Haryana Vet. (Dec., 2017) **56 (2),** 129-134

Research Article

INVESTIGATIONS INTO THE HAEMATO-BIOCHEMICAL PROFILE AND OXIDATIVE STRESS INDICES IN CATTLE NATURALLY INFECTED WITH BOVINE TROPICAL THEILERIOSIS

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ABSTRACT

The present study was planned to investigate the haemato-biochemical profile and oxidative stress indices in cattle naturally infected with bovine tropical theileriosis. A total of 18 cross-bred dairy cattle were selected based upon clinical signs consistent with tropical theileriosis and confirmed by detection of piroplasm infected erythrocytes in blood smears and schizonts in lymph node aspirate smears. The haematological profile revealed normocytic, normochromic anaemia with relative lymphocytosis in infected animals. Serum biochemical profile revealed significant increase in gamma-glutamyl transferase activity and levels of indirect bilirubin, total bilirubin and urea with significant lower levels of total protein in infected animals, indicating hepatic and renal dysfunction. Oxidative stress indices both in plasma and haemolysate revealed a significant increase in malondialdehyde level, glutathione peroxidase activity and a non-significant increase in superoxide dismutase activity in infected animals as compared to healthy control. The present data may be utilized for suggesting the therapeutic regimen of the disease in dairy cattle.

Key words: Cross-bred cattle, haematology, Theileriosis, Oxidative stress, Biochemistry

Bovine tropical theileriosis caused by *Theileria annulata,* causes heavy economic losses in large parts of Asia. The parasite causes lethal infections in exotic cattle and considerable mortality in indigenous and crossbred cattle. *Hyalomma anatolicum* (3-host tick vector) is an important vector for *T. annulata.* The disease is mainly characterized by fever and lympho-proliferative disorders, associated with varying degrees of leucopaenia and/or anaemia (Radostits *et al*., 2007; Verma and Singh 2016). Progressive anaemia is the main pathological feature of theileriosis which occurs due to increased fragility and oxidative damage in erythrocytes, damage to susceptible erythrocytes by parasites in the reticuloendothelial system, autoimmune reactions and intra-erythrocytic piroplasms (Basbug *et al*., 2011). Hepatic tissue damage evident by the increased level of AST (aspartate transaminase), ALT (alanine transaminase), direct bilirubin, total bilirubin, gamma glutamyl transferase and decreased level of total protein, cholesterol and triglycerides have been reported (Ganguly *et al*., 2015; Ellah, 2015). Along with hepatic dysfunction, *T. annulata* causes hypoproteinaemia, hypoalbuminaemia and hypophosphataemia along with renal dysfunction (Ellah, 2015).

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There are several reports suggesting role of lipid peroxidation in pathogenesis of anaemia in *theileriosis.* Lipid peroxidation is a general mechanism through which free radicals induce tissue damage and implicated under several pathological conditions (Halliwell and Gutteridge, 1999). The degree of lipid peroxidation is mainly used as an indicator of damage, caused by reactive oxygen species and the concentration of malondialdehyde (MDA) in blood and tissues (El-Bahr, 2013). Glutathione peroxidase enzymes remove hydrogen peroxide (H_2O_2) by using it to oxidize reduced glutathione (GSH) to oxidized glutathione (GSSG). Glutathione reductase, an FAD containing enzyme, regenerates GSH from GSSG, by using NADPH as a source of reducing power (Flohe, 1982). Superoxide dismutase, another antioxidant enzyme neutralizes superoxide ions by going through successive oxidative and reductive cycles of transition metal ions at its active site. The level of antioxidant enzymes such as superoxide dismutase (SOD) (Razavi *et al*., 2011) and glutathione peroxidase (GPx) (Nazifi *et al*., 2009; Razavi *et al*., 2011) may be altered by the parasite *T. annulata*. Considering the pathological effects of the disease, the present investigation was planned to investigate the haemato-biochemical profile and oxidative stress indices in cattle naturally infected with tropical theileriosis.

