Drug resistance in recent isolates of *Trypanosoma brucei* and *Trypanosoma congolense*

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JOSHUA (R. A.). Résistance médicamenteuse d'isolats récents de *Trypanosoma brucei* et *Trypanosoma congolense*. *Revue Élev. Méd. vét. Pays trop.*, 1988, **41** (4) : 359-364.

Des études ont été menées sur des souris pour déterminer la sensibilité aux médicaments d'isolats récents de *Trypanosoma brucei* et *Trypanosoma congolense*. Chacune des onze souches de *T. congolense* et des cinq souches de *T. brucei*, toutes isolées sur des bovins, ont été testées pour sa sensibilité à la dose normale thérapeutique de chlorure d'isometamidium, d'acéturate de diminazène et de chlorure d'homidium. Simultanément des examens de contrôle ont été effectués sur des souches de laboratoire caractérisées de *T. brucei* et *T. congolense*. Six souches de *T. congolense* étaient résistantes à l'acéturate de diminazène à la dose de 3,5 mg/kg ; une seule souche s'est révélée résistante à 7 mg/kg. Dix isolats du groupe de *T. congolense* étaient résistants au chlorure d'homidium à 1 mg/kg. Tous les isolats de *T. congolense* étaient sensibles au chlorure d'isometamidium à 0,5 mg/kg. Du groupe de *T. brucei*, deux étaient résistants à l'acéturate de diminazène à 7 mg/kg alors que tous étaient résistants au chlorure d'homidium, même à 3 mg/kg. Tous les isolats de *T. brucei* étaient sensibles au chloride d'isometamidium à 0,5 mg/kg. Les trypanosomes témoins étaient immédiatement sensibles aux trois médicaments aux doses thérapeutiques normales. *Mots clés* : *Trypanosoma brucei* - *Trypanosoma congolense* - Résistance aux médicaments - Trypanocide - Bérénil - Novidium - Samorin.

INTRODUCTION

Animal trypanosomiasis has nearly the same hold on the African continent today as it had about three decades ago (10). The provision of drugs for the treatment of the disease in animals has remained static in the last twenty five years (17, 18) and hopes of new drugs coming into the market are very dim. The distribution of the disease therefore changes from time to time as efforts are made to control it. In the treatment of trypanosomiasis of cattle only isometamidium (Samorin™) and less toxic diminazene aceturate (Berenil™) have survived the waves of resistance development which followed the introduction of quinapyramine (Antrycide™) and homidium (Ethidium™) and led to their restricted use in large areas of Africa (7, 19).

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Although chemoprophylaxis against bovine trypanosomiasis is widely practised in Africa, relatively few attempts have been made to assess its effectiveness. Available data indicate that the overall situation with regard to African trypanosomiasis in domestic livestock is deteriorating (9, 10). Current trypanosomiasis control measures which involve tsetse eradication or the use of trypanocidal drugs on domestic animals have been limited in their efficacy. Recent developments in the strategic use of drugs suggest that these approaches must be constantly evaluated with a view to improving the therapeutic efficacy in the control of trypanosomiasis in domestic livestock. A surveillance of drug resistance in recent isolates of trypanosomes gives a useful feedback in formulating chemoprophylactic control measures.

The present investigation was carried out to assess the drug susceptibility of recent isolates of trypanosomes obtained from domestic cattle in three different locations in the same ecological zone.

MATERIALS AND METHODS

Animals and parasites

Blood samples were collected monthly during a two-year study period on herds of cattle kept at three different locations in the Jos Plateau *i.e.* Binchin, Ganawuri and Ryom.

At least 5 cc of heparinized blood was collected from each animal at each visit. Heparin was used at 10 I.U. per cc of bovine blood.

One Giemsa-stained thin film was made from each sample and the trypanosomes present were identified as described by HOARE (5). From each sample of cattle blood, 0.5 cc was inoculated into each of two mice. Parasitaemia in each mouse was monitored for twenty-five days.

Each infected mouse was bled from the orbital plexus as previously described by RILEY (13). Trypanosome infected blood was cryo-preserved as previously described by CUNNINGHAM *et al.* (2). One monomorphic stock of each of *T. brucei* and *T. congolense* which has been maintained in the laboratory for over five

years was included as control for each species of trypanosome.

Drugs

Berenil™ (diminazene aceturate, Hoechst Nig. Ltd.) : Two stock solutions were prepared. The first stock drug solution was 159 mg of drug granules dissolved in 100 cc of distilled water ; 0.1 cc was injected intramuscularly per mouse. This corresponds to 3.5 mg/kg. The second stock solution was made by dissolving 159 mg in 50 cc of distilled water and 0.1 cc of the solution was injected intramuscularly into each mouse. This corresponds to 7 mg/kg body weight.

