Investigations on the transmissibility of *Trypanosoma congolense* by the tsetse fly *Glossina morsitans morsitans* during its development in a mammalian host

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Abstract

Experiments were conducted to investigate the effect of the developmental stage of a monomorphic *T. congolense* IL1180 strain, in a vertebrate host, on its transmissibility by the tsetse fly *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae). Batches of 160 male teneral tsetse flies were given a single bloodmeal on mice infected with this *T. congolense* strain 4, 5, 6, 7 or 10 days post-infection. The proportion of infected flies in each of those batches showed that the stage of development of the trypanosome does affect the proportion of flies that develop a mature or immature infection with immature and mature infection rates of flies infected on days 5 or 10 significantly higher. The proportion of infected flies was not affected by the parasitaemia at the moment of infection. Results show that tsetse flies can become infected at any phase of the development of the *T. congolense* IL 1180 strain but the ease with which trypanosomes develop in the fly depends on the phase in the parasite's development in the host. Those observations suggest that in analogy with the pleomorphic *T. brucei s.l.* adaptation of the monomorphic *T. congolense* to development in the fly may also determine the parasite's transmissibility. Moreover, the findings stress the importance of standardising experiments in which the vectorial capacity of tsetse flies is determined and compared.

Keywords: *Glossina*; Transmission; *Trypanosoma congolense*; Development

1. Introduction

African trypanosomes causing sleeping sickness in humans and “Nagana” in livestock are cyclically transmitted by tsetse fly, *Glossina* spp. Hence, the proportion of infected tsetse flies in a population is of considerable importance in the epidemiology of human and animal trypanosomiasis. Fly-related factors that affect the proportion of infected tsetse flies are well documented ([Kubi et al., 2006] and [Aksoy et al., 2003]). Trypanosomes are also known to undergo substantial metabolic changes to allow adaptation to the changing environment in the mammalian host or insect vector (reviewed by Matthews, 2005). In polymorphic trypanosome species such as *T. brucei s.l.*, considerable morphological and metabolic changes prepare the trypanosome for its survival and development in the tsetse midgut environment that is low in glucose and oxygen (reviewed by Seed and Wenck, 2003). Therefore, the proportion of tsetse flies in which a *T. brucei* infection develops is a function of the numbers of short stumpy forms of the parasite in the bloodmeal (Wijers and Willet, 1960). It thus seems that for *T. brucei s.l.*, parasite-associated processes prepare the parasite for its development in the tsetse fly and determine the ease with which the trypanosome is transmitted by tsetse flies. Although *T. congolense* is generally considered to be monomorph, similar pleomorphism has been described in some strains ([Godfrey, 1960] and [Nantulya et al., 1978a]) and has been associated with the parasite's transmissibility (Nantulya et al., 1978b). Nevertheless, with the exception of the work described by Nantulya et al. (1978b), little is known of the transmissibility of *T. congolense* during its development in the host. Here we investigate the transmissibility of a monomorphic *T. congolense* strain during its development in a vertebrate host.

2. Materials and methods

2.1. Tsetse flies

Teneral male tsetse flies *Glossina morsitans morsitans* Westwood (less than 32 h old) from the colony maintained at the Institute of Tropical Medicine (Antwerp, Belgium) were used in the experiments. The origin of

this tsetse colony and the conditions of maintenance are described by Elsen et al. (1993). This line of tsetse has a high vectorial capacity (Van Den Abbeele, 2001).

2.2. Trypanosome

Trypanosoma congolense IL 1180, a strain originating from Serengeti in Tanzania (Geigy and Kauffmann, 1973) was used for the infections. One cryostabilate of this strain was reactivated in three mice (strain OF1) that served for experimental mice passage.

2.3. Experimental design

Each mouse of a pool of 25 mice (strain OF1) was injected with 0.2 ml of blood containing $10^{6.9}$ trypanosomes per millilitre. From the third day after the infection onwards, the parasitaemia of each mouse was measured daily using the scale of Herbert and Lumsden (1976). On days 4, 5, 6, 7 or 10 post-infection, four mice were selected randomly from the pool. The selected mice were anesthetized with 0.06 ml per mouse of a mixture of Ketalar® and Rompun® (80/20, v/v) and one cage containing 40 teneral male tsetse flies was placed on each anesthetized mouse to allow the flies to take an infective bloodmeal. After the infective bloodmeal, only fully engorged flies were retained. These flies were fed on clean rabbits three times a week until 48 h before dissection. The mice used to infect the flies were euthanized. To avoid re-infection of the flies during the *in vivo* maintenance, rabbits were replaced at weekly intervals. All surviving flies were dissected 21 days after the infective bloodmeal using the method described by Llyod and Johnson (1924). The midgut and mouthparts were examined for the presence of trypanosomes.

