

Haematobiochemical Profiles as Indicators of *Trypanosoma evansi* Infection in Cattle of Selected Agro Ecological Zones of Thrissur and Palakkad Districts of Central Kerala

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ABSTRACT

The study investigated haemato-biochemical changes in *Trypanosoma evansi* infected cattle of Thrissur and Palakkad districts of central Kerala, during a one-year timeframe. Blood samples were collected from 252 randomly selected, apparently healthy cattle exhibiting no evident signs of anorexia or recumbency. Blood smear examination revealed flagellate organisms with central nucleus and an undulating membrane. Confirmation was done using PCR targeting VSG gene which produced a 227 bp product. Complete blood count (CBC) was performed using a three-part automated haematological analyzer on 10 healthy control animals, 24 animals infected with *Trypanosoma* spp. alone and 47 animals that were co-infected with *Anaplasma* spp. and *Theileria* spp., which revealed highly significant differences in the total erythrocyte count, haemoglobin, packed cell volume, mean corpuscular haemoglobin, mean corpuscular volume and mean platelet volume. The serum biochemical values estimated in 10 healthy control animals along with 13 animals infected with *Trypanosoma* spp. alone and 12 animals with *Theileria* spp. and *Anaplasma* spp. showed a highly significant difference in the alkaline phosphatase, albumin and indirect bilirubin in affected individuals. The cattle suffered from macrocytic hypochromic anaemia associated with *T. evansi* infection and had a significant clinical manifestation that results from multiple pathological mechanisms. The parasite's presence in the bloodstream leads to haemolysis, immune-mediated destruction of erythrocytes, and impaired erythropoiesis, contributing to reduced red blood cell count and haemoglobin levels. Regular monitoring and implementing effective vector control strategies are crucial in managing *T. evansi* infections and preventing anaemia in affected cattle.

Keywords: Anaemia, Cattle, Haematology, PCR, Serum biochemistry, *Trypanosoma evansi*

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INTRODUCTION

Trypanosoma evansi is a vector-borne parasite that can thrive in the blood of all classes of vertebrates and are generally transmitted by invertebrate intermediate host. Horse has been incriminated as the natural host for this haemoflagellate and cattle, buffalo and camel act as reservoir host exhibiting subclinical form of the disease (Ponnudurai *et al.*, 2015). It is a haemoflagellate protozoa of both intra and extra vascular fluid and are able to periodically change the major surface glycoprotein (VSG), causing relapses of parasitaemias (Singh *et al.*, 2018). Fever associated with parasitaemia and progressive anaemia due to extra vascular haemolysis and loss of condition and lassitude are the major clinical features of this disease Trypanosomosis or Surra being a wasting disease poses a significant threat to livestock causing economic losses through increased morbidity, mortality, abortion, infertility, reduced milk yield and indiscriminate use of trypanocides (Singh *et al.*, 2018). It is also associated with immunosuppression that led to vaccination failure against haemorrhagic septicaemia (Sivajyothi *et al.*, 2014). The disease is prevalent across India with high incidence in low-lying regions, peaking during the rainy and post-rainy seasons (Rani *et al.*, 2015). Transmission is mechanically by biting flies like *Tabanus*, *Stomoxys*, *Lyperosia*,

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Haematobia, and *Chrysops*, transmitting bloodstream trypomastigotes to a range of mammalian hosts (Sumba *et al.*, 1998).

Awareness on the incidence of *T. evansi* infection is crucial for assessing the herd health and reducing economic

and genetic loss in areas with endemic instability (Smith *et al.*, 2000). Also the asymptomatic carrier animals played a major role in the dissemination of the haemoprotozoan to other susceptible animals (Chandu *et al.*, 2021). The clinical symptoms associated the *T. evansi* infection vary with intensity of infection and species affected. The haemato-biochemical changes associated with local strain of *T. evansi* in cattle in Kerala has not yet been attempted till date. So, the current study was aimed to establish possible alterations in the haemato-biochemical values comparing the infected ones with the healthy cattle and also in case of concomitant infection of common haemoparasites like *Anaplasma* spp. and *Theileria* spp. of central Kerala, establishing a foundation for future research and improving diagnostic and management strategies for the disease in this region.

