Ability of Trypanosome-Infected Tsetse Flies (Diptera: Glossinidae) to Acquire an Infection with a Second Trypanosome Species

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ABSTRACT The epidemiology of human and animal trypanosomiasis is determined to a large extent by the number of infected tsetse flies in a specific area. In the field, a substantial proportion of infected flies carry mixed trypanosome infections. The way in which these tsetse flies acquire a mixed infection is not fully understood. In particular, the susceptibility of tsetse flies to sequential infection with trypanosomes is not well understood. Accordingly, laboratory studies were made of the effects of age and prior infection on the probability of *Glossina morsitans morsitans* (Westwood) developing an infection of *Trypanosoma congolense* and *Trypanosoma brucei brucei* after feeding on infected mice. Results of these experiments clearly showed that 20–30-d-old *G. m. morsitans* can still pick up and develop a mature infection in the mouthparts/hypopharynx for *T. congolense* or in the salivary glands for *T. b. brucei*. However, their ability to acquire infection was significantly lower compared with teneral flies. Furthermore, 20–30-d-old flies that already carry a mature *T. congolense* or *T. b. brucei* infection remained at least as susceptible to a secondary trypanosome infection compared with noninfected flies of the same age. The immunological and epidemiological repercussions of those findings are discussed.

KEY WORDS *Glossina*, infection, *T. congolense*, *T. b. brucei*, vector competence

TSETSE-TRANSMITTED TRYPANOSOMIASIS is a serious constraint to human and animal health in large parts of sub-Saharan Africa. Tsetse flies, the main vector of the trypanosomes, occur on ≈10 million km^2 of the African continent. The epidemiology of the disease in humans and livestock is determined by various factors, including the proportion of infected tsetse flies. The tsetse's susceptibility to an infection with cyclically transmitted trypanosomes is determined by a range of intrinsic and extrinsic factors (Maudlin 1991). Tsetse flies are usually more susceptible to infections with *Trypanosoma congolense*, a trypanosome species that has a shorter development cycle compared with *Trypanosoma brucei* s.l. Using highly sensitive and species-specific molecular diagnostic tools, several researchers have shown that in the field a substantial proportion of the infected tsetse flies carry mixed trypanosome infections (Majiwa and Otieno 1990, McNamara et al. 1995, Masiga et al. 1996, Woolhouse et al. 1996, Morlais et al. 1998, Lehane et al. 2000, Jamonneau et al. 2004). Such mixed infections can be contracted simultaneously from a single animal supporting a mixed trypanosome infection (Van den Bossche et al. 2004) or could be the result of sequential infections over a number of feeds on different infected animals. Moloo et al. (1982) and Gibson and Ferris (1992) have shown that teneral tsetse flies can become infected with different trypanosome species during consecutive bloodmeals. The question remains how the presence of an established midgut or mature trypanosome infection in older flies effects the development of a new, secondary trypanosome infection. Experiments were conducted to determine the competence of older, trypanosome-infected tsetse flies to acquire such a secondary infection.

Materials and Methods

Trypanosomes. A *T. b. brucei* strain derived from the stock EATRO 1125 AntAR1 (Le Ray et al. 1977) and *T. congolense* IL1180, a strain originating from the Serengeti region in Tanzania (Geigy and Kauffman 1973), were used in the experiments.

Tsetse Flies. Male *Glossina morsitans morsitans* (Westwood) from the colony maintained at the Institute of Tropical Medicine (Antwerp, Belgium) were used. The origin of the colony and rearing techniques are described by Elsen et al. (1993).

Experimental Design. Six groups of flies were infected once or twice by feeding on infected mice. Flies were then dissected to determine their infection status according to the schedule presented in Table 1. After

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Table 1. Overview of infection experiments of *G. m. morsitans* with *T. congolense* IL1180, *T. b. brucei* AntAR1, or both

Infection group	Age (d)					
	0	10	20	30	40	50
Tc_0	*Tc*	→	Dissection			
Tc_0-Tb_{20}	*Tc*	→	*Tb*	→	→	Dissection
Tb_{20}	–		*Tb*	→	→	Dissection
Tb_0	*Tb*	→	→	Dissection		
Tb_0-Tc_{30}	*Tb*	→	→	*Tc*	→	Dissection
Tc_{30}	–	→	→	*Tc*	→	Dissection

Teneral flies = day 0. *Tb*, infected with *T. b.brucei*; *Tc*, infected with *T. congolense.*

offering an infected meal, only fully engorged flies were retained. Infected flies were maintained on rabbits and offered the opportunity to feed three times weekly. The rabbits were replaced weekly to avoid cyclic transmission of the trypanosomes.

