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Trypanosome infection rates in tsetse midguts using a short-term in vitro culture technique

Short communication

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To assess the risk of trypanosome infection or 'challenge' which tsetse flies present to animal or human hosts, it is first necessary to determine the infection rates in the flies. Trypanosome infection rates in testse have customarily been assessed by dissection and microscopical examination of the proboscis, midgut and salivary glands (Lloyd and Johnson, 1924). Using this dissection technique infection rates in wild flies have usually been found to be very low (Jordan, 1974); this reflects both the inherent refractoriness of most flies to infection (Maudlin et al., 1986) and the insensitivity of dissection as a diagnostic technique.

In attempting to isolate trypanosomes from wild flies we placed tsetse midguts in culture medium, and during the course of these experiments, established procyclic cultures from flies which were found to be uninfected by the dissection method. Following these findings, we surveyed the infection rates in wild flies using the two diagnostic techniques – dissection and culture. We present here the results of this survey carried out in Liberia.

Glossina palpalis palpalis (n = 962), *G. nigrofusca* (n = 122) and *G. pallicera* (n = 28) were caught in the Liberian rain forest using biconical traps (Challier and Laveissière, 1973). Flies were dissected in the laboratory of the LRU within 24 h of capture. Dissection took place in a clean environment with flame sterilized instruments, on sterile slides in a few drops of sterile dissection and isolation medium for procyclics (DIMP). This solution was a mixture of 10–15% foetal calf serum (FCS), 100 µg/ml gentamycin and 50 µg/ml mycostatin in 50% Cunningham's Medium (Cunningham, 1977) and 50% SDM-79 (Brun and Schönenberger, 1979).

The proboscis (hypopharynx, labrum, labium), midgut and salivary glands was examined under a Zeiss-microscope (128× and 320× magnification). Immediately after microscopic examination each midgut was transferred into one well of a 96-microwell dish (Nunclon) containing approx. 100 µl DIMP. The guts were incubated for 3 days at 27°C and examined daily with a Wild-invertoscope (200× magnification).

For all three *Glossina* species the overall mature infection rates determined by dissection were: 1.3% for *Nannomonas* and 1.7% for *Duttonella*, *Trypanozoon* were not detected (Table 1). Immature (midgut) trypanosomes were diagnosed in 44 (4%) of the 1112 flies examined. In contrast, in vitro cultivation of the midguts from these same flies recovered 107 (9.6%) procyclic midgut infections (6.05% on day 0, 2.2% on day 1, 0.7% on day 2, 0.6% on day 3 and 0.1% after day 3 of cultivation). Various fungal contaminations in the cultures prevented a longer observation period.

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Table 1. Tsetse fly infection rates with trypanosomes determined by dissection and short-term in vitro culture technique

Glossina species	No. examined	Dissection			In vitro culture
		mature		immature	immature
		infections with			
		N.[1]	*D.*[2]	*N.* or *T.*[3]	*N.* or *T.*
G. p. palpalis	962	13 (1.4%)	12 (1.2%)	36 (3.7%)[4]	86 (8.9%)[4]
G. nigrofusca	122	2 (1.6%)	6 (4.9%)	5 (4.1%)[4]	17 (13.9%)[4]
G. pallicera	28	0	1 (3.6%)	3 (10.7%)[4]	4 (14.3%)[4]
Total	1112	15 (1.3%)	19 (1.7%)	44 (4.0%)[4]	107 (9.6%)[4]

[1] *N.* = *Nannomonas*, [2] *D.* = *Duttonella*, [3] *T.* = *Trypanozoon*
[4] Flies with mature infections *(Nannomonas)* included

These findings show that the in vitro culture technique is significantly ($p < 0.001$) more sensitive than the dissection method in detecting immature *Nannomonas* or *Trypanozoon* infections; the ratio of mature: immature infections being increased from 1:1.9 (dissection) to 1:6.1 (culture). Examination of flies by dissection alone may lead to an underestimation of the numbers of immature midgut infections present in the fly population. The short-term in vitro culture technique described here could be used routinely to more accurately determine infection rates. The detection and discrimination of *Trypanosoma* spp. in *Glossina*, particularly of *T. congolense*, *T. simiae*, and human-infective and non-infective *T. brucei*, would probably reflect the variety of the entire trypanosome population in the host-fly-host cycle better than analysis of bloodstream forms derived from mammals.

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