Theileria annulata infection: disruption of erythrocyte antioxidant defense and enhanced lipid peroxidation in bovine Theileriosis

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Abstract

Bovine theileriosis is a major haemoprotozoan, lymphoproliferative disease of cattle caused by the intracellular parasite *Theileria annulata*, leading to substantial economic losses due to reduced productivity and increased mortality. Anaemia and its associated complications in theileriosis are strongly influenced by oxidative stress. The present study was undertaken to evaluate haematological parameters, erythrocytic antioxidant status, inflammatory mediators, and serum biochemical alterations in cattle naturally infected with *T. annulata*.

The study included 20 clinically healthy cattle (control group) and 20 clinical cases of tropical theileriosis presented to the Veterinary Clinical Complex, Bihar Veterinary College, Patna. Diagnosis was confirmed by blood smear examination and polymerase chain reaction. Haematological evaluation included TEC, TLC, Hb concentration, MCV, and PCV. Oxidative stress and antioxidant status were assessed by estimating erythrocytic SOD, catalase, GSH, MDA, and circulating interleukins (IL-6 and IL-1β). Serum biochemical parameters such as AST, ALT, total bilirubin, total protein, and albumin were also analyzed. Cattle affected with theileriosis exhibited moderate to severe anaemia, evidenced by significantly reduced TEC, Hb, PCV, and MCV values compared to controls. Antioxidant enzyme activities (SOD, catalase, and GSH) were markedly decreased ($p<0.0001$), whereas MDA levels were significantly elevated ($p<0.0001$), indicating enhanced lipid peroxidation. A concomitant increase in IL-6 and IL-1β levels ($p<0.0001$) reflected heightened inflammatory responses. Serum biochemical analysis revealed significantly elevated AST and total bilirubin levels ($p<0.0001$), suggestive of hepatic dysfunction, while total protein and albumin levels remained unaltered. These findings indicate oxidative damage to erythrocytes and compromised antioxidant defenses, contributing to anaemia in bovine theileriosis.

Key words: Tropical Theileriosis; Oxidative stress; Antioxidant status; Peroxidation

Introduction

Bovine theileriosis, particularly Tropical theileriosis caused by *Theileria annulata*, poses a significant challenge to cattle health and the livestock industry in developing countries (Denizhan et al 2017; Farhang 2017). This disease is transmitted primarily by the tick *Hyalomma anatolicum*, which thrives in India's diverse climatic conditions (Krishnamoorthy *et al.,* 2021). Bovine theileriosis has a significant impact in India, leading to considerable economic losses due to reduced milk output, weight reduction, and increased mortality rates among infected cattle. In India, the prevalence of bovine theileriosis is heightened by the extensive distribution of tick carriers and the large population of susceptible indigenous and crossbred cattle. The disease can lead to mortality rates as high as 80% in vulnerable cattle populations. Following infection, *Theileria annulata* sporozoites invade bovine leukocytes where they differentiate into schizonts that induce host cell proliferation and subsequently release merozoites that invade erythrocytes, completing the parasite's life cycle and contributing to clinical disease manifestations (Elati *et al.,* 2024), with affected cattle typically suffering from varying levels of leukopenia and/or anemia (Verma and Singh, 2016). Progressive haemolytic anaemia is a key pathological feature of bovine theileriosis, resulting from parasite-mediated erythrocyte destruction, oxidative damage–induced membrane fragility, autoimmune reactions, and intraerythrocytic piroplasms. Parasite-induced oxidative stress enhances lipid peroxidation and weakens antioxidant defenses, leading to premature erythrocyte lysis and reduced haemoglobin, packed cell volume, and erythrocyte indices in infected cattle (Pandey *et al.,* 2017). Immune-mediated hemolysis also plays a significant role, with secondary autoimmune responses promoting erythrocyte clearance in infected animals (Jalali *et al.,* 2018). In addition, the proliferation of infected leukocytes and subsequent inflammation may exacerbate erythrocyte damage and contribute to anaemia severity (Agina *et al.,* 2020). Crossbred and exotic cattle generally show higher susceptibility to infection and more pronounced anaemic changes compared to indigenous breeds, likely due to breed-specific differences in immune regulation and host response to *Theileria annulata* (Valente *et al.,* 2022).

