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# The composition of the *Trypanosoma brucei* subgroup in nonhuman reservoirs in the Lambwe Valley, Kenya, with particular reference to the distribution of *T. rhodesiense**

J. ROBSON,[1] L. R. RICKMAN,[2] R. ALLSOPP,[3] & D. SCOTT[4]

*Identification by means of the blood incubation infectivity test (BIIT) of 159* Trypanosoma brucei *subgroup strains recently isolated from non-human hosts in the Lambwe Valley, Kenya, has defined the distribution in these hosts of both* T. brucei *and* T. rhodesiense *in an endemic sleeping sickness area. The presence of a small third group strongly suggestive of a population intermediate between these two species has also been revealed for the first time.*

*Repeated testing of a number of these strains has shown marked consistency in the results. Strains identified by the BIIT as* T. rhodesiense *have been isolated for the first time from a reedbuck and a sheep. There appears to be a direct relationship between the local prevalence rates of* T. rhodesiense *in non-human reservoirs and the incidence of sleeping sickness in man.*

Extensive surveys for human and animal trypanosomiasis have recently been undertaken in the Lambwe Valley, Kenya, as part of the field programme of the WHO/UNDP(SF) Trypanosomiasis Research Project. The prevalence of sleeping sickness (Rhodesian form) was very low in this endemic area. By contrast, the overall incidence of trypanosomiasis in livestock, as established by blood and lymph node examination and mouse inoculation, was 17% in cattle and 2.6% in sheep and goats.

The blood incubation infectivity test (BIIT) developed by Rickman & Robson (1970a, 1970b) was used to differentiate all strains of the *Trypanosoma brucei* subgroup isolated from domestic livestock during the surveys, as well as isolates from game and tsetse flies obtained simultaneously in the same area. The application of the BIIT made it possible for the first time not only to define the distribution pattern of *T. rhodesiense* in its non-human reservoirs in relation to a particular locality in a sleeping sickness endemic area, but also to identify elements of this reservoir not previously recognized. In conjunction with human trypanosomiasis surveillance, which was carried out concurrently, the test gave a new and more complete perspective of the natural distribution of the infection.

In the course of this study it was discovered that in addition to identifying strains of trypanosomes as *T. brucei*, or less frequently as *T. rhodesiense*, the BIIT defined a third group in which the strain responded in an unusual, but characteristic and regular manner, strongly suggesting the existence of a distinct population intermediate between *T. brucei* and *T. rhodesiense*.

## MATERIALS AND METHODS

In addition to tsetse (*Glossina pallidipes* Aust.), the following animals were studied for trypanosome infections:

| | |
|---|---|
| Cattle | East African zebu |
| Sheep | local East African variety |
| Goats | local East African variety |
| Dogs | 1 boxer, 1 mongrel (both sick) |

* From the WHO/UNDP(SF) Trypanosomiasis Project, Lambwe Valley Field Station, South Nyanza, Kenya.

[1] FAO Veterinary Investigation Officer.

[2] WHO Technical Officer, Epidemiology.

[3] FAO Game Ecologist.

[4] WHO Project Manager.

Waterbuck (*Kobus defassa* Neumann)
Reedbuck (*Redunca redunca* Pallas)
Bushbuck (*Tragelaphus scriptus* Pallas)

Other game:

Oribi (*Ourebia ourebi* Zimmerman)
Impala (*Aepyceros malampus* Lichtenstein)
Topi (*Damaliscus rerrigum* Ogilby)
Duiker (*Sylvicapra grimmia* L.)
Bushpig (*Potamochoerus porcus* L.)
Hyaena (*Crocuta crocuta* Erxleben)
Roan (*Hippotragus equinus* Desmarest)

*Methods of isolation*

Strains of the *T. brucei* subgroup from domestic animals or game were isolated by inoculating susceptible white rats or mice with jugular blood. When game animals had been shot and the veins were collapsed, cardiac blood was used. Infected salivary glands of wild-caught *Glossina pallidipes* were macerated in saline and inoculated intraperitoneally into mice.

