Examination of large mammals for trypanosomes

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Sleeping Sickness Survey in the Serengeti Area (Tanzania) 1971

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I. Examination of large mammals for trypanosomes

R. GEIGY [1] and M. KAUFFMANN [1]

Abstract

During November 1971, 95 mammals were examined by various methods for the presence of trypanosomes. All animals investigated inside the park, namely 31 spotted hyaenas, 43 lions, 1 waterbuck and 9 hartebeest, were immobilized with tranquillizers. A further 11 hartebeest were shot just outside the park. Forty strains of *T. brucei* subgroup were isolated in rats and preserved in liquid nitrogen. All strains were subsequently tested with the BIIT, where 4 strains gave a *T. rhodesiense*-like reaction.

Introduction

The results of the extensive survey on Rhodesian sleeping sickness, carried out in 1970 by E.A.T.R.O. and the Swiss Tropical Institute in and around the Serengeti National Park (SLEEPING SICKNESS SURVEY IN MUSOMA DISTRICT, TANZANIA, 1971) suggested further investigations on the reservoir and transmission problems. Therefore a second survey – this time on game, tsetse flies and hippoboscids only – was carried out during November-December 1971. Spotted hyaena, lion, waterbuck and Coke's hartebeest had been examined, because from each of these species BIIT positive strains were isolated in the previous survey (GEIGY et al., 1971). Although tsetse flies feed only rarely on hyaena and lion, these two species may be of importance due to the extremely high incidence of *T. brucei* subgroup infections. Hartebeest on the other hand, has already been proven to be capable of harbouring *T. rhodesiense* (GEIGY et al., 1972).

[1] Swiss Tropical Institute, Basle.

Material and Methods [2]

1. Immobilizing of carnivores

Lions were generally timid, and had to be approached slowly and circuitously to avoid their fleeing. They were darted from ranges of 15 to 30 metres, using a CO_2-powered Palmer Cap-Chur gun, firing Dist-Inject darts of 3 ml capacity. The dose used for an average 110 kg lioness was 100 mg Sernylan (Phencyclidine hydrochloride; Parke-Davis) together with 20 mg Acetylpromazine; the dosage was altered in proportion to estimated weight for animals of different size. Such dose rates generally immobilized the lion after about 15 minutes, and kept it down for about 3 hours.

Hyaenas could rarely be approached to within the range of the Cap-Chur gun. Most were therefore darted when in or fleeing from culverts under the road, using a Dist-Inject pistol model 30, firing darts similar to those for lions. It was impossible to anticipate the size of the animal to be darted, so dose rates of 20–25 mg of Succinylcholine were used regardless of size. Such doses took ½–4 minutes to immobilize the animal, which regained its feet usually between 10 and 20 minutes later. Several animals had to be restrained physically while blood was taken from the jugular vein; a few required artificial respiration.

2. Immobilizing of herbivores

Nine Coke's hartebeest *(Alcelaphus buselaphus cokei)* were immobilized using one of the analgetics, Etorphine (M-99) or Fentanyl in combination with one of the neurolepts, Azaperone or Acetylpromazine. The doses used on adult males (140 kg) were:

Etorphine	1.5 mg
or Fentanyl	18 mg
with Azaperone	75 mg
or Acetylpromazine	20 mg

As reported elsewhere (Pienaar, 1968) and on other animals, these combinations worked well: the animals never went down voluntarily and in some cases were difficult to catch. Two animals were darted from a stationary vehicle using the Dist-Inject powder charged rifle. However, only one group was found with a sufficiently short flight distance and the rest were darted from a moving vehicle using the powder charged pistol. Chases of more than 2 km were found to tire the animals excessively. The antagonists used were Nalorphine hydrobromide (2.5 mg for each 1 mg Fentanyl) or Cyprenorphine (2 mg for each 1 mg M-99). One waterbuck *(Kobus defassa defassa)* was immobilized, but unfortunately died. This species proved very difficult to immobilize in the Serengeti owing to the rocky hills and gallery forests in which the animals took refuge.

The remaining eleven of the hartebeest sample were shot in the same general area, but outside the Park, owing to the difficulty of immobilizing them.

