A STUDY OF THE PREVALENCE OF BOVINE TRYPANOSOMOSIS AND TRYPANOCIDAL DRUG RESISTANCE IN COASTAL TANZANIA

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SUMMARY

Although bovine trypanosomosis and trypanocidal drug resistance have been well documented at some sites in Tanzania, notably Mkwaja Ranch, there is little detailed information on the extent of the problem on an area-wide basis. A survey was conducted between May 1997 and December 1998 in 30 sampling sites in Tanga, Coast and Dar es Salaam Regions of Tanzania. Samples (n = 2761) from cattle over 6 months of age were collected. Buffy-coats were examined for presence/absence of trypanosomes using the haematocrit centrifugation buffy-coat technique and Giemsa-stained blood films. Blood was collected for preparation of trypanosome stabilates for drug sensitivity testing, and for preparation of sera for trypanocidal drug determination.

Trypanosome infections were found in cattle in 18 (60.0%) of the sampling sites. Prevalence at individual sampling sites varied between 0 and 43.0%. Overall mean prevalence was 13.0%, and 73.0% of the infections were *Trypanosoma congolense*, 23% *T. vivax* and 4% *T. brucei*. The highest prevalences were found at sampling sites in Tanga Region, and one site (Bunju) in Coast Region (mean 14.3%). Other sites in Coast and Dar es Salaam Regions had relatively lower prevalences (mean 1.7%).

Seventeen pools of up to 5 stabilates per pool (from a single site), of *T. congolense* from six sites (5 in Tanga Region and Bunju) were tested for sensitivity to isometamidium chloride (ISMM) and diminazene aceturate (DA) in mice. Using discriminatory doses of 1.0 mg/kg b.wt. ISMM, and 20 mg/kg b.wt. DA, only 1 (5.9%) pool was sensitive to both drugs, 5 (29.4%) were resistant to ISMM only, none was resistant to DA only, and 11 (64.7%) were resistant to both drugs. These results indicate the presence of resistance to ISMM at two sites, and resistance to both ISMM and DA at four of the sites.

Analysis of sera using isometamidium-ELISA showed wide variation in usage rates, and further evidence of isometamidium resistance; 18.4% of 359 trypanosome-infected cattle showed evidence of recent isometamidium treatment.

INTRODUCTION

Tanzania has a total cattle population of about 16.4 million, constituting approximately half the human population. The economy of Tanzania depends on the major, on Agricultural sector, of which Livestock subsector forms an integral part. Nevertheless, trypanosomosis continues to threaten the Agricultural sector as 600,000 km^2 (67.9%) of the 883,398 km^2 of Tanzania's land surface area become infested with seven tsetse fly species namely: *Glossina morsitans, G. pallidipes, G. swynnertoni* and *G. austeni.* Other species include *G. brevipalpis, G. fuscipes* and *G. longipennis*. While animal trypanosomosis is reported on every region of the 20 regions of the Mainland, human trypanosomosis is reported in 8 regions, the most affected being Kigoma. About 5 million cattle and 4 million people are at risk of contracting trypanosomosis.

The main method of control of cattle trypanosomosis is primarily through control of the protozoan parasites by treatment using a variety of trypanocidal drugs, the more often used ones in Tanzania being different brands of diminazene aceturate (DA) for chemotherapy and isometamidium chloride (ISMM) (SamorinÒMay & Baker Ltd.) and homidium for chemoprophylaxis, as well as chemotherapy. Diminazene aceturate was introduced into Tanzania in 1954 and isometamidium chloride later in early sixties to check sporadic cases of chemoresistance, as well as to offer prophylaxis so as to reduce the frequency of trypanocidal drug treatment.

One of the major reasons of development of trypanocidal drug resistance is frequent use of trypanocides, which may be necessitated by underdosing and improper administration of the same, so that insufficient drug is made available for clearance of trypanosomes. Trypanocidal drug resistance hitherto was not widespread in Tanzania and only sporadic cases were reported (Njau *et al.*, 1981; Njau *et al.*, 1986; Mbwambo *et al.*, 1988). However, recent work conducted in three regions of Tanzania indicates, that drug resistance was under-estimated or has developed since the earlier studies. The study was conducted by The Animal Disease Research Institute (ADRI) Temeke Dar es Salaam, in collaboration with The University of Glasgow, UK. The purpose of the study was to assess trypanosomosis prevalence and trypanocidal drug usage in three regions of Tanzania, and to test in laboratory the sensitivity of trypanosome isolates to ISSM and DA treatment. Thirty sampling sites were covered.