Before dissection, flies were starved for 48 h. The labrum, hypopharynx, salivary glands, and the midgut were examined for the presence of trypanosomes. Trypanosome species identification was conducted using the method described by Lloyd and Johnson (1924). Flies of which only the midgut was infected were considered to have an immature infection. Flies with infections in the midgut or salivary glands were considered to have a mature infection. The mature infection rate was calculated as the proportion of dissected flies that developed a mature trypanosome infection. Differences in infection rates were compared using the Fisher exact test.

Results

In total, 763 flies, divided over the six experimental groups, were dissected. The infection results are shown in Table 2. The *T. b. brucei* or *T. congolense* infection rate of flies infected when, respectively, 20 and 30 d old (groups Tb_{20} and Tc_{30}, respectively) differed little from the infection rate of the same parasites in flies that were previously infected with another trypanosome species (groups Tc_0-Tb_{20} and Tb_0-Tc_{30}). The *T. congolense* infection rate of flies infected when teneral (Tc_0) were comparable with the one observed in flies infected with *T. congolense* when teneral and subsequently infected at the age of 20 d with *T. b. brucei* (group Tc_0-Tb_{20}). Similarly, the *T. b. brucei* infection rate of flies infected when teneral (group Tb_0) was similar to that in flies that were infected with *T. b. brucei* when teneral and subsequently infected at the age of 30 d with *T. congolense* (group Tb_0-Tc_{30}). The *T. b. brucei* or *T. congolense* infection rate of flies infected when 20 or 30 d old were significantly lower ($P < 0.001$ for *T. b. brucei* or *T. congolense* infections) that the infection rate when infected as teneral (groups Tb_{20} compared with Tb_0 and Tc_{30} compared with Tc_0).

The *T. b. brucei* infection rate in *T. congolense* infected flies was 2.9% (two *T. b. brucei* infected flies out of a total of 69 *T. congolense*-infected flies) (group Tc_0-Tb_{20}) compared with 5.2% (five *T. b. brucei*-infected flies out of a total of 97 infected flies) in flies that were infected for the first time at the age of 20 d (group Tb_{20}). The differences were statistically not significant ($P = 0.7$). The *T. congolense* infection rate in *T. b. brucei* infected flies was 22.2% (six *T. congolense*-infected flies on a total of 27 *T. b. brucei* infected flies) (group Tb_0-Tc_{30}) compared with 9.2% (13 *T. congolense* infected flies out of a total of 141 infected flies) in flies that were offered an infective bloodmeal only at the age of 30 d (group Tc_{30}). The observed differences in infection rates were again not statistically significant ($P = 0.09$).

Discussion

The results from our experiments show that an infective bloodmeal ingested by trypanosome-infected tsetse flies can result in the establishment of a mixed mature trypanosome infection. However, the tsetse's susceptibility to pick up a trypanosome infection decreases substantially with age. Indeed, the *T. b. brucei* or *T. congolense* infection rates of flies that are offered an infective bloodmeal at 20 or 30 d of age are significantly lower than the infection rates of flies that ingested the parasites at teneral stage, just after emergence. This confirms previous reports (Wijers 1958,

Table 2. Midgut and mature infection rates of male *G. m. morsitans* with number of flies (*n*), infected according to the infection schedule presented in Table 1

Group	No. dissected	Total midgut infection	*Tb* mature only	*Tc* mature only	*Tb*+ *Tc* mature	Total *Tb* mature	Total *Tc* mature
Tc_0	99	0.35 (35)	NR	0.35 (35)	NR	NR	0.35 (35)
Tc_0-Tb_{20}	212	0.46 (98)	0.038 (8)	0.32 (67)	0.0094 (2)	0.047 (10)	0.33 (69)
Tb_{20}	97	0.27 (26)	0.052 (5)	NR	NR	0.052 (5)	NR
Tb_0	90	0.51 (46)	0.21 (19)	NR	NR	0.21 (19)	NR
Tb_0-Tc_{30}	124	0.38 (47)	0.17 (21)	0.048 (6)	0.048 (6)	0.22 (27)	0.097 (12)
Tc_{30}	141	0.10 (14)	NR	0.092 (13)	NR	NR	0.092 (13)

Tb, T. b.brucei; Tc, T. congolense; NR, not relevant.

