

# Sensitivity of animal-derived *Trypanozoon* stocks from sleeping sickness endemic foci of Nigeria to trypanocides and human plasma

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Twelve *Trypanozoon* stocks isolated from semi-nomadic cattle in known sleeping sickness foci of central and northern Nigeria were studied in terms of susceptibility to two trypanocides, diminazene aceturate (Berenil) and isometamidium chloride (Samorin) and human plasma. In infected small ruminants, three of the stocks were resistant to diminazene aceturate at doses of 7.0 -14.0 mg/kg body weight (b.w.) while isometamidium chloride at doses of 1.0 mg/kg b.w. or higher failed to effect parasitological cure of infections with two of the diminazene-resistant stocks. The two isometamidium-resistant stocks were also consistently resistant to the trypanolytic action of human plasma. It is suggested that cattle are reservoirs of *Trypanosoma brucei* subspecies potentially infective to man and resistant to the therapeutic action of the known sanative pair (diminazene and isometamidium).

**Key words:** Sleeping sickness - *Trypanosoma* - Cattle - Swine - Blood plasma - Nigeria.

## INTRODUCTION

In West Africa, *Trypanosoma vivax*, *T. congolense* and *T. b. brucei* are the most important trypanosome species for livestock. The last two enjoy a wide host range, infecting also laboratory animals, wildlife and pigs as reviewed by Losos and Ikede (18) and Anosa (3). *T. brucei gambiense*, the main cause of sleeping sickness in the subregion has been isolated from pigs (10, 19, 27), sheep (26), dogs (10), game animals (20) and domestic chickens (31).

Spot surveys, abattoir samples and occasional outbreaks have provided the main sources of epidemiological studies of trypanosomiasis in Nigerian livestock over several decades (2). Although the study of the prevalence and distribution of the disease in ruminants is currently benefiting from a European grant (4), knowledge of the role of domestic animals and wildlife in its transmission in man and livestock is limited to the report of Joshua *et al.* (14) on the potential of migratory cattle to harbour human-infective trypanosomes. Also, despite reports on drug resistance among haematic trypanosome species viz *T. vivax* and *T. congolense* (2, 5) few attempts have been made to study the phenomenon among *Trypanozoon* species under Nigerian field conditions. This study was

designed to assess the sensitivity to trypanocides of *T. brucei* subspecies isolated from ruminants and pigs in sleeping sickness zones of Nigeria and to test the potential infectivity of the stocks to man.

## MATERIALS and METHODS

### Sleeping sickness foci, survey areas

The sleeping sickness endemic areas surveyed included the primordial foci in Tiv Province of Benue State. This area has been regarded as one of the oldest permanent foci of sleeping sickness in Nigeria. Others were the more northern areas of Jema'a Local Government (Kaduna State) and Plateau State. The areas lie within 7°10' - 10°25' North and 8°00' - 9°45' East and extend from the Southern to the Northern Guinea vegetation zones (fig. 1).

### The livestock

Samples were taken from bovine and porcine hosts reared semi-intensively. The pigs were housed in piggeries in the vicinity of owners' houses, while the cattle were either provided with shelter or housed in open enclosures in front of owners' residence, after the day's grazing within a mean of 5 km radius, as described by Kalu *et al.* (16).