MATERIALS AND METHODS

Field activities

Selection of study sites

Based on information obtained from the Regional and District Veterinary Officers of Coast, Dar es Salaam and Tanga Regions on trypanosomosis control activities (frequency and dosage rates of trypanocidal drugs administration) thirty sites were randomly selected between May 1997 and December 1998. These sites included; Tanga (19 sites), Coast (5 sites) and Dar es Salaam (6 sites) comprising of representative areas with intensive use and with no or limited use of trypanocidal drugs. In each area, cattle populations belonging to small farmers and larger commercial farmers ranging from 10 to 437 were examined.

Sample collection

Blood was collected from a marginal ear vein directly into heparinised capillary tubes for trypanosomosis diagnosis using the darkground/ phase contrast buffy-coat technique (BCT) (Murray *et al.*, 1977) and Giemsa-strained blood films; and for Packed Cell Volume (PCV) determinations. In addition to stabilate preparation of BCT-positive blood in the field, 0.2 ml of *Trypanosoma brucei* and *T. congolense* blood was inoculated intraperitoneally, into two mice each, for subsequent preparation of trypanosome stabilates in the laboratory. Mouse inoculation was also done on buffy-coat-negative cattle of PCV values below 20. Where possible, a calf was infected with *T. vivax* blood via jugular vein, to overcome poor viability of *T.vivax* stabilates prepared from low parasitaemias in the field. Sera were collected for the determination by ELISA, of trypanocidal drug concentrations. Parasitaemia, PCV, geographic location and treatments, were obtained and entered initially in field data sheets and then later transferred to a database.

Laboratory activities

Preparation of trypanosome stabilates

Harvesting of trypanosomes from mice for sub-inoculation or stabilate preparation was done at peak parasitaemia ($5x10^5$ trypanosomes/ ml), using the parasitaemia-estimation method described by Paris *et al.*, 1982. Stabilate preparation was done by mixing 1.8ml of infected blood in a cryovial containing 0.2ml glycerol. Stabilates were cryopreserved for future use.

Isometamidium ELISA

Isometamidium (ISMM) ELISA was carried out using the methodology described by Eisler *et al.*, 1993 and Eisler *et al.*,1994, on 2761 sera, and ISMM concentrations in serum related to parasitological and drug sensitivity tests.

Drug sensitivity testing in mice

Sixty three *T. congolense* stabilates were pooled into 17 pools (up to 5 stabilates per pool) from six sites (5 in Tanga region and one from Bunju, Bagamoyo district) and tested for sensitivity to isometamidium chloride and diminazene aceturate in mice. All stabilates comprising a pool were from a single site. The stabilates were pooled due to insufficient supply of mouse colony. A simplified protocol for trypanocidal drug-sensitivity testing using 3 doses for either drug (0.1, 1.0 and 10.0mg/kg b.wt.) or diminazene aceturate (1.0, 20.0 and 40.0mg/kg b.wt.) was employed at the beginning of the studies and later simplified further to discriminatory doses of 1.0 mg/kg b.wt. isometamidium chloride and 20 mg/kg b.wt. diminazene aceturate, as described by Eisler *et al.* (2002). Treatment was effected 24h after infection. Wet films of tail blood were monitored after every other day for 60 days. Results were expressed on the basis of number of mice cured and the number treated in each group. A stabilate was considered sensitive to treatment if =>80% of the mice were cured.

Drug sensitivity testing in cattle

A total of 22 stabilates comprising 7 pools (5 pools of *T.congolense* and two pools of *T.vivax*), and 2 single stabilates (1Tc; 1Tv) were tested in steers in the laboratory. Naive Friesian x East African Zebu steers kept in fly-proof pens for at least two weeks prior to start of experiment, were infected intravenously with trypanosome stabilates and treated with intramuscular(i/m) injection of a 7.0% solution of DA 3.5 or 7.0mg/kg b.wt., at first peak of parasitaemia. Parasitaemia and PCV values were monitored after every other day for at least up to 100 days. If a relapse of the infection was noted during the period of observation, then DA 7.0mg/kg b.wt. was administered i/m in at least two weeks after the first dose. Cases reftactory to treatment with DA, were given ISMM, injected deep i/m at a dose rate of 0.5mg/kg b.wt. The dose of ISMM was raised to 1.0mg/kg b.wt. in at least 50 days of previous ISMM treatment in case a relapse of the infection occurred. The period of 100 days is regarded as the optimal period of observation in trypanosome infected cattle following trypanocidal drug administration(s) for susceptible strains of trypanosomes to trypanocidal drug treatment (Eisler *et al.*, 2002).