Distelmans et al. 1982, Mwangelwa et al. 1987, Welburn and Maudlin 1992), and more research is required to determine the molecular background of age-specific changes in the tsetse's susceptibility to trypanosome infections. Despite this, a substantial proportion of the population of adult tsetse flies can still acquire an infection, even when the fly is already carrying a trypanosome infection. This implies that, in the field, the proportion of infected flies will increase with increasing age as was already observed by various researchers (e.g., Woolhouse and Hargrove 1998). Based upon our infection data, two important conclusions can be drawn with regard to the development of sequential trypanosome infections in tsetse. Firstly, a secondary infection with a different trypanosome species has no effect on an already established mature infection. Second, previous exposure to an infected bloodmeal or the presence of a mature or immature trypanosome infection has no effect on the development and maturation rate of the subsequent secondary infection. Hence, there seems to be little interaction between the two trypanosome species during their development in the midgut and subsequent migration from the midgut to either the mouthparts or the salivary glands does not seem to be hindered by the presence of a mature infection. Moreover, our data seem to suggest that already infected flies are as susceptible to develop a secondary trypanosome infection compared with noninfected flies of the same age. Considering the small sample size in our experiment, new experiments with a larger sample size may be required to confirm this observation. The underlying molecular factors that modulate the susceptibility of a tsetse fly for a trypanosome infection are still under debate. Among these factors, components of the insect immune system are assumed to be involved in the natural refractoriness observed in the tsetse fly. Prior studies have clearly demonstrated an induced expression and synthesis of several antimicrobial peptides when teneral flies are offered a trypanosome-infected bloodmeal (Hao et al. 2001, Boulanger et al. 2002). Moreover, it was shown that 20-d-old midgut-infected flies have elevated expression levels of two important immune peptides, attacin and defensin, compared with noninfected flies that had cleared the trypanosomes. This demonstrated that these immune peptides did not affect an already established midgut trypanosome population. Based upon our results, we can hypothesize that this elevated level of defense peptides in midgut-infected flies also does not interfere with the establishment of a new, secondary infection in the midgut of a 20- or 30-d-old fly when these flies ingest a new, infective bloodmeal. This would mean that the efficacy of the immune system to interfere with a secondary establishment of trypanosomes in the tsetse midgut is significantly decreased when a fully grown procyclic trypanosome population is already present in fly's midgut, although it was shown in previous studies that these flies are fully immunocompetent (Hao et al. 2001).

Notwithstanding the observed vector competence of nonteneral and infected tsetse flies, the prevalence of trypanosome infections in a natural tsetse population is usually low. The low infection rates in adult flies in our experiments confirm that a majority of tsetse flies will not develop a mature infection with neither *T. b. brucei* nor with *T. congolense*, even when the infected bloodmeal was offered to teneral flies. This indicates that a high proportion of the tsetse population is refractory to a trypanosome infection. This heterogeneity in the susceptibility to trypanosome infections in tsetse and the apparent absence of competition between trypanosome species infecting a fly may result in the aggregation of infections in the susceptible part of the tsetse population and the large proportion of mixed infections observed in the field (e.g., Majiwa and Otieno 1990, McNamara et al. 1995, Masiga et al. 1996, Woolhouse et al. 1996, Morlais et al. 1998, Lehane et al. 2000, Jamonneau et al. 2004).

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References Cited

Boulanger, N., R. Brun, L. Ehret-Sabatier, C. Kunz, and P. Bulet. 2002. Immunopeptides in the defense reactions of *Glossina morsitans* to bacterial and *Trypanosoma brucei brucei* infections. Insect Biochem. Mol. Biol. 32: 369–375.

Distelmans, W., F. D'Haeseleer, L. Kaufman, and P. Rousseeuw. 1982. The susceptibility of *Glossina palpalis palpalis* at different ages to infection with *Trypanosoma congolense.* Ann. Soc. Belg. Med. Trop. 62: 41–47.

Elsen, P., J. Van Hees, and E. De Lil. 1993. L'historique et les conditions d'élevage des lignies de glossines (Diptera: Glossinidae) maintenues á l' Institute de Médicine Tropicale Prince Léopold d'Anvers. J. Afr. Zool. 107: 439–499.

Geigy, R., and M. Kauffman. 1973. Sleeping sickness survey in the Serengeti area (Tannzania) 1971. I. Examination of large mammals for trypanosomes. Acta Trop. 30: 12–23.

