Effect of multiple treatment of cattle with diminazene aceturate on the infectivity and transmissibility of drug-resistant *Trypanosoma congolense* for *Glossina morsitans centralis*

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Summary

Six Boran cattle were infected with the drug-resistant *Trypanosoma congolense* IL 3338. At the first peak of parasitemia different groups of 200 teneral *Glossina morsitans centralis* were fed once on each animal, just prior to treatment with diminazene aceturate at a dose of 3.5 mg kg^{-1} body weight (b.w.). Thereafter, all animals were monitored three times a week and retreated with the same drug dosage whenever the packed red blood cell volume (PCV) declined in three consecutive samples from one or more of the animals. After eight treatments at approximately two-week intervals the mean duration when parasites were not detected after each treatment did not increase, but remained at 7.8 ± 1.1 days. The mean PCV declined from 33.2 ± 0.6% at the time of the first treatment to a mean inter-treatment value of 23.7 ± 2.6% between the eighth and ninth treatment. Therefore, subsequent to the eighth treatment diminazene aceturate was administered as before but at a dose of 7.0 mg kg^{-1} b.w. After treatment with this higher dosage the mean inter-treatment PCV increased from 25.4 ± 2.4% following the first treatment to 32.9 ± 1.7% for the two-month period following the fifth treatment. At least 14 days after treatments with diminazene aceturate at 3.5 mg kg^{-1} b.w., and 30 days after treatments at 7.0 mg kg^{-1} b.w., similar numbers of flies as used for the first feed were fed on one occasion on each animal. Thereafter, 10 flies with mature infections from each group were fed individually on mice to determine the transmissibility index. In general, the midgut and hypopharynx infection rates in flies of all groups were not significantly lower than that of the control group. However, while tsetse groups that fed following the second and third treatments with diminazene aceturate at 7.0 mg kg^{-1} b.w. picked up infections from all six cattle, flies fed following the fourth treatment became infected from only two of the six animals. Thus, repeated treatment with diminazene aceturate at a dose of 7.0 mg kg^{-1} b.w. resulted in the apparent complete elimination of infection in four out of six animals. In contrast, the transmissibility index of *T. congolense* IL 3338 was not affected by multiple treatment with diminazene aceturate.

■ INTRODUCTION

In most of sub-Saharan Africa, control of trypanosomosis in domestic livestock relies on the administration of chemotherapeutic and chemoprophylactic agents. However, there are a limited number of trypanocides. Furthermore, the different compounds are chemically closely related and have all been used in the field for over 35 years.

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In association with the lengthy period of usage of anti-trypanosomal compounds, drug resistance appears to be an increasing problem in trypanosome species that are pathogenic for domestic livestock (1, 5, 6, 11-16, 18, 21, 23, 26, 27, 30, 31, 33, 35, 36, 40). In addition, in some countries resistance to all three of the anti-trypanosomal compounds recommended for use in cattle, sheep and goats (namely, diminazene, isometamidium and homidium) has been described (5, 6, 11, 12, 27, 35, 37, 40, 41). In such situations, depending on the prevalence and level of resistance and the pathogenicity of infections, control of the disease with only anti-trypanosomal compounds may lead to problems with drug toxicity in livestock (10, 43).

In a study on the epidemiology of drug resistance in trypanosomes in Southwest Ethiopia, 840 East African Zebu cattle in the Ghibe