[2] We are greatly indebted to B. C. R. Bertram for collecting and immobilizing the carnivores and to P. Duncan for doing the same for the herbivores. Only their great experience and skill made our survey possible.

3. Isolation and preservation of trypanosomes

From the immobilized animals blood was taken with a syringe by venous puncture, using a soft rubber tube as tourniquet. A first syringe, containing sodium citrate 3.8 % (final concentration 1 part–4 parts) was filled and used to inoculate immediately 5–6 rats[3] with 4 to 5 ml i.p. A second syringe without anticoagulant was then attached to the needle and the blood so collected used for serological purposes. After that, blood was collected from the same needle to fill 4–6 heparinized capillaries for immediate examination with the haematocrit centrifuge technique (HCT, Woo, 1970) and more blood to make several thick and thin films. In the case of lions, blood was usually taken from the median branch of the *vena saphena* (inner side of hindleg). After some trials of taking blood from similar veins from hyaena, it was found that much better results could be obtained by using the jugular vein. The blood was best taken from immobilized antelopes from the *v. saphena,* outer side of hindleg and from shot ones either from a vein or the heart.

Blood films were stained with Giemsa[4], using buffered distilled water at a pH of 7.5 and examined later for the presence of parasites. Tail blood from the inoculated rats was examined regularly for 3 to 6 weeks either by wet preparation or by HCT, whenever the haematocrit centrifuge was not used in the field. As soon as a rat showed a high parasitaemia with *T. brucei*-subgroup trypanosomes, stabilates were made and preserved in liquid nitrogen. About 25 capillaries were made per stabilate, with an addition of 10 I.U. Heparin per ml and 7.5 % Glycerol. The first stabilates were flame sealed, the later ones plasticine sealed. The capillaries were put in a glass tube surrounded by a plasticine jacket of a thickness of 5–8 mm and suspended for at least one hour in the vapour phase of a liquid nitrogen container and then transferred to a storage container. This procedure gives a slow cooling rate of about 2 °C per minute.

4. Blood Incubation Infectivity Test (BIIT)

The first 17 *T. brucei* subgroup strains isolated on rats were immediately tested with BIIT (RICKMAN & ROBSON, 1970), using rats from the isolation passage, i.e. the first passage, incubating for 5 hours with fresh human blood and ESG (WALKER, 1970) as control. As soon as a control rat became parasitaemic, it was killed to avoid a secondary infection of test rats. All strains were later tested and retested in Basel[5]. Whenever a test rat became positive, a stabilate was made as soon as the parasitaemia was high enough. Some of these derivatives were retested with BIIT.

Results

Considering all findings, the overall infection rate in the 95 large mammals examined between November 2nd and December 17th, 1971, was found to be 78 % (cf. Table 1).

Twenty-eight strains of *T. brucei* subgroup (SG), 23 of *T. congolense,*

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[3] The rats were obtained from the colonies of E.A.V.R.O., Muguga, and E.A.T.R.O., Tororo.

[4] Siegfried AG, Zofingen / Switzerland.

[5] Rat strain SIV 50.

Table 1. Incidence of trypanosomiasis in wild mammals, examined during November-December 1971 inside and near the Serengeti National Park

ecies	No ex-amined	isolated in rats				BF+HCT only	HCT only	Total infecte
		T. bru-cei SG	*T. con-golense*	mixed B+C	T.?	*T. vivax*	T.?	
ocuta crocuta	31	10	7	3	0	0	3	23
nthera leo	43	15	15	9 *	0	0	0	39
bus defassa	1	0	0	0	0	0	1	1
celaphus buselaphus	20	3	1	0	2	2	3	11
tal 4 species	95	28	23	12	2	2	7	74

* 2 more *T. congolense* were found in blood films only.
Overall infection rate 74/95 = 78 %.

Table 2. Comparison of the infection rates of trypanosomiasis obtained with the 3 methods used

T. species	inocula-tion of rats i.p. alone	Blood films only	HCT only	inoculation of rats			BF+ HCT	Total
				+B F	+HCT	+BF/ HCT		
T. brucei SG	7	0	0	0	24	9	0	40
T. congolense	8	0	0	7	4	16	2	37
T. vivax	0	0	0	0	0	0	2	2
T. spec.?	0	0	7	0	2	0	0	9
Total	15	0	7	7	30	25	4	88

Mixed infections included.