MATERIALS AND METHODS

The study was conducted in cattle from selected agroecological zones of Thrissur and Palakkad districts of central Kerala from August 2023 to August 2024. 252 cattle which were apparently healthy at the time of examination were selected for the study. Initially all the cattle were tested for the presence of haemoprotozoans by peripheral blood smears examination using Giemsa staining. Blood samples were collected in 5 mL EDTA coated vacutainers and transported in ice boxes to the lab for PCR analysis. The genomic DNA extraction was carried out by modified high salt method according to Montgomery and Sise (1990). The extracted DNA was stored in -20°C until the PCR reaction was carried out. The PCR was performed with species specific primers of Variant surface glycoprotein gene (VSG gene) with an amplicon size of 227 bp (Kumar *et al.*, 2012; Azhahianambi *et al.*, 2018).

Along with this, 4 mL whole blood in vacutainers coated with clot activator and 2 mL blood in EDTA coated vials were collected aseptically from the study population and brought to the University Veterinary Hospital, Kokkalai, Thrissur, for haematological analysis in automatic haematology analyser (Orphee mythic 18 vet). The haematological analysis included 10 healthy control animals, 24 animals infected with *Trypanosoma* spp. alone and 47 animals that were co-infected with *Anaplasma* spp. and *Theileria* spp. The serum biochemistry was measured in Semi-automatic biochemistry analyser (Alpha technologies, Chennai). For the biochemistry analysis, 10 healthy control animals along with 13 animals infected with *Trypanosoma* spp. alone and 12 animals which were simultaneously infected with *Theileria* spp. and *Anaplasma* spp. were taken. Statistical analysis for comparison of each parameter was done by one way ANOVA.

RESULTS AND DISCUSSION

On detailed examination of the study cattle population, the wet film examination revealed the presence of motile trypanosoma organisms between the RBCs in 18 out of 252 animals. Mixed infections where *Anaplasma* spp. and

Theileria spp., were observed in blood smears, affecting 4.7 % (12/252) of the samples examined. Micrometry revealed that the *T. evansi* organisms were approximately $28 \pm 4 \mu\text{m}$ in size (Kumar *et al.*, 2012), (Fig. 1 a,b). John *et al.* (1992) recorded the average size of the parasite as 28.5 to 35 μm . The results of PCR analysis yielded a 227 bp product (Fig. 2). The haematological analysis revealed that TEC, PCV and Hb were reduced significantly ($p < 0.01$) compared to control group indicating anaemia which is multifactorial in origin (Sivajothi *et al.*, 2014).

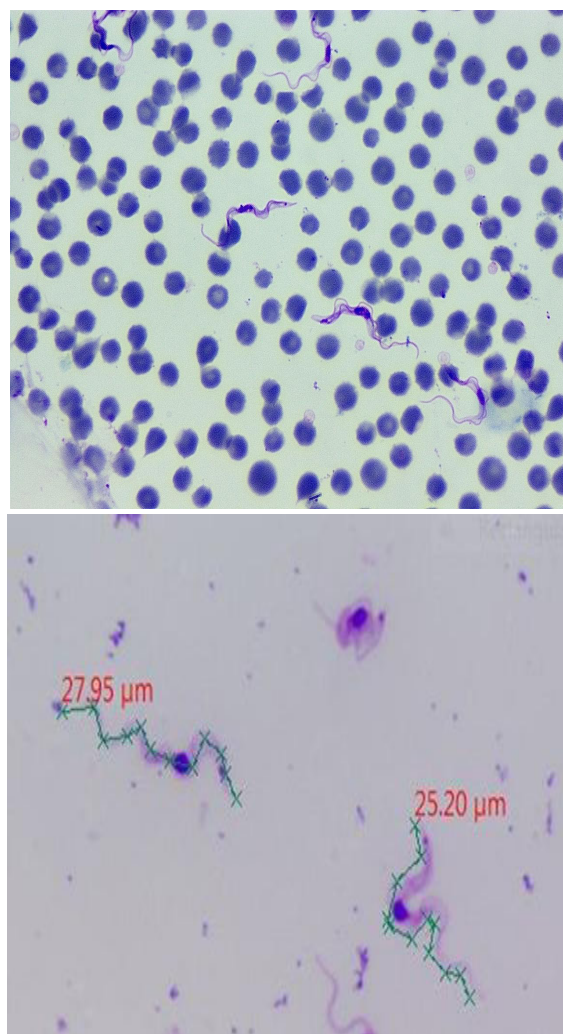


Fig. 1: (a) Giemsa stained blood smear showing *T. evansi*; (b) Micrometry of *T. evansi*

