Acta Tropica 113 (2010) 195–198

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Short communication

Transmissibility, by *Glossina morsitans morsitans*, of *Trypanosoma congolense* strains during the acute and chronic phases of infection

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ARTICLE INFO

Article history:
Received 29 May 2009
Received in revised form 31 August 2009
Accepted 8 September 2009
Available online 16 September 2009

Keywords:
Trypanosoma congolense
Glossina morsitans morsitans
Acute phase
Chronic phase
Transmissibility

ABSTRACT

In order to verify whether chronic trypanosomal infections can affect the transmissibility of *Trypanosoma congolense* by tsetse flies, batches of *Glossina morsitans morsitans* were fed on mice infected with the same level of parasitemia ($10^{8.1}$ trypanosomes/ml of blood) of two cloned low virulent *T. congolense* strains during the acute and the chronic phases of infection. Results showed that the proportions of procyclic infections in flies that were fed during the acute phase (32.6% and 45.4% for isolates 1 and 2, respectively) were significantly higher ($\chi^2 = 4.7$, $P < 0.05$ and $\chi^2 = 23.7$, $P < 0.0001$, respectively) compared to the proportions of procyclic infections of flies fed during the chronic phase of infection (18.8% and 14.9% for isolates 1 and 2, respectively). Similarly the proportions of metacyclic infections in flies fed during the acute phase (32.6% and 45.4% for isolates 1 and 2, respectively) were significantly higher ($\chi^2 = 6.3$, $P < 0.05$ and $\chi^2 = 23.7$, $P < 0.0001$, respectively) compared to the proportions of metacyclic infections in flies fed during the chronic phase of infection (16.8% and 14.9% for isolates 1 and 2, respectively). No significant difference was found in the maturation rate of both strains during the acute phase compared to the chronic phase of infection ($P > 0.05$). The results of this study suggest that *T. congolense* loses part of its transmissiblity by tsetse flies during the chronic phase of infection.

1. Introduction

In sub-Saharan Africa, *Trypanosoma congolense* is the most pathogenic trypanosome species infecting livestock. Most vertebrates can be infected with this species, but cattle remain the most susceptible. In this livestock species, the course of infection can vary but, in the field, most animals suffer from a more chronic form of the disease with an acute phase lasting for about one or two months followed by a chronic phase that can last for many months before the animal either clear or succumb from the infection (Stephen, 1986).

Along with *Trypanosoma brucei*, *T. congolense* is largely dependent on tsetse flies for its transmission. Once ingested, both species undergo metabolic variations and face tsetse immunological defense whereby the infection can either establish in the midgut or be cleared (Matthews, 1999). In flies where trypanosomes established, the infection can either remain in the midgut (immature infection) or mature in the salivary glands (*T. brucei*) or the mouth parts (*T. congolense*) (Leak, 1999). The ease with which trypanosomes are transmitted is affected by several factors among which the tsetse fly genome plays an important role. Tsetse flies belonging to the three groups can transmit the infection but, in most cases, fly species belonging to the *morsitans* group are more effective in transmitting trypanosomes compared to species of the *palpalis* or *fusca* group (Reifenberg et al., 1997). Moreover, similar variations in the vector competence exist within a fly group, between different fly species or subspecies and even between colonies of the same species (Kazadi et al., 1998; Moloo et al., 1995).

Apart from these tsetse-related factors, the transmissibility of trypanosomes can also be influenced by the trypanosome itself. Results from various field and experimental studies revealed that, in most cases, a high transmissibility is generally observed in *Trypanosoma vivax* compared to *T. congolense* or *T. brucei* (Woolhouse et al., 1994; Mohamed-Ahmed et al., 1989). However, significant differences in the transmissibility have also been reported within a trypanosome species. For example, *T. b. brucei* produces high infections in tsetse flies compared to *T. b. rhodesiense* (Welburn et al., 1995). Similarly, high transmissibility is observed in *T. congolense* belonging to the Savannah subgroup compared to the riverine-forest subgroup (Reifenberg et al., 1997). Similar variations have also been reported among various strains belonging to the same Savannah subgroup of *T. congolense* (Masumu et al., 2006a).

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0001-706X/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.actatropica.2009.09.002

Table 1
Procyclic infections, metacyclic infections and maturation rate (%, C.I.) of *G. m. morsitans* infected with *T. congolense* isolates during the acute and the chronic phases of infection in mice.

