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Epidemiology of animal trypanosomiasis on a cattle ranch in Kilifi, Kenya

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Summary

A study of the epidemiology of animal trypanosomiasis was carried out on a 2500 ha cattle ranch, with a history of trypanosomiasis, in the Coast Province of Kenya in 1982. The tsetse survey on the ranch revealed one breeding focus of *Glossina austeni* in a thicket of approximately 50 ha. Trypanosomes were detected in 20% of the 46 dissected tsetse. During the study period of 9 months, 0.8% of the 3315 samples collected from 2300 Ayrshire × Sahiwal crossbred cattle were found infected with trypanosomes; 32% of 5909 samples collected from the same cattle had a packed cell volume (PCV) of 30% or less. Animals with a PCV of 30% or less were treated with a trypanocide (Berenil, Ethidium or Novidium). Antibody to trypanosomes was detected in 22.1% of the 343 sera collected from the cattle. A sentinel herd of 20 cattle was exposed for 182 days inside the tsetse infested thicket. All animals became infected with *Trypanosoma congolense,* on average after 53 days; they were subsequently treated with Berenil (6 mg/kg). A second, third and fourth *T. congolense* infection was diagnosed in 17, 11 and 1 animals, respectively. The cattle were treated similarly with Berenil after each of these infections. *T. vivax* and *T. brucei* were not diagnosed in the sentinel cattle. The results suggest that acquired immunity to *T. congolense* infection did not play a significant role in the sentinel cattle.

Key words: trypanosomiasis; epidemiology; cattle; *Glossina austeni; Trypanosoma congolense;* serodemes; trypanocides.

Introduction

There is evidence suggesting that *Bos indicus* breeds of cattle in East Africa acquire some degree of resistance to trypanosomiasis when maintained under chemotherapy (Whiteside, 1962; Wilson et al., 1975, 1976; Murray et al., 1982).

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This may be related to the acquisition of immunity to a limited number of trypanosome serodemes, i.e., antigenically different trypanosome populations, circulating in the area. In attempting to develop immunological control measures against trypanosomiasis, it is essential to know the number of serodemes present (Murray et al., 1981). Various techniques have been used to characterize *Trypanosoma congolense* stocks and serodemes. These include serotyping of metacyclic trypanosomes (Nantulya et al., 1980), the use of local skin reaction, "the chancre", as a marker for previous exposure to tsetse-transmitted challenge with trypanosomes of different serodemes (Dwinger, 1985), karyotyping (Majiwa et al., 1985) and characterization of isoenzyme patterns (Young and Godfrey, 1983).

The present study was initiated to describe the tsetse situation in the area, to quantify the number of trypanosome serodemes in a defined area, to define the role of acquired immunity to metacyclic variable antigen types (VATs) in the development of resistance to trypanosome infections and to indicate the role of strategic drug therapy in the induction of resistance under low tsetse challenge. The major criteria for the selection of Kilifi Plantations as the preferred study area were as follows. The ranch had a well documented trypanosomiasis situation, in a cattle population with records on individual animal identification, productivity and breeding performance. The tsetse challenge and game population were known to be low and the game migration as well as cattle movement was minimal. The good collaboration with the ranch owners was a prerequisite for the initiation of the study in this area.

This paper describes the epidemiology of trypanosomiasis on the ranch and provides details of the circumstances under which trypanosome isolates were acquired. Results of laboratory studies on the characterization of these isolates will be presented in subsequent publications.

Materials and Methods

Study area

Kilifi Plantations is a cattle ranch of 2500 ha, located 60 km north of Mombasa in the Coast Province of Kenya. The ranch is bounded by the Indian Ocean coast to the east, Kilifi Creek to the north and the Sinawe river flowing into Takaungu creek to the south (Fig. 1). The main Mombasa-Malindi road divides the ranch into eastern and western sections. The annual rainfall averaged 1106 mm over the last 10 years, while during the 12 months study period, from February 1982 to January 1983, the total rainfall was 1684 mm, with the main rainy season in April through July (average monthly rainfall 315 mm) and a short rainy period from September to November (average monthly rainfall 127 mm). An estimated 245 ha of bush exists along stream gulleys within or around the pastures and the perimeter of the ranch. The Taratibu stream, a tributary of the Sinawe river, flows eastwards from the western margin of the ranch and has been dammed to form a reservoir. Small areas of bush, having *Lantana camara* as a major component, occur on the coastal border to the farm. The areas of thicketed woodland bordering Kilifi Creek are extremely dense and slope steeply to the coast where mangroves predominate along the tidal zone. The areas surrounding Kilifi Plantations to the west and south consist of traditional settlement amongst coconut and cashew plantations, partly overgrown with low bush and interspersed with areas of shifting cultivation. Tsetse surveys

