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Review Article

Virulence in *Trypanosoma congolense* Savannah subgroup. A comparison between strains and transmission cycles

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SUMMARY

Trypanosoma congolense strains have been shown to differ in their virulence both between subgroups and within the Savannah subgroup between strains. This review revisits these findings and complements them with information on the virulence of T. congolense Savannah subgroup strains isolated from cattle (domestic transmission cycle) in different geographical areas and of strains isolated in protected areas where trypanotolerant wildlife species are the reservoir of the trypanosomes (sylvatic transmission cycle). The virulence of a total of 62 T. congolense Savannah subgroup strains (50 domestic and 12 sylvatic), determined using a standard protocol in mice, was compared. Virulence varied substantially between strains with, depending on the strain, the median survival time of infected mice varying from five to more than sixty days. The proportion of highly virulent strains (median survival time <10 days) was significantly (P = 0·005) higher in strains from the sylvatic transmission cycle. The analysis highlights repercussions of the domestication of the trypanosomiasis transmission cycle that may have to be taken in consideration in the development of trypanosomiasis control strategies.

Keywords *livestock,* Trypanosoma*, virulence, wildlife*

INTRODUCTION

Human and animal tsetse-transmitted trypanosomiases are important diseases affecting people and livestock in extensive areas of sub-Saharan Africa. Human African trypanosomiasis is caused by infections with *Trypanosoma brucei gambiense* or *T. b. rhodesiense*. Infections with *T. b. gambiense* usually give rise to a chronic form of human sleeping sickness in West and Central Africa that may persist for several years, whereas *T. b. rhodesiense* usually causes an acute infection in East Africa (1). Nevertheless, a diversity of clinical evolutions from asymptomatic to acute forms has been described in *T. b. gambiense* infections. Similarly, in *T. b. rhodesiense*, the disease has a rather chronic character in southern countries such as Malawi and Zambia (1) but can also present an acute profile with rapid progression to the late stage as in Uganda (2). *Trypanosoma vivax* is a pathogen of livestock in Africa and in South America. It is transmitted cyclically by tsetse flies and mechanically by biting flies. Differences in virulence are recognized between East and West African *T. vivax* strains, the West African strains being generally regarded as more pathogenic to cattle (3). Nevertheless, there are also reports of a severe haemorrhagic disease caused by *T. vivax* in East Africa (4). In South America, most *T. vivax* infections are chronic and asymptomatic, with rare outbreaks of severe disease (5). The salivarian trypanosomes belonging to the subgenus *Nannomonas* (*T. congolense* and *T. simiae*) are major pathogens of livestock in sub-Saharan Africa. Contrary to the *T. brucei* group, *T. congolense* has been much less studied. Currently, two major clades are distinguished within the *Nannomonas* subgenus with one containing the *T. congolense*: Savannah, Forest and Kilifi subgroups and the other containing *T. simiae*, *T. godfreyi* and *T. simiae* Tsavo (6). Limited experiments, comparing the virulence of one strain of each subgroup in mice and cattle, have shown differences between the subgroups with the *T. congolense* strain of the Savannah subgroup being the most virulent (7,8). However, experiments conducted by Masumu *et al.* (9) have shown substantial variations in the virulence of

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T. congolense strain belonging to the Savannah subgroup. These findings were based on *T. congolense* stains isolated from susceptible livestock species (i.e. the domestic transmission cycle) and may not represent the natural trypanosome population as it is present in trypanotolerant wildlife (i.e. the sylvatic transmission cycle). This paper reviews the virulence profiles of *T. congolense* Savannah subgroup strains isolated from livestock and compares their virulence with the virulence of strains circulating in wildlife.

MATERIALS AND METHODS

Considering the dynamic nature of the epidemiology of tsetse-transmitted trypanosomiasis (10), trypanosome strains were isolated in a livestock production area where susceptible cattle constitute the main reservoir of trypanosomes (domestic transmission cycle) and in protected areas where trypanotolerant wildlife is the reservoir of trypanosomes and tsetse flies do not have contact with livestock (sylvatic transmission cycle).

Isolation of trypanosomes belonging to the domestic transmission cycle

A total of 45 *Trypanosoma congolense* strains were isolated from communal cattle (Ngoni breed) reared in a trypanosomiasis endemic area located in the Katete and Mambwe Districts of the plateau areas of eastern Zambia (9). The area is highly cultivated with a cattle population of approximately 8–10 animals/km^2. Cattle constitute the main host of the tsetse flies and are the main reservoir of trypanosomes (11). Large game animals are absent.

Another five *T. congolense* strains were also isolated from communal cattle (Ngoni breed) kept in the Siyavonga District in the Southern Province of Zambia. The area is separated from the tsetse-infested wildlife area between Chirundu and Kariba in Zimbabwe by the Zambezi River.

In both areas, cattle infected with *T. congolense* were identified using parasitological diagnostic tests (12). For each infected bovine, a volume of 0·3 mL of the infected blood was injected intraperitoneally (IP) into each of two OF1 mice. The injected mice were monitored for development of parasitaemia, with each positive mouse considered as an isolate. Parasitaemic mice were euthanized and the blood collected for stabilate production.

