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# DOT - ENZYME LINKED IMMUNOSORBENT ASSAY IN THE DIAGNOSIS OF EXPERIMENTAL *Trypanosoma evansi* INFECTION IN SHEEP*

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Diagnosis of *Trypanosoma evansi* infection in sheep is usually made by parasitological technique, chemical tests and serological tests (Gill, 1977). Serological tests viz., Agar gel immuno diffusion (AGID) (Carpenter, 1965), Counter-Immuno electrophoresis (CIEP) (Wallis & Melnick, 1971), Indirect fluorescent antibody technique (IFAT) (Williams *et al.*, 1963), Passive haemagglutination test (PHAT) (Krupp, 1969) and Enzyme linked immunosorbent assay (ELISA) (Voller, *et al.*, 1975) are in use. A simple and sensitive serological test-the Dot-Enzyme linked immunosorbent assay (DOT - ELISA) is described in this paper.

## Materials and methods

Twelve Madras red sheep of 16 - 24 months age, free from *T. evansi* infection were used for the experiment. Two animals were kept as controls.

The experimental animals were inoculated with 1.5 ml of *T. evansi* suspension with a concentration of $15^5$ organism per millilitre intraperitoneally.

Blood was collected from each sheep at weekly interval from one week prior to the inoculation till the end of the experiment and sera was separated. Sera samples in various dilutions were prepared and 1:100 was found to give optimum results for the test.

Nitro-cellulose paper (NC paper) (Sigma, USA) pieces bound to plastic strip in the form of dip stick were used to conduct the study.

Antigen:

*T. evansi* was separated from heavily parasitaemic rat blood using the mini-anion exchange method as described by Lanham and Godfrey (1970). The organisms were washed thrice in PBS (PH 7.2) and sonicated (SONITRON, IMECO, Ultrasonic, UK) for 5 minutes at 100w. The parasitic debris were removed by high speed centrifugation (10,000 x g for one hour at 4°C). The protein was estimated as 10 $\mu$g/ml by the method of Varley (1980). The antigen solution was aliquoted into 0.5 ml vials and stored at - 20°C.

A serial two fold dilution of purified *T. evansi* antigen was prepared in PBS (PH 7.2) and 1:10 dilution was found to give optimum results for the test.

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**Peroxidase conjugated Rabbit Anti-Sheep Immunoglobulin**

Peroxidase conjugated Rabbit Anti-Sheep immunoglobulin was prepared in PBS in various dilutions and optimum result was given by 1:1000 dilution.

**Test procedure:**

One micro litre of an optimal dilution (1:10) of purified *T. evansi* antigen in PBS (PH 7.2) were dotted on the NC paper using Dot well apparatus. The NC paper was then allowed to dry at room temperature and incubated at 37°C for 30 minutes. Unsaturated sites of the NC paper were blocked by a 5 per cent solution of milk powder (AMUL, India) in PBST solution (PH 7.4) for 1 hour at 37°C. The dip sticks were rinsed in PBST solution and were incubated at 37°C for 45 minutes in test serum (dilution 1:100). The unbound proteins were removed by rinsing the dipstick thrice for 10 minutes each time in PBST solution. The dipsticks were then dipped in peroxidase conjugate (1:1000 for 30 minutes at 37°C and subsequently washed thrice for 10 minutes each time in PBST solution. The dipsticks were then dipped in freshly prepared Diamino benzidine tetrahydrochloride (DAB) substrate solution for 3 minutes. The dipsticks were air dried and stored.

**Results and Discussion**

Development of a brownish dot at site of antigen coating on NC paper was taken as positive case.

Out of the samples screened, 83.6 per cent samples were positive for *T. evansi* antibodies. The test detected the antibodies from 2nd week onwards till the end of the experiment. Dot - ELISA had been used for diagnosis of Chaga's disease (Araujo, 1985 and Pappas, 1988). Giannini (1987) studied early immune response to *T. lewsi* infection in rate using Dot-ELISA.

Present study revealed that Dot-ELISA could be one of the best qualitative and more specific test than AGID and CIEP and can be performed in the field condition.

**Summary**

A new serological test Dot-ELISA has been described and found more sensitive and simple for detecting *T. evansi* antibodies in sheep at 2 week of infection.

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