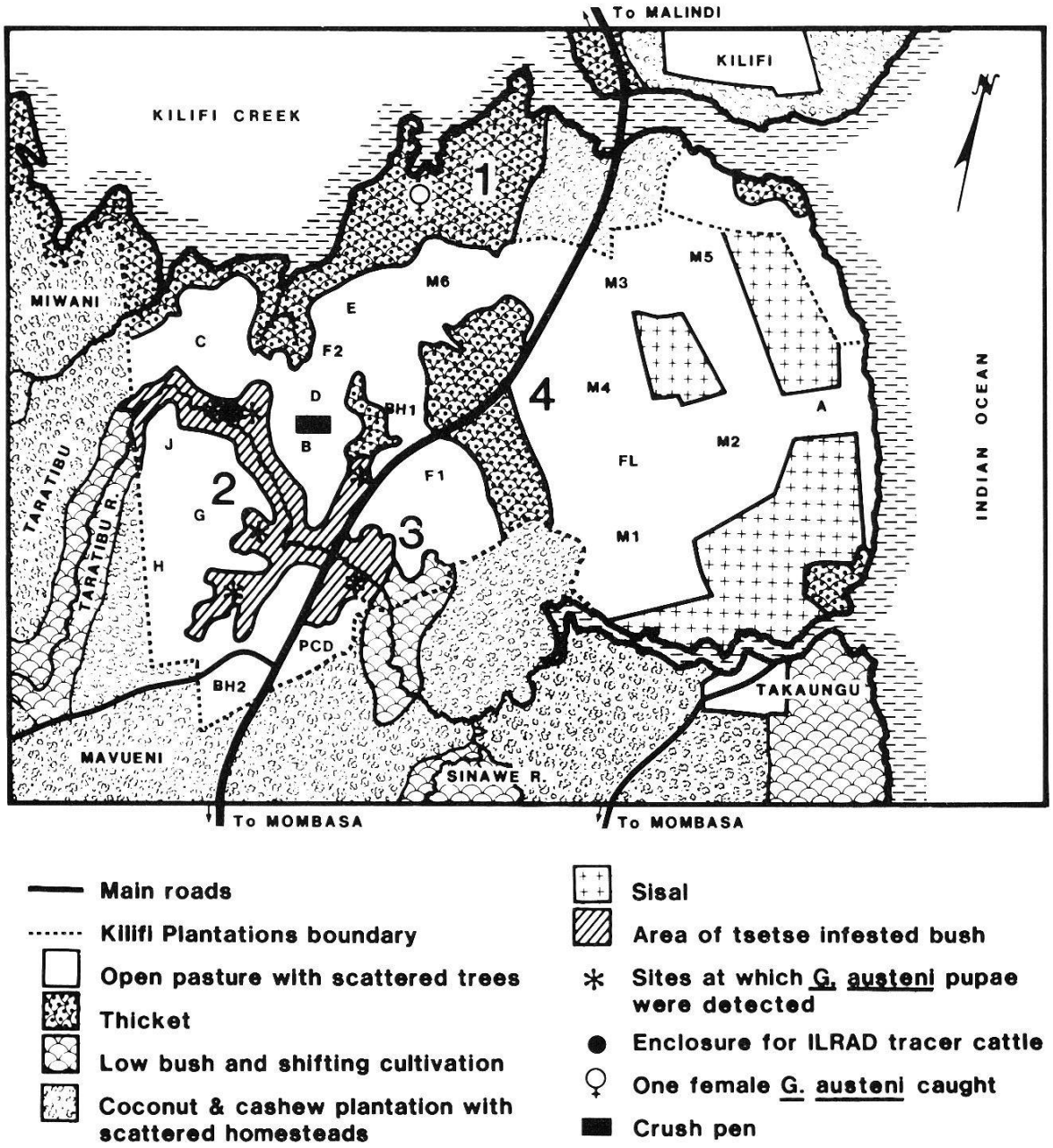

Fig. 1. Map of Kilifi Plantations showing the distribution of *G. austeni* and the cattle herds (1982). M1–M6 = milking cows; A–J = young stock; BH-PCD = bulls, heifers and dry cows; FL = feedlot; 1–4 = areas of tsetse survey.

have indicated the presence of *Glossina pallidipes* and *G. austeni* in the Kilifi area and trypanosomiasis has been a constant problem (Kenya Veterinary Department, Annual Report, 1962, 1963, 1972).

Tsetse survey

Four areas of bush on Kilifi Plantations were selected as possible tsetse habitat (Fig. 1). Initially, tracks were cut through each area of bush to allow access for bait-oxen fly-rounds and for trapping. Two surveys were carried out, one between 1 April and 30 June and the other between 27 October and 26 December 1982.

Biconical traps (Challier and Laveissiere, 1973) were fixed along the access tracks at intervals of approximately 100 meters. The traps, covering selected areas of bush in rotation, were checked daily. Nineteen traps were used during the first period and twenty-six during the second. During the second

survey period acetone, as odour attractant, was used in open 125 ml wash bottles, taped to the base of the supporting poles of the traps.