Effect of diminazene on drug-resistant T. congolense *in cattle*

valley were monitored from 1986 to 1990. During this period the animals were examined on a monthly basis and blood samples collected for detection of trypanosomes and determination of the packed red blood cell volume (PCV). Cattle found to be parasitemic and with a PCV less than 26% were treated with diminazene aceturate (Berenil®) at a dose of 3.5 mg kg^{-1} body weight (b.w.). The apparent tsetse challenge was also determined throughout the entire experimental period (19). In 1986 the prevalence of trypanosome infections in the cattle was approximately 18%. However, by April 1990 it had increased to approximately 40%, with the majority of infections due to *Trypanosoma congolense*. In association with an increase in the tsetse challenge the prevalence of recurrent (diminazene-resistant) infections, on the basis of field parasitological data, rose significantly from 6% in 1986 to 14% in 1990 (40). In order to confirm the presence of diminazene-resistant infections, 12 trypanosome isolates were collected from cattle at Ghibe in July 1989 and characterized in Boran (*Bos indicus*) cattle for their sensitivity to recommended doses of diminazene aceturate, isometamidium chloride (Samorin®) and homidium chloride (Novidium®). All 12 isolates were *T. congolense* and all were resistant to diminazene aceturate at a dose of 7.0 mg kg^{-1} b.w. Eleven of the same isolates were also resistant to isometamidium chloride at a dose of 0.5 mg kg^{-1} b.w. and homidium chloride at a dose of 1.0 mg kg^{-1} b.w. (6). Since clones derived from one of these isolates were multiple-drug resistant (6) it was concluded that chemotherapy on its own was not sufficient to control trypanosomosis in the cattle. As a result, a tsetse control program was implemented in April 1990 in which deltamethrin-impregnated targets were used across the valley. During the year following initiation of the tsetse control campaign there was a significant reduction in the apparent density of the tsetse population. Furthermore, despite a high prevalence of multiple-drug resistant infections in the cattle, this was associated with a significant reduction in the prevalence of parasitemic cattle on the basis of examination of the blood buffy coat (29), from a mean of 38% for the 12-month period preceding implementation, to a mean of 15% for the 12-month period following initiation of the tsetse control campaign. The reduction in tsetse density was also associated with a reduction in the apparent prevalence of drug-resistant trypanosome infections in cattle, and an increase in livestock productivity (38, 39). Thus, in the presence of a reduced tsetse challenge it appeared that cattle harboring drug-resistant trypanosomes were able to control the level of parasitemia and pathogenicity of such infections. However, it was not clear whether the animals eliminated the infections or whether they remained infective for tsetse flies. The effect of repeated treatment of cattle with diminazene when infected with a drug-resistant *T. congolense* was therefore evaluated in the work described here using animals maintained under controlled laboratory conditions. The infectivity and transmissibility of the drug-resistant *T. congolense* for *Glossina morsitans centralis* after repeated exposure to diminazene was also evaluated.

■ MATERIALS AND METHODS

Experiment animals

Six Boran cattle, approximately 18 months of age at the start of the experiment, were obtained from Kapiti Plains Estate, Machakos District, Kenya, an area free from tsetse and trypanosomosis. Adult male castrated goats (East African Maasai X Galla), weighing 20-25 kg, were purchased from a region of the Kenyan Rift Valley known to be free from trypanosomosis. Throughout the experiment the animals were housed in loose

boxes within fly-proof isolation units and supplied daily with a commercial pelleted ration (Unga Feeds Ltd., Nairobi). Water and hay were provided *ad libitum*.

Half-lop rabbits weighing approximately 2 kg and outbred Swiss white mice weighing approximately 30 g were obtained from the ILRI breeding colony. All small animals were maintained on a commercial pelleted ration (Unga Feeds Ltd., Nairobi).

Trypanosome population

Trypanosoma congolense IL 3338 is a stock that was derived from *T. congolense* IL 3330 by one passage in sublethally irradiated mice. *Trypanosoma congolense* IL 3330 is a primary isolate that was collected in July 1989 from an East African Zebu cow in the Ghibe valley, Ethiopia (6). In cattle, *T. congolense* IL 3330 has been shown to be resistant to treatment with diminazene aceturate, isometamidium chloride and homidium chloride at doses of 7.0, 0.5 and 1.0 mg kg^{-1} b.w., respectively (6). Thus, by comparison to drug-sensitive trypanosomes (34), the population is highly resistant to all three compounds.

Tsetse flies

Glossina morsitans centralis originated from Singida, Central Tanzania, and were from the production colony bred at ILRI (28). In order to obtain infected flies a goat was infected via the jugular vein with *T. congolense* IL 3338, then monitored for PCV and parasitemia. At the first peak of parasitemia, 220 teneral male *G. m. centralis* were maintained on the goat on a daily basis for 23 days. Thereafter, following two days of starvation, the flies were all made to probe (4) and those extruding metacyclic trypanosomes in their saliva used to infect the cattle.

Infection and monitoring of animals

Each of the Boran cattle was challenged by allowing 5 *G. m. centralis* infected with *T. congolense* IL 3338 to feed singly on their flanks. Thereafter, three times a week, blood samples were collected from the jugular vein into evacuated tubes containing potassium ethylenediamine tetra-acetate. The PCV was then measured and the level of parasitemia estimated using phase-contrast examination of the blood buffy coat (29).

