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Case Report

Occurrence of *Trypanosoma evansi* Infection in Rottweiler Dog from Cauvery Delta Region of Tamil Nadu, India: A Case Report

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Abstract

Trypanosomes are the extraerythrocytic haemoflagellate protozoan parasites of domestic and wild animals including dogs. A male Rottweiler dog was presented with the history of anorexia, cloudy eyes and vomiting for a week. The dog was examined both physically and clinically. Blood was collected and subjected to haematological and biochemical analysis. Echocardiography of the thoracic cavity and ultrasonography of the abdominal cavity were performed. Molecular confirmation of parasite was carried out by PCR and DNA was sequenced to identify strain variations. Upon clinical examination, the dog revealed emaciation with high fever, unilateral corneal opacity, anemia, enlarged lymph nodes and distended abdomen. Echocardiography of the thoracic cavity showed ventricular hypertrophy and cardiomyopathy. Ultrasonography of the abdominal cavity showed splenomegaly and renomegaly. Haematological findings revealed decreased haemoglobin and erythrocyte count and biochemical findings revealed increased BUN and creatinine, hypoglycemia and hypoalbuminemia. Wet film and blood smear examination revealed actively motile trypanosomes and massive infection of trypanosomes, respectively. Molecular confirmation of species was carried out by amplification of 227 bp partial VSG gene of *Trypanososma evansi*, subsequently sequenced and phylogenitically analysed. The infected dog was treated intramuscularly with diminazene aceturate @ 3.5 mg /kg b.wt along with the supportive therapy. The study described the occurrence and diagnosis of trypanosomosis in dog. Early diagnosis and timely treatment is the most reliable way to safeguard the companion animals. It is the first report on trypanosomosis in dog from Cauvery delta region of Tamil Nadu, India.

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Introduction

Trypanosomosis is an economically important haemoprotozoan diseases affecting wide range of domestic and wild animals including dogs (1). *Trypanosoma evansi* causes chronic disease (Surra) in camels and horses, but it is generally an acute and fatal disease in dogs and the infected dog may die within 2 - 4 weeks of infection if untreated (2). It is transmitted mainly by ingestion of infected herbivores meat and use of contaminated needles (3).

In India, the prevalence of *T. evansi* in dog was recorded in Madhya Pradesh (7.69%) (4), Andhra Pradesh (2.40%) (5), Kerala (2.7%) (6) and outbreak of canine trypanosomosis were also well documented in Calcutta, West Bengal (7). However, studies on trypanosomosis of dogs in Tamil Nadu are scanty.

We discuss about the diagnosis, clinical and therapeutic management of trypanosomosis in Rottweiler dog from Cauvery delta region of Tamil Nadu, India.

Case Report

An adult male Rottweiler dog was admitted to the Veterinary Clinical Complex (VCC) of Veterinary College and Research Institute (VCRI), Orathanadu, Thanjavur, Tamil Nadu, for the treatment with the history of anorexia, cloudy eyes and vomiting for a week. Upon physical examination, the dog was appeared dull and depressed, emaciated with high body temperature (40.1°C), unilateral corneal opacity in the left eye, pale conjuctival mucous membrane, palpable prescapular lymph nodes, oedematous swelling of the hind limbs and distended abdomen. Echocardiography of the right parasternal short axis showed ventricular hypertrophy and cardiomyopathy (Fig. 1). Abdominal ultrasound examination of the kidney showed enlarged left kidney (Fig. 2) and cortical and medullary hyper echogenicity of the right kidney (Fig. 3), sagital view of the spleen showed splenomegaly with hypoechoic mass near the free border of the spleen (Fig. 4).

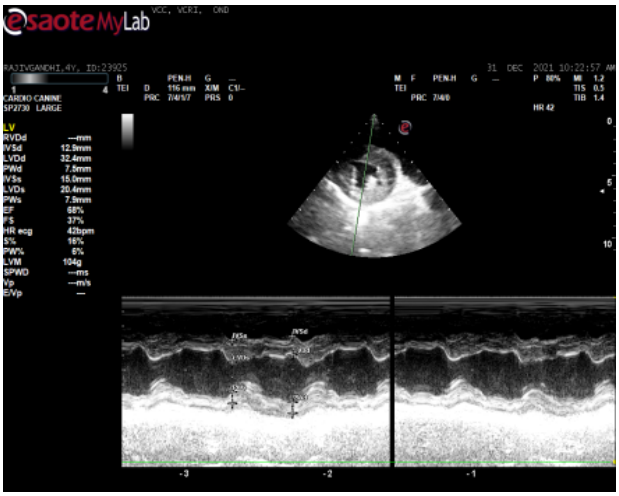

Fig. 1: M Mode Echocardiography - Right parasternal short axis- ventricular hypertrophy

