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# Infection rates in sterile males of *morsitans, palpalis* and *fusca* groups *Glossina* for pathogenic *Trypanosoma* species from East and West Africa

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## Summary

Infection rates in sterile male *Glossina morsitans centralis, G. austeni, G. palpalis palpalis, G. p. gambiensis, G. fuscipes fuscipes, G. tachinoides* and *G. brevipalpis* for *Trypanosoma vivax, T. congolense* and *T. brucei* isolated from East and West Africa, were studied. Five groups of the sterile males, together with the five groups of sexually fertile males, of each of the respective species and subspecies were allowed to feed for 24 days on a Boran calf or goats infected with *T. vivax* or *T. congolense,* or with *T. brucei* for 34 days, after which they were dissected. The results showed that the infection of the pathogenic *Trypanosoma* species became better established in some tsetse species than in others. Also, the infection rates of *T. vivax, T. congolense* and *T. brucei* for sterile and sexually fertile males of any of the above *Glossina* did not differ significantly. These results indicate that releases of sterile male tsetse in the tsetse control programme will potentially increase the risk of trypanosomiasis during the period of tsetse releases in the affected areas, unless in the areas with low tsetse density the sterile male tsetse are rendered refractory to trypanosome infection prior to their releases while in the areas with medium to high tsetse densities, the resident tsetse populations are initially reduced with insecticides, traps and/or targets.

**Key words:** *Glossina morsitans centralis; G. austeni; G. palpalis palpalis; G. p. gambiensis; G. fuscipes fuscipes; G. tachinoides; G. brevipalpis;* gamma-irradiation; sterile males; infection rates; *Trypanosoma vivax; T. congolense; T. brucei;* ox; goats.

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# Introduction

Sterile insect release (SIR) is a practical method for eradication or control of tsetse populations (Simpson, 1958; Dame and Schmidt, 1970). This genetic method was used to eradicate *Glossina palpalis gambiensis* from an area in Burkina Faso (Cuisance et al., 1980; Politzar and Cuisance, 1982) and *G. morsitans morsitans* from Mkwaja Ranch in Tanzania (Williamson et al., 1983). In both these attempts to eradicate tsetse, the resident tsetse populations were initially reduced with the aerial application of insecticides. The SIR method was also used in combination with biconical traps and insecticide-impregnated targets for the eradication of *G. p. palpalis* in a 1500-km² area of central Nigeria (Takken et al., 1986). However, it has been shown that sterile male *G. m. morsitans, G. m. centralis, G. austeni* and *G. tachinoides* are as efficient vectors of pathogenic *Trypanosoma* species as sexually fertile males (Moloo, 1982; Moloo and Kutuza, 1984). This paper reports on further investigations on the infection rates in gamma-irradiated sterile male tsetse for *T. vivax, T. congolense* and *T. brucei* isolated from East and West Africa. Seven species and subspecies of *Glossina* belonging to the three main taxonomic groups, *morsitans, palpalis* and *fusca*, were studied.

## Materials and Methods

The *morsitans* group tsetse used were *G. m. centralis* originating from mainland Tanzania and *G. austeni* from Zanzibar; the *palpalis* group were *G. p. palpalis* from Nigeria, *G. p. gambiensis* from Burkina Faso, *G. fuscipes fuscipes* from the Central African Republic and *G. tachinoides* from Chad; the *fusca* group tsetse used was *G. brevipalpis* from Kenya. All seven tsetse species and subspecies were from the production colonies bred at ILRAD. They were maintained under controlled climatic conditions at 25°C, with 12 h of subdued indirect light during the day and 12 h of darkness at night. Tsetse in the *morsitans* group were kept at 70% relative humidity, and the others at 85%. All the breeding tsetse colonies were allowed to feed on lop-eared rabbits daily except at weekends.

*T. vivax* IL 2241 is a derivative of KETRI 2375 which was isolated from a naturally infected cow at Likoni, Coast Province, Kenya in 1978 (Wellde et al., 1983). *T. vivax* IL 1392 is a derivative of Zaria Y486 which was isolated from a Zebu cow in Nigeria in 1973 (Leeflang et al., 1976). *T. congolense* IL 2047 is a derivative of STIB 212 which was isolated from a lion in Tanzania in 1971 (Geigy and Kauffmann, 1973). *T. congolense* IL 2281 is a cloned stock of TREU 1290 (Luckins and Gray, 1979) which was derived from Zaria/67/LUMP/69 isolated from a cow in Nigeria in 1967 (Luckins et al., 1986). *T. brucei* IL 923 is a derivative of STIB 247 isolated from a Coke's hartebeest in Tanzania in 1971 (Geigy and Kauffmann, 1973). *T. brucei* IL 2380 is a derivative of TREU 1317 isolated from a naturally infected pig in Nigeria in 1962 (Killick-Kendrick and Godfrey, 1963).

