Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 142 (2006) 179–186
veterinary parasitology
www.elsevier.com/locate/vetpar

Short communication

Evaluation of antioxidant status and oxidative stress in cattle naturally infected with *Theileria annulata*

S. Asri Rezaei *, B. Dalir-Naghadeh

Department of Clinical Sciences, School of Veterinary Medicine, Urmia University, Urmia, P.O. Box 1177, Iran

Received 8 February 2006; received in revised form 10 May 2006; accepted 29 May 2006

Abstract

To assess the antioxidant status and oxidative stress in bovine theileriosis due to *Theileria annulata* blood samples were collected from 35 clinically affected cattle referred to Veterinary Teaching Hospital, School of Veterinary Medicine, Urmia University, Urmia, Iran. Complete blood count, piroplasm parasitemia percentage, erythrocyte glutathione peroxidase, superoxide dismutase, catalase and glucose-6-phosphate dehydrogenase activities, malondialdehyde concentration, osmotic fragility test and median corpuscular fragility were determined and the results were compared with those of 50 healthy controls. Of 35 affected cattle, 12 (34.28%) had severe anemia and 23 had mild to moderate anemia and parasitemia varied from 5 to 40%. The activities of erythrocyte glutathione peroxidase, superoxide dismutase and glucose-6-phosphate dehydrogenase were significantly lower ($P < 0.0001$) and the activity of catalase was significantly higher in the affected cattle than in healthy ones ($P < 0.001$). Malondialdehyde concentration in erythrocytes of affected cattle was significantly more than those of healthy cattle ($P < 0.001$). The affected cattle showed increased fragility of erythrocytes, so that median corpuscular fragility (MCF) in affected group was significantly lower than those of healthy group ($P < 0.0001$). Median corpuscular fragility showed a positive correlation with the severity of parasitemia ($r = 0.81$, $P < 0.0005$) and a negative correlation with the activities of GSH-Px ($r = -0.78$, $P < 0.0001$), SOD ($r = -0.71$, $P < 0.0005$), catalase ($r = -0.53$, $P < 0.018$) and G6PD ($r = -0.58$, $P < 0.0005$). The results of this study suggest that oxidative damage to RBCs may contribute to the pathogenesis of anemia in bovine theileriosis.

Keywords: *Theileria annulata*; Glutathione peroxidase; Superoxide dismutase; Catalase; Glucose-6-phosphate; Dehydrogenase; Malondialdehyde; Oxidative stress

1. Introduction

Tropical theileriosis is a progressive lymphoproliferative disease of cattle caused by protozoan parasite *Theileria annulata* (Omer et al., 2003a,b; Taylor et al., 1992). The parasite acts as a serious constraint to cattle production in endemic areas, causing lethal infections in exotic cattle and considerable mortality in indigenous and crossbred stocks (Forsyth et al., 1997).

The significant feature of the disease is hemolytic anemia (Gill et al., 1977; Aulakh et al., 1998; Omer et al., 2002), caused by an immune-mediated hemolysis which indicated by the presence of a hemagglutinin (Hooshmand-Rad, 1976). Although, various evidences have been presented to explain the mechanism of the anemia, but the exact underlying mechanism is currently unknown (Shiono et al., 2004). Some hematological changes in red blood cells (RBCs) including, increased osmotic fragility of RBCs, acceleration of erythrocytes clearance and the presence

* Corresponding author. Tel.: +98 441 3650186; fax: +98 441 3650186.
E-mail address: s.asri@mail.urmia.ac.ir (S. Asri Rezaei).

0304-4017/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetpar.2006.05.033

of hemolytic activity in highly infected cattle are some of the suggested possible mechanisms in inducing anemia (Shiono et al., 2003b).

There are some evidences that oxidative stress and lipid peroxidation incorporate in pathogenesis of anemia in *theileriosis*. Lipid peroxidation is a general mechanism whereby free radicals induce tissue damages, and implicated under several diverse pathological conditions (Halliwell and Gutteridge, 1999; Knight, 1995). Malondialdehyde (MDA), an end product of polyunsaturated fatty acid oxygenation, is a reliable and commonly used biomarker for assessing lipid peroxidation (Moore and Roberts, 1998). In recent years, using MDA as a marker of lipid peroxidation, there has been growing interest in studying the role played by lipid peroxidation in various kinds of diseases (Sheu et al., 2003).

Grewal et al. (2005) showed increased oxidative stress and a significant increase in lipid peroxidation in erythrocytes of cattle infected with *T. annulata*. They concluded that this might be the cause of increased erythrocyte fragility due to membrane lysis. Recently, it has been documented that the levels of methemoglobin, used as an index of erythrocytes oxidation, markedly increase at the onset of anemia in experimental *Theileria sergenti* infection (Shiono et al., 2003a) and an inverse relationship has been observed between methemoglobin levels and PCV (Shiono et al., 2001). In addition, increased oxidation of proteins in the membrane of erythrocytes at the advanced stage of anemia in *T. sergenti*-infected cattle has been reported (Yagi et al., 2002). Shiono et al. (2003b) indicated that the levels of antioxidants in RBC decreased during the progression of anemia in cattle infected with *T. sergenti*. They suggested that oxidative damage of RBC has a close relationship with the onset of anemia in bovine theileriosis. These results strongly support the hypothesis that oxidative changes in erythrocytes are closely related to the pathogenesis of anemia in theileriosis.