Isolate identification	Phase of infection	Number of flies dissected	Procyclic infections in % (95% C.I.)	Metacyclic infections in % (95% C.I.)	Maturation rate in % (95% C.I.)
1	Acute	86	32.6 (22.8–43.5)	32.6 (22.8–43.5)	100 (87.7–100)
	Chronic	101	18.8 (11.7–27.8)	16.8 (10.1–25.6)	89.5 (66.9–98.7)
2	Acute	119	45.4 (36.2–54.8)	45.4 (36.2–54.8)	100 (93.4–100)
	Chronic	101	14.9 (8.6–23.3)	14.9 (8.6–23.3)	100 (78.2–100)

There is also evidence that the transmissibility of trypanosomes by tsetse flies is not a stable character. In this context, considerable changes in the transmissibility of *T. vivax* and *T. brucei* have been reported as a result of the evolutionary process. It is well known that the mode of transmission of the parasite can modify its transmissibility for tsetse flies. For example, loss or reduced transmissibility by tsetse flies has been reported in *T. vivax* circulating outside the tsetse belt areas (i.e. South America) (Stephen, 1986) or *T. brucei* infecting humans (Welburn et al., 1995), respectively. In *T. congolense* such variations have also been observed as a result of changes in some biological properties such as the increased resistance to trypanocidal drugs that is associated with an increased transmissibility by tsetse flies of resultant resistant trypanosome strains (Van den Bossche et al., 2006). In the same context, *T. congolense* strains of high virulence profile were shown to exhibit high potential of transmissibility by tsetse flies compared to trypanosome strains of moderate or low virulence profile (Masumu et al., 2006a).

Despite the observations mentioned above, very few studies have been conducted to assess changes in the transmissibility of trypanosomes that are related to the interactions between trypanosomes and the hosts. In a previous study, different infection rates were observed in flies fed on different host species infected with the same *T. congolense* strain (Moloo, 1981). Further studies revealed that changes in transmissibility can also be observed in *T. congolense* during its development in the same mammalian host (Akoda et al., 2008; Nantulya et al., 1978a). In their study, Akoda et al. (2008) observed that the transmissibility of *T. congolense* is temporarily reduced at the first peak of parasitaemia but then restored during the descending stage to a level comparable to that of the ascending stage of parasitaemia. Since this study was conducted during the first phase of infection (acute phase) only, it is not known whether the observed restoration of the transmissibility after the first peak of parasitaemia is definitive or further changes occur in the parasites that further affect their transmissibility during the chronic phase of the infection. Since in the field many animals are chronically infected with *T. congolense*, the transmissibility of this parasite during the chronic phase of infection is of considerable epidemiological interest. Hence, the objective of this study was to investigate the transmissibility of *T. congolense* during the chronic phase of infection and compare its transmissibility with that observed during the acute phase of infection.

2. Materials and methods

2.1. Trypanosome isolates

Two cloned *T. congolense* strains were used in this study. These strains were collected from a trypanosomosis endemic area in eastern Zambia and cloned in mice (Masumu et al., 2006b). Their subgroup identity was determined using the PCR-RFLP developed by Geysen et al. (2003) and was Savannah type. Genetic analysis using a modified amplified fragments length polymorphism developed by Masumu et al. (2006c) revealed that both strains belonged to different genotypes. Their virulence profiles were characterized in mice as described by Masumu et al. (2006b). Both strains had low virulence.

2.2. Tsetse flies

A total of 480 teneral male *G. m. morsitans* (less than 32 h old) were use. The flies originated from the colony of the Institute of Tropical Medicine, Antwerp/Belgium. Their origin and rearing conditions were previously described by Elsen et al. (1993).

2.3. Experimental design

For each strain, two mice (OF1 strain) were used to multiply the trypanosomes from the stabilates. These mice were checked for the development of parasitaemia three times a week by microscopic examination of a drop of blood collected from the tail. After the development of parasitaemia, trypanosomes belonging to each of the strains were diluted in phosphate buffered saline glucose (PSG) and inoculated into two groups of six OF1 mice each, respectively (10^5 trypanosomes/mouse). These mice were followed up three times a week for the development of parasitaemia as previously stated. At the first peak of parasitaemia (10 days post-infection, acute phase) and for each of the two trypanosome strains, three mice with a parasitaemia of $10^{8.1}$ trypanosomes/ml blood according to the scale of Herbert and Lumsden (1976) were used to feed three batches of 40 flies. The remaining mice were followed up for 60 days after which, for each of the two trypanosome strains, three mice with again a parasitaemia of $10^{8.1}$ trypanosomes/ml blood were used to feed three other batches of 40 flies (chronic phase). In all cases, mice were anaesthetized by intraperitoneal injection of 60 μl of a 8/3 (v/v) mixture of Anesketin® (diluted to 5% ketamine in physiological water composed of 0.85 g NaCl + 100 ml water) and Rompun® before the fly feeding. For each batch of 40 flies, a single meal was provided on a single mouse. After the infective meal, only engorged flies were retained, maintained on clean rabbits and fed three times per week. In order to avoid re-infection of the flies during the maintenance feeding, the rabbits were replaced at weekly intervals. All surviving flies were dissected 21 days after the infective blood meal using the method described by Lloyd and Johnson (1924). The midgut and mouthparts were examined for the presence of trypanosomes. This experiment was approved by the Ethics Commission of the Institute of Tropical Medicine, Antwerp/Belgium (Ref DG001-PD-M-TT).

