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