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Abstract

A survey was conducted to determine the prevalence of *T. evansi* in domestic animals in Rayalaseema region of Andhra Pradesh during the year 2009-10. A total of 1166 blood and sera samples from cattle, buffaloes and sheep were collected and screened by Wet blood film examination and Indirect ELISA. Overall, 1.54 and 24.52 per cent animals were found positive for *T. evansi* by Wet blood film (WBF) examination and indirect ELISA, respectively. Out of 320 cattle and 382 buffaloes examined for *T. evansi* infection, 2.50 and 2.61 per cent and 31.87 and 36.12 per cent were found positive by WBF examination and indirect ELISA, respectively. Out of 464 sheep examined, 9.91 per cent were found positive for *T. evansi* infection by indirect ELISA and none was found positive by WBF examination.

Keywords: Andhra pradesh, domestic animals, India, indirect elisa, prevalence, *Trypanosoma evansi*.

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PREVALENCE OF *TRYPANOSOMA EVANSI* IN DOMESTIC ...  

**Introduction**

*Trypanosoma evansi*, a blood protozoan parasite causes a serious disease known as ‘surga’ in domestic animals and wild animals. It is an arthropod borne disease and *Tabanus* spp has been implicated as the main vector. It is widely distributed in the animals of Asia, Africa and central and south Americas. In India, *T. evansi* infection is widely prevalent in different parts and is of significant economic importance in livestock production (Juyal et al., 2007; Sivajothi et al., 2012).Due to high degree of morbidity associated with decreased milk production, diminished working capacity and mortality of animals in fatal cases.

The host species affected by surra vary geographically. However buffaloes, cattle, camels and horses are mainly affected and dogs, tigers, sheep, goat, elephant, deer, cats and pigs can also be infected. Milk is presently a better avenue to make a living in many parts of the world (Ekeocha1, 2012). Due to *T. evansi* low milk production was observed. The disease in acute and sub-acute forms has been recorded from different parts of the world (Gebreyohannes and Legesse, 2014) and in India (Prasad et al., 1997; Sivajothi et al., 2013a). The studies on the prevalence of *T. evansi* in domestic animals in Andhra Pradesh are lacking, hence the present investigation was carried out to record the prevalence of *T. evansi* in domestic animals in Rayalaseema region of Andhra Pradesh.

**Materials and Methods**

**Blood and Serum Samples**

Blood samples from naturally infected or suspected cattle, buffaloes and sheep of Rayalaseema region of Andhra Pradesh were collected from ear vein or Jugular vein in separate vials with and without EDTA. The blood with anti-coagulant was used to detect *T. evansi* by wet blood film examination (WBF) as per methods (OIE, 2008; Sivajothi et al., 2013b). The blood without anti-coagulant was used for the serum collection. The serum was collected in sterilized vials with one or two drops of 1: 10,000 sodiumazide solution and stored at -20°C till used.

**Antigen Preparation**

*T. evansi* parasites were separated from experimentally infected Wistar rat’s blood by using DEAE-cellulose column chromatography, as per technique followed by Lanham and Godfrey (1970). Separated trypanosomes were washed twice in PSG, (pH 8.0 with glucose @1:1) by repeated centrifugation. The purified parasites were then sonicated at 150W for 3-4 cycles of 30 seconds each by ultrasonic disintegrator. The sonicated material was centrifuged at 2400 x g for 20 min at 4°C. The collected supernatant was designated as whole cell lysate (WCL) antigen (Ag) and it was partially purified after precipitating with 50 percent saturated ammonium sulphate followed by extensive dialysis against PBS, pH 7.4 (Singh et al., 1994). The protein content of WCL Ag of *T. evansi* was estimated as per the method of Lowry et al., (1951). The protein concentration of WCL Ag was adjusted to 1.0 mg/ml in PBS, pH 8.0 and stored at -20°C in 1.0 ml aliquots.