MATERIALS AND METHODS

Selection of Animals : A total of 18 cross-bred dairy cattle presented for treatment at Teaching Veterinary Clinical Complex (TVCC) along with animals from University Cattle Farm and a Private Cattle Farm of Rohtak having history of ticks infestation, pyrexia, lymph node enlargement and anaemia were included in this study. Animals having haemoglobin level <6 g/dl and showing signs of severe pneumonia, severe anaemia and recumbency were excluded from this study. Eighteen cross-bred cattle (9 adults and 9 calves) living under identical management conditions and found uninfected for *T. annulata* upon screening, were considered as healthy control.

Clinical Observations : The clinical vital parameters recorded were rectal temperature (°F), pulse rate (per minute) and respiration rate (per minute). The superficial lymph nodes were palpated and the enlargement graded as 1 to 4 (Rakha and Sharma, 2003). In control animals where the lymph node was not palpable, was graded as '0', 1 means 'very small', 2 means 'small', 3 means 'large' and 4 means 'very large'. The visible mucous membranes were examined to assess the degree of anaemia and graded as 1 to 4. In control animals where the mucous membrane was normal, graded as '0', 1 means 'mild anaemic', 2 means 'moderate anaemic', 3 means 'severe anaemic' and 4 means 'very severe anaemic'.

Parasitological Observations : Thin blood smear and lymph node aspirate smear were stained by Giemsa staining and percent parasitaemia was estimated microscopically by counting the numbers of piroplasm infected erythrocyte in total of about 1,000 erythrocytes. Presence of schizonts in lymph node aspirate smears was semi-quantitatively measured on the scale of 1 to 4 (Rakha and Sharma, 2003). It was a subjective measurement where in '1' stands for rare, '2' means sparse, '3' means high and '4' means very high in numbers in the aspirate smear.

Haemato-biochemical Examinations : The blood samples collected in EDTA vacutainers were used for complete haematological examination using fully automated Haematology Cell Counter (MS4s, Melet Schlosing Lab.). The serum samples were analyzed for estimation of biochemical profile using fully automated random access Clinical Chemistry Analyzer (EM Destiny 180, Erba Diagnostics Mannheim GmbH). Blood samples collected in heparin were used for measurement of oxidative stress indices both in plasma and haemolysate. Lipid peroxidation in terms of malondialdehyde (MDA) levels was assessed by method of Ohkawa *et al*., (1979). Glutathione peroxidase (GPx) activity was measured by method of Hafeman *et al*., (1974). Superoxide dismutase (SOD) activity was measured by method of Madesh and Balsubramanian, (1998). The haemoglobin in the haemolysate was estimated spectrophotometrically by the cyano-methaemoglobin method (Vankampen and Ziglstra 1961). The plasma, haemolysate and serum samples were stored at -20°C in aliquots till analysis.

Statistical Analysis: The data generated was analyzed statistically by suitable statistical methods using statistical software package (SPSS 16.0).

RESULTS AND DISCUSSION

All diseased animals revealed high rectal temperature, increased pulse rate and respiration rate (Table 1). Pyrexia is due to the liberation of endogenous pyrogens attributed to cellular lysis and parasitaemia leading to the stimulation of thermoregulatory centre in the hypothalamus (Glass *et al*., 2003). Increase in respiration rate might be due to the accumulation of oedematous fluid inside the lungs and thoracic cavity (Muraguri *et al*., 2006). Mean lymph node size in diseased animals was 2.83±0.17 (ranging from 1 to 4) i.e. very small to very large. The enlargement of superficial lymph nodes can be explained on the basis of lymphoid hyperplasia in early stage of the disease that occurs due to proliferation of schizonts inside the mononuclear cells and causes inflammatory reaction in the infected lymph node (Jabbar *et al*., 2008). Mean grade of colour of mucous membrane in diseased animals was observed as 1.05±0.05 (ranging from 1 to 2), indicating mild to moderately anaemic condition. All

Table 1
Clinical vital parameters of cattle infected with bovine tropical theileriosis (Mean ± S.E.)

