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# Impact of Theileria annulata Infection on Oxidative/Nitrosative Stress and Liver and Bile Duct Function in Cattle: Insights from Kırıkkale, Turkey

**Ali ŞENOL**
Kirikkale University: Kirikkale Universitesi https://orcid.org/0000-0003-4080-7776
**Elisha Apatewen Akanbong**
akanbongelisha605@gmail.com
Kirikkale University: Kirikkale Universitesi https://orcid.org/0000-0002-2556-7236
**Haceli ÖCAL**
Kirikkale University: Kirikkale Universitesi
**Sami GÖKPINAR**
Kirikkale University: Kirikkale Universitesi

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# Abstract

Bovine tropical theileriosis (BTT), caused by the hemoprotozoan parasite *Theileria annulata* (*T. annulata*), poses a significant threat to cattle health and productivity, leading to substantial economic losses worldwide. This study investigates the impact of *T. annulata* infection on oxidative and nitrosative stress, and on liver and bile duct function, in naturally infected cattle in Kırıkkale, Turkey. A total of 40 cattle, including 15 molecularly negative and 25 molecularly positive animals, were evaluated in this study. The serum samples obtained from the cattle were analysed for metabolic parameters (CRE, UREA, TBIL, ALB, TP, CHO, TRI, GLU, IP, CA) and for liver and bile duct function indicators through enzyme activities (AST, ALT, GGT, and ALP). To evaluate oxidative stress, we quantified total oxidant status (TOS), total antioxidant status (TAS), oxidative stress index (OSI), malondialdehyde (MDA), nitric oxide (NO), and total sialic acid (TSA). Results indicated altered levels of biochemical parameters, elevated TOS, OSI, MDA, NO, and TSA, and altered enzyme levels in molecularly positive cattle, reflecting oxidative and nitrosative stress and compromised liver and bile duct function associated with the infection. This study underscores the importance of continuous monitoring of BTT and highlights the role of oxidative stress in its pathogenesis, strengthening the need for antioxidant supplementation as an adjunctive therapy to mitigate its adverse effects and promote cattle health.


# Introduction

Bovine tropical theileriosis (BTT), caused by the apicomplexan haemoprotozoan parasite *Theileria annulata (T. annulata)*, is a vector-borne disease that affects cattle health and productivity. The disease results in severe economic losses due to decreased milk and/or meat production, high treatment and control costs, as well as high morbidity and mortality (about 80% in susceptible cattle) (Abdel-Hamied et al., 2020; Kumari et al., 2022; Waghmare et al., 2026). Among the *Theileria* species infecting cattle, one of the most pathogenic and economically important is *T. annulata* (Ganesan et al., 2024). *T. annulata* is primarily transmitted by the Ixodidae ticks *Hyalomma anatolicum*, particularly (Gokpinar & Aydenizoz, 2016; Behera et al., 2026; Waghmare et al., 2026), widely distributed in the tropical and sub-tropical regions and most profoundly found in the Mediterranean basin, the Middle East and South Asia (Kumari et al., 2022) and is characterised by a complex life cycle (Waghmare et al., 2026).

The pathogenesis of BTT commences with the transmission of salivary sporozoites by an infected tick into the bovine bloodstream, leading to the rapid invasion of leukocytes by the sporozoites, which differentiate into schizonts that transform the host cell, induce uncontrolled lymphoproliferation, and immunosuppression. The schizonts multiply asexually and eventually differentiate into merozoites, which are released into circulation and invade erythrocytes upon leukocyte lysis (Behera et al., 2026; Waghmare et al., 2026), resulting in clinical manifestations such as pyrexia, swelling of superficial lymph nodes, anaemia and/or leukopenia (Abdel-Hamied et al., 2020; Behera et al., 2026).

While the pathology of the disease comes with alterations in haematological and biochemical parameters, the abundance of membrane lipids in erythrocytes makes the erythrocytes more susceptible

to oxidative damage (Abdel-Hamied et al., 2020). *T. annulata* infection instigates the generation of reactive oxygen species (ROS) by the host, primarily to destroy the parasite; however, ROS are unable to discriminate between the host cells and the *T. annulata*, resulting in damage to host cells and organs. To ameliorate the damage, the host initiates antioxidative defences. However, the antioxidative defence can be overwhelmed by the enormously generated ROS, leading to oxidative stress (Molayi-Jabdaragi et al., 2020). Oxidative stress, through lipid peroxidation of erythrocytes' membranes, causes increased membrane permeability, increased erythrocyte fragility and subsequently anaemia (Abdel-Hamied et al., 2020). Thus, oxidative stress tremendously contributes to the pathogenesis of BTT.