Two black oxen, 2 years old were used in the bait-oxen fly-rounds. Each ox was led along one of the fly-round tracks accompanied by two catchers with handnets. The oxen were stopped at intervals and observed closely for tsetse; those which alighted were caught. Bait-ox fly-rounds commenced at 0530 h and continued up to 0800 h. Evening fly-rounds were carried out from 1700 h to 1900 h. All areas of bush were covered in rotation, using the bait-oxen fly-rounds between 1 April and 10 May.

All areas of bush were searched for puparia between 10 May and 30 June and between 27 October and 26 December. During the second period more attention was given to area No. 2 (Fig. 1). Soil and leaf litter from possible larviposition sites, to a depth of approximately 5 cm, was collected and washed through sieves with water. Intact and broken puparia were collected. Intact pupae were preserved until adult flies emerged.

The tsetse caught were dissected and the proboscides, midguts and salivary glands were examined to determine the infection rate and the trypanosome species, based on the location of the parasites in the tsetse (Lloyd and Johnson, 1924).

Bloodmeals were collected from tsetse by expressing gut contents on to sodium azide coated filter papers. Bloodmeals were then tested by an enzyme-linked immunosorbent assay (ELISA) (Lindquist et al., 1982; Rurangirwa et al., 1986) to determine the host species.

Cattle and the chemotherapeutic regime

The cattle of Kilifi Plantations were introduced in 1962 and originated from Ulu in Machakos District of Central Kenya. In 1982 the cattle herd numbered 2300. The breeding females are mainly Ayrshire (*B. taurus*) × Sahiwal (*B. indicus*) crossbreds. Recently, further cross-breeding with Brown Swiss (*B. taurus*) has been introduced (Trail and Gregory, 1981). Herds of approximately 100 animals were formed, based on age, sex and lactation status (Table 1). A routine chemotherapeutic strategy has been developed over the years and consists of treatment of all animals with a packed cell volume (PCV) of 30% or less with a trypanocide (Berenil 3.5 mg/kg, Ethidium 1.0 mg/kg or Novidium 1.0 mg/kg). Each animal was bled and examined 4 or 5 times a year. In addition pregnant cows were treated on 2 or 3 occasions during the last 6 months of pregnancy with one of the above trypanocides. Ticks and tick-borne diseases were controlled by spraying the animals with an acaricide at 10-day intervals.

A sentinel herd of 20 two-year-old Boran steers (*B. indicus*) was introduced to the ranch from outside the tsetse area on 26 May 1982. During the first 60 days these animals were herded through bush area No. 2 (which was identified as a breeding area of *G. austeni,* Fig. 1) from 0700 h–1100 h and from 1500 h–1800 h, but they did not become infected with *T. congolense, T. vivax* or *T. brucei.* In order to bring them into closer contact with tsetse, the animals were kept overnight in this bush in an enclosure from 26 July 1982 (day 0) to 24 January 1983 (day 182) and were left to graze on the pastures only from 1100 h–1500 h. Body temperatures of the sentinel animals were recorded daily at 0630 h and the animals were inspected daily for clinical signs of disease. Animals infected with trypanosomes were treated with Berenil (Hoechst AG, Frankfurt am Main, Fed. Rep. of Germany) (6 mg/kg) on average 9 days after the initial detection of parasitaemia. Tick control was not applied to these animals and no disease other than trypanosomiasis was encountered in the herd.

Parasite diagnosis

Apart from the routine PCV measurements, 2302 resident animals were also tested on 1, 2, 3 or 4 occasions (Table 1) for the presence of trypanosomes by the haematocrit centrifugation technique (HCT) (Woo, 1970) during the study period. The identity of the parasites was determined by the examination of Giemsa stained thin blood films. Stabilates were prepared of infected blood and stored in liquid nitrogen. The sentinel herd of 20 animals was closely observed by examining, twice a week, two micro-haematocrit capillary tubes per animal by the above mentioned method. Stabilates of infected blood were prepared each time an animal was found positive. Serum was collected from a subsample of about $\frac{1}{5}$ of the resident animals. Sera of the sentinel cattle were collected weekly for 4

Table 1. Herd structure of the cattle at Kilifi Plantations and sampling frequency in 1982

Section	Herd	Description	Herd size	Sampled in 1982
East[1]	A	Calves up to 4 months	245	2×
	Feedlot A	Bulls and oxen over 2½ years	88	1×
	Feedlot B	Bulls and oxen over 2½ years	71	1×
	M1	Adult dairy cows	140	4×
	M2	Adult dairy cows	140	4×
	M3	Adult dairy cows	129	4×
	M4	Adult dairy cows	136	3×
	M5	Adult dairy cows	100	3×
West	M6	Adult dairy cows	78	3×
	B	Calves, females 4–8 months	73	3×
	C	Calves, males 4–10 months	35	2×
	D	Heifers 8–12 months	49	3×
	E	Heifers 12–18 months	114	3×
	F1	Heifers 18–24 months	114	3×
	F2	Heifers 18–24 months	133	2×
	G	Bulls and oxen 10–18 months	159	2×
	H	Bulls and oxen 18–24 months	92	2×
	J	Bulls and oxen 24–36 months	152	2×
	BH	Bulls and heifers	143	4×
	PCD	Pregnant cows dry	111	4×
		Total	2302	

[1] East of the Mombasa–Malindi road and north of Takaungu Creek

weeks after the detection of a trypanosome infection and then at monthly intervals. Sera were stored at –20°C for 4 weeks and thereafter at –70°C and then tested for the presence of antibodies to *T. congolense, T. vivax* and *T. brucei* by the indirect fluorescent antibody test (IFAT) (Wilson, 1969; Katende et al., in press).

