Available online at www.sciencedirect.com
ScienceDirect
Research in Veterinary Science 86 (2009) 43–48
www.elsevier.com/locate/rvsc

Erythrocytic oxidative damage in crossbred cattle naturally infected with *Babesia bigemina*

Mostafa A. Saleh *

Biochemistry Unit, Regional Animal Health Research Laboratory, Animal Health Research Institute, EL-Kharga, El-Wadi El-Gadid, 725211, Egypt

Accepted 9 May 2008

Abstract

This study aimed to determine the erythrocytic lipid peroxidation and haemoglobin oxidation as contributory factors causing anaemia in cattle (Friesian × Egyptian native breed) infected with *Babesia bigemina*. Blood was collected from 32 cows infected with *B. bigemina* along with 18 healthy cows as controls for determination of erythrocytic malondialdehyde (MDA), blood methaemoglobin (MetHb), plasma free haemoglobin (PHb), corpuscular osmotic fragility (COF), red blood cell count (RBC), total haemoglobin (Hb) and packed cell volume (PCV). Percentage of parasitaemia varied from 14% to 36%. MDA, MetHb, COF and PHb were significantly increased ($P < 0.001$) in infected cows versus controls. Parasitaemia was positively correlated ($P < 0.001$) with MDA, MetHb, COF and PHb. MDA was positively correlated ($P < 0.001$) with COF and PHb and negatively correlated ($P < 0.001$) with RBC, Hb and PCV. MetHb was negatively correlated ($P < 0.001$) with RBC, Hb and PCV and positively correlated ($P < 0.001$) with COF. In conclusion, *B. bigemina* infection in cattle is associated with a parasitic burden-dependent corpuscular oxidative damage as indicated by membrane lipid peroxidation and methaemoglobin formation, which are contributed to COF and intravascular haemolysis.

Keywords: *Babesia bigemina*; Cattle; Oxidative stress; Anaemia

1. Introduction

Babesiosis is economically the most important arthropod-borne disease of cattle worldwide (Bock et al., 2004). The two species of greatest economic importance are *Babesia bovis* and *Babesia bigemina*, which affect cattle industries in tropical and subtropical parts (Böse et al., 1995). Previous studies provide information on the relative susceptibility of various breeds of cattle to *Babesia* infection (Bock et al., 1997). Marked differences in pathogenicity and clinical manifestations of babesiosis may occur between the different geographic areas (Vial and Gorenflot, 2006). Mahoney (1977) reported that the Australian *B. bigemina* rarely causes disease, whereas *B. bigemina* in Africa is highly pathogenic.

The erythrocyte stage of *B. bigemina* results in severe clinical symptoms in the infected cattle such as fever, anaemia and haemoglobinuria (Radostits et al., 2000). Haemolytic anaemia caused by destruction and removal of parasitized and non-parasitized erythrocytes from the bloodstream is a contributory factor to the weakness and loss of condition seen in *babesia*-infected cattle that survives the acute phase of the disease (Bock et al., 2004). In babesiosis, erythrocytes are destroyed by the physical effect of parasite multiplication (Wright, 1981), the increase of phagocytosis of erythrocytes by activated macrophages (Shoda et al., 2000; Court et al., 2001), the production of an anti-erythrocyte antibody (Goés et al., 2007) and the increase in the erythrocytic membrane permeability (Alkhalil et al., 2007).

Oxidation of the erythrocytes includes membrane injury, methaemoglobin formation, osmotic fragility and destruction of the cell (Harvey, 1997). Lipids especially polyunsaturated fatty acids are sensitive to oxidation, leading to the

* Tel.: +20 927920813; fax: +20 927920866.
E-mail address: salehmahmad@yahoo.com

0034-5288/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rvsc.2008.05.005

term lipid peroxidation or the thiobarbituric acid reactive substances (TBARS), of which, malondialdehyde (MDA) is the most abundant (Janero, 1990). Oxidative damage to haemoglobin has been shown to cause changes in its structure and function, resulting in denaturation, precipitation and methaemoglobin formation inside erythrocytes (Jaffé, 1981). In *Babesia gibsoni* infection, there is a strong evidence of the role of erythrocyte oxidation (as indicated by lipid peroxidation, haemoglobin oxidation and increased osmotic fragility of erythrocytes) in the pathogenesis of anaemia shown in infected dogs (Morita et al., 1996; Murase et al., 1996 and Otsuka et al., 2001, 2002). Little is known about erythrocyte oxidation in bovine babesiosis. However, enhanced production of MDA associated with methaemoglobinaemia was reported during *B. bovis* infection in cattle (Commins et al., 1988).