The proportion of immature (procyclic) infections was calculated as the proportion of dissected flies that had a trypanosome infection in the midgut whereas the proportion of mature (metacyclic) infections was calculated as the proportion of dissected flies infected in midgut and mouthparts. The maturation rate was calculated as the proportion of midgut infection that developed into mature infection. Statistical analyses of data were carried out in Stata software version 9.2 using a logistic regression to compare proportions of immature and mature infections in different experimental fly groups. Discrete explanatory variables were the days following mice infection, the parasitaemia of the mouse on which the infective bloodmeal was taken and the interaction between the two. Simplified models (from which non-significant explanatory variables (parasitaemia) were dropped) were selected when the likelihood ratio test had a p value > 0.05. Cluster effects resulting from flies infected on the same mouse and maintained in the same cage were taken into account in simplified robust models.

3. Results

Of a total of 800 male teneral flies, 70.5% (564/800) took the infective bloodmeal and were retained for the experiment. The mortality rate was 0.9%. A total of 559 tsetse flies were dissected to determine their infection status. The proportion of infected flies was significantly higher ($p < 0.05$) in teneral tsetse flies infected on day 5 or 10 post-infection compared to the proportion of infected flies that received an infective bloodmeal on the first day that parasites were observed in the blood of the mice (day 4 post-infection) (Table 1 and Table 2 and Fig. 1). At the peak of the parasitaemia (around day 7 post-infection), immature and mature infection rates were not significantly different from the infection rates observed at the onset of the parasitaemia (day 4 post-infection) (Table 1 and Table 2 and Fig. 1 for mature infection). The effect of parasitaemia on the mature and immature infection rates was not significant ($p = 0.130$ for immature and $p = 0.281$ for mature infections). The maturation rate of the midgut infection was high and not affected by the day of infection (Table 1). The design effect, reflecting the importance of intra-cluster correlation was small (DEFT range between 0.55 and 1.25) indicating that individual mouse used to infect the flies had little effect within experimental groups (day post-infection).

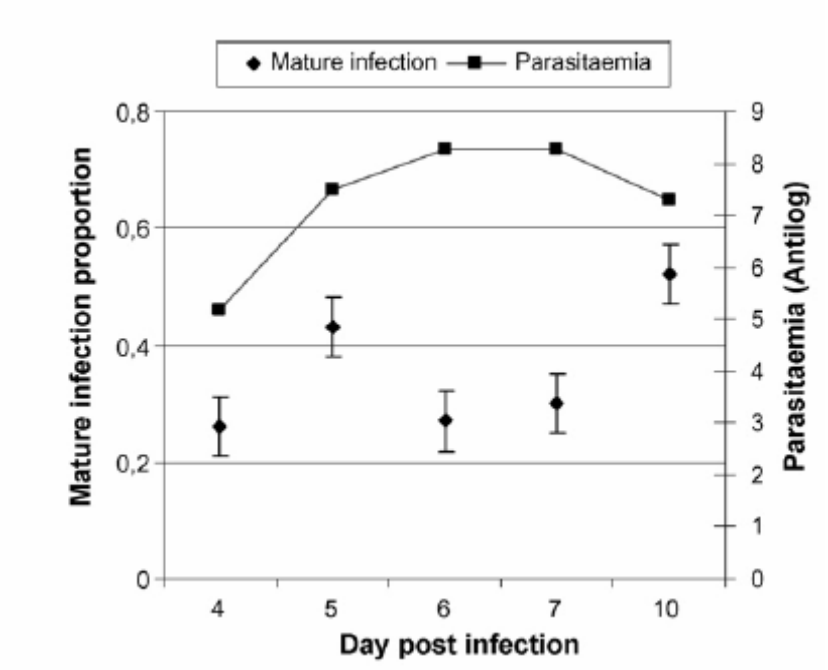
Table 1: *Immature and mature infection rates of male G. m. morsitans given a single infective bloodmeal on various days of the development of T. congolense IL1180 in mice*