While a substantial part of the disease pathology is linked to alterations in hemato-biochemical variables, recent studies indicate that anaemia in haemoprotozoan infections such as bovine theileriosis is also associated with oxidative damage to erythrocytes, where reactive oxygen species and lipid peroxidation compromise red blood cell integrity. Enhanced erythrocytic oxidative stress, marked by increased malondialdehyde (MDA) and reduced antioxidant defenses (such as decreased superoxide dismutase and catalase activity), has been documented in cattle affected by *T. annulata*, correlating with increased erythrocyte fragility and reduced hematological indices. These oxidative changes disturb membrane stability and promote erythrocyte destruction, contributing significantly to anaemia in infected animals (Pandey *et al.,* 2017). Recently, the application of MDA as a marker for lipid peroxidation has become increasingly popular in studies exploring its role in various diseases (Mohideen *et al.,* 2023). Although antioxidant vitamins are reported to mitigate oxidative stress in parasitic infections such as bovine tropical theileriosis, their role in protecting erythrocytes from oxidative damage remains unclear. Furthermore, the involvement of pro-inflammatory cytokines in the immunopathogenesis of *T. annulata* has been inadequately explored. Integrated assessment of hematological changes and variations in biochemical indices, antioxidant enzymes, and inflammatory cytokines offers meaningful insight into the severity of disease. The objective of this study was to assess the activity levels of erythrocyte glutathione peroxidase, superoxide dismutase, catalase, and glucose-6-phosphate dehydrogenase, which serve as vital indicators of antioxidant status, along with the level of malondialdehyde, serving as a biomarker of oxidative damage to erythrocytes in cattle exhibiting clinical symptoms of theileriosis.

Materials and Methods

Animals and Sample Collection

Twenty adult cattle (>1 year of age) suffering from Theileriosis and referred to Veterinary Clinical Complex, Bihar Veterinary College during the monsoon season when the cases of bovine theileriosis is highly prevalent in several districts of Bihar in and around Patna region were selected. For control group, 20 clinically healthy parasitologically free cattle from Livestock Farm Complex of Bihar Veterinary College were sampled during the peak incidence of theileriosis. Infected cattle were screened through a combination of clinical examination, positive results from blood smears based on piroplasm percentage, and cyto b1 target-based PCR testing. The clinical symptoms of *T. annulata* infection were monitored and documented. The recorded clinical signs of *T. annulata* infection included fever, lymph node swelling, nasal discharge, corneal opacity, and increased tearing. Thin blood smears were collected from the ear veins of all cattle. These smears were stained with 8% Giemsa stain and then examined using an Olympus microscope (Olympus, Japan) with an oil immersion lens at 1000x magnification for parasitemia percentage.

Molecular diagnosis with cyto b1 target based PCR assay

The genetic confirmation of *T. annulata* infection was conducted using PCR targeting the cyto b1 gene. DNA extraction from whole blood was performed following the manufacturer's instructions of the APSLABS Genomic DNA Minikit, (Catalog no-MAGSPIN-50). The extracted DNA was suspended in 50 µL of rehydration

buffer/ nuclease free water and then stored at -20°C. Cytochrome b sequences of *T. annulata* (accession number XM949625.1) were obtained from the National Center for Biotechnology Information (NCBI). The primer sequences utilized in the study, listed in the 5' to 3' orientation, are as follows: Forward primer Cytob1 - ACTTTGGCCGTAATGTTAAAC, and Reverse primer Cytob1 – CTCTGGACCAACTGTTTGG. PCR amplification was performed in a total reaction volume of 25 µL using standard PCR reagents. The amplification was carried out in a Bio-Rad MJ Mini Gradient Thermal Cycler with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 50 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. The amplified products (10 µL) were resolved on 1.5% agarose gel containing ethidium bromide in TAE buffer, electrophoresed at 75 V for 1 h, and visualized using a Bio-Rad XR+ Gel Documentation System. Additionally, both positive and negative control samples were included during the amplification process. The positive control contained DNA extracted from *Theileria annulata*-infected lymph, while the negative control contained no DNA at all.

Blood sampling for biochemical assays and routine haematological analysis

Blood samples were drawn from the jugular vein and placed in evacuated vacutainer tubes with EDTA-K2 (ethylene diamine tetra acetic acid dipotassium salt) for routine blood testing, as well as in heparinized glass-stoppered tubes for further analysis. An automated hematology analyzer (Nihon Kohden) was used to perform a complete blood count, which included hemoglobin concentration, packed cell volume (PCV) values, differential WBC counts, and counts of red and white blood cells (RBC and WBC), as described by Schalm *et al.* (1986). Diagnosis was confirmed by microscopic examination of Giemsa-stained thick blood smears prepared from ear vein samples for the presence of intraerythrocytic piroplasms. Piroplasm parasitemia was quantified as described by Shiono et al. (2003b) and expressed as the percentage of parasitized erythrocytes per 100 RBCs. For lipid analysis, 5 mL of blood was collected and centrifuged at 700 g for 15 min at 4 °C using a refrigerated centrifuge (Beckman Coulter Allegra X-30R). Plasma was separated, and the erythrocyte pellet was washed thrice with normal saline and lysed with distilled water under continuous stirring. The resulting hemolysate was used for the estimation of lipid peroxidation and superoxide dismutase activity.

Biochemical assays and analysis

The blood samples collected were allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 minutes to separate the serum, which was aliquoted and stored at −20 °C until analysis. Serum biochemical parameters, including total protein, albumin, total bilirubin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT), were quantitatively estimated using a DRI-CHEM NX500 automatic clinical chemistry analyzer (Fujifilm Corporation, Tokyo, Japan).

