



Original Research

Erythrocytic Oxidative Stress and Haemato-biochemical Changes in Cattle, Affected with Bovine Tropical Theileriosis

S. M. Nayak*, S. K. Senapati, S. Meher, P. Samal, K. Sethy¹ and R. C. Patra

Department of Clinical Veterinary Medicine , College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha, INDIA

¹Department of Animal Nutrition

*Corresponding author: sudhanshumohannayak1992@gmail.com

Rec. Date:	Mar 03, 2018 06:00
Accept Date:	May 20, 2018 16:06
DOI	10.5455/ijlr.20180303060002

Abstract

Bovine Tropical Theileriosis (BTT) is caused by blood protozoa *Theileria annulata* in cattle, which causes high mortality among the animals and incurs huge economic losses to the farmers. It decreases the milk yield and impairs reproductive performances. Measurement of erythrocytic oxidative stress is a need of hour for effective treatment and management of this disease. Oxidative stress and lipid peroxidation in erythrocytes of cattle infected with the intra erythrocytic protozoa results in increased fragility and membrane lysis of erythrocytes which is the major concern of this study. A group of 10 cattle affected with bovine tropical theileriosis were taken as Group II along with another 10 animals (apparently healthy animal) as Group I. The erythrocytic oxidative stress indices along with the haemato-biochemical parameters were measured for both the groups. It was found that there was significant increase ($p < 0.05$) of Malonaldehyde (MDA) value and significant decrease ($p < 0.05$) in super oxide dismutase (SOD) and Catalase in Group II as compared to Group I. In haemato-biochemical parameters, there was significantly decreased ($p < 0.05$) Hb, TEC, PCV, TLC, neutrophil, total protein, Albumin/Globulin ratio and increased AST and lymphocyte count was found in Group II than Group I. So it is concluded that Bovine Tropical Theileriosis (BTT) produces significant oxidative stress along with anemia and alterations in other haematobiochemical parameters.

Key words: Catalase, MDA, Oxidative stress, SOD, Theileriosis

How to cite: Nayak, S., Senapati, S., Meher, S., Samal, P., Sethy, K., & Patra, R. (2018). Erythrocytic Oxidative Stress and Haemato-biochemical Changes in Cattle, Affected with Bovine Tropical Theileriosis. International Journal of Livestock Research, 8(10), 219-225. doi: 10.5455/ijlr.20180303060002

Introduction

The protozoan parasite *Theileria annulata* bestowing the tick-borne disease bovine tropical theileriosis (BTT), intricate enormous economic losses about US\$ 384.3 million annually in Indian livestock sector



(Minjauw and McLeod, 2003). It is transmitted through *Hyalomma anatolicum* ticks which survive better and multiply faster in hot and humid climatic conditions like Odisha.

Oxidative stress is a very good indicator of health status in animals. There is excessive production of reactive oxygen species (ROS) when the body undergoes certain state of disease condition which is countered by natural antioxidant mechanism exist in the body (Samal *et al.*, 2017). This includes antioxidant enzymes like SOD, Catalase, GSH-PX and antioxidant vitamins like vitamin-A, E and C. (Sugino, 2006). Lipid peroxidation is a general mechanism whereby ROS induce tissue damage and are implicated under several diverse pathological conditions (Samal *et al.*, 2016; Halliwela and Gutteridge, 1999). The increased level of oxidative stress and lipid peroxidation in erythrocytes of cattle infected with *T. Annulata* causes increased erythrocyte fragility and membrane lysis which leads to severe anaemia and death (Grewal *et al.*, 2005).

Keeping in view the above facts, the present study was undertaken to study the changes in erythrocytic oxidative stress indices and haemato-biochemical parameters in the BTT affected cattle with respect to healthy cattle.

Materials and Methods

Present study was carried out in the cattle affected with BTT of Bhubaneswar, Khurda, Puri and the presented cases of Teaching Veterinary Clinical Complex, College of Veterinary Science & Animal Husbandry, OUAT. Clinico-pathological investigation for detection of the blood protozoa *Theileria annulata* was carried out for the affected animals showing typical clinical symptoms. 10ml blood was collected from jugular vein with EDTA from 35 suspected cows and blood smear examination was carried out by standard staining method (Kelly's method, 1979) to differentiate the positive cases. The study was carried out by taking 10 number of positive animals into Group II and 10 apparently healthy animal showing blood smear negative for protozoa in Group I. Different investigations like detection of blood protozoa, estimation oxidative stress (MDA, SOD, Catalase), haematological parameters (Hb, PCV, Neutrophil, Lymphocyte, TLC, TEC) and biochemical parameters (AST, ALT, Total protein, A-G ratio) were carried out from collected serum samples. Estimation of oxidative stress parameters MDA, SOD, Catalase was carried out by manual method using double beam UV-VIS spectrophotometer (Samal *et al.*, 2016). The haematobiochemical parameters were estimated by fully automatic analyzer using standard kit method. Data were analyzed by one-way analysis of variance (ANOVA) with post-hoc analysis by Duncan's multiple comparison tests using SPSS 16 software. Results were expressed as mean \pm SE with $p < 0.05$ considered statistically significant.