Tropical theileriosis caused by *T. annulata* is one of the most prevalent and fatal diseases of cattle in Iran. The work described here was undertaken to determine the activities of erythrocyte glutathione peroxidase, superoxide dismutase and glucose-6-phosphate dehydrogenase as important profiles of the antioxidant status and the level of malondialdehyde, as a biomarker of oxidative damage to erythrocytes in cattle clinically affected with theileriosis. In addition, the interrelationship of these markers with degree of parasitemia and anemia has been evaluated.

2. Materials and methods

2.1. Source of animals and samples

The study was carried out in the north west of Iran (west Azerbaijan), in a region where theileriosis due to *T. annulata* is very prevalent during warm seasons. Data were from an observational clinical study conducted in the Veterinary Teaching Hospital, School of Veterinary Medicine, Urmia University, Urmia, Iran. The study group was comprised of 35 crossbred cattle (Holstein Friesian X local native breeds) clinically affected with theileriosis caused by *T. annulata*. Cattle included in the study ranged from 1 to 5 years old (mean $\pm$ S.D. 3.6 ± 1.21 years old) of both sexes. As a control group, 50 clinically healthy cattle from several farms in the region of study during the peak period of theileriosis occurrence were also sampled.

2.2. Blood sampling and routine hematological examination

Blood samples were collected from jugular vein, in evacuated tubes contaminated ethylenediamine-tetra-acetic acid dipotassium salt (EDTA-K2) for routine blood tests and into heparinized glass-stoppered tubes for other analysis. Complete blood count including, RBC and WBC counts, differential WBC counts, PCV values, and hemoglobin concentration were made by automated hematology analyzer (Autolyser AL 820, Swiss) (Schalm et al., 1986). Thick and thin blood smears from the ear veins and enlarged lymph nodes aspirates were prepared for confirmation of the disease in the basis of observation of piroplasms in erythrocytes and schizonts in lymphocytes. Piroplasm parasitemia (parasitized RBC rate) was quantified by microscopic examination of blood films stained with giemsa, as the number of piroplasm-infected erythrocytes in 100 cells and expressed as the percentage of parasitized RBCs, according to Shiono et al. (2003b).

For evaluation of MDA and estimation of antioxidant enzymes, blood samples were centrifuged at $700 \times g$ for 15 min, plasma separated and packed cells was washed three times with normal saline solution and then haemolysate prepared by adding cold distilled water.

2.3. Osmotic fragility test (OFT)

The osmotic fragility of freshly taken erythrocytes reflects their ability to absorb water without rupturing and lysis. This test was done according to Chanarin (1989). Briefly, washed erythrocytes incubated with

saline buffer in a series of hypotonic solutions ranging in concentration from 1.0 g/L (0.1%) to 9.0 g/L (0.9%) sodium chloride. The percentage of haemolysis at each concentration of NaCl was calculated and a graph of haemolysis percent against concentration of NaCl was plotted. The results were expressed as the concentration of NaCl causing 50% haemolysis, i.e. the median corpuscular fragility (MCF).

2.4. Biochemical assays and analysis

The activities of erythrocyte glutathione peroxidase (GSH-Px, EC 1.11.1.9.) and superoxide dismutase (SOD, Ec 1.15.1.1) were determined in washed red blood cells obtained immediately after sampling from heparinized blood. Hemolyzed cells were stored frozen at −70 °C awaiting analysis. Glutathione peroxidase activity was measured according to Paglia and Valentine (1967) by commercially available kits (Ransel test kit, Randox Laboratories Ltd. G.B.). For evaluation of activity of superoxide dismutase, superoxide radicals generated by the xanthine oxidase reaction convert 1-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride quantitatively to a formazan dye (Ransod test kit, Randox Laboratories Ltd. G.B.). Conversion of superoxide radicals to hydrogen peroxide by superoxide dismutase inhibits dye formation and serves as a measure of superoxide dismutase activity.

The activity of catalase (EC 1.11.1.6) was determined by colorimetric method, described by (Slaughter & O'brien 2000), that involves two steps. Since the rate of dismutation of hydrogen peroxide to water and oxygen is proportional to the concentration of catalase, samples were first incubated with a known amount of hydrogen peroxide. The remaining hydrogen peroxide, following a fixed incubation period, was then determined by the oxidative coupling reaction of 4-aminophenazone (4-aminoantipyrene, AAP) and 3,5-dichloro-2-hydroxy-benzenesulfonic acid (DHBS) in the presence of H_2O_2 and catalyzed by horseradish peroxidase. The resulting quinoneimine dye was measured at 520 nm (Catalase Assay Kit, Oxford Biomedical Research, Inc., USA). Activities of the enzymes were expressed as U/mg Hb.