A one-year old Boran calf, reared at ILRAD and without previous exposure to haemoparasitic infections, as well as adult East African Galla crossbred goats, weighing 20–25 kg, we used. The goats were obtained from an area of Kenya which is free of trypanosomiasis and were screened for antibodies to trypanosomes by the indirect immunofluorescent antibody test (Wilson, 1969) and shown to be negative. All the animals were housed in maximum insect-proof isolation units, and were allowed access to water and hay with concentrate ration.

Teneral male *G. m. centralis, G. austeni, G. p. palpalis, G. p. gambiensis, G. f. fuscipes, G. tachinoides* and *G. brevipalpis* were exposed to 12 krad ± 2.5% (dose rate, 580 rad/min) in a ¹³⁷Cs radiation source under ambient conditions. The irradiated teneral males and about the same number of unirradiated teneral control males of the respective species were allowed to feed concurrently on the

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Boran calf infected with *T. vivax* IL 2241, daily except Sundays. On day 25 post-emergence, all the surviving males were dissected to determine the infection rates. Four additional groups of irradiated and of unirradiated teneral males of each of the above seven tsetse species and subspecies were treated as above, but on different days, and their infection rates determined. This experimental procedure was followed to determine the infection rates of gamma-irradiated sterile males and the unirradiated control males of all the seven species and subspecies of *Glossina*. They were fed on the goats infected with *T. vivax* IL 1392, *T. congolense* IL 2047, *T. congolense* IL 2281, *T. brucei* IL 923 or *T. brucei* IL 2380; five different goats were used for each stock but the test and the control groups of each batch were fed on the same goat and at the same time. Tsetse infected with *T. congolense* were dissected on day 25 while those infected with *T. brucei* were dissected on day 35 after emergence.

## Results

Table 1 shows that the infection rates of *T. vivax* stocks IL 2241 isolated from Likoni, Kenya, were not significantly different between the sexually sterile and fertile males in each of the seven *Glossina* species and subspecies. However, the mature infection rates for this *T. vivax* stock were very high in G. m. centralis (55.9%, 61.1%) and *G. brevipalpis* (69.7%, 75.3%) compared to the rates in *G. austeni* (4.2%, 1.8%), *G. p. palpalis* (0%, 0%), *G. p. gambiensis* (1.8%, 1.3%), *G. f. fuscipes* (0.3%, 0.5%) and *G. tachinoides* (0%, 0.3%). Table 2 shows that the infection rates of *T. vivax* IL 1392 from Nigeria also were not significantly different between the sterile and fertile males of any of the seven tsetse species and subspecies. In contrast to the Kenyan *T. vivax,* the mature infection rates for the Nigerian *T. vivax* were very high in all the seven tsetse species and subspecies examined (range 39.9–81.6%).

Table 3 shows that there were no differences in the infection rates of *T. congolense* IL 2047 from Tanzania, between the sterile and fertile males in any of the seven tsetse species and subspecies. However, the mature infection rates were very high in G. m. centralis (44.0%, 35.5%), lower in G. brevipalpis (18.3%, 15.7%) and very low in G. austeni (0%, 2.0%), *G. p. palpalis* (0.5%, 0.5%), *G. p. gambiensis* (0%, 0.4%), *G. f. fuscipes* (1.6%, 6.0%) and *G. tachinoides* (0.3%, 0.2%). A similar pattern of infection rates was observed for *T. congolense* IL 2281 from Nigeria (Table 4).

Table 5 shows that there were some differences in the mature infection rates of *T. brucei* in the sterile and fertile males of the seven tsetse species and subspecies but the differences were small and inconsistent and probably not significant. Again, there were species and subspecies differences in the infection rates. In *G. m. centralis,* the mature *T. brucei* infection rates were very high (50.9%, 40.4%) whereas in the other six tsetse species and subspecies, they were extremely low (range 0–2.1%). A similar pattern for the mature infection rates was observed for *T. brucei* IL 2380 from Nigeria; the infection did not mature to metacyclics in the salivary glands of *G. austeni, G. f. fuscipes* and *G. brevipalpis* (Table 6).

