Oxidant markers and their impact on antioxidant status and erythrocyte fragility in theileriasis in calves

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ABSTRACT
The present study has been carried out on the calves less than one month of age suffering from clinical Theileriosis. All the cases came to Referral veterinary polyclinic, IVRI, Izatnagar, Bareilly for treatment during 2015. Erythrocytic LPO in clinically affected calves were significantly (P<0.05) higher than healthy animals. GSH, and SOD values in ailing calves were significantly (P<0.05) lower than healthy control and posttreated calves. Catalase was also higher in infected calves but non significant. The hemoglobin concentration was significantly (P<0.05) lower in ailing animals. The erythrocyte fragility at 0.9%, 0.8% and 0.6% NaCl concentration was significantly (P<0.05) higher than the control group. However, there were less erythrocytic fragility observed in NaCl 0.4% and 0.2% concentration in infected animals. In conclusion, anemia occurs in *Theileria annulata* infection is due to corpuscular oxidative damage as revealed from lipid peroxidation and antioxidant status which contribute to RBC fragility and consequent anemia.

Key words: Antioxidant, Catalase, Glutathione peroxidase, Oxidative stress, Peroxidation, *Theileria annulata.*

INTRODUCTION
Theileriosis is a hemoprotozoal disease caused by various species of *Theileria* in various animals. It is a progressive bovine lymphoproliferative disorder attributed to intracellular protozoan parasite *Theileria annulata* (Omer et al., 2003). It is prevalent in Middle East, India, middle Asia and even in China (D’Oliveira et al., 1995). In the beginning of infection *Theileria annulata* proliferate in lymphoid tissues followed by invasion in erythrocytes to complete their life cycle (Soulsby, 1982), leading to severe anemia (Jain, 1993). Although this mechanism of entering the erythrocytes has been the subject of considerable attention, but still exact underlying mechanisms of anemia yet to be explained (Shino et al., 2004). As per earlier report, it may be due to haemagglutinin (Hooshmand-Rad, 1976), autoimmune reaction (Stockham and Scott, 2002). But some recent studies suggest that it may be due to oxidative damage to the erythrocytes (Shino et al., 2003; Nazifi et al., 2008). From this point of view, the activities of various antioxidant marker like superoxide dismutase (SOD), glutathione peroxidase (GPX) (Nazifi et al., 2009) and catalase (Grewal et al., 2005) may be altered by the parasite, resulting in the enhancement of the erythrocyte clearance by phagocytic cells (Shino et al., 2003). On the other hand, some reports suggest that RBC destruction during oxidative stress was related to lipid peroxidation of RBCs (Friedman, 1979; Grewal et al., 2005). Various pro-inflammatory cytokines are produced from mononuclear cells in hemoprotozoal diseases by activated mononuclear phagocytic cells such as oxygen and reactive nitrogen species (Brown 1998; Brunet et al., 2001 and Woldehiwet et al., 2010) leading to oxidative damage in the animal body (Zaidi et al., 2005). Oxidative stress occurs as a result of imbalance between radical-generating and radical scavenging activity, leading to oxidative tissue damage, when the cellular oxidant state is overwhelmed by excess production of reactive oxygen species (Saleh et al., 2011). Augmentation of median corpuscular fragility in erythrocytes of affected cattle indicates injury to RBC membrane leads to increase in permeability of these cells. Such alteration can results from oxidative stress and lipid peroxidation. Alteration of membrane stability results in augmentation of RBC osmotic fragility, because of morphological changes in the erythrocytes (Wagner et al., 1988; Saluja et al., 1999). This work has been undertaken to evaluate the activities of the key antioxidant enzymes (SOD, GPX and catalase), lipid peroxidation of erythrocytes, mean corpuscular fragility of RBC in calves naturally infected with *T. annulata.*

MATERIALS AND METHODS
Total six calves of less than one month of age were included in this study. All the cases brought to Referral veterinary polyclinic, IVRI, Izatnagar, Bareilly for treatment during last year. All the cases were showing the signs of high fever, pale mucus membrane, tick infestation, bilateral prescapular lymph node enlargement. Blood samples were collected from the jugular vein into EDTA containing tubes for measuring hematological parameters and with heparin