Serum reduced glutathione (GSH) was estimated using the method of Prins and Loos (1969). Serum proteins were precipitated using sulfuric acid and sodium tungstate, followed by centrifugation. The supernatant was reacted with DTNB in Tris buffer, and absorbance was measured at 412 nm against a reagent blank. GSH concentration was calculated using the extinction coefficient (EC = 13,100 M^{-1} cm^{-1}) and expressed as mM/mL of serum using the formula:

GSH (mM/mL of serum) = (OD of test × Total reaction volume) / (EC × Volume of supernatant × Dilution factor)

Serum catalase activity was determined spectrophotometrically following Cohen et al. (1970) by monitoring the decomposition of hydrogen peroxide at 240 nm. The decrease in absorbance was recorded at 20-second intervals for one minute. Since a decrease in absorbance of 0.05 corresponds to the disappearance of 3.45 µmoles of H_2O_2, catalase activity was calculated as:

Catalase activity (units/mL) = 1380 × A, where A is the change in absorbance per minute.

The estimation of Superoxide dismutase (SOD) and Lipid peroxidation was carried out in both serum and haemolysate samples. Lipid peroxidation was assessed by estimating malondialdehyde (MDA) using the thiobarbituric acid reactive substances (TBARS) method described by Stock and Dormandy (1971) and modified by Jain (1988). The reaction mixture was incubated and heated with thiobarbituric acid, and absorbance was measured at 535 nm. MDA concentration was calculated using the molar extinction coefficient of the MDA-TBA complex (1.56×10^5 $mmol^{-1}$ cm^{-1}) and expressed as nmol/g Hb using the formula: MDA (nmol/g Hb) = (OD of test × Total reaction volume) / (EC × Volume of supernatant × Hb concentration)

Erythrocytic superoxide dismutase (SOD) activity was measured using the pyrogallol-MTT inhibition method described by Madesh and Balasubramanian (1997) with minor modifications. The reduction of MTT to formazan was measured at 570 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit MTT reduction by 50%. SOD activity was calculated as: SOD activity (units/g Hb) = $(2 \times 100 \times A^T / A^B)$ × Dilution factor × (1/g Hb), where A^T is the absorbance of the test and A^B is the absorbance of the blank.

Serum concentrations of pro-inflammatory cytokines IL-6 and IL-1β and their corresponding antibodies were estimated using commercially available ELISA kits (Immunotag, Gbiosciences) according to the manufacturer's protocol. Optical density was measured at 450 nm using an ELISA plate reader (Bio-Rad), and cytokine concentrations were expressed as pg/mL by comparison with standard curves.

Statistical Analysis

Statistical differences between diseased and healthy counterparts were determined using unpaired T- tests to compare variances using GraphPad Prism 9 software (version 9.1.0 (221)). Results were presented as mean values with their corresponding standard error of the mean (SEM). A significance level of $P < 0.0001$ was applied to indicate statistically significant differences.

Results and Discussion

Patna, located on the southern bank of the Ganges, has a humid subtropical climate with hot summers (late March–June), monsoon rains (late June–September), and mild winters (November–February) (Singh & Singh, 2017). These climatic conditions particularly high temperature and humidity during the summer–monsoon period create a favorable environment for the proliferation of ixodid tick vectors, thereby contributing to the increased prevalence and endemicity of bovine theileriosis in the region. Based on the findings of parasitological, PCR and clinical examinations, the animals used in this study were classified as either positive or negative for tropical theileriosis (TT). On clinical examination, the group affected by theileriosis displayed typical TT findings, including fever (42.25 °C), depression, congested mucous membranes, loss of condition, nasal discharge, inappetence, corneal opacity and most evidently enlarged pre scapular superficial lymph nodes. Giemsa-stained peripheral blood films from cows infected with theileriosis showed intra-erythrocytic piroplasms (Fig 1), which is a hallmark of Theileria infection. In cattle naturally infected with Theileria spp., clinical signs commonly include pyrexia (fever) (Sujatha *et al.,* 2025). Tissue damage and clinical manifestations in TT may arise from uncontrolled proliferation and metastasis of schizont-infected lymphoid cells (Agina *et al.,* 2020) and increased production of pro-inflammatory cytokines by parasitized monocytes (Sujatha *et al.,* 2025).