T. spec.?: only very few forms found in blood films and HCT or fleeting infection in rats.

HCT: only very rarely a differential diagnosis could be made by controlling the haematocrit tubes, which means that in the case of *T. brucei* subgroup only 9 of the 40 strains isolated would have been diagnosed by HCT and bloodfilms (BF) only without inoculation of rats.

Table 3. Distribution of the 26 localities where game was examined in relation to the areas studied in 1970

Animals examined species	No.	Number infected with tryp. total	B	C	B+C	viv.	T ?	
Lion	10	10	0	3	7		0	all 12 localities within or on the border of area E
Hartebeest	7	3	0	1	0		2	
Hyaena	1	1	1	0	0		0	
Lion	4	4	1	2	1		0	
Hyaena	1	0	0	0	0		0	
Hyaena	3	3	2	1	0		0	
Lion-cubs. *	2	0	0	0	0		0	about 3 miles south of Seronera
Lion	5	4	0	2	2		0	near northern limit of area D
Waterbuck	1	1	0	0	0		1	between area B and C
Hartebeest	2	1	0	0	0		1	about 4,5 miles east of Seronera
Hyaena	1	1	0	0	0		1	10 miles ESE from Seronera } open plain, expected to be free of tsetseflies
Lion	4	4	0	4	0		0	13 miles ESE from Seronera } open plain, expected to be free of tsetseflies
Hyaena	25	18	7 **	6	3 **		2	culverts along road to Kirawira, starting about 20 miles west of Seronera
Lion	3	3	2	1	0		0	Northern part of area C
Hartebeest	3	1	0	0	0		1	West of Ikoma-Gate } Outside the Park all animals shot
	5	5	3 **	0	0	1	1	West of Ikoma-Gate
	3	1	0	0	0	1	0	North of Ikoma-Gate
Lion	3	3	1	2	0		0	Musabi Plain, south of area B
	5	5	4	0	1		0	
Lion	7	6	5 **	1	0		0	3 miles east of Banagi-Lobo road, 17 miles NE from Banagi

Only 2 rats inoculated per cub.
Among them one strain BIIT *T. rhodesiense*-like.