While there is evidence that *T. annulata* induces oxidative stress in small ruminants and cattle (Molayi-Jabdaragi et al., 2020, Mohammed et al., 2025, Behera et al. 2026). There is a need for continuous surveillance, especially in the Middle East, Turkey, where *T. annulata* is profoundly prevalent, to detect potential shifts in its pathogenesis. In view of that, the present study investigated the impact of *T. annulata* on: 1) oxidative/nitrosative stress by measuring total oxidant status (TOS), total antioxidant status (TAS), malondialdehyde (MDA), nitric oxide (NO), and total sialic acid (TSA); 2) liver and bile duct function by measuring aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP); and 3) relevant metabolic related biochemical parameters.

# Materials and Methods

**Ethical Approval:** Ethical clearance for the study was obtained from the Kırıkkale University Local Ethics Committee for Animal Experiments on 12/03/2026 (E-60821397-010.99-412252).

**Animals:** This study included 15 molecularly negative and 25 molecularly positive cattle, naturally infected with *T. annulata* in Kırıkkale province (İç Anadolu region, Turkey). The animals were from different districts within the same province (Çelebi, Delice, Balışeyh, Keskin, Yahşihan and the city center), different breeds (crossbreed, Native Anatolian Black, Brown Swiss, and Simmental), females, and ranged in age from 1 to 8 years.

**Blood Sample Collection:** Blood samples were collected from the jugular veins of all animals using gel-serum vacuum tubes. These samples were transported in a cold chain to the Department of Biochemistry, Faculty of Veterinary Medicine, Kırıkkale University. Upon arrival, the blood samples were centrifuged at 3,000 rpm for 10 minutes. The separated serum samples were then transferred to labelled, sterile microcentrifuge tubes and stored at -20°C until analysis.

**Determination of *T. annulata*-infected Samples:**

This study included cattle sampled under field conditions from different districts within the same province (Kırıkkale, İç Anadolu region, Türkiye), including Çelebi, Delice, Balışeyh, Keskin, Yahşihan, and the city center. Blood samples were collected from both clinically healthy animals, showing no apparent signs of disease, and from animals with clinical signs of theileriosis (dullness, anorexia, enlargement of

the lymph nodes, anaemia (pallor of mucous membranes), rise in rectal temperature (40.1–41.3 °C). All samples were analysed for the presence of *Theileria annulata* using microscopic examination, polymerase chain reaction (PCR), and Reverse Line Blotting (RLB) methods. Animals that tested positive by all three diagnostic methods were included in the positive group (*T. annulata*-infected). In contrast, animals that tested negative by all methods and were considered clinically healthy were assigned to the negative group (control group).

**Microscopic Examination:**

Blood smears were prepared from the ear tip of each animal for microscopic examination. The smears were fixed in methanol for 5 minutes and then stained with Giemsa for 40 minutes. The slides were examined under 100× magnification for the presence of *Theileria* spp. piroplasms by randomly assessing 200 microscopic fields where erythrocytes were evenly distributed. The presence of at least one piroplasm was considered a positive result.

**DNA Extraction and PCR**

Genomic DNA was extracted from blood samples using a commercial extraction kit, following the manufacturer's instructions. The extracted DNA was stored at −20°C until further use. PCR amplification targeting the V4 hypervariable region of the 18S rRNA gene of *Theileria* spp. was conducted using the primers RLB-F2 (5'-GAC ACAGGG AGG TAG TGA CAA G-3') and RLB-R2 (5'-biotin-CTA AGA ATT TCA CCT CTG ACA GT-3'), as described by Georges et al. (2001). These primers amplify fragments ranging from 460 to 520 bp. The PCR reaction was performed in a total volume of 50 µl, which included 5 µl of 10× PCR buffer (500 mM KCl, 100 mM Tris-HCl [pH 9.1], 1% Triton X-100), 1 µl of 5 mM $MgCl_2$, 500 µM of each dNTP, 5 U/µl Taq DNA polymerase, 5 µl of each primer (50 pmol/µl), and 5 µl of template DNA. Amplification was carried out in a thermal cycler under the following conditions: initial denaturation at 94°C for 2 minutes, followed by 40 cycles of denaturation at 94°C for 20 seconds, annealing at 57°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension step was performed at 72°C for 7 minutes. The PCR products were stored at +4°C until further analysis by Reverse Line Blotting (RLB).

