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Autor: Sones, K.R. / Njogu, A.R. / Holmes, P.H.

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[1] RMB Animal Health Ltd, Rainham Road South, Dagenham, Essex, RM10 7XS, England
[2] KETRI, P.O. Box 362, Kikuyu, Kenya
[3] University of Glasgow Veterinary School, Glasgow G61 1QH, Scotland

Assessment of sensitivity of *Trypanosoma congolense* to isometamidium chloride: a comparison of tests using cattle and mice

K. R. Sones[1], A. R. Njogu[2], P. H. Holmes[3]

Summary

The sensitivities of 3 strains of *Trypanosoma congolense* to isometamidium chloride (Samorin) were determined in mice and cattle, with the objective of evaluating sensitivity testing in mice as a means of predicting curative doses in cattle. Comparison of mouse effective dose 80% (ED_{80}) or curative dose 80% (CD_{80}) values with cattle minimum curative dose (MCD) values demonstrated a wide variation between trypanosome strains. Although a mouse test may give a broad indication of the sensitivity of a strain, it cannot be used to predict curative doses for cattle. It was concluded that care should be exercised in extrapolating the results of a mouse test to cattle.

Key words: *Trypanosoma congolense;* cattle; mice; isometamidium; diminazene aceturate; drug-sensitivity.

Introduction

Bovine trypanosomiasis is acknowledged to be one of the major constraints limiting livestock production in sub-Saharan Africa (Trail et al., 1985). In the absence of an effective vaccine, and lacking a coherent strategy for controlling the insect vectors, the livestock industry is highly dependent on the small number of drugs currently available for the treatment and prevention of the disease. Amongst that limited range of drugs, isometamidium chloride (Samorin, May and Baker Ltd, England; Trypamidium, Rhône Mérieux, France) is the most widely used chemoprophylactic.

Correspondence: Professor P. H. Holmes, Department of Veterinary Physiology, University of Glasgow Veterinary School, Bearsden Road, Glasgow G61 1QH, Scotland

Although examples do occur in the literature of demonstrable field resistance to isometamidium, and indeed to the other trypanocidal drugs, the term "drug resistance" is frequently used to explain any therapeutic or prophylactic failure, often with little or no supporting evidence. The existence of drug resistant strains, though important, is only one of many possible explanations for such failures. This makes it particularly important to be able to investigate reported incidences of drug resistance from the field, and ideally to be able to evaluate the sensitivities of the strains of trypanosomes implicated.

The mouse sensitivity test has for many years been the standard method of assessing the sensitivity of strains of trypanosomes, although *Trypanosoma vivax,* because it is generally not rodent infective, presents a special problem. A mouse test has obvious advantages over a comparable cattle sensitivity test in terms of the space and facilities required and the cost involved, but is only of value if the results obtained can be related in a predictable manner to the results that would have been achieved in a cattle experiment. Surprisingly, little effort has been expended in evaluating the mouse sensitivity test. Hawking (1963) presents comparative cattle and mouse sensitivity data for several strains of *T. congolense,* and a range of trypanocidal drugs including metamidium, the predecessor of isometamidium. His data for metamidium are limited, and the experimental design can be criticized. The original strains were prepared by Whiteside in Kenya where they were tested in grade (i.e. zebu × exotic) cattle, and were then passaged up to 20 times in mice before being transferred to Hawking in London for testing in mice. Comparisons of cattle and mouse data are possible for 3 strains of *T. congolense,* although for none of the strains was the curative dose of metamidium in cattle precisely determined.

The objectives of the present experiment were to determine mouse minimum effective dose (MED) and minimum curative dose (MCD) values, together with comparative data in cattle, for 3 strains of *T. congolense* treated with isometamidium, in order to evaluate the mouse sensitivity test as a means of predicting curative doses in cattle.

Materials and Methods

Experimental animals

Cattle: Trypanosome-naive, Boran steers, originally obtained from a tsetse-free region of Kenya, were housed in individual stalls in fly-proof accommodation. Cattle were maintained on a diet of hay, ad libitum, supplemented with concentrates. Water and a mineral lick were always available. Prior to the start of the experiment cattle were treated with an anthelmintic (Nilzan, Wellcome Kenya Ltd, Kenya) and, as a precaution against tick-borne disease, cattle were sprayed with an acaricide (Delnav, Wellcome Kenya Ltd, Kenya) once a week.