The comparison of haematological parameters between infected and control animals are given in Table 1. The mean TEC value ($3.85 \pm 0.25 \times 10^6/\mu\text{L}$) was significantly lower in *T. evansi* positive animals when compared to the control group ($8.31 \pm 0.13 \times 10^6/\mu\text{L}$). The mean Hb ($7.05 \pm 0.46 \text{ g/dL}$) and PCV ($22.0 \pm 1.34 \%$) of the *T. evansi* infected cattle and that in concomitant infections ($7.44 \pm 0.26 \text{ g/dL}$; $21.97 \pm 0.78 \%$) were significantly different from that of the control groups ($10.5 \pm 0.53 \text{ g/dL}$) and ($33.7 \pm 1.6 \%$). A significant decrease in Hb, PCV and TEC values in cattle were also

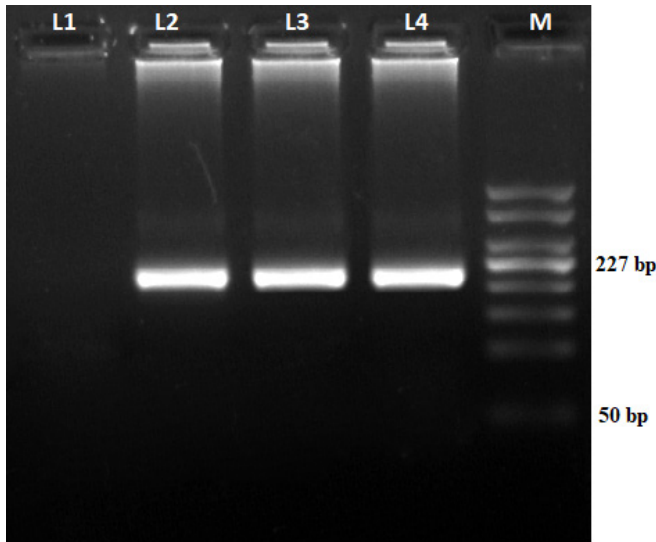


Fig. 2: Amplicons of VSG gene in *T. evansi*
L1- Negative control; L2-L4- Positive samples ; M- 50 bp Ladder

noted by Chaudhri *et al.* (2013), Sivajyothi *et al.* (2014), Pandya *et al.* (2017) and Apsari *et al.* (2024). The decreased RBC count, PCV and morphological anomalies of RBC were the best indicators associated with anaemia in cattle trypanosomiasis (Rossi *et al.*, 2017). They also noticed that adhesion of *T. evansi* to one or more erythrocytes led to the formation of microthrombi occluding capillaries causing necrosis of visceral organs and concluded that the injuries to RBCs promote haemolysis and erythrophagocytosis, which were directly linked to parasitaemia. High densities of trypanosomes in the bloodstream increased the likelihood of parasite-RBC interactions. This association was evident from the parasitaemia that coincides with a sharp decline in haematocrit during experimental infections in cattle (Rossi *et al.*, 2017).

Severe anaemia recorded in this study could be due to the haemolytic factors - haemolysin secreted by trypanosomes and the sialidase enzyme produced by the live or dead pathogens (Walia *et al.*, 1996). These enzymes are capable of RBC lysis and also adhesion of the parasite on to the RBC surface leads to morphological and biochemical changes and its subsequent erythrophagocytosis. This enzyme cleaves sialic acid on the surface of erythrocytes exposing underlying galactosyl residues. These exposed galactosyl residues are then recognized by beta-d-galactose-specific lectins on macrophages. The interaction between the lectins and the galactosyl residues leads to the phagocytosis of the erythrocytes ultimately resulting in anaemia (Sallau *et al.*, 2008). The sialidase and phospholipases damages the RBC membrane leading to production of reactive oxygen species which further causes oxidative stress and also increased erythrocytic lipid peroxidation. The increased production of peroxy radicals and consequent elevated MDA concentration renders the erythrocytes more fragile and prone to lysis (Pandey *et al.*, 2015). Mishra *et al.* (2017) reported a significant decrease in Hb and PCV levels in infected animals compared to healthy ones indicating increased erythrocytic oxidation. The reduced haematological indices and oxidative stress in their study suggested that trypanosome-infected cattle experienced severe anaemia. Additionally, extravascular removal of RBCs occurs in the spleen, bone marrow, and lungs due to erythrocytes being coated with trypanosomal antigens (Connor and Van Den Bussche, 2004). Mechanical injury to the red cells may be another reason behind the increased intravascular haemolysis and consequent highly significant increase noted in the mean corpuscular volume when compared with its control and mean corpuscular haemoglobin when compared to the low value of its control (Benjamin, 2010). Since MCV and MCH are ratios, they tend to increase as RBC count drops. A highly significant

