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Studies on transmission of two East African stocks of *Trypanosoma vivax* to cattle, goats, rabbits, rats and mice

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Summary

Transmission studies were conducted using two *Trypanosoma vivax* stocks isolated from bovines in Uganda. Parasitaemia was low and transient in rabbits and rats; it persisted for relatively longer in NMRI mice. The parasitaemia developed to a peak in a few A/J and Balb/c mice; in NMRI, C57B and C3H/He it was low and fleeting. Lethally irradiated A/J, C57B and C3H/He mice with caesium 137 at 900 Gy showed a high peak of parasitaemia; NMRI and Balb/c mice succumbed very rapidly to a similar radiation dose. Serial maintenance of one stock of *T. vivax* was achieved in normal NMRI and lethally irradiated A/J mice. Both stocks failed to develop in the proboscis of *Glossina morsitans morsitans* or of *G. m. centralis*, and hence cyclical transmission to goats also failed. However, non-cyclical transmission by tsetse from goat to goat, and from cattle to goats, was successful. The infection caused acute and fatal disease in goats which were anaemic at death. The Boran cattle used eventually suppressed the infection and recovered.

Key words: East African *Trypanosoma vivax;* non-cyclical transmission; cattle, goats, rabbits, rats, NMRI, A/J, Balb/c, C57B and C3H/He mice.

Introduction

Although *Trypanosoma vivax* is pathogenic to livestock and is therefore of great economic importance, it has been studied less than *T. congolense* and *T. brucei*, probably because the small laboratory animals in common use are refractory to this infection. *T. vivax* isolated from ruminants produced only transient parasitaemia in mice and rats (Desowitz and Watson, 1951; Godfrey

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et al., 1965) and its serial maintenance in rodents proved very difficult (Desowitz and Watson, 1952, 1953; Hull et al., 1971). Leeflang et al. (1976), however, isolated three stocks of *T. vivax* from cattle which were infective to mice and at least one of these can be cyclically transmitted by *Glossina* between rabbits, rats, mice and goats (Moloo, 1976, 1981).

In all this work, stocks of *T. vivax* isolated from ruminants in West Africa were used. However, Mwambu (1969) reported that during the cattle trypanosomiasis survey in Teso District, Eastern Uganda, the injection into mice of the blood of 1306 cattle resulted in 4 transient *T. vivax* infections. These parasites were not studied further in the rodent. The present study is concerned with transmission of East African stocks of *T. vivax* to cattle, goats, rabbits, rats and mice. Transmission of infection by *Glossina* to susceptible hosts has also been examined.

Materials

T. vivax ILRAD V-13 and V-4 used were derivatives of EATRO 1184 and 2058. They were isolated by Dr. P. M. Mwambu respectively in 1968 and 1972 from bovines in Teso District, Uganda.

The animals used were 7–12 months old Boran cows, East African adult Galla crossbred goats, New Zealand albino rabbits and Wistar albino rats. The strains of mice used were ILRAD bred NMRI, A/J, Balb/c, C3H/He and C57B.

Glossina morsitans morsitans and *G. m. centralis* were from the ILRAD R^6 colonies (Moloo, 1979). The experimental tsetse were kept at 25° C and 70% relative humidity.

To detect the infection, rats and mice were bled from the tail, and rabbits, goats and cattle from the ear, daily except Sundays; parasitaemia was determined by microscopic examination of unstained wet blood films, with phase-contrast illumination using a combination a Phaco 2 NPL Fluotar 40/0. 7 objective and Periplan GW 10× eye pieces (E. Leitz, Wetzlar, Germany). Packed cell volume (PCV) of all the hosts, except mice, was measured, and the buffy coat was examined for parasites using the haematocrit centrifugation technique (Woo, 1969).