Trypanosomes for the first BIIT of a strain were taken from a rat or mouse that had been inoculated directly from the field reservoir. When strains were re-tested the control rat from the previous test was sacrificed to provide the sample. In 11 instances re-tests were made on mice infected with stabilates.

## THE BLOOD INCUBATION INFECTIVITY TEST

In order that the outcome of these investigations may be clearly understood, it is necessary that the different responses given by the strains when tested by the BIIT should be described and the terms used in expressing the results defined.

It was a requirement of all tests that a persisting and increasing parasitaemia should develop in the control rat within a prepatent period of 12 days. Only 2 tests out of some 250 were rejected for failing to meet this criterion.

*Single test*

All individual tests gave one of three responses, which were as follows.

*Positive result* (BIIT-positive). The test rat developed a persisting and increasing parasitaemia within a prepatent period not exceeding 12 days.

*Negative result* (BIIT-negative). The test rat remained negative for at least 40 days.

*Late parasitaemia* (BIIT/LP). The test rat, which had previously been negative, developed parasitaemia between 15 and 19 days after inoculation. Two distinct subgroups of this phenomenon were observed repeatedly:

(1) *Transient late parasitaemia* (BIIT/TLP) where scanty trypanosomes were found on the 15th or 16th day. This lasted for only a few hours; the animal then became, and remained, negative.

(2) *Persistent late parasitaemia* (BIIT/PLP) where parasitaemia developed between the 15th and 19th day after inoculation, and thereafter it increased and persisted.

*Serial testing*

Three distinct patterns of response were observed when strains were tested repeatedly. Each strain tested on more than one occasion conformed precisely to one of these patterns.

(1) All strains that were BIIT-positive on the initial test were consistently positive throughout further tests and were identified as *T. rhodesiense*.

(2) All strains that were BIIT-negative on the first test were consistently negative on further tests and were identified as *T. brucei*.

(3) Strains showing late parasitaemia on the first test: when this was transient (BIIT/TLP) all re-tests were BIIT-negative; when it was persistent (BIIT/PLP) the first re-test was BIIT-negative in all instances except one, and subsequent tests gave the same results. One strain that responded to the BIIT initially as a BIIT/PLP gave a positive result in each of the three re-tests. All strains giving BIIT/LP results were identified as " intermediate ".

## RESULTS

Altogether, 159 strains of the *T. brucei* subgroup were isolated from animals and tsetse in selected areas of the Lambwe Valley and tested by the BIIT. Extensive re-testing had to be limited to a small number of strains since the work was undertaken in a field laboratory with only 9 months remaining before the end of the project.

*Identification of the strains*

The results of the initial tests of the 159 strains are shown in the following tabulation:

| *Identified as:* | *Number* | *Percentage of total* |
|---|---|---|
| *T. brucei* (BIIT-negative) | 129 | 81.1 |
| *T. rhodesiense* (BIIT-positive) | 17 | 10.7 |
| Intermediate group (BIIT/TLP 9 + BIIT/PLP 4) | 13 | 8.2 |
| | 159 | 100.0 |

Table 1. BIIT results obtained from initial testing of 159 fresh isolates of the *T. brucei* subgroup from nonhuman hosts in the Lambwe Valley, the identification of *T. brucei, T. rhodesiense,* and the intermediate group composing the *T. brucei* subgroup, and the results of re-testing a sample of the strains

| Identified by the initial response to BIIT as: | No. of strains tested | BIIT results | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | First response [a] | Re-test responses | | | | | | |
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| *T. brucei* | 1 | — | — | — | — | — | — | — | |
| | 1 | — | — | — | — | — | — | | |
| | 1 | — | — | — | — | — | | | |
| | 1 | — | — | — | — | | | | |
| | 17 | — | — | | | | | | |
| | 108 | — | | | | | | | |
| Intermediate group | 3 | TLP | — | | | | | | |
| | 6 | TLP | | | | | | | |
| | 2 | PLP | — | — | — | — | — | | |
| | 1 | PLP | — | | | | | | |
| | 1 | PLP | + | + | + | | | | |
| *T. rhodesiense* | 7 | + | | | | | | | |
| | 2 | + | + | | | | | | |
| | 1 | + | + | + | | | | | |
| | 1 | + | + | + | + | | | | |
| | 1 | + | + | + | + | + | | | |
| | 4 [b] | + | + | + | + | + | + | | |
| | 1 | + | + | + | + | + | + | + | + |

[a] TLP = transient late parasitaemia; PLP = persistent late parasitaemia.
[b] Including EATRO 1506.