Stock drug solutions were stored at -20 °C when not used.

Novidium™ (homidium chloride, Boots Nig. Ltd.) : Three stock solutions were prepared by dissolving 20 mg of drug in each of 100 cc, 50 cc, and 33 cc of distilled water respectively. Each mouse was given a single intramuscular injection of 0.1 cc of the appropriate drug dilution ; 0.2 cc of the second solution was injected into each mouse for the 4 mg/kg dose.

Samorin™ (isometamidium chloride, May and Baker, Nigeria Ltd.) : Two stock solutions were prepared by dissolving 10 mg of Samorin™ power in each of 100 cc and 50 cc of distilled water. Each mouse was given a single intramuscular injection of 0.1 cc of the appropriate drug dilution using a 1 cc hypodemic syringe and a 25 gauge needle.

Experimental animals

Balb/C mice that weighed between 18-20 g were used for trypanosome isolation and drug sensitivity tests.

Drug sensitivity test

Tests of drug sensitivity were carried out in batches of infected mice (generally 12).

Each sample of infected blood was retrieved from the cryobank and reconstituted into 3 ml of suspension in saline and inoculated intraperitoneally into each of twelve mice at 0.20 cc per mouse. Each group of infected twelve mice was divided into four subgroups of three mice each (A-D) and treated on day 5-8 post infection.

Subgroup A was treated with diminazene aceturate at 3.5 mg/kg.

Subgroup B was treated with homidium chloride at 1 mg/kg.

Subgroup C was treated with isometamidium chloride at 0.5 mg/kg.

Subgroup D was not treated with any drug but acted as controls.

All treatments for an isolate were carried out on the same day.

Parasitaemia in all of the mice was monitored for one month by microscopic examination of wet preparation from tail blood except in cases where death occurred before the expiration of the period. In the case of Berenil™ and Samorin™ repeat tests were carried out using the double the initial dose. Further drug trials with homidium were carried out in double, treble and quadruple the normal therapeutic doses. In all cases repeat tests were carried out in freshly infected mice rather than relapsed infections. Tail blood from mice that failed to show trypanosomes as from the third day till thirty days post treatment were regarded carrying trypanocide susceptible parasites. On the other hand wet blood preparations from mice that indicated the presence of motile trypanosomes within the same period were regarded as showing resistant organisms.

A careful note was also taken of the period in days at which the motile organisms were first detected in the blood of all treated mice.

RESULTS

Infectivity of isolates to laboratory animals

During the investigations, eleven stocks of *Trypanosoma congolense*, five stocks of *Trypanosoma brucei* and fourteen stocks of *Trypanosoma vivax* were detected in thirty of the cattle sampled. The *vivax* organisms did not establish any persistent infections in mice. Microscopic examination of Giemsa-stained thin film showed that one cattle had a mixed infection of *T. congolense* and *T. brucei*.

The *T. congolense* and the *T. brucei* readily established infections in mice, hence the drug tests were carried out on them. However, no drug tests were carried out on the sample with a mixed infection.

Test of drug sensitivity were carried out in eleven isolates of *T. congolense* and five isolates of *T. brucei*.

Resistance to homidium chloride

Ten out of the eleven recently isolated stocks of *T. congolense* were resistant to normal therapeutic dose

TABLE I Isolates found resistant to normal therapeutic doses in various herds.

Source of trypanosomes	Number isolated	*Trypanosoma brucei*			
		Diminazene		Homidium	Isometamidium
		3.5 mg/kg	7 mg/kg	1 mg/kg	0.5 mg/kg
Binchin	2	2	—	2	—
Ganawuri	2	2	2	2	—
Ryom	1	1	—	1	—
Laboratory stock	1	—	—	—	—
Total	6	5	2	5	—
		Trypanosoma congolense			
Binchin	4	2	—	3	—
Ganawuri	4	3	1	4	—
Ryom	3	1	—	3	—
Laboratory stock	1	—	—	—	—
Total	12	6	1	10	—

(1 mg/kg) of homidium chloride. When the dose was doubled five isolates were still resistant. Drug doses of 4 mg/kg however cured infections from nine isolates but two isolates were still resistant (Tables I, IV). All the *brucei* organisms were resistant to 3 mg/kg of homidium, increasing the drug dose to 4 mg/kg only resulted in a cure of one out of the five *brucei* isolates (Table III). The control *T. brucei* and *T. congolense* samples were readily susceptible to the drug at 1 mg/kg. It was also observed that the resistance was widespread in all the places sampled (Table II).

