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Transmissibility of *Trypanosoma brucei* during its development in cattle

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Summary Recent outbreaks of *Trypanosoma brucei rhodesiense* sleeping sickness in Soroti District of eastern Uganda have demonstrated the important role cattle can play as reservoirs of this parasite. To clarify the epidemiological importance of the cattle reservoir, experiments were conducted to determine the ease with which *T. brucei* is transmitted during the course of its development in Friesian cattle. The development of *T. brucei* in cattle is characterized by an acute phase with high levels of parasitaemia and a decline in PCV. The acute phase is followed by a chronic phase during which the PCV remains low but stable and the parasitaemia is low. Parasites are often difficult to detect using parasitological diagnostic tools during this chronic phase. Challenge of chronically infected cattle with *T. congolense* results in a sudden increase in the *T. brucei* parasitaemia. Despite significant differences in parasitaemia, the proportion of tsetse flies that developed metacyclic infections after a first bloodmeal on the infected cattle did not differ significantly between the acute and chronic phases or the phase of mixed *T. b. brucei*/*T. congolense* infection. This suggests that, throughout the observation period, the parasitaemia was above the threshold above which infection rates of tsetse are independent of the parasitaemia. The repercussions of the research findings for the understanding of the epidemiology, spread and the control of *T. b. rhodesiense* sleeping sickness are discussed.

keywords trypanosomiasis, cattle, sleeping sickness, *Trypanosoma brucei*, reservoir, transmission

Introduction

Bovine trypanosomiasis is a serious constraint to livestock development in large parts of sub-Saharan Africa. Infections with pathogenic trypanosome species affect various aspects of cattle productivity, often resulting in death (Swallow 2000). Impact of trypanosomes of the Trypanozoon subgroup on cattle is less straightforward. Usually, an infection in indigenous breeds of cattle with a *Trypanosoma brucei* sp. becomes chronic with limited impact on production (e.g. Wilde & French 1945; Doko *et al.* 1997a), but progressive inanition and death after a few months have been observed in some breeds of cattle (Wellde *et al.* 1989; Doko *et al.* 1997b). Because of the chronic nature of the disease, cattle can act as reservoirs of those trypanosome species and play a role in the epidemiology of sleeping sickness. Especially for *T. b. rhodesiense* sleeping sickness, the potential role of cattle in its epidemiology was demonstrated recently in Soroti District of eastern Uganda, where an outbreak was attributed to cattle infected with this parasite (Fèvre *et al.* 2001). The epidemiological importance of a *T. brucei* reservoir in cattle depends on the ease with which tsetse become infected when feeding on such infected cattle and subsequently transmit the parasite to humans. Previous experiments have shown that the parasitaemia of *T. brucei* in cattle varies substantially according to the phase of infection (Van den Bossche *et al.* 2004b). It is usually high in the acute phase and low in the chronic phase yet it increases substantially when chronically infected cattle are challenged with *T. congolense*. The effect of those changes in the parasitaemia on the *T. brucei* transmission rate is not known. To determine the transmissibility of *T. brucei* during the course of its development in cattle experiments were conducted whereby, as a precautionary measure, *T. b. brucei* rather than the human infective *T. b. rhodesiense* was used. Although both species are closely related, this infectivity to humans is linked to reduced transmissibility in tsetse (Welburn *et al.* 1995).

Material and methods

Experimental animals

Eight susceptible Friesian steers of Belgian origin of approximately 6 months age were used in the experiment.

They were housed in a fly-proof stable, dewormed and fed grass hay supplemented with a concentrate. Water was supplied *ad libitum*.

Trypanosomes

Trypanosoma b. brucei stock EATRO 1125 isolated from a bushbuck in Uganda (Van Meirvenne *et al.* 1975) was used to infect the cattle. *Trypanosoma congolense* stock IL 1180 originating from the Serengeti Region in Tanzania (Geigy & Kauffmann 1973) was used in the first *T. congolense* challenge. In the subsequent challenge use was made of *T. congolense* TRT 15 (Savannah-type) a clone of an isolate from cattle in Katete District, eastern Zambia.

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 colony and the rearing technique were described by Elsen *et al.* (1993). This line of tsetse has a high vectorial capacity (Van den Abbeele 2001).

