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Short Communication

MULTIPLE DRUG-RESISTANT BOVINE TRYPANOSOMES IN SOUTH DARFUR PROVINCE, SUDAN

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In an attempt to control bovine trypanosomosis in South Darfur Province, Sudan, it is planned to establish several trypanosomosis treatment stations along the northern limits of the Bahr El Arab fly belt (Hall *et al.*, 1984). These stations are to provide treatment of cattle with homidium bromide (Ethidium, CAMCO) and isometamidium chloride (Samorin, Rhône-Mérieux) during the dry season migration (November to June) in the tsetse belt. On the return of the herds (July to November) cattle will be treated at the stations for any residual infection with diminazene aceturate (Berenil, Hoechst) (Mohamed-Ahmed *et al.*, 1988).

Results obtained from a preliminary trial (Mohamed-Ahmed *et al.*, 1988) revealed that 5·9% of the treated cattle had remained infected in 1986. These findings suggested that resistance to the trypanocides employed might be a principal cause of persistent infections in cattle. Hence, experiments were conducted in the Regional Veterinary Research Laboratory, Nyala (see Hall *et al.*, 1983 for location) to find out if some trypanosome strains had developed resistance to homidium bromide, diminazene aceturate or isometamidium chloride.

Trypanosome strains were obtained from a herd of *c.* 2,000 nomadic cattle which is annually exposed to natural tsetse challenge in the Bahr El Arab fly belt from February to June. During the challenge period in 1986, approximately one half of the herd was regularly treated with isometamidium chloride at 0·5 mg/kg and the other half with homidium bromide at 1 mg/kg. The aim was to compare the curative and chemoprophylactic effect of these drugs under Sudan conditions (Mohamed-Ahmed *et al.*, 1988). On leaving the tsetse belt by July, cattle were mass-treated at Radom with diminazene aceturate at 3·5 mg/kg. Two months later when in the tsetse-free wet season grazing area at Domma, all cattle (*c.* 2,000) were examined for trypanosomosis using mainly the haematocrit centrifugation technique (HCT) (Woo, 1970; Paris *et al.*, 1982). In each case 5 ml of blood from each infected animal (with single or mixed infection) was injected into a goat. Out of the 15 positive animals, 7 single trypanosome infections (designated strains, Table I) were isolated and maintained by further passages in goats.

Later, 7 groups of 3 trypanosomosis-free male goats were used. Each group was infected with one trypanosome strain by intravenous injection of 5 ml of infective blood for each animal. When parasitaemia had reached one to 2 trypanosomes or more per microscopic field (× 400) in a monolayer wet film preparation, each of the infected animals was then treated with either one, 2 or 3 times the standard therapeutic field dose of 2·5% homidium bromide, 7% diminazene aceturate or 2% isometamidium chloride (Table I). Following treatment, animals were examined (Woo, 1970; Paris *et al.*, 1982) regularly at 3 hour intervals for 60 days to find: (a) the time required for initial clearance of parasitaemia from peripheral circulation, (b) relapse infections

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TABLE I

Time from treatment to clearance of seven trypanosome strains from peripheral circulation of goats following treatment with homidium bromide, diminazene aceturate and isometamidium chloride

	Time from treatment to clearance of trypanosomes from peripheral circulation (hours)								
	Homidium bromide (kg)			Diminazene aceturate (kg)			Isometamidium chloride (kg)		
Trypanosome strain	1 mg	2 mg	3 mg	3·5 mg	7 mg	10·5 mg	0·5 mg	1 mg	1·5 mg
T. congolense C989R	10	9	9	18	14	21	18	23	18
T. congolense B48	21	18	18	21	7	18	24	16	27
T. congolense B427	18	27	27	33	18	21	32	17	9
T. congolense B12	nc	nc	9	14	27	29	29	30	25
T. brucei BNVC	21	24	12	9[1]	12[1]	3[1]	16	14	14
T. brucei B51	39	–	–	–	15[1]	–	68	–	–
T. vivax B75D	67	–	–	–	–	–	–	–	–

[1] *T. brucei* infections were treated with diminazene aceturate at 7 mg/kg, 14 mg/kg and 21 mg/kg.
nc = Parasitaemia did not disappear before animals died.
– = Animals died before experiments were completed.

and the time between treatment and relapse and (c) permanent aparasitaemia (cure) within 60 days of treatment.