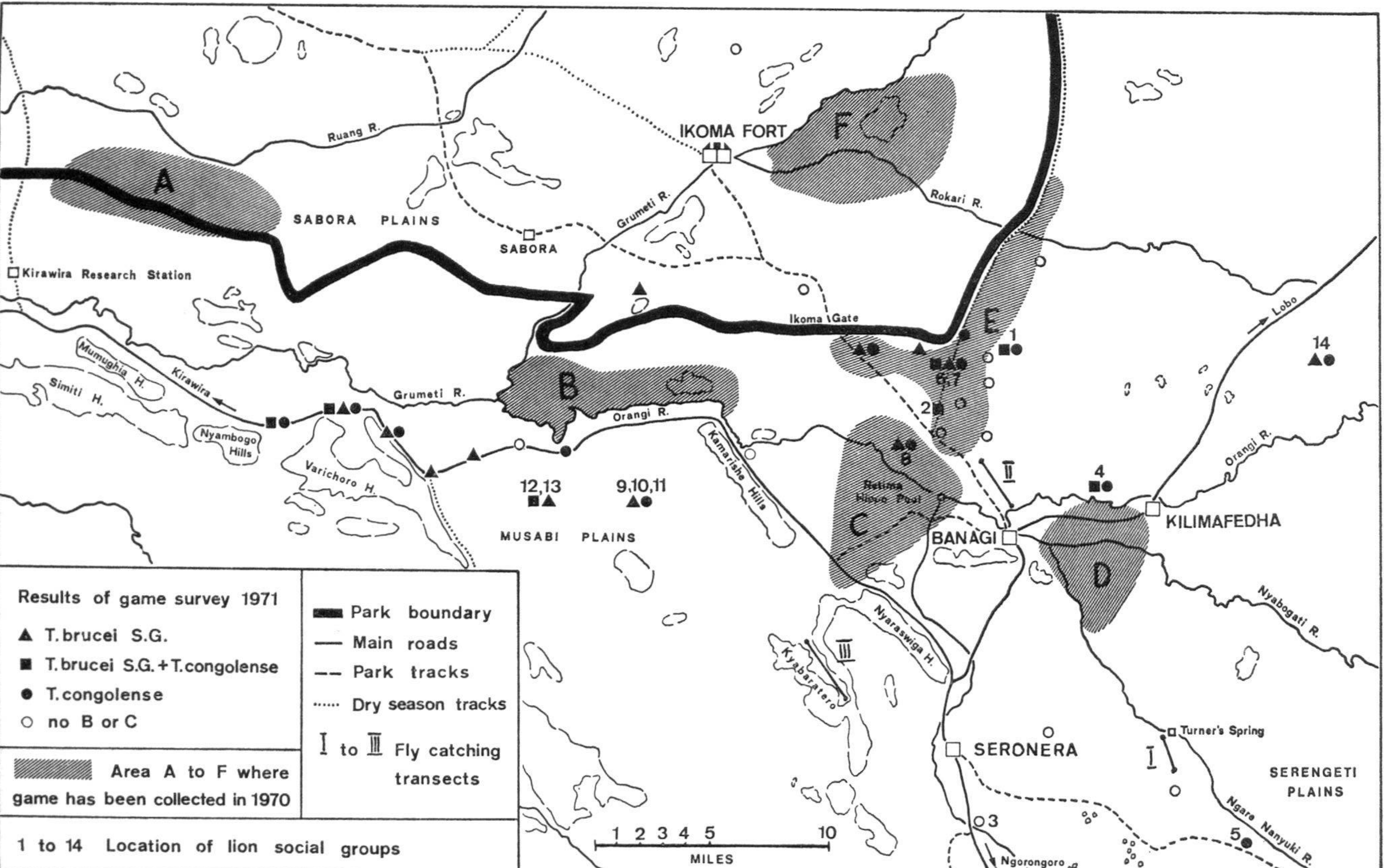

Fig. 1. Serengeti study area 1970/71.

12 mixed *T. brucei-congolense* and 2 T. indetermined (T. ?) could be isolated in rats. Furthermore, 2 cases of *T. vivax* were found in bloodfilms (BF) and with the Haematocrit centrifuge technique (HCT, Woo, 1970) as well as 7 unidentified trypanosome infections with the HCT only. Twice *T. congolense* was found in bloodfilms from lions, whereas in the inoculated rats only *T. brucei* subgroup could be identified (cf. table 2).

The prepatent period was 2–12 days for *T. brucei,* and all except 3 strains were detected during the first week after inoculation. The very early infections were usually found with HCT. The 23 pure *T. congolense* strains were found between day 4 and 19, 20 of them within the first two weeks, although individual rats showed a prepatent period of up to 29 days. We may therefore have missed a few infections, as rats remaining negative and inoculated with blood from 6 of the hartebeests could be examined for 4½ weeks only, from 1 of the lions for 3½ and from 4 of the hyaenas for 3 weeks. To avoid secondary infections of negative rats through licking or biting, each series of rats inoculated from one mammal were kept in separate cages.

Stabilates, preserved in liquid nitrogen, were made from all 40 *T. brucei* subgroup strains (including mixed infections) and from 4 of the 23 pure *T. congolense* strains.

Table 1 shows the incidence of trypanosomiasis, comprising all species, classified according to game species and method used.

Table 2 shows the number of infections of different species obtained by the three methods used.

Table 4. BIIT results of the 40 *T. brucei* SG strains isolated 1971

Species	No. of strains	BIIT results		
		+	+/0	0
Crocuta crocuta	13	2	1	10
Panthera leo	24	1	10	13
Alcelaphus buselaphus	3	1	2	0
Total	40	4 10 %	13 32.5 %	23 57.5 %

Submitted to the BIIT, 4 strains reacted *T. rhodesiense* like (10 %), 13 gave equivocal results (32.5 %) and 23 (57.5 %) were completely negative, i.e. reacting like *T. brucei sensu stricto.*

About 400 tests were carried out, in general 6 with completely negative stabilates and up to 20 or more with positive or equivocal ones. From all 3 hartebeest and the first of the lion strains, two stabilates were made from the isolation passage (different rats) and tested. For further details see GEIGY et al. (1972).