The activity of glucose-6-phosphate dehydrogenase activity (EC 1.1.1.49) was determined according to reaction described by Beutler (1984). In this reaction, glucose-6-P is oxidized to gluconate-6-P and $NADP^+$ is reduced to NADPH + H^+. The NADPH production in this reaction was determined by spectrophotometerically at 340 nm at 37 °C and was expressed as international units per gram of hemoglobin (IU/g Hb). Hemoglobin determination was carried out by the cyanomethemoglobin method (Chanarin, 1989).

Modified HPLC method based on Lykkesfeldt (2001) and Suttnar et al. (2001) was used to assess malondialdehyde (MDA). The measurement was based on MDA reaction with thiobarbituric acid (TBA) to form a colored MDA–TBA adduct (Sheu et al., 2003; Fukunaga et al., 1998). For determination of MDA in RBCs, erythrocytes were washed with phosphate-buffered saline (∼25% hematocrite) and then 40-μL sample was diluted with 100 μL of H_2O and mixed with 20 μL of 2.8 mmol/L butylated hydroxytoluene (BHT) in ethanol, 40 μL of 81 g/L sodium dodecyl sulfate and 600 μL of TBA reagent consisting of 8 g/L TBA diluted 1:1 with 200 ml/L acetic acid adjusted to pH 3.5 with NaOH. The mixture was immediately heated (60 min at 95 °C) and cooled with running water; 200 μL of H_2O and 1000 μL of butanol–pyridine (15:1, v/v) were then added. After vigorous mixing, the organic layer was separated by centrifugation (3 min at 16,000 × *g*). 1,1,3,3-Tetraethoxypropane was used as a standard, and malondialdehyde–thiobarbituric acid reactive substances (MDA–TBARS) values were expressed as MDA nanomoles per grams of hemoglobin (nmol/g Hb). The HPLC system used consisted of a solvent delivery pump (JASCO 980-PU, Tokyo, Japan), a reversed-phase column (Luna C18, 250 mm × 4.6 mm, Phenomenex, CA, USA), and a UV–vis detector (Jasco, UV-975, Tokyo, Japan) operated at 532 nm.

2.5. Statistical analysis

The results were analyzed by one way analysis of variance (ANOVA) followed by pair-wise comparisons using the Duncan tests. The relationship between antioxidant enzymes, MDA, severity of the parasitemia and anemia were assessed by using Pearson's correlation coefficient. Differences were considered significant when $P < 0.05$. The computer software, SPSS Version 9.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for analysis.

3. Results

3.1. Parasitological and hematological findings

The statistics of the measured parameters in healthy and affected cattle are presented in Table 1. Based on reference values for the PCV, affected cattle with PCV ≤ 12 were considered as severely anemic and those with PCV 13–24 were considered as mild to moderately anemic (Carlson, 1990; Schalm et al., 1986). Of 35 affected cattle 12 (34.28%) had severe and

Table 1
Statistics (mean ± S.E.M.) of hematocrite (L/L), level of malondialdehyde (nmol/g Hb), activities of glutathione peroxidase (GSH-PX) (U/mg Hb), catalase (katal/g Hb), superoxide dismutase (SOD) (U/mg Hb), glucose-6-phosphate dehydrogenase (G6PD) (U/mg Hb), median corpuscular fragility (MCF) (g/dL NaCl causing 50% hemolysis of RBC) in healthy and affected cattle with theileriosis

Parameter	Group I ($n = 50$)	Group II ($n = 23$)	Group III ($n = 12$)
Hematocrite (L/L)	30.86 ± 0.52^{a}	14.44 ± 0.58^{b}	9.75 ± 0.25^{c}
MDA (nmol/g Hb)	25.47 ± 0.59^{a}	95.78 ± 5.80^{b}	138.81 ± 2.62^{c}
GSH-PX (IU/mg Hb)	64.09 ± 1.39^{a}	26.17 ± 1.00^{b}	19.96 ± 0.94^{c}
Catalase (katal/g Hb)	26.69 ± 0.58^{a}	30.62 ± 0.97^{b}	26.65 ± 1.36^{a}
SOD (IU/mg Hb)	8.99 ± 0.17^{a}	7.72 ± 0.22^{b}	6.55 ± 0.18^{c}
G6PD (IU/g Hb)	22.19 ± 0.34^{a}	20.65 ± 0.70^{a}	15.28 ± 0.60^{b}
MCF (gr/dL)	0.49 ± 0.0018^{a}	0.57 ± 0.007^{b}	0.61 ± 0.006^{c}

Group I, healthy cattle; Group II, affected cattle with mild to moderate anemia (PCV = 13–24); Group III, affected cattle with sever anemia (PCV ≤ 12); means within a row with different superscript letters (a–c) denote significant differences ($P < 0.01$).

