Bovine trypanosomiasis risk in an endemic area on the eastern plateau of Zambia

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ABSTRACT

The control of bovine trypanosomiasis could be improved by using the available control tools during periods when the incidence of the disease is highest. The present study assessed the monthly risk of bovine trypanosomiasis in 85 sentinel cattle kept on the tsetse-infested eastern plateau of Zambia during a period of 19 consecutive months. To avoid problems associated with persistence of infections because of trypanocidal drug resistance and/or the time lag between sampling and molecular analysis, a survival analysis and the subsequent calculation of risk was used as an indicator of challenge. Results showed that the average monthly risk of infection (92.3% due to *Trypanosoma congolense*) was 6%. It was significantly higher (7.7%) during the beginning of the rainy season (December–February). According to the outcome of the study, bovine trypanosomiasis control in the study area can be improved through increasing control efforts during this period of highest challenge.

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1. Introduction

The importance of trypanosomiasis, a devastating disease affecting livestock populations in large parts of Africa, is often determined by calculating the monthly incidence of infection. The incidence of trypanosomal infections depends to a large extend on the level of challenge animals are subjected to. Trypanosomiasis challenge is determined by the tsetse density, the prevalence of trypanosomal infections in tsetse and the proportion of meals taken by tsetse from the host species of interest. On the plateau of eastern Zambia, most of these parameters have been quantified. The monthly average proportion of infected tsetse flies is about 9% (Kubi et al., 2007) and the proportion of meals taken from cattle amounts to 75% (Van den Bossche and Staak, 1997). Such a high preference for livestock is not surprising considering the almost complete absence of large game animals from the highly cultivated areas of the eastern plateau. Between the different livestock species, special preference goes to cattle. Such a high preference for cattle is reflected in the low prevalence of trypanosomal infections in other livestock species such as goats and pigs (Simukoko et al., 2007). Finally, the density of the tsetse population is known to undergo substantial seasonal variations (Van den Bossche and De Deken, 2002). Notwithstanding the high prevalence of trypanosomal infections and the important role of cattle in the epidemiology of livestock trypanosomiasis on the eastern plateau, possible seasonal differences in the incidence of infection could be exploited to focus further trypanosomiasis control strategies. Determining the incidence of infection is often difficult because of the low sensitivity of parasitological diagnostic tools (Marcotty et al., 2008) and the presence of trypanocidal drug resistance (Delespaux and de Koning, 2007). The latter is of particular importance on the plateau area of the Eastern Province (Delespaux et al., 2008) and makes it difficult to distinguish new from persisting infections. Molecular diagnostic tools could compensate for the low sensitivity of the parasitological diagnostic methods but results are usually delayed. To overcome the problems associated with resistance and molecular diagnosis, the monthly risk of infection was used as a measure of challenge or incidence. It was calculated as the monthly probability of a primo infection based on molecular diagnosis and used as the basis to improve trypanosomiasis control strategies.

2. Materials and methods

2.1. Study area

The study was conducted on the plateau of eastern Zambia between April 2004 and December 2005. The study area is situated between 31° 45′ and 32° 00′ E and between 13° 45′ and 14° 00′ S. The area is highly settled and cultivated and carried a cattle population of approximately 11 animals/km² (Doran and Van den Bossche, 1999). Two main vegetation types are present. Miombo

woodlands, an open one-storied woodland with tall trees of the genera *Brachystegia* and *Julbernadia*, dominates (Van den Bossche and De Deken, 2002). Most of the villages are located in miombo. Munga woodland, a one or two-storied woodland where the principal tree genera are *Acacia*, *Combretum* and *Terminalia* is found mainly in lower lying areas. The annual climatic cycle comprises three seasons; the warm rainy season (from early November to late April), the cold dry season (from early May to late August) and the hot dry season (from early September to late October). The main livestock species reared in the study area are cattle of the Angoni breed, goats, pigs and chickens. Cattle generally graze in the communal grazing areas. However, grazing patterns vary according to season (Van den Bossche and De Deken, 2002). During the rainy season, cattle are mainly found in miombo whereas from June onwards cattle disperse and are found in both munga and miombo. This distribution pattern is in accordance with changes in the management practices of communal cattle in eastern Zambia (De Clercq, 1997).