### *Trypanozoon* stocks

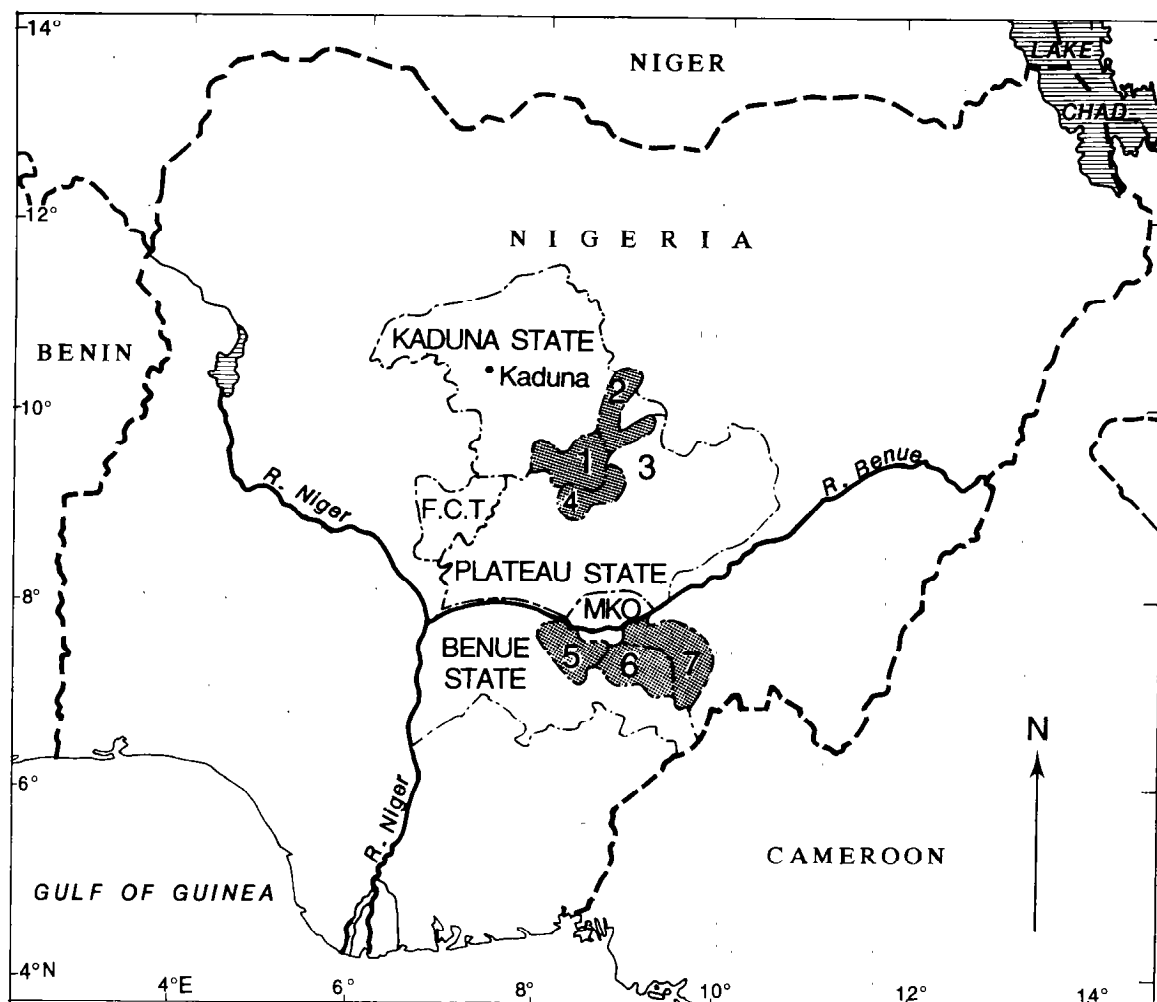
Primary isolates of *T. brucei* subspecies were made from the Livestock Investigation and Breeding Centre (Benue State) and other herds and piggeries in Gboko West and Katsina Ala Local Government Areas of Benue State. Zebu (Bunaji) cattle in Wamba and Jos (Plateau State) and Manchok/Kafanchan in Jema'a Local Government Area of Kaduna State formed the sources of the other stocks (see table III and fig. 1).

Control *Trypanozoon* stocks were obtained from the Nigerian Institute for Trypanosomiasis Research (NITR), Vom and were :

- *T. brucei brucei* 8/18, isolated from a pig at Nsukka, Nigeria (11) ;

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- |        |                           |  |             |
|--------|---------------------------|--|-------------|
| -----  | International boundary    |  | Sample area |
| -----  | State boundary            |  |             |
| F.C.T. | Federal Capital Territory |  |             |
1. Jemaa L.G.A. (Local Government Area), Kaduna State
  2. Bassa L.G.A., Plateau St.
  3. Barkin Lodi L.G.A., Plateau St.
  4. Akwanga L.G.A., Plateau St.
  5. Gwer L.G.A., Benue St.
  6. Gboko L.G.A., Benue St.
  7. Katsina Ala L.G.A., Benue St.

Figure 1 : Sleeping sickness endemic areas of Nigeria surveyed for animal trypanosomosis.

- *T. brucei gambiense* Kwa strain, isolated from the gland juice of a sleeping sickness patient in Shendam Local Government Area, Plateau State (28).

Isolates were maintained by stablation in liquid nitrogen at -196°C till required. Mixed populations were differen-

tially eluted through a DEAE cellulose (DE 52, Whatman Chemical Ltd, UK) anion exchange column with PSG according to Lanham and Godfrey (17), passaged through trypanosome naive suckling mice, and cloned derivatives were stablized. All trypanosome stablizes irrespective of source were passaged twice through mice

and their *Trypanozoon* morphology was confirmed by differentiation on Giemsa-stained thin films prior to the studies.