Results

Tsetse survey

During the two periods of trapping 47 adult *G. austeni* Newst. were caught in the biconical traps (34 females, 7 males, and 6 with damaged genitalia), 8 *G. austeni* by bait oxen fly-rounds (4 females, 4 males) and 1 female *G. pallidipes* Aust. by biconical trap (Table 2). Apart from a single female *G. austeni* caught in a trap in area No. 1, all other tsetse were caught in area No. 2 at the upper end of a seasonal river valley (Taratibu stream, Fig. 1).

During the first survey period 49 *G. austeni* puparial shells were found and 59 during the second, as well as 4 intact puparia, from which 2 adult female *G. austeni* flies emerged. All puparia were found in areas No. 2 and No. 3, none in area No. 4 or area No. 1 where a single fly was caught. The puparial shells were

Table 2. Catches of tsetse in biconical traps and by bait-ox fly rounds 1982

Sampling method	Period[1]	Species	Females	Males	Unidentified	Total
Biconical traps	a	*G. austeni*	13 (68%)	6	1	20
Bait-ox fly-round	b	*G. austeni*	4 (50%)	4	–	8
Biconical traps[2]	c	*G. austeni*	21 (95%)	1	5	27
Biconical traps[2]	c	*G. pallidipes*	1 (100%)	–	–	1
Total no. of tsetse			39 (78%)	11	6	56

[1] a = 1 April–30 June 1982 19 traps
b = 1 April–10 May 1982 2 cattle – 4 catchers
c = 27 October–26 December 1982 26 traps
[2] With acetone as attractant

found under fallen trees and branches or tree trunks growing at an angle to the ground. These sites appeared to be scarce but where they occurred within the tsetse infested bush, puparia could frequently be found. The tsetse infested thicket covered an area of approximately 50 ha (2000×250 m).

During the first period 26 *G. austeni* were dissected; 5 (19.2%) were infected with trypanosomes. Two had "*T. congolense*"-type and 2 had "*T. vivax*"-type infections. One fly had a gut infection only. During the second period 20 flies (19 *G. austeni,* 1 *G. pallidipes*) were dissected. Four *G. austeni* (20.0%) were infected. One had "*T. congolense*"-type, two had "*T. vivax*"-type infections and one had a gut infection (Table 3). The one *G. pallidipes* caught was uninfected.

Gut contens of 10 *G. austeni,* containing residual bloodmeal, were collected on filter papers. Their analysis showed the following source of bloodmeal: 3 cattle, 1 goat, 1 sheep, 2 men, 1 suni (*Nesotragus moschatus*), 2 bush-pigs (*Potamochoerus porcus*).

Cattle of Kilifi Plantations

Thirty-two percent of the samples collected in 1982 had a PCV of 30% or less (1890 out of 5909, Table 4); these cattle were treated with a trypanocidal drug. This percentage was 26% for the samples collected on the east side of the ranch (mainly calves up to 4 months and milking cows) and 36% for the samples collected on the west side (Table 5). Here a high percentage (over 50%) of low PCVs occurred in the herds of young stock C, D, E, G and H.

More than 50% of the 5909 samples examined for PCV were tested for the presence of trypanosomes. These include 1843 samples from animals with a PCV of 30% or less and 1472 with a PCV of over 30%. Twenty-six animals (0,8%) were found infected with salivarian trypanosomes; 25 with *T. congolense* and 1 with *T. vivax.* Twenty-four of the infections were detected in animals with a PCV of 30% or less. Twenty (77%) of the infections occurred during the months

Table 3. Trypanosome infection rates in *G. austeni* at Kilifi Plantations

	April–June	Oct.–Dec.	Total
Number of flies dissected	26	19	45
Number of females dissected	15	18	33
Number of males dissected	11	1	12
Number of flies infected	5 (19.2%)	4 (21.1%)	9 (20.0%)
Number of females infected	3 (20.0%)	4 (22.2%)	7 (21.2%)
Number of males infected	2 (18.2%)	0 (0.0%)	2 (16.7%)
"*T. congolense*" type[1]	2 (7.7%)	1 (5.3%)	3 (6.7%)
"*T. vivax*" type[2]	2 (7.7%)	2 (10.5%)	4 (8.9%)
Immature infections[3]	1 (5.3%)	1 (5.3%)	2 (4.4%)

[1] Proboscis and gut infection
[2] Proboscis infection
[3] Gut infection only

Table 4. Period prevalence of trypanosome infections in cattle at Kilifi Plantations (1982)