Gibson, W., and V. Ferris. 1992. Sequential infection of tsetse flies with *Trypanosoma brucei* and *Trypanosoma congolense.* Acta Trop. 50: 345–352.

Hao, Z., I. Kasumba, M. J. Lehane, W. C. Gibson, J. Kwon, and S. Aksoy. 2001. Tsetse immune responses and trypanosome transmission: implications for the development of tsetse-based strategies to reduce Trypanosomiasis. Proc. Natl. Acad. Sci. U.S.A. 98: 12648–12653.

Jamonneau, V., S. Ravel, M. Koffi, D. Kaba, D. G. Zeze, L. Ndri, B. Sane, B. Coulibaly, G. Cuny, and P. Solano. 2004. Mixed infections of trypanosomes in tsetse and pigs and their epidemiological significance in a sleeping sickness focus of Côte d'Ivoire. Parasitology 129: 693–702.

Lehane, M. J., A. R. Msangi, C. J. Whitaker, and S. M. Lehane. 2000. Grouping of trypanosome species in mixed infections in *Glossina pallidipes.* Parasitology 120: 583–592.

Le Ray, D., J. D. Barry, C. Easton, and K. Vickerman. 1977. First tsetse fly transmission of the 'Antat' serodeme of *Trypanosoma brucei.* Ann. Soc. Belg. Med. Trop. 57: 369–381.

Lloyd, L. L., and W. B. Johnson. 1924. The trypanosome infections of tsetse flies in Northern Nigeria and a new method of estimation. Bull. Entomol. Res. 14: 225–227.

Majiwa, P.A.O., and L. H. Otieno. 1990. Recombinant DNA probes reveal simultaneous infections of tsetse with dif-

ferent trypanosome species. Mol. Biochem. Parasitol. 40: 245–253.

Maudlin, I. 1991. Transmission of African trypanosomiasis: interactions among tsetse immune systems, symbionts and parasites. Adv. Dis. Vector Res. 7: 117–148.

Masiga, D. K., J. J. McNamara, C. Laveissière, P. Truc, and W. C. Gibson. 1996. A high prevalence of mixed infections in tsetse flies in Sinfra, Côte d'Ivoire, detected by DNA amplification. Parasitology 112: 75–80.

McNamara, J. J., C. Laveissière, and D. K. Masiga. 1995. Multiple trypanosome infections in wild tsetse in Côte d'Ivoire detected by PCR analysis and DNA probes. Acta Trop. 59: 85–92.

Moolo, S. K., G. W. Faiqa Dar, and G. W. Kamunya. 1982. The transmission of mixed infections of pathogenic *Trypanosoma* species to susceptible hosts by *Glossina morsitans morsitans.* Acta Trop. 39: 303–306.

Morlais, I., P. Grebaut, J. M. Bodo, S. Djoha, and G. Cuny. 1998. Characterisation of trypanosome infections by polymerase chain reaction (PCR) amplification in wild tsetse flies in Cameroon. Parasitology 116: 547–554.

Mwangelwa, M. I., L. H. Otieno, and G.D.F. Reid. 1987. Some barriers to *Trypanosoma congolense* development in *Glossina morsitans morsitans.* Insect Sci. Appl. 8: 33–37.

Van den Bossche, P., R. De Deken, J. Brandt, S. Geerts, D. Geysen, and D. Berkvens. 2004. The transmission of mixed *Trypanosoma brucei brucei*/*T. congolense* infections by tsetse (*Glossina morsitans morsitans*). Vet. Parasitol. 119: 147–153.

Welburn, S. C., and I. Maudlin. 1992. The nature of the teneral state in *Glossina* and its role in the acquisition of trypanosome infection in tsetse. Ann. Trop. Med. Parasitol. 86: 529–536.

Wijers, D.J.B. 1958. Factors that may influence rate of *Glossina palpalis* with *Trypanosoma gambiense.* 1. The age of the fly at the time of the infected feed. Ann. Trop. Med. Parasitol. 52: 385–390.

Woolhouse, M.E.J., J. J. McNamara, J. W. Hargrove, and K. Bealby. 1996. Distribution and abundance of trypanosome (subgenus *Nannomonas*) infections of the tsetse fly *Glossina pallidipes* in southern Africa. Mol. Ecol. 5: 11–18.

Woolhouse, M. E., and J. W. Hargrove. 1998. On the interpretation of age-prevalence curves for trypanosome infections of tsetse flies. Parasitology 116: 149–156.

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