Mice were infected by allowing individual flies with metacyclic trypanosomes in their salivary probes (8) to feed singly on the abdomen of each animal. They were then monitored twice a week for 40 days by examining wet films of tail blood at 400x magnification.

Administration of diminazene aceturate

At the first peak of parasitemia, diminazene aceturate (Berenil®, Batch No. 371A748, Hoechst, Germany) was administered intramuscularly to the cattle in the middle third of the neck at a dose of 3.5 mg kg^{-1} b.w., using a 7% (w/v) solution in sterile-distilled water. The same treatment regimen was also used for treatments 2-8, inclusive. However, for treatments 9-13, inclusive, diminazene aceturate was administered via the same route at a dose of 7.0 mg kg^{-1} b.w. All animals were weighed immediately prior to administration of drug.

Experiment design

Following infection of cattle with *T. congolense* IL 3338, 200 teneral *G. m. centralis* (100 males and 100 females) were fed on one occasion on each of the cattle at the first peak of parasitemia. On the same day, after feeding of tsetse flies, all six animals were

treated with diminazene aceturate. Thereafter, for a 12-month period, all six animals were retreated *en bloc* with the drug whenever the PCV declined in three consecutive samples from one or more of the animals. After eight treatments at a dose of 3.5 mg kg^{-1} b.w. the inter-treatment interval had not increased. Furthermore, the ability to control the development of parasitemia in the animals did not appear to have improved. As a result, treatment with diminazene aceturate was thereafter increased to a dose of 7.0 mg kg^{-1} b.w. for the rest of the experiment. The experiment was terminated 60 days after the 13th treatment with the drug.

Throughout the experiment, on approximately a monthly basis, similar numbers of tsetse flies as used for the first feed were fed on one occasion on each animal; different batches of flies were used each month. On the basis of data from previous work (8), flies were not fed on the cattle less than 14 days following treatment with diminazene aceturate at a dose of 3.5 mg kg^{-1} b.w. since this dosage was shown to inhibit the infectivity and cyclical development of *T. congolense* IL 3338 in *G. m. centralis* when fed on cattle at such intervals. When treatment with diminazene aceturate was increased to 7.0 mg kg^{-1} b.w. the minimum interval between drug administration and feeding of *G. m. centralis* was increased to at least 30 days since data from pharmacokinetic studies (3, 24) indicated that drug concentrations in blood at this interval should be less than or equal to those on day 14 following treatment with diminazene aceturate at 3.5 mg kg^{-1} b.w. Tsetse groups II, III, IV, V, VI, VII and VIII were fed immediately prior to treatments 2, 4, 6, 8, 11, 12 and 13, respectively (table I).

Monitoring of tsetse flies

After feeding tsetse flies on cattle on one occasion, each batch of flies was maintained on six rabbits for 28 days (two rabbits for days 2-14, two rabbits for days 15-21, two rabbits for days 22-28). Thereafter the flies were left unfed for two days, then induced to probe on to warmed slides at 37°C (4). The saliva was then examined for the presence of metacyclic trypanosomes by phase-contrast microscopy at 320x magnification, using a combination of Periplan 10x eyepieces and a long-distance L32 objective. Ten flies from each group, whenever available, were then fed singly on the abdomen of mice to determine the transmissibility index. All flies were subsequently dissected to determine the infection rates (22).

Statistical analysis

Data were processed using paired t-tests and one-way analysis of variance available in MINITAB-Release-9 (1993, Minitab Inc., PA, USA).

■ RESULTS

Development of infections in cattle

All six Boran cattle were first detected parasitemic by days 13-14 following infection with *T. congolense* IL 3338. On day 16, at approximately the first peak of parasitemia, the animals were all treated with diminazene aceturate for the first time at a dose of 3.5 mg kg^{-1} b.w. The evolution of parasitemia in the six cattle following each subsequent treatment with diminazene aceturate is summarized in table II. Treatments 1-8, inclusive, were given at a dose of 3.5 mg kg^{-1} b.w.; treatments 9-13, inclusive, were carried out at 7.0 mg kg^{-1} b.w. Following the 1st treatment the mean period of aparasitemia was 7.8 ± 0.6 days. Following the 8th treatment it was 7.8 ± 1.1 days. Thereafter, it increased to 10.7 ± 1.9, 14.0 ± 1.9, 15.0 ± 0.0 and 14.5 ± 1.5 days following treatments 9, 10, 11 and 12, respectively. After the 10th treatment