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for measuring antioxidant enzyme activities, lipid peroxidation and MCF. Thin blood and lymph node smears were prepared, fixed with absolute methanol (3 min), stained with 10% Giemsa solution (45 min) and examined under oil immersion (×1000) to observe abnormal RBCs and intraerythrocytic forms and Koch’s blue bodies of *T. annulata* in RBC and lymphocytes respectively. To measure the oxidative stress indices, whole blood sample was centrifuged at 750 g for 15 min. Plasma was aspirated off and the erythrocyte pellets were washed three times with phosphate buffer saline solution. 10% hemolysate was prepared by addition of chilled distilled water to RBC pellet i.e 1 ml of pellet and 9 ml of distilled water. Lipid peroxidation and antioxidant parameter were measured as per standard protocol. Lipid per oxidation (LPO) as per method described by (Placera et al., 1966), GSH as per method described by Butler et al. (1963), Superoxide dismutase (SOD) as per method described by Marklund and Marklund, (1974) and Catalase as per method described by Slaughter and O’Brien, (2000). Hemoglobin concentration (Hb) was measured by “Acid hematin method” (Baker et al., 1965). The Mean corpuscular fragility test was done as per method described by Chanarin (1989). Post-treatment blood after clinical recovery from calves was taken for the comparison between infected and healthy. Another six apparently healthy calves of same ages were screened for the above parameters as healthy control.

**RESULTS AND DISCUSSION**

Erythrocytic LPO in treated and control groups were 305.34±1.48 and 251.53±1.03 respectively and the corresponding values in clinically affected calves were 534.32±6.1. Similarly, GSH values in treated, control and ailing calves were 18.95±0.27, 12.48±3.4 and 43.40±2.41 respectively (Table 1). SOD value treated, control and infected were 27.18±0.36, 25.29±0.84 and 29.12±0.21 respectively. Catalase were also found 7.68±0.17, 7.24±0.72 and 7.49±0.15 in treated, control and infected respectively. The LPO and GSH are significantly (P<0.05) higher in clinically affected calves than the control and treated calves. However, there was no significant difference in catalase and SOD activities in all the groups. The Hemoglobin concentration was decline significantly to 4.98±0.65 in ailing calves from 10.85±0.25, 11.20±0.35 in treated and control groups. The maximum erythrocytic fragility was 91.87±0.41 at 0.2% NaCl concentration, while it was 4.73±0.33 at 0.9% NaCl concentration in ailing calves. The erythrocyte fragility at 0.9%, 0.8% and 0.6% NaCl concentration was significantly (P<0.05) higher in clinically infected calves than the treated and healthy control groups (Table 2).

Significant rise of LPO in clinically affected calves is an indicator of oxidative stress caused by the piroplasm in infected RBC. Oxidative stress results when there is increased production of reactive metabolites of oxygen occur and the disposal mechanism through antioxidant fails to neutralize them. The RBC membrane is rich in polyunsaturated fatty acids which is a primary target for free radicals mediated damage, and is very susceptible for lipid peroxidation (May et al., 1998; Devasena et al., 2001). The increased level of LPO seen during the present study were similar in RBC samples of cattle naturally infected with *Theileria* (Nazirogil et al., 1999; Saluja et al., 1999). However, in one study Sahoo and colleagues (2001) reported insignificant rise in LPO in theileriasis. Excessive quantity of LPO production in Theileria infected calves results in lipid peroxidation in erythrocytes and subsequent oxidative injuries to erythrocytes during parasitemia, and confirmed that parasites may disturb RBC antioxidant mechanisms during infection (Razavi et al., 2011).

In the present study it has been observed that a high level of GPX and SOD activities in calves infected with *T. annulata*. From this it has been concluded that the increased level of GSH during parasitemia could be due to the fact that this enzyme activity is the main mechanism for the intracellular destruction of lipid peroxides activity (Razavi et al., 2011). Grewal et al. (2005) also reported a significant increase in the activity of this enzyme in cattle naturally

*S*Means with different superscript differs significantly at P<0.05.