Table 1: Haematological parameters of Trypanosma infected and control cattle

| Parameter | Control group (n= 10) | Trypanosome positive alone (Group 1) (n=24) | Concurrent infections (Group 2) (n=47) | F value | P value |
|---|------------------------------|---|--|---------|---------------------|
| TLC ($\times 10^3/\mu\text{L}$) | 10.46 \pm 0.20 | 8.79 \pm 1.64 | 9.28 \pm 0.92 | 0.23 | 0.79 ^{ns} |
| TEC ($\times 10^6/\mu\text{L}$) | 8.31 ^a \pm 0.13 | 3.85 ^c \pm 0.25 | 4.11 ^b \pm 0.16 | 68.57 | <0.01** |
| Hb (g/dL) | 10.5 ^a \pm 0.53 | 7.05 ^b \pm 0.46 | 7.44 ^b \pm 0.26 | 12.40 | <0.01** |
| VPRC/PCV (%) | 33.7 ^a \pm 1.6 | 22.0 ^b \pm 1.34 | 21.97 ^b \pm 0.78 | 18.44 | <0.01** |
| Lymphocyte ($\times 10^3/\mu\text{L}$) | 3.8 \pm 0.46 | 6.54 \pm 1.57 | 6.69 \pm 0.86 | 0.93 | 0.40 ^{ns} |
| Monocytes ($\times 10^3/\mu\text{L}$) | 0.62 \pm 0.09 | 0.35 \pm 0.03 | 0.42 \pm 0.06 | 2.42 | 0.09 ^{ns} |
| Granulocyte ($\times 10^3/\mu\text{L}$) | 3.83 \pm 0.15 | 2.34 \pm 0.37 | 2.67 \pm 0.36 | 1.69 | 0.19 ^{ns} |
| RDW (%) | 16.75 \pm 0.63 | 17.30 \pm 0.69 | 23.56 \pm 3.31 | 1.32 | 0.27 ^{ns} |
| MPV (μM^3) | 5.83 ^b \pm 0.36 | 6.61 ^{ab} \pm 0.26 | 7.46 ^a \pm 0.19 | 7.80 | 0.001** |
| MCV (μM^3) | 45.7 ^b \pm 1.43 | 63.5 ^a \pm 3.51 | 55.6 ^a \pm 2.03 | 5.78 | 0.005** |
| MCH (Pg) | 14.4 ^c \pm 0.52 | 18.67 ^a \pm 0.47 | 18.53 ^a \pm 0.30 | 17.29 | <0.01** |
| MCHC (g/dL) | 32.34 \pm 0.28 | 31.61 \pm 4.68 | 34.42 \pm 0.86 | 2.5 | 0.08 ^{ns} |
| PLT ($\times 10^3/\mu\text{L}$) | 5.83 \pm 14.3 | 6.61 \pm 45.6 | 7.46 \pm 25.7 | 1.60 | 0.208 ^{ns} |

*Significant ($p < 0.05$), **Highly significant ($p < 0.01$), ^{ns}Non significant ($p \geq 0.05$)

increase between the mean platelet volume (MPV) of the infected cattle ($6.61 \pm 0.26 \mu\text{M}^3$) and control cattle ($5.83 \pm 0.36 \mu\text{M}^3$) were observed which could be attributed to anaemia, thrombocytopenia and altered haemopoiesis and subsequent bone marrow suppression.

There was no significance noted in the monocytes, granulocyte, lymphocyte, mean corpuscular haemoglobin concentration (MCHC) and platelet count (PLT) of the *T. evansi* infected ones and those with concomitant infections.

The comparison of biochemical parameters between infected and the control cattle are given in Table 2. The serum biochemistry analysis showed unaltered BUN and ALT level, but a highly significant increase in the alkaline phosphatase (IU/L) level of the trypanosome infected ones (55.15 ± 5.5) and mixed infections (38.54 ± 4.0) compared to control cattle (26.94 ± 1.5). Alkaline phosphatase, being an enzyme on the cell membrane to hydrolyze extracellular phosphate esters, it was elevated due to tissue damage which result in the leakage of the enzyme from intracellular store into plasma in liver damage (Egbe-Nwiy *et al.*, 2010), bone marrow disorders (Akinseye *et al.*, 2020) and host immune system's destruction by trypanosomes (Enwezor and Sackey, 2005).