Table I

Timetable of treatments of cattle with diminazene aceturate and feeding of *Glossina morsitans centralis*

Treatment	Day post infection	Dose of diminazene aceturate (mg kg^{-1} b.w.)	Group of tsetse flies (fed prior to treatment)
1	16	3.5	I
2	30	3.5	II
3	46	3.5	
4	60	3.5	III
5	77	3.5	
6	93	3.5	IV
7	112	3.5	
8	126	3.5	V
9	137	7.0	
10	163	7.0	
11	189	7.0	VI
12	217	7.0	VII
13	247	7.0	VIII

one of the six cattle remained aparasitemic until the end of the experiment. Subsequent to the 11th treatment three additional cattle remained aparasitemic until the end of the experiment. Finally, over a period of two months following treatment 13, all six cattle remained aparasitemic on the basis of the buffy-coat phase-contrast diagnostic technique.

Table II summarizes the mean inter-treatment PCV values for all six cattle. In all animals there was a progressive decline of the PCV throughout the period that treatments with diminazene aceturate were carried out at a dose of 3.5 mg kg^{-1} b.w. In contrast, a progressive increase in the value occurred when treatment was carried out at 7.0 mg kg^{-1} b.w.

Infection rates in tsetse flies

The mean infection rates in the midgut, labrum and hypopharynx of *G. m. centralis* fed on the six Boran cattle at various intervals following infection with *T. congolense* IL 3338 are summarized in table III. In order to demonstrate the between-animal variation in these data, table IV summarizes the midgut and hypopharynx infection rates in the tsetse flies fed on each Boran cow at each time point. Analysis of the mean infection rates in table III indicated that, except for the hypopharyngeal infection rate of group VI, the values for the midgut, labrum and hypopharynx in all groups were not significantly lower than those of the control group (group I). Finally, except for midgut infections in groups II and IV, labrum, midgut and hypopharyngeal infection rates did not differ significantly between male and female flies in all groups (data not shown).

Trypanosome transmission rates

Table V summarizes the mouse transmission rates for tsetse flies with mature infections in each group. Prior to statistical analysis the transmission rate data lying within the range of 90 to 100% were normalized following arc-sin transformation using Bliss table (42). One-way analysis of variance did not show a significant difference between any of these data and the corresponding value of the control group (P < 0.05). Thus, repeated administration of diminazene aceturate to cattle infected with *T. congolense* IL 3338 did not affect the transmissibility of the trypanosome population to mice by tsetse flies.

Effect of diminazene on drug-resistant T. congolense *in cattle*

Table II

Evolution of parasitemia and PCV in six Boran cattle infected with *Trypanosoma congolense* IL 3338
and repeatedly treated with diminazene aceturate

Diminazene treatment[a]	Mean PCV[b]	Animal number						Overall mean[c] ± SE
		BP 21	BP 22	BP 23	BP 24	BP 25	BP 26	
1	29.3 ± 1.8	9[c]	6	8	9	6	9	7.8 ± 0.6
2	28.1 ± 2.3	9	9	10	9	10	10	9.5 ± 0.2
3	26.3 ± 2.1	9	9	10	10	8	8	9.0 ± 0.4
4	25.6 ± 1.9	8	8	8	8	8	8	8.0 ± 0.0
5	24.7 ± 1.9	7	7	7	9	8	8	7.7 ± 0.3
6	22.9 ± 1.9	6	7	9	9	6	10	7.8 ± 0.7
7	22.5 ± 2.2	4	8	8	8	4	8	6.7 ± 0.8
8	23.7 ± 2.6	3	7	9	8	10	10	7.8 ± 1.1
9	25.4 ± 2.4	4*	8*	13*	17*	9*	13*	10.7 ± 1.9
10	26.0 ± 2.5	13*	9*	16*	X	12*	20*	14.0 ± 1.9
11	29.1 ± 2.2	X	15*	X	X	15*	X	15.0 ± 0.0
12	30.8 ± 2.5	X	13*	X	X	16*	X	14.5 ± 1.5
13	32.9 ± 1.7	X	X	X	X	X	X	X

[a] Treatments 1-8, inclusive, given at 3.5 mg kg^{-1} b.w.; treatments 9 onwards carried out at 7.0 mg kg^{-1} b.w.