**Table 1:** Erythrocytic oxidant marker and antioxidant in terms of mg Hb in clinically affected calves (n=6).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy control Mean±SE</th>
<th>Theileria Positive Mean±SE</th>
<th>Post treatment Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO (nmol/ml)</td>
<td>251.53±1.03*</td>
<td>534.32±6.1*</td>
<td>305.25±1.48*</td>
</tr>
<tr>
<td>GSH (mM/mgHb)</td>
<td>12.48±3.4*</td>
<td>43.40±2.41*</td>
<td>18.95±0.27*</td>
</tr>
<tr>
<td>SOD (unit/mgHb)</td>
<td>25.29±0.84*</td>
<td>29.12±0.21*</td>
<td>27.18±0.36*</td>
</tr>
<tr>
<td>Catalase (unit/mgHb)</td>
<td>7.24±0.72*</td>
<td>7.49±0.15*</td>
<td>7.68±0.17*</td>
</tr>
<tr>
<td>Hb (mg/ml)</td>
<td>11.20±0.35*</td>
<td>4.98±0.65*</td>
<td>10.85±0.25*</td>
</tr>
</tbody>
</table>

*Means with different superscript differs significantly at P<0.05.

**Table 2:** Erythrocyte fragility test in calves with clinical Theileriosis at various concentrations of NaCl (n=6).

<table>
<thead>
<tr>
<th>NaCl conc(%)</th>
<th>Healthy control Mean±SE</th>
<th>Theileria Positive Mean±SE</th>
<th>Post treatment Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9</td>
<td>0.039±0.03*</td>
<td>4.73±0.33*</td>
<td>0.04±0.01*</td>
</tr>
<tr>
<td>0.8</td>
<td>2.31±0.04*</td>
<td>13.43±0.41*</td>
<td>2.85±0.07*</td>
</tr>
<tr>
<td>0.6</td>
<td>14.23±0.07*</td>
<td>30.91±0.31*</td>
<td>15.42±0.09*</td>
</tr>
<tr>
<td>0.4</td>
<td>84.63±0.27*</td>
<td>84.67±0.16*</td>
<td>85.28±0.15*</td>
</tr>
<tr>
<td>0.2</td>
<td>96.0.54±0.56*</td>
<td>91.87±0.52*</td>
<td>95.14±0.75*</td>
</tr>
</tbody>
</table>
infected with *T. annulata* and concluded that such an increase could be due to a protective mechanism to protect the erythrocytes from oxidative stress due to increased lipid peroxidation in erythrocytes. However, Asri et al., (2006) reported reduced activities of SOD and GSH, because of dependence of the activities of these enzymes to NADPH levels in the cell.

The catalase activity in infected calves was found to be reduced than the healthy calves. It has been reported that catalase is of equal importance to GSH-Px in the defense of human erythrocytes against H₂O₂ generating reactions (Harvey, 1989). However, the results of the present study indicated that catalase might be acting in concert with GSH-Px to scavenger H₂O₂ for the protection of erythrocytes infected by *Theileria*. In the present study, the insignificant decrease in catalase activities was in agreement with the findings of Grewal et al. (2005) and (Razavi et al., 2011). The median corpuscular fragility (MCF) in affected cattle was significantly (P < 0.05) lower than those of healthy calves in 0.4% and insignificant lower in 0.2% NaCl concentration and showed significantly (P < 0.05) increased erythrocytes fragility in comparison with healthy calves in 0.9%, 0.8% and 0.6% NaCl concentration. This finding shows that erythrocytes from theileria affected calves are more susceptible to hemolysis. Increased median corpuscular fragility in erythrocytes of affected calves indicates injury to erythrocytes membrane and subsequent alteration in permeability of these cells. Such alteration can results from oxidative stress and lipid peroxidation. The present findings are in agreement with the findings of Asri et al., (2006), Yagi et al., (1989) and Haider (1992) in calves infected with *T. sergenti*. They reported that, increase in parasitemia causes significant augmentation of osmotic fragility of erythrocytes. Reduction in membrane stability leading to increased RBC osmotic fragility, because of morphological changes in the cell surface of erythrocytes (Wagner *et al*., 1988; Saluja *et al*., 1999). These morphologically altered erythrocytes are removed from the body by macrophages through a process of erythrophagocytosis, which commonly results in severe anemia, Winterbourn (1990).

**CONCLUSION**

In conclusion, the results of the present study showed significant increase in lipid peroxidation of the membrane of erythrocytes of calves suffering from theileriosis. Further it has been concluded that the increased level of GSH and SOD during parasitemia could be due to the fact that this enzyme activity is the main mechanism for the intracellular destruction of lipid peroxides activity (Razavi *et al*., 2011). However, a significant rise in the activities of the antioxidant enzymes SOD and GSH could not lower oxidative stress. The erythrocytic fragility was also found exaggerated in clinically affected calves consequently lead to augmentation of erythrophagocytosis resulting in anemia.

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**REFERENCES**


