the larva were triturated and injected into a clean rat and a transient parasitaemia developed.

Table 7. Survival of *Trypanosoma (T.) brucei* in *Auchmeromyia* sp. larvae

Time after bloodmeal	Result
30 min.	+
1 h	+
2 h	+
4 h	+
8 h	+
10 h	+
21 h	+ (transient)
34 h	–

+ = infection produced on inoculation into rats.  
– = no infection.

It is apparent that the *T.(T.) brucei* strain used remained infective to rats for at least 21 h and in some cases even longer.

#### c) Possible cyclical development of trypanosomes in *Auchmeromyia* sp.

Dissections of the salivary glands from wild caught larvae and larvae fed on *T.(T.) brucei*-infected rat blood revealed no parasites.

Larvae were fed on infected rat blood and 5 were killed daily for 8 days. The larvae were triturated and injected into a rat which was subsequently examined to see if a parasitaemia developed. Only those larvae injected into a rat on the day following feeding produced a parasitaemia, while rats injected with *Auchmeromyia* sp. larvae fed 2–8 days earlier did not produce any infection. Thus no evidence was produced of a cyclical development of trypanosomes in *Auchmeromyia* sp. It is possible that the time interval of 8 days was too short but time did not allow for it to be extended in this survey.

#### d) Mechanical transmission of trypanosomes by *Auchmeromyia* sp.

Two experiments were set up to test the possibility that mechanical transmission of *Auchmeromyia* sp. occurs. In the first experiment 15 second and third stage larvae were allowed to start a blood meal on a rat heavily infected with the hyaena strain of *T.(T.) brucei*. When the bloodmeal was approximately half completed the larvae were then

transferred to a clean rat to complete their meal. The blood of this rat was examined for the development of parasitaemia. The experiment was repeated using seven larvae. In both cases, no infection developed. The larvae were known to contain infective trypanosomes since, in both cases, when the batches were triturated in saline and injected into rats an infection developed. The larvae were unable to transmit the parasites in this interrupted feeding experiment.

### Discussion

The genus *Auchmeromyia* has not been extensively studied and in the last twenty years virtually nothing new has been learned. Two species were encountered in this survey, *A. luteola* and *A. bequaerti*. Most authors have considered *A. luteola* to be associated with man, feeding primarily on this host. However, there are some indications in the literature that this is not entirely true. WELLMAN (1906) for example discovered adult *A. luteola* half a mile from human habitation while SCHWETZ (1933) found adults, larvae and pupae in warthog burrows. We have been able to confirm that *A. luteola* populations survive and flourish in the complete absence of the human host and that in the Serengeti National Park it is largely dependent upon warthogs for its food. It is interesting to note that *A. bequaerti* was also found in close association with *A. luteola* and a further study is required to determine the exact ecological niches of these two species. Evidence from the survey of culverts suggested rapid repopulation of the culverts once a population had been removed, confirming an observation by RODHAIN & BEQUAERT (1913) that *A. luteola* could be found in newly constructed huts three weeks after their construction. Populations of *Auchmeromyia* sp. in the culverts must be considered in terms of rapid changes depending upon the conditions and a long term study of the dynamics of such populations would be valuable. It is apparent that the populations in the culverts do not tolerate damp or wet conditions, the larvae dying rapidly. This must occur regularly during the rainy season. In the latter part of this survey, many of the culverts became flooded due to unseasonal rain (Table 3) and the fly populations were wiped out.

Potential hosts in the culverts vary from day to day and a regular source of food is not always available. Along the Mwanza Road the *Auchmeromyia* sp. larvae are maintained almost entirely on warthogs. The larvae must feed when warthogs rest inside the culverts and since the warthogs do not use the resting sites every day it seems unlikely that feeding occurs at more than irregular intervals and certainly not daily as suggested by GARRETT-JONES (1950). Blood takes four or five

days to be digested by the larvae and so daily feeding would be unnecessary for maturation of a larva. It is well known that larvae of *A. luteola* can survive long periods without food (GARRETT-JONES, 1950) and so this should not provide a major difficulty. On several occasions, dead starved larvae were found in some of the culverts confirming the irregular habits of their hosts.