[b] Mean inter-treatment packed red cell volume in all six cattle following the treatment, e.g., the value given for diminazene treatment 1 represents the mean packed red cell volume in all six cattle between treatments 1 and 2

[c] Number of days following treatment that animals were aparasitemic using the blood buffy-coat phase-contrast technique

* Parasitemia became intermittent after relapse was detected

X: No parasites detected after treatment

Table III

Mean trypanosome infection rates in groups of *Glossina morsitans centralis* fed on six Boran cattle
infected with *Trypanosoma congolense* IL 3338 and repeatedly treated with diminazene aceturate

Group of tsetse[a]	Num. of tsetse fed	Num. of tsetse dissected	Midgut infection rates - %	Labrum infection rates - %	Hypopharynx infection rates - %
I	1200	1050	36.44 ± 1.94	25.71 ± 1.18	24.79 ± 1.34
II	1200	1093	48.94 ± 2.77	32.38 ± 1.71	28.53 ± 1.75
III	1200	1056	33.49 ± 1.94	29.11 ± 1.85	27.09 ± 2.07
IV	1200	1069	51.12 ± 1.74	40.29 ± 1.97	37.88 ± 2.05
V	1200	1081	34.54 ± 1.46	27.32 ± 1.28	26.37 ± 1.34
VI	1200	1079	29.61 ± 2.75	18.03 ± 4.53	15.93 ± 3.61*
VII	1200	1062	52.13 ± 2.63	38.06 ± 2.71	32.76 ± 2.52
VIII	1200	1097+	34.38 ± 7.15	29.13 ± 8.57	26.38 ± 8.60

[a] See table I for timing of feeding of tsetse groups

* Significantly lower than corresponding value of group I (P < 0.05), i.e., control group

+ Flies became infected from only two cattle

■ DISCUSSION

In the work described here the ability of Boran cattle to control a pathogenic drug-resistant *T. congolense* infection after repeated treatment with diminazene aceturate was evaluated over a 12-month period. Six cattle were infected with *T. congolense* IL 3338 and thereafter treated *en bloc* with the drug whenever the PCV in one or more animals decreased in three consecutive blood samples. For treatments 1-8, inclusive, the cattle were treated with the minimum recommended dose of diminazene aceturate: 3.5 mg kg^{-1} b.w. However, following each of these treatments the mean interval when trypanosomes were not detected by the buffy-coat phase-contrast technique (29) did not increase and was always less than 10 days. For example, following the 1st, 2nd and 8th treatments the mean (± standard error) aparasitemic duration was 7.8 ± 0.6, 9.5 ± 0.2 and 7.8 ± 1.1 days, respectively. Over the same period the mean PCV values of the animals progressively decreased from 33.2 ± 0.6% at the time of the 1st treatment (data not given) to a mean of 23.7 ± 2.6% for the inter-treatment period between the 8th and 9th treatments. Thus, since the aparasitemic interval following each treatment did not increase, while the PCV values progressively decreased, multiple treatment with

■ PATHOLOGIE PARASITAIRE

Revue Élev. Méd. vét. Pays trop., 1998, 51 (3) : 211-218

214

Effet du diminazène sur un T. congolense *chimiorésistant chez des bovins*

Table IV

Infection rates (%) in *Glossina morsitans centralis* fed on six Boran cattle
infected with *Trypanosoma congolense* IL 3338 and repeatedly treated with diminazene aceturate

Group of tsetse[a]	Animal number											
	BP 21		BP 22		BP 23		BP 24		BP 25		BP 26	
	GI	HI	GI	HI	GI	HI	GI	HI	GI	HI	GI	HI
I	38.4	22.2	35.9	23.0	41.6	20.8	39.8	28.7	34.7	28.2	28.2	25.8
II	43.1	29.9	43.5	25.4	61.6	24.9	49.5	33.9	46.8	24.2	49.2	33.0
III	42.9	36.0	33.1	24.3	29.4	23.9	32.4	29.8	31.3	22.3	31.7	26.3
IV	52.2	36.4	59.0	44.8	51.2	35.9	48.3	31.0	48.0	36.4	48.1	42.7
V	37.4	31.0	34.5	29.3	27.7	22.0	35.5	24.7	37.5	25.6	34.5	25.6
VI	35.7	22.9	39.3	27.7	29.6	12.3	22.4	2.3	23.3	13.9	27.3	16.5
VII	54.4	31.0	58.9	40.6	52.4	32.1	41.1	22.8	57.1	37.8	48.9	32. 3
VIII	0	0	41.5	40.0	0	0	0	0	27.2	17.8	0	0

