

Absence of serological markers of infection with *Trypanosoma brucei gambiense* in domestic animals in a sleeping sickness focus in South Congo*

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Abstract

A total of 33 domestic animals living in close contact with man in a human trypanosomiasis focus in South Congo were examined parasitologically and tested for serological markers of *Trypanosoma brucei gambiense* infection. 84.8% of the animals presented detectable *T. congolense* parasitaemia. The high rate of seropositivity observed with CATT (81.8%) contrasted with the low seroprevalence found with ELISA (< 13%). None of the 33 plasma samples showed lytic antibodies when analysed by immune lysis test against 10 distinct *T. b. gambiense* predominant variable antigen types (LiTat 1.1 to 1.10). The results demonstrate the lack of specificity of CATT, and to a lesser extent ELISA, in detecting *T. b. gambiense* infection in animals. The seropositivity may be due to cross-reaction with certain *T. congolense* antigens. The absence of serological markers specific to *T. b. gambiense* confirms the parasitological data which estimate the prevalence rate of animals infected with *Trypanozoon* as less than 1% in the region.

Introduction

The parasitological prevalence rate of *Trypanozoon* infections in domestic animals in central african villages where human trypanosomiasis is widespread is less than 1% (Noireau et al., 1986b; Makumyaviri et al., 1989). In parallel, the seroprevalence observed by the card agglutination test (CATT), which is considered to be specific to *Trypanosoma brucei gambiense* infection in man, is often over 50% in the same animals (Noireau et al., 1986b). The observed discrepancy has been explained in part by serological cross-reactions with *T. congolense* (Noireau et al., 1986a). The fact remains that CATT seropositivity may indeed indicate that the animal has been in contact with *T. b. gambiense*. The existence of such

"occult" infections is worth considering since they are likely to affect the epidemiology of the human disease. In order to determine the role of the animal reservoir, the immunological analysis of plasma was carried out to detect specific markers of *T. b. gambiense* infection.

Materials and methods

The study was carried out in February 1989 in the village of Kimbedi, situated in the Pool region (Congo). At that time, the prevalence rate of human trypanosomiasis was 5.5%. An entomological study showed that the vector, *Glossina palpalis palpalis*, was preferentially zoophilic and fed primarily on domestic animals living in semi-liberty in the village (Noireau et al., 1990). About 30% of the recorded livestock (11 pigs, 19 sheep and 3 goats) were investigated. Venous blood samples of all animals were taken. A thick blood film and a buffy coat, obtained by centrifuging the blood samples in capillary tubes (Woo, 1970), were prepared from each animal and examined for trypanosomes. A serological analysis was carried out on the plasma of the animals by CATT (Magnus et al., 1978). The quantitative test was considered positive at a titre ≥ 4 . The plasma was also analyzed by ELISA (Vervoort et al., 1978). The antigens used were the LiTat 1.6, a variant specific to the *T. brucei* group, and LiTat 1.3, considered to be specific to the *gambiense* sub-species. The threshold of positivity corresponded to the 400 titre. The immune lysis test (ILT), according to the method described by Van Meirvenne et al. (1975), was used to detect complement-activating lytic antibodies to antigenic variants (VAT). The plasma samples were tested against 10 *T. b. gambiense* predominant blood forms VATs (LiTat 1.1 to 1.10).

Results

Of the 33 animals examined parasitologically, 28 (84.8%) presented detectable *T. congolense* parasitaemia, whereas none was parasitized with the *T. brucei* spp.. Positive CATTs were observed in 27 animals (81.8%). However, few plasma samples gave positive ELISA tests, by LiTat 1.6 (12.1%) or LiTat 1.3 (9.1%). The few ELISA-positive samples presented low titres, equal to 400. All the CATT-negative animals were also ELISA-negative, whereas the 4 ELISA LiTat 1.6 positive corresponded to a CATT titre ≥ 8 . The ILT carried out on the 33 plasma samples did not reveal any lytic antibody to the 10 VATs tested, including the LiTat 1.6 which is a common antigenic type also expressed by the *T. brucei* spp..

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Discussion

The animals giving negative results with the ILTs can be considered as free from *T. b. gambiense* infection. Unlike in man, ELISA and especially CATT tests were found to be non-specific in animals in the Congo. Considerable discrepancies were however observed between CATT (81.8% positive) and ELISA (less than 13% positive). At the given thresholds of specificity, both tests may react to the presence of antibodies to certain antigenic types of *T. congolense*, a parasite which is found in most domestic animals in sleeping sickness foci (Noireau et al., 1986b). The estimation of the prevalence of *T. b. gambiense* animal infections based on the detection of trypanosomes is thus not corrected by testing for specific serological markers.

This low prevalence is all the more difficult to explain given that the animals live in close contact with man and that, in the region studied, they are the preferential feeding-host of tsetse-flies (Noireau et al., 1990). Three hypotheses can be put forward to account for the very low prevalence of the infection in these animals:

- The animals may not be receptive to most *Trypanozoon* stocks which are pathogenic to man (Noireau et al., 1989);
- After inoculation with metacyclic *T. b. gambiense* trypanosomes, the animals might eliminate the parasites at the first episode of parasitaemia, and recover without producing detectable trypanolytic antibodies. The few *Trypanozoon* stocks isolated in the animals in the Congo could originate from the transient episode of parasitaemia.
- As opposed to observations in West Africa (Croft et al., 1984; Kaminsky, 1986), the rate of infection of *Glossina* with *T. congolense* seems to be high in the Congo, sometimes exceeding 10% (Noireau, unpublished). Consequently, domestic animals are inevitably infected with *T. congolense* early in life, probably in the first days following birth. They may generate effective immunity against certain antigenic types common to both *Nannomonas* and *Trypanozoon*, and this may protect them later from infection with the latter sub-genus. Finally, only the animals infected initially with *T. b. gambiense* (unlikely given the low infection rate of the vectors) would be receptive to this species.

Although infection of the animals with *T. brucei brucei* cannot be categorically ruled out owing to the type of repertoire tested by ILT, it seems unlikely given the low ELISA titres observed with LiTat 1.6 and especially the absence of antibodies against the same antigen showed by ILT. Contrary to observations made in West Africa and in certain forest zones of Central Africa (Mehlitz et al., 1982; Makumyaviri et al., 1989), *T. b. brucei* does not seem to circulate in animals in savannah areas of the Congo.

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