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A Survey of Wild Animals as Potential Reservoirs of Trypanosomiasis in the Ulanga District (Tanzania)

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This survey was undertaken as the epidemiological situation in the Ulanga District strongly suggests the presence of a trypanosomiasis reservoir in wild animals. In a rather small area (about 1200 sq miles) around Kilosa-kwa-Mpepo, each year there occur between 20 to 70 cases of sleeping sickness. All cases spotted by sleeping sickness scouts or local dispensaries have to report to the Lugala hospital; there the diagnosis must be confirmed and treatment has to be carried out. The people in this area live rather scattered around a few centres, such as Kilosa-kwa-Mpepo, Malinyi and Mpanga (map). A close study of the case histories, kindly provided by the Lugala hospital, showed that most people who contracted the disease did so while farming for a short period on small plots in the middle of the miombo (Brachystegia-bushland), hunting for game, searching wild honey or collecting firewood outside the new settlement boundaries. On these occasions they are bitten by tsetse flies. As so few people are present in the areas infested with tsetse flies (mainly zoophilic *Glossina morsitans*) but quite a lot of game, an examination of the latter seemed indicated.

The survey was carried out between the middle of October and the middle of December 1966. We chose the Mission station at Ngoheranga as base for our excursions, as this station lies in about the centre of the area which we wanted to investigate and was also able to provide the necessary facilities, such as living quarters, room for a small laboratory as well as for the many rats and mice needed and for our provisions. The most frequent game at this time of the year were *Phacochoerus aethiopicus, Syncerus caffer, Redunca arundinum* (the form typical for this southern region) and *Hippotragus niger. Taurotragus oryx* and *Alcelaphus lichtensteini* were met in smaller numbers. *Loxodonta africana* live farther away, but invade the area during the rainy season.