[a] See table I for when tsetse were fed on the cattle

GI: midgut infection rate

HI: hypopharynx infection rate

Table V

Mouse transmission rates for mature
Trypanosoma congolense IL 3338 infections
in *Glossina morsitans centralis* fed on infected Boran
cattle repeatedly treated with diminazene aceturate

Tsetse group[a]	Num. of mice[b]	Num. of mice infected	Transmission rate[c] - %
I	60	57	95.0 ± 2.2
II	60	58	96.7 ± 2.1
III	60	60	100 ± 0.0
IV	60	58	96.7 ± 2.1
V	60	57	95.0 ± 2.2
VI	44	43	98.0 ± 2.0
VII	60	59	98.3 ± 1.7
VIII	20	20	100 ± 0.0

[a] See table I for when tsetse were fed on the cattle

[b] Number of mice on which tsetse flies were fed on one occasion

[c] Mean ± standard error

diminazene aceturate at a dose of 3.5 mg kg^{-1} b.w. did not appear to result in reduced virulence and pathogenicity of *T. congolense* IL 3338. This suggests that the animals did not acquire resistance to infection and disease, as might have been expected based on other works on *T. congolense* (7, 44).

Since treatment with diminazene aceturate at 3.5 mg kg^{-1} b.w. did not ameliorate the pathogenicity of infections, administration of the drug thereafter (i.e., treatment 9 onwards) was carried out at 7.0 mg kg^{-1} b.w. In association with this increase in drug dosage the mean PCV of the cattle increased from 25.4 ± 2.4% for the interval between the 9th and 10th treatments to 32.9 ± 1.7% for the 60-day period following the 13th treatment. Furthermore, the mean duration following treatment when trypanosomes were not detected rose from 10.7 ± 1.9 days after treatment 9 to 14.5 ± 1.5 days after treatment 12. In many animals detection of parasites became intermittent after the 9th treatment. Moreover, after the

10th treatment trypanosomes were not detected in one of the six animals until the end of the experiment. The same occurred in an additional three animals following treatment 11. The apparent "self-cure" status that developed following treatments 10 and 11 was not, however, associated with complete elimination of infections in the cattle since *G. m. centralis*, fed on the cattle after the 10th and 11th treatments (2nd and 3rd treatments, respectively, with diminazene aceturate at 7.0 mg kg^{-1} b.w.), picked up infections from all six animals (tsetse groups VI and VII, respectively, table IV). This continued carrier status, despite the lack of detection of trypanosomes with a routinely used diagnostic technique, is similar to observations with long-term *Trypanosoma brucei* infections in cattle (25). At present, there is no satisfactory explanation for the progressive development of aparasitemia in cattle infected with *T. congolense* IL 3338, particularly since pharmacokinetic data for diminazene (3) indicate that diminazene accumulation should not have occurred in the cattle at the dosage regimen that was used in this study. Development of aparasitemia and the associated recovery of normal PCV values may have been associated with increasing efficiency of immune responses to trypanosome antigens. However, the relative contribution of anti-variable surface glycoprotein and anti-invariant antigen antibody responses to this phenomenon is unclear and requires characterization.

In contrast to flies fed on cattle after the 10th and 11th treatments with diminazene aceturate, flies fed on cattle after the 12th treatment (4th treatment at 7.0 mg kg^{-1} b.w.; tsetse group VIII) became infected after feeding on only two of the six cattle. Thus, in four cattle infections were apparently eliminated. In light of the diminazene resistance of *T. congolense* IL 3338, and the stability of resistance to diminazene that is observed in trypanosome populations after long-term maintenance *in vivo* (12), elimination of infections would appear most likely to be associated with exhaustion of the trypanosomes' variable antigenic type repertoires (32).

