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Isolation of *Trypanosoma (Trypanozoon) rhodesiense* from Game and Domestic Animals in Musoma District, Tanzania

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A short abstract of these findings has been published in Acta Tropica, vol. 29, 1972, p. 199, under the heading "Additional Animal Reservoirs of *T. rhodesiense* Sleeping Sickness".

Although Rhodesian sleeping sickness is a zoonosis, the causative organism *Trypanosoma (Trypanozoon) rhodesiense* (HOARE, 1966) has been isolated only once from a naturally infected bushbuck (HEISCH et al., 1958) and once from a cow (ONYANGO et al., 1966). Both these isolations, believed to be the first incontrovertible evidence that game and domestic animals are reservoir hosts of *T. rhodesiense,* were obtained from animals inhabiting endemic and epidemic areas of Western Kenya, which lies to the North of Tanzania. Polymorphic trypanosomes of the *T. brucei* subgroup (Plimmer and Bradford, 1899) have been isolated from game animals in various parts of Tanzania but on three occasions when these trypanosomes have been inoculated into human volunteers to find out if any were *T. (T.) rhodesiense* none proved infective to man (ASHCROFT, 1958; BAKER et al., 1967, and GEIGY et al., 1967). In this paper an account of a successful isolation of *T. (T) rhodesiense* from a hartebeest *(Alcelaphus buselaphus)* resident in the Serengeti National Park and from a cow at Ikoma, both contiguous areas in Musoma District of Tanzania is reported.

A brief résumé of the history of Rhodesian sleeping sickness in this area is given. The disease was diagnosed for the first time in Ikoma in 1925. It is believed to have been brought in by people coming from Maswa where an epidemic of Rhodesian sleeping sickness broke out also for the first time in 1922 (FAIRBAIRN, 1948). The tsetse fly vector responsible for the transmission of the disease was found to be *Glossina swynnertoni.* Game and cattle were abundant in Ikoma at the time. The locality remained an endemic focus of sleeping sickness and cases continued to be notified from the area until 1954 when the disease seemed to have disappeared probably due to the closure of the gold-

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mines and the evacuation of the mining settlements which considerably reduced man-fly contact. The surrounding areas to the east and south of Ikoma which was little populated but contained a large number of game was designated the Serengeti National Park and Game Reserve just after the second world war. The decade that followed saw the development of the area as a tourist attraction and human population simultaneously increased. From 1964 a few cases of Rhodesian sleeping sickness reappeared and the number of yearly notifications gradually increased reaching 14 by 1968. Some of the cases were said to have contracted the disease at Ikoma and Shirati and some within the Serengeti National Park at Banagi and Seronera. In 1970 a survey was therefore carried out by us with the object of finding out factors responsible for the recrudescence of the disease in the area in general and possible animal reservoir hosts in particular (see Acta Tropica, vol. 28, 1971).

Materials and Methods

Trypanosome Strains: Four out of 12 *T. brucei* subgroup trypanosome stabilates, E.A.T.R.O. Nos 1811, 1822, 1857 and 1873, isolated from game (2 from lion, 1 hyena and 1 hartebeest, respectively) in the Serengeti National Park (GEIGY et al., 1971) and one out of 10 *T. brucei* subgroup stabilates, E.A.T.R.O. 1825, isolated from cattle in Ikoma (MWAMBU & MAYENDE, 1971) were selected for inoculation into human volunteers. These stabilates had been preserved in liquid nitrogen by the method of DAR et al. (1972). All the stabilates had been tested and gave more or less "equivocal" results with the Blood Incubation Infectivity Test – BIIT (RICKMAN & ROBSON, 1970) and had also been found sensitive to Antrypol, Berenil and Mel. B. The isolate from cattle, however, had been found to be slightly insensitive to Mel. B.

Laboratory Animals: Adult Swiss white mice and albino rats reared in the closed colony at E.A.T.R.O. were used in the passages of the stabilates before inoculation of volunteers.

Volunteers: Five male Tanzanian adults were selected from among nine volunteers. Their general condition was good and they were found to be free from any other infections hence medically fit to participate in the volunteer experiments. A full explanation, as to what they were being exposed to, was given by one of us (R. J. O.).

Drug Sensitivity Test for the Trypanosome Stabilates: Each test stabilate was inoculated into a group of 5 mice. When parasitaemia reached the extent of 50 parasites per high power microscope field

(magnification of 320) the mice were bled and their blood pooled. The pooled blood of each group was inoculated into 35 fresh mice and these were divided into 3 groups of 10 mice each and one group of 5 mice. As soon as parasitaemia became evident (6–10 trypanosomes per microscope field, $\times 40$) in all the subinoculated mice, those in the groups of tens received the test drugs intraperitoneally (single dose); one group getting Antrypol, the other Berenil and the third Mel. B. The mice in the groups of five were left untreated and served as controls. The dose of Antrypol administered was 50 mg/kg body weight and of Berenil 50 mg/kg body weight. As Mel B contains propylene glycol, which is toxic to rats and mice, 25 mg/kg of Melarsen Sodium dissolved in water was used. After the administration of the drugs the mice were examined daily for sixty days.