Period	Number sampled	Animals with PCV of 30% or less		Animals with trypanosome infection[1]					
				Group PCV of 30% or less			Group PCV over 30%		
		Number	%	Number tested	Number pos.	%	Number tested	Number pos.	%
March	752	159	21	159	1	0.6	71	0	0
May–July	1760	626	36	626	20	3.2	NT[2]		
July	1100	306	28	259	1	0.4	NT		
Sept.–Nov.	2297	799	35	799	2	0.3	1401	2	0.1
Total	5909	1890	32	1843	24	1.3	1472	2	0.1

[1] By haematocrit centrifugation technique (HCT) (Woo, 1970)
[2] Not tested

of May to July (rainy season) (Table 4). One *T. vivax* infection was diagnosed on 27 March 1982 in cow 783, belonging to herd M2 on the east side of the ranch (isolate: KILIFI/82/IL/2). All *T. congolense* infections occurred in animals under 3 years of age on the west side of the ranch, belonging to herds C, D, E, F2, G, H and J (Table 5, Fig. 1). Details of 24 *T. congolense* isolates are presented in Table 6. The percentage of animals infected, within the group of animals of under 3 years of age on the west side and having a PCV of 30% or less, was 2.4%. Fifteen animals were found infected with *T. theileri* (isolates: KILIFI/82/IL/22, 23, 39, 40, 61, 63, 71, 83 and 85).

Sera of 343 cattle of Kilifi plantations were tested for the presence of antibody to *T. congolense, T. vivax* and *T. brucei*. Antibody was detected in 22%

Table 5. Prevalence of low PCVs, trypanosome infections and IFAT antibody in different age groups of cattle at Kilifi Plantations in 1982

Group	Group size[1]	Average percentage of animals with PCV of 30% or less	Average percentage of animals with trypanosomes[2, 3]	Average percentage of animals with IFAT antibody
Calves (0–4 mo)	245	30	0.0	10
Feedlot (2½–4 years)	159	19	0.0	NT[4]
Milking cows	645	26	0.2 (1)	14
Total east	1049	26	0.1 (1)	14
Cows and heifers	332	14	0.0	20
Female calves and heifers (herds B, E, D, F)	483	41	0.9 (5)	28
Male calves, bulls and oxen (herds C, G, H, J)	438	47	4.4 (18)	30
Total west	1253	36	1.9 (23)	28
Total Ranch	2302	32	1.2 (24)	22

[1] Sample frequency in Table 1
[2] Within the group of animals with PCV of 30% or less; in brackets actual number
[3] By HCT (Woo, 1970)
[4] Not tested

of the samples; these included 16% *T. congolense* and 6% *T. vivax*. Antibody to *T. brucei* was not detected. The highest percentage of sera with a positive titre occurred in the herds of young stock on the western side of the ranch, 30% and 28% for 84 males and 111 females, respectively (Table 5). Antibody to *T. congolense* was predominant (94%) on the western side, while on the eastern side antibody to *T. vivax* was present in 76% of the 18 milking cows, which had a positive titre out of a total of 123 which were tested (Fig. 2).

Sentinel cattle

The exposure of the sentinel cattle during the first 60 days resulted in only 3 *T. theileri* infections, detected on days 33, 42 and 65 post-exposure (isolates KILIFI/82/IL/29, 30 and 41). However, when the animals were kept in the tsetse infested bush overnight from 26 July, 1982 (day 0), all the 20 cattle became infected with *T. congolense* but none with *T. vivax* or *T. brucei*. *T. congolense* infections were detected first between days 15 and 28 (10–23 August) and 14 out of the 20 animals became infected. Thereafter *T. congolense* infections were diagnosed from day 72 (6 October) onwards and this continued until the end of the study (day 182–24 January, 1983). During this period (days 72–182), 35 infections were detected; 6 were first infections, 17 were second infections (on

Table 6. *Trypanosoma congolense* isolates acquired from resident cattle at Kilifi Plantations (1982)

Isolate[1] Kilifi/82/IL/	Date of isolation	Animal number	Herd identification
10	21.5	4148	H
11	21.5	3842	H
12	21.5	3818	H
13	31.5	3840	H
(14)	28.5	3840	H
15	4.6	4166	H
16	10.6	4580	C
17	10.6	4696	C
18	10.6	4656	C
19	11.6	3604	]
(36)	12.7	3604	H
20	11.6	4.81	]
21	11.6	242	]
24	15.6	3969	E
25	15.6	1080	E
(37)	13.7	1080	BH
31	9.7	582	G
32	15.7	4530	G
38	27.7	3818[2]	H
57	10.9	4483	D
68	23.10	4221	F2
69	30.10	3852	]
(80)	6.11	3852	]
70	30.10	4148	]

[1] In brackets second isolations of the same infections
[2] Second infection of this animal