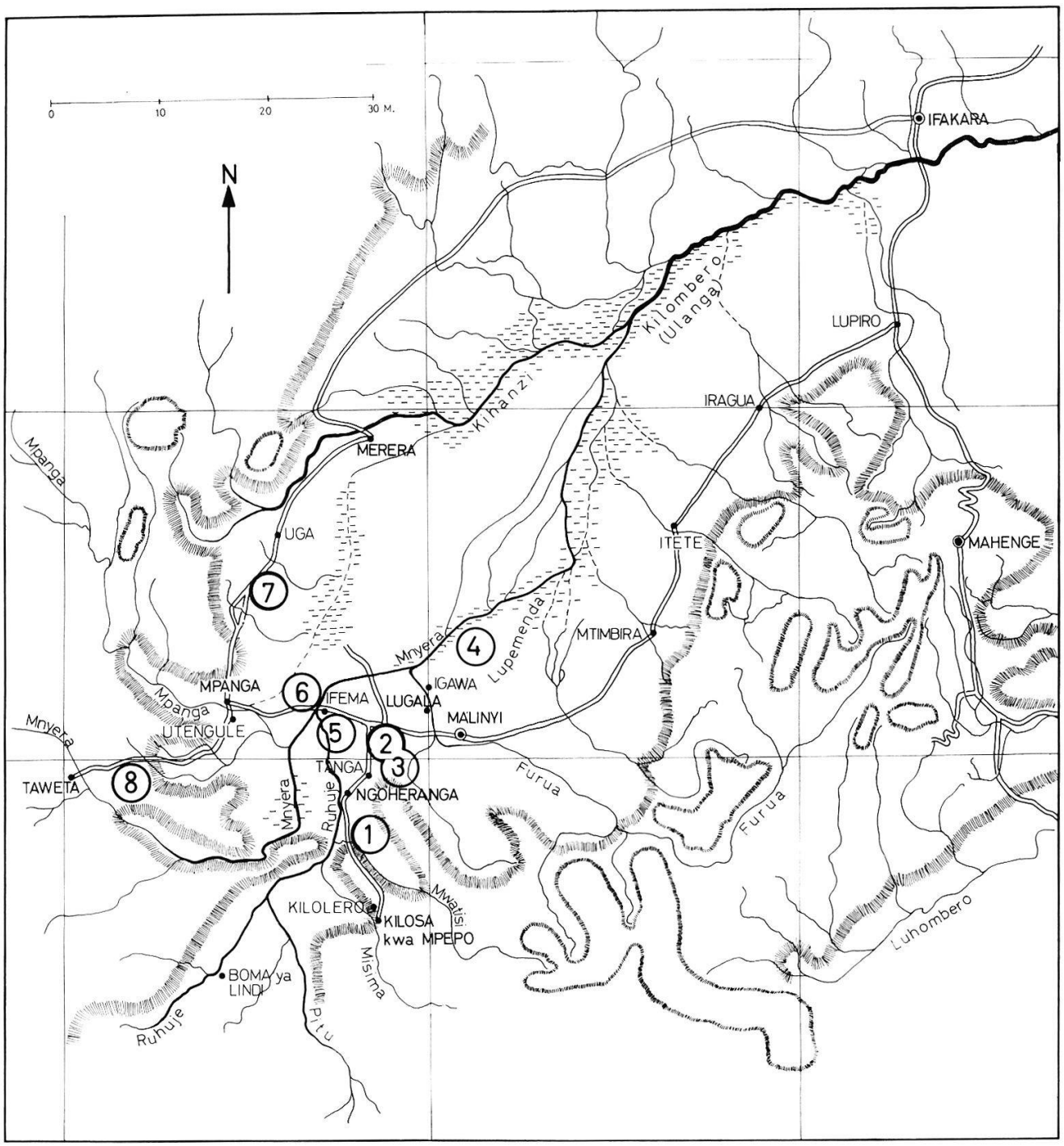
Material and Methods

The animals were shot in the areas numbered ① to ⑧ on the adjoining map, they were approached by the roads and tracks shown there. Most of the game was found about one to five miles off those and we tried to get as near as possible with landrovers to be able to take blood quickly after death of the animal, i.e. nearly always within 10 to 20 minutes. In most cases, we were able to take the blood from the vena jugularis. If we could not get enough blood from it, all the neck vessels were cut quickly with a sharp knife, the skin lifted, allowing a deep pool of fresh blood to form and the blood was then taken with a syringe without needle. To avoid clotting, we always filled the syringe up to one fifth with sterile 3.8% sodiumcitrate from ampoules. Several thin films were made with and without citrate and fresh preparations were examined immediately under the microscope. 5 to 6 white rats or mice (kindly reared for us by the Central Veterinary Laboratory, Dar es Salaam) were inoculated intraperitoneally (i.p.). Rats of about 120 to 160 g received 5 ml citrated blood each, mice of approx. 25 g 2 ml i.p.

We preferred rats to mice, as bigger quantities of blood can be injected into them. As emphasized by HEISCH et al. (1958), it is essential that enough blood should be injected into a sufficient number of animals. Otherwise, light infections may easily be missed. In one case, we found *brucei* group trypanosomes only in 1 out of 6 rats inoculated from a Hippotragus. During the last weeks we had to use smaller animals and could inject only 3 ml per rat and 1 ml per mouse. Before inoculation, rats were always slightly anaesthetized with ether.

Once a week the tail blood was examined for trypanosomes. A fresh preparation as well as a thin film were made each time. Whenever possible, a control was also made on the 11th day. Rats and mice were controlled for 6 to 8 weeks.

From each strain, which we were able to isolate in this way, one or two rats inoculated from the game were sent by air to the EATRO centre at Tororo for preservation by deep freezing. The first 4 strains of the *T. brucei* group were subinoculated into fresh rats to get enough material for trials with volunteers. The number of trypanosomes was counted like erythrocytes with an ordinary haemocytometer, the blood being diluted (1 : 100) with either Tuerk's solution or saline.



Map of the upper Kilombero Valley in South Ulanga with the hunting areas

① = Road Ngoheranga–Kilolero
② = Road Ngoheranga–Malinyi
③ = Mahuhu
④ = Lupemenda
⑤ = Ifema
⑥ = Utengule
⑦ = Road Mpanga–Merera
⑧ = Road Mpanga–Taveta

Results

During two months we were able to examine the blood of 35 animals belonging to 8 different species as shown in Table 1. Of these, 12 were found to harbour trypanosomes:

1 infected with *T. brucei* group only
4 infected with *T. congolense* group only
7 showing a mixture of both

By direct examination of thin films from the game itself, only two *T. congolense* group infections could be spotted, but no *T. brucei* or *T. vivax* group. As *T. vivax* does not multiply in white rats or mice, we were not able to spot any of these trypanosomes.

