Shifting from wild to domestic hosts: the effect on the transmission of *Trypanosoma congolense* to tsetse flies

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Abstract

The epidemiology and impact of animal African trypanosomosis is influenced by the transmissibility and the pathogenicity of the circulating trypanosome strains in a particular biotope. The transmissibility of 22 T. congolense strains isolated from domestic and wild animals was evaluated in a total of 1213 flies. Multivariate mixed models were used to compare infection and maturation rates in function of trypanosome origin (domestic or sylvatic) and pathogenicity. Both trypanosome pathogenicity and origin significantly affected the ability to establish a midgut infection in tsetse flies but not the maturation rates. The interaction between pathogenicity and origin was not significant. Since being pathogenic and having a domestic origin both increased transmissibility, dominant lowly pathogenic trypanosomes from domestic environments and highly pathogenic trypanosomes from sylvatic environments presented similar levels of transmissibility: 12% and 15%, respectively.

Blood meals with parasite concentration ranging from 0,05 to 50 trypanosomes/µl blood for 3 strains of T. congolense were provided to different batches of tsetse flies to evaluate the relationship between the parasite load in blood meals and the likelihood for a fly to become infected. A linear relationship between parasite load and transmissibility was observed at low parasitaemia and a plateau was observed for meals containing more than 5 trypanosomes / µl. Maximum transmission was reached with 12,5 trypanosomes/µl blood. About 50% of the flies were refractory to T. congolense, whatever their concentration in the blood meal. The results suggest that the dose – transmissibility relationship presents a similar profile for different *T. congolense* isolates.

Highlights

- Increased pathogenicity in mice is associated to higher transmissibility to tsetse
- At equal pathogenicity, domestic trypanosomes are better transmitted than wild ones
- Transmissibility of dominant sylvatic and domestic trypanosomes is equivalent
- Relation between trypanosome dose and transmission to tsetse has a constant profile

1 Introduction

Tsetse transmitted trypanosomosis is an important tropical disease affecting livestock (*nagana*) and humans (sleeping sickness). Tsetse flies (Diptera: Glossinidae) cyclically transmit several trypanosome species i.e. *Trypanosoma vivax*, *Trypanosoma congolense*, *Trypanosoma simiae* and *Trypanosoma brucei* (Aksoy et al., 2003) with *T. congolense* being the most pathogenic for cattle (Swallow, 2000). Animal trypanosomosis can present different clinical aspects ranging from acute and severe to chronic forms. Typically, three situations are observed in animals: (i) relative endemic stability in livestock in areas where wildlife was eliminated, (ii) relative endemic stability within the game reserves

(trypanotolerant animals) and finally (iii) severe outbreaks in livestock living in the vicinity of game reserves (Van den Bossche and Delespaux, 2011). Wild animals are both more resistant and tolerant to trypanosomes resulting in low parasite abundance in their blood and absence of clinical reactions. Yet, when the same trypanosomes are transferred to more susceptible hosts (livestock or laboratory animals), they appear much more pathogenic than trypanosomes isolated from livestock (Van den Bossche et al., 2011). Lowly pathogenic trypanosomes cycling in livestock cause much lower parasitaemia in their host. This is probably the result of the adaptation of trypanosomes to more fragile hosts. A conserved high pathogenicity would, indeed, lead to the death of the host and, consequently, of the parasite. A linear relationship was reported between the transmissibility of the parasite and the host parasitaemia at low parasite concentrations only (10^{-3} to 5 trypanosomes/µl) (Walshe et al., 2011). Moreover, for the same level of parasitaemia in mice, more pathogenic strains are more transmissible to tsetse flies (Masumu et al., 2006b). Hence the question: in a domestic environment how effective is the transmission of *T. congolense* to tsetse flies compared to trypanosomes cycling in a sylvatic environment if domestic strains present a lower pathogenicity and a lower proliferation capacity?

The aim of this study was thus to compare the transmissibility of *T. congolense* strains of high and low pathogenicity in mice isolated from wildlife and from domestic cattle and, eventually, propose hypotheses on the necessary adaptations required for the parasite to shift from one cycle to another. The receptivity of the tsetse flies used in the study was evaluated so as to confirm that the observed differences were not caused by the trypanosome infection doses in the blood meals.