Isolation of trypanosomes belonging to the sylvatic transmission cycle

Six *T. congolense* strains were isolated from tsetse flies in the South Luangwa National Park in Zambia. The South Luangwa National Park is a protected game area where wildlife acts as reservoirs of the trypanosomes. Tsetse flies (*Glossina morsitans morsitans* and *G. pallidipes*) were trapped using epsilon traps (13), and live flies were dissected to determine their infection status. The mouthparts of tsetse flies, infected with trypanosomes in both the midgut and mouthparts, were injected intraperitoneally (I.P.) into an immunosuppressed OF1 mouse (300 mg/kg Cyclophosphamide; Endoxan®, Baxter SA, Lessines, Belgium). The injected mice were then monitored for the development of parasitaemia, with each positive mouse considered as an isolate. Parasitaemic mice were euthanized and the blood collected for stabilate production.

Finally, six *T. congolense* strains were isolated from buffalos belonging to herds that were selected randomly for tuberculosis testing in the Hluhluwe-iMmfolozi Park located in the KwaZulu-Natal Province of South Africa. From each of the 132 buffalo sampled, a volume of 0·3 mL of jugular blood was injected IP into each of two OF1 mice. The injected mice were then monitored as described previously, and stabilates were prepared from the blood of positive mice.

Virulence testing

The virulence of the *T. congolense* isolates, all belonging to the Savannah subgroup (14), was determined using a standard protocol in OF1 mice (9). All strains were at their fifth or sixth passages in mice. Before infection, each of the strains was expanded into two OF1 mice. Wet tail-blood films of the infected mice were examined microscopically at 2-day intervals to estimate the parasitaemia (15). When the parasitaemia reached between 10^7 and 10^8 trypanosomes/mL, tail-blood was collected and diluted with Phosphate buffer Saline Glucose (PSG) to achieve a concentration of 10^5 parasites in a total volume of 0·2 mL. This volume was injected I.P. in six OF1 mice for each strain. A group of six mice, injected I.P. with 0·2 mL of PSG, was used as control.

For each strain, the prepatent period (number of days between the inoculation and the first appearance of parasites in the blood) and the survival time were recorded up to 60 days post-infection. Mortality in infected and control mice was recorded daily. An animal was considered parasitologically negative when no trypanosomes were detected in at least 50 microscopic fields. Animal ethics approval for the experimental infections was obtained from the Ethics Commission of the Institute of Tropical Medicine, Antwerp, Belgium (Refs DG001-PD- M-TTT and DG008-PD-M-TTT).

Statistical analysis

The median mice survival time of the infected mice was estimated in parametric survival models using a log-normal

hazard distribution in Stata 10. The strains for which none of the infected mice died during an observation period >60 days were discarded from the analysis. In a first model, the strains were used as discrete explanatory variables. In a second model, transmission cycle type (domestic or sylvatic) was used as explanatory variable. Data clustering in relation to the different isolates was taken into account using the frailty option (shared for strains).

Strains were subsequently allocated to three virulence classes according to their estimated median survival time (<10 days, 10–50 days and >50 days). Strains for which none of the infected mice died during an observation period of more than 60 days were allocated to the last class. An ordered multinomial regression was applied on the data using the cycle type as explanatory variable.

RESULTS

The virulence of a total of 62 *T. congolense* strains was tested and compared. Median survival time of infected mice differed substantially between strains with mice infected with the most virulent strains having a median survival time of <5 days and mice infected with the least virulent strains surviving for more than 50 days. An overview of the median survival time (95% C.I.) of mice infected with 60 of the 62 strains (survival time could not be calculated for two strains because survival was more than 60 days) is presented in Figure 1.

Based on the distinction made by Masumu *et al.* (9), strains were grouped into a high virulence (median survival time <10 days), a medium virulence (median survival time between 10 and 50 days) and a low virulence (median survival time between >50 days) category. Of the strains isolated in the sylvatic transmission cycle, 50% were extremely virulent compared to 16% for strains isolated in the domestic transmission cycle (Table 1). The difference was statistically significant ($P = 0{\cdot}005$). Among the six extremely virulent strains from the sylvatic cycle, two were sampled from the tsetse flies and four from the buffaloes. The median survival time of mice infected with strains isolated in the sylvatic transmission cycle was 7·9 (C.I. 6·9–9·0) compared to 11·1 (C.I. 9·9–12·4) for those from the domestic transmission cycle ($P < 0{\cdot}001$).

