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Effects of maintaining *Glossina morsitans morsitans* on different hosts upon the vector's subsequent infection rates with pathogenic trypanosomes

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Summary

The percentage infection rates of *Trypanosoma vivax* in *Glossina morsitans morsitans* maintained after the infected meal on a cow, goats, rabbit, rats or mice were 88.0, 86.7, 94.8, 76.4 and 6.1, respectively. There were no significant differences between the males and females in this respect. The mortality rates of the tsetse maintained on mice or sheep were relatively high; the infection rate of the few survivors (5%) maintained on the latter host was 44.4%. The rates of *T. congolense* infection in the vector maintained on different hosts after the infected blood meal also differed. Goats (infection rate, 12.6%) and rabbit (11.4%) proved superior as maintenance hosts while rats (2.8%) and mice (0%) were inferior; sheep (7.8%) and cow (6.5%) were intermediate. Again, the mortality rate of the tsetse maintained on mice and sheep was markedly high; the reasons for this are discussed. Whereas cows (11.5%) and rabbit (11.2%) were efficient hosts for maintaining tsetse after the *T. brucei* infected feed, rats (4.1%) were inferior. Goat (9.0%) and mice (7.6%) proved intermediate. It is suggested that the rabbit is the best host for maintaining tsetse after infected feeds since the infection rates of all three *Trypanosoma* species in the vector were quite high, and this host is also relatively easy to handle in routine feeding of tsetse.

Key words: Glossina morsitans morsitans; Trypanosoma (Duttonella) vivax, T. (Nannomonas) congolense, T. (Trypanozoon) brucei; maintenance hosts: cows, goats, sheep, rabbits, rats, mice; infection rates.

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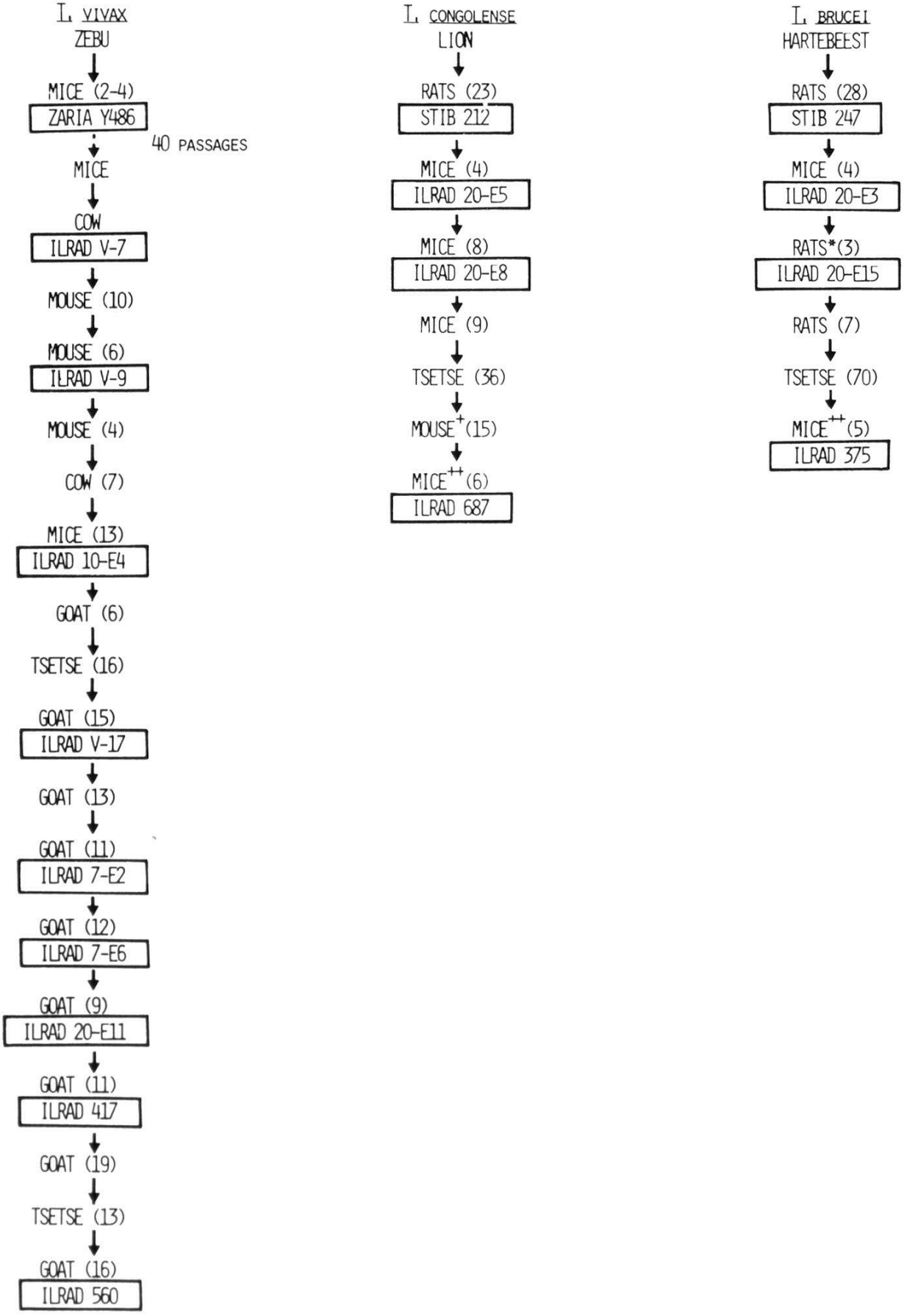