**Raising of Hyper Immune Sera**

The hyper immune sera (HIS) was raised in two healthy New Zealand white rabbits, weighing up to 1.5 to 2.0 kg body weight against whole cell lysate antigen of *T.evansi* as per the method of Singh and Chhabra (1993) with slight modifications. Antibody levels of HIS raised against WCL Ag of *T. evansi* was confirmed by Agar gel precipitation test and counter immuno electrophoresis. Hyper immune sera collected from the immunized rabbits was passed through 0.45μ membrane filter, aliquoted in sterile vials (1ml/vial) to avoid multiple freeze-thaw cycle and stored at -20°C until use and it was used as a positive control in standardization of Indirect ELISA for detection of circulatory antibodies of *T. evansi*. Pre immunized serum of these experimental rabbits was also stored at -20°C till use as negative control serum for standardization of Indirect ELISA in the present investigation.

**Indirect Enzyme Linked Immunosorbert Assay**

Indirect ELISA was standardized according to Shahardar et al., (2008) with some modifications to detect the *T. evansi* antibodies from 1166 serum samples of cattle (n=320), buffaloes (n= 382) and sheep (n= 464) collected during the investigation. The concentrations of the antigen (WCL Ag of *T.
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evansi), test sera and conjugate (rabbit anti bovine IgG-HRPO/ donkey anti sheep IgG-ALP) for optimum performance of the assay were determined by checker board titration. Flat bottom, high binding capacity 96-well EILSA plates (Nunc, Denmark) were used in the study. Unbound sites in the wells of the plate after antigen coating were blocked with 1% bovine albumin serum (BSA) in PBST. Antibody concentration was standardized by using known positive and negative sera. Reaction with anti-species antibody – enzyme-conjugate was developed by adding hydrogen peroxide (30%) in tetra methyl benzidine (TMB) solution in case of serum samples from bovines or p-nitrophenol phosphate in diethol amine (DEA) buffer for serum samples of sheep. The substrate/chromogen reaction was stopped by adding 5M H₂SO₄ / 3N NaOH solutions and the readings were taken immediately in the ELISA reader (Qualigens, India) at 450 nm and 405 nm for cattle and sheep samples, respectively. Cut-off value of optical density (OD) for the assay was established by mean of the OD values shown by the panel of negative serum samples plus three standard deviations. The sera having OD above the cut-off value were considered as positive. Standardization of Indirect ELISA in different animals was according with the Sivajothi et al., (2014a; 2014b).

Results

Prevalence of T. evansi in domestic animals in Rayalaseema region of Andhra Pradesh was studied during 2009-11. A total of 1166 blood and sera samples were collected from cattle (n=320), buffaloes (n=382) and sheep (n=464). Overall, 1.54 and 24.52 per cent animals were found positive for T. evansi by WBF and indirect-ELISA, respectively (Table 1 and Fig. 1).

Table 1: Prevalence of Trypanosoma evansi infection in domestic animals in Rayalaseema region of Andhra Pradesh.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Species</th>
<th>Number screened</th>
<th>T.evansi detected by</th>
<th>WBF</th>
<th>Indirect ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Number found</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>positive</td>
<td></td>
<td>positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Cattle</td>
<td>320</td>
<td>8</td>
<td>2.50</td>
<td>102</td>
</tr>
<tr>
<td>2</td>
<td>Buffalo</td>
<td>382</td>
<td>10</td>
<td>2.61</td>
<td>138</td>
</tr>
<tr>
<td>3</td>
<td>Sheep</td>
<td>464</td>
<td>Nil</td>
<td>-</td>
<td>46</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1166</strong></td>
<td><strong>18</strong></td>
<td><strong>1.54</strong></td>
<td><strong>286</strong></td>
<td><strong>24.52</strong></td>
</tr>
</tbody>
</table>

WBF: Wet blood film.
Indirect ELISA: Indirect enzyme linked immunosorbent assay.
* Not screened by Indirect ELISA.

Fig. 1: Prevalence of Trypanosoma evansi infection in domestic animals in Rayalaseema region of Andhra Pradesh.
PREVALENCE OF \textit{TRYPANOSOMA EVANSI} IN DOMESTIC ... 