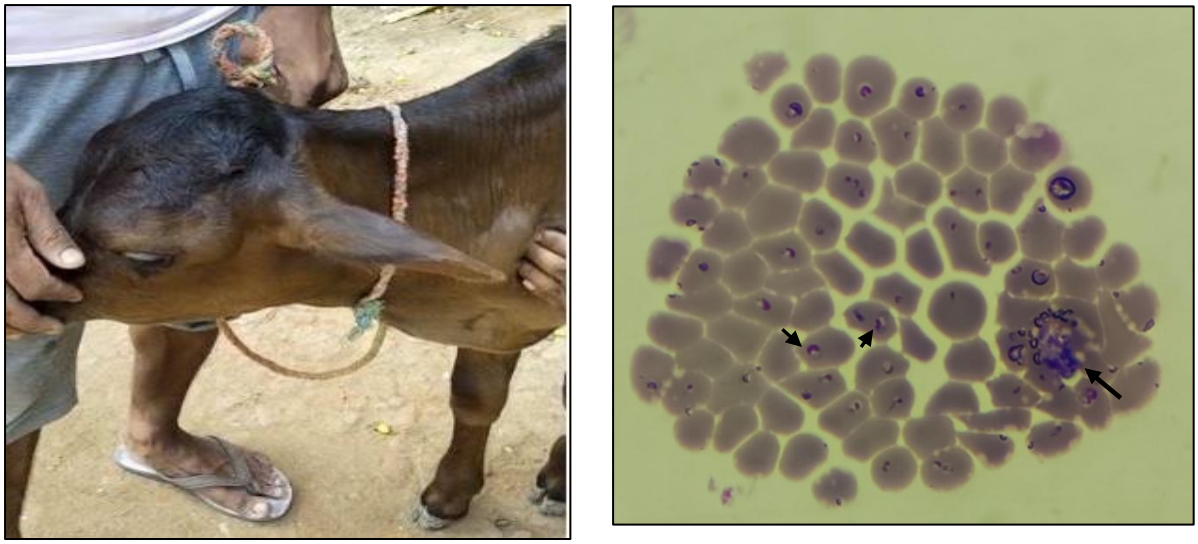
Blood films obtained from infected cattle also exhibited various abnormalities in RBCs, including macrocytosis and anisocytosis. On average, one to six piroplasmic forms were observed in the erythrocytes of affected cattle, with parasitemia levels ranging from 5% to 60%. Among the twenty cattle used in the study, the majority (75%) showed moderate (1-3%) to high (5-8%) levels of parasitaemia. On the other hand, there were no developmental stages of the protozoan in the smears from control cows. Anemia in TT can be attributed to various factors, including the destructive effect of intra erythrocytic piroplasms (Ugalmugle *et al.,* 2010) and persistent blood loss due to blood-sucking ticks (Tripathi and Jaiswal, 2022).

PCR analysis of blood samples from the theileriosis-affected group (Fig 2) revealed the presence of the specific 312 bp amplicon using the *T. annulata*-specific cyto b1 gene, whereas samples from the control group tested negative by PCR. These findings confirmed that *T. annulata* was responsible for the disease pathogenesis in the affected group, whereas the control group was negative for pathogen. Table 1 shows the statistics for the measured haemogram parameters in healthy and affected cattle. The cytological characteristics of erythrocytes were significantly altered, with a notable decrease observed in the mean corpuscular volume compared to controls in animals, a significant ($p<0.05$) decrease in hemoglobin content, hematocrit, TEC and TLC was also observed in diseased cases compared to controls. These findings suggest that cattle infected with TT experience significant normocytic hypochromic anemia throughout the disease course. Similar results were reported by Khan *et al.* (2011) and Ganguly *et al.* (2015), highlighting anemia as a prominent clinical feature in affected cases (Nazifi *et al.,* 2011).

Erythrocyte antioxidant defenses were markedly compromised in cows infected with *Theileria annulata*. The invasion of erythrocytes by parasites can significantly disrupt crucial antioxidant defense mechanisms, leading to damage to red blood cells and ultimately causing extravascular hemolysis.

Activities of CAT were significantly reduced in infected animals compared to healthy controls (CAT: 20.7 vs. 141.7 U/ml, $P < 0.0001$). Similarly, serum GSH levels were significantly lower in the diseased group (0.05301 mM/ml) than in controls (0.07651 mM/ml; $P < 0.0001$), indicating substantial oxidative stress associated with infection. The invasion of erythrocytes by parasites can significantly disrupt crucial antioxidant defense mechanisms, leading to damage to red blood cells and ultimately causing extravascular hemolysis.

SOD levels were assessed in both serum and haemolysate samples. SOD activity in haemolysate samples was significantly reduced in Theileria annulata-infected cows (866.9 U/ml) compared to healthy controls (3045 U/ml; $P < 0.0001$), indicating compromised intracellular antioxidant defense. In serum samples, SOD levels were lower in the diseased group (−460.6 U/ml) than in controls (735.6 U/ml), but this difference was not statistically significant ($P > 0.1$). These results suggest of notable erythrocytic changes with enhanced scavenging activity which might be attributed by increased osmotic fragility of RBCs throughout the progression of the disease. The significant decrease in GSH activity and the concurrent reduction in SOD and catalase levels observed in affected cattle align with recent reports of disrupted antioxidant defenses in bovine tropical theileriosis, where infected animals exhibited markedly lower activities of key antioxidant enzymes compared to healthy controls (Pandey *et al.,* 2017). Oxidative stress associated with T. annulata infection has been shown to impair erythrocyte antioxidant systems, leading to enhanced lipid peroxidation and compromised cellular protection. Furthermore, similar declines in antioxidant enzyme activities, including GSH, catalase, and SOD, have been documented in T.