Fig. 1. Flow diagram showing history of the stocks of *Trypanosoma* species used in the present study. (Numbers in brackets indicate days after challenge.) * Irradiated at 900 rads gamma-radiation; + A/J mice; ++ BALB/c mice.

Introduction

In experiments involving the infection of *Glossina* with *Trypanosoma* species, different host animals are used in various laboratories to maintain the vector after they have been given the infected blood meal. However, little data are available about the effect of maintaining *Glossina* on different hosts in terms of infection rates after the vector have been fed on animals infected with *Trypanosoma (Duttonella) vivax*, *T. (Nannomonas) congolense* or *T. (Trypanozoon) brucei*. The present study is concerned with this subject.

Materials and methods

Tsetse

Glossina morsitans morsitans were from the ILRAD R^6 colony (Moloo, 1979). The experimental tsetse were kept at 25° C and 70% r.h.

Trypanosomes

Trypanosoma vivax (ILRAD 417) was a derivative of Zaria Y486 which was isolated from a Zebu in Nigeria in 1973 (Leeflang et al., 1976). *T. congolense* (ILRAD 687) was derived from STIB 212 which was isolated from *Panthera leo* (lion) and *T. brucei* (ILRAD 375) was derived from STIB 247 which was isolated from *Alcelaphus buselaphus* (Coke's Hartebeest). Both these stocks were isolated in Serengeti, Tanzania in 1971 (Geigy and Kauffmann, 1973). The history of the three stocks used is summarised in Fig. 1.

Hosts

The animals used to infect or maintain tsetse were 7- to 12-month old ILRAD bred Boran cows, East African adult Galla crossbred goats, adult female Merino crossbred sheep, Himalayan rabbits, Wistar derived albino rats bred in ILRAD from stock orginating from MRC in England, and the NMRI, A/J and Balb/c mice were also ILRAD bred.

