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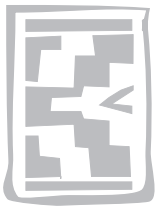

The susceptibility of *Trypanosoma congolense* isolated in Zambézia Province, Mozambique, to isometamidium chloride, diminazene aceturate and homidium chloride

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ABSTRACT

JAMAL, S., SIGAUQUE, I,. MACUAMULE, C., NEVES, L., PENZHORN, B.L., MARCOTTY, T. & VAN DEN BOSSCHE, P. 2005. The susceptibility of *Trypanosoma congolense* isolated in Zambézia Province, Mozambique, to isometamidium chloride, diminazene aceturate and homidium chloride. *Onderstepoort Journal of Veterinary Research*, 72:333–338

Resistance to trypanocidal drugs has been detected in various African countries and is a serious impediment to the control of livestock trypanosomosis. To determine whether drug resistant trypanosome strains are present in the Zambézia Province of Mozambique a study was initiated. To assess the effect of the farming system and the drug-use regimen on the development of drug resistance, trypanosome isolates were collected from cattle from subsistence and commercial livestock production systems. The susceptibility of seven isolates against isometamidium chloride, diminazene aceturate and homidium chloride was tested in mice using a multiple-dose test. In four of the seven isolates high levels of drug resistance to diminazene aceturate and isometamidium chloride were detected. In most cases the observed levels of drug resistance correlated with the drug-use practices in the particular livestock production system.

Keywords: Diminazene aceturate, homidium chloride, isometamidium chloride, resistance, *Trypanosoma congolense*

INTRODUCTION

Tsetse-transmitted trypanosomosis constitutes a major constraint to livestock development in Africa. Over 10 million km^2 of sub-Saharan Africa is infested by tsetse flies. Trypanosome infections in livestock seriously impede livestock production in particular and rural development in general (Swallow 1998). Trypanosomosis can be controlled by controlling the vector, the parasite or a combination of both. Despite the availability of effective vector-cotrol methods, it is very likely that curative and prophylactic trypanocidal drugs will continue to contribute significantly to the control of the disease in livestock.

Only a small group of chemoprophylactic and chemotherapeutic compounds are currently in use and new compounds are unlikely to become available in the near future (Peregrine 1994). Geerts & Holmes (1998) estimated that in Africa about 35 million doses of trypanocidal drugs are administered each year. Furthermore, there is growing concern

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that the effectiveness of this control method will be severely reduced by the widespread development of resistance in trypanosomes.

In Mozambique, trypanosomosis in livestock is controlled mainly by treatments with diminazene aceturate as curative and/or isometamidium chloride as prophylactic drug. Treatment intensity differs greatly between farming systems and, within a farming system, between livestock owners. Despite the current and future importance of trypanocides in Mozambique no information is available on the prevalence of resistance to these drugs.

The aim of this study was to determine the sensitivity of *Trypanosoma congolense* isolates collected from cattle from commercial and subsistence livestock management systems in three districts of Zambézia Province to diminazene aceturate, isometamidium chloride and homidium chloride. The standardized method for trypanocidal drug resistance testing described by Eisler, Brandt, Bauer, Clausen, Delespaux, Holmes, lemobade, Machila, Mbambo, Mcdermott, Mehlitz, Murilla, Ndung'u, Peregrine, Sidibé & Geerts (2001) was used.

MATERIALS AND METHODS

Study area

The study was conducted in the Chinde, Nicoadala and Maganja da Costa districts of Zambézia Province of Mozambique (Fig. 1). Zambézia Province is located in the central part of the country. It covers about 103 130 km^2 and has a population density of 32.2 inhabitants per km^2. About 14 300 head of cattle (predominantly Brahman breed) are present. They are distributed mainly in the palm plantations of the littoral part of the province. The littoral zone constitutes an extensive and excellent grazing area. Although a large-scale commercial ranch is present, cattle in the Province are kept mainly under an extensive husbandry system.

Sample collection

A total of eight sampling sites were identified in the three Districts. At each sampling site, 165 animals were randomly selected and sampled. Blood was collected from an ear vein into heparinized microhaematocrit centrifuge capillary tubes and onto glass slides on which thick and thin blood smears were made for trypanosome species identification. The capillary tubes were sealed with "Cristaseal" (Hawksley) and immediately centrifuged in a microhaematocrit centrifuge for 5 min at 9 000 rpm. After centrifugation, the buffy coat and the uppermost layer of red blood cells of each specimen were extruded onto a microscope slide and examined for the presence of motile trypanosomes (Paris, Murray & McOdimba 1982).