The time required for initial clearance of parasitaemia from peripheral circulation at each dosage rate of homidium bromide, diminazene aceturate or isometamidium chloride is shown in Table I. Homidium bromide at 1 or 2 mg/kg did not clear the parasitaemia of the B12 strain before infected goats died after 5 and 7 days of treatment, respectively. The *Trypanosoma brucei* infections were treated with diminazene aceturate at 7 mg, 14 mg and 21 mg/kg with subsequent initial clearance time for the BNVC strain after 9, 12 and 3 hours, respectively.

Following the initial clearance of parasitaemia (Table I) relapse infections were noted for all trypanosome strains treated with up to 3 times the recommended dose of homidium bromide or diminazene aceturate (Table II). Relapses due to *T. congolense* B427 and *T. brucei* B51 treated with homidium bromide at 1 mg/kg, and *T. congolense* B48 treated with diminazene aceturate at 3·5 mg/kg (Table II), occurred after a short period (2 days) of clearance of parasites and therefore might not have been real relapses. Furthermore, 4/6 (66·7%) and 1/5 (20%) of these trypanosome strains relapsed after treatment with isometamidium chloride at 0·5 mg or 1 mg/kg, respectively. The BNVC *T. brucei* strain relapsed within 25 and 15 days after treatment with diminazene aceturate at 7 mg and 14 mg/kg, respectively, but was permanently cured with the same drug at 21 mg/kg. Permanent cure was also achieved in 5/5 (100%), 4/5 (80%) and 2/6 (33·3%) of these strains after treatment with isometamidium chloride at 1·5 mg, 1·0 mg or 0·5 mg/kg, respectively (Table II).

However, a curative dose of isometamidium chloride assessed in goats may not be equally effective for cattle infected with the same trypanosome strains. For this reason, a random group of 544 cattle from the original herd of 2,000, some of which (13·3%) had been shown to harbour trypanosome strains resistant to at least the standard field doses of homidium bromide and diminazene aceturate, were mass-treated with isometamidium chloride at 1·5 mg/kg during the wet season in 1987 (Mohamed-Ahmed *et al.*, 1988). Treated cattle were examined for trypanosomosis

TABLE II

Time from treatment to relapse of infections in goats with 7 trypanosome strains following treatment with homidium bromide, diminazene aceturate and isometamidium chloride

	Time from treatment to relapse (days)								
	Homidium bromide (kg)			Diminazene aceturate (kg)			Isometamidium chloride (kg)		
Trypanosome strain	1 mg	2 mg	3 mg	3·5 mg	7 mg	10·5 mg	0·5 mg	1 mg	1·5 mg
T. congolense C989R	6	8	8	5	11	18	16	22	cured
T. congolense B48	10	10	8	2	5	9	cured	cured	cured
T. congolense B427	2	7	8	5	5	15	26	cured	cured
T. congolense B12	nc	nc	11	10	16	8	cured	cured	cured
T. brucei BNVC	6	25	31	25[1]	15[1]	cured[1]	20	cured	cured
T. brucei B51	2	–	–	–	7[1]	–	16	–	–
T. vivax B75D	15	–	–	–	–	–	–	–	–

[1] *T. brucei* infections were treated with diminazene aceturate at 7 mg/kg, 14 mg/kg and 21 mg/kg.
nc = Parasitaemia did not disappear before animals died.
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85 days later just before they entered the tsetse belt. None of the 544 treated animals was found infected, indicating the complete disappearance of the drug resistant trypanosomes.

The data presented have established the existence of some trypanosome strains resistant to the most commonly used trypanocides in Africa. These strains were controlled in cattle by a single high dose of isometamidium chloride. However, it is probable that such drug-fast strains are widespread in herds in South Darfur Province and neighbouring areas inside and outside the country. Should this be the case, the benefits anticipated from the proposed trypanosomosis treatment stations in South Darfur Province may not be fully achieved.

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