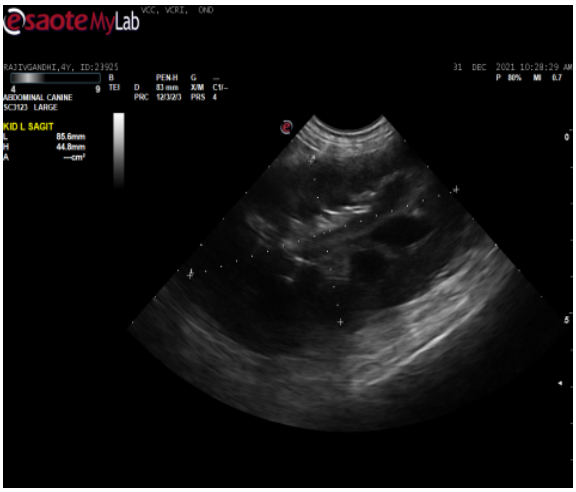

Fig. 2: Left kidney - Sagittal view – Renomegaly (85.6 X 44.8mm)

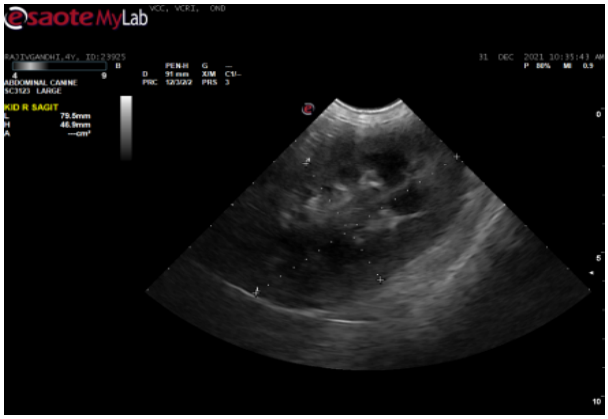

Fig. 3: Right kidney - Sagittal view - Cortical and medullary hyper echogenicity (79.5 X 46.9 mm)

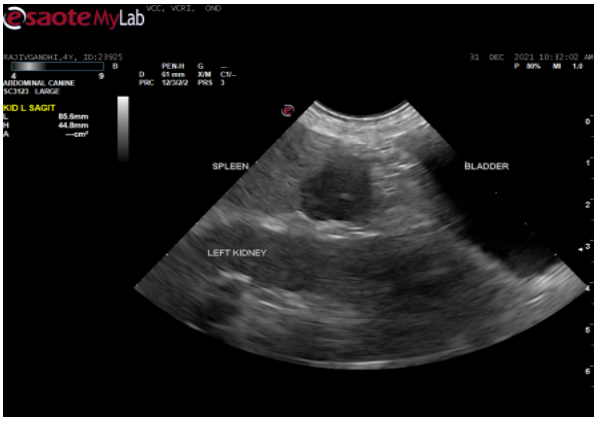

Fig. 4: Spleen - sagittal view - Splenomegaly with hypoechoic mass noticed on tail of the spleen, bladder and left kidney on same window

After clinical examination, blood was collected from cephalic vein and subjected to haematological and biochemical analysis. Hematological findings revealed severe anaemia as indicated by profound decrease in the haemoglobin value (4.2 g /dL) and total erythrocyte count of $2.7x10^6/mm^3$ whereas the total leucocyte count ($14.6x10^3/mm^3$) and differential leukocyte count (neutrophils - 74%, lymphocytes - 17% and monocytes - 6% were found normal (Table 1). The biochemical values of the serum samples revealed increased BUN (248 mg/dL) and creatinine (2.93 mg/dL) and decreased glucose (60.5 mg/dL) and albumin (2.16 g/dL) levels (Table 2).