Isolation of trypanosomes in the field

From the infected cattle, 1 mℓ of blood was inoculated intraperitoneally into outbred albino male and female mice. The experimental mice had been immunosuppressed 24 h previously by intraperitoneal injection of 300 mg/kg cyclophosphamide (Endoxan®, Asta Medica). The parasitaemia of the mice inoculated in the field was monitored twice weekly by examining the buffy coat of tail blood. Stabilates of the blood of the ones that became parasitaemic were prepared in liquid nitrogen using glycerol (10 % v/v) as a cryopreservative (Dar, Ligthart & Wilson 1972).

All trypanosome infections in cattle were due to *T. congolense*. Seven *T. congolense* isolates, three from Chinde District, three from Nicoadala District and one from Maganja da Costa District, were used for resistance testing. The three isolates (Isolates 1, 2 and 3) from Chinde District originated from cattle from a commercial ranch (Madal Estate). On that ranch, all cattle were treated with diminazene aceturate followed by isometamidium chloride treatment two weeks later four times per year. Sick animals were treated with diminazene aceturate. Since 2002, however, diminazene aceturate has been replaced by quinapyramine. Despite this treatment frequency, the prevalence of trypanosome infections is high. One of the three isolates from Nicoadala District (Isolate 4) was collected from cattle belonging to a subsistence farmer based at Namutungurine. The farmer treated his animals three times per year with diminazene aceturate followed by isometamidium chloride two weeks later. Isolate 5 came from cattle kept at Botao in Nicoadala District. Animals of this herd were treated once a year with diminazene aceturate followed by isometamidium chloride treatment. One isolate (Isolate 6) was collected from cattle belonging to a subsistence farmer based at Licuare (Nicoadala District) who only treats sick animals with diminazene aceturate. Finally, one isolate (Isolate 7) was collected from cattle belonging to a commercial livestock owner based at Cangu (Maganja da Costa District). Here, animals were treated once per year with diminazene aceturate followed by isometamidium chloride two weeks later. Sick animals were treated with diminazene aceturate.

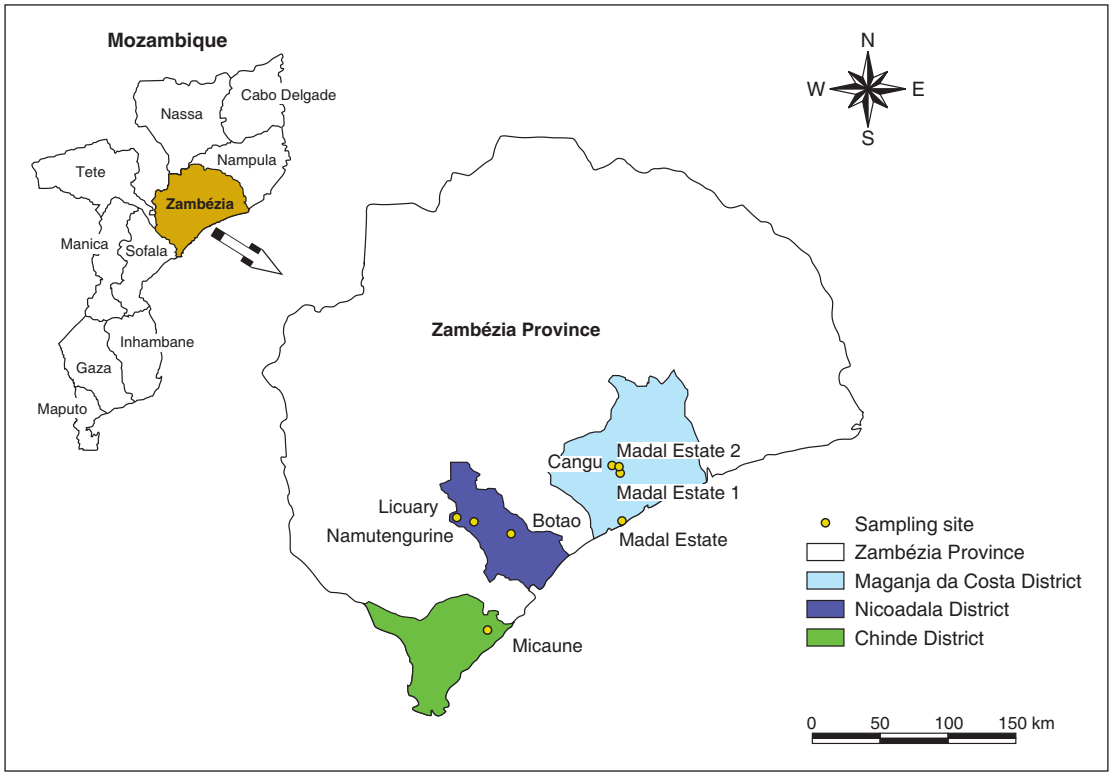

FIG. 1 Map of Zambézia Province indicating the location of the seven sampling sites in the three districts