RESULTS

Parasitological diagnosis

A total of 2761 samples were tested from 30 study sites. Of the total samples screened, 359(13.0%) were positive for trypanosomes. Of the total samples screened, 359 (13.0%) were collected and screened for trypanosomes. Out of positive samples 33 (9.2%) (Bunju 7, Tanga Dairy 5, Duga-Vumilia 1, Mivumoni 8, Mkwaja 7 and Sakura 5) were trypanosome-buffy coat-

negativesamples of cattle with PCV values <=24 which became positive for trypanosomes when inoculated into mouse. *T. congolense* constituted 262 (73.0%) followed by *T.vivax* 83 (23.1%) and *T.brucei* 14 (3.9%) of total positive cattle (Table 1). Results obtained from Tanga region indicate a overall trypanosomosis prevalence of 313/2098 (14.9%), followed by Coast region 41/502 (8.2%) and Dar es Salaam region 5/161 (3.1%). High prevalence was recorded at three sites in Tanga region namely, Mivumoni, 98/400 (24.5%), Mkwaja 99/437 (22.7%) and Sakura 59/308 (19.2%) and at one site in Coast region; Bunju 38/247 (15.4%). A summary of trypanosome infections and PCV is reported per Region and district, and is presented in Tables 2 and 3, respectively. One hundred sixty three trypanosome stabilates were successfully prepared in the field and expanded in mice (*T. congolense* 120 and *T.brucei* 9) and in cattle (*T.vivax* 33) in the laboratory (Table 4).

Drug determination (Isometamidium ELISA)

Studies on the use of trypanocides indicate, that use of drugs is variable from intensive use to little or rare usage. Of the 2761 sera screened for presence of drug, 442 (16.0%) showed evidence of isometamidium chloride (Table 5). Out of 359 cattle that were trypanosome-infected only 66(18.4%) showed evidence of drug (above 0.4ng/ml) -Table 6.

Drug Sensitivity Testing

Drug Sensitivity Testing in mice

Of the 17 pooled stabilates tested, 35.3% were sensitive to DA and, 29,0% to ISMM whereas 94.1% were resistant to ISMM and 64.7% to DA. Multiple resistance occurred among 11 (64.7%) pools tested from 4 sites namely Mkwaja, Sakura and Mivumoni in Pangani district Tanga region, and 2 pools from Bunju, Coast region (Table 7).

Table 1. Summary of results showing trypanosomosis prevalence in eight districts of Coast, Dar es Salaam and Tanga regions

Region	District	Total	Trypanosome species			Total
		tested	*T. brucei*	*T. congo*	*T. vivax*	Positive (%)
Coast	Bagamoyo	356	0	33	6	39 (10.9%)
	Kibaha	146	0	1	1	2 (0.6%)
Dar	Ilala	67	0	0	4	4 (1.1%)
	Kinondoni	94	0	0	1	1 (0.3%)
Tanga	Korogwe	38	1	1	0	2 (0.6%)
	Muheza	437	0	13	2	15 (4.2%)
	Tanga	414	3	19	15	37 (10.3%)
	Pangani	1208	10	193	56	259 (72.1%)
	Total	2760	14 (3.9%)	260 (72.4%)	85 (23.7%)	359

Table 2. Summary of trypanosome infections and Packed Cell Volume (PCV) values of samples taken, by region

Region	Total tested	Total infected[1]	% infected	Average PCV
Coast	502	41	8.2	29.2
Dar es Salaam	161	5	3.1	26.7
Tanga	2098	313	14.9	29.1
Total	2761	359	13.0	28.3

Drug Sensitivity Testing in Cattle

Out of 7 pools and two single trypanosome stabilates tested for sensitivity in cattle, 44.4% were sensitive to DA treatment and 55.6% resistant to DA, whereas 66.7% were sensitive to ISMM treatment and 33.3% were resistant to ISMM (Table 8). The maximum dose used for DA and ISMM was 10.0 and 1.0 mg/kg body weight(b.wt.), respectively.

