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Anti-Oxidant Status for the Oxidative Stress in Blood of *Babesia* Infested Buffaloes

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Abstract: Blood smears; whole blood and serum samples were taken from both *Babesia* infested and clinically healthy buffaloes to investigate the antioxidant status and oxidative stress. The data recorded a positive correlation between babesiosis and malondialdehyde (MDA) and a negative correlation with reduced glutathione (GSH), total superoxide dismutase activity (t-SOD), glutathione peroxidase activity (GSH-Px), glutathione reductase activity (GR-ase), glutathione S-transferase activity (GST) and glucose 6-phosphate dehydrogenase activity (G6PD). In addition, a significant ($P<0.05$) increase in serum aldolase activity, lactate, cortisol and iron were observed. We can conclude that babesiosis can induce a great oxidation and stress in infested animals.

Key words: Babesia · Anti-oxidant · Oxidative Stress

INTRODUCTION

Cattle and buffaloes are one of the main sources of milk and meat. Their general health conditions were impaired by blood parasites specially *Babesia* which is considered the most important blood parasites affecting cattle and buffaloes in Egypt [1, 2]. Members of the genus *Babesia* cause one of the most common parasitic infestations worldwide in wild and domestic animals. Economically, bovine babesiosis caused by *Babesia bovis*, *Babesia bigemina*, *Babesia major* and *Babesia divergens* forms. Results in high mortality rates among susceptible cattle and causes a virulent disease characterized by fever, anemia, anorexia and hypotensive shock syndrome [3, 4]. Parasitized erythrocytes are often sequestered in the capillary beds of the brain and lung, resulting in low peripheral parasitemia, but causing severe pathology like cerebral babesiosis and respiratory distress which eventually can lead to death [5]. Babesiosis is one of the most important diseases in Egypt because it occurs sometimes in acute forms with serious recognized clinical manifestations, yet lowering the productive performance of the affected animals [1]. *Babesia* in Port Said governorate and highlights a marked contribution of these parasites to the illness of livestock in Egypt [6]. Wherever, no *Babesia* species primarily infect humans, some of the species, like *Babesia divergens*, can be transmitted to humans [7]. The improvement of babesial infection diagnosis tools will facilitate the understanding of the disease and the development of improved methods for control [8, 9].

Oxidative stress has a critical role in the pathophysiology of several diseases, in physiological conditions the reactive oxygen species (ROS) produced in the course of normal conditions are completely inactivated by cellular and extracellular defense mechanisms [10]. Normally there is a balance between prooxidant and antioxidant defense systems and increased generation of ROS, depletion of antioxidant defense system or both leads to enhanced ROS activity and oxidative stress, resulting in tissue damage which induced by different mechanisms, including lipid peroxidation, DNA damage and protein modification [11]. The antioxidant endogenous defense system consists of a variety of extracellular and intracellular antioxidants able to protect tissue injuries from ROS and reactive nitrogen species (RNS) [12]. Copper, manganese, zinc, iron and selenium are required for the activity of SOD,

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catalase and GSH-Px, respectively. In addition, the non-enzymatic defense mechanisms include glutathione, albumin, vitamin E, vitamin C,β-carotene and dietary fish oil [13-15].

Erythrocyte and serum associated biochemical changes in *Babesia* infested buffaloes is the strict aim of this study. This will be evaluated by measurement of malondialdehyde (MDA); reduced glutathione (GSH); total superoxide dismutase activity (t.SOD) (EC.1.15.1.1); glutathione peroxidase activity (GSH-Px) (EC. 1.11.1.9); glutathione reductase activity (GR-ase) (EC.1.6.4.2); glutathione S-transferase activity (GST) (EC.2.5.1.18) and glucose 6-phosphate dehydrogenase activity (G6PD) (EC. 1.1.1.49). Moreover, serum aldolase activity (EC. 4.1.2.13), Lactate, cortisol and iron were determined.

MATERIALS AND METHODS

Animals: This investigation was performed on fifty buffaloes, 1-2 years of age in private farms located in Al-Beheira governorate, Damanhour city.

Clinical Examination: All animals were subjected to clinical and parasitological examinations. Eighteen infected animals with case histories of infection showed clinical signs such as fever (41°C), anorexia, depression, weakness, icteric mucous membrane, emaciation, weight loss, hemoglobinuria and accelerated heart and respiratory rates. Anemia was evident in advanced stages of the infections.

