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PREVALENCE OF TRYPANOSOMA CONGOLENSE IN EAST AFRICAN ZEBU CATTLE UNDER HIGH TSETSE CHALLENGE

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Studies began in 1986 in the Ghibe River Valley in South West Ethiopia to obtain information on the health and productivity of East African Zebu cattle, managed in village herds and under levels of comparatively high tsetse challenge. Ghibe is one of a number of sites which are part of the African Trypanotolerant Livestock Network coordinated by the International Livestock Centre for Africa. The Network was established in 1983 to achieve, among other things, a better understanding of genetic and acquired resistance to trypanosomiasis and of the environmental factors that affect susceptibility. The East African Zebu are susceptible to trypanosomiasis and one of the objectives of the study was to compare aspects of their health and productivity at this site with those of trypanotolerant breeds at network sites in other countries in Africa. Preliminary results for the first 18 months have been presented by Mulatu et al. (1988). However, as the project continued it became apparent that many individuals were repeatedly being detected parasitaemic despite treatment. These observations together with the fact that overall trypanosome prevalence was increasing and appeared to be higher than might be predicted from the level of tsetse challenge suggested that trypanocidal treatment was becoming less effective. Indeed, there were more infections detected in animals that had been treated in the previous month than in animals that had not been treated (Woudyalew Mulatu et al., 1990).

This analysis of the data has been undertaken in an attempt to distinguish between new and recurrent infections. The paper summarises these findings and also describes the results of a trial designed to compare the efficacy of two levels of the trypanocidal drug used in these herds.

MATERIALS AND METHODS

Baseline Survey

East African Zebu cattle from 7 traditionally managed village herds in the Ghibe Valley in South West Ethiopia are being sampled and weighed monthly. Blood collected from the ear is used to determine the packed cell volume (PCV) by micro-haematocrit centrifugation and to detect the presence of trypanosome species Trypanosoma congolense, Trypanosoma vivax or Trypanosoma brucei by the dark ground/phase contrast buffy coat method (Murray et al., 1977). When parasitaemia is detected in a blood sample and PCV is less than 26%, or when clinical signs of

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trypanosomiasis are apparent without parasitaemia being detected, the animal is treated with an intramuscular injection of 3.5 mg/kg diminazene aceturate*. When parasitaemia is detected but PCV is 26% or above the animal is not treated.

Data from samples collected between January 1988 and June 1989, the period when trypanosome prevalence was highest, are reported in this paper. An average of about 600 cattle were sampled each month.

Field trial

Two of the 7 herds, containing 39 and 60 animals respectively, were selected for more frequent sampling in June 1989 to compare the efficacy of the 3.5 mg/kg dose of diminazene aceturate (Berenil) with that of a 7 mg/kg dose. All animals were sampled on 13th June 1989 and then at 13 approximately 10-day intervals until 22nd October 1989. On each occasion blood samples were analysed for PCV and presence of trypanosomes using the methods already described. At the 1st sampling animals found to be parasitaemic and to have a PCV of 20% or greater were given one of 3 treatments: no Berenil, 3.5 mg/kg or 7 mg/kg Berenil. Animals found to be parasitaemic and with a PCV less than 20% were treated with either 3.5 or 7 mg/kg Berenil. The same treatment protocol was used at 20 day intervals, i.e. at samplings 3, 5, 7, 9, 11 and 13, irrespective of any treatment given at a previous sampling. At the 1st sampling the first animal entering the crush, tested and found positive was given 7 mg/kg, the next 3.5 mg/kg, the next left untreated and so on. The order in which treatments were given was randomised on each subsequent occasion. Thus, assignment of a treatment to an animal was as far as possible at random. Treatment with 7 mg/kg was also permitted if clinical signs of trypanosomiasis were apparent without parasitaemia being detected, but these cases were not considered in the statistical analysis. On intermediate sampling occasions 2, 4, 6, 8, 10, 12 and 14 blood samples were analysed for PCV and tested for presence of trypanosomes, but no treatments were given.

For statistical analysis, cases of parasitaemia which occurred in samples 1, 3, 5, 7, 9 and 11 (defined as day 0) were assembled together, and a subset of records defined for which samples were available on both the following 2 sampling occasions (days 10 and 20). Berenil is reputed to provide protection against reinfection for up to a maximum of about 22 days (Rogers, 1985), but this varies both with the level of dose and level of tsetse challenge. A new infection results in a detectable parasitaemia from about 7 days. Theoretically, therefore, any parasitaemia detected at 10 or 20 days after treatment is likely only to be a consequence of an uncured infection.