2 Materials and methods

2.1 Tsetse flies

A total of 2,200 teneral male *Glossina morsitans morsitans* Westwood (aged between 15 and 28 hours post eclosion) were used to compare the transmissibility of *T. congolense* strains isolated from domestic and sylvatic cycles. Additional 1,200 flies were used to confirm the dose-transmission relationship. The flies originated from the colony of the Institute of Tropical Medicine, Antwerp, Belgium where the colony was maintained. This colony originated from pupae collected in Kariba (Zimbabwe) and Handeni (Tanzania). The rearing and maintenance of this colony was previously described by Elsen *et al* (Elsen et al., 1993).

2.2 Trypanosome strains from domestic and sylvatic cycles

Twelve trypanosome strains were isolated from a sylvatic cycle (6 from Hluhluwe game reserve, South Africa and 6 from South Luangwa National park, Zambia) whilst another ten were isolated from cattle (7 from Eastern Province and 3 from Southern Province, Zambia). The isolation of the sylvatic and the domestic strains was described by Van den Bossche *et al* (2011) and Masumu *et al.* (2006a), respectively. The *T. congolense* isolates were categorized as highly pathogenic (median survival time of infected mice < 10 days) and lowly pathogenic strains (median survival time of infected mice > 30 days) (Masumu et al., 2006b).

The IL1180 strain was also used, as well as one of the domestic and one of the sylvatic strains above, to confirm the relationship between the parasite load in blood meal and the chance to infect tsetse flies.

2.3 Mice and rabbits

Outbred OF1 (Oncins France 1) mice and Hycole rabbits were used in the experiment and maintained on water and feed pellets ad-lib. Animal ethics approval for experimental infections (mice) and feeding of tsetse flies (rabbits) was obtained from the Ethics Committee of the Institute of Tropical Medicine, Antwerp (PAR-012 & DG-008).

2.4 Experimental design

2.4.1 Comparison of the transmissibility in the sylvatic and domestic cycles

For each of the 22 isolates, two OF1 mice were used to revive and multiply trypanosomes from stabilates. The parasitaemia in mice was monitored by examining tail blood microscopically. Parasitaemia was quantified by the Uriglass® method (A. Menarini Diagnostics Srl). Briefly, parasitaemic mouse blood was diluted 1:200 in phosphate buffered saline (PBS) and transferred into 3 Uriglass® counting chambers. Counts were done under microscope (X25) in all 160 cells of each chamber. The average count of the 3 chambers was then used to estimate the trypanosome concentration in the blood. The infections were standardised so that all the flies had a blood meal containing 50 trypanosomes/µl of defibrinated horse blood, the trypanosomes being harvested 4 days after the inoculation of the mice (Akoda et al., 2008; Masumu et al., 2010). Hundred flies were then exposed to each of the 22 trypanosome isolates and allowed to feed for a minimum of 10 minutes after which they were anaesthetized using nitrogen gas and the unfed flies were discarded from the experiment. The fully engorged flies were then maintained on uninfected rabbits for the first week, after which they were maintained by feeding on defibrinated horse blood via a membrane. All the live flies were then dissected after 21 days using the method described by Lloyd and Johnson (1924). The infection status of each dissected fly, in both midgut and

mouthparts, was recorded. The percentage of procyclic (immature) infections was calculated as the proportion of flies that had a midgut infection whilst the percentage of metacyclic (mature) infections was calculated as the proportion of flies that had infections in both the midgut and mouth parts. Maturation rate was defined as the percentage of flies with a midgut infection that developed an infection in the mouth parts.

Fly batches were infected over a period of two weeks. Each infection day, one sylvatic and one domestic strains were randomly selected (independently of their pathogenicity) to infect tsetse flies. This was done to reduce the risk of bias caused by different production, feeding and maintenance conditions of the tsetse flies.