Mice: For the mouse sensitivity tests, female random-bred Swiss mice (20–30 g bodyweight) were used. Female, outbred mice obtained from the KETRI closed colony were used for routine subinoculation of cattle blood.

Trypanosomes

The 3 strains of trypanosomes used in this experiment were obtained as cryopreserved stabilates. Prior to use the strains were expanded in sublethally irradiated rats, and then stored as stabilates in liquid nitrogen until required. The strains were:

- *T. congolense* KETRI 2885 (IL Nat 3.1), a doubly cloned derivative of an isolate originally made from a lion in Serengeti National Park (Geigy and Kauffman, 1973; Nantulya et al., 1984), known to be sensitive to isometamidium at 0.5 mg/kg in mice (Whitelaw et al., 1986).
- *T. congolense* KETRI 2883 (ILRAD 3035), a derivative of an isolate made from a steer (M1068) maintained under isometamidium prophylaxis at Muhaka, Coast Province, Kenya.
- *T. congolense* KETRI 2880 (ILRAD 2856), a derivative of an isolate (Banankeledaga/83/CRTA/67) made from a steer in Burkina Faso, and reported to be partially sensitive to isometamidium at 4 mg/kg in mice (Pinder and Authié, 1984).

Methods

Cattle and mice were each infected with approximately 1×10^5 trypanosomes, the former by intravenous inoculation and the latter by intraperitoneal inoculation (ip). After infection cattle parasitaemias were monitored three times a week by the buffy coat method (Paris et al., 1982), but with direct rather than phase-contrast illumination, and using a peripheral blood sample collected from a marginal ear-vein. Packed cell colume (PCV), determined from a jugular blood sample, and rectal temperature were also recorded three times a week, and bodyweight recorded once a week. Mice were monitored by examination of tail-blood wet-films. In addition, at 2 weekly intervals, jugular blood samples were collected from all cattle judged trypanosome-negative by the buffy coat method, and 0.5 ml aliquots were inoculated into mice (3 per steer). After subinoculation mice were monitored by tail-blood wet-film examination for 8 weeks. No infections were detected by subinoculation of blood that were not also detected by the buffy coat technique, although in some cases infections were detected earlier by subinoculation.

For both cattle and mice, treatment with isometamidium was administered at first detection of trypanosomes, at the dose rates shown in Table 1. Initially 10 cattle were infected with each strain; 3 cattle were subsequently treated, by deep intramuscular injection in the middle third of the neck, at each of 0.1, 0.5 or 1.0 mg/kg bodyweight, while one animal was left untreated as an infection control. In the case of KETRI 2885 for which 0.1 mg/kg isometamidium proved to be curative, 2 further cattle were infected and treated at either 0.01 or 0.001 mg/kg, and an additional steer was also infected with KETRI 2880 and treated at 2 mg/kg.

When the PCV of the infection control steers declined to 17%, they were withdrawn from the experiment and treated with diminazene aceturate (Berenil, Hoechst, W. Germany) initially at 3.5 mg/kg, increasing the dosage in increments of 3.5 mg/kg when relapse infections were encountered.

For all cattle in which relapse infections occurred after initial treatment with isometamidium, parasitaemias were monitored for 7 days, and then the cattle were retreated with the same isometamidium dose. When a second relapse infection occurred, cattle were again monitored for 7 days and then treated with diminazene aceturate. An exception was the steer infected with KETRI 2880 and treated at 1 mg/kg isometamidium, which relapsed twice to this dose (Steer 895). This animal was then treated at 2 mg/kg isometamidium.