23 had mild to moderate anemia. An average of one to five piroplasmic forms was observed in erythrocytes of affected cattle with a range of 5–40% parasitemia. Schizont parasitosis in lymph nodes was generally high (more than 15%). There was a negative significant correlation ($r = -0.83$, $P < 0.001$) between degree of parasitemia and hematocrite (Table 2).

3.2. Evaluation of lipid peroxidation and antioxidant enzymes activity

MDA evaluation indicated that lipid peroxidation in erythrocytes of affected cattle was significantly more than those of healthy cattle. In addition, in severely affected cattle the levels of MDA were significantly

Table 2
Correlation between activities of antioxidant enzymes, oxidative stress markers and parasitemia in cattle affected with theileriosis

Parameter	PCV	Parasitemia	MDA	GSH-PX	Catalase	SOD	G6PD	MCF
PCV								
PC[a]	1.000	−0.940	−0.924	0.750	0.249	0.720	0.703	−0.781
P-value	–	<0.0005	<0.0005	<0.0005	0.149	<0.0005	<0.0005	<0.0005
Parasitemia								
PC		1.000	0.980	−0.838	−0.219	−.0795	−0.769	0.813
P-value		–	<0.0005	<0.0005	0.207	<0.0005	<0.0005	<0.0005
MDA								
PC			1.000	−0.831	−0.232	−0.794	−0.791	0.833
P-value			–	<0.0005	0.179	<0.0005	<0.0005	<0.0005
GSH-PX								
PC				1.000	0.322	0.718	0.619	−0.782
P-value				–	0.059	<0.0005	<0.0005	<0.0005
Catalase								
PC					1.000	0.167	0.177	−0.532
P-value					–	0.366	0.308	0.018
SOD								
PC						1.000	0.662	−0.706
P-value						–	<0.0005	<0.0005
G6PD								
PC							1.000	−0.583
P-value							–	<0.0005
MCF								
PC								1.000
P-value								–

PCV, packed cell volume; MDA, malondialdehyde; GSH-PX, erythrocyte glutathione peroxidase; SOD, superoxide dismutase; G6PD, glucose-6-phosphate dehydrogenase; MCF, median corpuscular fragility.
[a] Pearson correlation.

more than those of mild to moderately affected cattle ($P < 0.01$; Table 1). There was a positive significant correlation ($r = 0.98$, $P < 0.0005$) between degree of parasitemia and MDA levels in the erythrocytes (Table 2). It seems that, *T. annulata* stimulates oxidative stress and induces malondialdehyde production.

As Table 1 shows, the activities of erythrocyte glutathione peroxidase ($P < 0.0005$) and superoxide dismutase ($P < 0.0005$) were significantly lower in the affected cattle than those of the healthy ones, and the activities showed significant decrease in severely anemic cattle in comparison with the mildly to moderately anemic cattle ($P < 0.01$).

There was no significant difference ($P > 0.05$) in erythrocyte catalase activity between healthy and severely anemic cattle, but the activity in mild to moderately anemic cattle was significantly ($P < 0.01$) more than those of healthy and severely anemic cattle (Table 1).

There was a significant inverse relationship of severity of parasitemia with activity of erythrocyte glutathione peroxidase ($r = -0.838$, $P = 0 < 0.0005$) and superoxide dismutase ($r = -795$, $P < 0.0005$)). There was also an inverse relationship between the activities of these antioxidant enzymes and MDA levels ($r = -0.831$, $P < 0.0005$; $r = -0.794$, $P < 0.0005$, respectively, for erythrocyte glutathione peroxidase and superoxide dismutase, Table 2).

A significant reduction ($P < 0.01$) in the activity of erythrocytes glucose-6-phosphate dehydrogenase was only detected in the severely anemic cattle (Table 1). G6PD as a major enzyme of hexose monophosphate pathway is involved in production of $NADPH + H^+$ (Beutler, 1966), needed directely for the activity of SOD and indirectly for GSH-Px activity. Decreased activity of G6PD in affected erythrocytes results in a reduction in the activities of the $NADPH + H^+$-dependent SOD and GSH-Px. The results also showed a negative significant correlation ($r = -0.77$, $P < 0.0005$) between G6PD activity and severity of parasitemia (Table 2).

3.3. Osmotic fragility of erythrocyte

The median corpuscular fragility (MCF) in affected cattle was significantly ($P < 0.01$) lower than those of health cattle and severely anemic cattle showed significantly ($P < 0.01$) increased erythrocytes fragility in comparison with mild to modernly anemic cattle (Table 1). This finding shows that erythocytes from theileriosis-affected cattle are more susceptible to hemolysis. Furthermore, median corpuscular fragility showed a significant positive correlation with severity of parasitemia ($r = 0.81$, $P < 0.0005$) and a negative correlation with activities of GSH-Px ($r = -78$, $P < 0.0005$), SOD ($r = -0.71$, $P < 0.0005$), catalase ($r = -0.53$, $P < 0.018$) and G6PD ($r = -0.58$, $P < 0.0005$) (Table 2).

