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Recirculation of *Trypanosoma brucei brucei* in cattle after *T. congolense* challenge by tsetse flies

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Abstract

The effect of challenging cattle, chronically infected with *Trypanosoma brucei brucei*, with *T. congolense* on the development of the *T. b. brucei* infection was investigated. For this purpose, nine experimental animals were first infected with *T. b. brucei* through the bites of infected tsetse flies. Once the *T. b. brucei* had developed into a chronic infection, that was difficult to detect using routine parasitological diagnostic tools, seven of the experimental animals were challenged by tsetse flies infected with *T. congolense*. Two of the animals infected with *T. b. brucei* were kept as control. The infection with *T. congolense* resulted in a sudden increase in the parasitaemia of *T. b. brucei*. In the *T. b. brucei* control animals, on the other hand, the parasitaemia remained below the level of detection. The epidemiological repercussions of this increase in the parasitaemia of *T. b. brucei* after infection with *T. congolense* are discussed.

Keywords: *Trypanosoma brucei*; Cattle; *Trypanosoma congolense*

1. Introduction

The development and pathogenicity of trypanosome infections in cattle differ greatly between trypanosome species. Infections with *Trypanosoma congolense* and *T. vivax* may result in acute trypanosomosis with significant impact on the health of the infected animals. Infections with *T. brucei*, on the other hand, have been described as being chronic and subpatent with minimal impact on the health of the infected cattle (Killick-Kendrick, 1971). It is thus not surprising that cattle may act as important reservoirs of human pathogenic *T. brucei* species and can play an important role in the epidemiology of human sleeping sickness (Fèvre et al., 2001). The importance of a bovine carrier in the epidemiology of *T.*

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brucei trypanosomosis will be determined to a large extent by the behaviour of the parasite. Of particular interest is the behaviour of the *T. brucei* when such reservoirs are challenged and become infected with other trypanosome species such as *T. congolense*. This is likely to occur frequently in the field considering the high infection rate of tsetse flies with *T. congolense* compared to *T. brucei* (Woolhouse et al., 1994). To investigate the effect of such challenge on a chronic *T. brucei* infection in cattle an experiment was conducted.

2. Materials and methods

2.1. Experimental animals

A total of nine steers (Belgian breed), between 6 and 12 months old, were used in the experiment. They were housed at the experimental stable of the Veterinary Department of the Institute of Tropical Medicine (Antwerp, Belgium). They were fed grass hay supplemented with a concentrate. Water was supplied ad libitum.

2.2. Trypanosomes

T. brucei brucei stock EATRO 1125 isolated from a bushbuck in Uganda (Van Meirvenne et al., 1975) and a *T. congolense* stock TRT 15 (savannah-type) isolated from a cow in eastern Zambia were used (Van den Bossche, pers. commun.).

2.3. Infection of cattle

All experimental animals were infected through the bites of infected tsetse flies. Teneral male *Glossina morsitans morsitans* (less than 32 h old), from the colony maintained at the Institute of Tropical Medicine, were used in the experiments. The origin of this tsetse fly colony and the rearing technique were described by Elsen et al. (1993). Tsetse flies were given their first bloodmeal on anaesthetised mice, either infected with *T. b. brucei* or *T. congolense*, with a parasitaemia of at least $10^{8.4}$ trypanosomes/ml of blood. After the infective bloodmeal, only fully engorged flies were retained and kept in cages of approximately 40 flies each for a period of 30 days.

In a first phase of the experiment, nine experimental animals were infected with *T. b. brucei* by allowing one cage of infected flies to feed on the flank of each animal. Sixty days after the animals infected with *T. b. brucei* became parasitaemic, seven were challenged with tsetse infected with *T. congolense*. The remaining two animals were kept as (*T. brucei*) controls.

At the end of the experiment, all experimental animals were treated with diminazene aceturate (Berenil®, Hoechst) at 7 mg/kg body weight.

2.4. Parasitological follow-up

Three times per week jugular blood was collected in EDTA-coated vacutainer tubes. The packed cell volume was measured as an estimation of the level of anaemia by the capillary microhaematocrit method. The buffy coat and Giemsa-stained thin smear were used as

parasitological diagnostic tests (Paris et al., 1982). Rectal temperature was taken before blood sampling.

Relative changes in the parasitaemia of *T. b. brucei* were determined by counting the number of parasites observed on the Giemsa-stained thin smear. A maximum of 250 microscopic fields were examined per smear using a 40× objective lens. The animals were considered aparasitaemic when no trypanosomes could be detected in the 250 fields.