Evaluation of the effect of repeated treatment with diminazene aceturate on the tsetse infectivity and transmissibility of *T. congolense* IL 3338 indicated that 13 treatments affected neither the

Revue Élev. Méd. vét. Pays trop., 1998, 51 (3) : 211-218

215

Effect of diminazene on drug-resistant T. congolense *in cattle*

transmissibility nor the infection rates in the midgut and hypopharynx of *G. m. centralis*. It should be noted, however, that the mean mature infection rate in flies of group VI (fed on cattle prior to treatment 11) was significantly less (P < 0.05) than the corresponding value of the control group (group I). The reason for this is unclear.

In summary, this study has shown that when Boran cattle are infected with drug-resistant *T. congolense* IL 3338, from Ghibe, Ethiopia, repeated treatment with diminazene aceturate at a dose of 3.5 mg kg^{-1} b.w. was not associated with an improved ability of the cattle to control parasitemiae below the limit of detection of the buffy-coat phase-contrast technique. Furthermore, such treatment was associated with a progressive decline in PCV values. This observation is in contrast with data from cattle at Ghibe, in which there was a high prevalence of multiple-drug resistant *T. congolense* infections (6); treatment with diminazene aceturate at a dose of 3.5 mg kg^{-1} b.w. when animals were detected parasitemic with the buffy-coat phase-contrast technique, at a time of low tsetse challenge, was associated with a substantial reduction in the apparent prevalence of parasitemic cattle and an increase in their PCV values (20, 35). The reason for the different result in the work reported here is unclear. It is possible that although the cattle at Ghibe and in this study were both *Bos indicus,* differences in the level of innate resistance of the two populations to trypanosome infections may have been responsible for the different results (9). Alternatively, and more likely, the longer exposure of cattle at Ghibe to *T. congolense* infections may have resulted in the expression of higher levels of acquired resistance (2, 44) than in the cattle reported here. Finally, this work has also shown that when cattle infected with *T. congolense* IL 3338 are repeatedly treated with diminazene aceturate at 7.0 mg kg^{-1} b.w. the ability of the animals to control the level of parasitemia appears to progressively increase. In light of the high level of diminazene resistance of *T. congolense* IL 3338 relative to the level of resistance described in other *T. congolense* populations (6, 17) it is recommended that the efficacy of multiple treatment with this drug dosage should be evaluated in field situations where treatment with diminazene aceturate at 3.5 mg kg^{-1} b.w. is not efficacious.

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Revue Élev. Méd. vét. Pays trop., 1998, 51 (3) : 211-218

217

Effect of diminazene on drug-resistant T. congolense *in cattle*

Résumé

Diack A., Moloo S.K., Peregrine A.S. Traitements multiples par l'acéturate de diminazène chez les bovins : effets sur l'infectivité d'une souche chimiorésistante de *Trypanosoma congolense* et sur sa transmissibilité par *Glossina morsitans centralis*

Six bovins de race Boran ont été infectés par la souche chimiorésistante IL 3338 de *Trypanosoma congolense*. Au premier pic de parasitémie, 200 *Glossina morsitans centralis* ténérales organisées en plusieurs groupes ont été nourries sur chaque animal, juste avant leur traitement à l'acéturate de diminazène à la dose de 3,5 mg/kg de poids corporel (p.c.). Les animaux ont ensuite été suivis par prélèvement sanguin trois fois par semaine et ont été traités comme précédemment chaque fois que l'hématocrite baissait dans trois prélèvements consécutifs, pour au moins un des animaux. Après huit traitements à approximativement deux semaines d'intervalle, la longueur moyenne de la période prépatente après chaque traitement n'a pas augmenté, restant à 7,8 ± 1,1 jours. La valeur moyenne de l'hématocrite est descendue de 33,2 ± 0,6 p. 100 lors du premier traitement, à 23,7 ± 2,6 p. 100 entre les huitième et neuvième traitements. En conséquence, à la suite du huitième traitement, l'acéturate de diminazène a été administré comme précédemment, mais à la dose de 7,0 mg/kg p.c. Résultant de l'augmentation de la dose, la valeur moyenne de l'hématocrite entre deux traitements est montée de 25,4 ± 2,4 p. 100 sitôt après le premier traitement à 32,9 ± 1,7 pour les deux mois qui ont suivi le cinquième traitement. Au moins 14 jours après les traitements à l'acéturate de diminazène à la dose de 3,5 mg/kg p.c., et 30 jours après les traitements à la dose de 7,0 mg/kg p.c., un nombre de mouches équivalent à celui utilisé lors de l'infection initiale a été nourri une fois sur chaque animal. Afin de déterminer l'indice de transmissibilité, 10 mouches de chaque groupe présentant une infection arrivée à maturité ont été nourries individuellement sur des souris. En général, les taux d'infections de l'intestin moyen et de l'hypopharynx des mouches de tous les groupes n'étaient pas significativement inférieurs à ceux du groupe témoin. Par contre, tandis que les mouches du groupe nourri après les deuxième et troisième traitements à l'acéturate de diminazène à 7,0 mg/kg p.c. se sont infectées sur tous les six bovins, celles nourries après le quatrième traitement se sont seulement infectées sur deux des six animaux. Ainsi, un traitement répété à l'acéturate de diminazène à la dose de 7,0 mg/kg p.c. s'est soldé par une élimination apparente totale de l'infection chez quatre des six animaux. En revanche, l'index de transmissibilité de *T. congolense* IL 3338 n'a pas été affecté par des traitements multiples à l'acéturate de diminazène.

