Drug Resistant Trypanosomes: a Threat to Cattle Production in the Southwest of Ethiopia

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Key words

Cattle – Zebu – *Trypanosoma congolense* – Drug resistance – Ethiopia.

Summary

Trypanosomosis is an important disease of cattle in the southwest of Ethiopia. At present chemotherapy and chemoprophylaxis are the only practical methods available for the control of animal trypanosomosis, but their effectiveness is being eroded by the emergence of drug resistant trypanosomes. Of the drugs available for the treatment of animal trypanosomosis, Berenil® (diminazene aceturate) and Trypamidium® (isometamidium chloride) have been used the most because of their availability and relatively low toxicity to cattle. In this study, four stocks of *Trypanosoma congolense,* originally isolated from cattle in the southwest of Ethiopia (Ghibe, Bedelle, Sodo and Arbaminch), were tested for their Berenil and Trypamidium sensitivity using Swiss white mice and indigenous zebu cattle. The results on the limited number of stocks indicated the existence of drug resistant strains of *T. congolense*. Isolates from Ghibe, Bedelle and Sodo were resistant to a therapeutic dose of diminazene aceturate (3.5 mg/kg) and to standard therapeutic and prophylactic doses of isometamidium chloride (0.5 and 1 mg/kg). However, all three stocks were found to be sensitive to 7 mg/kg diminazene aceturate. The fourth, the Arbaminch stock, was found to be resistant to the manufacturers' recommended doses of diminazene aceturate and isometamidium chloride.

■ INTRODUCTION

Trypanosomosis is caused by several species of blood and tissue-dwelling protozoan parasites encountered throughout the southwest of Ethiopia (1). The most important trypanosome species affecting livestock in Ethiopia are *Trypanosoma congolense*, *T. vivax* and *T. brucei* in cattle, sheep and goats, *T. evansi* in camels, and *T. equiperdum* in horses (5, 6, 10). Trypanosomosis in cattle, locally referred to as Gendi, is a serious constraint to livestock production in areas of the southwest of Ethiopia at altitudes lesser than 1700 m above sea level (6). Animals bitten by tsetse flies develop fever, anemia, lose weight, and progressively become weak and unproductive. Breeding animals frequently abort or may become infertile. Severely affected animals die of anemia, congestive heart failure or intercurrent bacterial infections that frequently take advantage of the weakened immune system. If trypanosomosis could be controlled in Ethiopia, much of the best-watered and most fertile lands of the southwest could be utilized. At present, there are two principal approaches to control trypanosomosis in Ethiopia. These are control or elimination of tsetse flies and prevention or treatment of animals using trypanocidal drugs. These approaches, however, have many drawbacks such as the high cost of drugs and insecticides, possibilities of undesirable environmental pollution by insecticides, the increasing development of resistance in the parasites to the existing drugs and the lack of new drugs to replace them. Because of the potential threat drug resistance in trypanosomes poses to livestock production, there is an urgent need to conduct a drug sensitivity test on trypanosome field isolates. The aim of this study was therefore to assess the degree of resistance of *T. congolense* in the southwest of Ethiopia, where trypanocidal drugs are routinely used.

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■ MATERIALS AND METHODS

Experimental animals

Eight male indigenous zebu cattle aged 15 to 20 months were purchased from a market in the tsetse free area of Ethiopia. Prior to the experiment the animals were dewormed using a broad spectrum anthelmintic, and sprayed with acaricide. The animals were ear tagged, housed in a fly-proof isolation facility and maintained on *Eragrostis tef* straw, concentrates and water *ad libitum.*

Swiss white mice, weighing 20–25 g, were purchased from the breeding colony of the Ethiopian Health and Nutrition Research Institute and maintained on a commercial pelleted ration and water *ad libitum*. The mice were housed in a fly-proof stable at the Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit.

Trypanosome field isolates

In the southwest of Ethiopia, namely at Ghibe, Bedelle, Sodo and Arbaminch, blood samples were collected from cattle failing to respond to trypanocidal drug treatments and injected into laboratory mice for isolation. Mice incubating trypanosomes were transported to the laboratory at the Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, where series of passages and species identification were done.

Trypanocidal drugs

Diminazene aceturate (Berenil®, Lot No 518W742; Hoechst, Germany), isometamidium chloride (Trypamidium®, Lot No H0197A; Rhône Mérieux, France) were used for drug sensitivity studies. Each drug was either administered intramuscularly to cattle or intraperitoneally to mice.

