Identification by the blood incubation infectivity test of "Trypanosoma brucei" subspecies isolated from game animals in the Luangwa Valley, Zambia

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Identification by the blood incubation infectivity test of *Trypanosoma brucei* subspecies isolated from game animals in the Luangwa Valley, Zambia

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Summary

A total of 7 stocks of *Trypanosoma brucei* subspecies, isolated from naturally infected game animals in the Luangwa Valley, Eastern Province, Zambia were examined using a modified version of the Blood Incubation Infectivity Test (BIIT). One stock giving consistent BIIT responses typical of *T. b. rhodesiense*, was obtained from warthog (*Phacochoerus aethiopicus*). Four other stocks, 2 from hyaena (*Crocuta crocuta*), 1 from a waterbuck (*Kobus ellipsiprymnus*) and 1 from a lion (*Panthera leo*) responded like *T. b. brucei*. One stock from a waterbuck and 1 from a giraffe (*Giraffa camelopardalis*) failed to infect mice after incubation in human serum for 30 min at 37° C when first tested, but after 5 or 6 further serial passages in mice and even with serum incubation time increased to 5 h, they retained infectivity.

Key words: Trypanosoma brucei subspecies; blood incubation infectivity test; game animals.

Introduction

The identification of *T. b. rhodesiense* in a bushbuck (*Tragelaphus scriptus*) by Heisch et al. (1958) and later in cattle by Onyango et al. (1966) provided the first evidence of the significance of game and domestic animals as reservoirs of trypanosomes pathogenic for man.

However, the lack of a suitable laboratory test with which to differentiate human-infective trypanosomes among the morphologically identical members of the *T. brucei* complex, when these were isolated from a non-human host, has precluded both quantitative and qualitative assessment of the game reservoirs

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Table 1. BIIT responses of 7 stocks of *Trypanosoma brucei* subspecies, isolated from naturally infected game animals in the Luangwa Valley, Zambia, after incubation in human serum in vitro at 37° C for 30 min or 5 h

			BIIT													
			Passages before testing	30 min incubation						5 h incubation						
				Sample A (Test)				Sample B (Control)				Sample C (Test)				Sample D Control)			
Stock number	Date of isolation	Host		Mouse number 1	2	3	Mean PP	Mouse number 1	2	3	Mean PP	Mouse number 1	2	3	Mean PP	Mouse number 1	2	3	Mean PP
J 10	4. 11. 1973	Hyaena (Crocuta crocuta)	3	–	–	–	–	+	+	+	1.0	–	–	–	–	+	+	+	1.66
J 11	8. 11. 1973	Hyaena (Crocuta crocuta)	9	–	–	–	–	+	+	+	2.33	–	–	–	–	+	+	+	1.0
H 6	21. 8. 1974	Waterbuck (Kobus ellipsiprymnus)	6	–	–	–	–	+	+	+	4.66	–	–	–	–	+	+	+	5.6
H 3	13. 8. 1974	Lion (Panthera leo)	1	–	–	–	–	+	+	+	1.33	–	–	–	–	+	+	+	1.0
H 18	25. 11. 1974	Warthog (Phacochoerus aethiopicus)	1	+	+	+	8.33	+	+	+	6.0	+	+	+	6.0	+	+	+	1.0
H 1	21. 7. 1974	Waterbuck (Kobus ellipsiprymnus)	5	–	–	–	–	+	+	+	1.0	not tested							
H 1	21. 7. 1974	Waterbuck (Kobus ellipsiprymnus)	11	–	not tested							+	+	+	5.0	+	+	+	1.0
H 15	6. 10. 1974	Giraffe (Giraffa camelopardalis)	1	–	–	–	–	+	+	+	1.33	not tested							
H 15	6. 10. 1974	Giraffe (Giraffa camelopardalis)	6	not tested								+	+	+	3.66	+	+	+	2.0

– = indicates mouse did not become parasitaemic within 40 days
\+ = indicates mouse developed parasitaemia within 40 days
PP = prepatent period in days

of *T. b. rhodesiense* in East and Central Africa. The discovery and development of the blood incubation infectivity test (BIIT) by Rickman and Robson (1970a, b) provided such a test which has led to the study of several *T. brucei* sub-group stocks isolated from game, domestic stock, human patients and tsetse flies (Awan 1971; Dillmann and Awan, 1972; Robson et. al. 1972; Awan and Dillmann, 1973; Robson and Rickman, 1973; Targett and Wilson, 1973; Geigy et al., 1975).