(a) **(b)**

Figure 1: Clinical findings confirming *T. annulata* infection in affected cattle. (a) Cattle showing symptoms of swollen prescapular lymph node. (b) Giemsa stained blood smear confirming the presence of intra-erythrocytic stage of *Theileria annulata*.

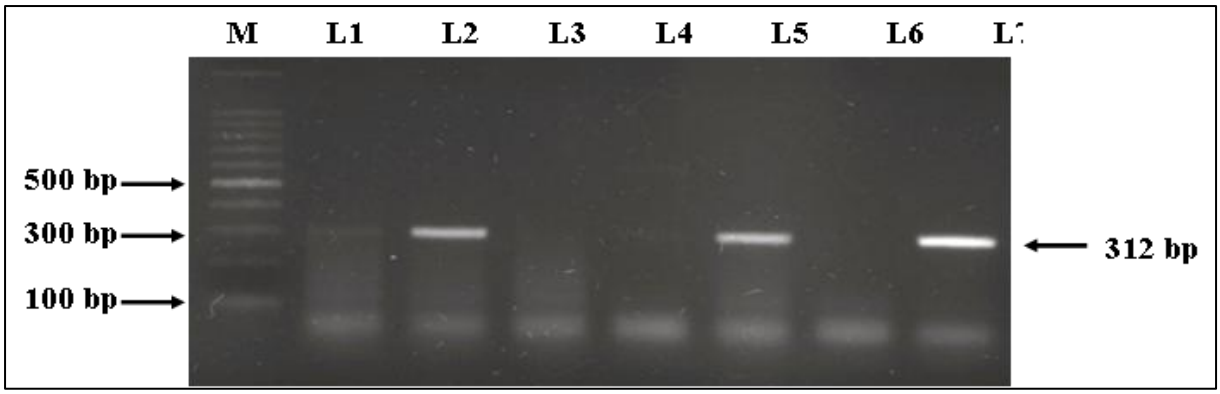


Figure 2: PCR based confirmation *T. annulata* in blood samples amplified by cyto b1 gene.M: 100 bp DNA ladder. Lane 1, 2, 5, 7: Presence of the specific 312 bp amplicon in affected group using the *T. annulata*-specific cyto b1 gene Positive control; Lane 3, 4, 6: Negative amplification for the control group

Table 1: Erythrocytic indices in healthy control and theileria affected cattle. Results shown are expressed as mean values)

Parameters	Healthy Control (mean values) (n=20)	Theileria Infected Cows (mean values) (n=20)
Hb (g/dl)	10.51 ± 1.8	6.16 ± 1.3
TEC (X 10^6 /µl)	10.24 ± 0.52	4.8 ± 0.61
TLC (X 10^3 /µl)	8.5 ± 0.39	3.1 ±0.91
MCV (μm^3)	45.61 ±5.9	38.43± 4.3
PCV (%)	28 ± 0.26	18.7 ± 0.82

Table 2: Biochemical parameters in healthy control (n=20) and Theileria naturally infected cattle (n=20). Results are expressed as mean values.

Parameters	Healthy Control (mean values) (n=20)	Theileria Infected Cows (mean values) (n=20)
Alanine Transaminase (ALT) (U/L)	15.48	16.14
Aspartate Transaminase (AST) (U/L)	21.58	36.03
Total Protein (g/dl)	5.306	5.106
Total bilirubin (mg/dl)	0.624	2.647
Albumin	3.754	4.162

annulata–infected buffaloes and other livestock, supporting the role of oxidative damage in the pathogenesis of hemoprotozoan diseases (Molayi-Jabdaragi *et al.,* 2020). Conversely, cattle infected with *T. annulata* showed a significant increase in lipid peroxidation, as reflected by elevated malondialdehyde (MDA) levels in both serum and haemolysate samples. Serum MDA levels in the diseased group were 17.31 µmoles/ml, significantly higher than in healthy controls (7.13 µmoles/ml; $P < 0.0001$). A similar trend was observed in haemolysate samples, with infected cattle showing MDA levels of 292.1 µmoles/ml compared to 87.31 µmoles/ml in controls ($P < 0.0001$), indicating enhanced oxidative damage to erythrocytes during infection.

As MDA is a key biomarker of oxidative stress, elevated levels indicate ROS-mediated lipid damage and impaired membrane integrity. In *T. annulata*-infected cattle, we observed a significant increase in MDA alongside decreased activities of antioxidant enzymes (SOD, GSH, and catalase), reflecting substantial oxidative stress. These findings are consistent with recent studies reporting heightened lipid peroxidation and compromised antioxidant defenses in livestock infected with hemoprotozoan parasites (Powlowska *et al.,* 2023). Collectively, the results suggest that excessive ROS generation, coupled with inadequate enzymatic antioxidant responses, contributes to oxidative damage of erythrocytes and may play a critical role in the development of anaemia during infection.