(i) T. vivax infection rates in G. m. morsitans

Six capillaries of *T. vivax* stabilate ILRAD 417 were diluted in 3 ml of phosphate-buffered saline-glucose (PSG), pH 8.0 (Lanham and Godfrey, 1970), and material containing about 4 parasites per field of ×400 (4/F) was injected intramuscularly (i.m.) into a goat. This host was bled from the ear daily, except Sundays, and the parasitaemia was determined by examination of unstained wet blood films. The parasites were examined by phase-contrast microscopy using a combination of Phaco 2 NPL FLUOTAR 40/0.7 and Periplan GW 10× eye pieces (E. Leitz, Giessen, Germany). Packed cell volume (PCV) of the peripheral blood was also measured using the haematocrit centrifugation technique (Woo, 1969). When the parasitaemia rose to 40+/F on day 12, sixty groups containing altogether 1,200 teneral tsetse, mostly 20 per cage, were allowed to feed on the clipped and cleaned flanks of the goat. Nineteen tsetse did not feed and were discarded. The tsetse were then separated into 6 groups and maintained on six different uninfected host species, namely, a cow, 2 goats, a sheep, a rabbit, 5 rats or 10 NMRI mice. The large animals were kept and used in Isolation Units having maximum fly proofing while the small animals were in the Tsetse Vector Laboratory. The female tsetse were mated on day 3 post-emergence with 8-day old males from the colony. For the first 3 hosts, tsetse were fed on their clipped and cleaned flanks. In view of its thick fleece, the sheep was washed with water relatively more thoroughly after clipping. Tsetse were fed on the ears of the rabbit, or the belly of the rats or mice. The rats and mice were bled from the tail, and the parasitaemia was determined as described above. The other four hosts were bled from the ear and the parasitaemia and PCV were determined as described previously. Any mainte-

nance host that showed infection in the peripheral blood was not used further for tsetse feeding. Tsetse which were being maintained on sheep showed abnormally high mortality, and the few survivors were dissected on day 18 after the infected food intake. Their labra and hypopharynges were examined for parasites by phase-contrast microscopy to determine the infection rate; the remaining tsetse were dissected on day 21.

(ii) T. congolense infection rates in G. m. morsitans

Eight capillaries of *T. congolense* stabilate ILRAD 687 were diluted in 15 ml of the PSG, and the material containing 2 parasites/F was injected intraperitoneally (i.p.) into 60 NMRI mice, 0.2 ml per mouse. Sixty groups of teneral tsetse (600 males and 600 females) were allowed to feed on 48 mice on day 8 or 9 after the injection, when the parasitaemia in the host was 40 + /F. Fourteen tsetse which did not feed were discarded. The tsetse were separated into six groups and maintained on a cow, 2 goats, a sheep, a rabbit, 10 rats or 10 NMRI mice. The rest of the procedure was as described in (i) above. Again, the mortality of tsetse maintained on sheep was abnormally high and all were dead by day 14 after the infected blood meal intake. This experiment was repeated as described previously, but another sheep was introduced. The 2 sheep were clipped as closely as possible and were washed several times to remove any contaminant having insecticidal properties. The sheep were used on alternate days for tsetse feeding, and their flanks were washed thoroughly before the tsetse cages were strapped onto them. On days 20 and 21 after the infected feed, all the tsetse were dissected and their labra, hypopharynges and midguts were examined for trypanosomes.

(iii) T. brucei infection rates in G. m. morsitans

Six capillaries of *T. brucei* stabilate ILRAD 375 were diluted in 3 ml of PSG, and the material containing 2 parasites/F was injected i.m. into a goat. On day 22 after the injection when parasitaemia had risen to 35/F, 503 tsetse were allowed to feed on its clipped and cleaned flanks. They were then separated into 5 groups, each of 5 cages, and maintained on 2 cows, a goat, a rabbit, 20 rats or 50 NMRI mice. Two rats and 5 mice were used for 3 days after which they were discarded and new ones used. Sheep were not used since previous experience had shown that the mortality of tsetse maintained on this host was abnormally high. The rest of the experimental procedure was as described previously (see [i]). The surviving tsetse were dissected on days 31–33 after the infected feed, and the midgut, proventriculus, labrum, hypopharynx and salivary glands of each were examined to determine the trypanosome infection rates. The crops of tsetse maintained on cows and goats were also examined for parasites.

Results

a) T. vivax infection rates in G. m. morsitans

Table 1 shows the mean *T. vivax* infection rates in the above groups of tsetse. There was no significant difference when tsetse were maintained on cow or goat, but the infection rate was only just significantly higher when they were fed on rabbit compared with goat ($t_{diff.}$ 2.11, $p < 0.05$). Comparison between cow and rabbit as hosts revealed no significant difference ($t_{diff.}$ 1.26, $p > 0.05$). The difference was highly significant between rabbit and rats used as hosts. There was no significant difference between cow and rats ($t_{diff.}$ 1.89, $p > 0.05$) and the difference was only just significant between goat and rats ($t_{diff.}$ 2.11, $p < 0.05$). When NMRI mice were used as the maintenance host, the infection rate in the vector was very low and the mortality rate very high. The tsetse maintained on sheep showed abnormally high mortality. On day 18 after the blood meal intake, only 2 males out of 100 (2%) and 7 males out of 101 (6.9%) were alive. The