Experiments and Results

Susceptibility of mice, rats, rabbits and goats

A goat having initial PCV of 33% was injected intra-muscularly (i.m.) with *T. vivax* ILRAD V-13. The prepatent period was 6 days. When the parasitaemia had risen to about 15 parasites per field (15/F), 10 NMRI mice, 10 rats and 2 rabbits were injected intra-peritoneally (i.p.) with heparinised blood collected from the jugular vein of this goat; mice and rats were given 0.5 ml each whereas each rabbit received 1.5 ml. In 8 mice the infection became patent on day 3; parasitaemia in 6 mice rose to a peak between days 4 and 7. The mice suppressed the infection by day 14, and all were killed on day 40. Only 4 rats became infected; parasitaemia was very low and transient. All rats were killed on day 60. The prepatent period in both rabbits was 4 days; parasitaemia was low and both suppressed the infection by day 11 and were killed on day 90. The

infected goat died on day 77. It had a PCV of 15% at death, a drop of 54.5% of the original value.

In an attempt to determine infectivity of *T. vivax* ILRAD V-4 to mice, rats and rabbits, a similar experimental procedure was used. The results were similar; parasitaemia in mice was high and the animals eventually suppressed the infection; in rats and rabbits it was low and transient. The prepatent period in the goat was 8 days and it died 44 days later, with a PCV drop from the initial value of 37% to 16% at death.

Serial maintenance in mice and goats

A goat was injected i.m. with *T. vivax* stabilate ILRAD V-4. The prepatent period was 6 days, and the parasitaemia peaked on day 11. On this day, heparinised infected blood from jugular vein was injected i.p. into 5 NMRI mice, 0.5 ml per mouse. The infection from the rodent was serially subpassaged in goats and in groups of 5 NMRI mice as shown in Fig. 1. The parasitaemia in mice was high, but the animals eventually suppressed the infection. With increasing numbers of subpassages in mice from each successively infected goat the level of parasitaemia decreased until finally the rodent became resistant to infection. However, the number of successful serial subpassages in mice was highest from the tenth goat; parasites from the 25th subpassage were cryopreserved in liquid nitrogen. Also, from the second group of serially infected mice, the parasites were finally subpassaged twice in rats. The infection in the latter was low. All the rodents suppressed the infection and were killed 50 days after injection. The prepatent periods in the goats varied between 4 and 8 days. All the goats succumbed to the disease between days 36 and 132 following injection and were anaemic at death.

Susceptibility of different strains of mice

T. vivax ILRAD V-4 was injected i.p. into 5 individuals of the following strains of normal or lethally irradiated mice: NMRI, A/J, Balb/c, C57B and C3H/He. Whole body irradiation (900 Gy) was given using a caesium 137 radiation source one hour before injecting the mice. All mice were examined for parasites as previously described; those which survived for 40 days were killed. The results are given in Table 1. Whereas in normal A/J and Balb/c the parasitaemia peaked in some, in the remaining strains of normal mice it was markedly low. All mice suppressed the infection and were killed on day 40 after injection. Lethally irradiated NMRI and Balb/c mice died very rapidly, probably from the effect of radiation, while C3H/He survived the longest; A/J and C57B mice were intermediate. Four A/J and 4 C3H/He showed a high peak of parasitaemia; this was observed in only 2 C57B. In the infected mice of the other two strains, parasitaemia was markedly low and transient. It is clear that although normal A/J and Balb/c were more susceptible than the other three strains in terms of parasitaemia, they invariably suppressed the infection. Lethally irra-