In relation to the proinflammatory cytokines, there has been a significant elevation in both the levels of IL-6 and IL-1β ($p<0.0001$) (Fig 4). A remarkable increase in the IL-6 values of diseased (mean value of 0.016pg/ml) in comparision with non-infected group (0.01464 pg/ml) as well as IL-1β values (diseased- 0.0153 pg/ml; healthy control-0.01364 pg/ml) can be correlated with the results of Razavi et al., 2010 and El-Sebaei et al., 2014. IL-6, originating from monocytes and macrophages, plays a critical role in activating B- and T-cells and initiating the acute phase response (Hunter & Jones, 2015). In acute inflammation, IL-6 is the primary cytokine triggering the production of C-reactive protein (CRP), serum amyloid A and fibrinogen in hepatocytes (Reinhart *et al.,* 2012). IL-6 has a longer plasma half-life and is more reliably detectable in plasma compared to IL-1β. The rise in the cytokine levels can be correlated with host susceptibility as well as the virulence of Theileria species as described by Tümer and Kızıl, 2023.

Additionally, the findings from biochemical analysis are listed in Table 2. The findings indicated a non-significant decrease in serum total protein ($p>0.05$) and serum albumin ($p>0.05$) in diseased cattle compared to controls (Abubakar *et al.,* 2019). The decrease in albumin synthesis in diseased cattle may stem from reduced feed intake and impaired liver function (Ganguly *et al.,* 2019). Liver damage in bovine theileriosis could result from the toxic effects of protozoan metabolites, inflammatory changes due to trapped infected cells, and degenerative changes due to anemic hypoxia. A significant increase in total bilirubin levels ($p<0.0001$) was observed in the diseased group. These findings are consistent with those reported by Ayadi *et al.,* 2017. Significant increases in AST ($p<0.0001$) activities were noted in the sera of diseased cows, consistent with findings reported by Kachhawa *et al.*, 2016. They attributed the elevated AST activity to hepatic injury during the disease. Additionally, since AST is present in red blood cells (RBCs), the increased RBC lysis during the disease could also contribute to the heightened serum AST activity (Latimer, 2011). However, the levels of ALT in the diseased sera did not show considerable changes and is similar to the findings of Devadevi *et al.,* 2018).

A key highlight of the present study is the observed interplay between oxidative stress and proinflammatory cytokines, particularly their association with erythrocyte antioxidant defense mechanisms during natural Theileria infection. To elucidate these relationships, Pearson correlation analysis was performed between proinflammatory cytokines (IL-6 and IL-1β), oxidative stress markers [MDA and GSH], and erythrocytic antioxidant enzymes [catalase and SOD].The analysis revealed a significant positive correlation between IL-6 and MDA levels ($r = 0.455$, $p < 0.05$; Fig. 5A), indicating that increased inflammation is associated with elevated lipid peroxidation. Moreover, IL-6 levels positively correlated with reduced glutathione ($r = 0.606$, $p < 0.01$; Fig. 5B), suggesting a compensatory upregulation of the glutathione antioxidant system in response to inflammatory stress. However, no significant correlations were observed between IL-6 and catalase or SOD activity, nor between IL-1β and any of the oxidative or antioxidant parameters assessed. These findings underscore a specific and biologically relevant link between IL-6 and oxidative stress markers, particularly lipid peroxidation and glutathione balance. The positive correlation between IL-6 and MDA suggests a mechanistic interaction in which oxidative stress, specifically, lipid peroxidation may enhance IL-6 expression via redox-sensitive transcription factors such as NF-κB (Manful *et al.,* 2025). Given MDA's role as a marker of lipid damage and inducer of proinflammatory pathways, its association with IL-6 underscores the link between oxidative stress and inflammation. While overall GSH levels were reduced, its positive correlation with IL-6 may reflect a compensatory response. In contrast, catalase and SOD showed no correlation with IL-6, likely due to their erythrocyte localization, susceptibility to oxidative inactivation, and disruption from Theileria-induced hemolysis. These findings highlight a selective interplay between inflammation and oxidative damage in infected cattle, particularly involving IL-6, lipid peroxidation, and glutathione. This interplay reinforces the notion that oxidative and inflammatory pathways are intricately linked in the pathophysiology of Theileria infection and may offer insights into potential therapeutic targets aimed at modulating redox balance and immune responses.