### Experimental animals

Adult Wister mice ( $18.2 \pm 1.5$  kg) were obtained from the Laboratory Animal Unit of the Parasitology Division, NITR, Vom. Red Sokoto goats (mean weight  $14.6 \pm 2.2$  kg) and West African dwarf sheep ( $12.5 \pm 1.8$  kg) were purchased at various times from the local markets in Mangu LGA on the tsetse-free Jos plateau. They were screened for trypanosomes and other haemoparasites, dewormed with thiophanate (Nemafax, May & Baker, U.K.) and given doses of iron dextran (Myofer 100, Farbwerke Hoechst, Germany) during a 3 month acclimatization period. The animals were fed concentrates supplemented by a grass/legume mixture and Acha hay (*Digitaria exilis*) *ad libitum*. Only parasite-free animals were used in the experiments.

### Infection of goats and sheep

Cardiac blood of donor mice infected with *Trypanozoon* stocks was pooled and the trypanosomes counted in a Neubauer haemocytometer. Each experimental small ruminant was then syringe-inoculated, via the intramuscular route, with approximately  $1 \times 10^7$  bloodstream trypanomastigotes contained, after dilution in phosphate buffered saline (PBS; pH 7.4), in 2 to 3 ml of the inoculum.

### Drug susceptibility trials

Drug treatment started at different days following prepatency (table I), depending on the pathogenicity of the strains. Diminazene aceturate (Berenil, Farbwerke Hoechst AG, Germany) and isometamidium chloride (Samorin, May & Baker Ltd, U.K.) were used at the recommended doses for ruminants of 3.5 mg and 0.5 mg/kg body weight, respectively or higher (tables I, II). Cures and relapses of infection following treatment were monitored daily by detection of parasites in ear vein blood using the dark ground buffy coat examination (23) and the haematocrit centrifugation technique (29), as described by Kalu *et al.* (15).

### Blood incubation infectivity test (BIIT)

The potential infectivity of *Trypanozoon* isolates to man was evaluated by the blood incubation infectivity test. The technique employed was that described by Rickman (24). Each incubated sample was inoculated into 5 test mice as recommended by ILCA's manual (22). Experimental animals were housed in separate cages to prevent oral transmission (21). Stocks were designated sensitive or resistant to human plasma according to the criteria of Hawking (12).

## RESULTS

### Susceptibility to trypanocides

Twelve *T. brucei* isolates were each tested for susceptibility to Berenil and Samorin. Out of these, 3 were consistently resistant to Berenil at 7.0 mg/kg b.w. or higher doses (table I). Also 2 *T. brucei* stocks were resistant to Samorin at 1.0 mg/kg b.w. (or more) (table II). The two Samorin-resistant stocks (ICS/CT 40 and GBS 2/CT 18) were among the Berenil-resistant ones. All the stocks susceptible to Berenil were also cleared from small ruminant hosts by Samorin at 0.5 mg/kg b.w.

### Sensitivity to human plasma

Two of twelve stocks tested were repeatedly resistant to normal human plasma (table III). Morphologically, the *Trypanozoon* stocks were pleomorphic like the others, susceptible to human plasma, had longer prepatent periods and also exhibited lower parasitaemia in caprine/ovine hosts (table III).

## DISCUSSION

Drug resistance among trypanosomes has been reported mostly among the haematic trypanosomes (*T. vivax* and *T. congolense*) which are more pathogenic and prevalent among ruminants in Nigeria (3, 4, 18). Trypanosomes of the *brucei* group require higher doses to effect a cure (especially with Berenil) as they invade tissues and cause relapse infections from their locations *e.g.* the brain (3, 4, 13). They may also become more pathogenic under stress conditions and in the areas where other trypanosome species have been effectively reduced by chemotherapy. The finding of a high proportion of stocks of this species resistant to trypanocides indicates the necessity of more judicious drug use and suggests that adequate diagnosis should be made prior to any therapy under field conditions.

It has long been believed that human infective *T. brucei gambiense* and *T. brucei rhodesiense* also occur in animal hosts (8, 30). Direct evidence using human volunteers (9) and indirect evidence by the blood incubation infectivity test (6, 20, 25) support this belief. Also, the role of domestic animals as reservoirs of trypanosomes infective to man has been reviewed by Mehlitz *et al.* (20) while, in West Africa, various domestic animals including cattle, pig, sheep, dog and chicken have been incriminated by various workers (10, 19, 20, 26, 27, 31).

