# Haematological and Biochemical Profiles, Including Vitamin A and Zinc Status, in Clinical and Subclinical Oriental Theileriosis in cattle




## ABSTRACT

**Aim:** The present study investigated the clinical, haematological and biochemical profiles in cattle infected with *Theileria orientalis*, correlating disease severity with various parameters.

**Study design:** A total of 32 clinically ill, 10 subclinically infected, and 10 healthy control animals were included.

**Place and Duration of study:** Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala, between November 2023 to September 2024.

**Methodology:** *Theileria* piroplasms, observed via Giemsa staining, appeared as thick and thin rods with trailing cytoplasm and annular, pyriform, garland, and signet-ring shapes. PCR amplification of the *T. orientalis* major piroplasm surface protein (MPSP) yielded a specific 776 bp product in clinically and subclinically infected animals. **Results:** Haematological analysis revealed significant anaemia and thrombocytopenia in infected groups, without notable changes in leukocyte counts. Serum protein analysis indicated hypoproteinemia and hypoalbuminemia, while liver enzymes, particularly AST, were elevated in clinically infected cattle, suggesting hepatic involvement. Additionally, serum vitamin A and zinc concentrations were significantly lower in infected cattle, underscoring a possible role for oxidative stress in disease pathogenesis.

**Conclusion:** These findings suggest that *T. orientalis* infection is associated with multi-systemic impacts, including haematological abnormalities, liver damage, and nutrient deficiencies, which are crucial for accurate diagnosis and effective management strategies.


**Keywords:** *T. orientalis*, anaemia, haematology, vitamin A, zinc, serum biochemistry

## 1. INTRODUCTION

Tick-borne diseases represent a significant threat to livestock production worldwide, warranting urgent attention. Among these, babesiosis, theileriosis, and anaplasmosis are

critical diseases that substantially affect cattle farming in endemic regions. Theileriosis, in particular, poses a considerable economic burden on high-yielding cattle, especially in tropical and subtropical climates. It is primarily transmitted by arthropod vectors and is caused by various species within the genus *Theileria*, notably *Theileria parva* and *Theileria annulata*. While *Theileria orientalis* was historically regarded as benign and asymptomatic, it has been increasingly recognized for its potential to cause severe clinical disease in cattle.

In India, a meta-analysis by Krishnamoorthy *et al*. (2021) estimated a 20% prevalence of theileriosis, with *T. annulata* (21%), *T. orientalis* (16%), and other *T. species* (17%) identified as contributing factors. Notably, in Kerala, a study by Kariyappa *et al*. (2017) reported prevalence rates of 40% for *T. orientalis* in northern regions and 39.28% in central Kerala. Of the 11 identified genotypes of *T. orientalis*, outbreaks have predominantly been linked to the Chitose and Ikeda genotypes.

The identification of *T. orientalis* in asymptomatic cattle underscores the importance of subclinical infections as reservoirs for disease transmission via tick vectors. The rapid spread of clinical theileriosis is further exacerbated by the movement of these subclinically infected cattle and vectors without adequate quarantine measures. Factors such as stress, immunosuppression, transportation, pregnancy, calving, poor nutrition, and adverse climatic conditions can precipitate clinical illness in these animals. Recent years have seen a troubling increase in clinical cases of oriental theileriosis among cattle and buffaloes in Kerala, characterized by elevated morbidity and mortality rates. However, research investigating the pathogenesis of anemia and its associated clinical and immunological changes in oriental theileriosis remains limited.

This study aims to assess the haematobiochemical changes, including serum vitamin A and zinc levels, associated with oriental theileriosis in cattle from the Wayanad district of Kerala, contributing to a deeper understanding of this emerging threat to livestock health.

## 2. MATERIALS AND METHODS

Cattle with clinical signs such as pyrexia, anorexia, anaemia and enlarged lymph nodes were selected for the present study from different parts of Wayanad district.

Peripheral blood smears from ear veins were collected and stained using Giemsa stain for microscopic examination. Whole blood and serum samples were collected from jugular vein and 2 mL was transferred to EDTA vial and 4 mL to clot activator vial. Polymerase chain reaction was done to confirm the cases of clinical and subclinical oriental theileriosis and also to select healthy control animals (Ota *et al*., 2009). Blood in clot activator vials were then centrifuged at 1000- 2000xg for ten minutes to separate serum. Whole blood samples were subjected to haematological analysis and serum samples were subjected to biochemical analysis and estimation of Vitamin A and zinc.