Glossina morsitans morsitans is the only tsetse species present in the area. It takes 75% of its bloodmeals from cattle (Van den Bossche and Staak, 1997). *Trypanosoma congolense* is the most prevalent trypanosome species. The prevalence of infection in cattle is about 30% whereas in pigs and goats the prevalence of trypanosomal infections is low 6% and 3%, respectively (Simukoko et al., 2007).

2.2. Animal selection

A total of 85 head of cattle, representing four age and sex categories (i.e. oxen, cows, young stock and bulls) were selected randomly from their respective herds. The number of animals in each category was proportional to the normal herd structure in the study area (Doran, 2000) and constituted of 34 oxen, 25 adult females (>3 years old), 13 young males and females (between 1 and 3 years of age), 11 calves (<1 year) and two bulls. Animals were selected from herds that graze together and thus, theoretically, are subjected to the same tsetse challenge. All sentinel animals were ear-tagged and, 2 months before the start of the study, treated with a double dose of diminazene aceturate (i.e. 7 mg/kg body weight (BW), Berenil©, Hoechst) to clear any trypanosome infections acquired prior to the study. The animals were followed for a period of 19 consecutive months. Livestock owners whose animals were part of the study were advised not to treat their animals. Confirmed trypanosomiasis cases were treated with 3.5 mg/kg body weight of diminazene aceturate (Berenil©, Hoechst).

2.3. Sampling and diagnosis

Sentinel animals were sampled monthly to determine their infection status. From each animal, jugular blood was collected in a vacutainer tube with ethylenediaminetetraacetic acid (EDTA) as anticoagulant. After sampling, the vacutainer tubes were placed in a cool box containing ice packs and transported to the laboratory within 4 h of collection. From each vacutainer tube, blood was transferred into three capillary tubes which were sealed at one end with "Cristaseal" (Hawxley). The capillary tubes were spun in a microhaematocrit centrifuge for 5 min at 9000 rpm. After centrifugation, the packed cell volume (PCV) was determined. The buffy coat and the uppermost layer of red blood cells of one capillary tube were extruded onto a microscope slide and examined for the presence of motile trypanosomes. Samples were examined with a phase-contrast microscope at ×400 magnification (Murray et al., 1977). At least 50 fields were observed before declaring a slide as negative. Blood samples that were positive were further processed as blood smears for trypanosome species identification. Giemsa-

stained thick and thin blood smears were examined under ×100 oil immersion objective lens (×1000 magnification).

The buffy coats of the two remaining capillary tubes were extruded onto a labelled filter paper (Whatman no. 3, Whatman®). Filter papers were stored in sealed plastic bags containing silica gel at −18 °C. The samples were further analysed using the polymerase chain reaction–restriction length polymorphism (PCR–RFLP) described by Geysen et al. (2003).

2.4. Statistical analysis

To analyse the data, use was made of a parametric survival model in Stata 10 assuming an exponential survival distribution. A failure (i.e. an animal becoming infected with trypanosomes) was recorded when an animal was found to be infected using the PCR–RFLP diagnostic tool. Only animals that were PCR–RFLP negative at the start of the study were included in the sentinel herd. To avoid problems associated with drug resistance (thus excluding trypanosomal infections that were a result of treatment failure rather than tsetse challenge) and considering the delay between sampling and obtaining results from the molecular analyses, only the first infections or primo-infections were taken into account. Hence, once an animal has become infected it was excluded from further analyses. The overall risk of infection was calculated in a model without explanatory variables. The significance of the months as explanatory variable was estimated in a separate model.

The PCV data were analysed using linear regressions in Stata 10. Cross-sectional models were used to account for the repeated sample collection from individual animals. The square-root of PCV values ranging between 0 and 1 were arcsin transformed to assure normality (Obsborne). Discrete explanatory variables were the trypanosome infection status determined by PCR–RFLP and the time of sampling. The interaction between the two explanatory variables was tested and ignored if the likelihood ratio test was not significant ($P > 0.05$). The normal distribution assumption was verified in non cross-sectional models using the same response and explanatory variables. Residual quantiles plotted against the quantiles of a normal distribution quantile–quantile plot (Q–Q plot) were visually assessed and the heteroskedasticity was tested (Breusch-Pagan/Cook-Weisberg test for heteroskedasticity in Stata 10).