In rats as well as mice, the first trypanosomes were usually found after 1 or 2 weeks, in a few cases after 3 or 4 weeks. We decided therefore after some time that it should be sufficient to examine these animals for 6 weeks only. Trypanosomes of the *brucei* group appeared usually earlier than those of the *congolense* group. Pure infections of the two groups were easy to diagnose,

TABLE 1

Incidence of trypanosomiasis found in wild animals—October to December 1966

Species	No. examined	No. infected	*T. brucei* group	*T. congolense* group	*Brucei* + *congolense*
Loxodonta africana	2	–	–	–	–
Phacochoerus aethiopicus	6	1	1	-	–
Syncerus caffer	6	–	–	–	–
Taurotragus oryx	3	1	–	1	–
Redunca arundinum	6	5	4	5	4
Hippotragus niger	6	4	2	4	2
Alcelaphus lichtensteini	5	1	1	1	1
Manis temminckii	1	–	–	–	–
8 species	35	12	8	11	7

26. 12. 1966	35	12 = 34.3%

TABLE 2

List of Ulanga strains isolated from wild animals—October to December 1966

Strain	Trypanosome group	No.	isolated from species	Date of isolation	Area of isolation
U I U II	*brucei* * *congolense*	9	*Hippotragus niger*	22. 10. 1966	⑦ road Mpanga–Merera
U III	*congolense*	5	*Hippotragus niger*	20. 10. 1966	⑦ road Mpanga–Merera
U IV U V	*brucei* * † *congolense*	14	*Redunca arundinum*	4. 11. 1966	① road Ngoheranga–Kilolero
U VI	*brucei* * †	16	*Phacochoerus aethiopicus*	7. 11. 1966	⑤ Ifema
U VII U VIII	*brucei* * † *congolense* **	24	*Hippotragus niger*	23. 11. 1966	① road Ngoheranga–Kilolero
U IX	*congolense*	19	*Taurotragus oryx*	19. 11. 1966	⑥ Utengule
U X U XI	*brucei* *congolense*	31	*Redunca arundinum*	2. 12. 1966	① road Ngoheranga–Kilolero
U XII U XIII	*brucei* *congolense*	32	*Redunca arundinum*	3. 12. 1966	① road Ngoheranga–Kilolero
U XIV	*congolense*	35	*Hippotragus niger*	4. 12. 1966	① road Ngoheranga–Kilolero
U XV U XVI	*brucei* *congolense*	29	*Alcelaphus lichtensteini*	1. 12. 1966	① road Ngoheranga–Kilolero
U XVII U XVIII	*brucei* *congolense* **	38	*Redunca arundinum*	10. 12. 1966	① road Ngoheranga–Kilolero
U XIX	*congolense*	39	*Redunca arundinum*	10. 12. 1966	① road Ngoheranga–Kilolero

Strains U I to U XVIII were sent to Tororo for preservation by deep freezing.

* Inoculated into human volunteers. † Inoculated into domestic animals.

** Trypanosomes found in thin films from game.

but as trypanosomes of the *congolense* group show many morphological variations, it was often difficult to spot the first *T. congolense* forms in mixed infections.

19 strains, namely 8 of the *T. brucei* group and 11 of the *T. congolense* group were isolated on rats or mice. We called them Ulanga (U) I to XIX in order of their appearance (Table 2). The infection rate for the game in the small area studied, proved to be rather high, i.e. 34.3%, for the *T. brucei* group alone 22.8%.

As regards the 8 *T. brucei* group strains found, the question of their character remains open. In the Ulanga District they might be either *T. brucei* or *T. rhodesiense*. Up to now, only trials with human volunteers as well as domestic animals may elucidate this point (HEISCH et al., 1958; ASHCROFT, 1958; ONYANGO et al., 1965; BAKER et al., 1967).

Trials with Human Volunteers

With the help of the local authorities we were able to recruit 18 African volunteers from Malinyi and Ngoheranga. A first batch of 5 were inoculated at Lugala hospital with strain U I, isolated from *Hippotragus niger*. They received between 1.2 and 2.9 ml blood from subinoculated rats i.m. in the forearm (Table 3). The parasitaemia of these rats had been controlled daily from the third day onward. The rats were bled as soon as they showed a great number of trypanosomes (+++) and while their number was still rising. The infections of the rats were of similar strength as the ones applied later in Ifakara, where we were able to evaluate the number of trypanosomes present.