4. Discussion

The goal of this study was to evaluate the cytochemical alteration of RBCs in anemic cattle suffering from bovine theileriosis. Our findings showed that oxidative damage to RBCs may be involved in the pathogenesis and onset of anemia in theileriosis caused by *T. annulata*.

In accordance with the findings from other studies (Naziroglu et al., 1999; Saluja et al., 1999; Grewal et al., 2005), our results indicated that the lipid peroxidation in erythrocytes of affected cattle increases MDA production. Increased MDA concentration in erythrocytes of affected cattle may be an indication of elevated oxidative stress in theileriosis. Oxidative stress results when the production of the free radicals and reactive metabolites of oxygen exceeds their safe disposal by antioxidant mechanisms. Free oxygen radicals cause lipid peroxidation and the end product of lipid peroxidation is MDA. Determination of MDA allows detection of the degree of lipid peroxidation and level of free oxygen radicals indirectly (Esterbauer, 1996; Yagi, 1998; Owen, 1996). The erythrocytes membrane is rich in polyunsaturated fatty acids, a primary target for reactions involving free radicals, and is very susceptible to lipid peroxidation (May et al., 1998; Devasena et al., 2001).

Of interest in connection with increased MDA production, was the relationship between erythrocytes infection rate with *T. annulata* and the severity of anemia. A similar finding has been reported in theoleriosis caused by *T. sergenti*. Shiono et al. (2003a) reported that in cattle with *T. sergenti* during the onset of anemia, levels of MDA began to increase remarkably in proportion to the decrease of packed cell volume and increase of parasitemia. During the serious stage of anemia, this oxidative index reached to its maximum value. They concluded that oxidative damage to the RBCs might play an important role in the pathogenesis of anemia in bovine theileriosis. Based on results of the present study, we would suggest that same pathogenic mechanism may also be involved in cattle infected with *T. annulata*.

Increased median corpuscular fragility in erythrocytes of affected cattle indicates injury to erythrocytes' membrane and consequently altered permeability of

these cells. Such alteration can results from oxidative stress and lipid peroxidation. A positive correlation of the MCF with the rate of parasitemia and MDA concentration; and a negative correlation with the activities of GSH-Px, SOD, catalase and G6PD may suggest contributory role of oxidative stress in development of hemolytic anemia in affected cattle. These results are in accordance with the findings of Yagi et al. (1989) and Haider (1992) in calves infected with *T. sergenti*. They reported that, as parasitemia progressed, the osmotic fragility of erythrocytes increased significantly. Loss of 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).

The significant decrease in the activity of G6PD in affected cattle suffering from severe anemia (PCV $< 12\%$) is an indicator of a metabolic disturbance in the erythrocytes. This enzyme has a key role in the pentose phosphate pathway, which has critical significance in the survival of erythrocytes (Beutler, 1984). G6PD enzyme is the principal source of NADPH, which helps in maintaining glutathione in the reduced state, thus protecting erythrocytes from oxidative stress. G6PD serves as an antioxidant enzyme and decreased activity of G6PD has been associated with increased hemolysis in buffaloes affected with theileriosis (Singari et al., 1991) and increased oxidative stress in endothelial cells (Leopold et al., 2003). In the present study, the significant decrease in G6PD activities was not in agreement with the findings of Grewal et al. (2005). They reported a significant increase in the activity of this enzyme in cattle naturally infected with *T. annulata* and concluded that such an increase could be due to a safeguard mechanism to protect the erythrocytes from oxidative stress in response to increased lipid peroxidation in erythrocytes. The different results of the two studies regarding G6PD activities may be related to the grouping of the affected animals by severity of the anemia in the present study. It should be noted that decreased activity of G6PD was only observed in affected cattle with PCV less than 12%.

Decreased G6PD activity can be followed by reduced activities of SOD and GSH, because of dependence of the activities of these enzymes to NADPH + H levels in the cell. In agreement with Agar and Board (1983) we also found a direct relationship between erythrocyte G6PD activity and the activities of GSH-Px and SOD in infected cattle Also to be considered was the significant decrease in the activity of GSH-Px in affected cattle, which is in agreement with the findings of Ozan and coworkers (1999) in cattle naturally infected with *T. annulata*. However, in contrast with our results, Grewal et al. (2005) Grewal and colleagues (2005) reported a significant rise in the activity GSH-Px in infected cattle. GSH-Px activity is a major mechanism for intracellular decomposition of lipid peroxides (Christophersen, 1966; Flohe, 1971). Hafeman and colleagues (1974) also proposed that GSH-Px plays a crucial role in preventing membranes from peroxide damage induced by lipid peroxides. Reduced glutathione is required for the disposal of H_2O_2 from erythrocytes by a reaction catalyzed by GSH-Px. This reaction is important because accumulation of H_2O_2 might decrease the lifespan of erythrocytes by increasing the rate of oxidation of hemoglobin to methemoglobin (Winterbourn, 1985).

