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Sleeping Sickness Survey in Musoma District, Tanzania

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

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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

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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.

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