**Reverse Line Blotting (RLB)**

All oligonucleotide probes were synthesised by SynGen in Sacramento, CA, USA. They were modified at the 5' end with an N-terminal N-trifluoroacetamidohexyl-cyanoethyl N, N-diisopropyl phosphoramidite (TFA)-C6 aminolinker. This modification allows for binding to the negatively charged Biodyne C membrane, as described by Gubbels et al. (1999). Membrane preparation and hybridisation were carried out following the protocols established by Gubbels et al. (1999) and Georges et al. (2001). The probes were diluted to final concentrations of 100–400 pmol in 150 µl of 500 mM $NaHCO_3$ to optimise hybridisation conditions.

**Serum Biochemistry Analyses**

Using a biochemistry analyser (Gesan, Chem200, Italy) and commercial test kits, we measured the concentrations of the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP). Additionally, we assessed the biochemical parameters: creatinine (CRE), urea (URE), total bilirubin (TBIL), albumin (ALB), total protein (TP), cholesterol (CHO), triglycerides (TRI), glucose (GLU), inorganic phosphate (IP), and calcium (CA). To evaluate oxidative stress, we quantified the total oxidant and antioxidant status (TOS-TAS) in serum samples using a spectrophotometer (Multiskan GO, Thermo, USA) and commercially available assay kits (Rel Assay Diagnostics, Turkey), in accordance with the manufacturer's guidelines (Erel, 2004; Erel, 2005). The oxidative stress index (OSI) was determined as the percentage ratio of TOS to TAS, with results presented in arbitrary units (AU) (Devrim et al., 2020; Sak et al., 2018). Furthermore, the serum concentrations of malondialdehyde (MDA), nitric oxide (NO), and total sialic acid (TSA) were evaluated using a spectrophotometer (Thermo Scientific Multiskan GO), employing the methods described by Buege and Aust (1978), Miranda et al. (2001), and Sydow (1985), respectively.

### Statistical Analysis

The data were analysed using SPSS 20 statistical software (SPSS Inc., Chicago, IL, USA). First, we conducted a normality assessment using the Shapiro-Wilk test. For datasets that showed a normal distribution, we used the Independent Samples T-test. In contrast, the Mann-Whitney U test was applied to data that did not follow a normal distribution. A P-value < 0.05 was deemed statistically significant between the two groups. Parametric data are presented as mean ± standard error (x ± SE), while nonparametric data are reported as mean ± standard error, together with median and mean rank.

## Results

### Enzymatic Activities and Biochemical Parameters:

The biochemical analyses indicate significantly lower serum levels of CRE, TP, ALB, and GLU in the molecularly positive group than in the molecularly negative group (P < 0.05; **see Table 1**). While the concentrations of TBIL, URE, CHO, and TRI did not vary significantly, TBIL and TRI were higher in the molecularly positive group than in the molecularly negative group; URE was nearly the same between the groups, whereas CHO was lower in the molecularly positive group.

In terms of enzyme activities, except for ALP, all enzymes showed higher activity levels in the molecularly positive group compared to the molecularly negative group, with only the activity of ALT being statistically significant.

**Table 1.** Levels of enzymatic activities and biochemical parameters

| Parameters | | Group 1 (Molecularly negative cattle) | Group 2 (Molecularly positive cattle) | P value |
|---|---|---|---|---|
| **AST (U/L)** | Mean±SE | 52.16±1.84 | 57.12±2.24 | 0.096 |
| **ALT (U/L)** | Mean±SE | 23.53±1.21 | 32.54±1.09 | 0.000 |
| **GGT (U/L)** | Mean±SE | 65.40±4.77 | 73.91±2.69 | 0.134 |
| **ALP (U/L)** | Mean±SE | 241.47±27.39 | 229.41±23.33 | 0.665 |
| | Median | 202 | 197 | |
| | Mean rank | 19.93 | 18.36 | |
| **TBIL** | Mean±SE | 2.38±0.11 | 2.46±0.12 | 0.670 |
| **UREA** | Mean±SE | 108.20±0.88 | 108.90±1.02 | 0.626 |
| **CRE** | Mean±Sx | 5.19±0.40 | 4.12±0.35 | 0.030 |
| | Median | 5.10 | 3.57 | |
| | Mean rank | 23.67 | 15.82 | |
| **TP (g/dl)** | Mean±SE | 5.96±0.10 | 5.60±0.06 | 0.005 |
| **ALB (mg/dl)** | Mean±SE | 2.22±0.04 | 2.08±0.02 | 0.003 |
| **GLU (mg/dl)** | Mean±SE | 76.13±2.74 | 88.18±2.37 | 0.002 |
| **CHO (mg/dl)** | Mean±SE | 396.85±22.71 | 342.80±24.83 | 0.137 |

| | | | | |
|---|---|---|---|---|
| **TRI (mg/dl)** | Mean±SE | 173.40±1.59 | 178.49±3.07 | 0.491 |
| | Median | 173.40 | 174.47 | |
| | Mean rank | 17.97 | 20.50 | |