Proportions of cases detected parasitaemic again on days 10 and 20 were compared between treatment by the Chi-square test. Mean PCVs on days 10 and 20 were compared by analysis of variance which also allowed for effects of sampling occasion and age.

In order to evaluate any possible bias due to variations in animal susceptibility to trypanosomiasis, a subset of cases was also formed so that only the first case recorded was used for each animal. These were subjected to the same statistical analyses.

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*Berenil (Hoechst)

RESULTS

Baseline survey

The mean prevalences of T. congolense, T. vivax and T. brucei observed in monthly samples from cattle in 7 herds between January 1988 and June 1989 are shown in Table 1. T. congolense was the predominant species and its prevalence gradually increased with age up to about 32 months of age (Table 2), remaining constant thereafter. T. vivax, on the other hand, showed no increase in prevalence after 8 months of age and its incidence was much lower than that of T. congolense. The prevalence of T. brucei was lower still. Ninety four per cent of detected parasitaemias were associated with a PCV less than 26% and were treated. These represented 91% of all treatments, the remainder being for cases where clinical signs were apparent, but where no trypanosomes were detected.

Table 1. Mean monthly trypanosome prevalence in 7 herds of cattle sampled between January 1988 and June 1989.

	Age (months)		
	< 9 (1369)	9 - 32 (3714)	> 32 (6370)*
T. congolense	3.0	14.1	30.7
T. vivax	2.1	5.6	4.2
T. brucei	0.3	0.9	1.2
Mixed infections	0.1	0.2	0.4

*Number of samples.

Table 2. Mean monthly prevalence of T. congolense in 7 herds of cattle sampled between January 1988 and June 1989 and classified by age.

Age (months)	No. of samples	Prevalence of T. congolense
0 - 4	659	1.8
5 - 8	710	4.2
9 - 12	781	10.8
13 - 16	821	11.1
17 - 20	622	12.5
21 - 24	523	16.2
25 - 28	538	21.4
29 - 32	429	18.4
33 - 36	404	30.7
37 - 40	392	26.8
41 - 44	290	30.7
45 - 48	214	32.2

Since T. congolense was the predominant species and the one giving most repeated infections (Woudyalew Mulatu et al., 1990), subsequent statistical analysis was confined to this species alone and samples containing either T. vivax or T. brucei were ignored. In order to devise a method for distinguishing between possible new and recurrent infections, the distribution of parasitaemic blood samples with varying levels of PCV was first examined for the two age groups : 9-32 and > 32 months (Table 3). Two thirds of samples from animals over 32 months of age with PCV less than 21%, but less than 5% of samples with PCV greater than 26%, were found to contain T. congolense. Less than 2% of samples with PCV greater than 26% were detected with T. congolense in the younger age group. It was decided, therefore, that a blood sample with a PCV greater than 26%, and without T. congolense detected, would be considered 'negative'; it was felt that the occurrence of a T. congolense infection following a series of 'negative months' defined in this way would be more likely to indicate a new infection rather than a recurrent one.

Table 3. Distribution of PCV and percentages of samples detected with T. congolense in cattle between 9 and 32 months and greater than 32 months of age.

	Age (months)			
	9 - 32		> 32	
PCV (%)	No. of samples*	Proportion parasitaemic	No. of samples*	Proportion parasitaemic
< 17	129	0.65	389	0.84
17 - 18	137	0.55	420	0.73
19 - 20	274	0.44	865	0.58
21 - 22	202	0.34	535	0.49
23 - 24	228	0.23	556	0.35
25 - 26	950	0.10	1596	0.19
27 - 28	678	0.02	821	0.05
29 - 30	448	0.01	482	0.04
31 - 32	184	0.00	146	0.02
> 32	235	0.01	194	0.00

*Samples included only if either no trypanosome detected or T. congolense detected alone.

The data were re-analysed taking into account for each animal and each sample in turn the numbers of consecutive months found to be negative prior to the month in question. Table 4 shows values for prevalence of T. congolense derived when samples were restricted to those preceded by zero, one, two, three and four consecutive negative months respectively. T. congolense prevalence decreased from 32.8 to 16.6% and from 15.3 to 7.5% respectively in the two age groups as the number of preceding negative months increased from 0 to 2. When 3 negative months were used the prevalence in the older age group was reduced yet further to 14.1%, but thereafter a small reduction was at the expense of a corresponding reduction in sample size. A 'new infection' was consequently defined as one occurring in an animal which had had 3 consecutive samples with PCV > 26% and parasitaemia not detected. From Table 4 these values were 7.8 and 14.1% in the younger and older age groups respectively.