Table 1. Infection rates of *Trypanosoma vivax* IL 2241 from East Africa in irradiated and unirradiated control males of seven different *Glossina* species and sub-species

| Tsetse species | Treatment | Number infected | Number dissected | Infection rates (%) ±SE | |
| --- | --- | --- | --- | --- | --- |
| | | | | Labrum | Hypopharynx |
| *G. m. centralis* | Irradiated | 500 | 338 | 58.0±12.6 | 55.9±13.0 |
| | Control | 500 | 379 | 66.9±9.3 | 61.1±7.8 |
| *G. austeni* | Irradiated | 500 | 360 | 4.9±4.2 | 4.2±3.6 |
| | Control | 500 | 374 | 2.5±1.6 | 1.8±1.2 |
| *G. p. palpalis* | Irradiated | 500 | 414 | 0.2 | 0.0 |
| | Control | 500 | 344 | 0.0 | 0.0 |
| *G. p. gambiensis* | Irradiated | 500 | 370 | 2.5±1.4 | 1.8±1.5 |
| | Control | 500 | 416 | 3.4±1.9 | 1.3±1.0 |
| *G. f. fuscipes* | Irradiated | 490 | 342 | 0.5 | 0.3 |
| | Control | 490 | 332 | 0.8 | 0.5 |
| *G. tachinoides* | Irradiated | 500 | 369 | 0.4 | 0.0 |
| | Control | 500 | 389 | 0.5 | 0.3 |
| *G. brevipalpis* | Irradiated | 435 | 354 | 76.8±8.2 | 69.7±8.6 |
| | Control | 436 | 391 | 81.1±6.3 | 75.3±6.8 |

The results given as mean ±SE are from five independent experiments.

Table 2. Infection rates of *Trypanosoma vivax* IL 1392 from West Africa in irradiated and unirradiated control males of seven different *Glossina* species and sub-species

| Tsetse species | Treatment | Number infected | Number dissected | Infection rates (%) ±SE | |
| --- | --- | --- | --- | --- | --- |
| | | | | Labrum | Hypopharynx |
| *G. m. centralis* | Irradiated | 500 | 386 | 66.0±10.4 | 49.5±6.2 |
| | Control | 500 | 403 | 63.1±8.9 | 46.3±8.4 |
| *G. austeni* | Irradiated | 500 | 410 | 62.7±7.7 | 50.0±4.9 |
| | Control | 500 | 427 | 70.8±4.5 | 54.8±4.2 |
| *G. p. palpalis* | Irradiated | 500 | 455 | 52.4±8.5 | 45.3±7.8 |
| | Control | 500 | 437 | 45.3±10.2 | 39.9±9.7 |
| *G. p. gambiensis* | Irradiated | 500 | 406 | 64.0±7.2 | 58.1±6.7 |
| | Control | 500 | 436 | 69.8±5.0 | 62.8±4.0 |
| *G. f. fuscipes* | Irradiated | 486 | 384 | 47.7±7.5 | 42.2±6.5 |
| | Control | 486 | 403 | 46.3±5.1 | 41.2±4.9 |
| *G. tachinoides* | Irradiated | 500 | 423 | 50.6±11.9 | 41.7±12.3 |
| | Control | 500 | 457 | 51.5±9.4 | 41.5±9.0 |
| *G. brevipalpis* | Irradiated | 385 | 344 | 86.8±8.3 | 75.4±12.6 |
| | Control | 385 | 348 | 92.1±5.7 | 81.6±7.5 |

The results given as mean ±SE are from five independent experiments.

Table 3. Infection rates of *Trypanosoma congolense* IL 2047 from East Africa in irradiated and unirradiated control males of seven different *Glossina* species and sub-species