Table 1. The mean infection rates of *T. vivax* in *G. m. morsitans* which were maintained on five different hosts post-infective feed

Hosts	Cow		Goat		Rabbit		Rats		Mice
$\bar{x} \pm se$	88.0 ± 5.1		86.7 ± 3.5		94.8 ± 1.7		76.4 ± 3.5		6.1 ± 3.4
n	10		10		10		10		10
df		18		18		18		18	
$t_{diff.}$		0.21		2.11		4.79		14.52	
p		>0.05		<0.05		<0.01		<0.01	

$\bar{x} \pm se$ = mean infection rates ± standard error.

total infection rate of these survivors was 44.4%. The subject of high mortality will be discussed later.

There were instances when parasites were present in the labrum alone. It is possible that in such tsetse the cycle of development was not completed at the time of dissection. It is equally possible that a few trypanosomes located in the hypopharynx of such tsetse were all extruded at the pre-dissection feed. In some tsetse trypanosomes were sited only in the hypopharynx and were attached anteriorly to the inner wall of the latter. Since the labrum lacked epimastigotes, it is probable that in such tsetse the trypanosomes in the hypopharynx would eventually be lost with subsequent feeds.

Table 2 shows the comparison of *T. vivax* infection rates between male and female tsetse maintained on different hosts. There were no significant differences between the sexes of tsetse in terms of the infection rates.

b) T. congolense infection rates in G. m. morsitans

Table 3 shows the mean infection rates of *T. congolense* in *G. m. morsitans* which were maintained on 6 different hosts post-infected feed. The mature infection rates were highest in tsetse maintained on goat or rabbit and lowest in those fed on rats; those maintained on sheep or cow were intermediate in this respect. Of the 198 tsetse maintained on mice, only 20 (10.1%) were left at dissection and only 1 had parasites in the midgut and labrum. Again there were examples of parasites being present only in the labrum and none in the hypopharynx. In the present study there was not a single tsetse with parasites sited in the hypopharynx alone, though in other studies with the same stock of *T. congolense* such infections were encountered. These observations can be explained in the same way as stated in a) above. In six groups out of 12, the incidence of trypanosome infection in the midgut was higher than that in the proboscis. This is currently being investigated. The observed differences in the infection rates between male and female tsetse were inconsistent.

As before, the mortality of males maintained on sheep was markedly higher than that of females (Fig. 2 a–c). In the first attempt when tsetse were

Table 2. Comparison of the *T. vivax* infection rates between the male and female *G. m. morsitans* which were maintained on four different hosts after the infected blood meal intake

Hosts	Cow		Goat		Rabbit		Rats	
Sex of tsetse	♂	♀	♂	♀	♂	♀	♂	♀
Number used	100	102	102	107	101	97	80	112
Number dissected	95	92	97	96	99	92	78	97
Number of groups (n)	5	5	5	5	5	5	4	6
Infection rates % $\bar{x} \pm se$	92.6 ± 2.1	83.4 ± 4.8	86.6 ± 4.4	86.9 ± 5.8	94.0 ± 2.9	95.6 ± 2.1	80.5 ± 5.0	73.7 ± 5.0
$t_{diff.}$	0.89		0.04		0.44		0.97	
d.f.	8		8		8		8	
Significance p	>0.05		>0.05		>0.05		>0.05	

Table 3. Infection rates of *T. congolense* in *G. m. morsitans* when the vectors were maintained on six different hosts after the infected blood meal intake