According to the results of this study, catalase activity in erythrocytes of mildly to moderately anemic cattle increased significantly, while there was a significant decrease in the activity of this enzyme in the erythrocytes of cattle with severe anemia. It has been reported that catalase is of equal importance to GSH-Px in the defense of human erythrocytes against H_2O_2 generating reactions (Harvey, 1989). However, the results of the present study indicated that catalase might be acting in concert with GSH-Px to scavenger H_2O_2 for the protection of erythrocytes infected by theileria.

Evaluation of SOD activity in affected cattle showed that by increasing the severity of parasitemia and oxidative stress in parasitemized erythrocytes (increased MDA concentration), activity of this enzyme significantly reduced. Reduced SOD activity was accompanied by decreased G6PD activity in infected erythrocytes. It appears that, during theileriosis, SOD similar to GSH-Px, playes an important role in protection of erythrocytes against oxidative stress. Similar findings were reported in other parasitic infections. It has been reported that *Plasmodium*-infected erythrocytes show decreased capacity of their antioxidant enzymes, including superoxide dismutase (Friedman, 1979; Wozencraft, 1986; Erel et al., 1997), catalase, glutathione peroxidase (Greve et al., 1999), G6PD (Roth et al., 1988), methemoglobin reductase (Stocker et al., 1985) and antioxidant substances such as Vitamin E (Griffiths et al., 2001).

In conclusion, the results of the present study showed significant increase in lipid peroxidation of the membrane of erythrocytes of cattle suffering from theileriosis. The levels of the antioxidant enzymes in the erythrocytes of affected cattle decreased as severity of

the anemia and parasitemia increased. It seems that antioxidant mechanisms of erythrocytes that protect them against oxidative damage may be disturbed by *Theileria* infection.

References

Agar, N.S., Board, P.G., 1983. Red Blood Cells of Domestic mammals, first ed. Elsevier Science Publishers, Amsterdam, pp. 227–270.

Aulakh, R.S., Gill, I.P.S., Johal, H.S., Juyal, P.D., Joshi, D.V., 1998. Haematological observation in *T. annulata* infected cross bred cattle and their treatment with buparvaquone. J. Res. Punjab Agric. Univ. 35, 66–88.

Beutler, E., 1966. A series of new screening procedures for pyruvate kinase deficiency, glucose-6-phosphate dehydrogenase deficiency and glutathione reductase deficiency. Blood 28, 553–562.

Beutler, E., 1984. Glucose-6-Phosphate Dehydrogenase (G6PD) and 6-Phosphogluconate Dehydrogenase (6-PGD), Red cell metabolism. A Manual of Biochemical Methods, third ed. Grune and Startton, Orlando, pp. 68–71.

Carlson, G.P., 1990. Disease of the hematopoitic and hemolymphatic systems. In: Bradford, P., Smith, (Eds.), Large Animal Internal Medicine. The C.V. Mosby Co., St. Louis, pp. 1068–1084.

Chanarin, I., 1989. Laboratory Haematlogy: An Account of Laboratory Techniques. Churchill Livingstone Publisher, pp. 13–14.

Christophersen, B.O., 1966. Oxidation of reduced glutathione by sub cellular fractions of rat liver. Biochem. J. 100, 95–101.

Devasena, T., lalith, S., Padma, K., 2001. Lipid peroxidation, osmotic fragility and antioxidant status in children with acute post-streptococcal glomerulonephritis. Clin. Chim. Acta 308, 155–161.

Erel, O., Kocyigit, A., Avci, S., Aktepe, N., Bulut, V., 1997. Oxidative stress and antioxidative status of plasma and erythrocytes in patients with vivax malaria. Clin. Biochem. 30, 631–639.

Esterbauer, H., 1996. Estimation of peroxidative damage. A critical review. Pathol. Biol. (Paris) 44, 25–28.

Flohe, L., 1971. Glutathione peroxidase: enzymology and biological aspects. Klinische Wocllenschrift 49, 669–683.

Forsyth, L.M.G., Jackson, L.A., Wilkie, G., Sanderson, A., Brown, C.G.D., Preston, P.M., 1997. Bovine cells infected in vivo with *Theileria annulata* express CD11b, the C3bi complement receptor. Vet. Res. Commun. 21, 249–263.

Fukunaga, K., Yoshida, M., Nakazono, N., 1998. A simple, rapid, highly sensitive and reproducible quantification method for plasma malondialdehyde by high-performance liquid chromatography. Biomed. Chromatogr. 12, 300–303.