**Oxidative and Nitrosative Stress Findings:**

Oxidative and nitrosative stress assessment revealed significantly higher levels of TOS, MDA, NO, and TSA in the molecularly positive group compared to the molecularly negative group. This resulted in a higher calculated OSI value for the infected group, although the difference was not statistically significant (**see Table 2**). Additionally, the molecularly positive group exhibited a relatively higher TAS level, but this difference was also not statistically significant.

**Table 2.** Levels of oxidative and nitrosative stress parameters

| Parameters | | Group 1 (Molecularly negative cattle) | Group 2 (Molecularly positive cattle) | P value |
|---|---|---|---|---|
| **TAS (mmol Trolox Eq/L)** | Mean±SE | 0.47±0.04 | 0.51±0.02 | 0.440 |
| **TOS (µmol H2O2 Eq/L)** | Mean±SE | 6.26±0.36 | 9.01±0.51 | 0.000 |
| **OSI (Arbitrary units)** | Mean±SE | 1.49±0.17 | 1.89±0.15 | 0.054 |
| | Median | 1.26 | 1.79 | |
| | Mean rank | 15.20 | 24.54 | |
| **MDA (µmol/L)** | Mean±SE | 1.44±0.04 | 1.74±0.11 | 0.033 |
| | Median | 1.42 | 1.57 | |
| | Mean rank | 14.40 | 22.14 | |
| **NO (µmol/L)** | Mean±SE | 32.94±0.37 | 37.25±0.55 | 0.000 |
| | Median | 32.84 | 38.23 | |
| | Mean rank | 10.23 | 25.54 | |
| **TSA (mg/dl)** | Mean±SE | 39.96±0.53 | 43.85±0.64 | 0.000 |

# Discussion

The present study investigated the impact of *T. annulata* on oxidative and nitrosative stress and biochemical parameters in cattle in Kırıkkale, Turkey. The findings indicate that *T. annulata* significantly induces oxidative and nitrosative stress, as evidenced by increased levels of TOS, MDA, NO, and TSA, culminating in a higher OSI in the molecularly positive group. Additionally, considering the overall results on enzyme activities, *T. annulata* appears to affect liver and bile duct function, as evidenced by increased levels of AST, ALT, and GGT in the molecularly positive group. Notably, these increases did not reach statistical significance, except for ALT. The significantly increased ALT activity in the molecularly positive group is considered an indicator of hepatocellular damage, and this finding is consistent with the study

by Khan et al. (2010). In contrast, the lack of statistical significance in the observed increases in AST and GGT levels may be related to disease severity, infection stage, or biological variations among individual animals. Indeed, while the literature reports that enzymes such as AST and GGT are generally significantly increased in cases of *T. annulata* infection (Hasanpour et al., 2008; Col and Uslu, 2006; Aslam et al., 2023), some studies have also reported that these increases were limited or did not reach statistical significance (Mathai et al., 2025). These differences are thought to be due to factors such as the severity of the infection, the level of parasitaemia, and the clinical course. Overall, the findings indicate that *T. annulata* infection has varying effects on liver and biliary tract functions, and these effects may differ depending on the clinical and pathophysiological characteristics of the disease.

When serum protein concentrations were evaluated, the hypoproteinaemia and hypoalbuminemia observed in the molecularly positive group were consistent with studies reported in the literature (Col and Uslu, 2006; Kumari et al., 2022; Alnahari et al., 2026). The main reason for this decrease is the reduced protein synthesis capacity due to hepatocellular damage caused by *T. annulata* infection in the liver. Indeed, hepatocellular necrosis and loss of function lead to a significant decrease, particularly in albumin synthesis. Additionally, albumin, which is a negative acute-phase protein, decreases in concentration during the acute phase of an inflammatory response (Aydın et al., 2025). In addition, lymph node lesions accompanying the disease and the leakage of protein-rich fluids into extravascular spaces because of increased vascular permeability also contribute to the decrease in serum total protein and albumin levels. Furthermore, increased protein loss through the kidneys (especially albuminuria) can be considered another important factor supporting this decrease (Col and Uslu, 2006; Kumari et al., 2022; Alnahari et al., 2026). Therefore, the reduced levels of TP and ALB observed in this study may be attributed to the deleterious effects of inflammation induced by the toxic metabolites of *T. annulata* or hepatic dysfunction (Alnahari et al., 2026; Aziz et al., 2022).