None of the volunteers showed any reaction except some slight swelling on the day after inoculation on the site of the injection. They were controlled for 3 weeks. As the temperature remained normal during this period and no trypanosomes could be found, the experiment was considered to be negative and the 5 volunteers received a full course of treatment with Antrypol, as suggested by the medical authorities at Dar es Salaam and as is usual in Lugala.

Another 13 volunteers were then recruited and brought to Ifakara hospital for further trials. Three more strains were tested, U IV, mixed with U V *congolense,* isolated from *Redunca arundinum,* U VI isolated from *Phacochoerus aethiopicus* and U VII mixed with U VIII *congolense,* isolated from *Hippotragus niger.* Between 1.4 and 5 ml blood containing from 135 to 280 million trypanosomes were injected subcutaneously (s.c.) into the forearm (Table 3). Two volunteers (U. and L.) showed a local reaction on

TABLE 3

Trials with volunteers

Strain	Vol.	ml inoculum	ml blood	Number of trypanosomes in millions
U I	E	i.m. 2.5	2.0	
	F	2.2	1.2	
Hippotragus niger	G	2.5	2.0	not determined
	H	3.5	2.9	
	I	2.8	2.2	
U IV *	K	s.c. 3.5	2.8	140
mixed with	O	3.5	2.7	135
U V *(congolense)*	U **	5.2	3.7	222.9
Redunca arundinum	V	7.0	5.0	150
U VI	M	s.c. 2.5	2.0	260
	N	3.0	2.4	198
Phacochoerus				
aethiopicus	Q	2.5	2.0	260
	R	4.0	3.1	255.7
U VII *	L **	s.c. 5.0	4.0	280
mixed with	P	3.2	2.4	276
U VIII *(congolense)*	S	2.1	1.4	149
Hippotragus niger	T	2.5	2.0	200
	X	2.5	2.0	200

* *Brucei* predominant.

** Local reaction on 7th day after inoculation.

the seventh day, but no parasites were found in material aspirated by local punction. The body temperature was taken twice daily from the beginning of the experiment. Blood was controlled on the seventh day and then daily from the 10th day onward.

As here again no rise of temperature occurred in any of the volunteers and not a single trypanosome could be found, treatment with Antrypol was given after three weeks.

Trials with Domestic Animals

Three of the four Ulanga strains tested on human volunteers were also applied to cattle, sheeps and 1 horse (Table 4). EATRO stabilates of the strains U IV + (V), U VI and U VII + (VIII) were thawed up and injected i.p. into white rats. Tail blood of these rats was examined from the fourth day on, and as soon as a suitable parasitaemia with predominant *T. brucei* was reached, the

TABLE 4

Trials on domestic animals

Passage-chain: i.p. game→rat EATRO stabilate i.p. →rat i.v. →H/S/C

Strain	Animal No.	Animal Weight kg	Rat No.	Rat D. I.	Number of trypanosomes in millions	First positive on D. I.
U IV + (V) *	S 1 ♂	35	19	5th	250	10th
Redunca	S 4 ♂	32	18	10th	315	8th
Stabilate 1097	H 7 ♂	620	18	10th	665	3rd
	C 8 ♀	590	19	5th	450	8th
U VI	S 2 ♀	26	23	7th	178.5	7th
Phacochoerus	S 3 ♀	27	24	7th	450	9th
Stabilate 1094	C 9 ♀	710	23	7th	312.4	11th
	C 10 ♀	730	24	7th	750	10th
U VII + (VIII)	S 5 ♂	35	27	11th	191.25	4th
Hippotragus	S 6 ♂	30	28	11th	293.5	6th
Stabilate 1100	C 11 ♀	670	27	11th	318.75	3rd
	C 12 ♀	800	29	11th	587	5th

S: sheep, H: horse, C: cow. D.I.: Day of infection.
* In mixed infections *brucei* predominant, ♀ not pregnant.