Hosts	Number used	Sex	Number dissected	Age at dissection	Infection rate in			Total mature infection rate %
					Gut (%)	Labrum (%)	Hypopharynx (%)	
Cow	100	♂	72	21	5 (6.9)	3 (4.2)	3 (4.2)	6.5
	95	♀	66	21	9 (13.6)	6 (9.1)	6 (9.1)	
Goat	100	♂	59	21	12 (20.3)	8 (13.6)	7 (11.9)	12.6
	100	♀	60	21	11 (18.3)	8 (13.3)	8 (13.3)	
Sheep	100	♂	5	21	1 (20.0)	1 (20.0)	1 (20.0)	7.8
	100	♀	59	21	4 (6.8)	4 (6.8)	4 (6.8)	
Rabbit	96	♂	82	21	5 (6.1)	5 (6.1)	5 (6.1)	11.4
	97	♀	67	21	12 (17.9)	12 (17.9)	12 (17.9)	
Rats	100	♂	47	20	2 (4.3)	0 (0.0)	0 (0.0)	2.8
	100	♀	60	20	8 (13.3)	4 (6.7)	3 (5.0)	
Mice	98	♂	15	20	1 (6.7)	1 (6.7)	0 (0.0)	0.0
	100	♀	5	20	0 (0.0)	0 (0.0)	0 (0.0)	

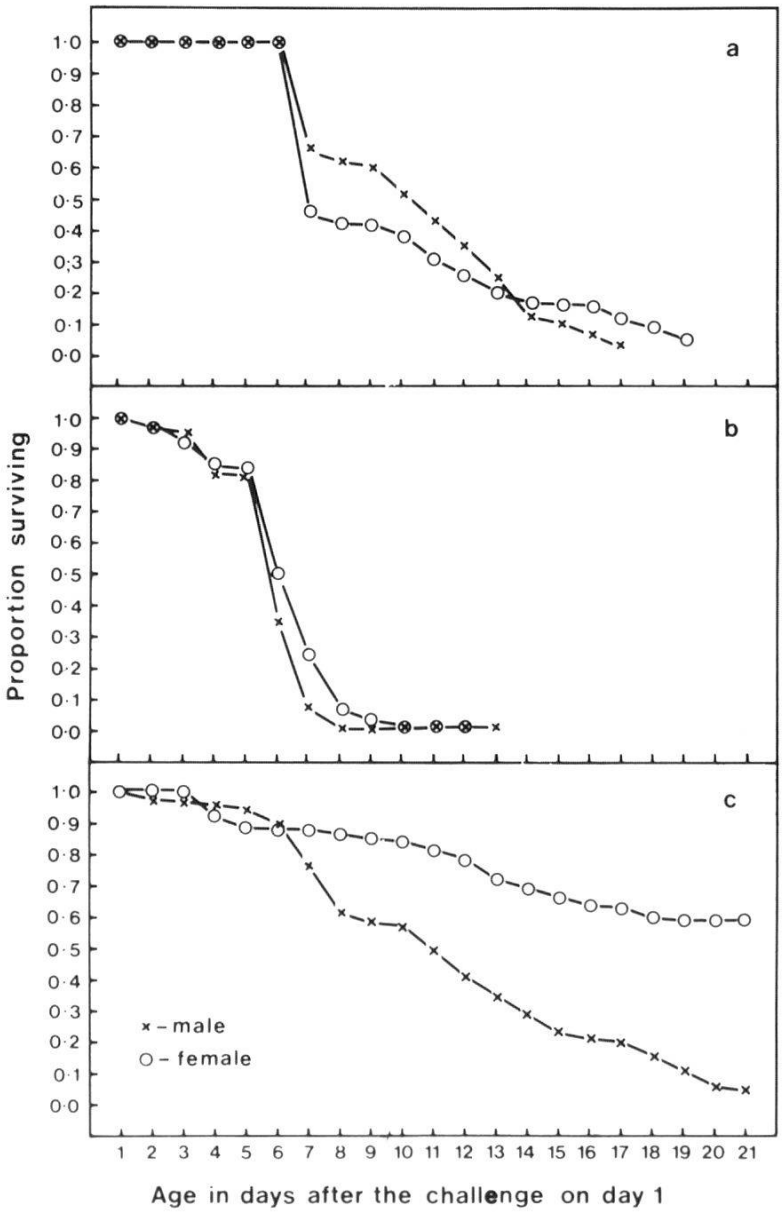

Fig. 2. Survival of males and females of tsetse when maintained on sheep after *T. vivax* (a) and *T. congolense* (b and c) infected blood meal.