Mice were treated at one of a range of isometamidium doses up to 20 mg/kg by ip injection, with 5 mice in each treatment group. The parameters ED_{80} and CD_{80} were determined for mice, that is the minimum dose which brought about either temporary clearance of circulating trypanosomes (ED_{80}), or a permanent cure (CD_{80}), in at least 80% of mice, i.e. 4 out of 5. In the case of KETRI 2880 the assessment of ED_{80} was complicated by characteristic remission of infection, even in untreated mice, following the first parasitaemic peak. The ED_{80} value for KETRI 2880 was therefore taken to be the minimum dose which resulted in temporary clearance of circulating trypanosomes in 4 or more mice, of at least twice the mean duration observed in the infection controls.

Cattle and mice were monitored for at least a 100 day post-treatment observation period.

Table 1. Allocation of experimental animals to treatment groups (number of animals per group)

Samorin mg/kg	Cattle			Mice		
	T. congolense KETRI			*T. congolense* KETRI		
	2885	2883	2880	2885	2883	2880
0	1	1	1	5	5	5
0.001	1			5		
0.01	1			5		
0.1	3	3	3	5	5	
0.5	4	3	3	5	5	
1.0	3	3	3	5	5	5
2.0			2	5	5	5
5.0				5	5	5
10.0				5	5	5
20.0				5	5	5

Table 2. Responses of *T. congolense*-infected cattle to treatment with isometamidium

Strain	Dose mg/kg	Effect of treatment (number of cattle)			
		Number in treatment group	No effect[a]	Temporary[b] clearance	Cure[c]
2885	1.0	3	0	0	3
	0.5	4	0	0	4
	0.1	3	0	0	3
	0.01	1	0	0	1
	0.001	1	0	0	1
	0	1	1	0	0
2883	1.0	3	0	0	3
	0.5	3	0	0	3
	0.1	3	1	2	0
	0	1	1	0	0
2880	2.0	2	0	0	2
	1.0	3	0	3	0
	0.5	3	0	3	0
	0.1	3	1	2	0
	0	1	1	0	0

[a] Animals remained parasitaemic after treatment.

[b] Parasitaemia dropped below limit of detection following treatment, but trypanosomes reappeared during observation period.

[c] Trypanosomes disappeared from the circulation following treatment and did not reappear during a 100 day observation period.

Results

Prepatent period

For KETRI 2885 the mean prepatent periods (±SD), following needle challenge, were 7 (±0) days in cattle and 5 (±0) days in mice; for KETRI 2883, 7.1 (±0.3) and 5.9 (±0.5) days and for KETRI 2880, 10.6 (±0.7) and 9.5 (±1.4) days, respectively.

Virulence and pathogenicity

Sixty percent (3/5) of mice infected with either KETRI 2885 or 2880 which received no treatment, died during a 100 day observation period. All mice (5/5) infected with KETRI 2883 and left untreated died, with a mean number of days to death of 28.6 (±22.6). Deaths of mice which were infected with KETRI 2885 or 2880 tended to be after a more chronic infection with mean numbers of days to death of 72.7 (±19.6) and 84.3 (±6.0), respectively.

Infection control cattle were prevented from dying by intervention with diminazene aceturate when their PCV had dropped to 17%. PCV values at infection were similar for all three cattle (33–34%). In the case of the KETRI 2880 infection control the PCV had declined to 17%, 33 days after infection. For the KETRI 2883 infection control this point had been reached 34 days post-infection and for the KETRI 2885 infection control, 38 days post-infection.

Responses to treatment with isometamidium

Cattle: The responses of cattle infected with the 3 strains of *T. congolense* and treated with isometamidium, are shown in Table 2. Cattle infected with KETRI 2885 were cured by treatment at 0.001 mg/kg isometamidium, the lowest dose used.

KETRI 2883 was temporarily cleared from the circulation of 2 out of 3 cattle treated at 0.1 mg/kg. All cattle treated at 0.5 or 1.0 mg/kg were permanently cured.

No cattle infected with KETRI 2880 and treated with a single injection of isometamidium at doses up to 1 mg/kg were cured, although 2 out of 3 steers treated at 0.1 mg/kg and all 3 treated at 0.5 or 1.0 mg/kg experienced temporary remission of infection.

Cattle which either relapsed after initial treatment with isometamidium, or for which initial treatment had no effect, were retreated at the same dose (Table 3).

