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Short communication

The transmissibility of *Trypanosoma congolense* seems to be associated with its level of resistance to isometamidium chloride

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Abstract

In large parts of Africa the control of livestock trypanosomiasis relies on the use of trypanocidal drugs. Resistance against the available compounds is developing rapidly in the trypanosome population. The effect of the development of drug resistance on the fitness of the trypanosome is not well known. To determine the effect of the development of resistance to isometamidium chloride on the trypanosome's transmissibility, transmission experiments were conducted. Use was made of three isogenic clones of *Trypanosoma congolense* with different susceptibility to the drug. The infection rate in *Glossina morsitans morsitans* differed significantly between clones and was significantly higher in tsetse flies infected with the *T. congolense* clone with the highest level of drug resistance.

Keywords: Transmission; *T. congolense*; Resistance; Isometamidium

The control of bovine trypanosomiasis relies, to a large extend, on the use of trypanocidal drugs. Only a small group of chemoprophylactic and chemotherapeutic compounds are currently in use and new compounds are unlikely to become available in the near future (Peregrine, 1994). Geerts and Holmes (1998) estimated that in Africa about 35 million doses of trypanocidal drugs are administered each year. Furthermore, there is growing concern that the effectiveness of this control method will be severely reduced by the widespread development of resistance in trypanosomes. This is especially so for trypanocidal compounds with a prophylactic effect, such as isometamidium chloride. There is thus increasing emphasis in trypanosomiasis control on rational management of drug use in an attempt, if not to prevent, at least to delay the spread of resistance in the parasite population (Geerts and Holmes, 1998). Effective drug use management should be based on a good understanding of the factors contributing to the development, maintenance and spread of trypanocidal drug resistance in a population. In this respect, an important question remains the relative fitness and

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competitiveness of resistant trypanosome strains. As a general rule, mutant forms of an organism are likely to be less fit than their wild-type strains in the absence of selection. Although information on the fitness of resistant trypanosome stains is scarce there are indications that resistance reduces infectivity (Gray and Roberts, 1971; Mutugi et al., 1995; Coleman and McDermott, 2000). Another factor that may significantly affect the spread of resistant trypanosome strains is their transmissibility. Studies aiming at determining the transmissibility of resistant trypanosome strains have been conducted previously (Gray and Roberts, 1971). However, in previous experiments use was made of genetically different strains that were either susceptible or resistant. In this case it is difficult to distinguish any effect of the development resistance on transmissibility from differences in transmissibility before resistance developed. To avoid such confounding effects, the transmissibility of three isogenic *T. congolense* IL1180 clones with different levels of resistance to isometamidium chloride was compared. The origin of the isogenic clones is described by Delespaux et al. (2005). They are isogenic apart from the mutation(s) underlying the isometamidium chloride resistance phenotype. The susceptible clone had a CD50 (the curative dose that gives complete cure in 50% of the animals) in mice of 0.018 mg/kg. The resistant clones had a CD50 of 1.8 and 3.6 mg/kg for the low and high resistant clone, respectively.

To compare transmissibility each clone was injected into four to six outbred mice (OF1) (Table 1), each mouse being considered as a replicate for each clone. Infection of teneral male *Glossina morsitans morsitans* (Elsen et al., 1993), less than 32 h old, was carried out by feeding batches of 40 flies on one of those anaesthetized mice with a parasitaemia of $10^{8.4}$. Only fully engorged flies were retained. After the infected bloodmeal, flies were maintained on rabbits. To avoid the chance of re-infection of the flies, rabbits used for fly maintenance were changed at weekly intervals. Thirty days after the infected bloodmeal, all tsetse were dissected using the method described by Lloyd and Johnson (1924) and their infection status was determined. The data were analysed using a logistic mixed model (Stata, 2003). The infection in flies was used as response variable whereas the resistance of trypanosomes to isometamidium chloride was used as a categorical explanatory variable and the individual mouse (or replicate) as random effect.

A total of 380 tsetse flies, infected with one of the three isogenic *T. congolense* clones, were dissected. Infection rate was lowest in the flies infected with the susceptible clone and highest in the flies infected with the clone showing the highest level of resistance (Table 1). Within the groups, the random variability caused by individual mice on infection rates was not significant ($p = 1$). The observed increase in transmissibility was only significant ($p < 0.05$) in the clone with the highest level of resistance. Other experiments have shown no relationship between transmissibility and resistance (Gray and Roberts, 1971). However, comparisons were made between strains of a different genotype and not isogenic clones. Furthermore, the level of resistance may not be sufficiently high to significantly affect transmissibility. The observed change in transmissibility may have important repercussions for the understanding of the epidemiology of trypanocidal drug resistance in trypanosomes. Since differences in the relative growth rate or competitiveness could also affect transmissibility, more studies are required to determine transmissibility of resistant trypanosome strains in a mixed infection.

Table 1
Comparison of the pooled infection rates in the mouthparts and midguts of *G. m. morsitans* infected with one of the isogenic clones of *T. congolense* IL1180 with different levels of resistance to isometamidium chloride

T. congolense clone	Number of mice (replicates)	Number		Number of infection in		Infection rate (%)
		Infected	Dissected	Midgut	Mouthparts	
Susceptible	5	124	119	64	64	53.8
Resistant (low)	6	131	128	73	71	55.5
Resistant (high)	4	125	122	83	82	67.2

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