In Nigeria, the only evidence of the involvement of domestic livestock in the transmission of sleeping sickness is that provided by Joshua *et al.* (14) from nomadic cattle grazing on the Jos plateau. The observation by Hawking (12) that all forms of Gambian (West African) type human-infective trypanosomes are resistant to human plasma has been

TABLE I  
Sensitivity of *Trypanosoma brucei* subsp. to Berenil® (diminazene aceturate)\*

Test Animal	<i>T. brucei</i> Stock Number	Prepatent period (days)	Days of infection before treatment	Parasitaemia on treatment day	Dose (mg/kg body weight)	Days between treatment and relapse	Remarks (P.f days)
Goats (6)*	ICS/CT 6	7	8	2/1 <sup>a</sup>	7.0	10	Relapsed/resistant
Goats (6)	ICS/CT 40	—	13	L	10.5	32	Relapsed/resistant
		9	4	1/5	7.0	21	Relapsed/resistant
		9	3	1/1	10.5	(5 : dead)	Relapsed/resistant
Sheep (2)	ICS/CT203	9	16	10/1	7.0	—	Sensitive (57)
Goat (2)	ICS/CT690	5	21	6/1	7.0	—	Sensitive (24)
Goat (2)	GBS 12/PG 30	4	3	1/1	7.0	—	Sensitive (60)
Goat (2)	GBS 12/PG 32	12	28	12/1	7.0	—	Sensitive (92)
Goat (2)	GBS 12/PG 33	8	15	M	7.0	—	Sensitive (45)
Goat (2)	GBS 12/PG 40	3	7	L	7.0	—	Sensitive (28)
Sheep (1)	GBS 2/CT 9	6	4	L	7.0	—	Sensitive (63)
Goat (3)	GBS 2/CT 18	11	8	1/1	7.0	7	Relapsed/resistant
		—	5	1/5	14.0	(9 : dead)	Relapsed/resistant
Sheep (2)	WA/CT 46	4	3	15/1	7.0	—	Sensitive (84)
Sheep (1)	KAF 2/SH 5	7	1	5/1	7.0	—	Sensitive (30)
Goat (1)							

\* All figures are given as mean of the number of ruminants used for each trypanosome stock.

Parasitaemia : wet film examination at low power (10 x 40) magnification.

<sup>b</sup> P-f = mean parasite - free days in test host before end of observation.

<sup>a</sup> Bracketed values indicate number of experimental animals used.

<sup>c</sup> Number of parasites/Number of microscopic fields.

L = light ; M = moderate.

TABLE II  
Susceptibility of Berenil®-resistant *Trypanozoon* stocks to Samorin (isometamidium chloride)

Test host (Number used)	<i>Trypanozoon</i> stock	PPP <sup>1</sup> (Days)	Treatment day after PPP	Parasitaemia on treatment day	Dose (mg/kg)	Relapse (Day after treatment)	Remarks (P-f days)
Goat (2)	ICS/CT 6	14	6	1/2 <sup>a</sup>	0.5	—	Susceptible (60)
Goat (2)	ICS/CT 40	11	5	1/1	0.5	15	Relapse
		8	3	3/1	1.0	20	Resistant
Goat (2)	GBS 2/CT 18	14	7	1/2	0.5	18	Relapse
(2)	"	18	5	4/1	1.0	17	Resistant
(1)	"	11	4	1/2	1.5	32	"

<sup>1</sup> PPP = prepatent period.

<sup>b</sup> P-f : mean parasite-free days in test host before end of observation.

<sup>a</sup> Number of parasites/number of microscopic fields.

confirmed by Brun and Jenni (7). This study provides evidence that semi-nomadic cattle, in close proximity to herd-owners, harbour trypanosomes potentially infective to man in sleeping sickness endemic areas of Nigeria. The Nigerian control *Trypanozoon* strains were either inactivated by the normal human plasma (*T. brucei brucei* 8/18) in line with the trypanolytic action of human plasma, serum or blood, or resistant to it (*T. brucei gambiense* Kwa) (6, 9).

In addition to being resistant to human plasma and trypanocides, the low parasitaemia of the 2 stocks in experi-

mental ruminants, close contact between livestock and their owners in known sleeping sickness endemic foci is highly suggestive of the reservoir status of cattle in this area. *G. tachinoides*, a riverine tsetse fly species with high vectoral capacity for the sleeping sickness parasite, has recently been reported to be the only vector of hyperendemic ruminant trypanosomosis in Gboko, Benue state (16) - one of the areas covered by this study. These findings, under conditions where transmission could be essentially from animal to man (1), are of epidemiological importance for human sleeping sickness.

