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International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya

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

S. K. MOLOO, S. B. KUTUZA, J. DESAI

### **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-km<sup>2</sup> 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  $\pm$  2.5% (dose rate, 580 rad/min) in a <sup>137</sup>Cs 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

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 metacyclids 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 (%) $\pm$ SE	
				Labrum	Hypopharynx
<i>G. m. centralis</i>	Irradiated	500	338	58.0 $\pm$ 12.6	55.9 $\pm$ 13.0
	Control	500	379	66.9 $\pm$ 9.3	61.1 $\pm$ 7.8
<i>G. austeni</i>	Irradiated	500	360	4.9 $\pm$ 4.2	4.2 $\pm$ 3.6
	Control	500	374	2.5 $\pm$ 1.6	1.8 $\pm$ 1.2
<i>G. p. palpalis</i>	Irradiated	500	414	0.2	0.0
	Control	500	344	0.0	0.0
<i>G. p. gambiensis</i>	Irradiated	500	370	2.5 $\pm$ 1.4	1.8 $\pm$ 1.5
	Control	500	416	3.4 $\pm$ 1.9	1.3 $\pm$ 1.0
<i>G. f. fuscipes</i>	Irradiated	490	342	0.5	0.3
	Control	490	332	0.8	0.5
<i>G. tachinoides</i>	Irradiated	500	369	0.4	0.0
	Control	500	389	0.5	0.3
<i>G. brevipalpis</i>	Irradiated	435	354	76.8 $\pm$ 8.2	69.7 $\pm$ 8.6
	Control	436	391	81.1 $\pm$ 6.3	75.3 $\pm$ 6.8

The results given as mean  $\pm$  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 (%) $\pm$ SE	
				Labrum	Hypopharynx
<i>G. m. centralis</i>	Irradiated	500	386	66.0 $\pm$ 10.4	49.5 $\pm$ 6.2
	Control	500	403	63.1 $\pm$ 8.9	46.3 $\pm$ 8.4
<i>G. austeni</i>	Irradiated	500	410	62.7 $\pm$ 7.7	50.0 $\pm$ 4.9
	Control	500	427	70.8 $\pm$ 4.5	54.8 $\pm$ 4.2
<i>G. p. palpalis</i>	Irradiated	500	455	52.4 $\pm$ 8.5	45.3 $\pm$ 7.8
	Control	500	437	45.3 $\pm$ 10.2	39.9 $\pm$ 9.7
<i>G. p. gambiensis</i>	Irradiated	500	406	64.0 $\pm$ 7.2	58.1 $\pm$ 6.7
	Control	500	436	69.8 $\pm$ 5.0	62.8 $\pm$ 4.0
<i>G. f. fuscipes</i>	Irradiated	486	384	47.7 $\pm$ 7.5	42.2 $\pm$ 6.5
	Control	486	403	46.3 $\pm$ 5.1	41.2 $\pm$ 4.9
<i>G. tachinoides</i>	Irradiated	500	423	50.6 $\pm$ 11.9	41.7 $\pm$ 12.3
	Control	500	457	51.5 $\pm$ 9.4	41.5 $\pm$ 9.0
<i>G. brevipalpis</i>	Irradiated	385	344	86.8 $\pm$ 8.3	75.4 $\pm$ 12.6
	Control	385	348	92.1 $\pm$ 5.7	81.6 $\pm$ 7.5

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

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

The results given as mean  $\pm$  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 (%) $\pm$ SE	
				Gut	Salivary glands
<i>G. m. centralis</i>	Irradiated	482	208	80.5 $\pm$ 5.1	50.9 $\pm$ 3.7
	Control	481	357	80.7 $\pm$ 4.9	40.4 $\pm$ 2.7
<i>G. austeni</i>	Irradiated	500	427	10.2 $\pm$ 2.1	1.0 $\pm$ 0.6
	Control	500	426	5.4 $\pm$ 1.9	0.2
<i>G. p. palpalis</i>	Irradiated	426	350	5.5 $\pm$ 0.9	0.4
	Control	427	353	3.7 $\pm$ 1.3	0.4
<i>G. p. gambiensis</i>	Irradiated	481	413	2.0 $\pm$ 0.6	0.0
	Control	481	436	2.3 $\pm$ 0.3	0.4
<i>G. f. fuscipes</i>	Irradiated	371	329	6.0 $\pm$ 1.2	1.6
	Control	371	302	5.6 $\pm$ 1.1	2.1 $\pm$ 0.8
<i>G. tachinoides</i>	Irradiated	425	313	5.0 $\pm$ 1.2	1.5
	Control	421	355	3.3 $\pm$ 0.3	1.0
<i>G. brevipalpis</i>	Irradiated	481	342	64.8 $\pm$ 2.3	0.0
	Control	482	402	59.3 $\pm$ 3.4	0.2

The results given as mean  $\pm$  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 (%) $\pm$ SE	
				Gut	Salivary glands
<i>G. m. centralis</i>	Irradiated	494	216	45.0 $\pm$ 9.6	12.3 $\pm$ 3.4
	Control	494	440	36.4 $\pm$ 5.2	6.8 $\pm$ 1.7
<i>G. austeni</i>	Irradiated	482	385	0.3	0.0
	Control	486	419	0.9	0.0
<i>G. p. palpalis</i>	Irradiated	386	319	1.2 $\pm$ 0.6	0.4
	Control	385	353	1.5 $\pm$ 0.6	0.0
<i>G. p. gambiensis</i>	Irradiated	459	379	0.5	0.2
	Control	459	391	0.4	0.0
<i>G. f. fuscipes</i>	Irradiated	345	289	0.0	0.0
	Control	350	279	0.8	0.0
<i>G. tachinoides</i>	Irradiated	427	284	5.0 $\pm$ 1.4	1.3 $\pm$ 0.6
	Control	418	372	2.2 $\pm$ 0.6	0.7 $\pm$ 0.4
<i>G. brevipalpis</i>	Irradiated	500	386	23.5 $\pm$ 5.6	0.0
	Control	500	402	18.1 $\pm$ 5.0	0.0

The results given as mean  $\pm$  SE are from five independent experiments.

## 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  $12 \mu\text{g ml}^{-1}$  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, it 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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