Three bloodmeals from culvert 68 contained mixed hyaena and warthog blood. This indicates that larvae will feed whenever a suitable host is present. It should be noted that multiple feeds on warthogs taken on separate occasions would not be shown up by the identification procedures used. The finding of hyaena blood in *Auchmeromyia* sp. proves an additional host for this genus which had not previously been suspected. It is also the first hairy mammal to be implicated as a food source.

The finding of a single larva on a hyaena several hundred metres from the culverts confirms the blood meal results and suggests that dispersal of the species can occur by passive transfer of larvae by their hosts as well as the dispersal of adult flies.

Several authors have suggested that *Auchmeromyia* sp. may be involved in disease transmission but there is no conclusive evidence to support this. MORRIS (1932) concludes that in Northern Rhodesia (now Zambia) myiasis or fly affection of cattle is frequent and is caused by *Lucilia*, *Chrysomya* and *Auchmeromyia*. However, in South Africa, *Calliphora* and *Auchmeromyia* do not appear to infest domestic stock (CURSON 1924). Obviously, affection by *Auchmeromyia* sp. is not a major problem as there is little reference to it in the literature. Another disease which has been suggested to be transmitted by *Auchmeromyia* is onchocerciasis (BLACKLOCK 1926). However, if *Onchocerca volvulus* is taken in with a bloodmeal by *A. luteola* it is rapidly digested and cannot be transmitted on a subsequent bite (BLANCHARD & LAIGRET, 1924). The third disease which has been suggested is trypanosomiasis (LAMBORN, 1927; 1933). Various experiments were carried out to try to determine the role of *A. luteola* in trypanosome transmission. For example, larvae were fed to repletion on a dog heavily infected with *Trypanosoma rhodesiense* and allowed to take 28 subsequent feeds on a white rat 39–92 days later. No infection resulted. Similarly larvae were allowed to feed on a guinea pig after an initial meal on an infected dog and, again, no infection resulted although, in one case, the guinea pig was bitten 30 times between days 2 and 30. Interrupted feedings of *A. luteola* on an infected dog and subsequently on an uninfected rat also produced no infection.

MCHARO (personal communication) found, on dissection, a single *Auchmeromyia* sp. larva containing a flagellate which he took to be a trypanosome. This larva was collected in the Serengeti National Park.



Unfortunately, it was not possible to make a permanent preparation or confirm the identification. This record must therefore be treated with considerable caution since the possibility that it was an insect flagellate cannot be ruled out. ROUBAUD (1913) has described a parasitic flagellate in the intestine of adult *A. luteola* but nothing is known about larvae.

In our experiments we have been able to show that trypanosomes remain viable in the gut of *Auchmeromyia* sp. for about 21 h but that the larvae are incapable of transmitting the infection by interrupted feeding. Also no evidence of cyclical development was found although little attempt was made to look at this histologically. These experimental findings together with the absence of isolation of any trypanosome strains from the 502 blood fed larvae caught in the culverts leads us to the conclusion that *Auchmeromyia* sp. probably do not transmit trypanosomes mechanically in nature or, if they do, it is not important for the epidemiology of the disease. In a restricted habitat, such as a culvert, where resting, infected animals are found together with large numbers of blood sucking *Auchmeromyia* sp. larvae, mechanical transmission of trypanosomes might be expected, should it occur.

If *Auchmeromyia* sp. is involved in disease transmission the most likely pathogens to be transmitted are the viruses. To date no studies on this subject have been undertaken but a study of African Swine Fever Virus would be worthwhile.

It is interesting to speculate on how the hyaenas become infected with trypanosomes since they appear to be one of the major reservoirs of *T. (T.) brucei* organisms in the Serengeti. It has previously been shown that *G. swynnertoni* only very occasionally feeds on hyaenas (MOLOO et al., 1971) and since there is such a high infection rate in this host other possibilities must be considered. Non-cyclical transmission by *Auchmeromyia* sp. in the culverts seems unlikely, but other biting insects could be involved. However, no live trypanosomes were found in over 200 *Hippobosca longipennis* collected from darted lions and hyaenas (ROGERS & BOREHAM, 1973). The most likely mechanism would appear to be direct infection by eating infected game (MOLOO et al. 1973). In addition to eating carrion, the hyaena is known to actively hunt and kill game and could well become infected by this means.

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