

The polymerase chain reaction (PCR) was used to identify trypanosomes in *Glossina pallidipes* and *G. longipennis* caught in Kenya. Of 3826 flies dissected, 188 (4.9%) were parasitologically positive overall. The infection rate in *G. pallidipes* was 5.7% (187 of 3301 flies), but only one of 525 *G. longipennis* was infected (infection rate 0.2%). There was a higher infection rate in female *G. pallidipes* flies than male flies ($\chi^2 = 18.5$, $P < 0.001$) and odds ratio = 2.5 (95% 1.6, 3.7). The infected flies were analysed by PCR using 10 sets of primers specific for species and subgroups within the subgenera *Nannomonas*, *Trypanozoon* and *Duttonella*. Of 188 parasitologically positive samples, PCR identified 137 (72.9%), leaving 51 (27.1%) non-identified. We recorded infection rates of 47.2% for *Trypanosoma congolense* savannah, forest and kilifi subgroups, 20.9% for *T. simiae*/*T. simiae tsavo*/*T. godfreyi*, 14.9% for *T. brucei* ssp. and 13.8% for *T. vivax*. Thirty-nine (26.7%) flies had mixed infections, with a minor association between *T. congolense* savannah/*T. simiae tsavo*/*T. godfreyi* ($\chi^2 = 6.93$, d.f. = 1, $P < 0.05$). The relative proportion of each trypanosome species or subgroup varied between fly belts with *T. congolense* (all subgroups) being the most abundant and *T. godfreyi* the least. Statistical analysis showed that dissection method and PCR test classified infections independently ($\chi^2 = 10.5$, d.f. = 1, $P < 0.05$ and kappa = 0.38). This study shows that pathogenic trypanosomes are widespread in all sampled testes fly belts with *G. pallidipes* as the main vector. Further, PCR test is more reliable in detecting and identifying trypanosomes than dissection method.