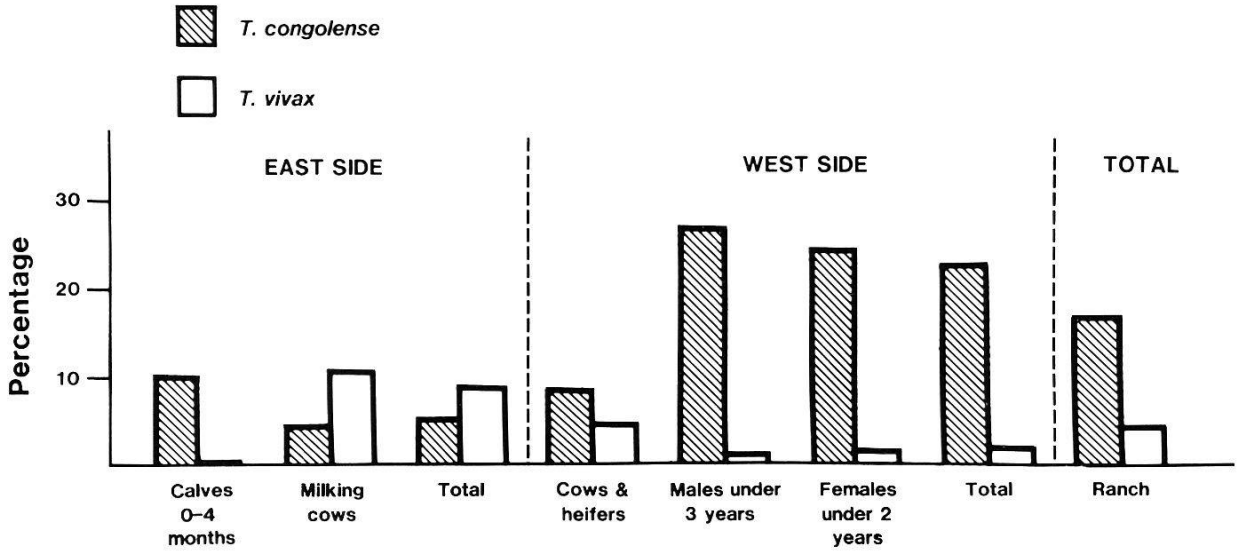

Fig. 2. Percentage of cattle in each age group with IFAT antibodies to *T. congolense* and *T. vivax* on Kilifi Plantations (1982).

Table 7. Infections of *T. congolense* in 20 sentinel cattle at Kilifi Plantations, exposed for 182 days in a *G. austeni* infested thicket (26 July 1982–24 January 1983)

Animal number	First infection		Second infection			Third infection			Fourth infection			Final isolate[3]
	Days p.e.[1]	Isolate Kilifi/82/IL/[2]	Days p.e.	Isolate Kilifi/82/IL/[2]	Days post Berenil treatment	Days p.e.	Isolate Kilifi/82/IL/[2]	Days post Berenil treatment	Days p.e.	Isolate Kilifi/83/IL/	Days post Berenil treatment	Kilifi/83/IL/
560	28	51	72	58+62	36	119	86	40	175	101	46	
561	15	42	101	78	80	173	96	61				
562	15	43+47	98	73	69	173	97	66				
563	28	52	105	81	69	173	98	61				
564	21	48	98	74	68							107
565	133	90										112
567	28	53	173	93	137							
568	15	44	84	67	63	129	89	35				113
569	28	55										114
573	72	59	122	87	43	178	109+115	49				
574	112	84	173	99+111	51							
575	98	75	129	88	22							116
576	as bait ox[4]	8	21	49	85	173	94+105	–				
577	21	50	98	76	69	148	92	41				117
578	28	56	101	79	65	175	102	62				
579	15	45	72	60	51	178	110	99				
580	15	46	81	66	60	173	100+106	85				
581	133	91	175	103+108	26							
582	173	95										
583	28	54	105	82	69	175	104	63				

[1] Post exposure

[2] Isolate nominations from 93 onwards should read Kilifi/83/IL/93 etc.

[3] Prepared on day 182, no trypanosomes diagnosed by HCT (Woo, 1970)

[4] On 19 April 1982

Table 8. Intervals of subsequent *T. congolense* infections in 20 sentinel cattle exposed for 182 days at Kilifi Plantations

	First infection	Second infection	Third infection	Fourth infection
Number of animals infected	20	17	11	1
Average interval between exposure and infection in days (range)	53 (15–173)	115 (72–175)	163 (119–178)	175
Average interval between treatment and re-infection in days (range)		61 (22–137)	60 (35–99)	46