Inoculation of Volunteers: The five volunteers, I, II, III, IV, and V, received E.A.T.R.O. 1873, 1811, 1857, 1822, and 1825, respectively. The first four strains were inoculated into volunteers after 3 passages in mice and one in rats. The fifth (1825) was inoculated after 2 passages in mice and one in rats. Each volunteer was inoculated subcutaneously on the medial aspect of the left forearm with about 1 ml of heavily infected rat blood. The inocula were prepared as in the previous experiments (ONYANGO et al., 1966) and contained more than 10^8 motile trypanosomes as estimated by a haemocytometer count (improved Neubauer ruling) under a microscope. The inoculation was carried out under strict aseptical condition. After the inoculation the volunteers were admitted into hospital for observation as in-patients.

Experimental Results

Volunteers II, III, and IV inoculated with E.A.T.R.O. 1811, 1857, and 1822, respectively, showed no evidence of trypanosome infection during 3 weeks' period of observation in hospital. They spent 2 weeks more at their homes after which they were recalled for clinical and laboratory examinations. These examinations again revealed no evidence of infection.

The results of the inoculation of volunteers I and V are tabulated in Tables 1 and 2, respectively.

Discussion

In one of the earlier experimental infections of volunteers, it had been estimated that the minimum requisite number of motile trypanosomes in an inoculum which will cause overt infection in man is about

Table 1. Results of the inoculation of volunteer I (E.A.T.R.O. 1873)

Day after inoculation	Temperature	Blood film examination	Clinical state	Other remarks
9th day	99 °F	Negative	Headache and joint pains	Primary chancre the size of a pea, axillary glands palpable but not tender. Mice subinoculated
11th day	102 °F	*P. falciparum* positive	Quite ill	Oral chloraquine treatment given. Swelling of chancre
14th day	104 °F	Negative	Still ill	subsiding
15th day	99 °F	Negative	Much improved	– Chancre healed
16th day	98 °F	Negative	Feels very well	Salm. typh. (O) and Salm. typh. (H) negative; Proteus Ox 19, neg.; Br. Abor. neg.
16th–19th day		Patient discharged home at his own request		
20th day	100 °F	Negative	Complains of fever and headache	Volunteer returned for admission on his own voliation
21st day	102 °F	Morning: negative; afternoon: trypanosomes present	Febrile and quite ill	Mice subinoculated in the morning
22nd day	103 °F	–	Still ill	I.M. Phenergan 50 mg and
23rd day	98 °F	Negative	Feels much improved	I.V. Antrypol 0.25 g given I.V. Antrypol 1 g given
24th to 40th day	98 °F	Negative	Good	Complete course of I.V. Antrypol given
41st day	98 °F	Negative	Good	C.S.F.: 3 cells/cm^3; 19 mg % protein; no trypanosomes
43rd day		Volunteer discharged home		

Mice subinoculations: (a) Mice subinoculated on the 9th day after the inoculation of the volunteer showed parasitaemia after a prepatent period of 32 days. (b) Mice subinoculated on 21st day were positive for trypanosomes after a prepatent period of 20 days.

N.B.: I.M. = intramuscular, I.V. = intravenous, C.S.F. = cerebro-spinal fluid.

80,000 (WILLET, 1956). The number of trypanosomes in each of the inocula used for the inoculation of volunteers in the experiments described in this paper is very much greater than this minimum. The identity of trypanosome strains in the inocula that failed to infect the

Table 2. The results of the inoculation of volunteer V (E.A.T.R.O. 1825)

Day after inoculation	Temperature	Blood film examination	Clinical state	Other remarks
7th day	101 °F	Negative	Febrile with constitutional disturbances. Primary chancre definite. Axillary glands enlarged and tender	Chancre 1 cm diameter, painless and not tender. Mice subinoculated
8th day	103 °F	Morning: trypanosomes positive; afternoon: parasitaemia increasing	Very ill	Tepid sponging
9th day	104 °F	Positive for tryps	Still very ill	Tepid sponging continued. I.M. Phenergan 50 mg, I.V. Antrypol 1 g, E.S.R. 26 mm/1 h
10th day	99 °F	Negative	Improving, chancre subsiding. Urine clear	I.V. Antrypol 1 g
11th to 36th day	Normal	Negative	Good	Complete course of I.V. Antrypol
37th day	Normal	–	Good	C.S.F. no cells, 15 mg % protein, no tryps. E.S.R.: 13 mm/1 h
38th day		Volunteer discharged home		

Mice subinoculation: Mice subinoculated on 7th day after inoculation of volunteer were found parasitaemic after a prepatent period of 7 days.

N.B.: I.V. = intravenous, I.M. = intramuscular, C.S.F. = cerebro-spinal fluid, E.S.R. = erythrocyte sedimentation rate.

three volunteers may therefore be regarded as *T.(T) brucei.* These are E.A.T.R.O. 1811, 1822, and 1857, which had given equivocal results with BIIT.

