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Autor: Moloo, S.K. / Dar, Faiqa / Kamunya, G.W.
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International Laboratory for Research on Animal Diseases (ILRAD), Nairobi, Kenya

The transmission of mixed infections of pathogenic *Trypanosoma* species to susceptible hosts by *Glossina morsitans morsitans*

S. K. MOLOO, FAIQA DAR, G. W. KAMUNYA

Summary

Studies have been conducted on mixed infections of *T. brucei*, *T. congolense* and/or *T. vivax* in *Glossina morsitans morsitans* using in vivo feeding on infected hosts or in vitro feeding upon mixed infected heparinised blood. The vector can become infected with double or triple trypanosome infections and can transmit such mixed infections to susceptible hosts.

Key words: *Glossina morsitans morsitans*; *Trypanosoma vivax*, *T. congolense*, *T. brucei*; mixed infection and transmission; goats; mice.

Introduction

Field studies have revealed that many wildlife species which serve as natural hosts of *Glossina* and also the susceptible livestock inhabiting tsetse infested areas of tropical Africa, often carry mixed infections of the *brucei*-complex, the *congolense* type and/or the *vivax*-type parasites (Ashcroft, 1959; Godfrey et al., 1965; Baker, 1968; Geigy et al., 1971; Geigy and Kauffmann, 1973; Mwambu and Mayende, 1973). However, it is not known whether an individual tsetse can acquire and eventually transmit such double or triple infections to susceptible hosts. This latter aspect is of considerable epidemiological interest, and the present experimental study was undertaken to clarify the role of an individual tsetse in the transmission of mixed infections.

Materials and Methods

Glossina morsitans morsitans were from the ILRAD bred colony. The history of the stocks of *T. vivax* (ILRAD 417), *T. congolense* (ILRAD 687) and *T. brucei* (ILRAD 375) used has been described elsewhere (Moloo, 1981). The animals used to infect or maintain tsetse were East African adult Galla crossbred goats, New Zealand white rabbits and ILRAD bred A/J or Balb/c mice.

Correspondence: Dr. S. K. Moloo, ILRAD, P.O. Box 30709, Nairobi, Kenya

Mixed infections using in vivo system

Three hundred teneral tsetse were fed on *T. brucei* infected goat. Thereafter, the tsetse were divided into three equal groups; one group was fed on *T. congolense* infected A/J mice and the second on *T. vivax* infected goat on day 3 post-emergence. The third group was fed on *T. congolense* infected A/J mice on day 3 and then on *T. vivax* infected goat on day 5. A fourth group comprising 97 teneral tsetse were fed on *T. congolense* infected A/J mice and then on *T. vivax* infected goat on day 3. These four groups of tsetse were maintained on different rabbits.

On day 22 after emergence the salivary probes of the 4 groups were examined for metacyclics. The tsetse which extruded these infective forms were used to challenge A/J mice. These mice were bled from the tail daily except Sundays, and the parasitaemia was determined by examination of unstained wet blood films at $400\times$ magnification using a phase-contrast microscope. Giemsa stained thin blood films were prepared of those animals in which infection had become patent. The surviving tsetse were dissected on day 63, and the types of trypanosome infection determined. The *Trypanosoma* species in the tsetse were identified according to their location and morphology, and in the hosts by their morphology using the above methods.

Mixed infections using the in vitro system

T. brucei, *T. congolense* and *T. vivax* infected goats were bled from the jugular vein, and equal volumes of their heparinised blood were mixed. Two hundred teneral tsetse were fed in vitro (Bauer and Wetzel, 1976) and then maintained on a rabbit. On day 38, the salivary probes of the surviving 175 tsetse were examined for metacyclics. Twenty-five tsetse showed these forms in their saliva and were used singly to challenge A/J mice. These mice were examined as described previously.

All these mice became infected; two showed mixed infections in wet films, one with *T. brucei*/*T. congolense* and the other with all three species. The remaining 23 mice showed *T. brucei* infection only. The tsetse which transmitted the double infection was allowed to feed on one goat for 16 days, and on another goat for 23 days to determine if it had also acquired and could transmit *T. vivax* infection. The goats were bled from the ear and Giemsa stained slides were examined for the parasites and for their morphological characteristics. The remaining 23 tsetse which had transmitted *T. brucei* infection alone to A/J mice were dissected on day 57; one showed *T. brucei*/*T. vivax* mixed infection. Its salivary glands, gut and proboscis were macerated and injected individually into Balb/c mice. The latter were examined for parasites as described previously. The surviving 148 tsetse were dissected on day 35 and their labra, hypopharynxes, midgut and salivary glands were examined for the parasites.