In this paper we report further investigations of the game animal reservoirs of *T. b. rhodesiense* and *T. b. brucei* and describe the BIIT responses given by 7 stocks isolated from naturally infected game animals in the Luangwa Valley, Zambia.

Materials and methods

Trypanosome stocks. All the stocks originated from an area on the eastern bank of the Luangwa River, between Milyoti Gate and Nyamaluma Pontoon (i.e. between latitudes 13–13°30′ S and between longitudes 31° 30′–32° E) at an altitude of 600–800 metres; they were isolated in mice and preserved in liquid nitrogen using 7.5% glycerine as cryoprotectant.

Blood Incubation Infectivity Test (BIIT). A stabilate of the stock to be tested was brought to ambient temperature and inoculated intraperitoneally to 3 mice. Cardiac blood from a single donor mouse provided all the samples for any one test.

Identification of the test stock as *T. brucei* subspecies was confirmed before testing by morphological examination of a Giemsa stained, thin tail-blood film. Each stock was tested by incubating 2 samples (A and B) for ½ h and 2 samples (C and D) for 5 h at 37° C (Awan and Dillmann, 1973). Only the "test" samples were incubated in the human serum, while the "control" samples were incubated in the absence of human serum. Samples were examined by wet-film microscopy before and after incubation at 37° C, and three mice were then inoculated with aliquots of each sample. Post-inoculation microscopy of recipient animals, by wet-film examination of tail blood samples, was continued up to 40 days if aparasitaemic.

Results

Wet-film examination of samples. No difference could be detected in trypanosome motility in samples examined before and after incubation at 37° C, in the absence of human serum. By contrast, in most test samples of BIIT positive stocks, after incubation in serum, trypanosome number and motility were both markedly reduced. This adverse effect of human serum in vitro was reflected in a significant prolongation of the prepatent periods in the recipient animals (Table 1).

BIIT responses (Table 1). Of the 7 stocks tested, 4 (i.e. J10, J11 from hyaena, H6 and H3 from waterbuck and lion, respectively) were uninfective to mice after serum incubation of 30 min or 5 h. One stock, H18 (warthog) retained infectivity in each of the 2 tests, i.e. with incubation of 30 min or 5 h, although one of the 3 test mice inoculated with the sample of stock H18 incubated for 5 h remained negative up to day 38.

The remaining 2 stocks, H1 (waterbuck) and H15 (giraffe), were uninfec-

tive after 30 min incubation, but both retained infectivity when tested after 6 and 5 further passages (respectively) in mice, although the incubation time was increased to 5 h.

Discussion

Because of its consistent infectivity it is likely that the stock from warthog was *T. b. rhodesiense.* This is the first time that this parasite has been identified in these animals. In a preliminary study also the warthog stock retained its infectivity (Dillmann and Townsend, 1976). Similarly, the 4 stocks consistently loosing infectivity were almost certainly *T. b. brucei.* The loss of infectivity also seems to confirm the earlier findings of Awan (1971), that incubating *T. b. brucei* trypanosomes in human serum in vitro at 37° C for 30 min is sufficient to inhibit the normal infectivity of these organisms for small laboratory rodents.

The inconsistency in the results of stocks H1 and H15 seems not to be due to the duration of incubation of the samples in human serum. It may be due to the possible presence of mixed populations of *T. b. rhodesiense* and *T. b. brucei* in newly isolated uncloned stocks from naturally infected game animals (Geigy et al., 1975). During the course of passage in mice the proportion of the two populations probably fluctuates and the resultant BIIT responses are dependent on the dominating population type in a donor mouse.

In our experiments there appears to be some in vitro activity of human serum on *T. b. rhodesiense* stocks, as evidenced by prolongation of the subsequent prepatent periods.

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