2.4.2 Effect of the parasite load of a blood meal on the transmissibility

The relationship between the trypanosome abundance in the blood meal and the chance for *G. m. morsitans* tsetse flies to be infected was determined for three *T. congolense* strains: one internationally characterised strain with medium pathogenicity (IL 1180), one lowly pathogenic domestic strain (Kapeya 357) and one lowly pathogenic sylvatic strain (MF 1). At day 7 post infection, the parasitaemia of the mice were estimated using an Uriglass® counting chamber as outlined above. The volume of a blood meal was assumed to be 20 µl and defibrinated horse blood with the following parasite concentrations was used to feed tsetse flies through feeding membranes: 0,05 - 0,25 - 0,5 - 1 - 2,5 - 5 - 25 and 50 trypanosomes/µl. Eight batches of 50 flies were fed with each parasite load of each of the 3 strains. After 10 minutes of feeding, the flies were anaesthetized and only the engorged flies were retained for the experiment. The flies were then maintained on rabbit 3 times a week. After 10 days, the flies were dissected and their mid-guts checked for the presence of trypanosomes using the protocol described by Lloyd & Johnson (1924). Metacyclic infections

in the mouth parts were not considered for fear of confounding effect caused by the maturation.

This experiment took place several months after the former. Fly batches were infected over a period of one week and were randomly allocated to the different trypanosome strains and doses to avoid systematic biases.

2.5 Statistical analysis

For the comparison of the trypanosome transmissibility in the sylvatic and domestic cycles, mixed logistic models were applied on tsetse fly procyclic and metacyclic infection binary data, using individual strains as random effects. Binary explanatory variables were the pathogenicity level (high or low), the cycle type (domestic or sylvatic) and the interaction between the two. For the sake of simplification, the interaction term was removed from the model if it was not significant ($p>0.05$). For the maturation, metacyclic infections were analysed using an identical model but the data was restricted to the flies that had a midgut infection.

A multivariate quadratic logistic regression was applied on midgut binary infection data to evaluate the effect of parasite load of blood meals on the proportion of procyclic infections in tsetse flies. Trypanosome strains were used as categorical explanatory variables whereas the logarithm and the squared logarithm of the trypanosome concentration in blood meal were used as continuous explanatory variables. The interaction terms between the strains and the logarithm of the dose and between the strains and the squared logarithm of the dose were also tested as explanatory variables and dropped if non-significant (likelihood ratio test with $p > 0.1$). The mode of the relationship between the proportion of infected flies and the trypanosome concentration in blood meals corresponded to the value of

trypanosome concentration for which the derivate equalled zero. The confidence interval of the logarithm of the corresponding number of trypanosomes was calculated using a non-linear combination of estimators in Stata.

3 Results

3.1 ResultsComparison of the transmissibility and maturation rate between cycles and pathogenicity level

A total of 1213 flies were dissected. The overall maturation rate was 93% with 202 (16.7%) flies that were found infected in the midgut (procyclic infections) and 187 of them (15.4%) that were found infected in the mouthparts (metacyclic infections). The number and proportion of flies with a procyclic and metacyclic infection are summarized in table 1 for each of the 22 *T. congolense* strains. Neither the trypanosome pathogenicity (OR = 0.37; 95% CI: 0.08 - 1.7; p = 0.21) nor the environment (OR = 0.41; 95% CI: 0.09 - 1.9; p = 0.25) had a significant effect on the maturation of trypanosome in tsetse flies.

The interaction between pathogenicity and cycle categories was neither significant on procyclic nor on metacyclic infections (odds ratio = 1.4; 95% CI: 0.36 – 5.4; p = 0.63 and odds ratio = 1.3; 95% CI: 0.38 – 4.8; p = 0.64, respectively), indicating that the pathogenicity effect was not significantly different in the domestic and the sylvatic cycles. In simplified models (without the interaction terms), both cycle and pathogenicity were significant as explanatory variables. Higher pathogenicity was correlated with higher transmissibility in both cycles (odds ratio = 0.40; 95% CI: 0.21 – 0.75; p = 0.004 and odds ratio = 0.37; 95% CI:

Table 1 Strains used in the study and their pathogenicity level

Strain	Transmission cycle	Virulence category	No. of flies dissected	No. (%) of infected flies	
				Procyclic	Metacyclic
MF1 CL1	SYLVATIC	LOW	80	7 (8.8)	6 (7.5)
MF2 CL5	SYLVATIC	HIGH	65	2 (3.1)	2 (3.1)
MF3 CL1	SYLVATIC	HIGH	54	4 (7.4)	4 (7.4)
MF4 CL8	SYLVATIC	HIGH	82	24 (29.3)	22 (26.8)
MF5 CL3	SYLVATIC	HIGH	52	11 (21.2)	10 (19.2)
MF6 CL1	SYLVATIC	HIGH	44	3 (6.8)	3 (6.8)
BT0106	SYLVATIC	HIGH	54	8 (14.8)	8 (14.8)
BT0206	SYLVATIC	HIGH	55	9 (16.4)	9 (16.4)
BT0306	SYLVATIC	HIGH	59	13 (22.0)	11 (18.6)
BT0306 ISM	SYLVATIC	HIGH	65	16 (24.6)	15 (23.1)
BT0406	SYLVATIC	HIGH	25	3 (12.0)	3 (12.0)
BT0506	SYLVATIC	HIGH	44	8 (18.2)	7 (15.9)
S1 CL1	DOMESTIC	LOW	57	9 (15.8)	9 (15.8)
S3 CL3	DOMESTIC	LOW	48	7 (14.6)	7 (14.6)
S4 CL3	DOMESTIC	LOW	55	5 (9.1)	5 (9.1)
MSORO M7 C3	DOMESTIC	LOW	52	10 (19.2)	9 (17.3)
MSORO M19 C1	DOMESTIC	LOW	41	6 (14.6)	6 (14.6)
CHIPOPELA 37 C3	DOMESTIC	LOW	55	6 (10.9)	6 (10.9)
KAPEYA 357 C2	DOMESTIC	LOW	50	3 (6.0)	3 (6.0)
ALICK 589 C1	DOMESTIC	LOW	51	10 (19.6)	6 (11.8)
ALICK 339 C6	DOMESTIC	HIGH	64	25 (39.1)	24 (37.5)
YOBO 2038 C2	DOMESTIC	HIGH	61	13 (21.3)	13 (21.3)

0.21 – 0.65; p = 0.001 for the procyclic and metacyclic infections, respectively), whereas belonging to the domestic group increased the transmissibility in the two pathogenicity categories (odds ratio = 0.48; 95% CI: 0.26 – 0.89; p = 0.019 and odds ratio = 0.45; 95% CI: 0.26 – 0.9; p = 0.005 for the procyclic and metacyclic infections, respectively). The estimated proportions of flies with a metacyclic infection (in the mouthparts) for the two pathogenicity categories in the domestic and sylvatic cycles, with their 95% confidence intervals, are shown in table 2.

Table 2 Proportions of infected flies as predicted by a mixed logistic model using pathogenicity, cycle type and the interaction between the two as explanatory variables and the strains as random effect

Cycle	Pathogenicity in mice	Maturation (95% C.I.)	Proportion of mature metacyclic infections (95% C.I.)
Sylvatic	High	92% (83 – 97%)	15% (11 – 19%)
Sylvatic	Low	86% (40 – 98%)	7% (3 -18%)
Domestic	High	97% (83 – 100%)	29% (19 – 42%)
Domestic	Low	92% (77 – 97%)	12% (9 -17%)

These results indicate that for the strains most commonly found in the field (lowly and highly pathogenic strains in mice, isolated from the domestic and sylvatic environment, respectively), the proportion of mature metacyclic infections was equivalent (OR = 0.81; 95% CI: 0.51 – 1.28; p = 0.37) in flies infected with *T. congolense* strains isolated in a domestic (12%) and a sylvatic environment (15%) (see table 2).