Two out of 3 cattle infected with KETRI 2883 and treated at 0.1 mg/kg experienced a temporary clearance of circulating trypanosomes, and all 3 were temporarily cleared by repeat treatment at the same dose.

Although KETRI 2880 was temporarily cleared from the circulation of 2 out of 3 cattle treated at 0.1 mg/kg, repeat treatment at the same dose had no effect. Repeat treatment at the same dose for cattle initially treated at 0.5 mg/kg

Table 3. Aparasitaemic intervals following treatment and retreatment with isometamidium

Steer number	Dose mg/kg	Days to relapse			
		1st treatment	mean	Repeat treatment	mean
KETRI 2883					
899	0.1	0		7	
902	0.1	14	8.3	9	9.3
893	0.1	11		12	
	≥0.5	>100			
KETRI 2880					
803	0.1	0		0	
884	0.1	3	2.3	0	0
881	0.1	4		0	
877	0.5	21		16	
818	0.5	13	15.7	14	15.7
801	0.5	13		17	
819	1.0	37		>100	
895	1.0	19	44.3	72	
870	1.0	77		>100	
895	2.0	>100			
805	2.0	>100			

again resulted in temporary aparasitaemia. Of the 3 cattle originally treated at 1.0 mg/kg, one steer relapsed to repeat treatment at the same dose while the remaining 2 steers did not relapse during a 100 day post-retreatment observation period. The steer which relapsed twice after treatment at 1.0 mg/kg was then treated at 2 mg/kg, and did not relapse during the following 100 days. An additional steer infected with KETRI 2880 and treated at 2 mg/kg was also cured, corroborating this observation.

These results indicated that the MCD for KETRI 2885 was less than or equal to 0.001 mg/kg isometamidium, for KETRI 2883 was 0.5 mg/kg and for KETRI 2880 was 2 mg/kg.

The intervals from treatment to relapse for KETRI 2880 and 2883 in cattle are shown in Table 3. Cattle infected with KETRI 2883 and treated with isometamidium at a dose of 0.1 mg/kg relapsed in a mean of 8.3 days, and after retreatment at the same dose in a mean of 9.3 days.

For KETRI 2880 the mean number of days to relapse following treatment at 0.1 mg/kg was 2.3 days. Retreatment at the same dose had no effect. Mean number of days to relapse after both treatment and retreatment at 0.5 mg/kg was 15.7 days. Cattle treated at 1.0 mg/kg relapsed after a widely varying timespan (19–77 days post-treatment) with a mean of 44.3 days. Only one steer

Table 4. Responses of *T. congolense*-infected mice to treatment with isometamidium (5 mice per treatment group)

Strain	Dose mg/kg	Effect of treatment (number of mice)		
		No effect[a]	Temporary[b] clearance	Cure[c]
2885	>0.1	0	0	5
	0.1	0	0	5
	0.01	0	4	1
	0.001	5	0	0
	0	5	0	0
2883	20.0	1	0	4
	10.0	0	1	4
	5.0	1	1	3
	2.0	0	5	0
	1.0	3	2	0
	0.5	4	1	0
	0.1	5	0	0
	0	5	0	0
2880	20.0	0	1	4
	10.0	0	4	1
	5.0	0	5	0
	2.0	0	5	0
	1.0	3	2	0
	0	0	4	1

[a] [b] [c] as Table 2

retreated at 1 mg/kg relapsed (day 72), and this animal remained aparasitaemic during a 100 day observation period following treatment at 2 mg/kg.

Mice: The responses of mice infected with one of the 3 strains of *T. congolense* and then treated with one of a range of isometamidium dosages are shown in Table 4. For KETRI 2885 the ED_{80} was 0.01 mg/kg isometamidium and the CD_{80} value was 0.1 mg/kg. For KETRI 2883 and 2880 the corresponding values were 2 and 10 mg/kg and 2 and 20 mg/kg, respectively.

Comparison of mouse ED_{80} or CD_{80} and cattle MCD values

Mice ED_{80} and CD_{80} values and cattle MCD values are compared in Table 5. The ratios in both cases show a wide range of variation ($\geqslant$10 fold) between the 3 different strains.

