Survey of cattle for the evidence of "T. rhodesiense" infections

Sleeping Sickness Survey in Musoma District, Tanzania

III. Survey of Cattle for the Evidence of *T. rhodesiense* Infections

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Abstract

During a sleeping sickness survey of the Musoma District, particularly the Serengeti National Park and its environs, 798 head of cattle in the Ikoma area (just outside the park) were examined for evidence of *T. rhodesiense* infections. Four of the isolations of *T. brucei* subgroup organisms gave an equivocal result with the BIIT.

The results have revealed a 3.5% infection in cattle with *T. brucei* subgroup organisms, a group which includes *T. rhodesiense*. The BIIT for differentiating *T. brucei* (sensu stricto) from *T. rhodesiense* has, however, shown majority of the isolates to be *T. brucei* with others giving inconclusive BIIT results.

Introduction

The area where the examination of cattle was carried out lies immediately outside and north of the western extension of the Serengeti National Park (see map in Fig. 1, part I). This area is inhabited by Waikoma people who keep only the East African short-horned zebu cattle. These cattle are grazed on communal basis, and modern methods of livestock husbandry are not practised. The cattle while grazing come into frequent contact with tsetse flies and game animals from the Serengeti National Park and Game Reserve. Other times they are in close contact with the people in the homesteads (Bomas) where they are tethered for the nights and part of the mornings.

Previous records of cattle trypanosomiasis in Ikoma are difficult to trace and the infecting *Trypanosoma* species are not clearly known. The Tanzania Veterinary Department, however, is aware of the existence of the disease and mention is made in the departmental Annual Reports of treatment of cattle against trypanosomiasis.

Material and Methods

Blood samples

All cattle examined were resident and bred in the area. It had been arranged to collect blood samples from the animals at a public crush, but the latter was found unsuitable for the work and so sampling was performed at the cattle owners' homesteads.

Each of 798 cattle presented was bled from a vein in the ear and thick and thin blood films were made. Drops of blood were also collected from 765 cattle and absorbed on Whatman No. 4 filter paper.

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Among the herd 260 cattle randomly selected were aseptically bled from the jugular vein for immediate, intraperitoneal inoculation into mice. Two Swiss white mice were inoculated with blood from each animal.

The thick and thin blood films were allowed to dry and then carried back to the laboratory in slide boxes. Blood samples on filter paper were dried in the sun, after which they were bundled together, wrapped in envelopes and preserved in a deep freeze (–20°C).

Examination of the blood samples

Thick and thin blood films were stained with Giemsa's stain. Stained thick blood films were examined under an oil immersion objective (×100) with ×8 oculars until parasites were found, or until at least 200 microscope fields had been scanned. Where parasites were present, the respective thin blood films were examined for the identification of the trypanosome species.

The dried blood samples on filter paper were processed and examined as outlined in previous work by the indirect fluorescent antibody test (IFT) (CUNNINGHAM et al. 1967). Three antigens (i.e. *T. brucei, T. vivax* and *T. congolense*) were used.

Wet films made from the tails of the inoculated mice were examined on every working day starting on the fourth day after inoculation, for four weeks. Thereafter the mice which were aparasitaemic were examined once weekly up to 60 days after inoculation. Mice that were positive were left to attain good parasitaemias, then thin blood films were made, stained and examined for the identity of the infecting trypanosomes. Where the trypanosomes were found to be of the *T. brucei* subgroup, the mice were bled from the heart and the infected blood preserved in liquid nitrogen.

Blood Incubation Infectivity Test (BIIT)

Ten *T. brucei* subgroup stabilates isolated in mice and preserved in liquid nitrogen were tested to differentiate those that were *T. brucei* sensu stricto from those that were likely to be *T. rhodesiense* using BIIT (RICKMAN & ROBSON 1970). *T. brucei* and *T. rhodesiense* are morphologically indistinguishable members of the *T. brucei* subgroup and yet the former is non pathogenic to man. Stored human whole blood obtained from Tororo Hospital blood bank was used in the test. The experimental and control samples were incubated at 37°C for 5 hours before the inoculation of rats. All the white rats used in this test were mature. Apart from the initial test, two additional tests were performed for each trypanosome stabilate. BIIT negative and positive

controls (a man-tested *T. brucei,* E.A.T.R.O. 999, and two proven *T. rhodesiense,* one man-tested E.A.T.R.O. 1135 and the other isolated from man-E.A.T.R.O. 1084) were included during the test. The rats used in the test were examined daily for 40 days.