3.2 Parasite load and transmissibility

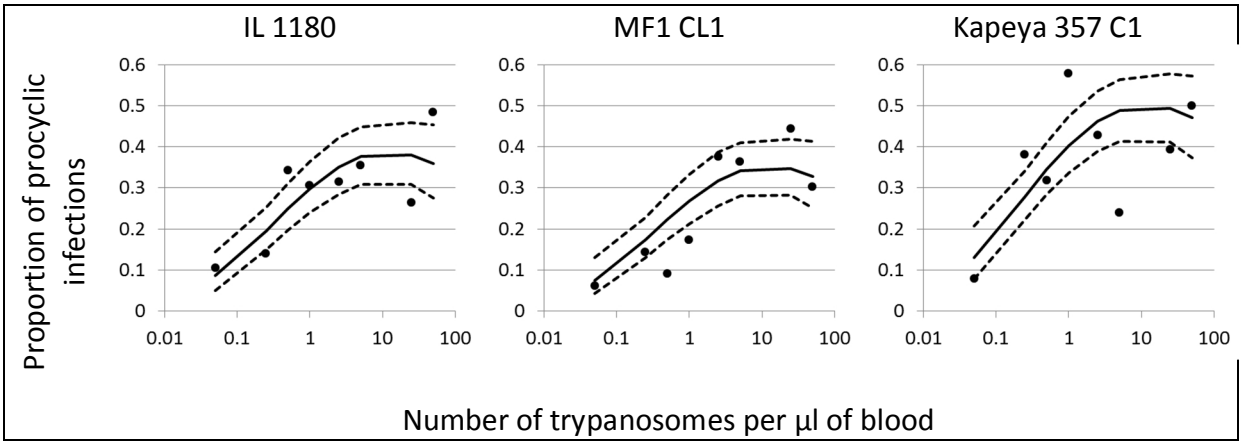

Figure 1 Relation between the parasite load of the blood meal and the infection rate in tsetse flies. Observed (dot symbols), predicted proportions (plain lines) and 95% confidence intervals (dashed lines) of procyclic infections in function of the three trypanosome strain loads in blood meals

A total of 841 flies were dissected. We observed a linear relationship between parasite load and transmissibility up to a value of about 5 trypanosomes/µl blood (+/- 100 trypanosomes per blood meal). The dose – transmissibility relationship presented a similar profile with the 3 strains since the interaction terms between the dose and the logarithm of the dose (squared or not) were not significant (likelihood ratio test: $p = 0.37$). Yet, at an equal dose, the sylvatic MF 1 strain was significantly less transmissible to tsetse flies than IL 1180 ($p = 0.015$) and the domestic Kapeya 357 strain ($p = 0.002$). The transmissibility of IL 1180 was not significantly lower than that of Kapeya 357 ($p = 0.44$). In the absence of interaction terms, the estimated maximum transmission was reached with 12,5 trypanosomes/µl blood (95% CI: 4 – 38) (+/- 250 trypanosomes per blood meal), with, on average, less than 50% of the flies infected (figure 1).

4 Discussion

The comparison of the transmissibility in the sylvatic and domestic cycles presented in this article shows that both pathogenicity and cycle types (domestic or sylvatic) of *T. congolense* had a significant effect on the transmissibility of trypanosomes to tsetse flies. We acknowledge the fact that few isolates belonging to the low pathogenicity sylvatic and high pathogenicity domestic trypanosome categories were available for testing. The mixed statistical model used accounted for possible random effects related to the different strains and, consequently, limited the risk of underestimating the standard errors. Mixed models do evaluate intra-cluster correlation of the data and reduce the risk of wrongly rejecting the null hypothesis (absence of differences) when the clusters contain high numbers of observations (Neuhaus, 1992). In spite of this, both domestic and sylvatic trypanosome strains were found significantly better transmitted if they are more pathogenic to mice, even when the number of trypanosomes present in the blood meal is standardized. This observation is in agreement with the findings of Masumu *et al.*(2006b). The parasite dose used in our study (50 trypanosomes/µl) was well above the dose we observed to cause a threshold (12.5 trypanosomes/µl), thus indicating that this difference was not due to parasite density as assumed by the simple trade-off model of Anderson and May (1982). This suggests that pathogenicity and transmissibility are genetically linked: a pathogenic trypanosome adapts better to tsetse flies than a mild one. This better adaptation must concern the procyclic form of the parasite since the maturation of trypanosomes did not vary significantly across pathogenicity and cycle types.