Resistance testing of trypanosome isolates

Resistance testing in mice was done using the multi-dose protocol described by Eisler *et al*. (2001). In short, groups of six mice were each inoculated intraperitoneally with a fresh inoculum containing 1 x 10^5 trypanosomes of a particular isolate. About 24 h after trypanosome inoculation, the mice were treated intraperitoneally with a dose of one of the trypanocidal drugs in 0.2 mℓ of sterile distilled water. For each of the seven isolates, five different dose levels varying between 0.01 and 20 mg/kg body mass (bm) of isometamidium chloride (Samorin®, Merial), between 0.01–10 mg/kg bm of homidium chloride (Novidium® , Merial) and between 1–30 mg/kg bm of diminazene aceturate (Berenil®, Intervet) were used. A dose level of 60 mg/kg bm of diminazene aceturate caused high mortality in the mice and was not included. Control mice were treated in a similar way but received 0.2 mℓ of distilled water only. After inoculation and treatment tail blood of each individual mouse was examined twice weekly. Blood was collected from the tail tip into a heparinized capillary tube, centrifuged and the buffy coat was examined. Blood of the trypanocide-treated mice was examined until a relapse occurred or until 60 days post treatment. Parasitaemia of the mice that relapsed was estimated using the method described by Murray, Trail, Turner & Wissocq (1983). If at least five mice in the group of six were cured, the isolate under test was considered sensitive to the drug dose. If one mouse in a treated group of six died without becoming parasitaemic, at least four of the remaining five mice must be cured before the parasite population could be classified as sensitive. At least five of the six control mice had to become parasitaemic, otherwise the test was repeated for all three groups.

Analyses of results

Interpretation of results was conducted according to Eisler *et al.* (2001). Whenever possible, the sensitivities of each isolate to isometamidium chloride, diminazene aceturate and homidium chloride were expressed as 50 % curative dose (CD_{50}) using a logistic regression.

RESULTS

The outcome of the trypanocidal drug resistance tests in the mice clearly showed the presence of resistant trypanosome isolates (Table 1). All seven isolates were not susceptible to diminazene aceturate at doses between 1 and 10 mg/kg bm. At the

TABLE 1 Number of mice (out of a total of six) that relapsed after treatment with a trypanocide at various doses for each of the seven isolates

Drug	Dose (mg/kg)	Number of mice positive per isolate						
		4	6	5	1	2	3	7
Diminazene aceturate	1	6	6	5	5	5	5	5
	3	6	5	6	6	6	6	6
	10	3	4	5	4	6	5	4
	20	0	1	5	2	6	5	3
	30	0	1	1	2	6	5	3
	0 (control)	6	5	6	6	5	5	6
Isometamidium chloride	0.01	6	0	5	6	6	5	6
	0.1	6	0	5	6	6	4	5
	0.5	2	0	4	6	4	5	5
	3	0	0	4	5	5	5	5
	20	0	0	0	2	5	4	4
	0 (control)	6	5	6	6	6	6	6
Homidium chloride	0.01	3	5	6	6	6	5	6
	0.1	5	3	6	6	3	4	5
	0.5	3	3	6	6	4	5	6
	3	4	0	2	5	5	2	5
	10	0	0	1	0	0	1	1
	0 (control)	6	6	6	5	6	6	6

TABLE 2 Sensitivity (expressed in mg/kg bm) of *T. congolense* isolates from Zambézia Province (Mozambique) to isometamidium chloride, diminazene aceturate and homidium chloride in mice

Isolate	Diminazene		Isometamidium		Homidium	
	CD_{50}	95 % C.I.	CD_{50}	95 % C.I.	CD_{50}	95 % C.I.
4	10.00	9.4–10.6	0.48	0.4–0.5	2.74	0.5–5.0
6	13.49	6.9–20.1	<	–	0.44	0.03–0.8
5	23.69	15.8–31.6	3.39	1.1–5.7	4.79	2.1–7.5
1	19.41	11.7–27.1	8.32	5.4–11.3	4.69	1.9–7.4
2	>	–	>	–	3.84	1.3–6.4
3	>	–	>	–	3.82	1.1–6.6
7	25.06	13.0–37.1	>	–	9.59	3.6–9.5