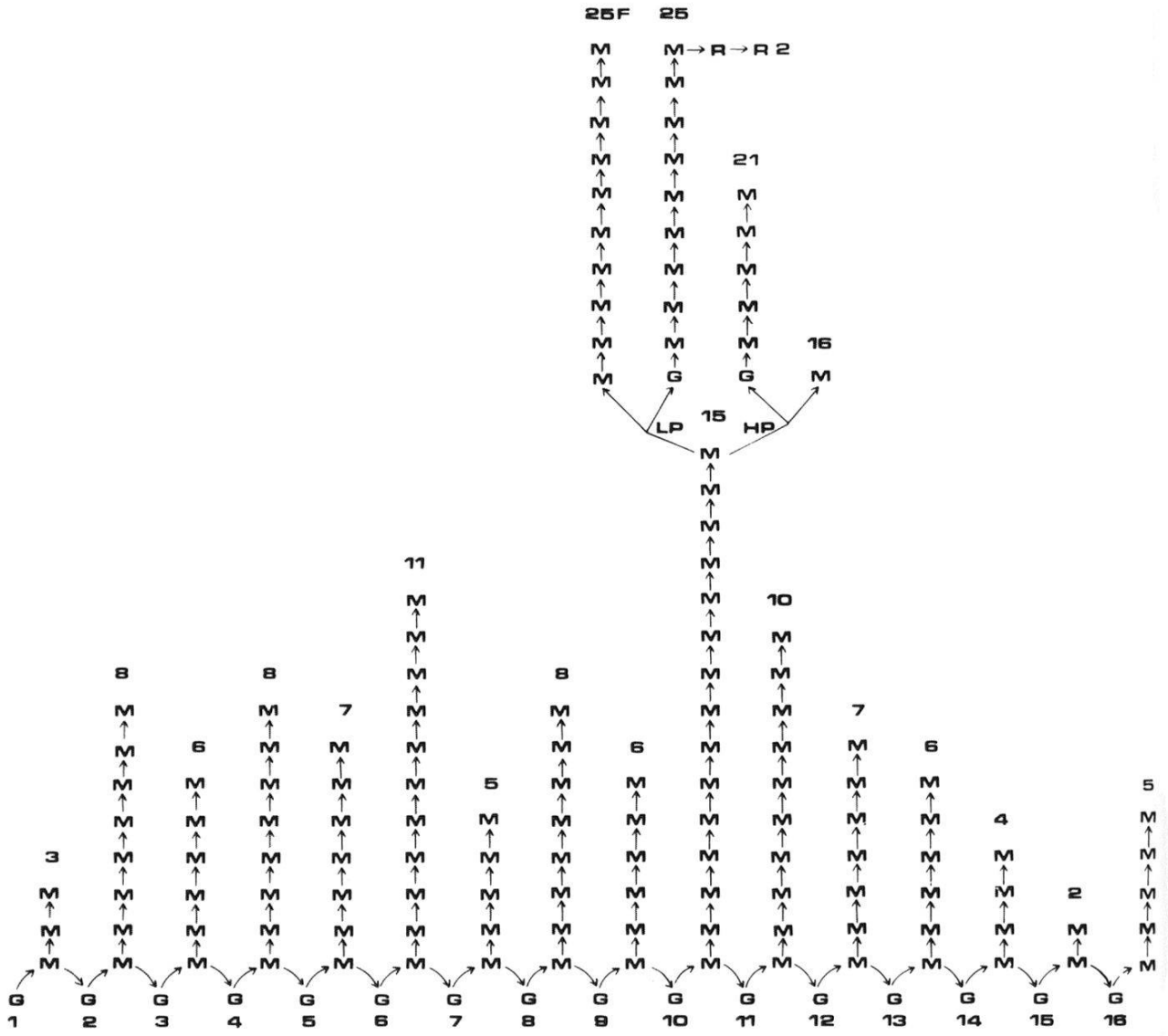

Fig. 1. Serial maintenance of *T. vivax* (ILRAD V-4) in mice and goats (G, goats; M, mice; R, rats; LP, low parasitaemia; HP, high parasitaemia; F, frozen; numbers indicate subpassages).

diated A/J, C57B and C3H/He mice died of the infection or radiation sickness or both, while the other two strains of mice succumbed to the radiation very rapidly.

Serial maintenance in lethally irradiated A/J mice

To determine the effect of serial maintenance of *T. vivax* ILRAD V-4 in lethally irradiated A/J mice upon the parasitaemia pattern, 3 mice were injected i.p. with the stabilate. These mice became parasitaemic 3 days after injection. One was killed on day 7 when the parasitaemia was 20/F, for second passage into 2 mice. The other was found dead on day 12; on the previous day the infection was 28/F. The third mouse showed a high peak parasitaemia of 40 + /F on day 12. This level of infection continued up to day 20; thereafter it declined and by day 39 the animal had suppressed the infection. It was killed on day 60.