3. Results

A total of 19 monthly samplings was conducted. Three animals died due to suspected trypanosomiasis during the study period. One died during the first year of observation while the other two died during the second year. During the samplings, 155 new trypanosomal infections were detected when diagnosis was based on the results of the PCR–RFLP technique. Of those 155 trypanosomal infections, only 85 (54.8%) were detected using parasitological diagnostic tools (buffy coat method). A total of 143 (92.3%) infections were due to *T. congolense*, 7 (4.5%) to *Trypanosoma vivax* and 5 (3.2%) to mixed infections with *T. congolense* and *T. vivax*. The majority of the single or mixed *T. vivax* infections (11 out of 12% or 91.7%) was detected during the hot dry seasons. The remaining two *T. vivax* infections were detected during the month of July (cold dry season). The *T. congolense* incidence during the study period is summarised in a Kaplan–Meier survival curve (Fig. 1). Throughout the observation period the four weekly average risk of infection was 6.0% (95%, confidence interval (CI): 4.6–7.7%). However, the risk of infection varied significantly between months ($P = 0.017$) with a higher risk (7.7%) between December and February (i.e. the beginning of the rainy season) (Fig. 2).
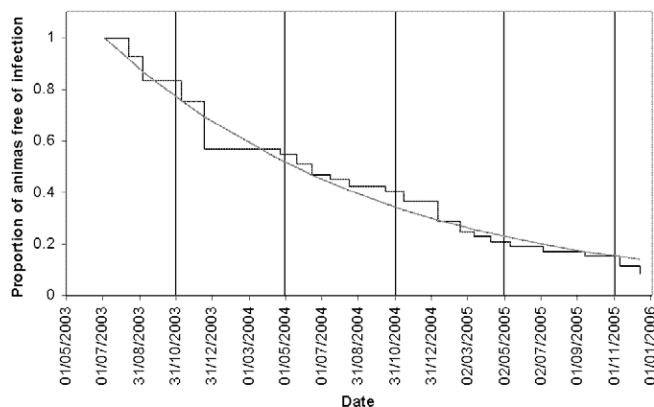
Fig. 1. Kaplan–Meier survival curve (between July 2003 and January 2006) of sentinel cattle subjected to tsetse challenge on the plateau area of eastern Zambia.
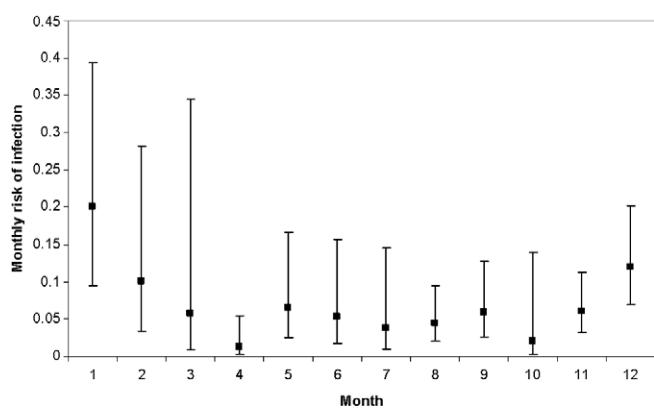

Fig. 2. Monthly variations in the predicted monthly risk of trypanosomiasis (*Trypanosoma congolense*) transmission to cattle on the plateau of eastern Zambia and 95% confidence intervals.

Infection with trypanosomes was significantly correlated with a reduction in the value of the PCV ($P < 0.001$). Monthly average PCV values ranged between 19.5% and 24.9% in infected animals and between 27.7% and 30.8% in uninfected animals (Fig. 3). In spite of the low amplitude of the monthly variations, the effect of time
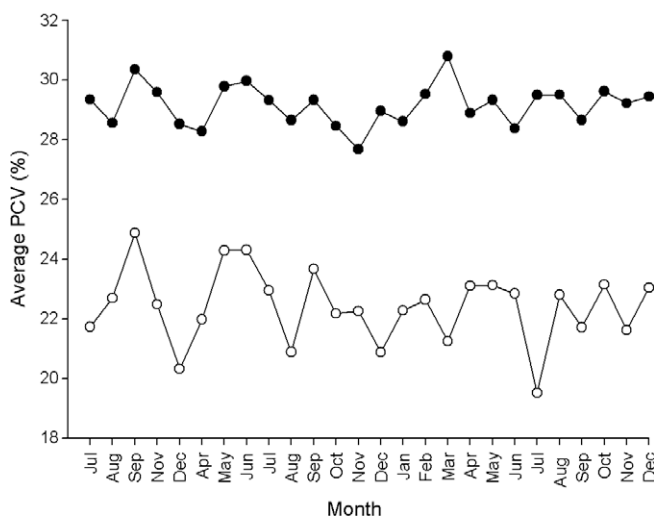