rats were bled. From each rat one sheep and one cow or horse were infected by intravenous (i.v.) injection. Temperature was taken daily and frequent thin films examined. Trypanosomes appeared in the blood of all twelve animals. They were first found between the third (horse) and the eleventh day (cow). The number of trypanosomes in sheep and cattle was always very low, often only one trypanosome could be found after a long search. Only the horse (H7) infected with U IV + (V) showed a greater number of trypanosomes in the blood with a peak (++) on the 8th day. All animals developed a primary rise of temperature between the 3rd and 5th day after inoculation as typical for experimental trypanosomiasis. Afterwards the horse (H7) and sheeps (S1–6) showed a characteristic fever curve. In cattle, only the initial peak could be observed. Otherwise the behaviour of all 12 animals remained normal: no signs of distress, appetite and faeces normal. 12 to 24 days after inoculation the animals were treated with Berenil (7 mg per kg i.m.). Before treatment, venous blood was taken from each animal and injected i.p. into 2 to 4 rats for further studies of the strains concerned.

TABLE 5

Incidence of trypanosomiasis in the different hunting areas

① Road Ngoheranga–Kilolero

Species	No. examined	No. infected	*T. brucei* group	*T. congolense* group	*Brucei* + *congolense*
Phacochoerus aethiopicus	3	–	–	–	–
Redunca arundinum	5	5	4	5	4
Hippotragus niger	4	2	1	2	1
Alcelaphus lichtensteini	4	1	1	1	1
4 species	16	8	6	8	6

④ Lupemenda

Redunca arundinum	1	–			
Syncerus caffer	1	–			
Alcelaphus lichtensteini	1	–			
3 species	3	–			

⑤ Ifema

Phacochoerus aethiopicus	3	1	1	–	–

⑥ Utengule

Syncerus caffer	5	–			
Taurotragus oryx	3	1	–	1	–
2 species	8	1	0	1	0

⑦ Road Mpanga–Merera

Hippotragus niger	2	2	1	2	1

⑧ Road Mpanga–Taveta

Loxodonta africana	2	–			

Ngoheranga

Manis temminckii	1	–			

Total

8 species	35	12	8	11	7

Discussion

The incidence of 34.3% of trypanosomiasis found in 35 wild animals is rather high and we still have to suppose that the true incidence will be even higher, as our results are based on a single examination after death of each animal. As theoretically a good reservoir animal is expected to show parasitaemia over a longer period of time and the number of parasites goes up and down, it seems improbable that all infections can be spotted by single examination. If we consider Table 5, where our data are grouped according to the different hunting areas, the results become even more striking. We are well aware that the number of animals examined in the different areas is very small, but the fact remains that for example off the Kilolero road ① 8 out of 16 animals harboured trypanosomes, whereas only one out of 8 was found positive near Utengule ⑧ and no infection could be spotted in Lupemenda ④. At the same time, we could observe that tsetse flies were usually present in area ①, frequently met in area ⑧ but that area ④ proved to be on the border of the tsetse belt. This was also the reason, why we did not continue to examine game from the Lupemenda area, where in the absence of tsetse flies only *T. vivax* was to be expected. Of course, one has also to consider the fact that many game species move around considerably according to the seasons and water and food conditions. On the other hand, *Redunca arundinum* for example, which showed the highest incidence, seems to be rather stationary. During our stay we could confirm this for Redunca and also observe always in the same areas small herds of between 4 to 15 *Hippotragus niger*.

As regards the species infected, we found contrary to former surveys (see ASHCROFT et al., 1959) rather a high incidence in *Hippotragus niger* (4 animals out of 6 examined). So far, no trypanosomiasis has been reported in these animals in East Africa, only a few cases in Mozambique (3 out of 332). *Redunca arundinum* too shows a remarkably high incidence, 5 out of 6 animals were positive. All 5 from the Kilolero road (area ①) showed *congolense* group trypanosomes and four of them *T. brucei* group as well. The only negative Redunca came from Lupemenda (area ④,) which as already mentioned lies on the border of the tsetse fly belt. This high incidence in Redunca seems specially interesting, as it has been shown experimentally that it could be an ideal reservoir: 'it shows blood positive periods of considerable length' and 'is commonly bitten by tsetse flies' (ASHCROFT, 1959).

To elucidate the nature of the 8 *brucei* strains isolated, we started to test them on human volunteers and domestic animals.