A complete blood count including the following parameters such as total erythrocyte count (TEC), haemoglobin (Hb), volume of Packed Red Cells (VPRC), thrombocyte count, total leucocyte count (TLC), lymphocytes (L), monocytes (M), granulocytes (G) were estimated

using a four-part fully automated haematology analyser (Mindray BC-30Vet) within 12 hours of collection of samples.

Biochemical parameters were estimated using semi-automatic serum biochemical analyser (MISPAVIVA 2578-10/17). Commercially available biochemical kits of Serum total protein (TP), albumin, aspartate aminotransferase (AST), creatinine, gamma-glutamyl transferase (GGT), total bilirubin and direct bilirubin were used for estimation. Serum vitamin A was estimated by high performance liquid chromatography (HPLC) (Dionex Ultimate 3000 UHPLC) and serum zinc level by atomic absorption spectroscopy (AAS) (PerkinElmer, USA).

## 2.1 Statistical analysis

Data obtained were entered into a Microsoft Excel Spreadsheet and subjected to statistical analysis in IBM-SPSS software version 24.0. Comparison of haematological and serum biochemical parameters were done using one-way ANOVA followed by Duncan Multiple Range Test (DMRT) for finding out which of the groups shows significant difference between each other.

## 3. RESULTS AND DISCUSSION

A total of 32 animals were confirmed to be clinically ill due to oriental theileriosis, while 10 were identified as subclinically infected. Ten healthy animals served as the control group. Morphologically, Theileria piroplasms predominantly presented as thick and thin rods with trailing cytoplasm, along with other forms such as annular, pyriform, garland, and signet-ring shapes (fig.1). PCR analysis successfully amplified a ~776 bp fragment specific to *T. orientalis* MPSP, confirming the presence of the parasite in both clinically and subclinically infected animals (fig.2).

Haematological analysis revealed significant anaemia and thrombocytopenia in both groups of infected animals, with marked reductions in red blood cell count (RBC), haemoglobin concentration, and packed cell volume (PCV) compared to healthy controls (Table 1). These findings align with previous research indicating erythrocyte destruction as the primary cause of anaemia, likely due to direct parasitic invasion (Kawamoto *et al*., 1990). Notably, total leukocyte count (TLC) and differential leukocyte count (DLC) showed no significant changes, suggesting variable leukocyte responses based on the infection stage, consistent with earlier studies (Izzo *et al*., 2010; Haron *et al*., 2014).

Biochemical analysis indicated a significant reduction in total serum protein and albumin levels in infected cattle (Table 2), potentially linked to lymphadenopathy and impaired lymphatic drainage (Goud *et al*., 2021). Elevated aspartate aminotransferase (AST) levels, primarily in clinically infected animals, suggest hepatic damage likely resulting from parasite-induced hepatocellular injury (Shivakumar *et al*., 2022). Although gamma-glutamyl transferase (GGT) levels were elevated, the increase was not statistically significant, which may reflect hypoxic liver injury secondary to anaemia (Forshaw *et al*., 2020).

The serum analysis of vitamin A and zinc showed a significant decrease in vitamin A and zinc concentrations in clinical and subclinical cases compared to controls (Table 3). This depletion

of vitamin A, a crucial antioxidant, may exacerbate oxidative stress and contribute to the severity of anaemia (Razavi *et al*., 2011). The decrease in vitamin A likely reflects depleted liver stores, as Thurnham and Singkamani (1991) suggested. Similarly, the reduced zinc levels could impair the animal's antioxidant defenses, potentially worsening oxidative damage from the parasite (Carmo *et al*., 2022).

The findings underscore the complex multi-systemic impacts of *T. orientalis* infections. Anaemia and thrombocytopenia are prominent haematological abnormalities, likely due to direct destruction of erythrocytes and potential consumption of platelets from parasite products (Çöl, 2006). The biochemical disturbances, particularly hypoalbuminemia and elevated AST levels, reinforce the notion of hepatic involvement as part of theileriosis pathogenesis, possibly exacerbated by hypoxia-related liver damage.