maintained on sheep following the *T. vivax* infected feed (see [i] above), all were alive for the first 6 days but on day 7 a large number died; the proportion of female deaths being higher than that of males. Thereafter, the mortality rate of the females dropped less steeply with time compared with that of males. In the second attempt using *T. congolense*, the mortality rate was much higher particularly after day 5 with the female survival being only marginally better than that of the males. In the third attempt the survival rate was relatively much better, probably because of the greater care taken in clipping and washing the flanks of sheep before each feed. However, the mortality rate of males increased greatly with time while that of females increased only gradually. At the time of dissection on day 21 there were only 5% of the males surviving against 59% of the females.

Table 4. Infection rates of *T. brucei* in *G. m. morsitans* when the vectors were maintained on five different hosts after the infected blood meal intake

Hosts	Number used	Sex	Number dissected	Age at dissection	Infection rates in				Total mature infection rates %
					G/P (%)	Labrum (%)	Hypopharynx (%)	S. G. (%)	
Cow	73	♂	68	32	15 (21.4)	10 (14.3)	10 (14.3)	9 (12.9)	11.5
	30	♀	28	32	8 (32.0)	2 (8.0)	2 (8.0)	2 (8.0)	
Goat	68	♂	61	33	13 (21.3)	6 (9.8)	6 (9.8)	6 (9.8)	9.0
	30	♀	28	33	8 (28.6)	4 (14.3)	2 (7.1)	2 (7.1)	
Rabbit	66	♂	59	31	10 (17.0)	7 (11.9)	6 (10.2)	6 (10.2)	10.3
	30	♀	29	32	10 (34.5)	3 (10.3)	3 (10.3)	3 (10.3)	
Rat	70	♂	50	31	12 (24.0)	4 (8.0)	3 (6.0)	3 (6.0)	4.1
	30	♀	24	31	8 (33.3)	2 (8.3)	0 (0.0)	0 (0.0)	
Mice	49	♂	20	31	3 (15.0)	3 (15.0)	3 (15.0)	3 (15.0)	7.6
	49	♀	33	31	8 (24.2)	2 (6.1)	1 (3.0)	1 (3.0)	

G/P = Midgut and proventriculus; S.G. = Salivary glands.

c) T. brucei infection rates in G. m. morsitans

Table 4 shows the mean infection rates of *T. brucei* in *G. m. morsitans* which were maintained on 5 different hosts after the infected feed. The mature infection rates of tsetse maintained on cow, goat or rabbit were higher than those maintained on rats or mice. The observed differences between the former hosts were too small and hence of doubtful significance, since experience with other studies has shown that such differences are common at least with this stock. The tsetse maintained on rats had the lowest infection rate while those on mice were intermediate. The tsetse maintained on the latter host again showed higher mortality. Males in the main showed higher infection rates than females. There were examples of tsetse with parasites located in the labrum or labrum and hypopharynx but with salivary glands not infected. All such tsetse had infection in the gut and proventriculus. Also, in all groups the incidence of infection in the gut and proventriculus was much higher than the mature infection rates. The crops of tsetse maintained on cow or goat were also examined. The infection rates in this organ were the same as those for gut/proventriculus.

Discussion

The present study has demonstrated that the species of hosts on which *G. m. morsitans* are maintained after the infected feed can have a significant influence on the vector's subsequent infection rates with pathogenic trypanosomes. The infection rate of *T. vivax* in tsetse was lowest when maintained on mice. It is known that the stock of *T. vivax* used can infect mice (Leeflang et al., 1976; Moloo, 1976), but the transmission rate via tsetse to this host species is very low compared with that to goats (Moloo, in press). It is possible that the blood of mice contains some factor/s deleterious to this *T. vivax* stock and hence only a small proportion of the trypanosomes manage to become established in the vector. However, the cyclical transmission rate to rats is also very low (Moloo, in press), yet the infection rate in vectors maintained on rats was as high as in those maintained on cow. The use of mice to maintain tsetse after the latter have been given a blood meal containing *T. vivax* is not recommended. The rabbit is efficient for this purpose since tsetse maintained on this host showed a very high infection rate and also in view of the ease in handling this animal species. The present study has also shown that there is no difference between the sexes in terms of the *T. vivax* infection rates in the vector.

