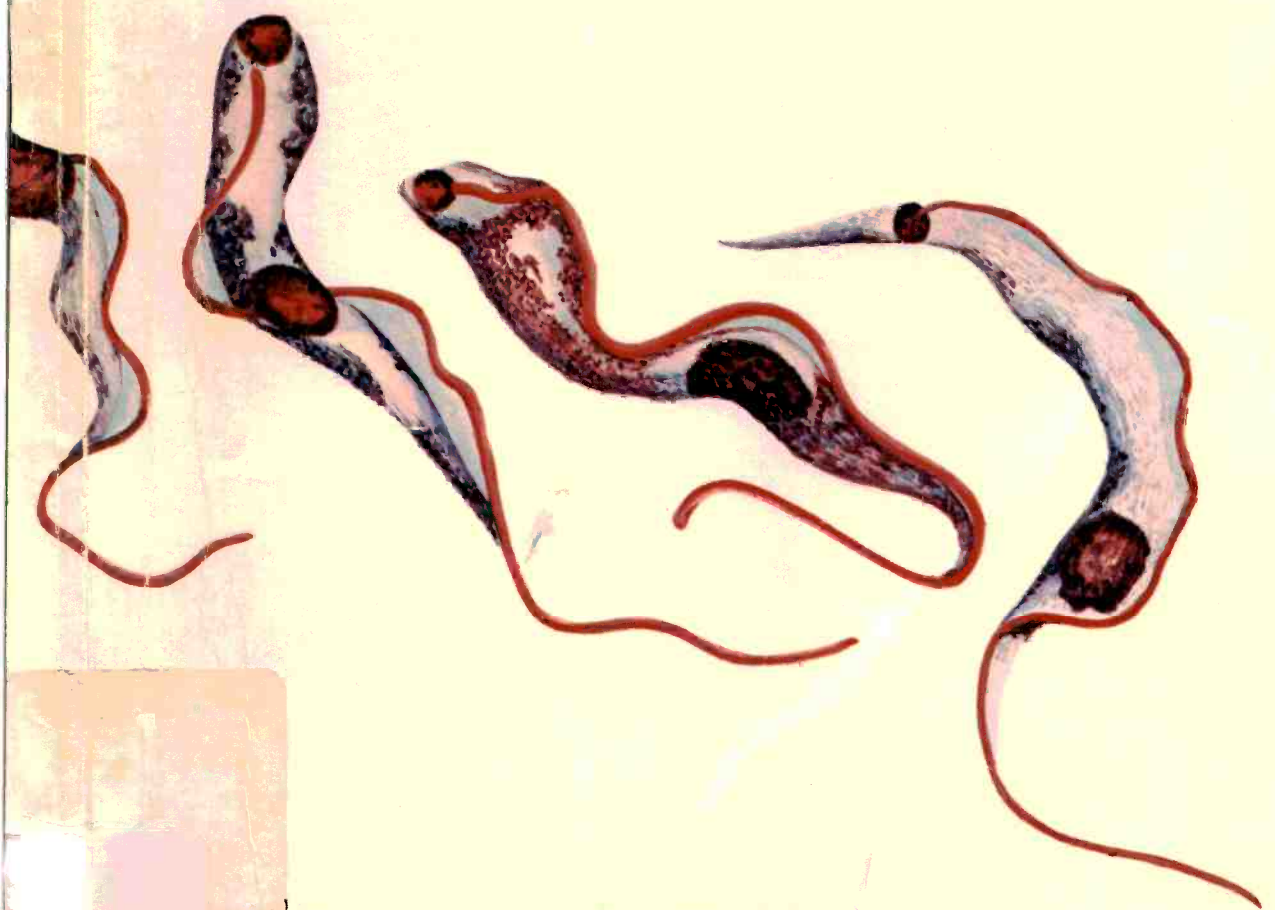
IDRC-132e

Pathogenicity of Trypanosomes

Proceedings of a workshop held at Nairobi, Kenya, 20-23 November 1978



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Editors: **George Losos[1] and Amy Chouinard[2]**

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Contents

Immunity in the bovine to *T. congolense* induced by self-cure or chemotherapy

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Abstract. We tested the hypothesis that cattle acquired immunity to trypanosomiasis after self-cure or chemotherapeutic cure. Using Hereford cattle from trypanosome-free areas of Kenya, we intravenously infected 42 animals with *T. congolense* and monitored their progress until they self-cured, required diminazene aceturate (Berenil) treatment for survival, or died. Later, the surviving animals and a control group were challenged with a homologous strain of *T. congolense*. Our results during primary infection indicated that animals aged 4 months to 1 year have a strong resistance to the pathogenic effects of trypanosomiasis — 10 of 11 animals in that age group experienced self-cure, whereas all 20 animals more than 2 years old either died or required drug therapy to survive. After the second challenge, adult animals that had survived the first infection exhibited some immunity; they showed no clinical signs of disease from a third challenge.