Moreover, the significant reductions in serum vitamin A and zinc levels indicate potential antioxidant deficiencies that could contribute to oxidative stress, further complicating anaemia and overall disease severity. Vitamin A deficiency has been linked to increased oxidative damage and impaired haemoglobin synthesis, highlighting its importance in the context of theileriosis (Razavi *et al*., 2011). Likewise, zinc deficiency may arise from the parasite's metabolic demands or the host's stress response. Zinc plays a critical role in the body's antioxidant defense, particularly as a cofactor for copper-zinc superoxide dismutase (SOD), which catalyzes the conversion of harmful superoxide radicals into hydrogen peroxide (Carmo et al., 2022).

In conclusion, *T. orientalis* infection induces profound haematological, biochemical, and antioxidant imbalances in affected cattle, complicating its clinical manifestations. These alterations are critical for developing effective diagnostic and therapeutic strategies and improving the management of theileriosis in endemic regions. Further investigation into the relationships among oxidative stress, micronutrient levels, and disease progression will be essential to fully elucidate the impact of *T. orientalis* on cattle health.

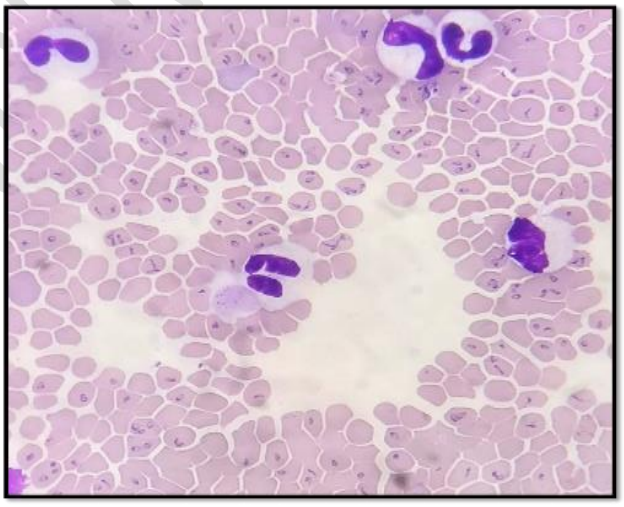

**Fig.1. Theilerial piroplasms in the erythrocytes (1000xmagnification)**

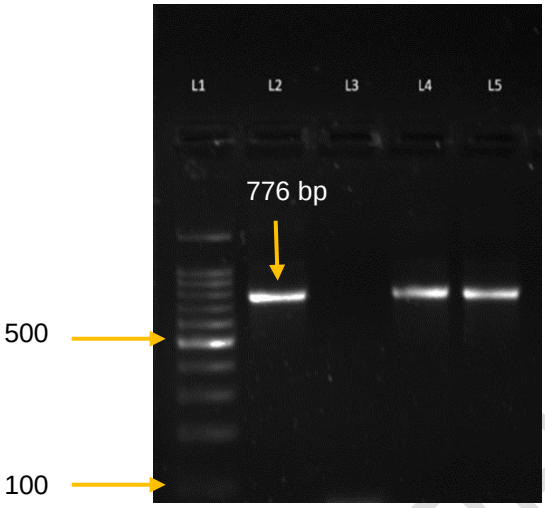


**Fig.2 Molecular detection of *T. orientalis***

**Table 1.** Comparison of different hematological parameters between clinically infected, subclinically infected and control groups

| Haematological parameters | Clinically infected cattle | Subclinically infected cattle | Control | F-value (P-value) |
|---|---|---|---|---|
| Total red blood cell count ($10^6$/µL) | $4.27^b \pm 0.28$ | $4.95^b \pm 0.41$ | $6.63^a \pm 0.39$ | 6.84** (0.003) |
| Total leucocyte count ($10^3$/µL) | 11.60 ± 0.81 | 9.37 ± 0.77 | 9.43 ± 0.33 | $1.282^{ns}$ (0.288) |
| Haemoglobin concentration (g/dL) | $6.42^b \pm 0.39$ | $7.72^b \pm 0.34$ | $10.18^a \pm 0.59$ | 9.445** (<0.001) |
| Granulocytes (%) | 43.64 ± 2.86 | 44.36 ± 2.47 | 42.30 ± 3.51 | $0.032^{ns}$ (0.969) |
| Lymphocytes (%) | 51.12 ± 2.89 | 50.94 ± 2.46 | 52.05 ± 3.29 | $0.012^{ns}$ (0.989) |
| Monocytes (%) | 5.20 ± 0.42 | 4.71 ± 0.42 | 5.63 ± 0.57 | $0.279^{ns}$ (0.758) |
| Thrombocyte count ($10^5$ /µL) | $342.84^b \pm 35.59$ | $353.17^b \pm 42.24$ | $570.50^a \pm 44.32$ | 3.972* (0.026) |
| Volume of packed red cells (%) | $20.44^b \pm 1.18$ | $29.78^a \pm 1.83$ | $30.60^a \pm 2.2$ | 10.466** (<0.001) |