Experimental design

Tsetse flies used to infect the experimental animals were given their first bloodmeal on anaesthetized mice, infected with either *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.

To mimic such a field situation in which most cattle are likely to have been subjected to previous trypanosomal infections with more common trypanosome species such as *T. congolense* before being infected with *T. brucei*, all eight experimental animals were in the first phase of the experiment infected with *T. congolense* IL1180. Twenty-six days later, when parasitaemia was high and the PCV below 18%, animals were treated with diminazene aceturate (Berenil®, Hoechst) at a dosage of 3.5 mg/kg. About 30 days after treatment, when the packed cell volume (PCV) had again reached a normal value, all animals were challenged with tsetse infected with *T. b. brucei*. Three months later, experimental animals where again challenged with tsetse infected with *T. congolense* TRT15 to allow the development of a mixed *T. brucei*/*T. congolense* infection. At the end of the experiment, all experimental animals were treated with diminazene aceturate at 7 mg/kg body weight. The experimental setup is summarized in Figure 1.

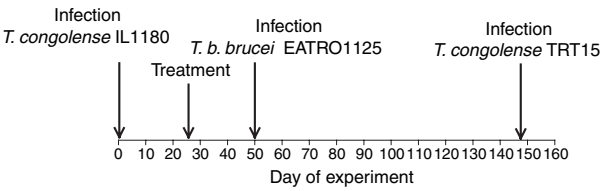

Figure 1 Chronological representation of the infections and treatments of the experimental animals.

Throughout the *T. b. brucei* infection (single and mixed), batches of 40 teneral tsetse were at weekly intervals offered a single bloodmeal on the flanks of one of four of the eight experimental animals. Only engorged flies were retained and maintained on uninfected rabbits. They were fed three times a week for a period of 30 days. To avoid cyclical transmission, the rabbits used for feeding were replaced at weekly intervals. Thirty days after the infected bloodmeal, all tsetse were dissected using the method described by Lloyd and Johnson (1924) and their infection status was determined.

Parasitological follow-up

Three times per week, ear vein blood was collected into heparinized capillary tubes from each of the experimental animals. The PCV 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. An animal with a rectal temperature above 39 °C was considered to have fever.

Relative changes in the parasitaemia of *T. b. brucei* were determined by counting the number of parasites observed in a total of 250 microscopic fields on the Giemsa-stained thin smear at 10×40 magnification. The animals were considered aparasitaemic when no trypanosomes could be detected in the 250 fields.

Statistical analyses

The overall infection rates were calculated as the intrinsic vectorial capacity (IVC) (Le Ray 1989). It was calculated as follows:

$$\mathrm{IVC} = p \times m,$$

where p is the proportion of flies which established a procyclic infection in the midgut, and m the proportion of procyclic infections that developed into a metacyclic infection in the salivary glands.

Statistical analyses were carried out in Stata 8 (Statcorp 2001). The parasitaemia was analysed using a cross-sectional Poisson regression in function of the phase (acute, chronic or mixed) and individual animals as random effect. The acute phase was the period after infection when high parasitaemias and fever were observed. The acute phase is followed by a chronic phase during which parasitaemia declines and fever was absent. The mixed phase was the phase of mixed *T. brucei* and *T. congolense* infection. Similarly, the proportions of flies infected in the midgut and in the salivary glands were analysed using cross-sectional logistic regressions in function of the pahses and, within a phase, day post-infection.

Results

Packed cell volume and rectal temperature

All animals developed trypanosome infections after challenge by the tsetse infected with either *T. congolense* or *T. b. brucei*. Those infections resulted in a significant decline in the PCV (Figure 2) in all animals with a more pronounced decline in PCV in *T. congolense* compared with *T. b. brucei* infections. *Trypanosoma b. brucei* infections resulted in a decline in the PCV of about 10% within 20 days after infection followed by a plateau of a low but stable PCV (between 24 and 26%) (Figure 2). Infections with *T. congolense*, on the other hand, caused a continuous decline in the PCV until animals were treated with diminazene aceturate (Figure 2). This drug treatment resulted in a fast recovery of the PCV.