Although many workers have attempted to induce immunity to trypanosomiasis under field conditions, their results have been contradictory. Reports of several investigators have shown no evidence of immunity in cattle maintained in endemic areas over long periods (Hornby 1941; Wilson, Paris, and Dar 1975). Other studies, however, claim that drug therapy induces a degree of protective immunity (Bevan 1928; van Saceghem 1938; Fiennes 1953; Soltys 1955; Smith 1958; Wilson et al. 1976). In some instances, these observations are difficult to interpret because of the nature of the drugs used to cure or suppress trypanosome infections.

It has also been postulated that young animals show an increased resistance to infection (reviewed by Fiennes 1970), possibly through transmission of an immune factor from the mother (Whiteside 1962). Other authors disagree and claim there is no evidence for naturally acquired immunity except in resistant breeds of cattle (Weitz 1970). No substantial laboratory investigations have been performed to resolve the conflicts concerning drug-induced or age resistance to trypanosomiasis. In this regard, we initiated a series of experiments to investigate the questions of natural and acquired immunity to trypanosomiasis in bovines.

Materials and Methods

Cattle, predominantly Hereford, were obtained from the veterinary department farm at Kabete or from other trypanosomiasis-free areas in Kenya. All animals were treated with phenamidine isethionate and oxytetracycline (Terramycin) to eradicate any latent babesia or anaplasma infections, dipped in an acaracide weekly and treated periodically with triclofenol piperazine (Ranizole) to limit helminthic infection.

We used the Trans Mara-I strain of *T. congolense*, which was isolated from an infected cow in the Trans Mara area near the Kenya-Tanzania border in 1966. The strain had been stored as a stabilate in dry ice and maintained in cattle by blood passage. We infected the cattle with trypanosomes obtained from infected bovines or mice. We enumerated the trypanosomes in a hemocytometer and diluted them with phosphate-buffered saline (pH 7.8) containing 5% glucose and 10% fetal calf serum. Cattle were infected intravenously with 1×10^4 *T. congolense*/227 kg body weight.

A second strain of *T. congolense* (Yoani-I) was used to test the specificity of immunity in animals immunized with the Trans Mara-I strain. This strain

Table 1. Effects of trypanosome dose on prepatent periods.

Dose (tryps/ 227 kg) (±2 SE)	Animals (no.)	Prepatent period (d) (±2 SE)
$3.0 (\pm 0.6) \times 10^8$	2	2.0 (±0.0)
$1.7 (\pm 0.5) \times 10^7$	2	3.0 (±0.0)
$7.0 (\pm 1.0) \times 10^6$	3	3.3 (±0.7)
$1.2 (\pm 0.2) \times 10^5$	9	4.6 (±0.5)
$1.1 (\pm 0.2) \times 10^4$	28	5.7 (±0.3)
$2.8 (\pm 0.1) \times 10^3$	2	7.0 (±0.0)

Table 2. Effect of age on *T. congolense* infections in cattle.

Age (y)	Animals (no.)	Median survival time (wk)[a]	Self-cures (%)
0.5–1	11	>78	10 (91)
1–2	11	25	2 (18)
2–3	11	12	0 (0)
3–4	5	6	0 (0)
4–5	2	7	0 (0)
5–6	2	8	0 (0)

[a]Based on time to treatment or day of death.

(Yoani-I) was isolated at Yoani, Kenya, in May 1977 from an infected dairy cow.

We reared *Glossina morsitans* by standard methods for fly-transmitted challenges. Newly emerged flies fed on an infected bovine donor 14 days. Thereafter, the flies fed for 5-day intervals on uninfected bovines until needed to induce infection.

We used diminazene aceturate (Berenil) to treat infected animals. The dose was 1.05 g active ingredient/300 kg body weight.

We obtained blood for smears from the tip of the tail 6 days a week and blood for hematological examinations from the jugular vein in disodium ethylenediaminetetracetic acid (EDTA) usually twice a week.

Parasitemias were estimated by counting the numbers of trypanosomes/100 leukocytes on thick blood smears and relating these values to the total leukocyte counts/mm^3. Thrombocyte numbers were determined by phase microscopy according to the method of Brecher and Cronkite (1950).

