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Short communication

Clinical and biochemical studies on *Theileria annulata* in Egyptian buffaloes (*Bubalus bubalis*) with particular orientation to oxidative stress and ketosis relationship

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ABSTRACT

This study was carried out on 68 *Theileria annulata* naturally infected buffaloes in addition to 25 parsitologically free buffaloes distributed in small herds at Dakahlia and Gharbya governorates, Egypt, to demonstrate the clinical picture associated with theileriosis in this buffaloes with particular emphasis to the oxidative stress and ketosis relationship. Clinical signs recorded in infected buffaloes were in the form of fever, enlargement of one or more lymph node, ocular discharge, corneal opacity, skin lesions, decreased milk yield, pale mucous membrane and anorexia. Blood and serum analysis revealed significant ($p \leq 0.05$) decrease in RBCS and or Hb concentration in infected animals compared to control ones. Moreover, significant increase ($p \leq 0.05$) in the levels of beta hydroxy butyric acid (BHBA) and non-esterified free fatty acid (NEFA) with a significant decrease ($p \leq 0.05$) in the levels of reduced glutathione (R.GSH), superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC), nitric oxide (NO), glucose and glucose-6-phosphate dehydrogenase (G6PD) in infected animals compared to control ones. It can be concluded that *T. annulata* plays an important role in the occurrence of anemia, oxidative and ketotic stressor in Egyptian water buffaloes.

1. Introduction

Theileria annulata, a protozoan parasite of cattle and domestic buffaloes, is transmitted by ticks of the genus Hyalomma, and causes a disease named Mediterranean or tropical theileriosis. It represents a major threat to Egyptian water buffaloes, where it causes significant economic losses as well as reduced production. There are some evidences that oxidative stress and lipid peroxidation incorporate in the pathogenesis of anemia in theileriosis. Lipid peroxidation is a general mechanism where by free radicals induce tissue damages, and are implicated under several diverse pathological conditions (Halliwell and Gutteridge, 1999). 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). Recently there has been growing interest in the use of MDA as a marker of lipid peroxidation in various kinds of diseases (Sheu et al., 2003).

Grewal et al. (2005) showed an increased in oxidative stress and lipid peroxidation in erythrocytes of cattle infected with *T. annulata*. They concluded that this might be the cause of increased erythrocyte fragility and 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

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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.

Diagnosis of *T. annulata* infection in buffaloes on the basis of clinical signs is difficult due to the wide variety in the clinical picture of the disease that may be mistaken with other diseases. Stained thin blood film and lymph node smears are accepted as a method of laboratory diagnosis in cattle and buffaloes (Ramazan and Ugur, 2006).

The prevalence of *T. annulata* infection in Egyptian buffaloes is high and there was a different clinical picture that may be mistaken with other diseases. So, this study is aimed to throw the light on the clinical picture with special reference to oxidative stress and ketotic state of such infection.

2. Materials and methods

2.1. Animals

This study was carried out in Dakahlia and Gharbya governorates, Egypt, on 68 water buffaloes located in small groups and in contact with cattle. In addition, 25 parasitologically free ones located in the same area and under the same levels of nutrition and hygiene were used as a control group. Infected buffaloes were selected on the basis of clinical examination and positive blood and/or lymph node smears.

2.2. Clinical examination

Clinical examination was performed on all animals. The signs of *T. annulata* infection were observed and recorded. Thin blood smears were prepared from the ear veins of all animals. Lymph node aspirates were collected from suspected cases suffered from enlarged superficial lymph nodes.

2.3. Sampling protocol

All animals under study were subjected to ear vein puncture and lymph node aspiration. Blood samples were collected from all infected buffaloes and parasitologically free control one through jugular vein puncture, in tubes contaminated with ethylenediamine-tetraacetic acid dipotassium salt (EDTA-K2) for routine blood tests and into heparinized glass-stoppered tubes for other analysis (Schalm et al., 1986).

2.4. MDA and NO estimation

MDA and NO levels were estimated using commercially available test kits supplied by Biodiagnostic-Egypt, according to the methods described by Satoh (1978) and Okawa et al. (1979) and Montgomery and Dymock (1961), respectively.

2.5. Beta hydroxy butyric acid (BHBA), non-esterified free fatty acid (NEFA), glucose-6-phosphate dehydrogenase (G6PD) and glucose levels

BHBA, NEFA, G6PD and glucose levels were carried out using commercially available test kits supplied by Biostc-Italy, Biodiagnostic-Egypt, and Spinreact-Spain, respectively, according to the methods described by Tietz (1999), Beutler (1984) and Young (2001), respectively.

2.6. Superoxide dismutase (SOD), reduced glutathione (R.GSH), catalase (CAT) and total antioxidant capacity (TAC)

The activity of SOD, R.GSH, CAT and TAC was carried out using commercially available test kits supplied by Biodiagnostic-Egypt according to the methods described by Nishikimi et al. (1972), Beutler et al. (1963), Aebi (1984) and Koracevic et al. (2001), respectively.

2.7. Statistical analysis

The obtained data was analyzed using Student's *t*-test according to the method described by Snedecor and Cochran (1989).

3. Results and discussion

The obtained data showed that, the clinical signs of theileriosis in Egyptian water buffaloes were fever, superficial lymph node enlargement (Fig. 1), lacrimation, respiratory manifestations, anorexia, skin lesion (Fig. 2), diarrhea, corneal opacity (Fig. 3), nasal discharge, pale

Fig. 1. Buffalo showing enlarged lymph node.

Fig. 2. Buffalo showing skin lesion.