| Tsetse species | Treatment | Number infected | Number dissected | Infection rates (%) ±SE | | |
|---|---|---|---|---|---|---|
| | | | | Gut | Labrum | Hypopharynx |
| *G. m. centralis* | Irradiated | 500 | 324 | 57.4±8.1 | 45.6±8.6 | 44.0±8.7 |
| | Control | 500 | 374 | 49.3±8.6 | 36.5±5.6 | 35.3±5.7 |
| *G. austeni* | Irradiated | 500 | 352 | 14.5±5.3 | 0.5 | 0.0 |
| | Control | 500 | 377 | 14.4±4.0 | 3.4±0.6 | 2.0±1.0 |
| *G. p. palpalis* | Irradiated | 447 | 362 | 8.8±4.2 | 0.8 | 0.5 |
| | Control | 447 | 340 | 10.2±4.7 | 0.8 | 0.5 |
| *G. p. gambiensis* | Irradiated | 493 | 315 | 6.9±2.6 | 0.2 | 0.0 |
| | Control | 493 | 339 | 5.9±2.9 | 0.4 | 0.4 |
| *G. f. fuscipes* | Irradiated | 434 | 253 | 13.8±5.0 | 2.5±1.2 | 1.6±0.5 |
| | Control | 434 | 273 | 13.2±5.5 | 6.7±3.4 | 6.0±3.4 |
| *G. tachinoides* | Irradiated | 445 | 313 | 8.6±5.7 | 0.3 | 0.3 |
| | Control | 446 | 357 | 7.2±2.7 | 0.2 | 0.2 |
| *G. brevipalpis* | Irradiated | 425 | 349 | 30.5±5.8 | 20.1±5.5 | 18.3±6.1 |
| | Control | 424 | 354 | 33.8±5.0 | 17.6±5.0 | 15.7±5.4 |

The results given as mean ±SE are from five independent experiments.


Table 4. Infection rates of *Trypanosoma congolense* IL 2281 from West Africa in irradiated and unirradiated control males of seven different *Glossina* species and sub-species

| Tsetse species | Treatment | Number infected | Number dissected | Infection rates (%) ±SE | | |
|---|---|---|---|---|---|---|
| | | | | Gut | Labrum | Hypopharynx |
| *G. m. centralis* | Irradiated | 500 | 403 | 69.1±3.6 | 56.4±3.4 | 55.8±3.8 |
| | Control | 500 | 421 | 63.5±5.4 | 49.9±4.2 | 49.2±4.0 |
| *G. austeni* | Irradiated | 489 | 408 | 14.0±4.9 | 3.1±0.5 | 2.1±0.6 |
| | Control | 490 | 419 | 14.6±6.7 | 3.4±1.2 | 3.0±1.0 |
| *G. p. palpalis* | Irradiated | 388 | 284 | 9.5±3.4 | 1.4±0.6 | 1.0±0.3 |
| | Control | 388 | 286 | 18.7±12.4 | 0.7±0.4 | 0.7±0.3 |
| *G. p. gambiensis* | Irradiated | 485 | 395 | 16.3±11.7 | 0.5±0.3 | 0.3 |
| | Control | 485 | 363 | 15.6±12.2 | 2.2±0.6 | 0.3 |
| *G. f. fuscipes* | Irradiated | 445 | 316 | 16.1±3.7 | 6.8±2.6 | 1.5±0.5 |
| | Control | 444 | 301 | 9.2±2.7 | 3.2±1.1 | 0.9±0.7 |
| *G. tachinoides* | Irradiated | 483 | 360 | 11.1±9.4 | 1.2±0.9 | 0.8±0.5 |
| | Control | 482 | 401 | 9.8±4.3 | 2.5±0.7 | 2.0±0.5 |
| *G. brevipalpis* | Irradiated | 459 | 369 | 42.0±7.8 | 17.1±4.9 | 8.8±3.3 |
| | Control | 459 | 331 | 40.3±11.4 | 15.4±4.0 | 6.3±2.0 |

The results given as mean ±SE are from five independent experiments.

Table 5. Infection rates of *Trypanosoma brucei* IL 923 from East Africa in irradiated and unirradiated control males of seven different *Glossina* species and sub-species

| Tsetse species | Treatment | Number infected | Number dissected | Infection rates (%) ±SE | |
|---|---|---|---|---|---|
| | | | | Gut | Salivary glands |
| *G. m. centralis* | Irradiated | 482 | 208 | 80.5±5.1 | 50.9±3.7 |
| | Control | 481 | 357 | 80.7±4.9 | 40.4±2.7 |
| *G. austeni* | Irradiated | 500 | 427 | 10.2±2.1 | 1.0±0.6 |
| | Control | 500 | 426 | 5.4±1.9 | 0.2 |
| *G. p. palpalis* | Irradiated | 426 | 350 | 5.5±0.9 | 0.4 |
| | Control | 427 | 353 | 3.7±1.3 | 0.4 |
| *G. p. gambiensis* | Irradiated | 481 | 413 | 2.0±0.6 | 0.0 |
| | Control | 481 | 436 | 2.3±0.3 | 0.4 |
| *G. f. fuscipes* | Irradiated | 371 | 329 | 6.0±1.2 | 1.6 |
| | Control | 371 | 302 | 5.6±1.1 | 2.1±0.8 |
| *G. tachinoides* | Irradiated | 425 | ˙313 | 5.0±1.2 | 1.5 |
| | Control | 421 | 355 | 3.3±0.3 | 1.0 |
| *G. brevipalpis* | Irradiated | 481 | 342 | 64.8±2.3 | 0.0 |
| | Control | 482 | 402 | 59.3±3.4 | 0.2 |

The results given as mean ±SE are from five independent experiments.