Table 4 also shows the prevalence of T. congolense one month following treatment, itself preceded by one or two negative months. Similar figures were obtained for the older age group when either one or two negative months preceding a treatment were considered. There were too few samples to consider 3 negative months and the values in the table are, therefore, the best possible estimates of the response to treatment of a 'new infection'. Table 4 shows that approximately 29% of 'new infections' in cattle over 32 months of age were detected parasitaemic again a month later. A small proportion of these parasitaemias may have arisen from new infections which occurred once the period of Berenil protection had elapsed. This would have been considerably lower than the monthly prevalence figure of 14% estimated above, and so the majority are likely to have been due to recurring infections which failed to respond to treatment. Similarly, a high incidence of recurrent infections are also indicated in the younger group.

Table 4. Mean prevalence of T. congolense from monthly samples collected between January 1988 and June 1989 in cattle over 8 months of age classified according to their PCV and parasitaemic status in previous months.

Previous months*				Age (months)			
				9 - 32		> 32	
4	3	2	1	No. of samples	Prevalence	No. of samples	Prevalence
.	.	.	.	3472	15.3	6028	32.8
.	.	.	-	1416	9.0	1530	20.2
.	.	-	-	790	7.5	662	16.6
.	-	-	-	461	7.8	340	14.1
-	-	-	-	304	6.3	217	13.4
.	.	-	+	89	20.2	233	28.8
.	-	-	+	42	16.7	89	29.2

* . all samples included irrespective of level of PCV or presence or absence of trypanosomes.
- no trypanosome detected and PCV > 26%.
+ T. congolense detected and treated with 3.5 mg/kg Berenil.

These observations led to the design of a trial to investigate in more detail the rates of relapse to the 3.5 mg/kg treatment and to compare its efficacy to that of a 7 mg/kg treatment.

Field trial

Six of the 99 animals were less than 7 months of age and these have been omitted from the statistical analysis. The remaining 93 were at least 15 months of age at the start of the trial. The average 10-day prevalence of T. congolense among these animals was 30%. Only 17 cases of T. vivax and 5 cases of T. brucei were recorded throughout the 14 sampling occasions.

Case records were restricted to the 151 involving T. congolense alone. These are shown in Table 5 classified by treatment group and with the numbers and proportions detected parasitaemic again over the next 20 days. There was no effect of age group on proportions detected parasitaemic when animals older and younger than 32 months were compared. Only 53% of controls (parasitaemic animals not treated) were detected parasitaemic again 10 days later, but by day 20, 81% had been detected parasitaemic on at least one of the two occasions. Cases treated with either dose of Berenil and with PCV $\geq$ 20% resulted in significant reductions in the numbers of detected parasitaemias at day 10 (3.5 mg/kg $P < 0.01$; 7 mg/kg $P < 0.001$). At day 20, however, the proportion of cases treated with 3.5 mg/kg and detected parasitaemic (.59) was not significantly different from the controls (.72) but the proportion of cases treated with 7 mg/kg and detected parasitaemic (.18) remained significantly lower than the controls ($P < 0.001$). Treatment of cases with PCV < 20% resulted in no significant differences between the effects of the two doses but numbers of cases were small. Table 5 also includes results based on one case per animal. It was possible to use 78 of the 93 animals which met the criteria for inclusion of a case record. These results were similar to those for all 151 cases suggesting that there was no apparent bias in the analysis of the data due to possible variations in animal susceptibility.

Table 5. Numbers of cases of T. congolense detected with T. congolense again over next 2 sampling occasions, classified by levels of PCV and levels of treatment of Berenil on day 0.

	PCV (%) at day 0	Dose of Berenil at day 0 (mg/kg)	No. of cases	No detected parasitaemic again at day 10	at day 20	by day 20*
All cases	$\geq$ 20	0	43	23 (.53)	31 (.72)	35 (.81)
		3.5	41	8 (.20)	24 (.59)	25 (.61)
		7	40	2 (.05)	7 (.18)	9 (.21)
	< 20	3.5	12	1 (.08)	3 (.25)	4 (.33)
		7	15	1 (.07)	4 (.27)	5 (.33)
	Total	0	43	23 (.53)	31 (.72)	35 (.81)
		3.5	53	9 (.17)	27 (.51)	29 (.55)
		7	55	3 (.05)	11 (.20)	14 (.25)
One case per animal+	Total	0	17	9 (.53)	12 (.71)	13 (.76)
		3.5	30	6 (.20)	14 (.47)	15 (.50)
		7	31	3 (.10)	6 (.19)	9 (.29)

* Parasitaemic at day 10 or day 20 or both.
\+ Only the first case recorded used for each animal.