Table 5. Other parasites found in thick and thin films

Species	Number examined	Babesia Nuttallia Theileria	Hepatozoon	Microfilariae
Crocuta crocuta	31	31	31	29 *
Panthera leo	43	42	42	3 **
Kobus defassa	1	0	0	0
Alcelaphus buselaphus	20	20	0	6 ***
Total	95	93	73	38

* All microfilariae without sheaths. The thick films of the 2 negative hyaenas were of very poor quality!

** Microfilariae with sheath. The 3 infected lions came from the same pride (locality 23, cf. Table 3 and Fig. 1).

*** Three without sheaths, 2 with sheath, 1 mixed. No obvious relation to locality.

In Table 3 in connection with map Fig. 1 the results are classified according to the localities, where the game was found; *T. vivax* and undetermined strains are neglected in map Fig. 1.

Table 4 gives the BIIT results.

Table 5 gives a preliminary list of other parasites found in thick and thin films.

Discussion

1. Relation to age and sex

No relation between the incidence of trypanosomiasis and age or sex of the lions examined was found, whereas SACHS et al. (1971) state that, analysing the results of trypanosome incidence in over 60 lions studied 1966/67 in the Serengeti National Park (S.N.P.), they found a rather higher infection rate in lions of 2–3 years than in the other age groups. The results obtained during the 1970 and 1971 surveys, covering 52 lions from different localities within the Park, rather suggest that from a certain age on, all lions are harbouring trypanosomes. In 1970 one cub of about 4 months was found negative and considered to be too young to have developed a patent parasitaemia. In 1971 five individuals between 7–8 and 10 months old, were examined, 3 of which

were negative. The earliest *T. brucei* SG infection was found in a cub of 8 months, the earliest *T. congolense* in a cub of 10 months. It seems now more likely that, in the 3 negative ones, trypanosomes were present but not detected (see below). The same may be said from the 4th negative lion, which was 5 years old.

As regards spotted hyaena, the age of 28 individuals could be determined, ranging from 9 months to 8 years. There appears a slight increase of trypanosome incidence with age:

Incidence of trypanosomiasis in different age groups of spotted hyaena

Age group in years	Number of animals examined	Number of animals infected	B	C	B + C	T.? HCT only
under 1	2	0	0	0	0	0
1 to 2	11	8	4	2	1	1
2 to 4	10	9	4	3	1	1
4 to 5	4	4	1	2	1	0
8	1	0	0	0	0	0
Total	28	21	9	7	3	2

B = *T. brucei* subgroup, C = *T. congolense*, T.? = T. species undetermined.
The above figures are, however, too small to draw any definite conclusions.

2. *Relation to localities*

In general, *T. brucei* SG as well as *T. congolense* were found in all areas where blood samples were collected. This was to be expected, as all but 6 localities were chosen in places where these trypanosome species had already been isolated in former surveys. Two localities (14 and 15) in the north-west corner of the Serengeti plains, just south of the Ngare Nanyuki River, were specially chosen as this area was supposed to be free of tsetse flies. Only 5 animals (1 hyaena and a group of 4 lions) could be immobilized there and small numbers of tsetse flies were found in the scarce fringing forests accompanying the many little streams in the area. *T. congolense* was isolated from all 4 lions of this group.

In locality 3, south of Seronera, only 2 lion cubs were examined and no trypanosomes found. This result is not significant, specially as, due to a temporary shortage of rats, only two were inoculated from each cub.

In the other localities, where neither *T. brucei* nor *T. congolense* were found, only single hyaenas or from one to three hartebeest could be examined (cf. Table 3 and map Fig. 1).