2.5. Statistical analyses

Statistical analyses were carried out in Stata 7 (Statcorp., 2001).

3. Results

All the experimental animals became infected with *T. b. brucei* after a single challenge with tsetse flies. Similarly, all animals infected with *T. b. brucei* and challenged once by tsetse flies infected with *T. congolense* developed a mixed *T. b. brucei*/*T. congolense* infection.

3.1. Temperature and PCV

In seven of the experimental animals challenged by tsetse flies infected with *T. b. brucei*, body temperature started to increase about 4 days after challenge. It reached a peak (on average $40.03 \pm 0.39\,^{\circ}C$) 3 days later. Rectal temperature remained high for a period of about 14 days post-infection (Fig. 1). Challenge with *T. congolense* resulted in a slight increase in rectal temperature. The peak temperature developed during the initial *T. b. brucei* infection was significantly higher ($P > 0.001$) than the peak temperature reached after challenge with *T. congolense* (on average $39.2 \pm 0.40\,^{\circ}C$).

The *T. b. brucei* infection resulted in a steep decline in the average PCV (Fig. 1) from 36.6% before the infection to 26.6% 22 days later. It stabilised but remained low afterwards. The infection with *T. congolense* resulted again in a steep decline in average PCV reaching an average of 18.5% on the day of treatment.

3.2. Parasitaemia of T. b. brucei

The development of fever coincided with the first observation of *T. b. brucei* in the buffy coat on day 7 post-infection. Most of the blood samples remained positive on buffy coat for about 35 days (Fig. 2). From day 45 post-infection onwards, the proportion of buffy coat positive animals started to decline. However, challenge with *T. congolense* resulted in a sudden increase in the *T. b. brucei* parasitaemia in all seven animals. In the two experimental animals that were not challenged with *T. congolense*, the buffy coat remained negative throughout (Fig. 2).

The observations made using the buffy coat were confirmed by the estimates of the parasitaemia of *T. b. brucei*. In the majority of the experimental animals, it took about 14 days before the first trypanosomes were detected on the Giemsa-stained thin smears. The *T. b. brucei* parasitaemia fluctuated with a first peak reached around day 16, and a second peak