Table 3. Summary of trypanosome infections and Packed Cell Volume (PCV) values of samples collected, at District

Region	District	Total tested	Total infected	Percentage infected	Average PCV
Coast	Bagamoyo	356	39	10.9	29.4
	Kibaha	146	2	1.4	29.0
Dar- es-	Ilala	67	4	5.9	30.6
Salaam	Kinondoni	94	1	1.1	30.1
Tanga	Korogwe	38	2	5.3	31.7
	Muheza	437	15	3.4	30.3
	Pangani	1208	259	21.4	25.4
	Tanga	415	37	8.9	29.0
Total		2761	359	13.0%	29.4

Table 4. Showing number of trypanosome stabilates, at Loci

District	Locus	Trypanosome species			Total
		T. brucei	*T. congolense*	*T.vivax*	
Ilala	Kipunguni	0	0	1	1
	Sitakishari	0	0	3	3
Kinondoni	Kiluvya	0	0	1	1
Bagamoyo	Bunju	0	31	7	38
Tanga	Tanga Dairy	2	14	8	24
Muheza	Moa	0	4	0	4
	Mwakijembe	0	4	0	4
	Duga-Vumilia	0	1	0	1
Pangani	Mivumoni	2	24	13	39
	Sakura	3	16	0	19
	Mkwaja	2	26	0	28
Total		9	120	33	162

Table 5. Showing a comparison of status infection of cattle screened for presence of isometamidium chloride (ISMM)

	ISMM concentration		Total
Infection	+ve	-ve	
+ve	66	293	359
-ve	376	2026	2402
Total	442	2319	2761

Table 6. Isometamidium (ISMM) and presence of trypanosome infections in cattle in selected Loci.

	Mkwaja	Sakura	Mivumoni	Tanga Dairy	Bunju	Else-where	All
ISMM							
>= 0.4*	24	18	4	2	15	3	66
< 0.4*	75	41	94	32	23	28	293
Total	99	59	98	34	38	31	359
>=0.4*	24.2%	30.5%	4.1%	5.9%	39.5%	9.7%	18.4%

ISMM = Isometamidium chloride; * = ISMM concentration in ng per ml

Table 7. Drug Sensitivity Testing of *Trypanosoma congolense* stabilates in mice; summary by Pool

	Location	Locus code	Pool No.	Sensitive		Resistant	
				ISMM	DA	ISMM	DA
1	Mkwaja	16	Mkw001			1	1
2	Mkwaja	16	Mkw002			1	1
3	Mkwaja	16	Mkw003			1	1
4	Mkwaja	16	Mkw004			1	1
5	Mkwaja	16	Mkw005			1	1
6	Mkwaja	16	Mkw006			1	1
7	Sakura	17	Sak001			1	1
8	Sakura	17	Sak002			1	1
9	Mivumoni	21	Miv001			1	1
10	Mivumoni	21	Miv002		1	1	1
11	Mivumoni	21	Miv003		1	1	1
12	Mivumoni	21	Miv004		1	1	1
13	Tanga D	2	Tan001		1	1	1
14	Tanga D	2	Tan002		1	1	1
15	Moa/Duga	31,10	DM001	1			
16	Bunju	34	Bn001			1	1
17	Bunju	34	Bn002			1	1
				1 (5.9%)	6 (35.3%)	16 (94.1%)	11(64.7%)

ISMM = Isometamidium chloride; DA = Diminazene aceturate

Table 8. Drug Sensitivity Testing of *Trypanosoma congolense* stabilates in cattle.

Locus	Code No.	Pool No	Tryps Species	Sensitive		Resistant	
				ISMM	DA	ISMM	DA
Kiluvya	26	Tv26SAK*	*T.vivax*	1	1		
S/Shari	22	Tv22PKJ	*T.vivax*	1	1		
Bunju	34	Tc34WAS*	*T.congo*	1	1		
Bunju	34	Tc34PMB	*T.congo*			1	1
Bunju	34	Tv34PS1	*T.vivax*	1	1		
Bunju	34	Tc34PS1	*T.congo*	1			1
Bunju	34	Tc34PMB2	*T.congo*			1	1
Bunju	34	Tc34PSP	*T.congo*	1			1
Bunju	34	Tc34PS2	*T.congo*			1	1
Total				6(66.7%)	4(44.4%)	3(33.3)	5(55.5)

= Single stabilate; ISMM = Isometamidium chloride; S/Shari = Sitakishari; DA = Diminazene aceturate; ( ) = Percent; *T.congo* = *Trypanosoma congolense*.