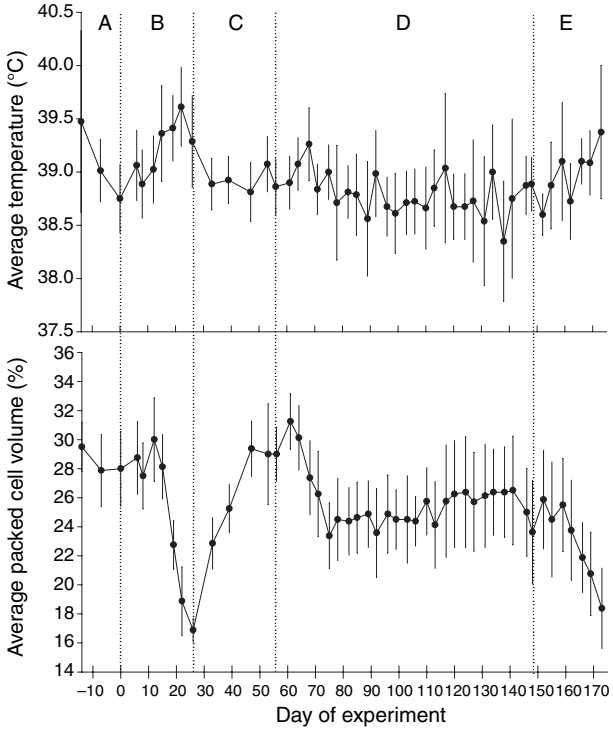

Figure 2 Average temperature and packed cell volume (±SD) of the eight experimental animals before infection (A), during the first *T. congolense* infection (B), after treatment with diminazene aceturate (C), after challenge with *T. b. brucei* (D) and during the mixed *T. b. brucei*/*T. congolense* infection (E).

The experimental animals developed fever throughout the *T. congolense* infection and during the early phase of the *T. brucei* infection (Figure 2). In the course of the subsequent chronic *T. b. brucei* infection rectal temperature was normal.

Parasitaemia of *T. b. brucei*

After challenge by tsetse infected with *T. b. brucei*, it took on average 11 days before the first parasites were detected in the peripheral blood. In most of those experimental animals an acute and chronic phase could be distinguished in the course of the *T. b. brucei* parasitaemia (Figure 3). The parasitaemia was high, on average 14.9 parasites (95% CI 9.5–23.4) per 250 microscopic fields for a period of 50 days following the prepatent period (acute phase of infection). During the chronic phase, about 60 days after challenge with *T. b. brucei*, the parasitaemia decreased substantially to an average of 4.5 parasites (95% CI 2.8–7.2). Challenge with *T. congolense* during the chronic phase resulted in a steep increase in the *T. b. brucei* parasitaemia [on average 40.4 parasites (95% CI 25.3–64.7)]. The observed differences in the parasitaemias were statistically significant ($P < 0.0001$). During the acute phase of infection, 7.8% (5/64 samples) of the blood samples collected from the experimental animals was parasitologically negative. In the chronic phase of the *T. b. brucei* infection, parasites could not be detected in 27.7% (12/44 samples) of the blood samples. Finally, during the mixed *T. b. brucei*/*T. congolense* infection 12.5% of the samples (2/16 samples) were parasitologically negative.

Trypanosoma b. brucei infections in *G. m. morsitans*

A total of 100 batches of teneral tsetse flies were offered their first bloodmeal on one of four experimental animals on various days of the acute and chronic or mixed (*T. b. brucei*/*T. congolense*) phase of the *T. b. brucei* infection. The IVC for each batch infected in the course of the *T. b. brucei* infection are presented in Figure 4. In total 1193 flies were dissected to determine their infection status (Table 1). A total of 348 flies (29.2%) developed a