Samples: Two blood samples were collected from the jugular vein by using a sterile sharp needle with wide pore. The samples that used for blood smear, RBCs count and separation of washed RBCs were collected in clean and dry test tube containing di-sodium EDTA as an anticoagulant. While, serum samples were collected in dry clean tubes and separated by centrifugation at 3000 RPM for 10 minutes, then clear supernatant serum aspirated carefully and subjected to biochemical analysis.

Blood Smear: Three thin blood films were prepared with peripheral blood withdrawn from the ear tip and left in the air to dry and fixed in absolute methyl alcohol for 1-2 minutes. Then stained with freshly prepared Giemsa stain for 30-45 minutes and then washed with distilled water to remove excess of stain. The slides were left to dry, then put one drop of cedar oil and examined under oil immersion lens [16]. The prepared blood films were examined [17]. Animals can be considered negative if three blood film slides were negative.

Biochemical Analysis: The RBCs numbers by collecting whole blood samples were done by automatic full digital cell counter, Exigo, Boule Medical AB, Sweden. The erythrocytes were washed by using physiological saline and erythrocyte hemolysate was prepared using digitonin [18]. Hemolysate were used for determination of MDA [19], GSH [20]; t.SOD [21]; GSH-Px [22], GR-ase [23], GST [24] and G6PD [25]. Collected serum samples were subjected to biochemical analysis of aldolase [26], lactate [27], cortisol [28, 29], iron [30] and hemoglobin content in the red blood cell lysate [31].

Statistical Analysis: Analysis of variance (one-way, ANOVA) was performed to compare between different groups at different weeks for the results of biochemical studies [32].

RESULTS

Babesia bigemina infection was confirmed with Giemsa stained smears prepared from the blood of infected animals revealed the presence of *Babesia bigemina* in the red blood cells. The percentage of parasitemia was nearly ranging from about 3.6%. The examined blood smears with an oil immersion lens revealed intra-erythrocytic double (pear shaped) of *Babesia bigemina*, differentiated it from other protozoan (Fig.1). In contrast, all control animals were free of those pathogens.

Erythrocytes oxidative stress markers associated with *Babesia bigemina* are expressed in Table (1) as mean and mean of standard error, values with different superscriptions (a and b) in rows differ significantly ($P<0.05$).

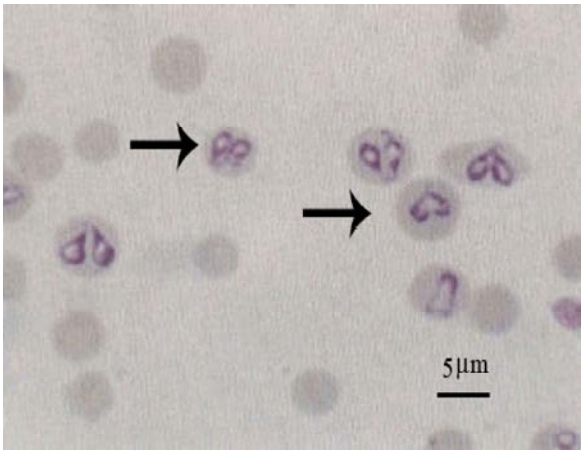

Fig. 1: Microphotography of *Babesia bigemina* inside buffalo erythrocytes

Table 1: Effect of infection with *Babesia bigemina* in buffalo calves aged 1-2 years on concentration of erythrocytic MDA, GSH, t.SOD, GSH-Px, GR-ase, GST and G6PD

	Healthy buffalo calves	Infected buffalo calves
MDA(nmol/ mg Hb)	0.27±0.01[b]	3.45±0.56[a]
GSH(mmol/ mg Hb)	2.60±0.06[a]	1.06±0.04[b]
t.SOD(U/mg Hb)	18.35±1.87[a]	9.20±1.57[b]
GSH-Px(U/mg Hb)	11.99±0.13[a]	6.50±0.74[b]
GR-ase(U/mg Hb)	2.98±0.17[a]	1.01±0.17[b]
GST(U/mg Hb)	2.90±0.05[a]	0.98±0.14[b]
G6PD(mU/10^9 RBCs)	114.46±0.72[a]	90.13±1.88[b]

Means within the same row carrying different letters are significantly different (P<0.05)

Table 2: Effect of infection with *Babesia bigemina* in buffalo calves aged 1-2 years on concentration of serum aldolase, lactate, cortisol and iron.