Results

The haemato-biochemical parameters like Hb, PCV, TEC, TLC, neutrophil, total protein and albumin-globulin ratio (Table 1) are found to be significantly ($P \leq 0.05$) lower in Group II animals than Group I animals, whereas lymphocyte count and AST values were significantly higher ($P \leq 0.05$) in Group II than Group I. no significant difference was found in the value of ALT in Group I and Group II.

Table 1: Haematobiochemical changes in Group II with respect to Group I

Haemato-biochemical Parameters	Group (n=10)	Mean \pm SE
Heamoglobin concentration (g/dl)	I	11.28 \pm 0.18 ^b
	II	06.72 \pm 0.37 ^a
Packed Cell Volume (%)	I	33.84 \pm 0.54 ^b
	II	20.16 \pm 1.10 ^a
Total Erythrocyte Count (*10 ⁶ / mm ³)	I	05.64 \pm 0.09 ^b
	II	03.36 \pm 0.18 ^a
TLC (*10 ³ / mm ³)	I	06.05 \pm 0.16 ^b
	II	03.76 \pm 0.26 ^a
Neutrophil (%)	I	41.17 \pm 0.95 ^b
	II	20.17 \pm 1.19 ^a
Lymphocyte (%)	I	53.83 \pm 0.95 ^a
	II	74.83 \pm 1.19 ^b
Total Protein (g/dl)	I	07.03 \pm 0.05 ^b
	II	05.22 \pm 0.11 ^a
A/G Ratio	I	01.25 \pm 0.03 ^b
	II	00.37 \pm 0.03 ^a
Aspartate Aminotransferease (IU/L)	I	93.30 \pm 4.57 ^a
	II	149.80 \pm 5.80 ^b
Alanine Aminotransferase (IU/l)	I	31.40 \pm 2.38
	II	33.70 \pm 3.01

Group I: Healthy control group; Group II: Cattle affected with bovine tropical theileriosis. Values are expressed as Mean \pm S.E. values. Mean values with different superscripts (^{a, b, c}) differs in a column between the groups are significant ($P \leq 0.05$)

The values of SOD and catalase were found to be significantly lower ($P \leq 0.05$) in Group II than Group I whereas the value of MDA was significantly higher ($P \leq 0.05$) in Group II than Group I (Table 2).

Table 2: Oxidative stress parameters of animals in different groups

Parameters	Groups (n=10)	Mean \pm SE
MDA (nmol/mg Hb)	I	1.12 \pm 0.04 ^a
	II	4.77 \pm 0.14 ^b
SOD (units /mgHb)	I	4.05 \pm 0.15 ^b
	II	1.87 \pm 0.12 ^a
Catalase (units /mgHb)	I	2.12 \pm 0.05 ^b
	II	1.47 \pm 0.12 ^a

Group I: Healthy control group; Group II: Cattle affected with bovine tropical theileriosis. Values are expressed as Mean \pm S.E. values. Mean values with different superscripts (^{a, b, c}) differs in a column between the groups are significant ($P \leq 0.05$)

Discussion

Theileriosis is one of the most important blood protozoan disease in cattle as there is every chance of death along with reduced quantity and quality of milk yield for a prolonged period, reduced reproductive performances leading to heavy economic losses to dairy farmers, immuno-suppression leading to high susceptibility to various diseases (Velusamy *et al.*, 2014). In this study, screening by blood smear examination revealed all forms of *Theileria annulata* piroplasms (cocci, rod, comma, signet-ring, and pear-shaped) with abnormalities in erythrocyte structure which was similar to the findings of Al-Emarah *et al.* (2012). The haematobiochemical and oxidative stress parameters of all animals in Group I and Group II are thoroughly investigated.