Parameter	Control animals (n=18)	Diseased animals (n=18)
Temperature (°F)	101.7±0.11	104.4 ± 0.12
Pulse rate (per min)	47.72±1.45	73.22 ± 1.70
Respiration rate (per min)	20.56±1.76	44.39 ± 1.56

Table 2
Haematological profile of cattle infected with bovine tropical theileriosis (Mean ± S.E.)

Parameter	Control animals (n=18)	Diseased animals (n=18)
Hb (g%)	9.51±0.43	8.13±0.36
TEC (M/mm^3)	8.20±0.41	7.04±0.23*
PCV (%)	31.63±1.11	30.91±1.46
MCV (fl)	39.92±2.02	44.52±1.88
MCH (pg)	10.99±0.55	11.60±0.38
MCHC (g/dl)	27.88±0.89	26.70±1.19
TLC (m/mm^3)	9.68±0.74	7.05±0.49
Lymphocytes (%)	38.17±2.61	84.95±4.32*
Monocytes (%)	14.87±1.16	4.88±1.59
Neutrophils (%)	40.76±3.43	8.34±2.68
Eosinophils (%)	5.80±1.69	1.68±0.47
Basophils (%)	0.40±0.06	0.14±0.41
THR (m/mm^3)	395.33±73.31	416.56±84.50
MPV (fl)	8.46±0.18	8.45±0.15

Value with superscript * differ significantly (P<0.05) in a row. Hb=Haemoglobin content; TEC=Total erythrocyte count; PCV=Packed cell volume; MCV=Mean corpuscular volume; MCH=Mean corpuscular haemoglobin; MCHC=Mean corpuscular haemoglobin concentration; TLC=Total leucocyte count; THR=Thrombocytes; MPV=Mean platelet volume.

diseased animals were anorectic, dull and having history of tick infestation, and 11 animals out of 18 were diarrhoeic. The anorexia usually accompanies with high fever. Dullness may be due to anorexia, fever, anaemia and deteriorating effect of parasite on body of the animal. Diarrhoea seen in infected cattle can be explained as a result to inflammatory reaction and ulceration of abomasum and gastro-intestinal tract (Radostits *et al*., 2007). Percent parasitaemia in diseased animals in blood smear was less than 1%, indicating the cases of mild theileriosis. Similarly, schizont density in diseased animals in lymph node aspirate smear was less than 1%, indicating mild theileriosis (Al-Temeimy, 1982).

Erythrocytic indices of cattle infected with *T. annulata,* revealed a significant decrease in total erythrocyte count and the result was in agreement with Al-Temeimy (1982), Nazifi *et al*. (2009) and Tuli *et al*. (2015). However, the mean values of Hb, PCV, MCH, MCHC and MCV in the present study were non-significantly altered than that of control animals (Table 2). This might be due to our selection criteria, as only animals having haemoglobin level more than 6 g/dl were selected. The overall erythrocytic indices were indicative of normocytic normochromic anaemia. Changes in erythrocytic indices occur due to harmful effect of toxic metabolites of *Theileria* parasite on bone marrow interfering with the process of erythropoiesis. The modern research explains the mechanism of anaemia might be due to the activity of antioxidant enzymes under the influence of parasite resulting into increased fragility of RBCs and thus, acceleration of erythrocytes clearance by phagocytic cells (Grewal *et al*., 2005). Leucocytic indices revealed a significant increase in relative lymphocyte count and non-significant decrease in total leucocyte count, neutrophils, monocytes, eosinophils and basophils count among infected animals when compared to healthy control (Table 2). Such changes in leucogram might be attributed to the persistent harmful effects of toxic metabolites of *Theileria* spp. on haemopoietic organs especially bone marrow and their interference with process of leuococytogenesis (Mbassa *et al*., 1994). Significant increase in the number of lymphocytes reflects a compensatory mechanism as target cells in response to invasion with *T. annulata* (Jabbar *et al*., 2008). Non-significant differences in thrombocyte count and mean platelet volume were observed in infected animals as compared to healthy control. In contrary, Schalm *et al.* (1975) and Ellah (2015) reported a significant decrease

Table 3
Serum biochemical profile of cattle infected with bovine tropical theileriosis (Mean ± S.E.)