	Healthy buffalo calves	Infected buffalo calves
Aldolase (U/ml)	0.64±0.14[b]	1.25±0.17[a]
Lactate (mg/dl)	17.97±4.17[b]	35.86±4.62[a]
Cortisol (ug/dl)	12.50±3.81[b]	22.11±1.16[a]
Iron (µg/dl)	124.63±1.02[b]	143.66±0.41[a]

Means within the same row carrying different letters are significantly different (P<0.05)

In general, the infected animals had a lower value of antioxidant parameters as (GSH, t.SOD, GSH-Px, GR-ase, GST and G6PD activities) when compared with healthy animals. On other side, values of oxidant markers (MDA; a product of lipid peroxidation) was significantly increased in the blood of the infected group as compared to the healthy group.

Infection with *Babesia bigemina* in buffalo calves aged 1-2 years, induced marked (P<0.05) elevation of serum aldolase activity, lactate, cortisol and iron levels in contrast to healthy buffalo calves (Table, 2).

DISCUSSION

The clinical feature in animals suffering from babesiosis include high temperature (40-41°C), loss appetite, cessation of rumination, anemia, labored breathing and hemoglobinuria, such finding could be due to destruction of large number of erythrocytes by blood parasite resulting in hemoglobinemia and consequently hemoglobinuria [33]. The sudden onset of high fever (40-41°C) as response to effect of unspecific toxic substances produced during the metabolism of *Babesia*. Then the heart rate was increased, marked dyspnea was then developed and visible mucous membranes were first congested but very soon became pale and in the terminal stages became icteric [34, 35]. Paleness of mucus membranes were exhibited the development of anemia and reduction of hemoglobin concentration and total erythrocytes count, was due to destruction and removal of infected erythrocytes by the reticulo-endothelial system. The icteric mucus membranes reflected the progressive anemia and bilirubinemia [36, 37].

The method of choice to detect *Babesia* in blood of infected animals especially in acute cases was blood film examination [38]. In the present study, examination of Giemsa stained blood smears revealed intra-erythrocytic double pyriform shape of *Babesia bigemina* inside RBCs of infected animals this is agreement with [39] added that round, oval and irregular forms may be observed depending on the develop-mental stage of *Babesia bigemina* inside erythrocytes [40].

Malondialdehyde is used as a trigger for the estimation of damage by reactive oxygen species and the major reactive aldehyde resulting from the peroxidation of biological membranes [41, 42].The present data revealed a significant increase MDA in infested animal group suggesting the oxidative damage to RBCs, this result is confirmed by Otsuka *et al.* and Esmaeilnejad *et al.* [43, 44]. To protect against the deleterious effects of ROS, the animal bodies have a complex system of endogenous antioxidant protection in the form of enzymes such as superoxide dismutase, catalase and glutathione peroxidase. Under normal, resting conditions reactive oxygen species are removed from the cell preventing any subsequent damage [45, 46]. The severity of parasitemia showed a significant decrease (P<0.05) of erythrocytic GSH levels and a significant decrease (P<0.05) of erythrocytic. SOD, GSH-Px, GR-ase, GST and G6PD activities in infested group as babesiosis depletes the antioxidant capacity of erythrocytes [44].

The damage RBCs were due to lipid peroxidation and decreased antioxidant capacity of it led to a significant increase in serum aldolase activity and iron levels. The present data revealed a significant increase in iron [47] such changes may be attributed to the hemolytic anemia induced by blood parasites and possibility of free radicals invading erythrocytes leading to distruction of their membrane [48].The significant decreased values of erythrocytic G6PD activity might be due to the disturbed glutathione redox system due to an important enzyme in cellular metabolism in the first and rate-limiting step of pentose-phosphate pathway. Among the functions of this pathway is the protection of cells from oxidative stress, through its role in conversion of NADP to NADPH, thereby replenishing the levels of reduced glutathione to protect against lipid peroxidation [49].

The results suggested that oxidative damage to RBCs may contribute to the pathogenesis of anemia [43]. Low levels of blood iron seem to have an additional role in the genesis of anemia and oxidative stress in dogs naturally infected with *Babesia gibsoni* [50]. The anemia produced from damage of RBCs led to hypoxia which concomitantly increased the serum level of lactate. Increased serum lactate levels are used as a marker of tissue hypoxia in critically ill patients [51]. A mild elevation of lactic acid in *Babesia* infested dogs was previously recorded [52].

All a previously mentioned stressful conditions in *Babesia* infested buffaloes were accompanied by a significant increase in serum cortisol. Basal cortisol was significantly higher in patients compared to control dogs [53] and *Babesia bovis* infected cross-bred cows [54].

CONCLUSION

From this study we can conclude that, babesiosis in Egyptian buffaloes induces critical oxidative and stressful agents on it which concomitantly lead to great losses in milk and meat production. Periodical examination and using of antioxidant medications along with anti-babesial drugs will affects the national economic income.

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