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Trypanosoma vivax, T. congolense or *T. brucei* infection rates in *Glossina morsitans* when maintained in vitro on the blood of goat or calf

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Summary

Tenerals of *Glossina morsitans morsitans* and *G. m. centralis* were infected with *Trypanosoma vivax*, *T. congolense* or *T. brucei* by feeding mainly on infected goats and then maintained either in vivo on uninfected calves, goats or rabbits, or fed in vitro upon heparinised or defibrinated blood of goats or calves for 21 days for *T. vivax* and *T. congolense* and 30 days for *T. brucei* and then dissected. The observed differences in the infection rates for all three trypanosome species maintained on different diets were small and/or inconsistent and possibly are of no significance. It is therefore likely that the in vitro feeding of the tsetse on these diets after infected blood meal has no adverse effect on the cyclical development of these trypanosome species in these vectors.

Key words: *Glossina morsitans morsitans; G. m. centralis; Trypanosoma vivax; T. congolense; T. brucei;* infection rates; in vitro maintenance; heparinised and defibrinated bloods; calves; goats.

Introduction

Following the succesful mass rearing of *Glossina* species in 1960s using in vivo feeding regimes (Nash et al., 1971), attempts were directed to develop an efficient in vitro feeding system to colonize this insect. This was achieved in 1970s with the development of Agar/Parafilm Membrane (Langley and Maly, 1969) which was later replaced with the more durable silicone rubber membrane (Bauer and Wetzel, 1976). In some laboratories colonies of tsetse are routinely fed through silicone rubber membranes upon the blood of pig or ox

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(Mews et al., 1976, 1977), but workers involved in laboratory studies of tsetse as vectors of trypanosomiasis largely use in vivo feeding systems. More recently, however, Evans (1979) has shown that *T. b. rhodesiense* and *T. congolense* procyclics can undergo cyclical development in tsetse using in vitro system, and Maudlin (1982) used culture of *T. congolense* procyclics to infect *G. morsitans*. Schöni et al. (1982) found that in vitro cultivated bloodstream and procyclic forms of *T. b. brucei* can complete cyclical development in tsetse when maintained on reconstituted freeze-dried pig blood. The present study was aimed to find if the incidence of cyclical development of the three pathogenic trypanosome species in tsetse are affected if they are maintained in vitro on the blood of goat or calf after feeding on infected hosts.

Material and Methods

Glossina morsitans morsitans and *G. m. centralis* used were from the colonies bred in ILRAD. The experimental tsetse were kept at 25° C and 70% relative humidity.

Trypanosoma vivax (ILRAD 417) was a derivative of Zaria Y486 isolated from a Zebu cow in Nigeria in 1973 (Leeflang et al., 1976). *T. brucei* (ILRAD 375) derived from STIB 247 was isolated from a Coke's hartebeest, and *T. congolense* (ILRAD 687) derived from STIB 212 was isolated from a lion in the Serengeti region of Tanzania in 1971 (Geigy and Kauffmann, 1973). The history of the three stocks has been described elsewere (Moloo, 1981).

The animals used to infect or maintain tsetse were 7- to 12-month-old Boran calves bred in ILRAD. East African adult Galla crossbred goats, ILRAD bred half-lop rabbits, and A/J mice.

To determine the degree of infection, goats, calves and rabbits were bled from the ear, and mice from the tail daily except Sundays, and wet blood films were examined for parasites with a phase-contrast microscope. The buffy coat was also examined for the parasites using the haematocrit centrifugation technique (Woo, 1969).

Infection of tsetse with T. vivax

Tenerals of male and female *G. m. morsitans* (306 ♂♂ and 306 ♀♀) were fed on the clipped flanks of a goat infected with *T. vivax* at peak of parasitaemia (>40 parasites/microscope field at 400×). They were then divided into six groups and each maintained for 21 days on one of the following six diets: (1) goat blood in vivo; (2) calf blood in vivo; (3) defibrinated goat blood using silicone membranes in vitro; (4) heparinised goat blood; (5) defibrinated calf blood; or (6) heparinised calf blood. The surviving tsetse were dissected and their labra and hypopharynges examined under a phase-contrast microscope to determine the infection rates. This experiment was repeated using 300 male and 300 female teneral *G. m. centralis*. One group was maintained in vivo on a rabbit while the other four were maintained by feeding in vitro using either defibrinated or heparinised goat or calf blood after feeding them on an infected goat at peak of parasitaemia.

Infection of tsetse with T. congolense

Tenerals of *G. m. centralis* (600 ♂♂ and 600 ♀♀) were fed for three days on a goat infected with *T. congolense*, the parasitaemia being 1, 4 and 10 parasites in 2 fields of 400× magnification, respectively. These tsetse were then divided into six equal groups comprising both sexes in each group and maintained for 21 days on six diets described above. The surviving tsetse were dissected and their midguts, labra and hypopharynges were examined to determine the infection rates. In a second experiment *G. m. centralis* (500 male and 500 female teneral tsetse) were fed on mice at peak of parasitaemia (>100 parasites/field at 400×) with *T. congolense*, were divided into five equal groups and maintained in vivo on rabbits or in vitro upon the defibrinated or heparinised blood of

goat or calf for 24 days. The surviving tsetse were dissected on day 25 post-emergence to determine the infection rates.