Parameter	Control animals (n=18)	Diseased animals (n=18)
ALT (U/L)	27.52±1.29	36.72±1.60
AST (U/L)	58.32±3.16	72.04±5.90
GGT (U/L)	18.11±0.83	25.45±1.98*
Bilirubin direct (mg/dl)	0.08±0.01	0.11±0.01
Bilirubin indirect (mg/dl)	0.03±0.00	0.05±0.01*
Bilirubin total (mg/dl)	0.11±0.01	0.16±0.02*
Alkaline phosphatase (U/L)	92.11±9.57	53.28±8.34
Albumin (g/dl)	3.44±0.09	2.95±0.10
Globulin (g/dl)	5.02±0.17	3.46±0.42*
Total protein (g/dl)	8.46±0.15	6.42±0.39*
Urea (mg/dl)	26.60±1.20	37.69±3.03*
Creatinine (mg/dl)	1.18±0.08	1.37±0.11
Total calcium (mg/dl)	10.02±0.26	9.10±0.16
Inorganic phosphorus (mg/dl)	7.15±0.39	6.68±0.35
Magnesium (mg/dl)	2.45±0.16	2.32±0.11

Value with superscript * differ significantly (P<0.05) in a row. ALT=Alanine transaminase; AST=Aspartate transaminase; GGT=Gamma glutamyl transferase.

in number of thrombocyte count attributed to depression of bone marrow in diseased animals.

Serum biochemical profile of infected animals showed significant (P<0.05) increase in levels of serum GGT, indirect and total bilirubin (Table 3). The serum levels of ALT, AST and bilirubin (direct) in diseased animals were non-significantly higher than those of healthy control which is in agreement with Ellah (2015). ALT and AST are involved in amino acid and carbohydrate metabolism. These enzymes are present in high concentrations in muscle and liver. Elevation of these enzymes in blood indicates necrosis or disease in these tissues (Murray *et al*., 1996). The findings of increase in bilirubin levels in serum are in agreement with Dhar and Gautam (1977) and Saber *et al*. (2008) who reported a significant increase in direct and indirect bilirubin in diseased animals. The increase in the levels of bilirubin might have resulted from the destruction of parasitized erythrocytes by erythro-phagocytosis in reticulo-endothelial system (Saber *et al*., 2008). There was significantly (P<0.05) lower levels of serum total proteins and globulin in infected animals as compared to healthy control while the albumin levels in diseased animals were non-significantly lowered than that of control animals. These findings are in agreement with Dede *et al*., (2014) and Ellah (2015). Hypoproteinaemia was likely due to hypoalbuminaemia induced by liver damage caused by *T. annulata* infection which includes coagulative necrosis, destruction of hepatic cords and heavy infiltration of lymphocytes in peri-portal area (Stockham *et al*., 2000). A significant (P<0.05) increase in serum urea levels was observed among infected animals while the serum creatinine levels in diseased animals showed a non-significant variation as compared to healthy control. It is in agreement with Sandhu *et al*. (1998) and Ellah (2015). It is probably because of renal dysfunctions associated with increase in haemoglobin catabolism. However, Omer *et al*. (2003) recorded relatively low creatinine concentration in naturally infected cattle. Non-significant (P<0.05) difference was found in serum total calcium, inorganic phosphorus and magnesium levels in infected animals as compared to healthy control, however, these values were in lower range than healthy control. These findings are in agreement with Omer *et al*. (2003) who reported significant decrease in levels of these macro-minerals. The decrease in serum calcium level might be due to hypoalbuminaemia and hypomagnesaemia while the decrease in serum phosphorus and magnesium levels in infected animals might be associated with diarrhoea and renal wasting (Agus *et al*., 1982).