Fig. 3. Monthly average PCV of infected (○) and not infected (●) sentinel animals on the plateau area of eastern Zambia.

of sampling on the PCV of infected and uninfected animals was statistically significant ($P < 0.001$, Fig. 3). However, the interaction between the infection status and the time of sampling was not significant ($P = 0.52$), indicating that seasonal variations was similar in infected and uninfected animals.

4. Discussion and conclusions

The results presented give a good picture of the trypanosomiasis challenge livestock undergo on the highly cultivated plateau of Zambia. The area is representative for large tsetse-infested cultivated areas in southern Africa where livestock constitutes the main host of tsetse and the main reservoir of trypanosomes (Van den Bossche, 2001). Presenting challenge as risk of infection with trypanosomes (i.e. infection with *T. congolense*) clearly avoids problems associated with the overestimation of the incidence of infection as a result of trypanocidal drug resistance, the time lag between sampling and the results of the molecular analysis and thus the delay in the treatment of animals that after parasitological diagnosis were false negatives. In this respect, about 50% of the infected animals could not be detected using parasitological diagnostic tools. This lack of sensitivity, in accordance with previous observations, questions the accuracy of trypanosomiasis incidence data based on parasitological diagnosis and stresses the need for diagnostic tools to improve the field diagnosis of trypanosomal infections in livestock (Marcotty et al., 2008).

Although the risk of infection with trypanosomes was constant throughout most of the year it increased significantly during the beginning of the rainy season. Sinyangwe et al. (2004) could not detect such seasonality in trypanosomiasis incidence on the plateau of eastern Zambia. This may not be surprising considering the fact that infections were diagnosed solely based on parasitological diagnosis. Since the infection rate of the tsetse population undergoes little variation (Kubi et al., 2007), the high incidence of trypanosomal infections at the beginning rainy season is explained by the high density of tsetse during this time of the year (Van den Bossche and De Deken, 2002). Such a close relationship between tsetse density and incidence of infection is attributed largely to the high proportion of bloodmeals taken from cattle by tsetse (Van den Bossche and Staak, 1997). The higher level of challenge at the beginning of the rainy season is reflected in the high frequency of trypanocidal drug treatments given to cattle during this period of the year (Van den Bossche et al., 2000).

T. congolense is the main trypanosome species in the study area but infections with *T. vivax* do occur (Simukoko et al., 2007). These *T. vivax* infections seem to be most prevalent during the time of the year when the survival of tsetse flies is lowest and, hence, favouring the development of trypanosome species (such as *T. vivax*) with a short development cycle. The relative role of mechanical transmission could also be higher during the dry season when tsetse density is at its lowest. Nevertheless, the almost absence of *T. vivax* infections during the dry season remains difficult to explain and confirms our limited knowledge of the epidemiology of *T. vivax* in tsetse-infested areas. The study again confirms the importance of *T. congolense* as the main trypanosome species in livestock in Zambia, in particular, and southern Africa, in general.

An infection with trypanosomes resulted in a significant decline in the PCV (Murray and Dexter, 1988; Marcotty et al., 2008). However, monthly variations in the average PCV of the infected and uninfected cattle did not differ significantly. The latter is in accordance with observations made by Van den Bossche and Rowlands (2001) indicating that factors such as nutrition affect the PCV of rural cattle.

In conclusion, the outcome of the longitudinal study suggests that further focussing and prioritising of bovine trypanosomiasis

control is possible. Indeed, more effort could be put in optimizing trypanosomiasis control through, for example, prophylactic treatment, during the period of highest challenge, i.e. especially the beginning of the rainy season.

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