Mouse	Day post-infection	Parasitaemia (antilog)	Number dissected	Proportion of infected flies		Maturation rate
				Immature	Mature	
1	4	6.9	35	0.20 (7/35)	0.20 (7/35)	1.00
2	4	6.9	29	0.34 (10/29)	0.34 (10/29)	1.00
3	4	<5.4	33	0.27 (9/33)	0.27 (9/33)	1.00
4	4	6.9	26	0.27 (7/26)	0.23 (6/26)	0.86
5	5	7.8	35	0.51 (18/35)	0.46 (16/35)	0.89
6	5	6.9	25	0.48 (12/25)	0.44 (11/25)	0.92
7	5	8.1	30	0.40 (12/30)	0.40 (12/30)	1.00
8	5	7.2	25	0.44 (11/25)	0.44 (11/25)	1.00
9	6	8.4	30	0.10 (3/30)	0.10 (3/30)	1.00
10	6	8.4	30	0.23 (7/30)	0.23 (7/30)	1.00
11	6	8.1	19	0.47 (9/19)	0.47 (9/19)	1.00
12	6	8.1	29	0.41 (12/29)	0.38 (11/29)	0.92
13	7	8.1	26	0.38 (10/26)	0.38 (10/26)	1.00
14	7	8.4	31	0.13 (4/31)	0.13 (4/31)	1.00
15	7	8.4	25	0.44 (11/25)	0.44 (11/25)	1.00
16	7	8.1	28	0.36 (10/28)	0.29 (8/28)	0.80
17	10	6.9	28	0.57 (16/28)	0.57 (16/28)	1.00
18	10	6.9	26	0.46 (12/26)	0.46 (12/26)	1.00
19	10	8.1	27	0.48 (13/27)	0.48 (13/27)	1.00
20	10	7.2	22	0.59 (13/22)	0.59 (13/22)	1.00

Table 2. *Significance (p value) of the difference between the proportion of male G. m. morsitans with a mature or immature infection and infected on days 5, 6, 7 or 10 post-infection compared to flies infected on day 4 post-infection (reference)*

Day post-infection	***p* values**	
	Immature infection	**Mature infection**
5	<0.001	<0.001
6	0.811	0.813
7	0.471	0.559
10	<0.001	<0.0001

Fig. 1*. Average proportion of mature infection of male G. m. morsitans infected on different days post-infection on mice infected with T. congolense IL1180.*



4. Discussion

Trypanosomes undergo a complex life cycle between the mammalian host and the insect vector. The mechanisms of refractoriness or susceptibility of tsetse flies to a trypanosome infection are not fully understood but parasite-related factors do play an important role in determining the transmissibility of the trypanosome.

Contrary to earlier observations by Nantulya et al. (1978b), high transmissibility of *T. congolense* does not seem to be associated with high parasitaemias. It is difficult to compare both experiments since the experimental setup

of Nantulya et al. (1978b) does not allow for a thorough analysis of variability between fly batches and variability between days on the rising, plateau or declining phase of the parasitaemia. Moreover, compared to Nantulya et al. (1978a) we did not observe the morphological changes in the parasite during its development.

Our results show that the transmissibility of the *T. congolense* strain used was not associated with differences in the parasitaemia of the mice at the moment of infection. Indeed, the infection rate of tsetse infected on day 6 or 7 of the infection is significantly reduced even when the parasitaemia is highest. This could be attributed to the effect of the host's immune system reducing the trypanosome's viability and, hence their capacity to infect tsetse flies (Morrison et al., 1985). On the other hand, the absence of a correlation between the infection rate of tsetse and the parasitaemia at the moment of infection may not be surprising considering the fact that a single trypanosome is sufficient to infect a tsetse fly (Maudlin and Welburn, 1989). In polymorphic *T. brucei s.l.* also, transmissibility is not correlated with the parasitaemia but with the proportion of non-dividing stumpy forms that constitute the predominant population during the remission and relapse of the parasitaemia ([Balber, 1972] and [Van den Bossche et al., 2005]). Such stumpy forms have the capacity to differentiate into procyclic forms either in the tsetse's midgut (Matthews, 1999) or *in vitro* (Breidbach et al., 2002) whereas long slender forms are not fit to survive in the insect vector and die rapidly after ingestion by the tsetse fly (Turner et al., 1988). Although such morphological changes are not observed in the monomorphic *T. congolense* strain used in this experiment, metabolic changes in the trypanosome could occur at each experimental stage and probably affect its capacity to infect tsetse flies. Such metabolic changes could make the trypanosome less susceptible to the elimination process in tsetse's midgut immediately after the bloodmeal ingestion (Van Den Abbeele et al., 1999). Although further research is required to determine the processes involved in adapting bloodstream forms of *T. congolense* to the tsetse fly's midgut environment, the observed differences in the trypanosome's transmissibility during its development in the mammalian host stress the importance of standardising trypanosome transmission experiments or experiments comparing the vectorial capacity of tsetse flies. Furthermore, it would be interesting to confirm these finding using infected bovines.

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