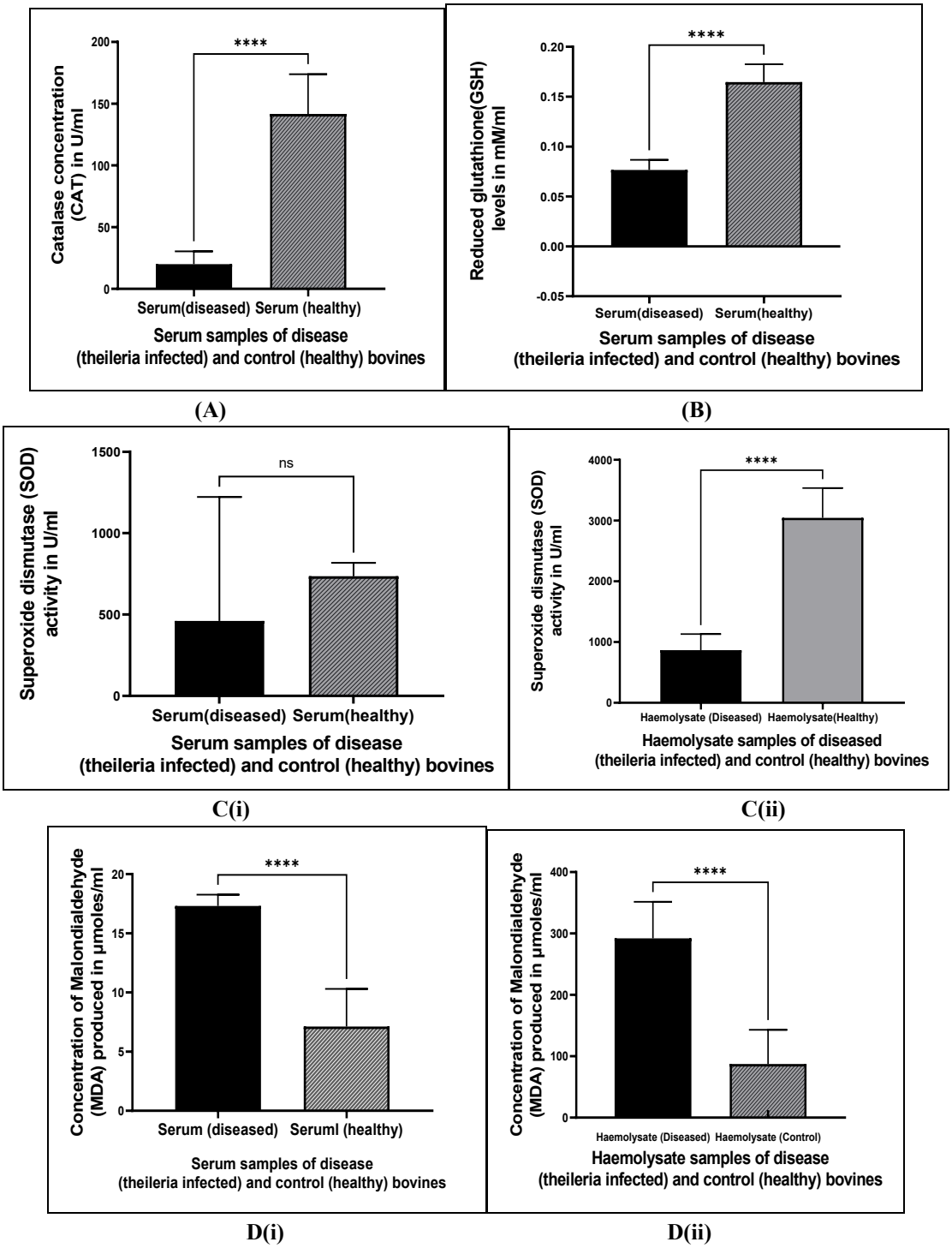
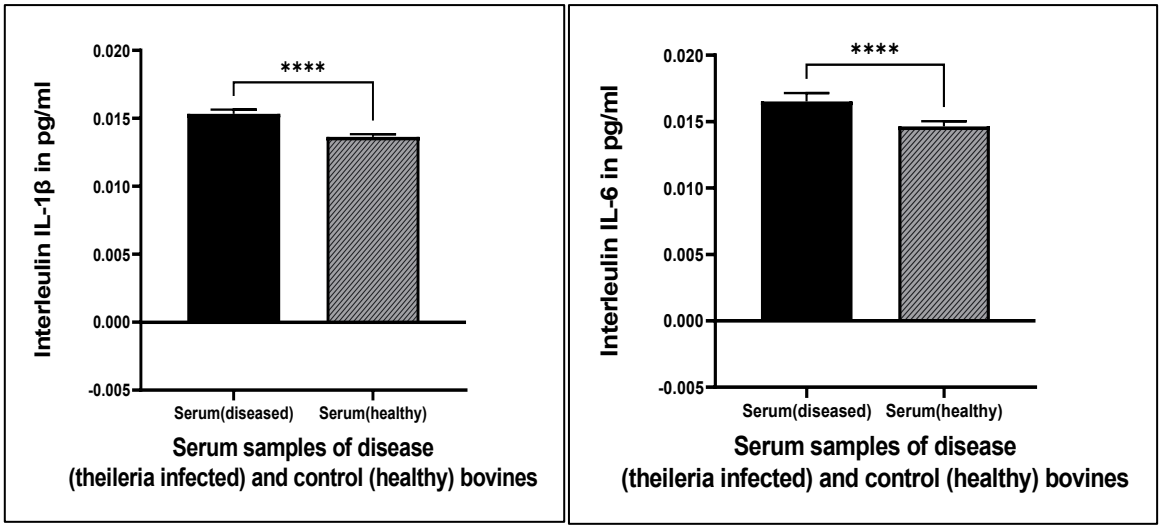