Table 1: Haematological analysis of blood collected from dog infected with *Trypanosoma evansi*

Cell type	*Subject's values*	*Reference values*	*Findings*
Haemoglobin(g/dL)	4.2	12 - 18	Severe anaemia
RBC ($106/mm^3$)	2.7	5.5 - 8.5	
WBC ($103/mm^3$)	14.6	4 - 15	Normal
Neutrophils (%)	74	60 - 70	
Lymphocytes (%)	17	12 - 30	
Monocytes (%)	6	2 - 8	

Reference range: The Merck Veterinary Manual, 11th Edn.

Table 2: Biochemical analysis of serum from dog infected with *Trypanosoma evansi*

Parameters	*Subject's values*	*Reference values*	*Findings*
BUN (mg/dL)	248	6 - 30	Uremia
Creatinine (mg/dL)	2.93	0.5 - 1.7	Azotemia
Total Protein (g/dL)	8.14	5.4 - 7.5	Normal
Glucose (mg/dL)	60.5	70 - 138	Hypoglycemia
Albumin (g/dL)	2.16	2.3 - 3.1	Hypoalbuminemia

Reference range: The Merck Veterinary Manual, 11th ed.

Additionally, wet film examination showed large numbers of motile trypanosomes, which was moving actively among the red blood cells (Fig. 5). Giemsa's-stained thin blood smear revealed a numerous long slender trypanosome with central nucleus, small sub terminal

kinetoplast, well developed undulating membrane and a long free flagellum. The length of the parasite was measured using micrometry and ranged between 19 - 31 µm. Severe parasitemia (+++) with the range of 9 -12 organisms per high power field was observed (Fig. 6).

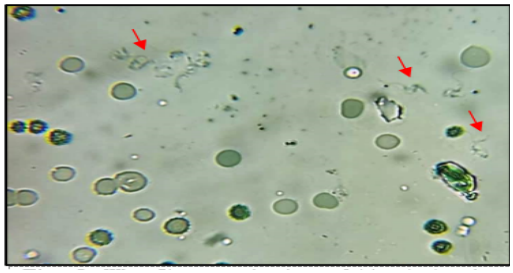

Fig. 5: Wet film examination of blood showing numerous Trypanosoma organisms (x400)

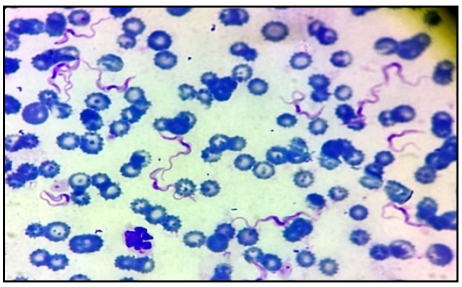

Fig. 6: Photomicrograph of Giemsa's-stained blood smear of dog showing numerous Trypanosoma organisms (x1000)

The clinical signs along with the microscopic examination of trypanosomes through blood smear examination suggested a case of trypanosomosis. However, it was difficult to distinguish the species of *Trypanosoma* by microscopical examination, hence, the species was confirmed by PCR amplification of 227 bp of the VSG gene of *Trypanosoma evansi* using species specific primers (Fig. 7), subsequently sequenced (Accession number - OP627198.1) and phylogenitically analyzed. Italics

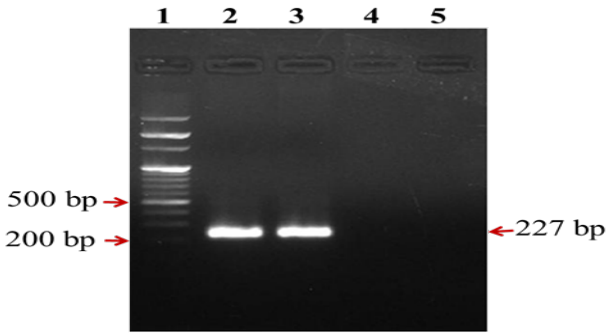

Fig. 7: Agarose gel electrophoresis of parasite DNA, L 1: 100 bp DNA ladder, L 2 & 3 : Amplification of 227 bp fragment of VSG gene of *Trypanosoma evansi*
L 4 & 5 : Negative control

Fig. 8: Maximum Likelihood phylogenetic analysis of VSG gene of *T. evansi*. Numbers at nodes represent bootstrap support (500 bootstrap replicates). Sequences obtained during this study are indicated with red dots

