Research Article

Detection of *Trypanosoma Evansi* by Different Methods in Bovines in Andhra Pradesh

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ABSTRACT

*Trypanosoma evansi* infection is the most important disease of bovines in India. The present work was carried out to evaluate the different diagnostic methods for field diagnosis of *T. evansi* infection in bovines in Rayalaseema region of Andhra Pradesh. Animals showing the clinical signs of intermittent fever, ocular discharges, chronic emaciation and corneal opacity were selected in this study. A total of 702 samples from four different districts of Rayalaseema regions were collected from 2009 to 2011 for *Trypanosoma evansi* infection. The tests used were wet blood film (WBF), Giemsa stained smear method and Indirect Enzyme–linked immunosorbent assay (Indirect ELISA). The prevalence of *T. evansi* infection in cattle was detected in 8, 33, 102 out of 320 cattle by wet blood film examination, Giemsa stained smears examination and by Indirect ELISA, respectively. In buffaloes infection was recorded in 10, 42 and 138 out of 382 buffaloes by wet blood film examination, Giemsa stained smears examination and by Indirect ELISA, respectively. Among the 702 bovines 2.56 %, 10.68 % and 34.18 % were found positive by wet blood film examination, Giemsa stained smears examination and by Indirect ELISA, respectively. In this study Indirect ELISA had the higher prevalence rate as compared with other tests. Observed clinical findings in the affected bovines were correlated with Indirect ELISA results in this study.

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INTRODUCTION

*Trypanosoma evansi* is a widely distributed flagellate protozoan that causes a disease called surra in domestic animals and is transmitted mechanically by biting flies such as *Tabanus* and *Stomoxys* spp. The incidence and the severity of the disease vary with the strain of the parasite as well as the species of host affected (Sivajothi et al., 2013a). Clinical signs in different animals vary based on the severity of the infection. Clinical signs and pathological lesions caused by *T. evansi* in animals are unreliable for definitive diagnosis. Diagnosis of *Trypanosoma evansi* infection (surra) in livestock is of considerable economic importance. Detection of trypanosomes in blood has been the “gold standard”; however, finding the organisms or establishing patency of parasitaemia has not always been possible even in symptomatic infections. As a consequence of low dose infection, the pre–patent period in *Trypanosoma evansi* may be longer and, even when symptoms have developed, trypanosomes may still not be demonstrable in blood, thus delaying treatment and thereby increasing morbidity and mortality (OIE, 2008). There are several techniques for parasite detection, which include direct microscopy, concentration techniques and animal inoculation (Sivajothi et al., 2012; 2013b). The most useful of these tests, in view of their sensitivity and specificity, is the enzyme immunoassay (Indirect ELISA) which is used for the diagnosis of *Trypanosoma evansi* infections (Sivajothi et al., 2014a). Hence the present investigation was designed to detect *Trypanosoma evansi* in bovines by different methods (Wet blood film method, Giemsa stained smears and Indirect ELISA) in Andhra Pradesh.

Figure 1: Buffalo suffering with *Trypanosoma evansi*

MATERIALS AND METHODS

The animals from the Rayalaseema region of Andhra Pradesh were screened for the infection by different methods such as wet blood film examination, Giemsa’s staining and Indirect ELISA (Enzyme–linked immunosorbent assay). Animals showing the clinical signs of intermittent fever, ocular discharges, chronic emaciation and corneal opacity were selected in this study (Figure 1).
Blood was obtained from the peripheral ear vein and jugular vein from suspected animals. Trypanosomes were recognized by their movement among the red blood cells (RBCs) in wet blood film examination. Blood samples collected into separate sterile vials without Ethylenediaminetetraacetic acid (EDTA) for serum collection. Serum samples were collected in sterile vials. Few drops of 1:10,000 sodium azide solution was added to the serum samples and stored at ~20°C until use.