The results obtained by repeated testing of selected strains were as follows:

(1) A total of 21 strains identified as *T. brucei* by the first BIIT were re-tested a total of 36 times. All gave negative results. (2) A total of 10 strains identified as *T. rhodesiense* by the first BIIT were re-tested a total of 39 times. All gave positive results.[1] (3) A group of 7 strains showing late parasitaemia on the initial test were selected: (*a*) 3 transient LP were each re-tested once and all gave BIIT-negative results; (*b*) 4 persistent LP strains were re-tested and 3 were found to be negative in all 11 tests to which they were subjected, while the fourth gave a positive BIIT reaction in all three re-tests.

The results of testing and re-testing are presented in Table 1.

*Distribution of the components of the* T. brucei *subgroup among non-human reservoirs*

The distribution of the components of the 159 strains of the *T. brucei* subgroup—namely, *T. brucei*, *T. rhodesiense*, and the intermediate group—among non-human hosts in the areas investigated in the Lambwe Valley is shown in Table 2.

*T. rhodesiense* was identified in cattle, the incidence being 0.8% (14 isolations, one of which (EATRO 1506) subsequently infected a human volunteer). One

[1] One of these strains (Magunga 16/3, preserved as EATRO 1506) was later tested in a human volunteer by the East African Trypanosomiasis Research Organization, Tororo (Uganda). The volunteer developed parasitaemia.

Table 2. The distribution of *T. brucei, T. rhodesiense,* and the intermediate group among nonhuman hosts investigated in the Lambwe Valley

| Host | No. examined | No. of infections | | | |
|---|---|---|---|---|---|
| | | *T. brucei* | *T. rhodesiense* | Intermediate [a] | |
| | | | | TLP | PLP |
| cattle | 1 686 | 109 | 14 (0.8 %) [b] | 7 (0.4 %) | 3 (0.2 %) |
| sheep | 208 | 4 | 1 (0.5 %) | — | — |
| goats | 636 | 4 | — | — | 1 (0.2 %) |
| dogs | 2 [c] | 2 [c] | — | — | — |
| waterbuck | 3 | 1 | — | — | — |
| reedbuck | 37 | 3 | 1 (2.7 %) | — | — |
| bushbuck | 6 | 1 | — | — | — |
| other game | 34 | — | — | — | — |
| *G. pallidipes* | 1 833 | 5 | 1 (0.1 %) | 2 (0.1 %) | — |

[a] TLP = transient late parasitaemia; PLP = persistent late parasitaemia.
[b] Including EATRO 1506.
[c] Both sick.

infection was found in a sheep and another in a reedbuck. One strain isolated from a goat gave a persistent late parasitaemia response on the first BIIT; it was re-tested three times and gave positive results on all occasions.

The strains of the *T. brucei* subgroup isolated from domestic livestock were obtained during the course of surveys carried out between April and October 1970 in three small localities of the Lambwe Valley (Otuok, Magunga/Wiga, and Obaluanda). In cattle, the prevalence of *T. rhodesiense* was 2.8%, 0.6%, and 0.0%, respectively, in the three areas. The prevalence of the intermediate group was 1.7%, 0.7%, and 0.0%, respectively. In sheep and goats neither *T. rhodesiense* nor the intermediate group was found in Magunga/Wiga (examinations of these animals were not made in Obaluanda). In Otuok the prevalences were 3.0% *T. rhodesiense* in sheep and 1.2% intermediate group in goats.

During the same period, 4 cases of sleeping sickness were diagnosed, 2 from Otuok, and 2 from Magunga/Wiga; with populations of 396 and 2 220, respectively, the prevalences of the disease during these 7 months were 0.5% and 0.1%. In Obaluanda, only 1 case has occurred within the last 3 years (March 1969) in a population of over 1 000. These data are presented in Table 3.