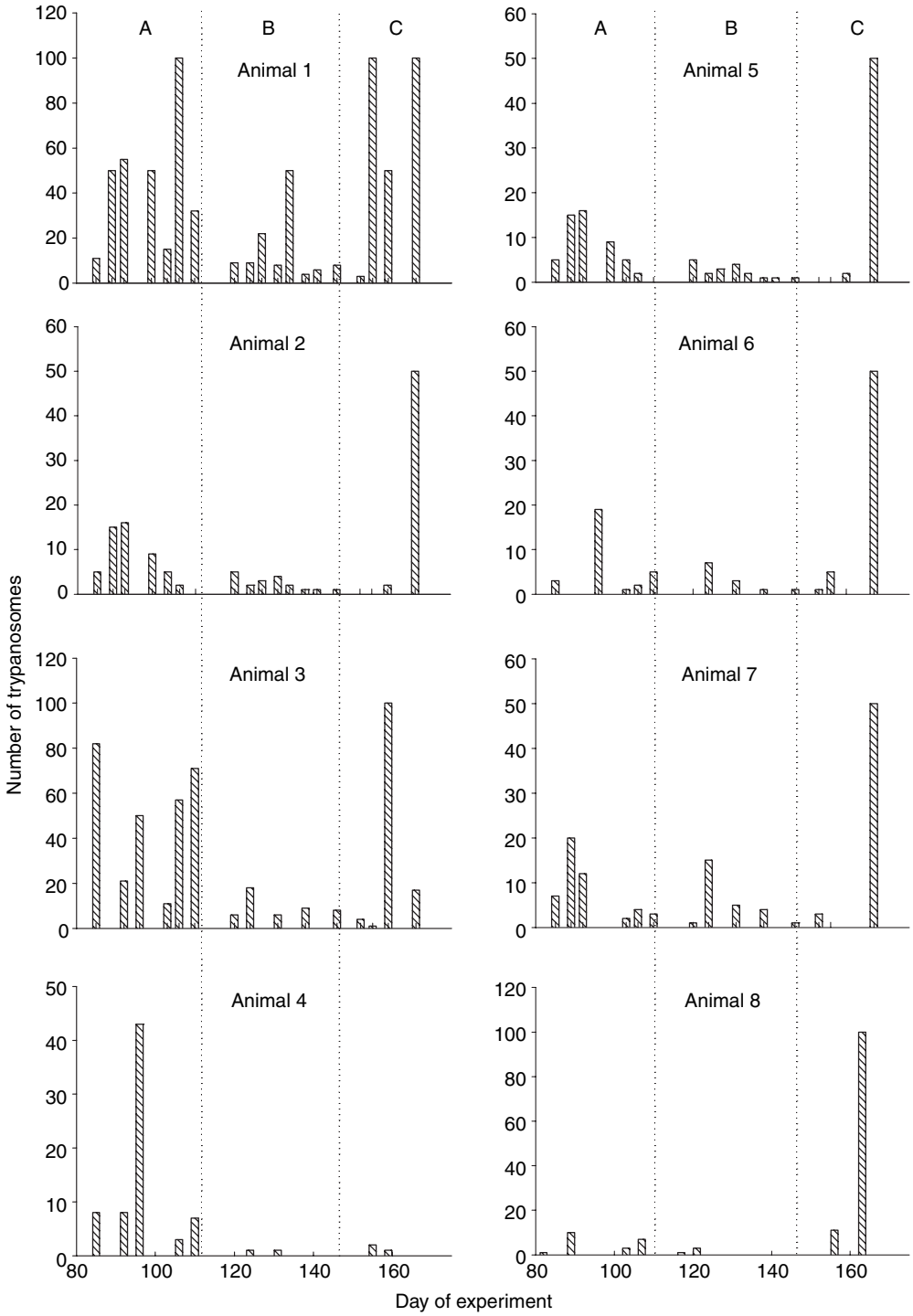

Figure 3 Number of *T. b. brucei* counted on 250 microscopic fields during the acute (A), chronic (B) and mixed (C) phase of the *T. b. brucei* infection in each of the eight experimental animals.