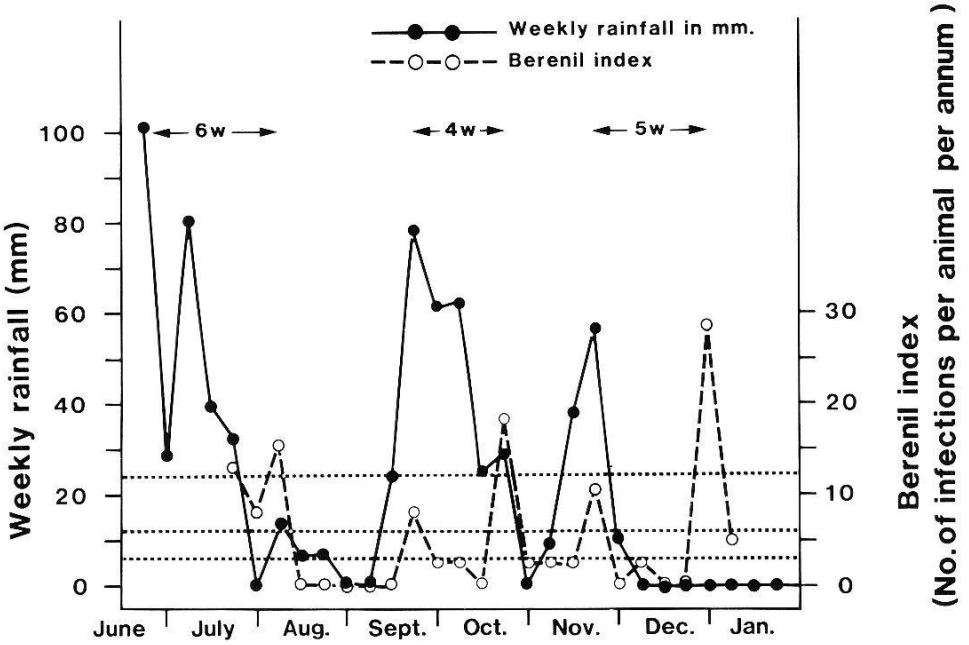

Fig. 3. Relation between the weekly rainfall and the Berenil index of the sentinel herd on Kilifi Plantations (1982). Berenil index: 0–3 = low challenge; 3–6.5 = medium challenge; 6.5–12 = high challenge; over 12 = very high challenge (Whiteside, 1962).

average 115 days after exposure and 61 days after Berenil treatment), 11 were third infections (on average 163 days after exposure and 60 days after Berenil treatment) and one animal became infected 175 days after exposure for a fourth time (48 days after Berenil treatment). Details of the infection and treatment intervals and the 63 *T. congolense* isolates obtained from the sentinel cattle are presented in Tables 7 and 8. The re-infections occurred with an interval varying from 22 to 137 days after Berenil treatment. The intervals between detection and treatment of the infections lay between 6 and 20 days (average 9 days). Clinical signs of trypanosomiasis were not observed. Body temperatures during

parasitaemia did not rise over 39.4° C and the average peak temperature for the first and second infections was 39.0° C and for the third 38.8° C; the normal body temperature averaged 38.5° C. PCVs dropped 11.4% from an average of 28.9% to 25.6%, after the animals became infected with *T. congolense.* The PCV drops during the first, second and third infections were 11.9%, 10.9% and 11.9% of the initial values, respectively. None of the animals had a PCV below 22.0% at any time. The trypanosome attack rate (TAR) measured by the Berenil index (BI), being the number of infections per animal per annum (Whiteside, 1962; Wilson et al., 1975), was 4.9 for the study period of 6 months, which was a medium challenge level using Whiteside's criteria. Periods with a very high challenge (Berenil index over 12.0) occurred on three occasions, 4–6 weeks after the rainfall peaks of June, September and November (Fig. 3). Periods with low challenge (B.I. under 3.0) were in the months of August, September, October, and December.

Discussion

It has been known for some time that tsetse inhabit the coastal region of Kenya including the Kilifi district. The relation between their distribution and the vegetation in the area has been described by Moggridge (1950). Surveys in 1962 and 1963 (Kenya Veterinary Department, Annual Reports) showed the presence of small foci of *G. austeni* and *G. pallidipes* in the areas directly bordering Kilifi Plantations. Surveys carried out in 1972 (Kenya Veterinary Department, Annual Report) and 1981 (W. F. Snow, personal communication), did not detect tsetse on Kilifi Plantations. However, the present survey, conducted in 1982, using three different catching methods, identified an area of approximately 50 ha along the Taratibu stream on the western part of the ranch, as a breeding focus of *G. austeni.* The number of *G. austeni* caught in biconical traps, with and without the use of acetone as an odour attractant, was low. However, the number of puparia collected was substantial. The capture of a single *G. pallidipes* possibly indicates that this species either bred on Kilifi Plantations or migrated into the farm from the surrounding area. The infection rate of 6.7% and 8.9% for "*T. congolense*" and "*T. vivax*"-type infections, respectively, recorded for the *G. austeni* population at Kilifi Plantations is relatively high, compared to 0.3 and 4.0% for *T. congolense* and *T. vivax,* respectively, recorded under experimental conditions for *G. austeni* (Moloo and Kutuza, 1984). A possible explanation for this finding is that a small population of flies fed on a small group of wild host animals such as bush-pig and small antelope, which were present inside the bush and which probably functioned as a reservoir of infection. It is equally possible that *T. congolense* and *T. vivax* stocks present on Kilifi Plantations became more readily established in *G. austeni* from the Kilifi area; Moloo and Kutuza (1984), in their study used *G. austeni* which originated from Zanzibar, *T. congolense* from Tanzania and *T. vivax* from Nigeria.