Responses to diminazene aceturate

Cattle which relapsed twice to their designated isometamidium dosage, and also the untreated infection control animals when their PCV had dropped to 17%, were treated with diminazene aceturate. Treatment was initially at-

Table 5. Comparison of mouse ED_{80} and CD_{80} with cattle MCD values (mg/kg)

Strain	ED_{80} mouse A	CD_{80} mouse B	MCD cattle C	Ratio A:C	Ratio B:C
2885	0.01	0.1	≤0.001	≥10	≥100
2880	2	20	2	1	10
2883	2	10	0.5	4	20

Table 6. Comparison of curative dose and subcurative treatment to relapse interval for cattle (treated at 0.1 mg/kg isometamidium) and mice (treated at 2 mg/kg isometamidium)

Strain	Cattle		Mice	
	MCD	Aparasitaemic interval (0.1 mg/kg)	CD_{80}	Aparasitaemic interval (2 mg/kg)
2885	≤0.001	>100	0.1	>100
2883	0.5	8.3	10	8.8
2880	2.0	2.3	20	6.0

tempted at 3.5 mg/kg, increasing the dosage in increments of 3.5 mg/kg if further relapse infections were encountered. Relapse infections were monitored for 7 days before treatment was attempted at a higher dose.

KETRI 2883. Four cattle infected with KETRI 2883 (3 initially treated at 0.1 mg/kg isometamidium, and the infection control) were treated with diminazene aceturate. All cattle relapsed to treatment at 3.5, 7.0 and 10.5 mg/kg diminazene aceturate, at mean intervals from treatment of 7.5 (±1.0), 14.3 (±3.3) and 17.0 (±6.0) days, respectively. At the time the experiment was terminated – up to 35 days after treatment – no cattle had relapsed to treatment at 14.0 mg/kg.

KETRI 2880. Seven cattle infected with KETRI 2880 (3 initially treated at 0.1 mg/kg isometamidium; 3 initially treated at 0.5 mg/kg isometamidium, and the infection control) were treated with diminazene aceturate. All cattle relapsed following treatment at 3.5 and 7.0 mg/kg diminazene aceturate, at mean intervals from treatment of 13.3 (±4.5) and 28.1 (±11.9) days, respectively. At the time the experiment was terminated, no cattle had relapsed to 10.5 mg/kg: for 5 of the 7 cattle this was in excess of 100 days from date of treatment.

Discussion

The 3 strains of *T. congolense* used in this experiment covered a wide range of sensitivities to the prophylactic drug isometamidium, with a difference of at

least 2000 fold in the cattle curative doses between the least and most sensitive strains. KETRI 2885 was extremely sensitive to isometamidium with a dose of 0.001 mg/kg proving to be curative in cattle, while KETRI 2880, the least sensitive strain, could still be cured by treatment at less than the maximum tolerated dose.

Hawking (1963) cautiously concluded that 'tests in mice apparently give a broad indication of the probable response of a strain in cattle'. Pinder and Authié (1984) suggested that his results showed a close relationship between mouse effective dose and cattle curative dose, with mouse curative doses being at least 10 times those for cattle.

The results of the present experiment show that there is considerable variation between strains in the relationship between mouse effective dose and cattle results. Thus while for KETRI 2880 and 2883 the mouse ED_{80} values were close to cattle MCD values, there was at least a 10 fold difference in the two values for KETRI 2885. Similarly while mouse CD_{80} values for KETRI 2880 and 2883 were approximately 10 times cattle MCD values, in the case of KETRI 2885 there was a difference of at least 100 times between the values for the two species. Based on these comparisons it is concluded that although the result of a mouse test may give a broad indication of the sensitivity of a strain, it cannot be reliably used to predict curative doses for cattle.

Mouse sensitivity tests can also be criticized on the grounds that they cannot be employed to investigate the sensitivity of strains of *T. vivax,* and that not all cattle isolates of *T. congolense* are rodent infective. The variable infectivity of strains of *T. congolense* to rodents may be related to the morphological type of trypanosomes present (Godfrey, 1961) – greater rodent infectivity being associated with the longer *dimorphon*-type rather than the shorter *congolense*-type –, and also, as has been reported for *T. vivax,* to the stage of infection in the donor (Desowitz and Watson, 1951).