Table 1. Infectivity of an East African stock of *T. vivax* ILRAD V-4 in five different breeds of normal and lethally irradiated mice

Breed	Normal			Irradiated		
	Transmission rate	Prepatent period days	Remarks	Transmission rate	Prepatent period days	Remarks
NMRI	4/5	3–5	In all 4 mice very low parasitaemia was detected on one day only; they suppressed the infection	1/5	3	Very low parasitaemia detected on one day only. All died between day 5 and 7
A/J	5/5	3	One showed a high peak on day 11, and 4 showed low peak between day 9 and 11. All suppressed the infection by day 16	5/5	3	Four showed a high peak and 1 a low peak on day 8. All died between day 11 and 16
Balb/c	4/5	3	Two showed a high peak on day 10 or 24. Very low parasitaemia detected in the other 2. Parasitaemia transient	2/5	3	Very low parasitaemia detected on one day only. All died between day 5 and 7
C57B	4/5	3–5	Parasitaemia pattern was similar to that observed in the NMRI mice	5/5	3–5	Two showed a high peak while the other 2 low peak between day 8 and day 13. In 1 very low infection was observed on one day only. All died between day 8 and 14
C3H/He	4/5	3–5	Parasitaemic pattern was similar to that observed in NMRI mice	4/5	3–7	In all 4 mice the infection peaked. The uninfected one died on day 11. Of the 4 infected mice, 2 died on day 15, 1 on day 36 and 1 on day 41

Both mice of the second passage became infected on the day following injection. In one, the infection peaked on day 6 when the mouse was killed for subsequent passage into 3 mice. In the second mouse the parasitaemia peaked on day 8; it died on day 10.

In the third passage, one mouse was used on day 5, when the parasitaemia was 36/F, for injection into 3 A/J mice. In the other mouse, the infection peaked on day 6, declined thereafter and the animal died on day 10. The infection in the third mouse peaked on day 10, remained high till day 13 and was then suppressed. This mouse was killed on day 60.

Mice of the fourth passage all became infected. One A/J mouse was killed on day 9 when the parasitaemia was 30/F, and the infected blood was injected into 5 A/J mice. The other 2 mice died without attaining a high peak of infection; one died on day 7 and the other on day 11.

In the last, fifth passage, all the 5 A/J mice became infected. Whereas in 3 mice the parasitaemia was low, in the remaining 2 it peaked; 80 teneral *G. m. morsitans* were allowed to feed on the latter. Both mice died on day 8 after the tsetse had fed. Two of the other mice died on day 10 and the third on day 13. The tsetse were maintained on a rabbit for 26 days and the surviving 63 tsetse were dissected. None was infected.

Attempts at cyclical and non-cyclical transmission

One goat was injected i.m. with *T. vivax* ILRAD V-4, another with ILRAD V-13. The prepatent periods were 19 and 10 days respectively. When the parasitaemia in both goats had risen to about 20/F, teneral *G. m. morsitans* were fed on their clipped and cleaned flanks, 240 per goat. Thereafter, the tsetse were maintained on two different rabbits. About 40 tsetse from each of the two groups were dissected on day 5, 10, 15 or 21 after the infected blood meal. The labrums and hypopharynges were examined for parasites, but they were not infected. Two groups of 40 tsetse each were allowed to bite two different goats on day 30. Both goats remained uninfected and none of the dissected tsetse was infected. The two rabbits remained uninfected for 90 days and were killed. The two infected goats died on days 44 and 41, respectively. Both were anaemic at death with PCV drop of 29% and 39%.

This experiment was repeated using a goat infected with *T. vivax* ILRAD V-4. Three hundred and eighty teneral males and females of *G. m. morsitans* and 600 of *G. m. centralis* were fed on the infected goat for 5 days when parasitaemias were between 1/F and 40+/F. The tsetse were maintained on a rabbit and the surviving 314 *G. m. morsitans* and 382 *G. m. centralis* were allowed to bite an uninfected goat and then dissected 35 days after emergence. There was no transmission to the goat and all the labrums and hypopharynges lacked trypanosomes. The rabbit did not become infected and was killed on day 90. The infected goat died 44 days after infection, and was anaemic at death.

Since *T. vivax* ILRAD V-4 and V-13 from the infected goats failed to com-

plete their developmental cycle in the tsetse, an attempt was made at the non-cyclical transmission. A group of 80 teneral male *G. m. morsitans* in 4 Geigy-20 cages was allowed to feed on the flanks of a goat infected with *T. vivax* ILRAD V-4. The feeding of most tsetse was interrupted and the cages strapped on to the flanks of an uninfected goat for the completion of engorgement. The parasitaemia in the infected donor goat was 10/F. Thereafter, the tsetse were maintained on a rabbit for 20 days and then allowed to feed on an uninfected goat. The surviving 72 tsetse were dissected; all lacked parasites in their labrums and hypopharynges. The rabbit used to maintain these tsetse and the challenged goat remained uninfected. Thus, cyclical development of the above *T. vivax* stock again failed, but non-cyclical transmission to the goat was successful. The prepatent period in the infected goat was 10 days and it died 27 days later. Its initial PCV was 30% and it was anaemic at death with PCV of 17%.