DISCUSSION

Although the use of prophylactic and therapeutic trypanocidal drugs has been well documented at some sites in Tanzania, notably Mkwaja Ranch (Trail *et al.*, 1985), there is little detailed information on the extent of the problem on an area-wide basis. Trypanocidal drug resistance has been reported in Tanzania (Njau *et al.*, 1981, Njau *et al.*, 1986; Mbwambo *et al.*, 1988), on sporadic cases. The present studies were comparatively intensive and covered three regions of Tanzania. The findings indicate that tryponosomal drug resistant poses a serious constraint to livestock development. In the present study trypanosome isolates were found not to respond to DA at 3.5 mg/kg bwt and ISMM at 1.0mg/kg bwt treatments. Results obtained on isometamidium ELISA confirmed presence of therapeutic levels of isometamidium chloride in the presence of trypanosome infections as confirmed by examination of buffy-coats using the MHCT-BC technique (Murray *et al.* 1977). The fact that only 16% of samples subjected to isometamidium ELISA were positive, two explanations can be put forward; firstly, insufficient doses of ISMM were administered or most cattle keeper administered ISMM at intervals longer than three months. The latter was found to be the case for this study.

Let it be emphasized that the integration of isometamidium ELISA with parasitological techniques further confirmed the presence of drug in the presence of trypanosome infections (drug resistance). Indeed these findings concur with the findings of Eisler *et al.*, 1997, that trypanosomes occurring in the presence of ISMM concentrations =>0.4ng/ml can be considered resistant to isometamidium treatment. One of the major reasons of development of trypanocidal drug resistance is frequent use of trypanocides, which may be necessitated by under-dosing and improper administration of the same, so that insufficient drug is made available for clearance of trypanosomes. It has been noted in the present studies, that farms with intensive use of chemotherapeutic and chemoprophylactic drug usage, particularly Mkwaja ranch Pangani district and Bunju Bagamoyo district have been much more affected than areas with limited drug usage like Kinondoni and Ilala districts of Dar es Salaam region. Mkwaja ranch was started in 1954 following availability of prophylactic drugs (Antrycide prosalt) and later (1964) isometamidium chloride Fox *et al.* (1993). Trypanocidal drug pressure may have been the cause of the multiple drug resistance recorded at Mkwaja, Sakura, and Mivumoni in Pangani district Tanga region and at Bunju Bagamoyo district Coast region. Variation on the degree of resistance to trypanocidal drug treatment has also been reported elsewhere (Peregrine *et al.*, 1991). It is postulated that the resistant isolate from Mivumoni Livestock

Multiplication Unit originated from Mkwaja ranch. Friesian x Boran F1 heifers from Mkwaja ranch are temporarily kept at Mivumoni LMU prior to distribution to small-scale dairy farmers.

Pangani District showed the highest trypanosome prevalence (21.4%) and lowest mean PCV values (25.4%) among the eight districts sampled, further confirmeing the influence of trypanosomosis on PCV values. On the contrary, Muheza District had a typanosomosis prevalence of 3.4% with mean PCV mean of 30.3.

Trypanocidal drug sensitivity tests carried out in mice and cattle indicate the presence of resistance to isometamidium at two sites, and resistance to both isometamidium and diminazene at four of the sites. Analysis of sera using isometamidium-ELISA showed wide variation in usage rates, and further evidence of isometamidium resistance; 18.4% of 359 trypanosome-infected cattle showed evidence of recent isometamidium treatment with ISMM.

CONCLUSION

Trypanosomosis and trypanocidal drug resistance constitute a serious problem to livestock keeping. As there are no prospects for alternative trypanocidal drugs, it is urged to exercise judicious use of the available trypanocides in order to achieve not only effective use of the drugs, but also delay development of trypanocidal drug resistance. It is also recommended to use parasitological techniques in combination with isometamidium ELISA and in-vivo drug sensitivity studies in order to confirm the presence of trypanocidal drug resistance.The development of other drug ELISAs (diminazene aceturate and Homidium that are also widely used in Tanzania) and continuation of studies on the Epidemiology of Resistance to Drugs used in the Control of Bovine Trypanosomosis to cover other areas of Tanzania are similarly urged.

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