

Clinical features and management of *Trypanosoma evansi* infection in buffaloes

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The haemoprotozoan parasite *Trypanosoma evansi* is endemic in most parts of the globe and can affect both domestic and wild animals (Desquesnes *et al.* 2013). In India, *T. evansi* is the primary cause of trypanosomiasis, commonly referred to as ‘Surra’. The disease has severe consequences in animal husbandry production system (Juyal *et al.* 2005). The bovine trypanosomiasis is primarily caused by *T. evansi* and transmitted by *Tabanid* flies. The disease negatively influences animal health and can cause a significant negative impact on public health (Singla *et al.* 2013). The clinical signs of the disease include inadequate draught power, abortion, reduced milk production, infertility, and neurological problems leading to the death of affected animals. The transmission of disease in herd causes devastating financial losses to dairy farmers (Ponnudurai *et al.* 2015).

In this study, six buffaloes who showed varied signs including, anorexia, emaciation, changes in body temperature (either normal or elevated), abnormalities in eye health (cloudiness or sudden blindness), behavioural changes (such as depression or unusual circling), specific localized or generalized swelling (e.g. leg swelling), normal or altered patterns of feed intake, water consumption, urination, and defecation were clinically examined for trypanosomiasis.

Further, the animals were also tested for *Trypanosoma* by combination of conventional and sero-molecular methods to detect the presence of the parasite. Primary screening was done by Giemsa-stained thin layer microscopy examination and Card Agglutination Test for Trypanosomiasis (CATT) as described earlier by Singla *et al.* (2013). Subsequently all the total blood samples were subjected to polymerase chain reaction (PCR) which precisely targeted repetitive nucleotide sequences (227 bp) of variable surface glycoproteins of *T. evansi* (Wuyts *et al.* 1994). The total DNA from packed cell volume was extracted using QIA (Qiagen India Pvt Ltd) total DNA extraction kit as per manufacturer’s instructions. The amplification was done

using Forward (TGCAGACGACCTGACGCTACT) and Reverse (CTCCTAGAAGCTTCGGTGTCTCT) primers. Amplified products were analysed by Agarose gel electrophoresis (1.5%) and observed under UV transilluminator. The diagnostic result for *Trypanosoma* detection is shown in Figs. 1 and 2.

In case 1, the animal was depressed, anorexic with

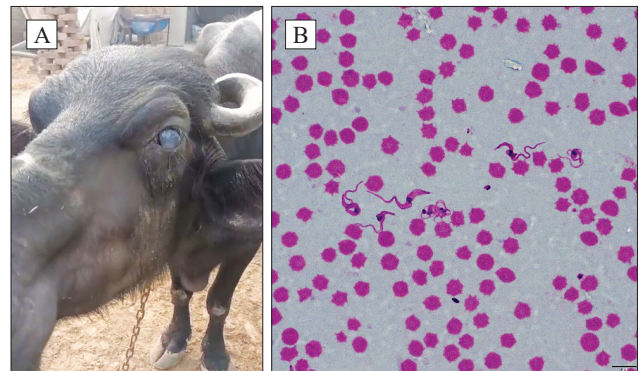


Fig. 1. (A) Representative buffalo showing corneal opacity; (B) Representative Giemsa stained thin-layer blood smear observed under microscope showing *T. evansi* (objective- 100×).

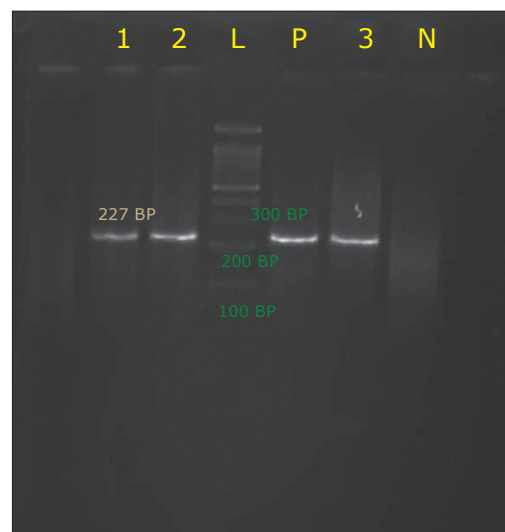


Fig. 2. Agarose gel (1.5%) electrophoresis showing amplified DNA from *T. evansi* (227 bp) [Lane L: molecular size marker 100 bp plus, Lane N: Negative control, Lane P: Positive control, Lane 1, 2, 3: DNA template of case 2, 3 and 5].