Table 6. Infection rates of *Trypanosoma brucei* IL 2380 from West Africa in irradiated and unirradiated control males of seven different *Glossina* species and sub-species

| Tsetse species | Treatment | Number infected | Number dissected | Infection rates (%) ±SE | |
|---|---|---|---|---|---|
| | | | | Gut | Salivary glands |
| *G. m. centralis* | Irradiated | 494 | 216 | 45.0±9.6 | 12.3±3.4 |
| | Control | 494 | 440 | 36.4±5.2 | 6.8±1.7 |
| *G. austeni* | Irradiated | 482 | 385 | 0.3 | 0.0 |
| | Control | 486 | 419 | 0.9 | 0.0 |
| *G. p. palpalis* | Irradiated | 386 | 319 | 1.2±0.6 | 0.4 |
| | Control | 385 | 353 | 1.5±0.6 | 0.0 |
| *G. p. gambiensis* | Irradiated | 459 | 379 | 0.5 | 0.2 |
| | Control | 459 | 391 | 0.4 | 0.0 |
| *G. f. fuscipes* | Irradiated | 345 | 289 | 0.0 | 0.0 |
| | Control | 350 | 279 | 0.8 | 0.0 |
| *G. tachinoides* | Irradiated | 427 | 284 | 5.0±1.4 | 1.3±0.6 |
| | Control | 418 | 372 | 2.2±0.6 | 0.7±0.4 |
| *G. brevipalpis* | Irradiated | 500 | 386 | 23.5±5.6 | 0.0 |
| | Control | 500 | 402 | 18.1±5.0 | 0.0 |

The results given as mean ±SE are from five independent experiments.

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# Discussion

The present study has demonstrated that male *G. m. centralis, G. austeni, G. p. palpalis, G. p. gambiensis, G. f. fuscipes, G. tachinoides* and *G. brevipalpis* rendered sexually sterile by gamma-irradiation are as susceptible to *T. vivax, T. congolense* and *T. brucei,* as sexually fertile corresponding males irrespective of whether the trypanosome isolate was from East or West Africa. These results confirm and extend our earlier observations that the use of gamma-irradiation to sterilize male *Glossina* prior to infection does not interfere with subsequent cyclical development of these parasites. By analogy with what has previously been shown for *G. m. morsitans* (Moloo, 1982), it is likely that the transmission characteristics of *T. vivax, T. congolense* and *T. brucei* to susceptible hosts by sterile males of the above tsetse species and subspecies remain unchanged by sterilization.

The results also demonstrated that some tsetse species are better vectors of the three pathogenic *Trypanosoma* species than others, but this selectivity is also unimpaired by gamma-irradiation.

The SIR method has been used in three previous campaigns to eradicate tsetse; *G. p. gambiensis* (Politzar and Cuisance, 1982) from an area in Burkina Faso, *G. m. morsitans* (Williamson et al., 1983) from Mkwaja Ranch in Tanzania, and *G. p. palpalis* (Takken et al., 1986) in an area in central Nigeria. In each of these campaigns, the tsetse populations were initially reduced by the application of insecticides prior to the sterile male releases. Consequently fewer sterile insects were required for release and so there was no associated increase in the risk of trypanosomiasis in the above areas. However, it has recently been shown that the incorporation of a trypanocidal drug, isometamidium chloride ('Samorin', May & Baker Ltd., Dagenham, England) in a bloodmeal completely suppressed the cyclical development within *G. m. centralis* of *T. vivax, T. congolense* or *T. brucei.* This was achieved by feeding the sterile teneral male tsetse on a bloodmeal containing 12 $\mu$g ml$^{-1}$ Samorin prior to a feed on infected goats (Moloo and Kamunya, 1987). Hence, inclusion in a SIR programme for tsetse eradication of a pre-release bloodmeal containing Samorin for the sterile teneral males would achieve two objectives. Firstly, is could effectively suppress the disease transmission by such tsetse and, secondly, in areas with medium or low tsetse densities it could reduce or eliminate the need for initial reduction of the resident tsetse populations with insecticides. However, in areas with high tsetse densities, the initial reduction of native tsetse populations will certainly have to be incorporated in SIR programme for tsetse eradication.

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