## CONCLUSIONS

Against the background of consistent BIIT results obtained with known strains of *T. brucei* and *T. rhodesiense* (Rickman & Robson, 1970b), the equally consistent response of the 159 isolates of the *T. brucei* subgroup derived directly from their field hosts leaves little doubt that strains initially BIIT-negative can be identified with certainty as *T. brucei*, and those initially BIIT-positive as *T. rhodesiense*. The remarkably constant behaviour of isolates under repeated testing is strong evidence that the BIIT is a valid method for differentiating these two species of trypanosome. Further support for this view comes from the outcome of the only test of one of the 159 strains of *T. brucei* subgroup isolated from the Lambwe Valley so far made in man. The strain selected by the Director of the East African Trypanosomiasis Research Organization was isolated from a domestic bovine in the Magunga area (EATRO 1506) and tested 6 times by the BIIT; the result was positive on all occasions. In the human volunteer the infection produced by the strain confirmed the BIIT identification of *T. rhodesiense*.

Three new facts in the epidemiology of Rhodesian sleeping sickness have been established by these first studies to exploit the recently developed BIIT. In

Table 3. The survey point prevalence of *T. brucei, T. rhodesiense,* and the intermediate group in nonhuman hosts, and the 7-month period prevalence of sleeping sickness in three localities of the Lambwe Valley

| Host | Percentage [a] prevalence | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Otuok | | | | Magunga/Wiga | | | | Obaluanda | | | |
| | Tb | Tr | I | Population | Tb | Tr | I | Population | Tb | Tr | I | Population |
| cattle | 23.7 | 2.8 | 1.7 | 177 | 3.2 | 0.6 | 0.7 | 1 019 | 2.4 | 0.0 | 0.0 | 408 |
| sheep | 3.0 | 3.0 | 0.0 | 33 | 0.7 | 0.0 | 0.0 | 152 | not examined | | | — |
| goats | 3.5 | 0.0 | 1.2 | 86 | 0.0 | 0.0 | 0.0 | 41 | not examined | | | — |
| *G. pallidipes* | 0.4 | 0.1 | 0.2 | 1 000 | not examined | | | — | not examined | | | — |
| man | — | 0.5 | — | 396 | — | 0.1 | — | 2 220 | — | 0.0 | — | 1 111 |

[a] Tb = *T. brucei,* Tr = *T. rhodesiense,* and I = Intermediate group.

addition to identifying the two components of the *T. brucei* subgroup, which were previously known to exist among non-human reservoirs in an endemic area of Rhodesian sleeping sickness, the BIIT has demonstrated the existence of a third component in the Lambwe Valley. At one end of the range of the *T. brucei* subgroup lies the larger element, *T. brucei*; at the other is the less numerous *T. rhodesiense*. The BIIT responses given by the newly observed group show a range in the capabilities of the component strains to infect rats after incubation in human blood. These vary from feeble in strains showing only late and transient parasitaemia in the initial tests and negative results on re-testing, to strongest in those that show a late but persisting parasitaemia initially and that are positive on re-testing.

This evident grading in the nature of the response can best be explained by the presence of a third group of strains lying between, and connecting, the two recognized species. Those strains defined by the test as TLP are closest to *T. brucei* and, perhaps reflecting the proportion of that species within the subgroup, form 70% of the intermediate community. The element most closely associated with *T. rhodesiense*, i.e., those strains with a PLP response on the first test and positive on re-test, contribute only 7%. It is significant that the BIIT/LP response, which serves to identify the intermediate group, has been given only in initial tests and never in re-tests.

In addition to the strains obtained from cattle and tsetse, two others, identified as *T. rhodesiense*, were isolated from a sheep and a reedbuck. This shows, for the first time, that these animals serve as reservoirs of the human infection. Of the 13 intermediate strains, the one closest to *T. rhodesiense* in its BIIT response (see Tables 1 and 2) was isolated from a goat, suggesting that these animals might also be a source of humain infection.