Results

Table 1 shows the types and total number of trypanosome infections in *G. m. morsitans* fed in vivo on infected goats and mice. The total infection rates in the four groups varied between 23.7% and 85.3%. Mixed trypanosome infections were encountered in all four groups. Only one tsetse was carrying a triple infection.

In group A, 8 tsetse showed metacyclics in the salivary probes. All these transmitted the infection to A/J mice; 2 mice showed mixed *T. brucei*/*T. congolense* infections while the other 6 had *T. brucei* alone. Two of the 8 tsetse which had transmitted mixed infections also showed double infection upon dissection while the other six had only *T. brucei*. In groupe B, 12 tsetse showed metacyclics in the saliva and they transmitted infections to A/J mice. Six mice showed *T. brucei*/*T. vivax* mixed infections while the other six showed *T. brucei* only.

Table 1. Types of trypanosome infections in *G. m. morsitans* fed in vivo on infected goats and mice

Group number	Types of infected meals given	Number used	Number dissected	Mature infections
A	<i>Tb/Tc</i>	100	38	<i>Tb</i> (6) <i>Tc</i> (1) <i>Tb/Tc</i> (2)
B	<i>Tb/Tv</i>	100	83	<i>Tb</i> (10) <i>Tv</i> (36) <i>Tb/Tv</i> (8)
C	<i>Tb/Tc/Tv</i>	100	35	<i>Tb</i> (2) <i>Tc</i> (3) <i>Tv</i> (10) <i>Tb/Tv</i> (1) <i>Tc/Tv</i> (2) <i>Tb/Tc/Tv</i> (1)
D	<i>Tc/Tv</i>	97	68	<i>Tc</i> (2) <i>Tv</i> (46) <i>Tc/Tv</i> (10)

Tb = *T. brucei*; *Tc* = *T. congolense*; *Tv* = *T. vivax*

The numbers in parentheses indicate the number of infected tsetse.

The tsetse which had transmitted the double infections to the above mice showed mixed infections upon dissection, while the other 6 tsetse showed only *T. brucei*. In group C, only 3 tsetse had metacyclics and they transmitted the infection to A/J mice. One tsetse transmitted *T. brucei* while the other 2 transmitted *T. congolense* infections only. No double or triple transmission was encountered. One tsetse showed *T. brucei/T. vivax* infection; the other 2 had *T. congolense/T. vivax* mixed infection when dissected. In group D, 5 tsetse had metacyclics in the saliva and they transmitted infection to A/J mice. All such infections were *T. congolense*. Three of the 5 tsetse showed *T. congolense/T. vivax* mixed infections upon dissection.

In the attempt at the triple mixed infections of *G. m. morsitans* using the in vitro system, one A/J mouse which showed *T. brucei/T. congolense* mixed infection in the wet blood film was found to be infected with triple infection when the stained blood slides were examined. The tsetse which transmitted the infection to the above mouse also transmitted double *T. brucei/T. vivax* infection to the first goat and triple infection to the second goat challenged. Dissection of 148 surviving tsetse revealed 10.5% mature *T. brucei*, 1.3% *T. congolense* and 0.7% *T. vivax* single infections. The tsetse which had shown *T. brucei/T. vivax* mixed infection at dissection, transmitted only *T. brucei* infection to Balb/c mice by injection of the macerated salivary glands, gut and proboscis.

Discussion

The present study has demonstrated for the first time that two or three of the pathogenic trypanosome species, namely *T. brucei*, *T. congolense* and *T. vivax*, can develop concurrently in *G. m. morsitans*, and that the vectors with double or triple mixed infections can transmit them to susceptible hosts. The

present study has also shown that tsetse with mixed infections may transmit only a single species of trypanosome to their hosts.

It is not known whether in the field some tsetse are vectors of mixed trypanosome infections and transmit such infections to their hosts. Whereas *brucei*-complex infection becomes established in the vector if the latter are given the infected meal when teneral (Wijers, 1958), field studies have shown that *T. congolense* and particularly *T. vivax* parasites can complete development when *Glossina* feed at any age upon the infected hosts (Moloo et al., 1973; Rogers and Boreham, 1973). These observations have been confirmed under laboratory conditions (Moloo, unpublished). Hence, it is possible for tsetse infected with *brucei*-complex trypanosomes to become infected also with the other two trypanosome species or combinations thereof. It would therefore be of interest to examine tsetse in the field for mixed infections. If they are encountered frequently, this factor would have to be considered in formulating a model to evaluate trypanosomiasis challenge.

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