In the grading of the results obtainable by the recently introduced BIIT which is supposed to deferentiate *T.(T.) rhodesiense* from *T.(T.) brucei* three categories are recognized: positive, negative, and equivocal (RICKMAN, 1971). The trypanosome strains in the positive category give consistently positive results in 3 consecutive tests and

are thought to be *T.(T.) rhodesiense*. In the negative category the test strains give consistently negative results and are considered *T.(T.) brucei*. Trypanosome strains which give "equivocal" results during the BIIT are positive in one or two of the tests and negative in the others. They are called "intermediate" strains. The reason for the behaviour of the "intermediate" strains is thought to be due to mixed infections of *T.(T.) brucei* and *T.(T.) rhodesiense* in any one mammalian host or tsetse fly. It is likely that some game and also cattle in Serengeti and Ikoma, respectively, may have had mixed infections with the two organisms. The occurrence of such mixed infections in nature is indeed very probable and may be quite widespread.

However, the results of the present work suggest that the BIIT must be more standardized than before and carried out over more blood passages in laboratory animals. When ever possible, more than one stabilate per strain should be tested: two derivatives of the hartebeest strain (E.A.T.R.O. 1810 and 1873), frozen on different occasions from two different mice inoculated simultaneously in the field, gave different results with the BIIT. E.A.T.R.O. 1810 behaved equivocal (cf. GEIGY et al., 1971) whereas E.A.T.R.O. 1873 gave quite consistently positive results, from 16 tests carried out over mouse-passage 2 to 9, only two were negative, one each in passage 3 and 8. E.A.T.R.O. 1873 was inoculated into man and proved to be *T.(T.) rhodesiense*.

The trypanosome isolated from the hartebeest though eventually caused in man an acute infection which could be easily diagnosed by ordinary methods in any good laboratory reminds us, by its long incubation period in the volunteer and also its long prepatent period in subinoculated mice, that some strains of *T.(T.) rhodesiense* do not always show their characteristic virulence. In contrast, the trypanosome strain isolated from a cow during the same period and in the same ecological zone caused *T. rhodesiense* characteristic infection after an incubation period of only 8 days in man and a prepatent period of 7 days in mice.

These two successful isolations of *T.(T.) rhodesiense* from a hartebeest and from a cow in the Serengeti National Park and Ikoma lead us to the conclusion that in this old focus of Rhodesian sleeping sickness, transmission of the parasite is still taking place and that some species of game and cattle act as the principal animal reservoir hosts. Since game and tsetse share the same ecosystem, particular game species must be regarded as the natural reservoirs and cattle as incidental one.

The eradication of the disease in this particular ecological setup is not a feasible proposition for very obvious reasons which we need not go into here. The control, on the other hand, is a real possibility. What seems to us as a reasonable approach in the control of the disease in

this locality is intensive health education aimed at making both the resident populations and the visitors aware of the existing hazard. The people should take adequate protective measures against tsetse and report to competent health authorities any febrile attacks which may occur during or after a visit to the area. To rid cattle of *T. rhodesiense* which is non-pathogenic to them, however, it may be preferred to adopt block-treatment of herds of cattle as compared to treatment of individual cases in a herd which are suspected of infection, or which have been positively diagnosed.

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Zusammenfassung

Bei Felduntersuchungen über die Epidemiologie der ostafrikanischen Schlafkrankheit im Serengeti-Ikoma-Gebiet (Tanzania) im Jahre 1970 konnten 10 Brucei-Stämme aus Rindern und 12 aus Wildtieren isoliert werden. Einer der Rinder- und 4 der Wildtier-Stämme, welche sich bei Laboratoriums-Untersuchungen als *T. rhodesiense*-artig erwiesen, wurden in Tanzania auf afrikanischen Volontären ausgetestet. Dabei erwiesen sich der Rinderstamm sowie ein Kuhantilopen-Stamm als menschenpathogen. Es gelang hier also der sichere Nachweis, daß neben dem Buschbock (*Tragelaphus scriptus,* untersucht von HEISCH et al., 1958) auch die Kuhantilopenart *Alcelaphus buselaphus T. rhodesiense* beherbergen und als Reservoir für menschliche Schlafkrankheit in Frage kommen kann. Material, Technik und klinische Befunde werden ausführlich beschrieben.

Résumé

Une enquête sur l'épidémiologie de la maladie du sommeil Est-africaine dans la région de Serengeti–Ikoma (Tanzania) a permis, en 1970, d'isoler des souches de trypanosomes du groupe Brucei; 10 à partir du bétail et 12 à partir de mammifères sauvages. Une des souches du bétail et quatre de celles des animaux sauvages ont été testées sur des volontaires africains après que des tests de laboratoire aient montré qu'elles avaient les mêmes caractéristiques que *T. rhodesiense.* Il est apparu que la souche du bétail et qu'une des souches provenant des Bubales étaient pathogènes pour l'homme. Comme cela a déjà été démontré par HEISCH et coll. (1958) pour le Guib hornaché il semble que le Bubale *(Alcelaphus buselaphus)* héberge *T. rhodesiense* et puisse jouer le rôle de réservoir dans la trypanosomiase humaine. Matériels, techniques et résultats cliniques sont décrits en détails dans le texte.