The two stocks of *T. vivax* used were isolated from bovines, and hence it was logical to study them in these hosts. Hence, two Boran cows were infected as before, one with ILRAD V-4 and the other with V-13. The prepatent periods were 6 and 10 days, respectively. One hundred teneral *G. m. morsitans* and 160 teneral *G. m. centralis* were allowed to feed for 2 days on the flanks of the cow infected with ILRAD V-4, when its parasitaemias were 18/F and 40 + /F. These tsetse were then maintained on a rabbit and the surviving 81 *G. m. morsitans* and 133 *G. m. centralis* were allowed to bite a goat 22 days after their infected blood meal and then dissected. There was no transmission to this goat and all the tsetse were uninfected. The rabbit was examined for 90 days but no infection developed and it was killed. The parasitaemia in the cow peaked on day 9 after injection and the animal suppressed the infection on day 62. Its PCV declined somewhat for the first 9 days from an initial value of 31% to 25%, but thereafter it increased rapidly to the normal value which was maintained up to day 200 when examination ceased.

Eighty teneral *G. m. morsitans* and 360 teneral *G. m. centralis* were allowed to feed on the flanks of the cow infected with ILRAD V-13 for 4 days, when the parasitaemia varied between 1/20F and 30/F. The tsetse were then maintained on a goat for 22 days, and the surviving 42 *G. m. morsitans* and 215 *G. m. centralis* were dissected. None was found infected. The goat was examined for 90 days; it was not infected. There was therefore no cyclical development and hence no transmission. However, when a group of 40 tsetse feeding on the infected cow were transferred to the flanks of an uninfected goat to complete engorgement, the goat became infected by non-cyclical transmission. The prepatent period in the goat was 10 days and it was anaemic at death 19 days later, with a PCV drop of 53.3% from the initial value of 30%. The parasitaemia in the cow peaked on day 21 but was in the main markedly low, being often undetectable even in the buffy coat. The animal's PCV declined for the first 3 weeks but thereafter it recovered. This animal suppressed the infection by day 104 and its examination ceased on day 175.

Discussion

The present study has demonstrated that the two stocks of *T. vivax* isolated from bovines in Uganda could infect rabbits, rats and mice. Whereas in rabbits and rats the parasitaemia was markedly low and transient, in mice it persisted for a significant length of time, and in some strains the infection developed to a high peak. Also, the attempts at serial maintenance of the parasites (ILRAD V-4) were successful in both normal NMRI and lethally irradiated A/J mice. Hence, a few *T. vivax* stocks, including the present ones isolated from East Africa, can infect mice, but the physiological system in these parasites that allows growth in the rodents and rabbit is unknown. Differences in susceptibility to *T. vivax* infection were observed between the 5 strains of mice. Similar differences in susceptibility were found between 8 strains of mice to infection with *T. congolense* and this was attributed to differences in the nature or quality of the immune response to the trypanosome (Morrison et al., 1978). It is probable that this trend demonstrates genetic differences in susceptibility between the different strains to the trypanosome infections.

It is of some interest that all attempts at cyclical transmission of the two stocks to susceptible goats failed. The parasites could not become established in the proboscis of either *G. m. morsitans* or *G. m. centralis* and complete their cycle of development. However, non-cyclical transmission was demonstrated. It is therefore logical to conclude that these two *T. vivax* stocks had become incapable of cyclical transmission by *G. m. morsitans* and *G. m. centralis*. Probably that they were being transmitted non-cyclically by blood sucking insects among cattle in Teso District of Uganda from where they were isolated. The present study thus confirms previous observations (Rodhain, 1941; van Hoof, Henrard and Peel, 1948; Hoare, 1957) that *T. vivax* is transmitted non-cyclically in some parts of Africa where the infection is endemic.

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