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Full Length Article

Clinical, hemato-biochemical alterations and oxidant–antioxidant biomarkers in *Babesia*-infected calves

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Abstract *Babesia* is one of the main causes of anemia in cattle, a lot of elucidations have been suggested to explain its pathogenesis. This study was designed to investigate clinical, hemato-biochemical and oxidant/antioxidant status and its relation with the resultant anemia in *Babesia*-infected calves. Seventeen (17) native breed calves were involved in this study, clinical signs and microscopic findings were recorded, also blood samples were taken to investigate hematologic changes, serum biochemical variations and oxidative stress biomarkers. The most commonly observed clinical signs were fever, emaciation, depression, icterus and hemoglobinuria. Significant reduction in PCV, HB, RBCs, MCHC, Total protein, and albumin along with significant increase in MCV, WBCs, monocytes and BUN were the most consistent hemato-biochemical changes. Oxidant/antioxidant and trace mineral assessment showed significant reduction in Superoxide dismutase "SOD", Glutathione peroxidase "GPx", Zn, Cu along with significant increase in malondialdehyde (MDA) activities. In the current investigation, oxidant/antioxidant imbalance along with the synchronized alterations in antioxidant trace minerals was detected in *Babesia*-infected calves. These findings support notion that *Babesia* infection associated with oxidative stress and this process may be linked to the resultant anemia.

1. Introduction

Livestock in Egypt plays a considerable role in country economy. Cattle is one of the main livestock population raised in Egypt, according to FAO [1], cattle population in 2009 ranged from 3.5 to 5 million head.

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Cattle industry can be plagued with abundant diseases, among which tick-borne ailments are of considerable importance. These diseases have a negative influence on animal health and subsequently the economy [2]. Bovine babesiosis is an important tick-borne ailment all over the world but mostly in subtropical and tropical territories [3]. *Babesia* is an intraerthrocytic hemoprotozoan affecting animal erythroctytes [4]. In Egypt, bovine babesiosis are caused by *B. bovis* and *B. bigemina* [5,6].

Babesiosis common presentation is pyrexia, anemia, icterus, emaciation, inappetence and hemoglobinuria, in pregnant cattle, diarrhea and even abortion may occur [7,8]. Bovine babesiosis was known to cause anemia, the type of anemia usually hemolytic, though, ranged from hypochromic to normochromic, this change is associated with reduction in erythrogram parameters and alterations in serum biochemical values [3]. The microscopic examination of blood smear considered the most applicable and the cheapest method for diagnosis, presence of the protozoan inside red cells considered confirmative especially during acute stage of the disease, this ability decrease sharply in other situations like carrier animals [9,10].

Oxidative stress is a disproportion between scavenging mechanism and radical generating mechanism [11]. The erythrocyte peroxidation occurs in hemo-protozoan infection may be correlated to disease pathogen [12]. Free radicals can harm tissue and cells, when Reactive oxygen species ''ROS'' exceed the ability of antioxidant system, oxidative process ensues [13]. Recently in blood parasites investigations, strong accumulated evidences were found to link the resultant anemia with oxidative damage due to lipid peroxidation of red cells [12]. Anti-oxidant elements such as SOD and GPx are vital in counteract ROS damage [11].

The purpose of this study is to describe the clinical and laboratory alterations in *Babesia*-infected calves with special emphasis on oxidant-antioxidant status associated with this disease.

2. Materials and methods

2.1. Animals

Seventeen (17) clinically-ill native breed male calves aged 1 year, located at Alexandria-Cairo desert road, Egypt were involved in this study. Criteria of inclusion were presence of tick infestation, clinical signs and positive microscopic blood smear. Giemsa-stained thin blood films were inspected under the light microscope for identification of intraerythrocytic stages of the hemoparasite [14]. Control calves ($n = 6$) from same farm with the same age and sex were involved. Calves were thoroughly clinically examined, fecal samples were taken and blood smears from each control calf was examined microscopically to ensure they are parasitological free. The criteria for inclusion in control group were also depended on no former or existing history of tick exposure, and absence of clinical signs.

Each calf was subjected to comprehensive clinical examination and clinical signs were recorded.

2.2. Blood sampling and microscopic detection

Blood samples were collected from jugular vein in EDTA-containing tubes and plain tube to separate serum samples; the blood in plain tubes were preserved in slanted spot for approximately 2 h and then refrigerated at 4 °C overnight for serum separation. Clinical hematology was performed within 2 h after the sample collection. Three Giemsa stained blood films from each animal were examined under oil-immersion lens, to confirm the *Babesia* infection.

2.3. Hematologic investigations

For hematological analysis, Haemo-cytometric method was used to determine erythrocyte and leukocyte counts. Hemoglobin was estimated using Drabkin colorimetric method. Hematocrit values were evaluated by micro-hematocrit centrifugation. Differential leukocyte counts were determined from smears stained by the Giemsa method. The erythrocyte indices of MCV and MCHC values were also estimated by appropriate formulas.

2.4. Estimation of oxidant-antioxidant status

EDTA whole blood samples were used to formulate erythrocytes lysates for estimation levels of glutathione peroxidase (GPX) and superoxide dismutase (SOD) enzymes using respective test kits (Bio-Diagnostic Company-Egypt). While resultant plasma was used to estimate catalase using respective test kit (Bio-Diagnostic Company-Egypt). Estimation of these parameters was done manually using spectrophotometer (APEL, PD-303S, Japan) according to manufacturer instructions.

2.5. Estimation of serum biochemical parameters

Serum samples were used to determine total protein, albumin, cholesterol, triglycerides, BUN, Malondialdehyde (MDA), zinc (Zn) and copper (Cu) (Bio-Diagnostic Company-Egypt, Spectrum Diagnostic, and Egypt). Estimation of these parameters was done manually using spectrophotometer (APEL, PD-303S, Japan) according to manufacturer instructions.

2.6. Statistical analysis

Results of diseased calves were compared to control data, calculation of mean ± SE, and data comparison using student t-test (STATISTICA for Windows, version 5.1., StatSoft, Inc.). $P \leqslant 0.05$ considered significant.

3. Results

The most consistent clinical signs recorded in diseased calves were fever, tachypnea, decreased weight, anorexia, inappetence and depression; icterus and hemoglobinuria were also observed. Tick infestation was detected on affected calves.

The examination of blood films revealed the presence of pyriform merozoites of *B. bigemina* at an acute angle inside the erythrocytes, and only the calves that showed positive on microscopic examination were included in this study.

Hematologic variations in *Babesia* infected calves are shown (Table 1 and Fig. 1). Significant decrease ($P \leqslant 0.05$) in RBCs, HB, PCV, MCHC, platelet count along with significant increase in MCV was observed in diseased calves