2.4. Statistical analysis

Procyclic infections were calculated as the proportion of infected flies that developed infections in the midgut while metacyclic infections were calculated as the proportion of infected flies that developed infections in the midgut and the mouthparts. The maturation rate was calculated as the proportions of midgut infections that matured in the mouthparts. Statistical analyses were carried out using NCSS software (Hintze, 2007). Data from the infection experiments were analyzed using 2×2 contingency tables (Chi square). Confidence intervals of the proportions and the differences

between the proportions were computed. All the statistical analyses were performed with a significance level set at 5%.

3. Results

The results of the infection experiment are summarised in Table 1. The proportions of procyclic infections in flies that were fed during the acute phase (32.6% and 45.4% for strains 1 and 2, respectively) were significantly higher ($\chi^2 = 4.7$, $P < 0.05$ and $\chi^2 = 23.7$, $P < 0.0001$, respectively) compared to the proportions of procyclic infections of flies fed during the chronic phase (18.8% and 14.9% for strains 1 and 2, respectively). Similarly the proportions of metacyclic infections in flies fed during the acute phase (32.6% and 45.4% for strains 1 and 2, respectively) were significantly higher ($\chi^2 = 6.3$, $P < 0.05$ and $\chi^2 = 23.7$, $P < 0.0001$, respectively) compared to the proportions of metacyclic infections in flies fed during the chronic phase (16.8% and 14.9% for strains 1 and 2, respectively). No significant difference was found in the maturation rate of both strains during the acute phase compared to chronic phase of infection ($P > 0.05$).

4. Discussion

Results from this study show that the transmissibility of *T. congolense* strains is reduced during the chronic phase of infection compared to the acute phase. Since cloned isolates were used in this study and infection of tsetse was conducted at the same level of parasitaemia, the observed reduced transmissibility is attributed to changes in the trypanosome during its development in the host. Similar changes in the transmissibility during different stages of the parasitaemia were observed by Akoda et al. (2008). The results described in this paper again confirm that transmissibility by tsetse is independent of the parasitaemia at the time of infection. Whether variations in transmissibility occur during the chronic phase of infection cannot be determined from our experimental setup. Nevertheless, in this experiment, flies were fed on mice at the peak of parasitaemia during the acute and the chronic phases of the infection.

These results indicate clearly that parasites circulating in animals during the chronic phase of infection are different from those initiating the infection (acute phase). It seems that variations occurring in trypanosomes during the course of infection induce changes, not only affecting their transmissibility, but also many other biological properties. Previous studies revealed that *T. vivax* isolates collected during the early stage of infection in cattle could infect mice while trypanosomes collected during the chronic phase of infection could not (Leeflang et al., 1976). It is not known whether these variations were related to changes in parasite properties or trypanosome populations since field isolates were used in these studies. However, using cloned *T. congolense*, Joshua (1990) reported high virulence profile in trypanosomes collected at the chronic phase compared to the acute phase of infection when inoculated into a naïve host.

The same variations in the phenotype expression were also related to the susceptibility of trypanosomes to drugs. In *T. cruzi* for example, parasites occurring during the chronic phase of the infection were shown to be less susceptible to drugs compared to those circulating during the acute phase (Caldas et al., 2008). Similarly, Mamman et al. (1993) reported an alteration in the resistance phenotype of *T. congolense* circulating in animals during chronic infections. According to Caldas et al. (2008), the reduced susceptibility to drugs of the parasites in chronically infected animals is related to the genetic adaptability of the parasites leading to the development of drug resistance.

The factors affecting the transmissibility of *T. congolense* during the course of infection are not clear. At least in *T. brucei*, the morphology of trypanosomes (i.e. the proportions of short-stumpy forms) present in the blood of the host at the time tsetse flies take their blood meal is related to their transmission (Wijers and Willet, 1960). Contrarily to what was previously reported (Nantulya et al., 1978b), similar changes in the morphology of *T. congolense* during its development in the host were not observed (Akoda et al., 2008). Metabolic changes in trypanosomes during their development in the host could be responsible for the observed variations in the transmissibility of the parasite during its development (Akoda et al., 2008). Further research is required to elucidate this process.

Acknowledgement

The authors acknowledge the DGDC (Directory General for Development Cooperation) for financially supporting this work.

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