TABLE III  
Susceptibility of *Trypanozoon* stocks to normal human plasma (NHS)

Stock Number	Sources		Incubation in plasma		Infection in mice		Remarks
	Host	Locality	Sheep	Human	Inoculated	Infected	
ICS/CT 6	Muturu (M)	Raav (Benue)	+ —	— +	5 5	5 0	Sensitive to NHS
ICS/CT 40	Zebu (Z)x N'Dama (N)	"	+ —	— +	5 5	5 3	Resistant
ICS/CT 203	N'Dama (N)	"	+ —	— +	5 5	5 0	Sensitive
ICS/CT 690	Zebu X N	"	+ —	— +	5 5	5 0	"
GBS 12/PG 30	Pig	Gboko (Benue)	+ —	— +	5 5	5 0	"
GBS 12/PG 32	Pig	"	+ —	— +	5 5	5 0	"
GBS 12/PG 40	Pig	Manchok (Kaduna)	+ —	— +	5 5	5 0	"
GBS 2/CT 9	White Fulani (Zebu)	Gboko	+ —	— +	5 5	5 5	Resistant
WAM/CT 46	White Fulani	Wamba (Plateau)	+ —	— +	5 5	5 0	Sensitive
KAF 2/SH 5	Sheep	Kafanchan (Kaduna)	+ —	— +	5 5	5 0	"
* <i>T. brucei</i> 8/18	Pig	Nsukka	+ —	— +	5 5	5 0	Sensitive
* <i>T. gambiense</i> (Kwa)	Man	Kwa	+ —	— +	5 5	5 5	Resistant

\* Reference stocks for animal (*T. brucei* 8/18) and human (Kwa) infective *Trypanozoon*.

## CONCLUSION

In the sleeping sickness endemic foci of Nigeria and particularly the Tiv Province primordial area, cattle harbour trypanosome strains potentially infective to man. These *Trypanozoon* strains are also resistant to both diminazene aceturate and isometamidium chloride which are two commonly used trypanocides. This phenomenon may be responsible for cases of reported drug resistance under field conditions.

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**La sensibilité de douze souches de *Trypanozoon* isolées de bovins semi-nomades provenant de foyers endémiques connus de maladie du sommeil situés dans le Centre et au Nord du Nigeria a été étudiée vis-à-vis de deux trypanocides, l'acéturate de diminazène (Berenil) et le chlorure d'isométymidium (Samorin), et du plasma humain. Chez les petits ruminants infectés, trois souches ont montré une résistance vis-à-vis de l'acéturate de diminazène administré à des doses de 7,0 - 14,0 mg/kg de poids vif, alors que le chlorure d'isométymidium administré à des doses de 1,0 mg/kg de poids vif ou à des concentrations supérieures n'est pas parvenu à guérir les infections dues à deux des souches résistantes à l'acéturate de diminazène. De même, les deux souches ayant manifesté une résistance vis-à-vis du chlorure d'isométymidium se sont montrées régulièrement résistantes à l'activité trypanolytique du plasma humain. Cela tend à indiquer que les bovins jouent le rôle de réservoir du sous-genre *Trypanosoma brucei*, lequel est une source potentielle d'infection pour l'homme et résiste à l'action thérapeutique de ces deux médicaments, l'acéturate de diminazène et le chlorure d'isométymidium.**

**Mots clés :** Maladie du sommeil - *Trypanosoma* - Bovin - Porcin - Plasma sanguin - Nigeria.

**KALU (A.U.).** Sensibilidad hacia tripanocidas y plasma humano de stocks de *Trypanozoon* de derivados animales, provenientes de focos endémicos de la enfermedad del sueño en Nigeria. *Revue Élev. Méd. vét. Pays trop.*, 1995, 48 (2): 139-144.

**Se estudió la susceptibilidad hacia dos tripanocidas, el aceturato de diminazina (Berenil) y el clorhidro de isometamidina (Samorin), así como del plasma humano, en doce stocks de *Trypanozoon* aislados a partir de ganado semi-nómada, proveniente de focos conocidos de la enfermedad del sueño, en Nigeria central y del norte. Tres de los stocks provenientes de pequeños ruminantes infectados, fueron resistentes al aceturato de diminazina, a dosis de 7,0 - 14,0 mg/kg de peso vivo, mientras que el clorhidro de isometamidina a dosis de 1,0 mg/kg de peso vivo, fue incapaz de producir un efecto parasitológico curativo de las infecciones en dos de los stocks de diminazina-resistentes. Los dos stocks isometamidina-resistentes, presentaron también resistencia a la acción tripanolítica del plasma humano. Se sugiere que este ganado representa un reservorio de sub-especies de *Trypanosoma brucei* potencialmente infeccioso al hombre y resistente a la acción terapéutica de los dos productos curativos mencionados (diminazina y isometamidina).**

**Palabras clave:** Enfermedad del sueño - *Trypanosoma* - Bovino - Cerdo - Plasma sanguíneo - Nigeria.