Caught in a trap: DNA contamination in tsetse xenomonitoring can lead to over-estimates of *Trypanosoma brucei* infection

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Abstract

Tsetse flies (*Glossina sp*.) are vectors of *Trypanosoma brucei* subspecies that cause human African trypanosomiasis (HAT). Capturing and screening tsetse is critical for HAT surveillance. Classically, tsetse were microscopically analysed to identify trypanosomes but this is increasingly replaced with molecular xenomonitoring. Nonetheless, sensitive *T. brucei*-detection assays, such as TBR-PCR, are vulnerable to DNA cross-contamination. This may occur at capture, when live tsetse are retained temporarily in the cage of a trap. This study set out to determine whether infected tsetse can contaminate naïve tsetse with *T. brucei* DNA via faeces when co-housed.

Insectary-reared teneral *G. morsitans morsitans* were fed an infectious *T. b. brucei*-spiked bloodmeal. At 19 days post-infection, infected flies and naïve flies were caged together in the following ratios: (T1) 9:3, (T2) 6:6 (T3) 1:11 and a control (C0) 0:12 in triplicate. Following 24-hour incubation, DNA was extracted from each fly and screened for parasite DNA presence using PCR and qPCR.

All insectary-reared infected flies were positive for *T. brucei* DNA using TBR-qPCR, however naïve flies also tested positive. Even at a ratio of 1 infected to 11 naïve flies, 91% of naïve flies had positive TBR-qPCR results. Furthermore, the quantity of *T. brucei* DNA detected in naïve flies was significantly correlated with cage infection ratio. With evidence of cross-contamination, field-caught flies from Tanzania were then assessed using the same screening protocol. End-point TBR-PCR assays predicted an infection rate of 24.77%. By employing qPCR and Cq cut-offs optimised on insectary-reared flies, a more realistic parasite prevalence for field-trapped flies was estimated at 0.47% (95% confidence intervals [0.36, 0.73]).

Our results show that infected tsetse can contaminate naïve flies with *T. brucei* DNA when co-caged, and that the level of contamination can be extensive. Whilst simple PCR may overestimate infection rates, quantitative PCR offers a means of eliminating false positives.