Wet blood film examination was done by placing a droplet of blood on a clean microscope slide and covering with a cover slip (22 × 22 mm). The blood is examined microscopically at (400×) total magnification approximately 50–100 fields per slide. A drop of blood was taken in the center on one end of a clean glass slide for smear preparation as suggested by Benjamin (Benjamin 1986). The blood smears were prepared and dried by waving in the air. The blood smears were fixed in absolute methyl alcohol for 3 min and stained with Giemsa's stain method of staining as described by Benjamin (Benjamin 1986). A drop of cedar wood oil was placed on the stained smear and the slide was examined under the oil immersion lens (1000×) of the microscope for the presence of Trypanosoma evansi. Approximately 50–100 fields of the stained thin smear are examined, before the specimen was considered to be negative. Even after a trypanosome has been detected, approximately 20 extra fields were investigated to determine if more than one species was present. The sharp extremities of the smears were extensively explored, because of their capillary properties; trypanosomes may be concentrated at this place (OIE 2004; Sivajothi et al., 2013b).

Isolate of T. evansi collected from the field case was maintained in the Wistar rats for bulk harvest of parasites. At the high of parasitaemia in rats, the rats were bled by heart puncture and the blood was collected and used for separation and purification of Trypanosoma evansi by using DE AE (Diethyl amino–ethyl cellulose anionexchange column chromatography).Whole cell lysate (WCL) antigen was prepared from purified trypanosomes. Protein concentration of the WCL Ag of T. evansi prepared in the present study was estimated as per Lowry et al. (1951) and it was adjusted to 1.0 mg/ml in PBS, pH 8.0 and stored at ~20°C in 1.0 ml aliquots. The hyper immune sera (HIS) was raised in two healthy New Zealand white rabbits, raised against WCL Ag was confirmed by agar gel precipitation test and counter immune electrophoresis. Pre immunized serum of these experimental rabbits was also stored at ~20°C till use as negative control serum for standardization of Indirect ELISA (Sivajothi et al., 2014a).

RESULTS AND DISCUSSION

In wet blood film examination Trypanosomes were recognized by their movement among the red blood cells (RBCs) (Figure 2). Examination of the Giemsa stained smear had Trypanosoma evansi; it was monomorphic in character, slender in shape, having an undulating membrane with a well developed free flagellum present outside the cell as reported previously by Soulsby (1982) (Figure 3). Serum samples were examined for the presence of T. evansi antibodies by Indirect ELISA (Figure 4). T. evansi was detected by using the different methods. The results of different diagnostic tests adopted for the detection of T. evansi in bovines in Andhra Pradesh was presented in Table 1. In the wet blood film examination 8 (2.50 %) cattle were positive out of 320 animals screened whereas 10 (2.61 %) buffaloes were positive out of 382 screened. In the blood smear staining methods, 33 (10.31%) cattle were positive out of 320 animals screened whereas 42 (10.99%) buffaloes were positive out of 382 screened. The ELISA detected antibodies against T. evansi in 102 (31.87%) cattle out of 320 screened whereas 138 (36.12 %) buffaloes were positive out of 382 screened.

Figure 1: Trypanosoma evansi in stained smears (100 X with 4x camera magnification)

Figure 2: Trypanosoma evansi in wet blood smears (10 X with 2x camera magnification) (A–Live Trypanosoma evansi)

Figure 4: Indirect ELISA for diagnosis of Trypanosoma evansi
Prevalence of 

**Table 1: Table showing the diagnosis of Trypanosoma evansi by different methods**

<table>
<thead>
<tr>
<th>Number screened</th>
<th>Method of Examination</th>
<th>Wet blood film</th>
<th>Staining Method</th>
<th>Indirect ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number found positive</td>
<td>Percent found positive</td>
<td>Number found positive</td>
</tr>
<tr>
<td>Cattle</td>
<td></td>
<td>320</td>
<td>8</td>
<td>2.50</td>
</tr>
<tr>
<td>Buffaloes</td>
<td></td>
<td>382</td>
<td>10</td>
<td>2.61</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>702</td>
<td>18</td>
<td>2.56</td>
</tr>
</tbody>
</table>

when using Indirect ELISA. This Indirect ELISA will make it possible to detect infections in bovines in the very early stages where microscope examination is unclear and to monitor groups of animals after trypanocidal treatment. Furthermore, this will not only be beneficial for diagnosis but also useful for epidemiological study and designing rational Trypanosomosis control program. Present study concludes that diagnosis of clinical and subclinical 

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CONFLICT OF INTEREST

No conflict of interest.

REFERENCES