Friedman, M.I., 1979. Oxygen damage mediates variant red cell resistance to malaria. Nature 280, 245–247.

Gill, B.S., Bhattacharyulu, Y., Kaur, D., 1977. Symptoms and pathology of experimental bovine tropical theileriosis (*Theileria annulata* infection). Annales de Parasitologie 52, 597–608.

Grewal, A., Ahuja, C.S., Singha, S.P.S., Chaudhary, K.C., 2005. Status of lipid peroxidation, some antioxidant enzymes and erythrocyte fragility of crossbred cattle naturally infected with *Theileria annulata*. Vet. Res. Commun. 29, 387–394.

Greve, B., Lehman, L.G., Lell, B., Luckner, D., Schmidt-Ott, R., Kremsner, P.G., 1999. High oxygen radical production is associated with fast parasite clearance in children with *Plasmodium falciparum* malaria. J. Infect. Dis. 179, 1584–1586.

Griffiths, M.J., Ndungu, F., Baird, K.L., Muller, D.P., Marsh, K., Newton, C.R.J.C., 2001. Oxidative stress and erythrocyte damage in Kenyan children with severe *Plasmodium falciparum* malaria. Br. J. Haematol. 113, 486–491.

Hafeman, D.G., Sunde, R.A., Hoekstra, W.G., 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in rat. J. Nutr. 104, 580–587.

Halliwell, B., Gutteridge, J.M.C., 1999. Free Radicals in Biology and Medicine, third ed. Oxford University Press, New York, pp. 936.

Haider, M.J., 1992. Hematological study of water buffalo (*Bubalus bubalis*) during theileriosis (*T. annulata*). Ann. NY Acad. Sci. 653, 191–193.

Harvey, J.W., 1989. Erythrocyte metabolism. In: Kaneko, J.J. (Ed.), Clinical Biochemistry of Domestic Animals. fourth ed. Academic Press, New York, pp. 185–233.

Hooshmand-Rad, P., 1976. The pathogenesis of anaemia in *Theileria annulata* infection. Res. Vet. Sci. 20, 324–329.

Knight, J.A., 1995. Diseases related to oxygen-derived free radicals. Ann. Clin. Lab. 25, 111–121.

Leopold, l.A., Zhang, Y.Y., Scribner, A.W., Stanton, R.C., Loscalzo, I., 2003. Glucose-6-phosphatedehydrogenase over expression decreases endothelial cell oxidant stress and increases bioavailable nitric oxide. Arterioscler. Thromb. Vasc. Biol. 23, 411–417.

Lykkesfeldt, J., 2001. Determination of malondialdehyde as dithiobarbituric acid adduct in biological samples by HPLC with fluorescence detection: comparison with ultraviolet–visible spectrophotometry. Clin. Chem. 47, 1725–1727.

May, J.M., Qu, Z.C., Mendiratta, S., 1998. Protection and recycling of alfa-tocopherol in human erythrocytes by intracellular ascorbic acid. Arch. Biochem. Biophys. 349, 281–289.

Moore, K., Roberts, L.J., 1998. Measurement of lipid peroxidation. Free Radic. Res. 28, 659–671.

Naziroglu, m., Saki, C.E., Sevgili, M., 1999. The effect of buparvaquone treatment on levels of some antioxidant vitamins, lipid peroxidation and glutathione peroxidase in cattle with theileriosis. J. Vet. Med. 46, 233–239.

Omer, O.H., El-Malik, K.H., Mahmoud, O.M., Haroun, E.M., Hawas, A., Sweeney, D., Magzoub, M., 2002. Haematological profiles in pure bred cattle naturally infected with *Theileria annulata* in Saudi Arabia. Vet. Parasitol. 107, 161–168.

Omer, O.H., El-Malik, K.H., Magzoub, M., Mahmoud, O.M., Haroun, E.M., Hawas, A., Omar, H.M., 2003a. Biochemical profiles in Friesian cattle naturally infected with *Theileria annulata* in Saudi Arabia. Vet. Res. Commun. 27, 15–25.

Omer, O.H., Haroun, E.M., Mahmoud, O.M., Abdel-Magied, E.M., El-Malik, K.H., Magzoub, M., 2003b. Parasitological and clinicopathological profiles in Friesian cattle naturally infected with *Theileria annulata* in Saudi Arabia. J. Vet. Med. B 50, 200–203.

Owen T. 1996. Fundamentals of modern UV–vis. spectroscopy. Hewlett-Packard Publication no. 12, 5965-5123E.

Ozan, S.T., Yaralioglu, S., Yilmaz, S., Ozer, E., Saki, C.E., Segili, M., 1999. GSH-Px, G6PD and arginase activities and some biochemical parameters in cattle infected with *Theileria annulata*. Tr. J. Vet. Anim. Sci. 23, 553–557.

Paglia, D.E., Valentine, W.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med. 70, 158–169.