Parasitological examination

Blood samples were collected from the ear vein of cattle. The dark ground/phase contrast buffy coat method (8) was used to assess the presence of trypanosome infections. The presence of trypanosomes in mice was determined by examining wet films of tail blood. All sampling, both in cattle and mice, were done three times a week for 90 days following infection.

Drug sensitivity test in mice

Each test was started from blood samples collected from a mouse infected with field isolates and with a high degree of parasitemia. Each member of a group of mice was injected intraperitoneally with 10^5 *T. congolense* (Ghibe, Bedelle, Sodo and Arbaminch isolates). Forty-eight hours after infection all mice were treated with diminazene aceturate at a dose rate of either 3.5, 7.0, 14.0 or 28.0 mg/kg body weight.

Drug sensitivity test in cattle

The experimental animals were divided into two treatment groups of four animals each. Each trypanosome isolate was inoculated into two animals one from each treatment group. Passage was undertaken from donor mice with high degrees of parasitemia (higher than 10 trypanosomes per x40 microscopic fields) previously infected with field isolates. Eight days after infection all animals were treated with 7% diminazene aceturate and 2% isometamidium chloride at a dose rate of 3.5 and 0.5 mg/kg body weight, respectively. The second treatment, at the double dose of the initial treatment, was given to all the animals immediately after showing relapse.

■ RESULTS

Development of infection

Following syringe challenge all the experimental animals showed parasitemia within 48 h in mice and 5 to 7 days in cattle. Parasitemia was first observed in cattle infected with the Bedelle isolate on day 5 postinfection, while the Arbaminch isolate was observed 7 days postinfection. The remaining two, the Ghibe and Sodo isolates, appeared in the blood of infected cattle on day 6 postinfection.

Drug sensitivity test in mice

All four isolates of *T. congolense* (Ghibe, Bedelle, Sodo and Arbaminch) were found to be resistant to 3.5 mg/kg body weight diminazene aceturate. Mice treated with 7 mg/kg and above showed no relapse throughout the experiment period with Ghibe, Bedelle and Sodo isolates. After treatment with diminazene at a dose ranging from 3.5 to 28 mg/kg body weight, trypanosomes reappeared in all mice infected with the Arbaminch isolate within 10 to 19 days (Table I).

Table I

Sensitivity to diminazene aceturate of *Trypanosoma congolense* field isolates in mice

T. congolense	Num. of mice relapsed	Dose (mg/kg)	Response	Relapse interval in days ± SD
Ghibe isolate	5/5	3.5	Relapsed	7.8 ± 1.1
	0/5	7.0	Cured	
	0/5	14	Cured	
	0/5	28	Cured	
Bedelle isolate	5/5	3.5	Relapsed	5.4 ± 0.4
	0/5	7.0	Cured	
	0/5	14	Cured	
	0/5	28	Cured	
Sodo isolate	5/5	3.5	Relapsed	7.7 ± 1.2
	0/5	7.0	Cured	
	0/5	14	Cured	
	0/5	28	Cured	
Arbaminch isolate	5/5	3.5	Relapsed	10.1 ± 0.9
	5/5	7.0	Relapsed	10.2 ± 1.1
	5/5	14	Relapsed	15.6 ± 2.0
	5/5	28	Relapsed	19.1 ± 2.1

Drug sensitivity test in cattle

Subsequent to treatment with diminazene aceturate at a dose of 3.5 mg/kg body weight, trypanosomes reappeared in all four animals within 9 to 11 days. When the same four animals were thereafter treated with 7 mg diminazene, all the animals, except No 810, infected with the Arbaminch isolate recovered from the infection. In animal No 810 trypanosomes reappeared in the blood 21 days after the second diminazene treatment. Following treatment with isometamidium chloride at a dose of 0.5 mg/kg body weight, all animals relapsed within 8 to 10 days. When the same animals were given double the dose of isometamidium (1 mg/kg) trypanosomes reappeared in all four animals within 5 to 16 days after the second treatment (Table II).