Results

Within the range of numbers of trypanosomes used in our experiments, we found no relationship between dose and animal survival time. Males and females did not differ in their response to infection or in survival time. The dose of trypanosomes, however, did affect the prepatent periods in experimental bovines (Table 1).

A marked resistance to infection was observed in animals aged 4 months to 1 year (Table 2). Ten of 11 animals 6 months to 1 year, and 2 of 11 animals aged 1–2 years survived infection. All animals more than 2 years old died or required treatment to survive.

When self-cured animals were challenged with the homologous strain of *T. congolense*, they did not develop detectable infections or any evidence of disease, whereas all control animals of comparable age required treatment to survive (Table 3).

Adult animals that required therapy to survive were also challenged with the homologous strain of *T. congolense* (Table 4). They had developed an appreciable immunity, and many of them self-cured the second infection. When the challenging infection was given about 2 years after treatment, the infections produced a chronic disease, and most animals had to be treated to survive. The infections

Table 3. Results of primary challenge of previously infected, self-cured animals.

	Initial infection						Primary challenge (1×10^4/227 kg)		
No.	Age	Sex[a]	Dose/ 227 kg	PP[b] (d)	Last patent parasitemia (wk-d)	Interval[c] (wk-d)	Age (y)	PP[b] (d)	Result (wk-d)[c]
1	0.5	M	2.8×10^3	8	54-4	25-0	2.0	NP	NDI
2	1.3	MC	1.0×10^4	5	61-1	31-6	3.0	NP	NDI
3	0.3	F	1.0×10^4	5	56-2	36-5	2.0	NP	NDI
4	0.5	M	2.9×10^3	6	30-5	48-5	2.0	NP	NDI
5	1.4	MC	1.0×10^4	5	38-2	54-5	3.1	NP	NDI
Average of 3 controls for primary challenge							3.1	4.7	T (9-3) [d]

[a]F = female; MC = Male castrated; M = male.
[b]PP = prepatent period; NP = not patent.
[c]Time between last patent parasitemia and challenge.
[d]Treated (time since challenge).

Table 4. Results of primary challenge of previously infected and treated cattle.

	Initial infection						Primary challenge (1 x 10^4/227 kg)		
No.	Age (y)	Sex[a]	Dose/ 227 kg	PP[b] (d)	Time to treatment (wk-d)	Interval[c] (wk-d)	Age (y)	PP[b] (d)	Result (wk-d)[d]
6	1.0	F	6.8×10^6	3	7-0	28-6	1.7	14	SC (17-0)
7	4.4	F	1.0×10^4	6	9-0	30-0	5.2	10	SC (11-5)
8	2.7	F	1.0×10^4	5	11-5	42-2	3.8	18	SC (15-5)
9	2.6	F	1.0×10^4	5	6-6	47-1	3.7	13	T (36-6)
10	1.9	F	1.3×10^5	5	28-0	71-4	3.9	14	SC (4-4)
11	1.6	MC	8.4×10^3	6	5-5	86-0	3.4	8	T (21-3)
12	1.9	MC	1.0×10^4	6	5-5	86-0	3.7	6	T (11-5)
13	2.3	MC	1.9×10^4	5	11-0	122-5	4.9	6	T (27-0)
14	3.4	F	1.3×10^4	5	5-1	128-4	6.0	6	SC (29-2)
Average of 8 controls for primary challenge							4.1	5.5	T (9-4)

[a] F = female; MC = male castrated.
[b] PP = prepatent period.
[c] Time between treatment and challenge.
[d] SC = self cure (time of last patent parasitemia after challenge); T = treated (time since challenge).

were less serious than those of controls, and treatment was required later than in the control group or during the primary infection. When animals were challenged a second time with a homologous infection, no detectable infections or clinical signs of disease were observed, whereas all controls developed parasitemia and needed treatment to survive (Table 5).

Animals immune to challenge with blood forms were also largely immune to tsetse challenge with the same strain (Table 6). Of 10 immune animals challenged by fly bite, 5 did not develop parasitemia or clinical evidence of disease. The others had limited periods of patent parasitemia and no serious clinical signs of disease (Table 7). All 10 immune animals survived. A severe infection developed in the eight controls, and seven required treatment. One control (aged 1.9 years) survived a severe infection.

No detectable immunity was observed when

Table 5. Results of the second challenge of bovines requiring treatment or self-curing after primary challenge.