The nucleotide sequences were aligned and edited using the BioEdit 7.0 software and compared with those available in the GenBank database using Basic Local Alignment Search Tool (BLAST). To investigate the evolutionary relationship, phylogenetic analysis of twelve deduced nucleotide sequences in GenBank was used to compare the relationship of *T. evansi* with other strains using the MEGA X software (8). The evolutionary relationship tree was inferred using the Maximum Parsimony method (9). The analysis revealed that *T. evansi* was grouped into two major clusters. It was observed that *T. evansi* sequences from the Philippines (JQ653273), and Orathanadu, TN, India (OP627198) were belonging to the

cluster II, and the remaining ten sequences were clustered together exclusively in cluster I (Fig. 8). In the cluster I, various subspecies of *T. evansi* from Nigeria (MZ394795), Indonesia (MW489882), Chennai-India (MW489880), Kerala-India (MG600142), Malaysia (MW489885), Iraq (MH697863), Jabalpur-India (OK210088), Israel (HM209054), and Gujarat- India (MW221259) were belonging to the sub-cluster I. The overall mean distance was 0.370. *T. evansi* sequences of cattle in Philippines (JQ653273) and camel in Iran (MK752393) showed 100% and 98 % similarity with our sequences (OP627198), respectively. However, the other strain sequences were dissimilar with *T. evansi* sequences from various countries (37 - 100%).

Discussion

Trypanosomosis is a highly pathogenic hemoprotozoan disease of vertebrate animals and is mechanically transmitted by biting insects such as *Tabanus* and *Stomoxys* species (3). The source of infection in dog is always plausible. In a survey conducted at Ludhiana, India, 4.68% (3/64) of dogs were sub-clinically infected through examination of blood smears during the rainy season and those dogs were kept mainly in an area near to the dairy cattle. The contamination could have been occurred either orally by ingestion of aborted placenta or foetuses eliminated by infected females (10). Therefore, livestock act as a reservoir and play an important role in the maintenance of trypanosomes in endemic areas. In our study, we also postulate that the infected dog might have contracted the infection from nearby livestock farms either through biting flies or by eating contaminated sheep and goat meat that was offered as regular diet. It is probable that the dog may be infected by *Stomoxys* spp. or *Hippobosca* spp. fly transmission from infected domestic animals such as cattle, buffaloes and horses, however, the epidemiological studies are needed to confirm this hypothesis.

The major clinical signs of canine trypanosomiasis were neurological, ocular and vascular. They occurred as acute, sub-acute and chronic infections, mostly seen in adult dogs and characterized by myocardial dilation and ventricular arrhythmias. This may be due to the release of proteolytic enzymes by the parasite such as proteases and phospholipases, which initiate a cascade of events leading to hemolytic anemia that eventually could lead to cardiovascular collapse (11). Anemia was the most characteristic feature of canine trypanosomosis (12). The mechanism of injuries on erythrocytes by contact movement of the flagellum was plausible and may be due to extravascular hemolysis because of adsorption of *Trypanosoma* antigen on RBC surface (3). Biochemical changes such as uremia and elevated creatinine observed in the study could be due to kidney dysfunction upon tissue damage caused by parasitemia. Hypoglycemia may be due to utilization of blood glucose by the parasites in the circulation. The decrease in serum albumin could be attributed to the decreased liver biosynthesis and progressive loss of albumin in urine.

Diminazene aceturate had been used as a drug of choice for the treatment of trypanosome infection in livestock, but its use was limited in canine and equine species (13). In the present study, the dog was treated with intramuscular injection of diminazene aceturate at the dose rate of 3.5 mg/kg b.wt along with the supportive therapy; however, the dog was collapsed two days later. This result was in contradictory with the other studies that the dogs were treated with a single dose of diminazene aceturate at 3.5 mg/kg b.wt and observed good clinical improvement (14). These effective results could be possible that the affected dogs may be treated at early stages of the infection. Mortality of the dog observed in the current study might be occurred due to multi-organ failure where the dog was brought for the treatment during an advanced stage of the *Trypanosoma* infection.

Conclusion

The present study described the occurrence and diagnosis of *T. evansi* in a Rottweiler dog. The etiological agent was confirmed by blood smear examination and further characterized by molecular technique. The standard treatment protocol was followed using diminazene aceturate and supportive therapy. Molecular techniques are warranted to differentiate species of *Trypanosoma* sp. in dogs. In spite of treatment, the dog found to have multiorgan failure and died two-days later. This study clarifies that prompt attention is needed to diagnose and treat trypanosomiasis in canines at earliest to overcome its systemic effects, multiorgan failure and death.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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