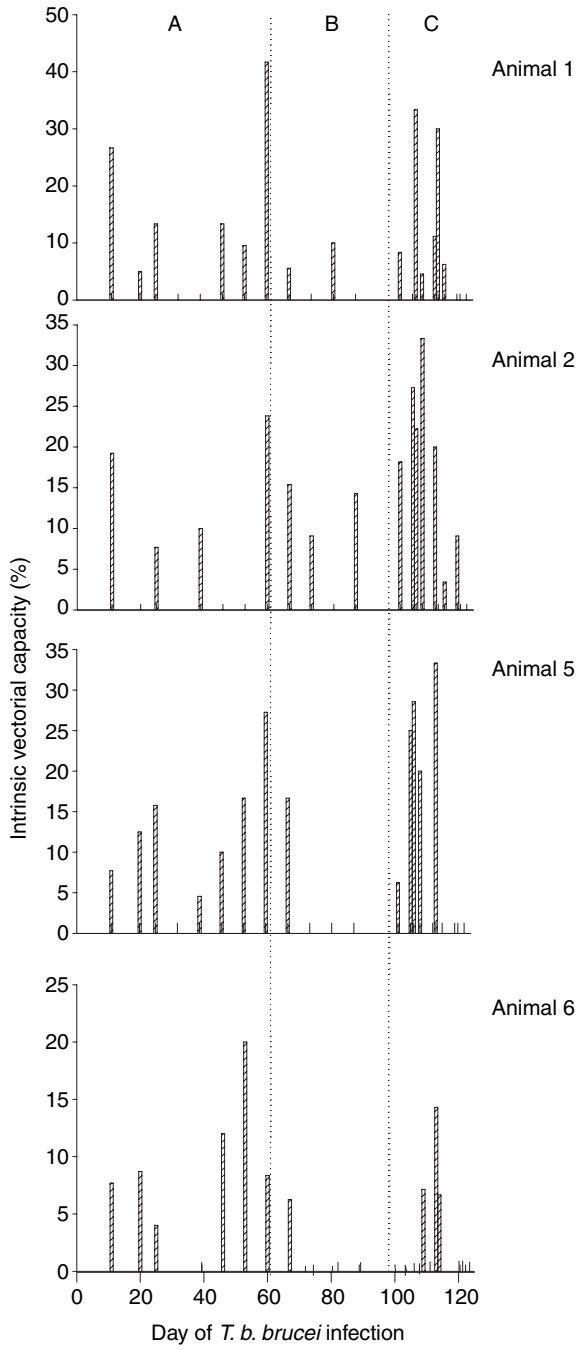

Figure 4 The intrinsic vectorial capacity of teneral *G. m. morsitans* after receiving one infected bloodmeal on one of four experimental animals during the acute (A), chronic (B) or mixed (*T. b. brucei* and *T. congolense*) (C) phase of the *T. b. brucei* infection. Each tick represents a day on which tsetse were offered a bloodmeal on the infected animals.

Table 1 Number of *G. m. morsitans* that took an infected bloodmeal on one of the experimental animals during the acute, chronic or mixed phase of the *T. b. brucei* infection and that developed a procyclic midgut or metacyclic salivary gland infection

		Number of flies infected		
Phase	*n*	Midgut	Salivary glands	IVC (%)
Acute	388	116	33	8.5
Chronic	318	83	30	9.4
Mixed	447	149	37	8.3

Table 2 Comparison of odds of a metacyclic infection between the different phases of the *T. b. brucei* infection in cattle

Phases of infection	Odds ratio (95% confidence interval)
Acute/chronic	1.02 (0.61–1.7)
Mixed (T. c/T. b)/chronic	0.99 (0.6–1.6)
Mixed (T. c/T. b)/acute	0.97 (0.59–1.6)

procyclic midgut infection. A metacylic infection was detected in a total of 100 flies.

The proportion of flies with metacyclic infections did not differ significantly between the acute and chronic phase and during the mixed *T. b. brucei*/*T. congolense* infection (Table 2). The proportion of midgut infections, on the other hand, was significantly higher ($P < 0.05$) during the acute phase and the mixed *T. b. brucei*/*T. congolense* infection than in the chronic phase of infection. The day of infection significantly reduced the metacyclic infection rate in flies infected during the chronic phase ($P < 0.001$) and during the mixed *T. b. brucei*/*T. congolense* infection ($P < 0.001$).

Discussion

Results from this experiment confirm that, by using body temperature, parasitaemia and the PCV as parameters, *T. b. brucei* infections in cattle can be divided in two phases (Van den Bossche *et al.* 2004b). The infection causes an increase in body temperature and a decline in the PCV in the early or acute phase. Afterwards, when the infection enters its chronic phase, the PCV remains relatively low but stable. Throughout the acute phase of infection, parasites are easy to detect in the blood with routine parasitological diagnostic methods. From about 60 days post-infection onwards or during the chronic phase, the parasitaemia decreases substantially and

parasites are detected at erratic intervals. Challenge with *T. congolense* during the chronic phase results in a significant increase in the *T. brucei* parasitaemia and a steep decline in the PCV. Since the chronic phase of infection is much longer than the acute phase, it is not surprising that under field conditions the parasitological prevalence of *T. brucei* infections in cattle is usually low and often underestimated (Picozzi *et al.* 2002).