An elevated total bilirubin (TBIL) level in the molecularly positive group is one of the most characteristic biochemical findings associated with tropical theileriosis. This hyperbilirubinemia can be attributed to two main mechanisms. First, haemolytic anaemia occurs due to the excessive destruction of parasitised erythrocytes by macrophages in the reticuloendothelial system (RES), particularly in the spleen and lymph nodes, a process known as erythrophagocytosis. Second, severe degeneration in the liver parenchyma impairs the processing and elimination of the released bilirubin (Col and Uslu, 2006; Khan et al., 2010; Alnahari et al., 2026).

The decrease in cholesterol (CHO) levels strongly aligns with findings in the literature linking anorexia, diarrhoea, and impaired fat metabolism in the liver to infected animals. However, the increase in triglyceride (TRI) levels is a particularly noteworthy finding. A study by Alnahari et al. (2026) found a

significant increase in triglyceride (and HDL) levels in infected cattle. This increase is considered a compensatory response of the liver to compensate for the severe protein loss experienced during the disease. Furthermore, rapid mobilisation of body fat stores due to severe negative energy balance may also have contributed to the increase in blood triglycerides (Ahmadi et al., 2020; Alnahari et al., 2026). These findings also align with the results reported by Mohamed et al. (2023). The elevated TRI level in the molecularly positive group may indicate a compensatory response aimed at generating energy and increasing glucose production (gluconeogenesis) to meet the glucose demands of *T. annulata*, as it utilises glucose. Again, the increased glucose (GLU) level in the molecularly positive group could result from gluconeogenesis from protein catabolism (Dutta et al., 2025), as evidenced by the lower protein levels.

Creatinine (CRE) is a marker of kidney function and muscle breakdown (Charitha et al., 2025). In this study, creatinine levels were significantly lower in the molecularly positive group. Typically, increased creatinine levels indicate kidney damage; however, these levels are also directly related to muscle mass and activity. In cattle with theileriosis, rapid and severe weight loss (cachexia), muscle wasting (atrophy), and prolonged recumbency due to physical inactivity may have led to a clinically significant decrease in blood creatinine levels. Additionally, the lack of a statistical difference in urea levels between the molecularly negative and molecularly positive groups suggests that no pathological conditions related to kidney function have developed.

Consistent with the findings of the present study, a study conducted by Alnahari et al. (2026) reported that *T. annulata* significantly decreased the levels of TP, ALB, and CHO in cattle. Additionally, significantly increased levels of AST, ALT, TRI, and TBIL were reported; however, the increase in TBIL did not reach statistical significance. Contrary to our findings, Alnahari et al. (2026) reported a significant decrease in GGT level in the infected group, while in our study, GGT level was higher in the molecularly positive group compared to the negative group. Based on the findings and existing literature, the elevated enzyme levels observed could partly be due to necrosis or injury to the hepatocytes and bile ducts, as evidenced by the higher level of TBIL in the molecularly positive group (Aziz et al., 2022; Charitha et al., 2025). Consequently, *T. annulata* has a marked impact on various biochemical parameters.

In *T. annulata* infection, piroplasms proliferating within erythrocytes and schizonts infecting macrophages trigger a severe inflammatory response. Haemolysis-induced anaemia leads to tissue hypoxia. Hypoxia, in turn, disrupts the mitochondrial respiratory chain and promotes the generation of reactive oxygen species (ROS) (Rezaei et al., 2006). The regulatory action of antioxidants protects organs and cells, including erythrocytes, from oxidative damage induced by ROS or oxidants. *T. annulata* infection elevates oxidant levels, leading to lipid peroxidation measured by MDA, a significant biomarker, which is often elevated in infected cattle, reflecting an increase in free radical activity and antioxidant depletion (Mohamed et al., 2023). Consistent with the findings of Mohamed et al. (2023), in the present study, the TOS level of the molecularly positive group was significantly elevated, causing an elevation in lipid peroxidation, as MDA level was significantly elevated in the molecularly positive group. Interestingly, the molecularly positive group had a relatively higher TAS level than the molecularly

negative group, and this is probably due to an attempt to neutralise the effects of the oxidants (compensatory increase).