Besides the obvious difference in bodysize between cattle and mice, there may also be differences in the pharmacokinetics and metabolism of trypanocidal drugs in the two species. Furthermore, even using the same stabilate for infection, the trypanosome population which becomes established in cattle may differ from that in mice. One, or more, of these factors may explain the observed differences in isometamidium dose rates required to cure infections in the two species. The lack of correlation between drug sensitivity values determined in the two species for different strains of *T. congolense,* could also be related to differences in parasite biology. For example, the propensity of a strain to sequester in drug inaccessible sites in one or other of the host species, or the relative susceptibility of a strain to bovine or murine products of drug metabolism.

If mouse sensitivity tests cannot be relied upon to predict curative dose rates of isometamidium for cattle, then other approaches need to be investigated. Williamson and Stephen (1960) developed a method to assess sensitivity

of strains of *T. vivax* to homidium. They had previously noted that in rodents, drug resistance was characterized by a reduction in the interval from subcurative treatment to subsequent relapse. Laboratory reared *Glossina morsitans* were fed on cattle infected with the trypanosomes to be investigated, and used to infect a batch of sheep which were then treated with a low dose of homidium. The interval from treatment to relapse was taken as an index of the degree of homidium resistance. A good indication of the relative sensitivities of the 3 strains of *T. congolense* used in the present study could be obtained by a similar approach, but using needle challenge (Table 6). For both cattle and mice, the subcurative treatment to relapse interval was shorter for the more resistant strain.

With improved techniques for the in vitro cultivation of trypanosomes, the possibility of using in vitro methods to assess sensitivity is now a reality. For example, a method has been developed by Borowy et al. (1985) for the in vitro assessment of drug sensitivities of a strain of *T. brucei brucei,* and this approach has been adapted for *T. congolense* (Borowy, personal communication). In this method trypanosomes are incubated in tissue culture plates for 24 h in the presence of one of a range of drug concentrations, using, for *T. congolense,* bovine aorta endothelial cells as a feeder layer. The rate of replication of trypanosomes in the drug-treated wells is compared to an untreated control, and the drug concentration which results in a 50% inhibition in replication rate (IC_{50} value) is recorded. The method is not without its problems however: it is unlikely that field isolates, or even laboratory strains, of *T. congolense* will immediately take to in vitro cultivation, and a period of adaptation is likely to be required. The conditions needed during this adaptation phase vary between strains (Ross et al., 1985). It is also possible that the adaptation process involves selection of the trypanosome population, although a recent experiment involving 3 strains of *T. congolense* demonstrated similar sensitivities in mice both before and after the adaptation process (Brown et al., 1987). Drugs may be toxic to the feeder layer at the concentrations needed to inhibit resistant strains, and it could therefore be difficult to distinguish between direct effects of the test drug on the trypanosomes, and indirect effects due to an impaired feeder layer. It is also possible that isometamidium is metabolized in the bovine to metabolites of enhanced trypanocidal activity, and therefore the use of raw isometamidium for in vitro tests may be questionable. It would however be of considerable interest to see how the results of cattle sensitivity tests correlate with in vitro estimates of sensitivity.

KETRI 2880 and 2883 were both resistant to diminazene aceturate, the former at dose rates up to 7 mg/kg and the latter at dose rates up to 10.5 mg/kg. Until recently (Authié, 1984) there has been no evidence of cross-resistance between isometamidium and diminazene, and the two drugs have been regarded as a 'sanative pair' (Whiteside, 1961). Finelle (1974) recommended the alternate use of isometamidium and diminazene, and, prompted by fears of

drug resistance, the management of Mkwaja Ranch in Tanzania adopted a regime based on the alternate use of isometamidium and diminazene aceturate in 1980 (Trail et al., 1985). In the present experiment KETRI 2880 has been shown to be resistant to the manufacturer's recommended doses of both isometamidium (0.5–1.0 mg/kg) and diminazene aceturate (3.5–7.0 mg/kg). The occurrence of strains of this type clearly has serious implications for the effective control of trypanosomiasis by trypanocidal drugs, and emphasises the need for reliable techniques for the assessment of sensitivity.