The potential role of this reservoir status in the epidemiology of *T. brucei* s.l. trypanosomiasis cannot be underestimated. Although trypanosome development in the tsetse fly is probably facilitated by the high vectorial capacity of the tsetse line used in this experiment, the high infection rates of tsetse even in the phase of low parasitaemia suggest that during the chronic phase of infection sufficient parasites are present in the tsetse's bloodmeal to infect the fly. Maudlin and Welburn (1989) have shown that a single trypanosome is required to infect a tsetse fly. This parasitaemia is well below the detection limit of the routinely used diagnostic methods (Paris *et al.* 1982). Above a threshold of at least seven trypanosomes per bloodmeal the infection rates of tsetse are independent of the parasitaemia. The latter is supported by the absence of a significant increase in the metacyclic infection rate during the mixed phase of *T. brucei*/*T. congolense* infection when the parasitaemia increases significantly and well-above the infection threshold. Furthermore, these observations confirm that the transmission of *T. brucei* is not influenced by the presence of another trypanosome species such as *T. congolense* (Van den Bossche *et al.* 2004a). On the days when flies were not infected, the parasitaemia was most likely below the threshold for flies susceptible to infection.

These findings suggest that the presence of *T. brucei rhodesiense* in cattle may have important repercussions on the epidemiology and control of sleeping sickness. First and foremost, the absence of a pathology or the chronic nature of the disease especially in indigenous breeds affects significantly the survival of the infected animal and thus makes cattle good reservoirs. Infection of cattle is likely to occur when cattle are kept adjacent to or introduced into tsetse-infested areas where *T. b. rhodesiense* is present in other hosts such as game animals. Because of the continuous pressure for land for agriculture and the subsequent encroachment of people and their livestock into tsetse-infested zones this situation is observed in many areas of eastern and southern Africa (Van den Bossche 2001). Transmission of the parasite to other cattle and to humans will depend on the tsetse's feeding preference. Nevertheless, in areas where tsetse are or will become increasingly dependent on cattle for their survival, the parasite is likely to spread quickly among the cattle population. The high transmission rate during the various phases of infection combined with close proximity between people and cattle can lead to human sleeping sickness. Cattle can thus play a crucial role in the creation and maintenance of sleeping sickness foci. Moreover, compared with game animals and tsetse flies with rather restricted movement patters, cattle are highly mobile and can thus be instrumental in the spread of the parasite from such foci to areas where human-infective parasites have been absent. This spread of parasites formed the basis for the sleeping sickness outbreak in the Soroti District of eastern Uganda (Fèvre *et al.* 2001). Although this study gives information on the possible spread and transmission of *T. brucei* s.l., it gives no additional cues on the factors which generate either an epizootic or an endemic sleeping sickness outbreak. Furthermore, the reduced transmissibility of *T. b. rhodesiense* will certainly result in lower infection rates in tsetse (Welburn *et al.* 1995). Challenge of chronically *T. brucei*-infected bovines with other trypanosome species seems to be able to drive the *T. brucei* parasites out of the tissues towards the blood circulation. However, this additional infection did not give rise to higher *T. brucei* infection rates in the tsetse flies fed on these animals.

Although *T. b. rhodesiense* infected cattle may constitute a substantial disease threat they form an easy target for effective control of sleeping sickness. Treatment of infected cattle with trypanocidal drugs will reduce significantly the importance of the reservoir but may, depending on the frequency of treatment, induce the development of resistance in trypanosomes. This strategy is hampered by the difficulty in detecting cattle infected with *T. brucei* s.l. More sensitive diagnostic methods may be required or prophylactic or curative treatment could be given to the whole cattle population irrespective of their infection status. Control of the tsetse population without addressing the parasite's reservoir will only result in a temporary solution.

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