

ETHIDIUM BROMIDE-RESISTANT TRYPANOSOMES IN SOUTHERN DARFUR

BY

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Introduction

Cattle trypanosomiasis is widespread in southern Darfur area. Since 1953 Ethidium bromide (2, 4 diamine-10 ethyl phenanthridium bromide) has been used as a curative drug of cattle trypanosomiasis in the Sudan (Karib, 1961). Although cattle owners have complained occasionally that treatment with Ethidium bromide was ineffective, the fact that such animals may die because of prevalent concurrent bacterial, viral or parasitic infections, stress or merely starvation make these complaints questionable.

Abdel Razig et al (1968) gradually induced in a strain of *T. congolense* from Malakal District, resistance to as high as 4 mg/kg body weight Ethidium bromide by treatment & relapse method. Abdel Gadir et al (1972) isolated a strain of *T. congolense* resistant to 1.0 mg/kg body weight Ethidium bromide from natural cases in southern Darfur. No cross-resistance between Ethidium, Samorin or Berenil could be established at the recommended dosage level for use in the field.

This preliminary study was undertaken to search for resistant trypanosomes in nomadic cattle in southern Darfur.

Materials and methods

During a survey of bovine trypanosomiasis in southern Darfur (Abdalla et al 1977), blood from naturally infected cattle was collected at El Radom and Sungo (Lat 9°N). The blood was inoculated subcutaneously S/C into sheep or goats which were transported to Nyala Regional Laboratory (about 150 miles north). Infected blood from each of these animals was subinoculated intravenously (IV) into 2 experimental calves (18-24 months) and 2 goats (9-24 months). The calves and goats were purchased from Nyala (a tsetse free area) and were examined for freedom of trypanosomiasis for 2 weeks before use.

Exactly 24 hours after the inoculation of the blood samples, two experimental animals for each isolate (one calf and one goat) were treated with Ethidium

bromide 1.0 mg/kg. The other calf and goat inoculated with the same isolate were left to develop parasitaemia and when this became in the order of 5-10 trypanosomes per high power microscopic field in the wet smear, the animals were treated with the drug. All animals relapsing after drug treatment were given Berenil at the rate of 3.5 mg/kg body weight.

The parasitaemia was monitored daily by microscopic examination of wet and Giemsa-stained blood films obtained from ear veins of the experimental animals.

Results

Blood examination of the sheep and goats subinoculated from naturally infected cattle at El Radom and Sungo, revealed the presence of seven *T. congolense*, three *T. brucei* and two *T. vivax*. When these twelve strains were injected into experimental calves and goats, and followed by drug treatment, nine trypanosome strains relapsed (Table 1).

Out of the twelve calves, treated with Ethidium bromide exactly 24 hours after inoculation with the isolates, three showed trypanosomes in their peripheral blood at periods of 10, 15 and 20 days. All the three calves were carrying *T. congolense*. Of the corresponding twelve goats similarly treated as above, five relapsed at periods varying from 9-38 days. The species of trypanosomes identified in them were two *T. brucei*, two *T. vivax* and one *T. congolense*.

Of the twelve calves treated with the drug at patent parasitaemia, five relapsed in a period of 10 to 17 days. The species of trypanosomes in the five relapsed calves were two *T. congolense*, two *T. brucei* and one *T. vivax*. From the corresponding twelve goats treated similarly, three relapsed at days 7, 11 and 14 and all were carrying *T. congolense* infection.

All the relapsed cases responded to treatment with Berenil at the rate of 3.5 mg/kg body weight and remained negative for more than sixteen weeks.

Discussion

To obtain reliable indication from the field that drug resistance exists is difficult. The results of this preliminary experiment gave reasonable evidence that Ethidium bromide resistant strains of trypanosomes do exist in cattle in the tsetse infected areas of southern Darfur. Although, no definite conclusion can be drawn on the frequency with which such

strains occur, due to the small number of isolates, it can be assumed that Ethidium bromide resistance is not exceptional. Out of twelve strains involved in the experiment, nine trypanosomes resisted Ethidium bromide at the recommended dosage (1.0 mg/kg body weight). This resistance was expressed by all the three species affecting cattle in Sudan: *T. congolense*, *T. vivax* and *T. brucei*. The possibility that trypanosomes in the early stages of infection are more sensitive to drug action than when there is already a well developed parasitaemia as has been discussed by Davey (1950), urged us to treat the experimental calves and goats 24 hours after infection with the isolates. Here again, the parasites proved to be equally resistant. This was found to be in agreement with the work of Jones-Davies and Folkers (1966) who showed that early treatment did not destroy the trypanosomes (drug-resistant) but rather prolonged the prepatent period.

In the field, when treatment is carried out with trypanocidal drugs, there often exists the possibility for the development of drug fastness, as under dosage may result from inaccurate weight estimation, accidents at injection, improper preparation of solutions or from exposure to injection in cattle with waning blood levels of prophylactic and curative drugs. Considering the fact that Ethidium bromide is in use for more than 25 years now, then drug resistance becomes expected in Darfur area.

Berenil at the rate of 3.5 mg/kg body weight can overcome Ethidium resistance, a conclusion which is in agreement with previous work (Abdel Gadir et al 1972). Abdel Razig et al (1968) stated that Berenil is a "sanative drug" in this country and he recommended the use of Berenil to destroy Ethidium resistant strains.

The results obtained proved the existence of Ethidium bromide resistant strains of trypanosomes

in cattle in tsetse infected areas of southern Darfur, but the important question whether or not such strains are also present in the tsetse fly (*Glossina*) population remains unanswered.

Summary

Ethidium bromide has been used since 1953 in southern Darfur for the treatment of cattle trypanosomiasis. A survey was carried out to investigate the prevalence of strains of trypanosomes resistant to Ethidium bromide (1.0 mg/kg body weight, the dosage used in the field).

Blood samples were collected from cattle that were presented for treatment at El Radom and Sungo, areas lying within the tsetse infected areas of southern Darfur. A total of twelve isolates of trypanosomes (seven *T. congolense*, three *T. brucei* and two *T. vivax*) were involved in this experiment. From these isolates, nine could be identified as Ethidium bromide resistant, from both camps. These include five *T. congolense*, two *T. brucei* and two *T. vivax*.

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Table (1)
The results of the prevalence of Ethidium bromide-resistant strains of trypanosomes in cattle in southern Darfur

Strain No.	Trypanosome	Local ity	Relapses (day) in calves treated :		Relapses (days) in goats treated:	
			24 h.PI ¹	P.P. ²	24 h.PI ¹	P.P. ²
1	<i>T. congolense</i>	Rado m	20	—	—	14
2	"	"	—	17	—	—
3	"	"	—	—	—	—
4	"	Sungo	—	13	38	—
5	"	"	15	—	—	11
6	"	"	—	—	—	—
7	"	"	10	—	—	7
1	<i>T. brucei</i>	Rado m	—	10	32	—
2	"	Sungo	—	—	—	—
3	"	"	—	13	23	—
1	<i>T. vivax</i>	Sungo	—	11	9	—
2	"	"	—	—	16	—

¹ PI = post infection

² P.P. = patent parasitaemia