The haemoglobin (Hb) concentration in theileriosis affected group was lower ($p < 0.05$) than the healthy control group (Ganguly *et al.*, 2015; Al-Emarah *et al.*, 2012). The mean packed cell volume and total erythrocyte count in Group II were at a significant ($p < 0.05$) lower level than Group I which is in agreement with Kumar *et al.* (2009). In contrast to present study, Vahora *et al.* (2012) recorded normal values of PCV in along with decrease in RBC count and Hb levels. The reduced PCV and TEC as reported in present study may be due to the extensive hemorrhages, abdominal ulcers and persistence of parasitic stages in erythrocytes leading to lower values of PCV, TEC and Hb (Madder *et al.*, 2001) or due to the removal of infected erythrocytes by spleen and liver and not due to the destruction of erythrocytes by the parasite (Modi *et al.*, 2015) or may be due to production of pro-inflammatory cytokines, especially tumor necrosis factor- α (TNF- α), which is implicated in the pathogenesis of anemia in bovine theileriosis by suppressing haemopoietic progenitors (Boulter and Hall, 2000; Kumar *et al.*, 2009) stated lysis of RBC may be due to the lipid peroxidation of membrane leading to low TEC count. Lower level of TLC was found in Group II, which was in accordance with the findings of Hasanpour *et al.* (2013) and Hussein *et al.* (2007) and in contrast Modi *et al.* (2015) and Ugalmugle *et al.* (2010) found significant ($P < 0.01$) increase in the White blood cell (WBC) counts in infected crossbred cattle as compared to healthy control group whereas Tehrani *et al.* (2013) demonstrated that the leucocyte count increased immediately following theileria infection and then significantly decreased within several days. Such changes in leucogram might be attributed to persistent harmful effects of toxic metabolites of *Theileria annulata* on the haemopoietic organs especially on bone marrow and their interference with the process of leucogenesis. Neutrophils level was also lowered ($P < 0.05$) in group-II and group-I which is supported by the findings of Al-Emarah *et al.* (2012) and Ananda *et al.* (2009) and contrasted by Ugalmugle *et al.* (2010) as they found neutrophilia with mild left shift in the theileriosis affected cattle. The mean lymphocyte count in Group II was at a significantly higher level ($p < 0.05$) than healthy control group, which is in agreement with the findings of Ganguly *et al.* (2015) and Muraleedharan *et al.* (2005) whereas several other researchers (Osman and Al-Gaabary 2007, El-Deeb and Younis 2009) have reported a pronounced lymphocytopenia in theileriosis affected cattle. Increase in the

number of lymphocytes reflects compensatory mechanism as target cells in response to their invasion with *Theileria* protozoan. According to Yamaguchi *et al.* (2010), the lymphocytosis may be due to intra-lymphocytic theilerial parasites transforming the host cells, leading to clonal growth of lymphocytes.

The mean AST values in theileriosis affected cattle had significantly ($p < 0.05$) higher level which is in agreement with the findings of Hasanpour *et al.* (2013) and Saber *et al.* (2008) and Ganguly *et al.* (2015). Increase in serum AST level in theileriosis affected animals may indicate the hepatic tissue damage that included coagulation necrosis, distortion of hepatic cords with heavy infiltration of lymphocytes in the periportal areas indicating severe damage to hepatobiliary system, (Modi *et al.*, 2015). Lower level of serum total protein and albumin level is in agreement with Ganguly *et al.* (2015), Saber *et al.* (2008), Hussein *et al.*, 2004, Omer *et al.*, 2003 and Singh *et al.*, 2001. Decreased albumin-globulin ratio are in accordance to Hussein *et al.* (2007) and Singh *et al.* (2001). The hypoproteinaemia and hypoalbuminaemia is possibly due to the harmful effect of toxic metabolites of *Theileria*.

In this present study, the oxidative stress was measured as lipid peroxidase (LPO) in form of malondialdehyde (MDA) n mol/mg of Hb. It was revealed that the cattle suffering from BTT in Group II were having significantly higher level ($p < 0.05$) of mean MDA value as compared to healthy control Group I. This proved that there was oxidative stress at a significant higher level in tropical theileriosis affected cattle. This finding is in agreement with Kumar *et al.* (2009), Radwan *et al.* (2013) and El-deeb *et al.* (2009). Theileriosis affected cattle undergoes oxidative stress and there is reduction in antioxidant enzymes due to their neutralization by the excessive production of free radicals which was revealed lower level of mean erythrocytic catalase values in Group II with respect to healthy cattle in Group I. There was significant ($p < 0.05$) decrease in mean erythrocytic superoxide dismutase concentration in Group II indicating another clue on reduction in antioxidant enzyme concentration, which reflects that theileriosis causes severe oxidative stress, which is in agreement with Rezaei and Dalir-Naghadeh (2006) and Razavi *et al.* (2011).

Conclusion

Based on the above finding, it was concluded that Bovine Tropical Theileriosis (BTT) produces significant oxidative stress along with anemia and alterations in other haematobiochemical parameters in affected cattle as compared to healthy cattle.

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