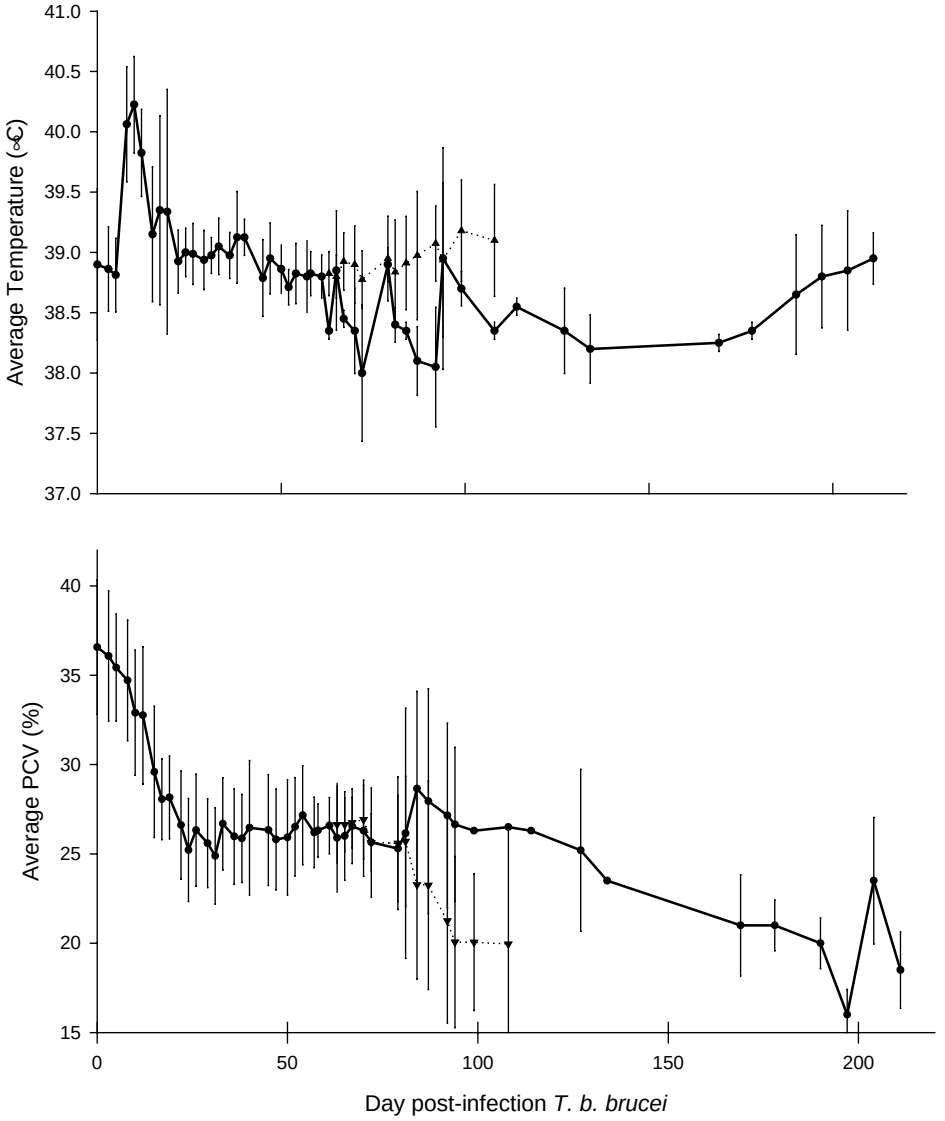

Fig. 1. Average rectal temperature and packed cell volume of experimental animals infected with *T. b. brucei* (solid line) and mixed *T. b. brucei*/*T. congolense* infections (dotted line).

around day 24 (Fig. 3). Afterwards, the parasitaemia declined and remained low. However, in all experimental animals the challenge with *T. congolense* resulted in a resurgence of the *T. b. brucei* parasitaemia to levels comparable to those observed during the acute phase of infection. In the *T. b. brucei* control animals, trypanosomes could not be detected on the thin smear. However, a proportion of the teneral tsetse flies that were fed on the control animals during this period developed metacyclic infections in their salivary glands.

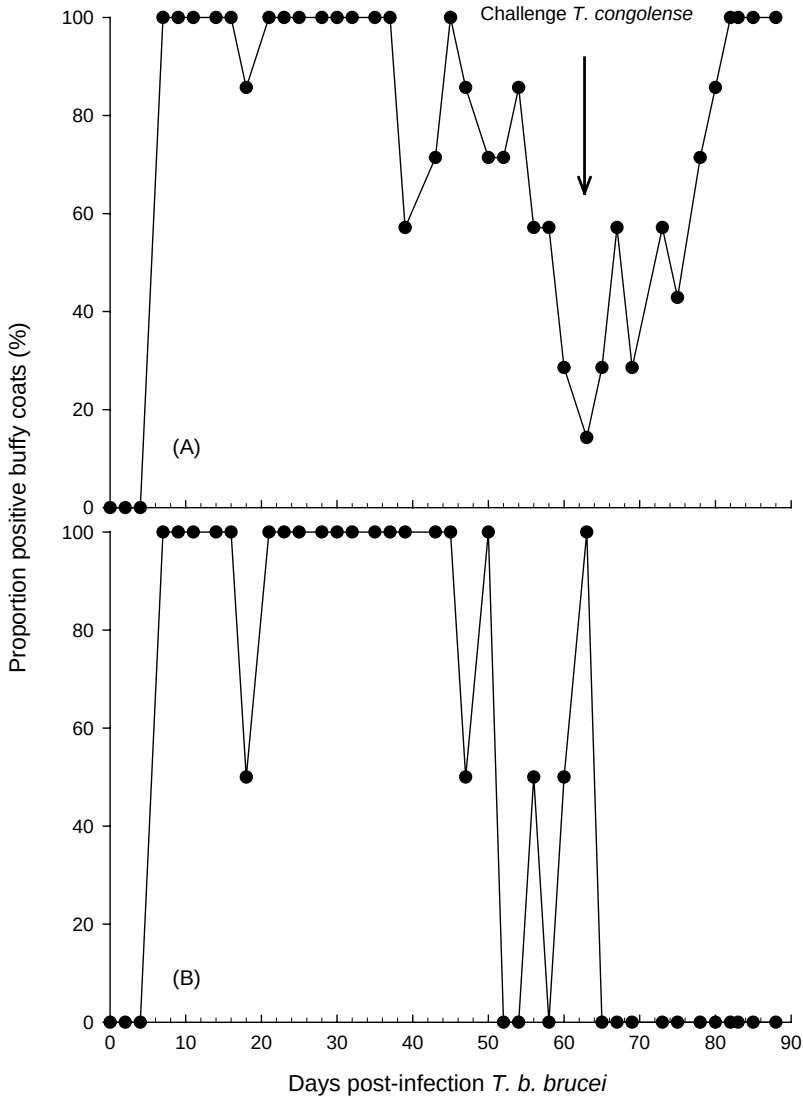

Fig. 2. Proportion of experimental animals with trypanosomes in the buffy coat in the animals infected with *T. b. brucei* and challenged with *T. congolense* (A) and the *T. b. brucei* control group (B).