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Table 1. Details of buffaloes tested for Trypanosomiasis

Case or animal no.	Age (in years)	Sex	Salient clinical sign			Laboratory diagnosis		
			Corneal opacity	Circling movement	Anaemia	Thin-layer microscopy	CATT	PCR
239	3	Female	+++	No	++	Positive	+++	Yes
240	7	Female	++	Yes	+	Positive	+++	Yes
241	3	Female	+	No	++	Negative	+++	Yes
242	4.5	Female	-	No	++	Negative	++	Yes
244	3	Female	++	Yes	No	Negative	++	Yes
246	8	Female	++	No	No	Positive	+++	Yes

normal temperature (101.6°F), from last 10 days with no passage of faeces for the same period and no ruminal motility was observed. The heart rate and respiratory rate was in the normal range and mucus membranes were pale. Acute peritonitis was observed on ultrasound examination of abdomen, showing peritoneal reaction at cranio-ventral junction of rumen and reticulum at 7th ICS on left side and also at middle of 13th ICS on right side of abdomen near liver. The CBC was normal with no manifestation of anaemia. The PCR analysis of whole blood and peritoneal fluid collected from above mentioned sites revealed the presence of genome of *T. evansi*, thus indicating the aberrant migration of trypanosomes in the peritoneum. Similarly for case 2, a 7-year-old buffalo exhibited symptoms such as anorexia, emaciation, and circling over a period of three days. Trypanosomes were clearly confirmed under thin-layer microscopy. Notably, the buffalo maintained a normal body temperature of 100.7°F. The animal was severely anaemic. The observed increase in total bilirubin (3.8 mg/dl), AST (205 U/L), GGT (24 U/L), BUN (35 mg/dl), and creatinine levels (3.4 mg/dl) suggested damage to both liver hepatocytes and renal parenchyma.

In case number 3, a 3-year-old buffalo was emaciated for duration of more than two months. There was bilateral corneal opacity. The buffalo had a normal body temperature of 100.2°F with severe anaemia. A comprehensive biochemistry panel further revealed several abnormalities, indicating systemic involvement. With respect to fourth buffalo examined, there was painful swelling in the legs over a 7-day period. The buffalo had preference for sitting, while maintaining normal feed and water intake. A comprehensive biochemistry panel revealed increased levels of liver enzymes, including AST (200 U/L), ALT

(67 U/L), and GGT (27 U/L), suggesting hepatocellular degeneration. Furthermore, the hypoalbuminemia (2.9 g/dl) in serum showed impaired liver function and protein metabolism. Haematological analysis showed an absolute neutrophilia with a severe left shift, indicative of an ongoing inflammatory response, possibly related to the underlying infection. In case 5 of a 3-year-old buffalo with a 15-day history of fever (104°F), reduced appetite, and decreased milk yield (15 to 6 kg), with bilateral eye cloudiness and generalized leg swelling, there was neutrophilic leukocytosis with a mild left shift, suggesting an ongoing inflammatory response. Microscopic examination was negative, but serological and molecular analyses confirmed the presence of *T. evansi*. In suspected sixth buffalo, sudden blindness was reported. Despite the blindness, the animal maintained normal patterns of feed intake, water consumption, urination, and defecation. A thorough diagnostic examination revealed that the buffalo was microscopically negative for *T. evansi*. However, the animal was seropositive and molecularly positive for *T. evansi*, indicating the presence of the parasite in its bloodstream. In addition to the ocular manifestation, a comprehensive biochemistry panel revealed abnormalities suggestive of liver dysfunction.

In developing countries like India, the economy heavily relies on agriculture and animal husbandry. Therefore, effective disease surveillance and epidemiological reporting are crucial, especially for veterinary diseases (Moody *et al.* 2000). Vector-borne diseases can exacerbate under stressful conditions. Serological assays help pinpoint high-risk areas that might otherwise be overlooked. PCR demonstrates superior sensitivity in detecting latent *T. evansi* infections (Muieed *et al.* 2010), crucial for timely treatment and reducing morbidity and mortality in animals. The CATT/

Table 2. Haematobiochemical parameters of *T. evansi* infected buffaloes

Parameter	Hb (g %)	TP (mg/dL)	Albumin (g/dL)	Glu (mg/dL)	AST (U/L)	TBIL (mg/dl)	GGT (U/L)	BUN (mg/dL)	Creatinine (mg/dL)
Case 1	ND	4.0	2.4	21	850	3.4	128	ND	ND
Case 2	4.3	5.2	1.8	25	205	3.8	24	35	3.4
Case 3	5.0	5.9	1.4	14	195	2.9	64	29	5.7
Case 4	12.00	ND	2.9	ND	200	ND	27	ND	ND
Case 5	11.2	6.0	1.5	40	125	1.2	11.0	17.	1.2
Case 6	10.8	8.2	2.3	59	210	0.6	47	16	ND

ND, nothing abnormal detected.