Four strains, Ulanga I, IV + (V), VI and VII + (VIII) have already been tried out on 4 to 5 volunteers each with negative results. This seems to indicate that they are rather *T. brucei* than *T. rhodesiense*. But the possibility remains that the strains are attenuated by the passage through a reservoir animal and might still regain their virulence for man by a further passage through their normal vector, the tsetse fly. Strains IV + (V), VI and VII + (VIII) were also tested on domestic animals and showed rather low pathogenicity for cattle, sheep and horse.

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Zusammenfassung

In der Gegend um Kilosa-kwa-Mpepo (Süd-Ulanga/Tanzania) werden jährlich 20 bis 70 neue Fälle von Schlafkrankheit gemeldet. Da dieses Gebiet dünn besiedelt, aber wildreich ist, vermutete man schon lange das Vorhandensein eines Wildreservoirs. Von Mitte Oktober bis Mitte Dezember 1966 gelang es den Autoren, dort das Blut von 35 Tieren 8 verschiedener Arten auf Trypanosomen zu untersuchen. Dabei wurden 12 infizierte Tiere gefunden, d. h. 34,3%. Im ganzen konnten auf Ratten oder Mäusen 19 Trypanosomenstämme (Ulanga I bis XIX) isoliert werden, und zwar 8 Stämme der *T. brucei*-Gruppe und 11

Stämme der *T. congolense*-Gruppe. Die meisten frisch isolierten Stämme konnten an das EATRO-Zentrum (Tororo/Uganda) geschickt werden, wo sie nach der dort üblichen Methode im Deep freeze konserviert wurden.

Was nun die Stämme der *T. brucei*-Gruppe betrifft, so kann nur durch Versuche an Volontären und Haustieren festgestellt werden, ob es sich dabei um *T. rhodesiense* oder *T. brucei* handelt. Die ersten vier dieser Stämme wurden bereits auf afrikanischen Volontären ausgetestet und drei derselben auf Haustieren in Europa. Die untersuchten Stämme ergaben keine Parasitaemie auf dem Menschen, erwiesen sich aber als schwach pathogen für Rinder, Schafe und Pferd. Die Untersuchungen über die Natur der isolierten Stämme müssen noch weitergeführt werden. Es ist durchaus möglich, daß sie durch lange Wildpassagen in ihrer Pathogenität abgeschwächt wurden und ihre Virulenz für den Menschen erst wieder durch eine erneute Glossinen-Passage zurückerhalten.

Résumé

Dans la région de Kilosa-kwa-Mpepo (Sud de l'Ulanga, Tanzanie) on enregistre chaque année 20 à 70 nouveaux cas de maladie du sommeil. Comme ce territoire est peu habité, mais riche en animaux sauvages, on soupçonnait depuis longtemps déjà l'existence d'un réservoir sauvage. Les auteurs ont pu rechercher, de la mi-octobre à la mi-décembre 1966, des trypanosomes dans le sang de 35 animaux appartenant à 8 espèces différentes. Douze animaux, c'est-à-dire 34,3 %, étaient infectés. Dix-neuf souches de trypanosomes (Ulanga I à XIX) purent être isolées sur rats et sur souris. Huit souches appartenaient au groupe *brucei* et onze au groupe *congolense*. La plupart des souches fraîchement isolées furent envoyées au centre EATRO (Tororo/Ouganda) pour y être congelées selon la méthode normale.

En ce qui concerne les souches du groupe *brucei*, seule l'injection à des volontaires et à des animaux domestiques pouvait permettre d'affirmer s'il s'agissait de *T. rhodesiense* ou de *T. brucei*. Quatre des premières souches furent expérimentées sur des volontaires africains, et, de ce même groupe, trois souches furent également injectées en Europe à des animaux domestiques. Aucune parasitémie ne fut observée chez l'homme. Par contre, les souches se révélèrent faiblement pathogènes pour le bœuf, le mouton et le cheval. Les recherches sur la nature des souches isolées doivent être encore poursuivies. Il est cependant fort possible, que par suite de longs passages chez les animaux sauvages, la pathogénie des souches ait été affaiblie et que leur virulence n'est pas assez forte pour assurer, par passage sanguin, une infection chez l'homme. Cette virulence pourrait être recouvrée après un nouveau passage par les glossines.