

Development of Slide Enzyme Linked Immunosorbent Assay (SELISA) for Detection of *Trypanosoma evansi* Infection in Bovines

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Abstract

The slide enzyme linked immunosorbent assay (SELISA) was standardized for detection of antibodies specific to *Trypanosoma evansi* and subsequently used for the screening of naturally infected bovine sera. A novel SELISA, a modification of the standard ELISA technique was used for the detection of antibodies against *Trypanosoma evansi* in bovines using positive and negative control sera. The test is based on immunostaining of the fixed whole *Trypanosoma evansi* organisms on microscopic glass slide, incubation with sera, anti-bovine IgG-HRPO conjugate and substrate Diaminobenzidine tetrahydrochloride (DAB). Finally the reaction was read under oil immersion of microscope. A total of 702 sera samples from bovines in Rayalaseema region of Andhra Pradesh were examined by SELISA and 192 were found positive for *Trypanosoma evansi* antibodies.

Keywords: *Trypanosoma evansi*; Bovines; Diagnosis; SELISA; India.

Introduction

Trypanosoma evansi causes a disease that is known as surra. Different mammalian species are susceptible for this disease like water buffaloes, cattle, horses, camels and pigs. (Herrera *et al.*, 2004). The disease has a great economic importance due to high degree of morbidity associated with decreased milk production, diminished working capacity and mortality of animals in fatal cases. Prevention by vector control and vaccination are not effective and chemotherapy is the only means of control of the disease at present. Therefore, definitive diagnosis of surra is of utmost importance. Conventional diagnosis in peripheral blood smear is difficult by direct microscopy because of scanty and irregular parasitaemia in chronically infected animals (Killick-Kendrick, 1968). Slide enzyme linked immunosorbent assay (SELISA) has been employed for the diagnosis of *Babesia bovis* infections and screening of *Babesia* specific monoclonal antibodies. It was reported to be as sensitive as existing non enzyme based serological tests for *Babesia bovis* (Kungu and Goodger, 1990). Additionally it has been applied for diagnosis of Toxoplasmosis

(Kumar, 2004) and *Babesia bigemina* infections (Ravindran *et al.*, 2007). The test procedure is simple and economical, slides can be kept for at least one week at room temperature and no need of ELISA reader. Therefore, the present study was undertaken to standardize SELISA in bovines.

Materials and methods

A total of 702 sera samples (320 cattle 382 buffaloes) collected from Rayalaseema region of Andhra Pradesh (India) were subjected to serological screening for *Trypanosoma evansi*. The serum samples collected from known infected animals were used as control positives where as the sera obtained from known negative animals and foetal bovine calf serum constituted the negative controls. An isolate of *Trypanosoma evansi* from cattle was maintained in the rats. *Trypanosoma evansi* parasites were separated from infected rat blood by using DEAE-cellulose anion exchange column chromatography method as described by Lanham and Godfrey (1970) with minor modifications described by Srivastava *et al.* (1988). The DEAE cellulose purified trypanosomes were suspended in PBS, pH 8.2 and thin smears of 5 mm diameter marked using a marker pencil were prepared on grease free glass slides. The smears were air dried, fixed in chilled acetone for 15 minutes, wrapped in

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aluminum foil and stored at 4°C till use.

The test was performed as described by Kungu and Goodger (1990) for *Babesia bovis*. The antigen coated slides were thawed at 37°C for 10 minutes (or) at room temperature for 30 minutes. Test sera were used as neat samples and about 15 microlitre of sample was applied to antigen coated zones on the surface of the glass slide. The slides were then placed in a moist chamber and incubated at room temperature for 90 minutes. The samples were washed by rinsing the glass slides in PBS, pH 7.1, 3 times for five min each and air dried. 10 microlitre of diluted rabbit antiovine horse radish peroxidase IgG (1:20 in PBS, pH7.4) was placed in each circle and the slides were again incubated at room temperature for 30 minutes in moist chamber. The slides were then washed twice with 0.01 M tris HCl (pH 7.6) and were air dried followed by addition of 10 microlitres of Diaminobenzidine tetrahydrochloride (DAB) to each circle. Finally, the slides were incubated at RT in moist chamber in dark for 10 minutes and the reaction was stopped by washing the slides with distilled water. The slides were air dried, mounted with glycerol-PBS and examined under oil immersion objective of bright field microscope.

Results

The control positive serum samples revealed typical dark brown coloured *Trypanosoma evansi* in SELISA (fig.1) where as no coloured *Trypanosoma evansi* were found in the control negatives (fig.2). Here source of antigen is acetone fixed DEAE cellulose purified *Trypanosoma evansi*. Then the test was employed in serum samples of bovines. Out of 702 test serum samples 240 were found positive with a percentage of 34.18. Same sero-reactivity was observed when the test sera were checked by Indirect ELISA.

Discussion

Blood smear examination is a practice under field condition but is very less sensitive (Killick-Kendrick, 1968). A sensitive serological test which is easy to perform and reliable is needed in countries like India where Trypanosomosis continues to be endemic and an important hindrance to livestock industry. The existing serological tests suffer from

the limitation of costly equipments which may not be available in the field conditions. In the present study, SELISA results can be viewed under ordinary light microscope and once standardized the test can easily be applied to field condition for primary screening of the animals.

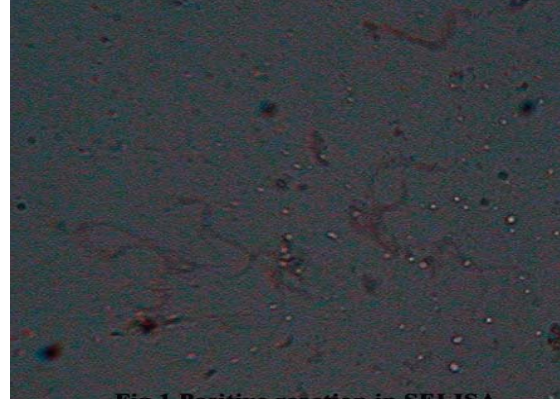


Fig. 1. Positive reaction to SELISA



Fig. 2. Negative reaction to SELISA

Another novel application of the test reported in literature pertains to use of the assay as a screening test in hybridoma antibody generation against *Babesia bovis* (Kungu and Goodger, 1990). These authors reported that the test was 79.94% and 89.47% sensitive in detecting *Babesia bovis* specific antibodies at 12 and 36 months after the single infection in cattle maintained under tick-free conditions. Although Kungu and Goodger (1990) reported that the test slides can be read microscopically for least a week at room temperature, but in the present study, the test reaction was found to be stable even up to 15 days after the test. Taking into considerations the requirements of the test, repeatability, simplicity, cost effectiveness and its acceptability in the field conditions, SELISA could emerge as a test of choice for the detection of antibodies against *T.evansi* infection in cattle and buffaloes in developing countries.

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