Roth Jr, E.F., Calvin, M.C., Max-Audit, I., Rosa, J., Rosa, R., 1988. The enzymes of the glycolytic pathway in erythrocytes infected with *Plasmodium falciparum* malaria parasites. Blood 72, 1922–1925.

Saluja, P.S., Gupta, S.L., Malhotra, D.V., Ambawat, H.K., 1999. Status plasma malondialdehyde in experimental *T. annulata* infected in cross bred bovine calves. Indian Vet. J. 76, 379–381.

Schalm, O.W., Jain, N.C., Carroll, E.J., 1986. Veterinary hematology, third ed. Lea and Febiger, Philadelphia, pp. 20–86.

Sheu, J.Y., Ku, H.P., Tseng, W.C., Chen, M.T., Tsai, L.Y., Huang, Y.L., 2003. Determination of thiobarbituric acid adduct of Malondialdehyde using on-line microdialysis coupled with high-performance liquid chromatography. Anal. Sci. 19, 621–624.

Shiono, H., Yagi, Y., Thongnoon, P., Kurabayashi, N., Chikayama, Y., Miyazaki, S., Nakamura, I., 2001. Acquired methemoglobinemia in anemic cattle infected with *Theileria sergenti*. Vet. Parasitol. 102, 45–51.

Shiono, H., Yagi, Y., Chikayama, Y., Miyazaki, S., Nakamura, I., 2003a. The influence of oxidative bursts of phagocytes on red blood cell oxidation in anemic cattle infected with *Theileria sergenti*. Free Radic. Res. 37, 1181–1189.

Shiono, H., Yagi, Y., Chikayama, Y., Miyazaki, S., Nakamura, I., 2003b. Oxidative damage and phosphatidylserine expression of red blood cells in cattle experimentally infected with *Theileria sergenti*. Parasitol. Res. 89 (3), 228–234.

Shiono, H., Yagi, Y., Kumar, A., Yamanaka, M., Chikayama, Y., 2004. Accelerated binding of autoantibody to red blood cells with increasing anemia in cattle experimentally infected with *Theileria sergenti*. J. Vet. Med. Ser. B 51, 39–42.

Singari, N.A., Bhardwaj, R.M., Chugh, S.K., Bhardwaj, S., 1991. Status of erythrocytic glucose-6-phosphate-dehydrogenase in phosphorus deficiency haemoglobinuria of buffaloes. Vet. J. 68, 226–230.

Slaughter, M.R., O'Brien, P.J., 2000. Fully-automated spectrophotometric method for measurement of antioxidant activity of catalase. Clin. Biochem. 33, 525–534.

Stocker, R., Hunt, N.H., Buffinton, G.D., Weidemann, M.J., Lewis-Hughes, P.H., Clark, I.A., 1985. Oxidative stress and protective mechanisms in erythrocytes in relation to *Plasmodium vinckei* load. Proc. Natl. Acad. Sci. U.S.A. 82, 548–551.

Suttnar, J., Masova, L., Dyr, J.E., 2001. Influence of citrate and EDTA anticoagulants on plasma malondialdehyde concentrations estimated by high-performance liquid chromatography. J. Chromatogr. B Biomed. Sci. Appl. 751, 193–197.

Taylor, S.M., Hunter A.G., Andrew A.H., Blowey R.W., Boyd H., Eddy R.G., 1992. Ch 47, Tick and arthropod-borne diseases. In: Bovine medicine, diseases and husbandry of cattle, Blackwell Scientific Publications Inc., 3 Cambridge Center, Cambridge, MA 02142, USA, pp. 722–757.

Wagner, G.M., Lubin, R.H., Chiu, D.T.Y., 1988. Oxidative damage to red blood cell. In: Chow, C.K. (Ed.), Cellular Antioxidant Defense Mechanisms, vol. I. CRC Press, Boca Raton, FL, pp. 185–195.

Winterbourn, C.C., 1985. Hemoglobin oxidation and interrelationship with lipid peroxidation in the red cell. Prog. Clin. Biol. Res. 195, 173–184.

Winterbourn, C.C., 1990. Oxidative denaturation in congenital hemolytic anemias: the unstable hemoglobins. Semin. Hematol. 27, 41–50.

Wozencraft, A.O., 1986. Damage to malaria infected erythrocytes following exposure to oxidant generation systems. Parasitology 92, 559–567.

Yagi, Y., Furuuchi, S., Takahashi, H., Koyama, H., 1989. Abnormality of osmotic fragility and morphological disorder of bovine erythrocytes infected with *T. sergenti*, Japan. J. Vet. Sci. 51, 389–395.

Yagi, K., 1998. Simple assay for the level of total lipid peroxides in serum or plasma. Methods Mol. Biol. 108, 101–106.

Yagi, Y., Thongnoon, P., Shiono, H., Chikayama, Y., 2002. Increase in oxidized proteins in *Theileria sergenti*-infected erythrocyte membrane. J. Vet. Med. Sci. 64, 623–625.