Table II

Drug sensitivity of *Trypanosoma congolense* field isolates in cattle

T. congolense	Tag No	First treatment	Relapse interval[1] (days)	Second treatment	Relapse interval[2] (days)
Ghibe isolate	813	Diminazene (3.5 mg/kg b.w.*)	10	Diminazene (7 mg/kg b.w.)	Cured
	811	Isometamidium (0.5 mg/kg b.w.)	8	Isometamidium (1 mg/kg b.w.)	5
Bedelle isolate	819	Diminazen (3.5 mg/kg b.w.)	10	Diminazene (7 mg/kg b.w.)	Cured
	812	Isometamidium (0.5 mg/kg b.w.)	7	Isometamidium (1 mg/kg b.w.)	12
Sodo isolate	817	Diminazene (3.5 mg/kg b.w.)	9	Diminazene (7 mg/kg b.w.)	Cured
	821	Isometamidium (0.5 mg/kg b.w.)	7	Isometamidium (1 mg/kg b.w.)	12
Arbaminch isolate	810	Diminazene (3.5 mg/kg b.w.)	11	Diminazene (7 mg/kg b.w.)	21
	815	Isometamidium (0.5 mg/kg b.w.)	10	Isometamidium (1 mg/kg b.w.)	16

[1] Days after the first treatment

[2] Days after the second treatment

* Body weight

■ DISCUSSION

The four isolates of *T. congolense* from Ghibe, Bedelle, Sodo and Arbaminch were found to be resistant to the curative action of diminazene (in mice and cattle) and isometamidium (in cattle) at a dose rate of 3.5 and 0.5 mg/kg body weight, respectively. The results showed that diminazene aceturate at a dose of 3.5 mg/kg and isometamidium chloride at a dose up to 1 mg/kg body weight failed to clear the infection in cattle infected with the four isolates of *T. congolense*. In addition, 7 mg of diminazene failed to clear the infection in animal No 810 infected with the Arbaminch isolate.

These results are in accordance with earlier reports from the southwest (4, 7, 9) and northwest (2) of Ethiopia. In a trial made to compare the therapeutic efficiency of diminazene aceturate at doses of 3.5 and 7.0 mg/kg body weight in two of the herds at Ghibe, Rowlands *et al*. (9) indicated that although the proportion of animals that relapsed by day 20 following treatment decreased in the higher dosage (25% vs 55%), it was not able to cure all the infections. This work was further substantiated in an experimental work by Codjia et al. (4) with 12 trypanosome isolates collected from cattle in Ghibe, where, except for one isolate sensitive to 0.5 mg/kg b.w. isometamidium chloride, all were found to be resistant to 7.0 mg/kg, 0.5 mg/kg and 1.0 mg/kg body weight of diminazene, isometamidium and homidium chloride, respectively. This multiple-resistant phenotype was even expressed at the clonal level. Mulugeta et al. (7) indicated a long-term occurrence of *T. congolense* resistant to diminazene, isometamidium and homidium in cattle of Ghibe, Ethiopia. Recently, Afewerk et al. (2) showed the presence of multiple-drug-resistant *T. congolense* in the village cattle of Metekel district, northwest Ethiopia. In the present study, *T. congolense* isolates from Ghibe, Bedelle, Sodo and Arbaminch showed a similar level of drug resistance to treatment with 3.5 mg/kg body weight diminazene and 0.5 and 1.0 mg/kg isometamidium.

The magnitude of drug resistant trypanosomes across Ethiopia is not well documented. However, the present study on a few isolates of *T. congolense* indicated the potential risk for the future in the greater part of tsetse infested areas, where the proportional infection rate of cattle by *T. congolense* is increasing (1) and where dependence on regular drug treatment for trypanosomosis control, which is a common practice now in Ethiopia, may lead to the risk of major drug resistance development. This has been observed by Bourn and Scott (3) at the Angar Guttin settlement area, Ethiopia, where during the introduction of working oxen to the area the proportion of infection rates by *T. vivax* was greater, but it was gradually overtaken by *T. congolense*. The authors also indicated that, at Anger Guttin settlement area, 36 days after prophylactic treatment with 1 mg/kg body weight isometamidium 66% of the oxen were found infected (positive for trypanosomosis), most of the infections being due to *T. congolense*. Rowlands et al. (9) for instance indicated the dynamic nature of the epidemiology of drug resistant infection in the Ghibe valley, which was 6% in 1986 and increased to 14% in 1989.

Chemotherapy and chemoprophylaxis are the common and practical methods available for the control of animal trypanosomosis, but their effectiveness is being eroded by the emergence of drug resistant trypanosomes. Of the drugs available for the treatment of animal trypanosomosis, diminazene aceturate and isometamidium chloride have been most used because of their availability and relatively low toxicity to cattle. Unfortunately, farmers can purchase a variety of trypanocidal drugs in most markets, although all trypanocidal drugs are supposed to be imported and supplied through the Ministry of Agriculture. The widespread use and misuse of drugs may have contributed to the development of drug resistance in the population of *T. congolense* parasites. The circumstances tending to produce resistant parasite populations include underdosing with trypanocidal drugs, irregular use of prophylactics, their discontinuation while cattle remain at risk, and the high incidence of trypanosomosis (11). Exposure of parasites to subtherapeutic drug concentrations, resulting from under dosing and uncontrolled use of trypanocidal drugs, and the lack of proper diagnosis, are considered the major causes of increasing drug resistance in Ethiopia.