Group	Animals (no.)	Interval[a] (m)	PP[b] (d)	Result[c]
Self-cure	3	5-30	NP	NDI
Treated	3	6-10	NP	NDI
Control	6	–	5-6	T (2.0 m)

[a] From last patent parasitemia or treatment.
[b] PP = prepatent period; NP = not patent.
[c] NDI = no detectable infection.

Table 6. Results of challenge of bovines immune to blood-form trypanosomes by tsetse fly bite (homologous strain).

Group	Animals (no.)	Patent (no.)	Median prepatent period (d)	Clinical signs	Survivors
Immune	10	5	>120	0	10
Control	8	8	10.5	8	1

Table 7. Clinical parameters of animals immune to blood form after challenge by tsetse flybite with the homologous strain of *T. congolense*.

Parameter	Group	Time after challenge (wk)								
		0	1	2	3	4	5	6	7	8
Packed cell volume (%)	Immune	31 (0.8)[a]	31 (1.0)	31 (1.0)	32 (0.7)	31 (0.9)	31 (0.9)	31 (0.9)	30 (0.9)	31 (0.9)
	Control	32 (0.5)	31 (0.6)	30 (0.6)	26 (0.8)	24 (0.7)	20 (0.5)	19 (0.3)	19 (0.7)	18 (0.5)
Thrombocytes ($\times 10^3/mm^3$)	Immune	466 (52)	432 (31)	410 (42)	415 (60)	398 (39)	415 (89)	464 (83)	369 (47)	333 (57)
	Control	533 (60)	608 (57)	258 (59)	120 (20)	130 (19)	196 (42)	151 (48)	168 (14)	187 (41)
Leukocytes ($\times 10^3/mm^3$)	Immune	13.7(1.2)	11.1(1.1)	12.7(1.4)	10.8(0.6)	10.6(0.6)	14.4(2.0)	12.6(1.4)	11.0(0.9)	11.8(0.6)
	Control	11.2(0.8)	10.5(0.7)	7.3(1.0)	6.9(1.4)	5.0(0.5)	5.6(0.6)	5.1(0.5)	5.2(0.9)	4.4(0.9)
Parasitemia (daily avg/mm^3)	Immune	0	0	147	470	117	137	299	0	524
	Control	0	0	4360	17163	3197	4039	7810	3781	3123
Animals patent/ animals remaining	Immune	0/10	0/10	3/10	2/10	2/10	2/10	2/10	0/10	3/10
	Control	0/8	0/8	8/8	8/8	8/8	8/8	7/7	6/6	6/6

[a] (1 standard error).

bovines, immune to the Trans Mara-I strain of *T. congolense*, were challenged with the Yoani-I strain, either by blood-induced or fly-induced challenge. Parasitemias and clinical evidence of disease in immune and control groups were undistinguishable, and all animals were treated during the 5th week after challenge, when packed cell volumes decreased to less than 20%.

Discussion

Our studies demonstrated an age resistance to *T. congolense* infection in bovines. Although young animals developed a relatively serious disease, almost all survived, whereas animals more than 2 years old invariably succumbed to infection. Although the mechanism(s) for the resistance is not known, it did not involve specific maternal antibody because mothers of our animals had never been infected. Our studies confirm and extend the observations of Fiennes (1970) whose results have not been widely accepted. Though Weitz (1970) asserted there was no evidence for an acquired protection in animals after recovery from the disease, we have shown that young surviving animals are resistant for extended periods to a challenge infection of the same strain by either syringe inoculation of blood forms or by tsetse fly bite (metacyclic forms).

Animals undergoing infection and cure were also resistant to challenge by the same strain. This protection was not premunitive, because the animals had been given curative therapy. The resistance correlated with the duration of infection, the time elapsing between treatment and challenge, and the number of infections to which the animals had been subjected. Our results support the field work of Wilson et al. (1976) who have shown convincing evidence that infected cattle develop immunity after treatment with diminazene aceturate (Berenil) in an endemic area. Effective levels of the drug persist in the blood of the bovine too short a time to complicate the studies. Trials in our laboratory (unpublished data) showed that diminazene aceturate (Berenil) (7 mg/kg) influenced infectivity for up to 12 days, the prepatent period up to 18 days but not later. These findings agree with previously published work (Cunningham et al. 1964).

Self-cure or cure by chemotherapy both largely protected animals against challenge by tsetse fly carrying the same strain of trypanosome. This fact indicates that cyclical transmission did not produce populations of parasites possessing different variant antigens from those expressed during the blood-induced infections.

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