Also consistent with the findings of the present study, Mohammed et al. (2025) reported that the parasite induced oxidative stress, causing lipid peroxidation, which resulted in a significant elevation in MDA level. NO, which has the potential of causing nitrosative damage to cells and tissues, was also found to be significantly higher in the molecularly positive group, indicating the presence of nitrosative damage. This finding aligns with that of Molayi-jabdaragi et al. (2020), who reported a positive correlation between the parasite and NO. Although the relationship between total sialic acid (TSA) and oxidative stress is not fully understood in veterinary medicine, a negative correlation between TSA and total antioxidant status (TAS) has been reported. Additionally, an increase in TSA levels has been observed in cases of oxidative stress induced by *T.  annulata* (Molayi-Jabdaragi et al., 2020), which aligns with the findings of the present study.

Our results indicate that the serum TSA concentration in cattle naturally infected with *T. annulata* is significantly different from that of the control group. This finding is consistent with previous studies that also noted increased serum sialic acid levels in animals infected with *T. annulata* (Karagenc et al., 2005; Esmaeilnejad and Froushani, 2016). The primary cause of the increase in sialic acid observed during infection is cellular destruction. As the disease progresses, lysis of mononuclear cells and erythrocytes infected by the parasite releases sialic acid from glycolipids and glycoproteins in the cell membranes into the bloodstream, resulting in elevated serum TSA levels (Dall'Olio, 2000; Çitil et al., 2004). Another mechanism, which plays a more crucial role in the disease’s progression, is the parasite's strategy for evading the host immune system (immune evasion). To ensure its survival within the host, *T. annulata* induces an excessive accumulation of sialic acid (hypersialylation) on the surface of the infected cells. This hypersialylation effectively masks the antigens on the cell surface, preventing natural killer (NK) cells, key players in innate immunity, from recognising and destroying the infected cells. As a result, this facilitates the invasion of erythrocytes by merozoites (Suzuki, 1995; Guzel et al., 2008; Esmaeilnejad and Froushani, 2016). Consequently, during *T. annulata* infection, the serum sialic acid profile changes dynamically, influenced by the severity of the disease, the level of parasitaemia, and the response of the immune system. Measuring sialic acid levels is important not only as an indicator of cellular damage but also for understanding the pathogenesis of the parasite and the mechanisms of immune evasion (Esmaeilnejad and Froushani, 2016).

## Conclusion and Recommendations

In conclusion, the present study, by highlighting the capacity of *T. annulata* to induce oxidative and nitrosative stress and its impact on biochemical parameters, provides essential insights into the biochemical impact of *T. annulata* infection in cattle. The findings demonstrate that *T. annulata* induces oxidative stress and deleterious effects on liver and bile duct function, evidenced by changes in enzyme activities. Additionally, the reductions in total protein and albumin levels observed in this study suggest a possible negative acute-phase inflammatory response and potential hepatic dysfunction resulting from

*T. annulata* infection. Despite the variations in some biochemical parameters, overall, the results support the notion that *T. annulata* significantly affects cattle health by disrupting both antioxidant-oxidant and metabolic homeostasis.

These findings highlight the need for antioxidant supplementation as an adjunct therapy for cattle infected with *T. annulata* to mitigate its adverse effects and enhance animal health. However, future research focusing on potential therapeutic interventions that could alleviate the oxidative stress and liver dysfunction associated with *T. annulata* infection and the role of TSA in *T. annulata*-induced oxidative stress.

## Limitations

The limitations identified in this study include: the absence of complete blood profile data, which limits our ability to thoroughly correlate the parameters. Secondly, although enzyme activities were measured in serum samples, we were unable to perform molecular quantification and gene expression analysis for the enzymes investigated in the liver. Finally, this study did not include data on inflammatory markers; thus, we were unable to fully connect the occurrence of oxidative stress and inflammation. Addressing these points in future research would provide a more comprehensive understanding of *T. annulata*-induced oxidative damage and its impact on liver and bile duct function.

## Declarations

### Competing interests

The authors declare that they have no competing interests.


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### Data availability

All authors declare that all data supporting the results reported in this article are included within it.

### Authorship contribution statement

**Ali Senol:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Supervision

**Elisha A. Akanbong:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation

**Haceli Öcal:** Writing – original draft, Investigation, Formal analysis, Data curation

**Sami Gökpınar:** Writing – review & editing, Writing – original draft, Methodology, Supervision

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