T. evansi test emerges as a valuable tool for early diagnosis and intervention in field conditions, effectively managing covert infections (Parashar *et al.* 2018).

The severity and occurrence of the disease caused by *T. evansi* depend on the parasite strain and the host species involved. Infections vary in intensity, influencing the clinical signs displayed by affected animals. Latent *T. evansi* infections can prolong the pre-patent period, during which trypanosomes may not be detectable in the blood even after symptoms appear. This delay in detection can contribute to increased morbidity and mortality (Singla *et al.* 2013).

Cattle typically experience subclinical infections and serve as reservoirs for this hemoprotozoan parasite (Sharma *et al.* 2019). Understanding the prevalence of *T. evansi* infection is crucial for assessing herd health and preventing economic and genetic losses in populations with endemic instability (Smith *et al.* 2000). Moreover, asymptomatic carriers of the parasite can spread infections to other animals (Chandu *et al.* 2021).

Infected animals often show increased GGT levels, possibly due to inflammation and tissue damage in organs like the kidney, liver, heart, and muscles as a result of trypanosome lysis during infection (Parashar *et al.* 2018, Sharma *et al.* 2019). Albumin and total protein levels tend to decrease in infected animals due to factors such as malnutrition, malabsorption, and breakdown of proteins during fever (Migri *et al.* 2016). Stress and dehydration can also contribute to hepatic depletion and reduced protein synthesis. Elevated levels of BUN and creatinine in infected groups may indicate nephrotoxicity and hepatotoxicity caused by toxins produced by parasites, or impaired excretion through the kidneys and increased production in the liver (Truc *et al.* 2013).

In animals testing positive for *T. evansi*, a slight increase in bilirubin levels may be associated with conditions like hemolysis, biliary obstruction, and hepatocyte damage during high parasitemia (Marques *et al.* 2000). A notable finding was the presence of *T. evansi* in the peritoneal fluid of infected buffaloes, similar to findings reported by Sood *et al.* (2011) with *T. theileri*, potentially leading to peritonitis in infected animals.

In conclusion, these case reports highlight the diverse clinical presentations of *T. evansi* infections. The absence of typical symptoms complicates early detection, while the chronic nature of the infection in cattle adds to management challenges. Enhanced surveillance, advanced diagnostic tools, and targeted intervention strategies are crucial to control the spread of *T. evansi*. Increasing awareness among livestock owners and implementing preventive measures are essential steps in mitigating the impact of this parasitic infection in endemic regions.

SUMMARY

Trypanosoma evansi is a haemoprotozoan that causes a severe disease called as *Surra* in domestic and wild mammals. The disease is endemic in India and the clinical

symptoms of disease can vary depending on the host species but generally include fever, anaemia, weight loss, weakness, neurological signs and decreased production capacity. In this study, the clinical observations of the six buffaloes that showed signs of emaciation, corneal opacity, oedema, and circling behaviour was done. All the suspected animals (n=06) were tested for *Trypanosoma* by a combination of methods to detect the presence of the parasite by microscopy, Card Agglutination Test for Trypanosomiasis (CATT) and Polymerase Chain Reaction (PCR). Only three animals were diagnosed positive by microscopy. All buffaloes were found positive for *Trypanosoma* infection by CATT and PCR assay. The haematological and biochemical test indicated significant fall in total haemoglobin (Hb), total erythrocyte count (TEC), packed cell volume (PCV), glucose, total protein, and albumin levels. Additionally, there were appreciable elevations in blood urea nitrogen (BUN), creatinine, gamma-glutamyl transferase (GGT), and total bilirubin levels, indicating liver and renal malfunction. One animal succumbed to infection and had acute peritoneal oedema in postmortem observations. The report underlines the significant clinical and biochemical changes linked to *T. evansi* infection in buffaloes. The study also highlights the need of early detection and treatment in reducing the negative effects of trypanosomiasis on animal health and production.

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