Comparative Evaluation of the Antibody-detection ELISA Technique Using Microplates Precoated with Denatured Crude Antigens from *Trypanosoma congoense* or *Trypanosoma vivax*

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**ABSTRACT**

Two FAO/IAEA indirect enzyme-linked immunosorbent assays (ELISA), which use microplates precoated with denatured crude *Trypanosoma congoense* or *Trypanosoma vivax* antigen for detecting anti-trypanosomal antibodies in bovine sera, were evaluated for their sensitivity, specificity and positive and negative predictive values, using 320 Ugandan field samples (known negative sera, n = 80; known positive sera, n = 80; cattle herds where control of tsetse and trypanosomosis was practised, n = 80; and cattle herds where there was no such control, n = 80). Cut-off points of 30% and 25% positivity were determined for the *T. congoense* and *T. vivax* assays, respectively, using a modified ROC (receiver operating characteristic) analysis. The *T. congoense* assay had estimated diagnostic sensitivity and specificity of 63.7% and 57.5%, respectively, while the *T. vivax* assay had estimated diagnostic sensitivity and specificity of 81.3% and 81.3%, respectively. The two assays conducted in parallel had estimated diagnostic sensitivity and specificity of 82.5% and 88.7%, respectively. Using the sera from the cattle in the area with control (detected prevalence of trypanosomosis 0%), both the *T. congoense* and *T. vivax* assays had negative and positive predictive values of 100% and 0%, respectively. Using the sera from the cattle in the area without control (detected prevalence of trypanosomosis 15%), the *T. congoense* assay had negative and positive predictive values of 91.1% and 33.8%, respectively, and the *T. vivax* assay had negative and positive predictive values of 93.0% and 27.0%, respectively. The *T. congoense* assay was in fair agreement with the buffy coat technique (BCT) (κ = 0.25), while the *T. vivax* assay was in substantial agreement with the BCT (κ = 0.625), and both assays conducted in parallel were in substantial agreement with the BCT (κ = 0.708). Both assays were found to be proficient and suitable for the diagnosis of bovine trypanosomosis, especially when used in parallel.

**Keywords:** antibody, antigen, cattle, diagnosis, ELISA, predictive value, serology, sensitivity, specificity, species, trypanosomes

**Abbreviations:** BCT, buffy coat technique; C⁺⁺⁺, C⁺, C⁻, strong, weak and negative controls, respectively; FAO/IAEA, Joint Food and Agriculture Organization and the International Atomic Energy Agency; OD, optical density; PCV, packed cell volume; ROC, receiver operating characteristic

**INTRODUCTION**

Animal trypanosomosis is an important disease constraining livestock production in Uganda (Okuna and Magona, 1997). It has been estimated that 98 500 km², 41% of the
entire landmass of Uganda, is infested with tsetse flies and 70% of the livestock population grazes under high challenge (Ndyabahinduka, 1993). Recent surveys carried out in 16 districts of Uganda, representing four major agroecological zones, revealed that the prevalence of trypanosomosis in indigenous cattle under the communal grazing system ranged from 0 to 25%, while that in exotic cattle under the intensive dairy farming system ranged from 0 to 11.9% (Okuna et al., 1996). Control of trypanosomosis in livestock in Uganda relies on chemotherapy, chemoprophylaxis, tsetse control using trapping, and the use of pour-on insecticides with livebait technology (Magona et al., 1998). However, effective monitoring of trypanosomosis control programmes, treatment of sick animals and seroepidemiological surveys of trypanosomosis require accurate diagnosis. The diagnosis of animal trypanosomosis presents special problems. Basing the treatment of sick animals for trypanosomosis on clinical signs and standard parasitological detection methods is acceptable under field conditions, but the clinical signs are not pathognomonic and the standard methods of parasitological detection have limited analytical sensitivity, i.e. low detection rates, and lead to underreporting of the prevalence of the disease (Paris et al., 1982). Antibody detection and antigen detection ELISA tests have been developed to overcome such shortcomings. The antigen detection ELISA is useful for assessing the effectiveness of tsetse and trypanosomosis control programmes and the efficacy of and strategic use of trypanocidal drugs (Nantulya and Lindqvist, 1992). However, more recent findings have shown that the antigen detection ELISA has poor sensitivity, although it displays a high specificity (Eisler et al., 1998). The antibody-detection ELISA is useful in assessing the magnitude and distribution of trypanosomosis in livestock, and hence it is valuable for seroepidemiological surveys (Luckins, 1977; Desquesnes, 1999). However, the proficiency and robustness of the antibody detection ELISA for trypanosomosis may be impaired by the instability of antigen preparations derived from crude trypanosome lysates, if a strict cold-chain (−80°C) is not maintained (Rebeski et al., 1999a). Hence, FAO/IAEA has developed antibody-detection ELISAs using plates precoated with denatured T. congolense and T. vivax antigen (detergent/heat-treated) (Rebeski et al., 1999b) in an attempt to improve the diagnosis of trypanosomosis in counterpart laboratories in Africa, including Uganda (Rebeski et al., 1999a). These two antibody assays were evaluated for their proficiency, sensitivity and specificity, using field sera from infected and uninfected cattle in Uganda, and for their negative and positive predictive values using field sera from cattle in two areas with a different prevalence of trypanosomosis.

MATERIALS AND METHODS

Cattle

Selection of the uninfected cattle population was based on epidemiological factors, such as the area being tsetse-free owing to ecologically unsuitable conditions for tsetse vectors, the clinical status of the cattle based on their PCV, and their infection status using the buffy coat technique (BCT) (Murray et al., 1977). The 80 uninfected cattle