■ CONCLUSION

This study showed the continued widespread occurrence of diminazene and isometamidium resistant populations of *T. congolense* in the southwest of Ethiopia. The increasing trend of drug resistant strains of trypanosomes is a serious threat to cattle production in Ethiopia. Therefore, there is an urgent need for detailed experimental work in the field to monitor the development of drug resistance in tsetse-infested areas of Ethiopia. Furthermore, strict supervision on the usage of trypanocidal drugs should be implemented in order to minimize the misuse. More attention should be given to the adoption of an integrated trypanosomosis control strategy, involving the vector as well as the parasite.

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Résumé

Chaka H., Abebe G. Trypanosomes résistants aux médicaments : une menace pour la production bovine du sud-ouest éthiopien

La trypanosomose est une maladie importante du bétail dans le sud-ouest de l'Ethiopie. Actuellement la chimiothérapie et la chimioprévention sont les seules méthodes pratiques de lutte contre la trypanosomose animale mais leur efficacité diminue à cause de l'émergence de chimiorésistances. Parmi les médicaments proposés pour le traitement de la trypanosomose animale, Bérénil® (acéturate de diminazène) et Trypamidium® (chlorure d'isométamidium) sont les plus utilisés du fait de leur disponibilité et de leur toxicité relativement faible pour le bétail. La sensibilité à ces médicaments de quatre souches de *Trypanosoma congolense* isolées à partir de bovins dans le sud-ouest de l'Ethiopie (Ghibe, Bedelle, Sodo et Arbaminch) a été testée sur des souris blanches (Suisses) et sur des zébus indigènes. Les résultats ont montré que, sur ce nombre limité de souches, des souches de *T. congolense* étaient résistantes aux médicaments. Les isolats de Ghibe, Bedelle et Sodo se sont révélés résistants aux doses thérapeutiques d'acéturate de diminazène (3,5 mg/kg) ainsi qu'aux doses thérapeutiques et prophylactiques standard de chlorure d'isométamidium (0,5 et 1 mg/kg). Ces trois isolats se sont cependant révélés sensibles à l'acéturate de diminazène à la dose de 7 mg/kg. La souche d'Arbaminch a été résistante aux doses recommandées par les fabricants pour les deux médicaments.

Mots-clés : Bovin – Zébu – *Trypanosoma congolense* – Résistance aux médicaments – Ethiopie.

Resumen

Chaka H., Abebe G. Tripanosomosis resistentes a las drogas: una amenaza a la producción de ganado en el sudoeste de Etiopía

La tripanosomosis es una enfermedad importante del ganado en el sudoeste de Etiopía. Actualmente, la quimioterapia y la quimioprofilaxis son los únicos medios prácticos al alcance para el control de la tripanosomosis animal, pero la eficacia está siendo erosionada por el surgimiento de tripanosomosis resistentes a las drogas. Entre las drogas disponibles para el tratamiento de la tripanosomosis animal, el Berenil® (aceturato de diminazina) y el Trypamidium® (cloridio de isometamidina) han sido las más usadas debido a la disponibilidad y a la relativamente baja toxicidad para el ganado. En el presente estudio, cuatro grupos de *Trypanosoma congolense*, aislados originalmente de ganado en el sudoeste de Etiopía (Ghibe, Bedelle, Sodo y Arbaminch), fueron analizados para la sensibilidad al Berenil y al Trypamidium, usando ratones blancos suizos y ganado cebú autóctono. Los resultados en este número limitado de grupos indican la existencia de cepas de *T. congolense* resistentes a las drogas. Los aislamientos de Ghibe, Bedelle y Sodo fueron resistentes a dosis terapéuticas de aceturato de diminazina (3,5% mg/kg) y a dosis estándar y terapéuticas de cloridio de isometamidina (0,5 y 1 mg/kg). Sin embargo, los tres grupos fueron sensitivos a 7 mg/kg de aceturato de diminazina. El cuarto, el grupo de Arbaminch, fue resistente a las dosis recomendadas por el fabricante de aceturato de diminazina y cloridio de isometamidina.

Palabras clave: Ganado bovino – Cebú – *Trypanosoma congolense* – Resistencia a medicamentos – Etíopia.