Mots-clés : Bovin - *Glossina morsitans centralis* - *Trypanosoma congolense* - Infection expérimentale - Transmission des maladies - Résistance aux produits chimiques - Kenya.

Resumen

Diack A., Moloo S.K., Peregrine A.S. Efecto del tratamiento múltiple de ganado con aceturato de diminasona sobre la infectividad y la transmisibilidad del *Trypanosoma congolense* resistente a las drogas por *Glossina morsitans centralis*

Se infectaron seis bovinos Boran con *Trypanosoma congolense* IL 3338 resistente a las drogas. Durante el primer pico de parasitemia, justo antes del tratamiento con aceturato de diminasona, a una dosis de 3,5 mg kg⁻¹ peso corporal (p.c.), se alimentaron diferentes grupos de 200 *Glossina morsitans centralis* tenerales en cada animal. En adelante, todos los animales se siguieron tres veces por semana y fueron tratados nuevamente, con la misma droga, cuando el hematocrito (PCV) disminuyó en tres muestras consecutivas, en uno o más animales. Después de ocho tratamientos, a intervalos de aproximadamente dos semanas, la duración media durante la cual los parásitos no se detectaron post tratamiento no aumentó, sino que se mantuvo en 7,8 ± 1,1 días. El hematocrito promedio declinó de 33,2 ± 0,6% al primer tratamiento hasta un valor promedio inter tratamiento de 23,7 ± 2,6% entre el octavo y el noveno tratamiento. Por lo tanto, después del octavo tratamiento, el aceturato de diminasona se administró de la misma manera, pero a una dosis de 7,0 mg kg⁻¹ p.c. Después del tratamiento a esta dosis elevada, el hematocrito entre tratamientos aumentó de 25,4 ± 2,4% al primer tratamiento a 32.9 ± 1,7% durante el período de dos meses después del quinto tratamiento. Mínimo 14 días post tratamiento con 3,5 mg kg⁻¹ p.c. de aceturato de diminasona y 30 días después del tratamiento con 7,0 mg kg⁻¹ p.c., se alimentaron, en una ocasión y en cada animal, cantidades de moscas similares a las utilizadas durante la primera alimentación. Seguidamente, diez moscas de cada grupo, con infecciones maduras, fueron alimentadas individualmente en ratones, para determinar el índice de transmisibilidad. En general, las tasas de infección en el intestino medio y la hipofaringe en todos los grupos de moscas, no fueron significativamente inferiores que los del grupo control. Sin embargo, mientras los grupos de tse-tse que se alimentaron después del segundo y tercer tratamiento con 7,0 mg kg⁻¹ de aceturato de diminasona se infectaron en todos los seis animales, las moscas alimentadas después del cuarto tratamiento se infectaron únicamente en dos de los seis animales. Por lo tanto, el tratamiento repetido con aceturato de diminasona a dosis de 7,0 mg kg⁻¹ p.c. resulta en una aparente eliminación total de la infección en cuatro de los seis animales. El índice de transmisibilidad de *T. congolense*, por el contrario, no se vio afectado por el tratamiento múltiple con aceturato de diminasona.

Palabras clave: Ganado bovino - *Glossina morsitans centralis* - *Trypanosoma congolense* - Infección experimental - Transmisión de enfermedades - Resistencia a productos químicos - Kenia.