In Table 3, a direct relationship is apparent between the prevalence of *T. rhodesiense* in domestic livestock and the occurrence of cases of sleeping sickness in the same area. In the two localities, Otuok and Magunga/Wiga, where active transmission to man took place at a prevalence of 0.5% and 0.1% respectively over a period of 7 months, the point prevalences of *T. rhodesiense* in cattle were 2.8% and 0.6% respectively while the respective figures in sheep were 3.0% and 0.0%.

Two patterns are observed in the distribution of the various prevalence rates of *T. brucei* subgroup infections among the three species of domestic livestock, and in the three areas investigated. The incidence rates were highest in Otuok, lower in Magunga/Wiga, and least in Obaluanda; in this distribution they reflected the local degree of contact with *G. pallidipes*. The second observation is that in a particular area the incidence of infection was higher in cattle than in sheep and goats; this is accounted for by the host preferences of the tsetse which is much less attracted to sheep and goats than to cattle. From the limited data available, it appears that the prevalences of *T. brucei* subgroup infections among the different hosts of the tsetse are dependent only on the local opportunities for transmission, and that over a fairly large area the composition of the subgroup, i.e., the proportions of its three components, *T. brucei*, *T. rhodesiense*, and the intermediate group, is similar in all hosts. The findings in one host species indicate the composition of the subgroup in the local environment as a whole.

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## RÉSUMÉ

### COMPOSITION DU SOUS-GROUPE *TRYPANOSOMA BRUCEI* CHEZ DES HÔTES NON HUMAINS DANS LA VALLÉE DE LA LAMBWE (KENYA) ET, EN PARTICULIER, RÉPARTITION DE *T. RHODESIENSE*

Les auteurs ont tiré parti de l'épreuve d'infectiosité après incubation en présence de sang humain, qu'ils ont récemment mise au point, pour différencier 159 souches du sous-groupe *Trypanosoma brucei* isolées au Kenya chez des hôtes animaux, sauvages ou domestiques, et chez des glossines. Ils définissent les termes utilisés pour exprimer les résultats obtenus.

Parmi les souches isolées, on comptait 81,1% de *T. brucei*, 10,7% de *T. rhodesiense* et 8,2% de souches donnant à l'épreuve d'infectiosité une réponse « intermédiaire ». Sur les 13 souches « intermédiaires », 9 ont provoqué une parasitémie tardive et passagère chez le rat; 3 d'entre elles ont été soumises à de nouveaux tests avec des résultats négatifs. Les 4 autres souches ont entraîné chez le rat une parasitémie tardive persistante; de nouvelles épreuves ont donné des résultats négatifs avec 3 d'entre elles et positifs avec la dernière.

Deux souches de *T. rhodesiense* identifiées par l'épreuve d'infectiosité ont été isolées pour la 1re fois chez une antilope et un mouton; 14 ont été isolées chez le bétail (incidence: 0,8%), y compris la souche EATRO 1506 qui a servi ultérieurement à infecter un volontaire; 1 souche a été isolée chez *Glossina pallidipes*.

L'identification chez une chèvre d'une souche « intermédiaire » donnant en épreuve d'infectiosité une réponse proche de celle de *T. rhodesiense* donne à penser que cette espèce animale pourrait être l'un des réservoirs de l'infection humaine.

On a constaté dans trois villages de la vallée de la Lambwe une relation directe entre le taux d'infection du bétail par *T. rhodesiense* et la fréquence de la trypanosomiase humaine causée par ce parasite. Dans deux localités, la prévalence de la trypanosomiase humaine était de 0,5 et 0,1% et celle de la trypanosomiase animale de 2,8 et 0,6% (bovins) et de 3,0 et 0,0% (moutons). Dans la dernière localité, on n'a décelé aucun signe de transmission active de l'infection chez l'homme et aucune souche de *T. rhodesiense* n'a été trouvée chez les animaux domestiques.

La remarquable régularité des résultats fournis par les divers isolats du sous-groupe *T. brucei* lors de l'épreuve d'infectiosité démontre la fiabilité de la méthode en tant que moyen de différencier *T. brucei* et *T. rhodesiense*.

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