4. Discussion

Results from our experiments confirm observations made by other researchers that *T. b. brucei* in cattle develops into a chronic infection. Only the initial phase of the infection, when the parasitaemia is still high, is characterised by a significant increase in body temperature followed by a substantial decrease of the PCV. In the chronic phase, parasitaemias are low

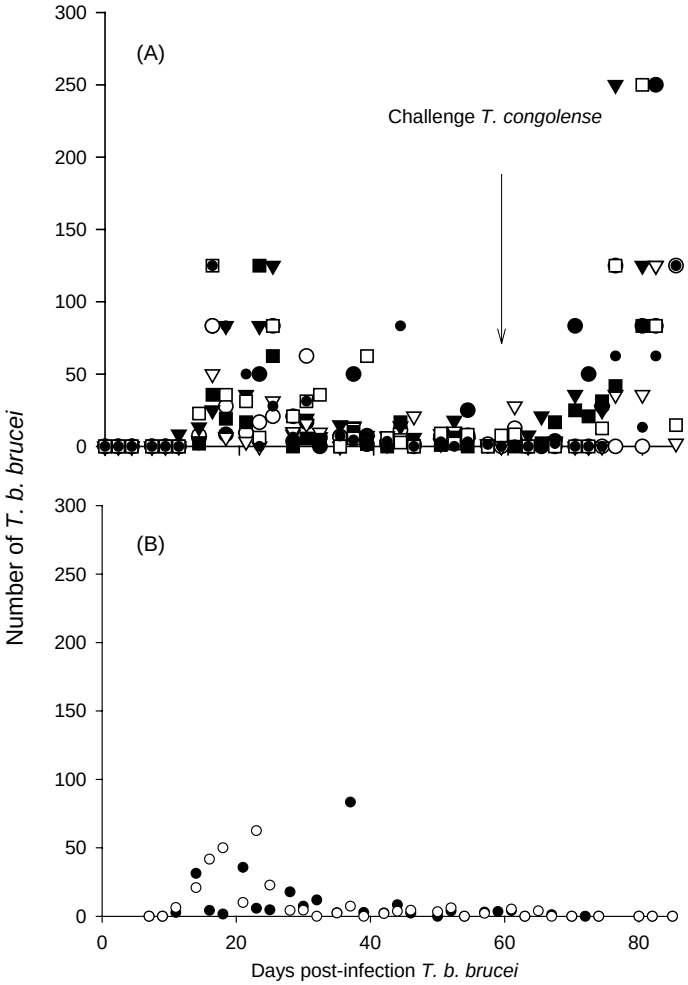

Fig. 3. Estimates of the parasitaemia of *T. b. brucei* on a Giemsa-stained thin smear in the seven experimental animals challenged with *T. congolense* (A) and in the two *T. b. brucei* controls (B).

and parasites become difficult to detect using routine parasitological diagnostic tools. It is, therefore, not surprising that the parasitological prevalence of *T. brucei* infections in cattle is usually low (Picozzi et al., 2002). Challenge with *T. congolense*, however, results in a steep increase of the parasitaemia of *T. b. brucei*. Reasons for this sudden increase in the parasitaemia of *T. b. brucei* after challenge with *T. congolense* remains as yet unexplained. This phenomenon will be subject to further investigations. Especially its impact on the epidemiology of human sleeping sickness is of significant importance. Indeed, it is probable that during the chronic phase of a *T. brucei* infection the level of parasitaemia is frequently below the threshold required to infect tsetse flies (Maudlin and Welburn, 1989). Under such circumstances transmission rate will be low. Any factor that increases the parasitaemia up to

a level above the threshold is likely to have a significant effect on the infection rate of tsetse and thus the epidemiology of *T. brucei*. This may be the case when an animal, chronically infected with *T. brucei*, is challenged with *T. congolense*. Experiments are being conducted to compare the transmission rate of *T. b. brucei* during the acute and chronic stage of infection and during the phase of increased parasitaemia after challenge with *T. congolense*.

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