Out of 320 cattle examined in different parts of Rayalaseema region of Andhra Pradesh for \textit{T. evansi}, 2.50, and 31.87 per cent were found positive by WBF and indirect ELISA, respectively. WBF detected 3.22, 2.81, 4.08, and 0 per cent cattle positive for \textit{T. evansi} infection and indirect ELISA detected 20.96, 41.54, 28.57, and 23.88 per cent positive for \textit{T. evansi} antibodies, respectively in Anantapur, Chittoor, Kadapa and Kurnool districts.

Table 2: Prevalence of \textit{Trypanosoma evansi} infection in cattle in Rayalaseema region of Andhra Pradesh.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>District</th>
<th>Number screened</th>
<th>T. evansi detected by WBF</th>
<th>T. evansi detected by Indirect ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Number found positive</td>
<td>Percent positive</td>
<td>Number found positive</td>
</tr>
<tr>
<td>1</td>
<td>Anantapur</td>
<td>62</td>
<td>2</td>
<td>3.22</td>
</tr>
<tr>
<td>2</td>
<td>Chittoor</td>
<td>142</td>
<td>4</td>
<td>2.81</td>
</tr>
<tr>
<td>3</td>
<td>Kadapa</td>
<td>49</td>
<td>2</td>
<td>4.08</td>
</tr>
<tr>
<td>4</td>
<td>Kurnool</td>
<td>67</td>
<td>Nil</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>320</td>
<td>8</td>
<td>2.5</td>
</tr>
</tbody>
</table>

WBF: Wet blood film.
Indirect ELISA: Indirect enzyme linked immunosorbent assay.

Fig. 2: Prevalence of \textit{Trypanosoma evansi} infection in cattle in Rayalaseema region of Andhra Pradesh.

In the present investigation, among 382 buffaloes examined in different parts of Rayalaseema region of Andhra Pradesh for \textit{T. evansi} infection, 2.61, and 36.12 per cent were detected positive by WBF and indirect ELISA, respectively. WBF detected 3.92, 0, 5.00, and 0 per cent and indirect ELISA detected 29.41, 40.47, 33.33, and 43.22 per cent samples positive in Anantapur, Chittoor, Kadapa and Kurnool districts, respectively.

The details of the prevalence of \textit{T. evansi} infection recorded in buffaloes in different parts of Rayalaseema region are shown (Table 3 and Fig. 3).
Table 3: Prevalence of *Trypanosoma evansi* infection in buffaloes in Rayalaseema region of Andhra Pradesh.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>District</th>
<th>Number screened</th>
<th>T. evansi detected by</th>
<th>WBF</th>
<th>Indirect ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number found positive</td>
<td>Percent found positive</td>
<td>Number found positive</td>
</tr>
<tr>
<td>1</td>
<td>Anantapur</td>
<td>102</td>
<td>4</td>
<td>3.92</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>Chittoor</td>
<td>42</td>
<td>Nil</td>
<td>-</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>Kadapa</td>
<td>120</td>
<td>6</td>
<td>5.00</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>Kurnool</td>
<td>118</td>
<td>Nil</td>
<td>-</td>
<td>51</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>382</strong></td>
<td><strong>10</strong></td>
<td><strong>2.61</strong></td>
<td><strong>138</strong></td>
</tr>
</tbody>
</table>

WBF: Wet blood film.
Indirect ELISA: Indirect enzyme linked immunosorbent assay.

Out of 464 sheep examined by WBF and indirect ELISA, 9.91 per cent were found positive for *T. evansi* infection by indirect ELISA and none was found positive by WBF. Indirect ELISA detected *T. evansi* antibodies in 13.11, 8.95, 9.09, and 7.81, per cent of sheep in Anantapur, Chittoor, Kadapa and Kurnool districts, respectively. Details of the prevalence of *T. evansi* infection recorded in sheep in the study are given in the (Table 4 and Fig. 4).

Table 4: Prevalence of *Trypanosoma evansi* infection in sheep in Rayalaseema region of Andhra Pradesh.