There was significant decrease in the levels of albumin (3.50 ± 0.09 g/dL) of infected cattle when compared with healthy control (3.83 ± 0.10 g/dL) (Table 2). Albumin being a crucial extracellular antioxidant that protects vital targets like erythrocytes from inhibiting iron-dependent free radical production (Mishra *et al.*, 2017) makes RBCs more fragile and more prone to lysis. Albumin, produced solely by the liver, regulates water flow between plasma and tissue fluids by colloidal osmotic pressure. Trypanosomes require albumin for growth and multiplication, so high parasitaemia in early infection can increase albumin usage. Combined with haemodilution, this leads to reduced protein synthesis, indicating hepatic dysfunction in trypanosomosis (Megahed *et al.*, 2012; Garjms, 2014). The drop in total serum protein could be due to its reduced synthesis due to liver damage, excessive protein breakdown from low feed intake, albumin catabolism, uptake by trypanosomes, or haemodilution. In chronic trypanosomosis, serum protein increases as globulin level increases in response towards the infection by the animal (Rajora *et al.*, 1986). Serum biochemical alterations

like hypoalbuminemia and an increase in ALP was observed by Ramakrishna and Yoganand (2008) as an indicative of hepatic dysfunction.

Significant difference was noted in the indirect bilirubin (mg/dL) of the trypanosome infected ones (0.64 ± 0.07) when compared to the control (0.34 ± 0.05) and mixed infections at 0.05 level, with the trypanosome-infected animals showing markedly higher values. Total bilirubin also showed similar trend, but the differences were statistically non-significant (Table 2). Hilali *et al.* (2006) observed a similar trend in the indirect bilirubin levels. Benjamin (2010) explained that the rise could be due to thrombocytopenia and increased bone marrow activity. The elevated levels of indirect bilirubin in cattle could be due to the increased rate of haeme protein (haemoglobin and myoglobin) catabolism in chronic trypanosomosis (Ogbu *et al.*, 2023).

The findings of the present study were highly consistent with the previous studies on the haemato-biochemical alterations in cases of trypanosome infected cattle, like, the decreased RBC, Hb, PCV, MPV, MCV, MCH, alkaline phosphatase, albumin and indirect bilirubin. But there was a deviation in the total leucocyte count (TLC) which showed a non-significant decrease in the trypanosome infected ones ($8.79 \pm 1.64 \times 10^3/\mu\text{L}$) when compared to control ($10.46 \pm 0.20 \times 10^3/\mu\text{L}$). The reason might be the polyclonal activation of circulating B-cells to eliminate the parasite (Hilali *et al.*, 2006). The usual severity in anaemia was observed in this haematological study which matched the previous studies.

CONCLUSION

Assessing haematological and serum biochemical changes is crucial for disease prognosis and identifying pathological alterations in the body. The trypanosome infected cattle and those with mixed infections of *Anaplasma* spp. and *Theileria* spp. exhibited drop in TEC, Hb, PCV, and a rise in MCH, MVC, alkaline phosphatase and indirect bilirubin. The decrease in the haematological values indicates anaemia which was macrocytic and hypochromic. Anaemia in trypanosomosis is opined to be multifactorial, involving extravascular and intravascular haemolysis, haemodilution, and inhibition of erythropoiesis, with the primary cause being red cell damage

Table 2: Biochemical parameters of the infected and control animals

| Parameters | Control group (n=10) | Trypanosome alone (Group 1) (n=13) | Concurrent infections (Group 2) (n=12) | F value | P value |
|----------------------------|-------------------------|------------------------------------|--|---------|--------------------|
| BUN (mg/dL) | 14.60±0.9 | 16.18±1.0 | 15.7±1.34 | 0.49 | 0.62 ^{ns} |
| ALP (IU/L) | 26.94 ^b ±1.5 | 55.15 ^a ±5.5 | 38.54 ^b ±4.0 | 10.43 | 0.01 ^{**} |
| ALT (IU/L) | 20.77±1.15 | 24.65±3.8 | 22.79±1.3 | 0.53 | 0.59 ^{ns} |
| Albumin (g/dL) | 3.83 ^a ±0.10 | 3.50 ^{ab} ±0.09 | 3.40 ^b ±0.14 | 3.46 | 0.04 [*] |
| Total bilirubin (mg/dL) | 0.45±0.05 | 0.94±0.19 | 0.88±0.21 | 2.09 | 0.14 ^{ns} |
| Indirect bilirubin (mg/dL) | 0.34 ^b ±0.05 | 0.64 ^a ±0.07 | 0.55 ^{ab} ±0.08 | 3.92 | 0.03 [*] |

*Significant ($p < 0.05$) **Highly significant ($p < 0.01$), ^{ns}Non significant ($p \geq 0.05$)



due to the haemolysins secreted by the trypanosomes. The significant differences in biochemical values indicated severe liver dysfunction and rise in indirect bilirubin levels accounted for haemolysis due to haeme protein catabolism.

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