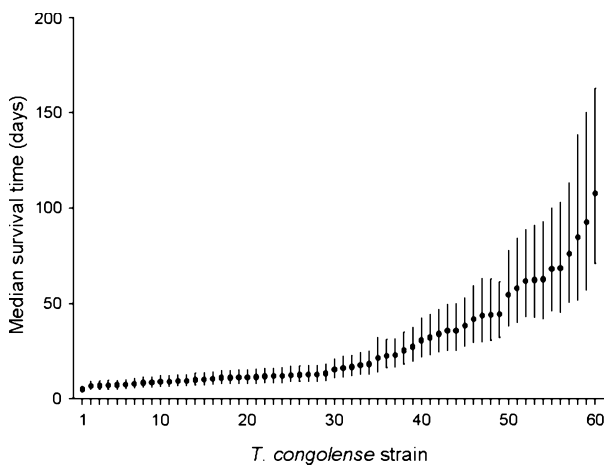

Figure 1 Median survival time (with 95% C.I.) of mice infected with one of the *Trypanosoma congolense* (savannah subgroup) strains isolated in Zambia and South Africa.

Table 1 Number of *Trypanosoma congolense* strains (savannah subgroup), isolated in the domestic or sylvatic transmission cycle, belonging to the low, medium and high virulence category

	Number of strains per virulence category		
Transmission cycle	Low	Medium	High
Domestic	22	20	8
Sylvatic	1	5	6

DISCUSSION

The comparison of the virulence of the 62 *T. congolense* strains belonging to the Savannah subgroup confirms the observation made by Masumu *et al.* (9) that virulence greatly differs from strain to strain. As experiments performed by Bengaly *et al.* (7,8) have shown concordance between virulence tests in mice and results of the same tests in cattle, our findings can be extrapolated to a field situation. Moreover, based on the limited number of strains from four geographical areas, the outcome of the analysis shows that virulent strains are not distributed evenly over the transmission cycles but that the proportion of highly virulent strains is significantly higher in the sylvatic transmission cycle. This may indicate that the evolution of trypanotolerance in wildlife has acted as an important selective pressure on trypanosomes by selecting for higher parasite replication rates to maximize the production of transmission forms and, at the same time, increasing the virulence of the strains in a susceptible host (16). The persistence of a relatively small proportion of strains with low virulence in the sylvatic cycle could be explained by variations in the susceptibility to trypanosomal infections in game animals with some species being more susceptible than others (17). The predominance of virulent trypanosome strains in wildlife may be the reason why livestock trypanosomiasis epidemics with high morbidity and high mortality are usually encountered when livestock is introduced in wildlife areas or when livestock is kept at a game/livestock interface and is thus exposed to tsetse flies transmitting highly virulent strains picked

from wild animals. For example, the restocking of cattle into tsetse-infested areas of northern, central and southern Mozambique after the civil war resulted in serious problems with livestock trypanosomiasis (18). Similarly, the introduction of livestock in the tsetse-infested zones of the Rift Valley in Ethiopia has resulted in important trypanosomiasis outbreaks with high mortality in the livestock population (19). Finally, the bovine trypanosomiasis epidemics in South Africa are all closely linked to the game/livestock interface of the Hluhluwe-iMmfolozi Game Park (20,21). Although the proportion of wild areas has reduced substantially over the past century, the current drive for reforestation, and the development and establishment of large trans-boundary conservation areas could result in the creation of important reservoirs of tsetse flies that can transmit trypanosomes from the wildlife to susceptible livestock and create trypanosomiasis epidemics in domestic animals at the interface. The management of the disease at such interfaces may require special attention and may be one of the major future challenges in the control of livestock trypanosomiasis.

Considering the threat posed by many of the trypanosome strains present in the trypanotolerant reservoirs, domestication of the transmission cycle seems to have considerable repercussions for the composition of the trypanosome population and its subsequent impact on livestock health. For each host–parasite interaction, there probably is an optimal level of host utilization that maximizes the balance between rapid transmission and the time before the host dies or is treated (22). This trade-off between virulence and replication is an example of how parasite fitness is influenced by the costs and benefits of host exploitation (23). A higher replication rate of a particular strain will allow for a more rapid dissemination of the alleles of this genotype compared to strains replicating slower. The relative fitness of those highly replicating strains will thus be higher as they will leave more alleles in the next generation of parasites relative to its competitor(s) (24). Inversely, a highly pathogenic strain may by killing the host decrease its spreading compared to its less pathogenic competitor(s), resulting thus in a lower relative fitness. Because susceptible hosts infected with virulent trypanosome strains will either be treated because of the acute illness (25) or die, virulent trypanosome strains are expected to have a low fitness in the domestic transmission cycle. These curative treatments or death will favour a selection against virulent strains and may result in a fast decrease in the proportion of virulent stains circulating in the livestock population. This explains the observed lower proportion of virulent strains in the domestic transmission cycle. Because infection with a low virulent strain protects animals against the adverse effects of a subsequent infection with a virulent strain, a number of virulent strains can persist in the susceptible livestock population (26). In conclusion, it thus seems that the observed variations in virulence in *T. congolense* strains belonging to the Savannah subgroup are largely the consequence of differences in the susceptibility of hosts to trypanosomal infections and the domestication of the transmission cycle. Further research is required to investigate how these variations can be exploited in the development of trypanosomiasis control strategies.

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