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>District</th>
<th>Number screened</th>
<th>T. evansi detected by</th>
<th>WBF</th>
<th>Indirect ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number found positive</td>
<td>Percent found positive</td>
<td>Number found positive</td>
</tr>
<tr>
<td>1</td>
<td>Anantapur</td>
<td>122</td>
<td>Nil</td>
<td>-</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>Chittoor</td>
<td>201</td>
<td>Nil</td>
<td>-</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>Kadapa</td>
<td>77</td>
<td>Nil</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>Kurnool</td>
<td>64</td>
<td>Nil</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>464</strong></td>
<td><strong>Nil</strong></td>
<td>-</td>
<td><strong>46</strong></td>
</tr>
</tbody>
</table>

WBF: Wet blood film.
Indirect ELISA: Indirect enzyme linked immunosorbent assay.
The prevalence of *Trypanosoma evansi* was recorded with indirect-ELISA as 36.12, 31.87 and 9.91 per cent in buffaloes, cattle and sheep, respectively. WBF detected *T. evansi* infection in 2.61, 2.50 and 2.36 per cent in buffaloes, cattle and sheep, respectively. However, none of the sheep examined in the study was found positive by WBF.

**Discussion**

Out of 1166 blood samples screened by WBF examination, 22 samples were found positive for *T. evansi* with an overall prevalence of 1.54 per cent. Out of 320 cattle and 382 buffaloes examined, 2.50, 2.61 and 6.76 per cent were found positive for *T. evansi* infection by WBF, respectively. Among 464 sheep examined, none was found positive for *T. evansi* infection by WBF. Indirect ELISA detected *T. evansi* antibodies in 286 (24.52%) out of 1166 sera samples processed. Prevalence *T. evansi* was recorded in 31.78, 36.12 and 9.91 per cent out of 320, 382 and 464 cattle, buffaloes and sheep examined.

Prevalence of *T. evansi* can be correlated with the vector population and favorable conditions for vector breeding, species of the host, host immune status etc. (Fentahun and Tekeba, 2013). All the species in the present study are more or less equally susceptible.

One obvious reason for low number of positive samples by WBF was the inherent low sensitivity of the test. Similar observations have been made by numerous workers over past decades in India (Sivajothi et al., 2013b) and in other countries (Desquesnes et al., 2009). Baghel et al., (1996) found positive in 13.26 and 76.53 per cent buffaloes by WBF and indirect ELISA. Sinha et al., (2006) reported *T. evansi* prevalence in 52.69 and 52.31 per cent of cattle and buffaloes in Patna. Molina et al., (2000) found 1.3 per cent and 9.0 per cent positive by WBF and Ab-ELISA, respectively. Desquesnes et al., (2009) reported 66.0 per cent and 83.3 per cent positive by parasitological examination and Ab-ELISA respectively in experimental infections. Saseendranath et al., (1994) reported 83.6 per cent of sheep were positive for *T. evansi* by indirect ELISA in an experimental infection. However these reports on prevalence of *T. evansi* in sheep are without exact epidemiological data and hence could not be compared with present findings.

Secondly, the number of infected animals should far exceed these figures in the areas where vector density is high during rainy and post rainy seasons and there are equal opportunities for the vector to bite on most of their hosts in these areas. The number of animals infected in the region should be higher than those detected by WBF. Because, latent infections with low parasitemia are common in cattle, buffaloes and camels (Sivajothi et al., 2014c; Losos, 1980), it is likely that many samples were missed by these tests. Antigenemia due to release of...
the antigens from the parasites hiding in some tissues has also been speculated. Victor et al., (2012) recorded the Hemorrhages and Prepuicial Inflammation in Pigs Experimentally Infected with Trypanosoma Congolense.

Another reason why WBF figures were low could be the indiscriminate treatment of suspected animals for trypanosomosis based only on signs and symptoms. Veterinary practice based on symptoms is quite common in the field in India. In such a situation, the recently treated animals would be declared positive by immunological tests. Parasite antibodies may remain in blood circulation for about few weeks to months after treatment (Wernery et al., 2001). Therefore, this fact would necessitate getting reliable history of the animal that received anti-trypanosome treatment during past few weeks before sample collection to make indirect-ELISA more dependable.

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