Infection of tsetse with T. brucei

Tenerals of male and female *G. m. morsitans* (586 tsetse) were fed on a goat showing *T. brucei* infection of 35 parasites per field of 400× magnification. These tsetse were divided into six groups and maintained in vivo on goat or calf, or in vitro upon the blood of these two hosts for 30 days. The survivors were dissected to determine the infection rates. In another experiment, 300 male and 300 female teneral *G. m. centralis* were fed on a goat for three days when *T. brucei* parasites were detectable in the buffy coat alone. They were then divided into five equal groups and maintained in vivo on a rabbit or in vitro upon the defibrinated or heparinised blood of goat or calf. The surviving tsetse were dissected to determine the infection rates.

Results and Discussion

Table 1 shows that there were some differences in *T. vivax* infection rates in *G. m. morsitans* and *G. m. centralis* which were maintained in vivo on a goat or a calf, or in vitro upon the blood of uninfected goat or calf after infected meals from a goat. In the repeat experiment, the mature *T. vivax* infection rates in tsetse were 42.6%, 26.0%, 37.4%, 56.1% and 59.8% when maintained in vivo on a rabbit, or in vitro on DGB, HGB, DCB and HCB, respectively. It would appear that the maintenance of tsetse in vivo or in vitro upon these diets have little effect on the incidence of cyclical *T. vivax* development in tsetse.

Table 2 shows *T. congolense* infection rates in *G. m. centralis* maintained in vivo on a goat or calf, or in vitro on the blood of goat or calf after feeding the tsetse as tenerals on infected goat. In the repeat experiment, the mature *T. congolense* infection rates in tsetse were 20.4%, 23.1%, 20.4%, 30.9% and 23.3% when maintained in vivo on a rabbit, or in vitro on DGB, HGB, DCB and HCB, respectively. These results show that though there were some differences, such differences were either small or inconsistent.

Table 1. Infection rates in *G. m. morsitans* when maintained on six different diets after blood meal intake from a goat infected with *T. vivax*

Maintained on	No. tsetse used	No. dissected	Infection rates (%) in	
			labrum	hypopharynx
Goat	97	94	74.5	67.0
DGB	91	88	64.8	64.8
HGB	98	93	72.0	68.8
Calf	110	108	79.6	65.7
DCB	110	107	57.0	53.3
HCB	106	103	49.5	49.5

Goat = Goat blood in vivo
DGB = Defibrinated goat blood in vitro
HGB = Heparinised goat blood in vitro
Calf = Calf blood in vivo
DCB = Defibrinated calf blood in vitro
HCB = Heparinised calf blood in vitro

Table 2. Infection rates in *G. m. centralis* when maintained on six diets after blood meal intake from a goat infected with *T. congolense*

Maintained on	No. tsetse used	No. dissected	Infection rates (%) in		
			midgut	labrum	hypopharynx
Goat	200	187	29.9	23.5	23.0
DGB	200	179	28.5	12.3	7.8
HGB	200	183	20.2	13.7	12.6
Calf	200	188	32.4	30.9	30.9
DCB	200	175	22.3	15.4	14.9
HCB	200	185	31.9	27.0	24.3

Table 3. Infection rates in *G. m. morsitans* when maintained on six different diets after blood meal intake from a goat infected with *T. brucei*

Maintained on	No. tsetse used	No. dissected	Infection rates (%) in	
			midgut	salivary glands
Goat	98	89	23.6	9.0
DGB	97	93	23.7	6.5
HGB	90	87	16.1	5.7
Calf	103	95	24.2	11.6
DCB	99	98	18.4	10.2
HCB	99	97	19.6	7.2

The results in Table 3 similarly show some differences in *T. brucei* infection rates in *G. m. morsitans* maintained on different diets. In the repeat experiment, the mature *T. brucei* infection rates in *G. m. centralis* were 9.7%, 9.0%, 11.9%, 16.7% and 3.2% when maintained in vivo on a rabbit, or in vitro on DGB, HGB, DCB and HCB, respectively. These differences in infection rates of the two different tsetse sub-species maintained on different diets are within the range of variability observed previously when *G. m. morsitans* infected with *T. vivax*, *T. congolense* or *T. brucei* were maintained on these host species in vivo (Moloo, 1981).

It is therefore possible that the cyclical development of *T. vivax*, *T. congolense* and *T. brucei* are unaffected by maintaining tsetse in vitro after the infected blood meal intake. It will therefore be economical to use in vitro feeding systems to maintain infected tsetse for experiments involving tsetse/trypanosome/host interactions since the blood of the same uninfected caprine or bovine can be used repeatedly for the maintenance of tsetse infected with any of these pathogenic trypanosome species, their different stocks and clones. It would be of interest to study the effect on the *T. vivax* and *T. congolense* infection rates in

tsetse when maintained in vitro after the infected meals, on the reconstituted freeze-dried bovine and caprine blood.

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