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Authié E.: Mise en évidence d'une résistance aux trypanocides parmi des souches de *Trypanosoma congolense* récemment isolées au Burkina. Appearance of trypanocide-resistant strains of *T. congolense* recently isolated in Burkina. Rev. Elev. Méd. vét. Pays trop. *37* (No. spécial), 219–235 (1984).

Borowy N. K., Fink E., Hirumi H.: Five commonly used trypanocides assayed in vitro with a mammalian feeder layer system for cultivation. Exp. Parasit. *60*,323–330 (1985).

Brown H. C., Ross C. A., Holmes P. H., Luckins A. J., Taylor A. M.: Adaptation of *Trypanosoma congolense* stocks to in vitro culture does not change their sensitivity to isometamidium. Acta trop. (Basel) *44*, 373–374 (1987).

Desowitz R. S., Watson H. J. C.: Studies on *Trypanosoma vivax*. I. Susceptibility of white rats to infection. Ann. trop. Med. Parasit. *45*, 207–219 (1951).

Finelle P.: Trypanosomiasis: chemotherapy and chemoprophylaxis. In: Les moyens de lutte contre les trypanosomes et leur vecteurs, p. 291. IEMVT, Maisons Alfort 1974.

Geigy R., Kauffman M.: Sleeping sickness survey in the Serengeti area (Tanzania) 1971. I. Examination of large mammals for trypanosomes. Acta trop. (Basel) *30*, 12–23 (1973).

Godfrey D. G.: Types of *Trypanosoma congolense*. II. Differences in the courses of infection. Ann. trop. Med. Parasit. *55*, 154–166 (1961).

Hawking F.: Drug resistance of *Trypanosoma congolense* and other trypanosomes to quinapyramine, phenanthridines, Berenil and other compounds in mice. Ann. trop. Med. Parasit. *57*, 262–282 (1963).

Nantulya V. M., Musoke A. J., Rurangirwa F. R., Moloo S. K.: Resistance of cattle to tsetse transmitted challenge with *Trypanosoma brucei* or *Trypanosoma congolense* after spontaneous recovery from syringe-passaged infections. Infect. Immun. *43*, 735–738 (1984).

Paris J., Murray M., Mcodimba F.: An evaluation of the sensitivity of current parasitological techniques for the diagnosis of bovine African trypanosomiasis. Acta trop. (Basel) *39*, 307–316 (1982).

Pinder M., Authié E.: The appearance of isometamidium resistant *Trypanosoma congolense* in West Africa. Acta trop. (Basel) *41*, 247–252 (1984).

Ross C. A., Gray M. A., Taylor A. M., Luckins A. G.: In vitro cultivation of *Trypanosoma congolense*: establishment of infective mammalian forms in continuous culture after isolation from the blood of infected mice. Acta trop. (Basel) *42*, 113–122 (1985).

Trail J. C. M., Sones K., Jibbo J. M. C., Durkin J., Light D. E., Murray M.: Productivity of Boran cattle maintained by chemoprophylaxis under trypanosomiasis risk. Research Report No. 9 ILCA, Addis Ababa, Ethiopia 1985.

Whitelaw D. D., Bell I. R., Holmes P. H., Moloo S. K., Hirumi H., Urquhart G. M., Murray M.: Isometamidium chloride prophylaxis against *Trypanosoma congolense* challenge and the development of immune responses in Boran cattle. Vet. Rec. *118*, 722–726 (1986).

Whiteside E.: Recent work in Kenya on the control of drug resistant cattle trypanosomiasis. ISCTR 8th Meeting, Jos, Nigeria, 1960. CCTA *62*, p. 141–154 (1961).

Williamson J., Stephen L. E.: A test for drug-resistant trypanosomes in experimental tsetse-fly challenge of cattle. Ann. trop. Med. Parasit. *54*, 366–370 (1960).