Results

A summary of the results of the tests is given in tables 6 and 7. Results in Table 6 show an overall infection in 28 cattle (3.5 %) with

Table 6. The number of cattle found infected with *T. brucei* subgroup organisms by thick blood films and mouse inoculation

Method of examination	No. of cattle examined	Positive results No.	Positive results % of 798
Thick films (a)	798	22 *	2.8
Mouse inoculation (b)	260	12 **	1.5
Combined (a) and (b)	798	28	3.5
IFT	765	251	31.5

* 5 of these were mixed (with *T. vivax*) infections.
** 2 of these were mixed (with *T. congolense*) infections.

Table 7. Results of the BIIT on the 10 isolated *T. brucei* subgroup organisms and on BIIT positive and negative controls

Stabilate No.	Results			Remarks
	Test	Retest 1	Retest 2	
E.A.T.R.O. 1801	0	0	0	BIIT negative
E.A.T.R.O. 1815	+	0	0	Equivocal result
E.A.T.R.O. 1817	0	0	0	BIIT negative
E.A.T.R.O. 1818	+	0	0	Equivocal result
E.A.T.R.O. 1820	0	0	0	BIIT negative
E.A.T.R.O. 1825	+	+	0	Equivocal result
E.A.T.R.O. 1828	0	0	0	BIIT negative
E.A.T.R.O. 1835	0	+	+	Equivocal result
E.A.T.R.O. 1843	0	0	0	BIIT negative
E.A.T.R.O. 1845	0	0	0	BIIT negative
E.A.T.R.O. 999 (Neg. control)	0	0	0	BIIT negative
E.A.T.R.O. 1135 (Pos. control)	+	+	+	BIIT positive
E.A.T.R.O. 1084 (Pos. control)	+	+	+	BIIT positive

T. brucei subgroup organisms, as diagnosed by the standard diagnostic techniques (SDT). The immunofluorescent test (IFT) which detects the common antibodies to any trypanosome infection, however, showed more positive cases. In addition to *T. brucei* subgroup infection *T. congolense* and *T. vivax* group infections were diagnosed by the SDT. The *T. vivax* and *T. congolense* infections will be discussed in a later publication.

Of the ten stabilates tested and retested (see Table 7), by the BIIT, six gave negative results. Of the remaining four, two (E.A.T.R.O. 1815 and 1818) were positive on initial test, but negative on the two retests. The positive result in each case was obtained in only one of the two rats used. The third (E.A.T.R.O. 1825) was positive on initial test and one retest but negative on a second retest. The positive result in this case was obtained in only one of the two rats. The fourth (E.A.T.R.O. 1835) was negative on initial test but positive on the two retests. Again as in the above cases, only one of the two rats used in each retest was parasitaemic.

The BIIT positive (E.A.T.R.O. 1084 & 1135) and negative (E.A.T.R.O. 999) control stabilates used gave the expected results, both rats in the case of the positive controls showing parasitaemia throughout the period they were alive.

Discussion

The results of the examination of blood samples obtained from cattle clearly indicate a high incidence of cattle trypanosomiasis in the Ikoma area. *T. brucei* subgroup, *T. congolense* and *T. vivax* groups are all involved. The results of mouse inoculation alone indicate that if this technique had been used in the examination of the entire 798 cattle, a higher number of *T. brucei* infections might have been diagnosed. Many of them could have given unequivocal results with the BIIT and therefore could have been supposed to be *T. rhodesiense*. Results of the investigation of sleeping sickness in the human population in the Ikoma area (Part I) and dissections of tsetse flies caught in areas contiguous to where cattle were examined (Moloo et al., Fig. 1, this paper, part II) revealed neither *T. rhodesiense* in particular nor *T. brucei* subgroup infections in general. The epidemiological significance of these results will be discussed in full in Part V in this issue.

Conclusion

Cattle in Ikoma, an area immediately bordering the National Park, were found to have a high infection rate of *T. brucei* subgroup,

organisms, and a few of these infections may have been *T. rhodesiense,* a pathogenic trypanosome to man. The four strains which gave inconclusive results by the BIIT need further retesting to ascertain their infectivity for man.

References

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