Mean PCVs at days 0, 10 and 20 for the 151 cases adjusted for age and month of sampling are shown in Table 6. Packed cell volume for cases treated with PCV $\geq$ 20% on the day of treatment showed an increasing trend at day 10 with increasing dose ($P < 0.001$) and this became even more marked at day 20. Whereas PCV in controls gradually decreased from 24.2 to 22.9% over the 20 day period, the 7 mg/kg dose resulted in an increase from 24.3 to 27.1%. Treatment of cases with PCV <

20% resulted in a rapid increase in PCV from 17.4% on day 0 to 23.2% on day 10. Cases treated with 7 mg/kg showed a further slight but non-significant increase in PCV on day 20.

Table 6. Mean values of PCV at days 0, 10 and 20 following cases of T. congolense, classified by level of PCV and level of treatment on day 0.

PCV (%) at day 0	Dose of Berenil at day 0 (mg/kg)	No. of cases	Mean PCV (%)		
			day 0	day 10	day 20
≥ 20	0	43	24.2	23.4	22.9
	3.5	41	24.0	25.1	24.0
	7	40	24.3	25.7	27.1
Average s.e. of difference between 2 means			0.69	0.65	0.83
< 20	3.5	12	17.4	22.8	23.0
	7	15	17.5	23.6	24.9
s.e. of difference between means			0.56	1.15	1.53

It was possible to extend 138 of the 151 records to 30 days, and when PCVs were compared at day 30 the above trends were maintained. By this day the 7 mg/kg dose had resulted in a significantly higher mean PCV being achieved (26.6%) than either the control or the 3.5 mg/kg treatment in both PCV groups (≥ and < 20%). The control and 3.5 mg/kg treatment groups had similar PCVs averaging 23.0%. As at day 20, proportions detected parasitaemic at least once over the 30 day period were similar between controls and 3.5 mg/kg treated cases and the proportion of the 7 mg/kg treated cases that were detected parasitaemic during the 30 day period (0.46) remained lower than the controls (0.86).

DISCUSSION

This paper has attempted to develop methods for distinguishing new and recurrent trypanosome infections in situations where only one monthly sampling is possible and has used 3 negative months prior to the sample in question as the criterion for the definition of a new infection. This method can only be approximate for it depends on a fixed threshhold for PCV for determining negative and positive samples and ignores individual animal variations in susceptibility which may change with age (Table 3). There is also the problem that parasitaemia may be detected in as little as 50% of infected cases (Table 5) due to the periodicity of waves of parasitaemia which frequently fall to indetectable levels. Nevertheless the results obtained suggest that a substantial proportion of the observed prevalence of T. congolense was due to recurrent infections.

The paper has also described the design and analysis of a simple field trial which, over the comparatively short period of 14 weeks, provided evidence of the relative efficacies of 2 doses of Berenil. The trial was conducted under conditions of high tsetse challenge and high trypanosome prevalence which allowed numbers of cases to be accumulated quickly. There is often debate over the most appropriate dosage of Berenil to be used in the treatment of animal trypanosomiasis in different situations, and, provided sufficient numbers of animals are tested, the approach

could also be used in situations where tsetse challenge is lower.

The high levels of recurring infections at the site coincided with lower levels of animal productivity. Mulatu et al. (1988) reported mean calf weight at 8 months of 62 kg and mean cow weight post-partum of 204 kg over the first 18 months of the study. An analysis of body weight from January 1988 onwards suggested reductions from these values of about 15% and 10% in calf and cow body weight respectively. Further statistical analyses are needed, however, to determine to what extent these reductions in productivity may be due to trypanosomiasis. An increase from 3.5 to 7 mg/kg of Berenil would likely increase average PCV and reduce the numbers of detected parasitaemias, and this in turn may result in some improvement in productivity. However, even at this dose relapses occurred (Table 5) suggesting the possible existence of drug resistance among certain stocks of T. congolense. Resistance by T. congolense to chemotherapeutic drugs has been reported previously in Ethiopia (Scott & Pegram, 1974). Consequently a number of field isolates have been obtained for testing for this. At the same time it is clear that additional measures are needed to reduce overall levels of trypanosomiasis. A tsetse control campaign is therefore being mounted using insecticide impregnated screens. Subsequent monitoring of trypanosome prevalence and animal health and productivity will allow the benefits of this campaign to be assessed alongside alternative chemotherapy regimes.

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