On the other hand, for a same level of pathogenicity in mice, domestic trypanosomes are better transmitted to tsetse flies than trypanosomes isolated from the wild. Since domestic trypanosomes are usually less pathogenic (Van den Bossche et al., 2011), dominant strains

in both transmission cycles i.e. domestic strains with low pathogenicity and sylvatic strains with high pathogenicity in mice have a similar level of transmissibility to tsetse flies. Thus, for an equivalent trypanosome density in the blood meal (parasitaemia), a similar proportion of tsetse flies would become infected when exposed to most trypanosomes of domestic or sylvatic origin.

In fact, we could hypothesize that the parasitaemia does not differ much in chronically infected hosts from the domestic and the sylvatic environments. Indeed, wildlife hosts express resistance against a trypanosome challenge which results in limiting the parasitaemia (Mulla and Rickman, 1988). In the domestic cycle, at least in Southern Africa, there is predomination of less prolific strains, thus ensuring survival of host and consequently parasite (Masumu et al., 2006a; Van den Bossche et al., 2011). These strains usually cause low parasitaemia in domestic animals to such an extent that, in the field, cattle parasitaemias often fall below microscopic detection limit (Marcotty et al., 2008). Low parasitaemia in cattle could result from a combination of low trypanosome proliferation capacity and development of immunity in domestic hosts (Masumu et al., 2009). Equivalent parasitaemia and transmissibility in domestic and sylvatic environments should result in equivalent proportions of infected flies. Yet, minor changes in the host parasitaemia might have an important impact on the transmissibility at such low parasitaemia level.

In tsetse habitat highly fragmented by agricultural activities, tsetse flies are less abundant (Van den Bossche et al., 2010; Ducheyne et al., 2009) but also more infected (personal observations from Zambia). Simukoko (2007) also reported, in Zambia, high trypanosomosis incidence in livestock despite a relatively low tsetse abundance. Some authors reported that starved and stressed tsetse flies were more receptive and more efficient vectors of the

parasite (Haines et al., 2010; Akoda et al., 2009b; Akoda et al., 2009a). It would be interesting to determine whether this is the main cause of increased transmissibility or whether the parasitaemia or intrinsic characteristics of the trypanosomes are responsible for this.

Infection rates in the second experiment were significantly higher than in the first. This is attributed to the fly breeding and maintenance conditions since the two experiments were carried out several months apart. Stressed or inappropriately fed flies are indeed known to be much more receptive to trypanosomes (Kubi et al., 2006). This variability is unlikely to have interfered with any of the two experiments because they were both completed within short periods (< 2 weeks) and the fly batches were randomly attributed to experimental groups. The relationship between the parasitaemia and the transmissibility of the trypanosomes confirmed that, at low parasitaemia, the chance for flies to be infected increases linearly with the trypanosome load of the blood meal. A plateau was observed around 12,5 trypanosomes/µl. These results are in agreement with the observations of Maudlin and Welburn (1989). The profile of the dose – transmissibility relationship was similar for the 3 strains tested, suggesting that, for a given variety of tsetse flies, the effect of the dose is the same with the different trypanosome strains. The effect of the dose on transmissibility is superimposed on that of trypanosome pathogenicity and domestication. The differences observed between the three strains tested were probably due to differences in terms of pathogenicity or adaptation to the domestic environment.

5 Conclusion

This study confirms that pathogenicity increases the chance of a trypanosome to be successfully transmitted to a tsetse fly but this relationship is only valid within the domestic

and the sylvatic environments, separately. Due to the trypanotolerant nature of wildlife highly pathogenic strains are expected to occur more in the sylvatic cycle. On the other hand, for the same level of pathogenicity, domestic trypanosomes are better transmitted to tsetse flies than trypanosomes isolated from the wild. As a result, for an equivalent trypanosome load in the blood meal, dominant trypanosomes from the sylvatic (highly pathogenic in mice) and the domestic (lowly pathogenic in mice) environments have an equivalent capacity to infect tsetse flies. Finally, the linear relationship between the parasite load in blood meals and the trypanosome transmissibility to tsetse flies at low parasitaemia and the plateau observed at higher parasitaemia presents similar profiles with trypanosomes of different origins.

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