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*

*Means having different letter as superscript differ significantly within a row*

**Table 2. Comparison of different serum biochemical parameters between clinically infected, subclinically infected and control groups**

| Serum biochemical parameters | Clinically infected cattle | Subclinically infected cattle | Control | F-value (P-value) |
|---|---|---|---|---|
| Total protein (g/dL) | $5.60^{b} \pm 0.20$ | $5.73^{b} \pm 0.21$ | $6.89^{a} \pm 0.11$ | 4.415* (0.018) |
| Albumin (g/dL) | $2.29^{c} \pm 0.08$ | $2.81^{b} \pm 0.1$ | $3.36^{a} \pm 0.07$ | 17.144** (<0.001) |
| Globulin (g/dL) | 3.30 ± 0.18 | 2.92 ± 0.12 | 3.54 ± 0.06 | $0.767^{ns}$ (0.471) |
| AST (IU/L) | $141.84^{a}$ ± 14.22 | $74.30^{b} \pm 3.86$ | $68.79^{b} \pm 6.02$ | 4.382* (0.019) |
| GGT (IU/L) | 20.62 ± 1.73 | 13.65 ± 0.90 | 14.23 ± 1.20 | $2.643^{ns}$ (0.083) |
| Creatinine (mg/dL) | 0.837 ± 0.042 | 0.960 ± 0.024 | 0.953 ± 0.046 | $1.375^{ns}$ (0.264) |
| Total bilirubin (mg/dL) | 0.783 ± 0.176 | 0.250 ± 0.034 | 0.302 ± 0.033 | $1.504^{ns}$ (0.234) |
| Direct bilirubin (mg/dL) | 0.392 ± 0.079 | 0.143 ± 0.021 | 0.120 ± 0.025 | $1.936^{ns}$ (0.157) |

*** Significant at 0.01 level; * Significant at 0.05 level; ns non-significant*

*Means having different letter as superscript differ significantly within a row*

**Table 3. Comparison of vitamin A and zinc between clinically infected, subclinically infected and control groups**

| Group | Vitamin A | Zinc |
|---|---|---|
| Control group | $0.057^{a} \pm 0.007$ | $178.35^{a} \pm 7.92$ |
| Subclinically infected cattle | $0.025^{b} \pm 0.007$ | $118.60^{b} \pm 11.00$ |
| Clinically infected cattle | $0.028^{b} \pm 0.005$ | $93.65^{b} \pm 14.39$ |
| F-value (P-value) | 8.180** (0.004) | 14.535** (<0.001) |

*** Significant at 0.01 level*

*Means having different letter as superscript differ significantly within a column*

## 4. CONCLUSION

In conclusion, the study highlights the significant impact of *T. orientalis* infection on cattle, revealing profound haematological and biochemical disturbances alongside antioxidant deficiencies. Clinically ill animals exhibited marked anaemia and thrombocytopenia, indicating the direct destruction of red blood cells likely due to parasitic invasion. The biochemical profiles further demonstrated impaired liver function and compromised protein levels, underscoring the multi-systemic effects of theileriosis. Notably, reductions in serum vitamin A and zinc levels suggest a link between oxidative stress and disease severity, as these micronutrients are vital for maintaining cellular health and antioxidant defences. These findings emphasize the need for effective diagnostic and therapeutic strategies to manage theileriosis in affected regions. Understanding the intricate relationships among parasitic load, nutritional status, and clinical outcomes will be crucial for enhancing cattle health and mitigating the effects of this disease. Future research should focus on the mechanisms underlying these alterations and explore potential interventions that could restore nutrient balance and improve resilience against *T. orientalis* infection. Overall, this study contributes valuable insights into the pathogenesis of theileriosis and its implications for cattle management in endemic areas.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies and text-to-image generators have been used during the writing or editing of this manuscript.

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