Figure 3: Assessment of antioxidant enzyme activity and oxidative stress in Theileria annulata-infected cattle and healthy controls (n = 20 per group). (A) Serum catalase (CAT) activity was significantly decreased in the infected group compared to healthy controls ($P < 0.0001$). (B) Reduced glutathione (GSH) levels were also significantly lower in the infected group ($P < 0.0001$). (C) Superoxide dismutase (SOD) activity was evaluated in both serum (C(i)) and hemolysate (C(ii)) samples. A non-significant reduction was observed in serum SOD levels ($P > 0.1$), whereas a significant decrease was noted in hemolysate samples ($P < 0.0001$). (D) Lipid peroxidation, measured as malondialdehyde (MDA) concentration, was significantly increased in both serum (D(i)) and hemolysate (D(ii)) of the infected group ($P < 0.0001$).



(E) **(F)**

Figure 4: Proinflammatory Cytokine Response in *T. annulata*-Infected Cattle

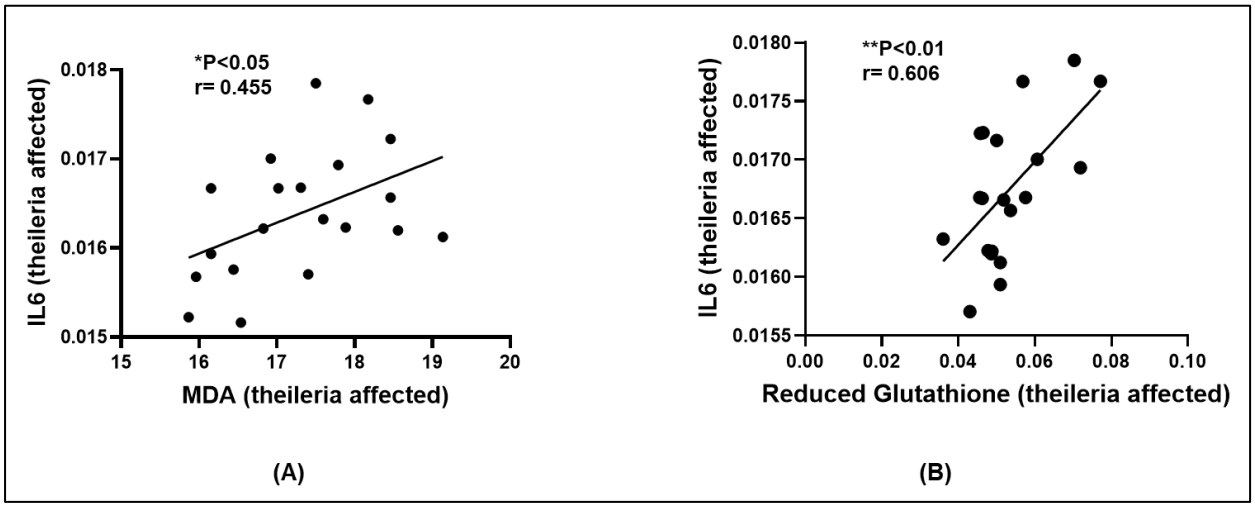


Fig 5: Correlation between proinflammatory cytokines and oxidative stress/antioxidant parameters in Theileria-infected cattle.(A –B) Scatter plots illustrating Pearson correlation analysis between inflammatory cytokine (IL-6) and oxidative stress markers (MDA, reduced glutathione). (A) Significant positive correlation were observed between IL-6 and malondialdehyde (MDA) suggesting a link between inflammation and oxidative imbalance. (B) Significant positive correlation were observed between IL-6 and reduced glutathione Data represent n = 20 samples. Pearson's correlation test, *p < 0.05, **p<0.01.

Data are expressed as mean ± SEM, and statistical comparisons were performed using two-tailed P values. The serum levels of proinflammatory cytokines IL-1β and IL-6 (pg/mL) were significantly elevated ($P < 0.0001$) in the diseased group compared to healthy controls, indicating enhanced immunopathogenic activity associated with *Theileria annulata* infection.

Conclusion

Our study aimed to assess the antioxidant status in erythrocytes, oxidative stress markers in serum and hemolysate, and lipid peroxidation in cattle naturally infected with *T. annulata*, in order to elucidate the mechanisms underlying anemia. Our findings unveiled a notable decrease in erythrocyte antioxidant defense mechanisms, indicating the presence of oxidative damage to red blood cells and consequent anemia. Theileriosis induces substantial hematologic alterations characterized by an overall poor blood picture, with anemia being a primary feature. Additionally, the disease leads to significant biochemical changes indicative of impaired liver function throughout its course. Theileriosis in cattle leads to a notable rise in lipid peroxidation within erythrocyte membranes, potentially affecting the levels of antioxidant enzymes in affected cattle. These enzyme levels

decrease as the disease advances, suggesting that Theileria infection may disrupt the erythrocytes' antioxidant mechanisms that defend against oxidative damage.

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