Susceptibility of diminazene aceturate

Drug dose of 3.5 mg/kg resulted in cure rate of five out of eleven isolates of *T. congolense* (Table I). Dose rate of 7 mg/kg resulted in a cure of ten out of eleven isolates. Thus one of the isolates was resistant to 7 mg/kg of the drug. The situation of *T. brucei* organisms are however different. All the isolates were resistant to diminazene aceturate at 3.5 mg/kg. However, only two were found resistant when 7 mg/kg drug was used.

Both control trypanosomes were readily susceptible to the drug at the recommended therapeutic dose.

Susceptibility to isometamidium

All isolates of *T. brucei* and *T. congolense* as well as control samples from the laboratory were readily susceptible to the drug at the recommended therapeutic dose (Tables I, II).

TABLE II Drug sensitivity of **T. brucei** *and* **T. congolense** *isolates.*

Drugs	Dosage	Number cured over number treated	
		T. congolense	*T. brucei*
Diminazene aceturate	3.5 mg/kg	5/11	0/5
Diminazene aceturate	7 mg/kg*	10/11	3/5
Isometamidium chloride	0.5 mg/kg*	11/11	5/5
Isometamidium chloride	1 mg/kg	11/11	5/5
Homidium chloride	1 mg/kg*	1/11	0/5
Homidium chloride	2 mg/kg	6/11	0/5
Homidium chloride	3 mg/kg	9/11	0/5
Homidium chloride	4 mg/kg	9/11	1/5

* Recommended therapeutic doses of drugs for all trypanosomes.

TABLE III Effect of increased doses of homidium chloride on relapses by **Trypanosoma brucei** *resistant to normal therapeutic doses.*

Drug doses	Number tested	Number resistant	Recrudescence of parasitaemia (days)
1 mg/kg	5	5	No remission
2 mg/kg	5	5	1.6 ± 0.5
3 mg/kg	5	5	3 ± 1
4 mg/kg	5	4	7.2 ± 2.3

Cross resistance

Infected mice were treated with only one drug. However, extrapolation of drug resistance results showed that some trypanosomes exhibited cross resistance. As shown in table V, one isolate of *T. congolense* and two isolates of *T. brucei* showed cross resistance to homidium chloride and diminazene aceturate. Such organisms were however readily susceptible to isometamidium.

Relapsed infection following drug treatment

As shown in tables III and IV increasing the dose of drug administered tended to lengthen the recrudescence of parasitaemia for both *T. congolense* and *T. brucei* in all treated mice with trypanocide resistant organisms.

TABLE IV Effect of increased doses of homidium chloride on relapses by **Trypanosoma congolense** *resistant to normal therapeutic dose.*

Drug doses	Number tested	Number resistant	Recrudescence of parasitaemia (days)
1 mg/kg	11	10	2.3 ± 0.7
2 mg/kg	11	5	6.4 ± 1.3
3 mg/kg	11	2	12.5 ± 2
4 mg/kg	11	2	15 ± 2.8

TABLE V Observed cross resistance in trypanosomes.

Drug pairs	Number found resistant	
	T. congolense	*T. brucei*
Homidium chloride and Diminazene aceturate	1	2
Homidium chloride and Isometamidium chloride	Nil	Nil
Diminazene aceturate and Isometamidium chloride	Nil	Nil

DISCUSSION

The present investigation has shown that one of the major problems encountered in the chemotherapy and chemoprophylaxis of animal trypanosomiasis is resistance to trypanocides. The very nature of disease control programme dictates that treatments must result in complete elimination of the targeted trypanosomes. Data from these tests agree with previously reported observation (6, 11, 14) that homidium resistant *T. congolense* are common in the field. In 1965 the Nigerian government's ten-year long policy of using homidium as standard therapy was abandoned in favour of Berenil™ as a consequence of the discovery of homidium resistant *T. congolense* during a survey (11). The present study extended the earlier observation of widespread resistance to homidium and to a degree, to diminazene aceturate twenty years after the adoption of the drug. Until recently isometamidium has a restricted use in Nigeria. The observed drug resistance was found in trypanosomes from all the three locations studied. Earlier observations (11) demonstrated cross resistance to both isometamidium chloride and homidium chloride, however no cross resistance to isometamidium was demonstrated in the present study. The present study corroborates that of TOURATIER (16). Previous studies by GRAY and ROBERTS (4) showed that drug resistant trypanosomes are readily transmitted by tsetse flies and that transmissibility was found to be prolonged in one stock of *T. congolense*. Repeated fly passage was without influence on drug resistance. The latter observation might explain why homidium resistant trypanosomes still persist. The circumstances tending to encourage resistance to diminazene might be principally under dosing.