Sentinel cattle became infected only after they were exposed inside the infested thicket on Kilifi Plantations during the nocturnal periods from 1800 h to 0700 h. The challenge inside the tsetse infested thicket, expressed as the Berenil index for the 182 days period, was medium following Whiteside's criteria (1962), which suggests that the number of *G. austeni* caught represented a tsetse population giving rise to a significant challenge to cattle.

Previous surveys showed that tsetse have been present on and around Kilifi Plantations since at least 1961. Since the introduction of cattle in 1962, trypanosomiasis has been a significant disease problem. However, this problem has in the course of time been reduced to an economically manageable size. The control strategies included the reduction of habitat suitable for tsetse through bush clearing, the application of insecticides and the systematic use of chemotherapeutic trypanocides in the cattle.

Statistical analysis of these trypanocidal drug treatment requirements for 800 breeding females, collected over 6 years was performed by Trail et al. (in preparation). Murray et al. (1982) refer to these analyses and presented evidence for the existence of genetic resistance in the Sahiwal crossbred cattle. Development of acquired immunity was also demonstrated, as it was shown that the more times an animal had been treated, the fewer treatments it required in the future. The study period of 9 months in 1982 was too short and tsetse challenge too low in the area where the breeding females were kept, to further elaborate the observations of Murray et al. (1982). To date, animal losses due to trypanosomiasis are reduced to incidental outbreaks of acute *T. vivax* infections in dairy cows (A. D. Wilson, personal communication). However, the presence of a small tsetse focus on this high producing ranch has important economic consequences as it requires a permanent, effective monitoring and treatment system. The present study showed that the systematic use of chemotherapeutic trypanocides for treatment of all animals with a PCV of 30% or less had a limiting effect on the number of animals with a detectable parasitaemia (0.8%), but also showed that a much larger proportion (22%) had been in contact with the parasite as indicated by a high number of positive IFAT titres. Although the drug strategy used seemed to result in a substantial number of Berenil treatments of animals without symptoms of trypanosomiasis, it was successful in the control of *T. congolense* infections, as 23 of the 25 *T. congolense* infections were detected in animals with a PCV between 26 and 30%. This success might also be attributed to the low pathogenicity of the *T. congolense* stocks present in the area. This observation was later confirmed by the results of experimental infections with the isolated parasites in cattle (Paling et al., in press; Masake, personal communication). Experimental infections in cattle using a tsetse-transmitted chancre-producing *T. congolense* clone indicated that immunity to tsetse-transmitted challenge with the homologous clone lasts for 6 months after treatment of the primary infection (Murray et al., 1982; Akol and Murray, 1985). However, in order to be of lasting significance, acquired immunity requires regular boosting

by the homologous serodeme. This situation may prevail at Kilifi Plantations where there is a small isolated tsetse focus with limited reservoir hosts and little chance for the introduction of additional serodemes.

Considering the location of the tsetse focus, the location of cattle herds at the time of sampling, the results of the parasitological and serological tests and the rotation system of the animals on the ranch, we can develop an epidemiological picture. Young stock of 4 months to 3 years, pregnant heifers and non-lactating cows were exposed on the west side to tsetse and mainly *T. congolense* infection. After calving, during the lactation period, the cows were located on the east side (Fig. 1). Here, tsetse were not encountered during the study period but might occur sporadically and the animals were mainly exposed to *T. vivax* infection. *T. vivax* could be introduced by tsetse, and the infection could subsequently be transmitted mechanically by other biting flies (Wells, 1972). The young calves of under 4 months of age were all fed with milk from bottle or bucket on the east side, away from any possible tsetse habitat. Trypanosome infections were not detected in this group. The antibody to *T. congolense* was probably of maternal origin, resulting from the exposure of their dams to tsetse on the west side during the 2–3 months before calving.

When a sentinel herd of 20 cattle was maintained inside the tsetse infested thicket for 182 days, all animals became infected with *T. congolense* and were subsequently treated. Second, third and fourth infections were diagnosed until day 175 as indicated in Tables 7 and 8. The means of the intervals between: exposure and first infection, treatment and subsequent second infection, treatment and subsequent third infection were very similar (53, 61 and 60 days, respectively). This finding is similar to that of Wilson et al. (1976), during a study with 20 Boran cattle in northern Kenya. Wilson et al. (1976) concluded that there was evidence for the development of immunity based on the changes in trypanocid drug requirements e.g. the periods between required drug treatments increased in the cause of 2 years exposure; however, this increase started to occur only after the fourth infection and treatment, which was on average after 242 days of exposure.

It can be concluded that the acquired immunity of the sentinel cattle at Kilifi Plantations did not play a significant role during the relatively short period of 182 days of contact with tsetse. Hence it seems not likely that the number of *T. congolense* serodemes present in the area is very limited.

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