> = CD_{50} could not be calculated but above resistance threshold
< = CD_{50} could not be calculated but below resistance threshold
C.I. = Confidence Interval

highest dose (30 mg/kg) relapses were observed in Isolates 4, 5, 6 and 7. For isometamidium, the results were similar, with the exception of Isolate 6, which was highly susceptible at all dose levels. Treatment with homidium chloride resulted in relapses for almost all isolates at doses between 0.01 and 3 mg/kg.

The isolates for which treatment with the various dose levels of a particular drug resulted in a high level of cure or a high level of relapse, the CD_{50} (95 % C.I.) could not be calculated. Using the cut-off of 20 mg/kg bm for diminazene and 1 mg/kg bm for isometamidium (Eisler *et al*. 2001), five of the Isolates (Isolates 1, 2, 3, 5 and 7) can be considered resistant to diminazene and isometamidium (Table 2). Isolate 6 is highly susceptible to isometamidium.

With the exception of isolates 6 and 7, susceptibility to homidium chloride did not differ much between isolates (Table 2). Isolate 6 was highly susceptible whereas Isolate 7 showed the highest level of resistance.

DISCUSSION

Notwithstanding the importance of trypanocidal drugs in the control of bovine trypanosomosis in Mozambique, there is resistance to the two most frequently used compounds. These findings confirm

field observations of relapses soon after treatment. The three isolates collected from cattle at Madal Estate (Isolates 1, 2 and 3) showed especially high levels of resistance to diminazene and isometamidium. To determine whether multiple resistance is present, isolates will have to be cloned and tested in mice for resistance to the same drugs. The levels of drug resistance in isolates from Madal Estate are perhaps not surprising since some of the major factors contributing to the development of drug resistance in trypanosomes were present (Geerts & Holmes 1998). Indeed, mass treatment with both drugs were conducted regularly and the introduction of quinapyramine may have contributed significantly to the multiple resistance to isometamidium and diminazene (Ndoutamia, Moloo, Murphy & Peregrine 1993). As with the other isolates, the level of drug resistance is to some extent correlated with the drug-use practices. This is certainly so for the isolate from cattle kept by a substance farmer in Licuare (Isolate 6), where only curative treatments with diminazene were given. This isolate had a slightly reduced susceptibility for diminazene but was highly sensitive to isometamidium. The same applies to the isolate collected from cattle belonging to a subsistence farmer in Namatungurine (Isolate 4). Here, treatments with isometamidium and diminazene were conducted at regular intervals but only animals from his and not the surrounding herds were treated. Hence, the selection pressure on the trypanosome population is expected to be low.

The susceptibility of *T. congolense* to homidium chloride in mice is not well known. However, the CD_{50}-values suggest differences in sensitivity between isolates. Isolate 6 was especially susceptible to homidium chloride treatment. This isolate was also very susceptible to treatment with isometamidium. The cross-resistance between isometamidium chloride and homidium chloride may explain this result. Indeed, all the other isolates that showed a decreased susceptibility to homidium chloride also had a reduced sensitivity to isometamidium chloride.

The findings of this study clearly show the presence of drug-resistant trypanosomes in Mozambique. Moreover, our study confirms the recommendations made by Geerts & Holmes (1998) with regard to type and combination of drug(s) used and number and frequency of treatments to retard the development of drug resistance. Using the outcome of our study, some general recommendations can be made with regard to trypanocidal drug-use practices and the management of trypanocidal drug resistance in Mozambique. In the case of single drug resistance, another drug can be used to eliminate the resistant trypanosome population. In the case of multiple drug resistance at the clonal level, an integrated approach including tsetse control and curative treatment of sick animals has proven to be effective and could be implemented (Leak, Peregrine, Mulatu, Rowlands & D'Ieteren 1996). If multiple drug resistance is present on Madal Estate, regular treatment with pyrethroid insecticides may significantly reduce the impact on production of resistant trypanosome strains. In conclusion, considering the important level of drug resistance in some areas and the ongoing cattle restocking programme that is taking place in Zambézia Province it is important to avoid the distribution of animals infected with resistant trypanosome strains to other areas where drug resistance is absent or not considered to be a problem.

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