The present study aimed to assess erythrocytic lipid peroxidation and blood methaemoglobin as indicators of oxidative damage of the erythrocyte and their contributory role as factors causing corpuscular osmotic fragility, cell lyses and anaemia in *B. bigemina* infected cattle in Upper Egypt.

2. Materials and methods

2.1. Animals

Thirty-two cows infected with *B. bigemina* along with 18 healthy cows were used in this study. These cows belonged to unorganized small-scale farming in the rural areas of El-Dakhla oasis (El-Wadi El-Gadid province, in the western desert of Egypt), where babesiosis due to *B. bigemina* is endemic in the summer season (June–August 2006). The selected healthy and diseased cattle were of crossbred type (Friesian × native Egyptian breed), and their age ranged from 2 to 3 years. All the control and infected cattles reared under the same management and environmental conditions. The common food available for these animals mainly consisted of Barseem (*Trifolium alexandrinum*), wheat or rice straw and concentrate mixture (1–2 kg/head/day).

On clinical examination, the infected cows showed signs of babesiosis including dullness, anorexia, red to brownish urine, signs of anaemia (pallor mucous membranes), rise in rectal temperature (39.7–41.2 °C), dyspnea and tachycardia. Cows with other simultaneous diseases were excluded. The control cows were healthy on clinical examination.

2.2. Blood sampling and parasitological examination

Thin blood smears were prepared from the ear vein, and stained with Giemsa for confirmation of the disease on the basis of microscopical observation of piroplasms in erythrocytes. From the infected cows, blood smears (and jugular blood samples for haematological and biochemical investigations) were taken at the phase of haemoglobinuria (during the first day of appearance of coloured urine). Because *B. bigemina* piroplasms are apparent and abundant in the blood film, only one sample (three slides) from each infected animal was satisfactory for identification of the infection and counting of the intra-erythrocytic piroplasms. For control animals, however, blood for smear preparation was sampled twice. During the first, jugular blood was sampled simultaneously for haematological and biochemical investigations. The second blood smear was prepared two weeks later to insure that these animals were parasite free.

Quantitative evaluation of parasitaemia was done by counting of the number of parasited erythrocytes present per 1000 cells; the parasited cells all count was then divided by 10 and expressed as parasitaemia percentage.

For haematological and biochemical investigations, 10 ml of jugular blood was drawn from each cow in centrifuge tubes containing disodium salt of ethylenediaminetetraacetic acid (Na_2-EDTA) as anticoagulant. Large needles were used in order to minimize the in vitro haemolysis from mechanical injury to erythrocytes.

2.3. Haematological investigations

The count of red blood cells (RBC) was determined using a haemocytometer, whereas packed cell volume (PCV) and haemoglobin concentration (Hb) were determined by microhaematocrit and cyanomethaemoglobin methods, respectively (Jain, 1986).

Concentrations of plasma free haemoglobin and blood methaemoglobin (MetHb; as percentage of total Hb) were measured spectrophotometrically by using their absorbance spectra and Allen correction (Noe et al., 1984; Fairbanks and Klee, 1994).

2.4. Preparation of erythrocyte haemolysate

Immediately after collection, blood samples were centrifuged at 1000 rpm for 15 min at 4 °C. The plasma and buffy coats were removed by aspiration. The sediment containing blood cells was washed three times by re-suspending in isotonic phosphate-buffered saline, followed by re-centrifugation and removal of the supernatant fluid and the buffy coats. The crude cells were lysed in nine volumes of ice-cold distilled water to prepare a 10% erythrocyte haemolysate.

2.5. Estimation of lipid peroxide (MDA)

Lipid peroxidation in the RBC haemolysate was determined as thiobarbituric acid reactive substances (TBARS) according to Placer et al. (1966). The method is depended on forming a colour complex between the products of lipid peroxidation and thiobarbituric acid (TBA). Briefly, 0.2 ml of the RBC haemolysate was added to 1.3 ml of 0.2 mol/l Tris, 0.16 mol/l KCl buffer (pH 7.4). TBA (1.5 ml) was added and the mixture was heated in a boiling water bath for 10 min. After cooling, 3 ml of pyridine–butanol (3:1 v/v) and 1 ml of 1 mol/l NaOH were added. The absorbance was read at 548 nm against bi-distilled water as a blank. In