The present investigation did not reveal any cross resistance between diminazene and isometamidium and thus corroborates the earlier observation (3, 19) that diminazene and isometamidium are the preferred drugs for *T. congolense* infection in ruminants. The observed susceptibility to isometamidium by these trypanosomes should not be construed as a condemnation of other trypanocides but as an observation from a limited survey. LEWIS and THOMSON (8), PINDER and AUTHIÉ (12), AUTHIÉ *et al.* (1) observed resistance to isometamidium by *T. congolense*.

The present results modify that of WILLIAMSON (19) that only isometamidium and diminazene have stood the wave of mass resistance by trypanosomes. It however confirms the observation of AUTHIÉ *et al.* (1) about resistance to diminazene aceturate and isometamidium in *T. congolense* isolates from Burkina.

The absence of proven and practical techniques of mass immunization underlines the continuing interest in the use of drugs for trypanosomiasis control.

Currently over 25 million trypanosomiasis treatments are given in Africa (15). The main constraints are cost and the need for suitable regimes to prevent the appearance of drug resistant trypanosomes. Regular surveillance of drug sensitivity of isolates will provide a useful pointer in the chemotherapy of trypanosomiasis. It is apparent from the present study that the alternate use of two drugs that do not cause mutual cross resistance might still provide reasonable prospect of control by chemotherapy.

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JOSHUA (R. A.). Drug resistance in recent isolates of *Trypanosoma brucei* and *Trypanosoma congolense*. *Revue Élev. Méd. vét. Pays trop.*, 1988, **41** (4) : 359-364.

Studies were carried out in mice to assess the drug sensitivity of recent isolates of *Trypanosoma brucei* and *Trypanosoma congolense*. Each of eleven stocks of *T. congolense* and five stocks of *T. brucei* all isolated from cattle was tested for sensitivity to normal therapeutic dose of isometamidium chloride, diminazene aceturate and homidium chloride. Contemporaneous control tests were carried out on authenticated laboratory stocks of *T. brucei* and *T. congolense*. Six stocks of *T. congolense* were resistant to diminazene aceturate at 3.5 mg/kg but only one stock was found resistant to 7 mg/kg. Ten isolates of the *T. congolense* group were resistant to homidium chloride at 1 mg/kg. All the *T. congolense* isolates were susceptible to isometamidium chloride at 0.5 mg/kg. Two of the *T. brucei* were resistant to diminazene aceturate at 7 mg/kg while all were resistant to homidium chloride at even 3 mg/kg. All *T. brucei* isolates were sensitive to isometamidium chloride at 0.5 mg/kg. The control trypanosomes were readily sensitive to the three drugs at normal therapeutic doses. *Key words* : *Trypanosoma brucei* - *Trypanosoma congolense* - Drug resistance - Trypanocidal drug - Berenil - Novidium - Samorin.

JOSHUA (R. A.). Resistencia medicamentosa de *Trypanosoma brucei* y *Trypanosoma congolense* aislados recientemente. *Revue Élev. Méd. vét. Pays trop.*, 1988, **41** (4) : 359-364.

Se utilizaron ratones para determinar la susceptibilidad a los medicamentos de *Trypanosoma brucei* y *Trypanosoma congolense* aislados recientemente. Se comprobaron cada una de las once cepas de *T. congolense* y de las cinco cepas de *T. brucei* aisladas a partir de bovinos para su susceptibilidad a la dosis normal terapeútica de cloruro de isometamidio, de aceturato de diminazeno y de cloruro de homidio. Simultaneamente, se efectuaron comprobaciones sobre cepas de laboratorio caracterizadas de *T. brucei* y *T. congolense*. Seis cepas de *T. congolense* eran resistentes al aceturato de diminazeno a la dosis de 3,5 mg/kg ; una sola cepa fué resistente a 7 mg/kg. Diez aislamientos del grupo de *T. congolense* eran resistentes al cloruro de homidio a 1 mg/kg. Todos los *T. congolense* aislados eran susceptibles al cloruro de isometamidio a 0,5 mg/kg. Dos *T. brucei* eran resistentes al aceturato de diminazeno a 7 mg/kg mientras que todos resistían al cloruro de homidio, incluso a 3 mg/kg. Todos los *T. brucei* aislados eran susceptibles al clorido de isometamidio a 0,5 mg/kg. Los tripanosomas testigos eran inmediatamente susceptibles a los tres medicamentos a las dosis terapeúticas normales. *Palabras claves* : *Trypanosoma brucei* - *Trypanosoma congolense* - Resistencia a los medicamentos - Tripanocida - Berenil - Novidium - Samorin.

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