3. Comparison of methods applied in this survey

The results of the current survey show clearly that as many methods as possible should be applied at the same time. For *T. brucei* subgroup, inoculation of rats or mice in sufficient numbers seems to be essential to reveal most of the infections with this species (cf. SACHS et al., 1971, and BAKER et al., 1967): 40 strains could be isolated in this way, but only 9 of them were definitely diagnosed in blood films. 24 of the cases were also HCT positive, but a differential diagnosis could be made by examining the haematocrit tubes in only a very few cases. Of the 37 strains of *T. congolense,* 8 were found in rats only, 7 in rats and BF, 4 in rats and HCT and 16 by all three methods. A further 2 *T. congolense* turned up in BF and HCT only (cf. Table 2). Seven infections were found with HCT exclusively and put down as T. ?, as the few trypanosomes seen in the haematocrit tubes did not allow a determination of the species. These may be either *T. vivax* or *T. congolense.*

Two trypanosome strains isolated in rats (from hartebeest) showed such a transient and low parasitaemia, only spotted by the HCT, that no trypanosomes were found in thin films made from tailblood of the infected rats. One of these two appeared only once on the 5th day after inoculation, the other on the 10th day, probably both *T. vivax,* as similar results have been obtained with experimental infections with known *T. vivax* strains (BLACKLOCK, 1912). We would like to mention here that in rats inoculated with blood from one hyaena, we even found once on the 16th day a microfilaria with the HCT.

Even by using all three methods mentioned, some infections will be missed by a single examination. In one case, a hyaena was darted twice with 3 days' interval. On the first occasion, *T. brucei* subgroup was found in 1 of 5 rats and *T. congolense* in 1 of the 3 thick films made at the same time. The second time, 4 of the 5 inoculated rats showed a pure *T. congolense* infection, but no trypanosomes were found in BF. As regards the HCT: on the first examination 3 capillaries were negative, 1 showed a single trypanosome and a fifth one microfilaria. On the second examination 1 capillary was completely negative, the other four showed microfilariae only. Several factors may be responsible for the equivocal results obtained:

1. The parasitaemia in reservoir hosts is usually very low, varying probably from day to day. As has been shown for *T. brucei* subgroup at certain times only tissue forms may be present (LOSOS & IREDE, 1970).

2. The distribution of the parasites (trypanosomes as well as microfilariae) in the peripheral blood is uneven.

3. The amount of blood examined by the three methods varies from about 4 ml per rat to 0.06 ml per capillary and 0.05 to 0.2 ml per thick film.

4. Differences in the susceptibility of laboratory animals to different species and strains of trypanosomes, even for *T. brucei* subgroup (GOEDBLOED et al., 1971).

All these findings seem to indicate that as much blood as possible with as many methods as feasible should be examined at he same time and also that, even then, more infections will be detected by repeated examination of the same animal.

4. Comparison of three surveys carried out in the Serengeti National Park from 1966 to 1971

It is of interest to combine some of the results obtained during the three surveys carried out in and around the S.N.P. in 1966/67 (BAKER et al., 1967; SACHS et al., 1971), 1970 and 1971. Only data obtained with the same methods, i.e. inoculation of rats or mice at the same time as bloodfilms, are taken into consideration.

Incidence of T. brucei-subgroup S.N.P. 1966–1971

Crocuta crocuta			*Panthera leo*			*Alcelaphus buselaphus*		
number examined	number infected	%	number examined	number infected	%	number examined	number infected	%
5 *	0	0	9 *	5	55.5	10 **	1	10
5	2	40	9	5	55.5	11	3	27
31	13	42	43	24	56	20	3	15
41	15	36.5	61	34	56	41	7	17

CHS et al., 1971.
KER et al., 1967.

The figures for lion from all three surveys correspond astonishingly well and give the infection rate which was calculated by SACHS et al. (over 50 %) analysing their figures. If we divide the lions examined into groups of 9, as studied in 1966/67 and 1970, we find 3 to 7 *T. brucei* infections per group. As to the infection rate in hyaena, in 1966/67 no *T. brucei* were found, whereas in 1970 and 1971 the results coincide

quite well. If we divide the 31 hyaenas (1971) into groups of 5 individuals, we find 1 to 3 infections per group. For hartebeest the results vary much more. In 1971 all three *T. brucei* found came from the same locality (21), where 5 animals were shot and belonged probably to the same herd.

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