NMRI mice as hosts were again inferior for the maintenance of the *T. congolense* infected tsetse. The same is true with rats. Whereas the cow and sheep were intermediate as regards the trypanosome infection rates in vectors maintained on them, goat and rabbit proved the best. It could be that the blood of different hosts has a different nutritional value for the trypanosomes in the midgut. The rabbits as maintenance hosts resulted in high infection rates in the

vector and the animal is easily manageable; hence it is recommended for this purpose.

The higher incidence of gut infection compared to the mature infection rates is notable. Possibly the developmental cycle in such tsetse was not completed at the time of dissection. It is equally possible that the trypanosome populations in such vectors lacked the capability to cross the physical and/or physiological barrier and complete the maturation cycle in the proboscis. The sporadic differences between the sexes in terms of the infection rates were probably due to chance.

To control tick infestation the cattle used were spray-raced and goats and sheep were dipped twice a week using 1.2% Delnav (Dr. R. C. Cook, pers. comm.) which is an organophosphorus compound (product of Wellcome Kenya Ltd – Cooper Veterinary Division). The activity of this acaricide is short against ticks and the sheep were put in the isolation unit 2–3 weeks before use and were not dipped during this period or thereafter during the experiment. Nevertheless, the high mortality of tsetse maintained on sheep even after thorough washing of their clipped flanks possibly suggests that there were still traces of the acaricide which was quite potent against tsetse. However, the goats were also similarly treated and yet the survival of tsetse maintained on this host was quite good. The structure and physiology of the skin of sheep is different from that of goat. It is conceivable that a relatively very high lanolin content in the sheep skin in combination with traces of Delnav was responsible for the high tsetse mortality. Whatever the reason involved, it is interesting to note that the males of tsetse were more susceptible than were the females. It is possible that the latter sex could detoxify the potent factor responsible for high death rate of tsetse. It is known that females of *G. morsitans*, particularly when pregnant, are more tolerant than males to organophosphorus (Burnett, 1961) and also to some other persistent insecticides (Burnett, 1962). The present results possibly confirm this. The high mortality rate of the tsetse maintained on mice could be due to the fact that, in view of their smaller size, the frequency of the vector feeding on them was relatively lower. But, great care was taken to ensure that they fed quite well, and hence the above explanation is unlikely. The alternative reason could be that the blood of mice is of lower nutritional value or somewhat deleterious to tsetse.

Whereas the cow, goat and rabbit appeared quite good hosts for maintaining tsetse after the *T. brucei* infected feed, rats and mice were inferior as the maintenance hosts in terms of the infection rates in the vector. Again, the rabbit is recommended for the same reasons discussed earlier for the *T. vivax* and *T. congolense*. The higher incidence of midgut/proventriculus infection in contrast to the mature *T. brucei* infection rates is interesting. It is possible that at the time of dissection the infection in such tsetse had not matured. However, it is equally possible that the populations of trypanosomes in the ectoperitrophic space and proventriculus of such tsetse lacked the capability to migrate to the

salivary glands and complete the cycle of development. The higher mature infection rates in males than in females is notable. The opposite was true with the midgut and proventriculus infection rates. The number of tsetse used was small in each group and hence could not be subjected to statistical analyses; thus the observed differences could be due to chance event. This subject is being investigated further.

Duke and Mellanby (1936) observed *T. rhodesiense* in the crops of *G. palpalis* 10 to 14 days post-infected meal, but these trypanosomes had lost their infectivity to rats. In the present study trypanosomes were seen in the crops 31 days after the infected meal intake. It is possible that the trypanosomes remain attached to the inner wall of the crop which had emptied its contents into the gut at the first infected meal as suggested by Robertson (1913) for *T. gambiense* in *G. palpalis*. But, in the present study the infected crops were observed only in tsetse with infected gut and proventriculus. It is therefore probable that some trypanosomes from the latter migrate to the crop possibly during the crop emptying process at diuresis (Moloo and Kutuza, 1970). Hence, the trypanosomes found in the crop migrated there during the pre-dissection feeds.

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