Oxidative stress indices of cattle infected with bovine tropical theileriosis showed a significant (P<0.05) increase in malondialdehyde level in plasma and a non-significant increase in haemolysate as compared to control animals (Table 4). Oxidative stress occurs when the production of the reactive metabolites of oxygen exceeds their safe disposal by antioxidant mechanisms. The erythrocytic membrane is rich in polyunsaturated fatty acids, a primary target for reactions involving free radicals and is very susceptible to lipid peroxidation (Devasena *et al*., 2001). The higher levels of lipid peroxidation (MDA) was in accordance with the earlier reports (Grewal *et al*., 2005; Rezaei and Dalir-Naghadeh, 2006; Kumar 2016). Glutathione peroxidase activity both in plasma and haemolysate was significantly (P<0.05) higher in infected animals. Superoxide dismutase activity was non-significantly (P<0.05) higher in infected animals as compared to control animals. These findings were contrary to the findings of Rezaei and Dalir-Naghadeh (2006) and Razavi *et al*. (2011) who reported activities of erythrocyte glutathione peroxidase and superoxide dismutase significantly lower in *T. annulata* infected animals as compared to that of healthy control. The significant rise in GPx activity in infected animals in the present study might be due to the fact that GPx activity constitutes the major mechanism for intracellular decomposition of lipid peroxides (Flohe 1971). Hafeman *et al*. (1974) also proposed crucial role of GPx for protection of membranes from peroxides damage induced

Table 4
Oxidative stress indices of cattle infected with bovine tropical theileriosis (Mean ± S.E.)

Parameter	Control animals(n=18)	Diseased animals(n=18)
MDA plasma (nmol/ml)	6.48±0.73	16.69 ±1.99*
MDA haemolysate (nmol/ml)	16.93±1.86	40.25±2.81
GPx plasma (U/mg protein)	0.37±0.05	3.47±0.20*
GPx haemolysate (U/mg Hb)	1.31±0.20	6.85±0.69*
SOD plasma (U/mg protein)	0.36±0.03	0.44±0.03
SOD haemolysate (U/mg Hb)	0.16±0.01	0.18±0.02

Value with superscript * differ significantly (P<0.05) in a row. MDA=Malondialdehyde level; GPx=Glutathione peroxidase activity; SOD=Superoxide dismutase activity.

by lipid peroxides. Reduced glutathione is required for the disposal of H_2O_2 from erythrocytes by a reaction catalysed by GPx. This reaction is important because accumulation of H_2O_2 might decrease the lifespan of erythrocytes by increasing the rate of oxidation of haemoglobin to methaemoglobin. Along with the important role of GPx, the activity of SOD could also be assigned as factor in preventing the RBC membrane from peroxide damage induced by lipid peroxides. Similar findings were reported by Grewal *et al*. (2005) and Kumar *et al*. (2016), who observed that infection with *T. annulata* led to increased oxidative stress in the animals. The increase in antioxidant enzymes could be a safeguard mechanism to protect the erythrocytes from oxidative stress in response to increased lipid peroxidation in erythrocytes in the earlier stages of the disease, however, the activity of the antioxidant enzymes would gradually decrease as a sequelae of insufficient capability of such mechanisms to neutralize the oxidative agents in the later stages of the disease.

It may be concluded from the present study that cattle infected with bovine tropical theileriosis manifests mild haemato-biochemical alterations suggestive of normocytic, normochromic anaemia, relative lymphocytosis, hepato-renal dysfunction while the oxidative stress indices reveal activation of compensatory antioxidant mechanism to combat oxidative damage. The present data may be utilized for suggesting the therapeutic regimen of the disease in dairy cattle.

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