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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 parasitologically 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.

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### 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-K<sub>2</sub>) 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

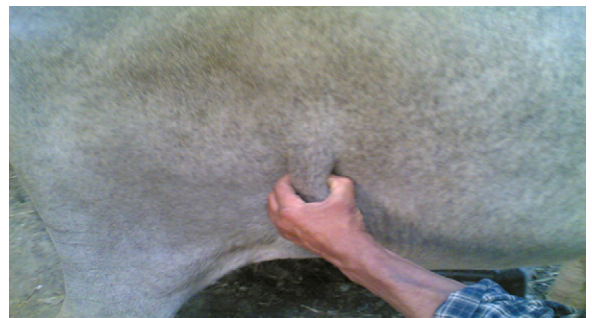


Fig. 1. Buffalo showing enlarged lymph node.



Fig. 2. Buffalo showing skin lesion.

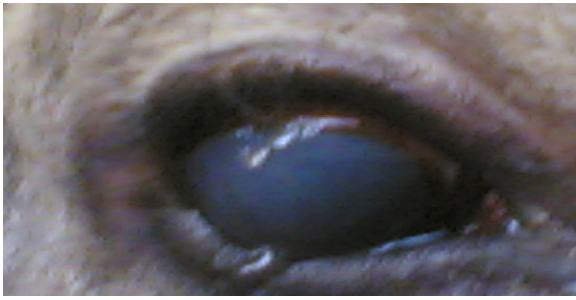


Fig. 3. Buffalo showing corneal opacity.

**Table 1**  
Percentage of different clinical signs in *Theileria annulata* infected buffaloes.

The clinical picture	Number of affected animals/total diseased number	%
Fever	68/68	100
Enlarged lymph node	68/68	100
Lacrimation	18/68	26.47
Respiratory manifestation	18/68	26.47
Anorexia	68/68	100
Skin lesion	2/68	0.3
Diarrhea	5/68	0.7
Corneal opacity	25/68	36.7
Nasal discharge	6/68	0.8
Pale mucous membrane	68/68	100
Decreased milk	18/68	26.47

mucous membrane, and decreased milk production (Table 1). These clinical signs are in agreement with those obtained by Osman and AL-Gaabary (2007).

Hematological examination (Table 2) revealed significant decrease ( $p \leq 0.05$ ) in the Hb content, PCV%, RBCs and WBCs counts in the diseased buffaloes compared to the control ones. Neutropenia, eosinopenia, lymphopenia, monocytopenia with significant increase ( $p \leq 0.05$ ) in the numbers of thrombocytes were recorded. These results are in agreement with those obtained by Osman and AL-Gaabary (2007).

The decrease in RBC counts could be due to increase in the levels of activated complement products (Omer et al.,

**Table 2**  
Blood picture in *T. annulata* free and infected buffaloes.

Variable	Parasitologically healthy buffaloes (no. = 25)	Infected buffaloes (no. = 68)
Hb (g/dL)	12.36 ± 0.29	5.2 ± 0.18*
RBCs ( $10^6/\mu\text{L}$ )	9.48 ± 0.13	5.82 ± 0.21*
PCV (%)	38.49 ± 0.042	27.11 ± 0.169*
TLC ( $10^3/\mu\text{L}$ )	8.65 ± 0.34	5.46 ± 0.33*
Neutrophils ( $10^3/\mu\text{L}$ )	2.8 ± 0.018	2.2 ± 0.047*
Basophiles ( $10^3/\mu\text{L}$ )	0.0128 ± 0.0001	0.0128 ± 0.0001
Eosinophils ( $10^3/\mu\text{L}$ )	0.98 ± 0.10	0.145 ± 0.0003*
Lymphocytes ( $10^3/\mu\text{L}$ )	3.78 ± 0.025	2.72 ± 0.028*
Monocytes ( $10^3/\mu\text{L}$ )	0.39 ± 0.001	0.35 ± 0.002*
Thrombocytes ( $10^3/\mu\text{L}$ )	277.25 ± 2.4	177.8 ± 3.2*

\* Means are significantly different at the level ( $p \leq 0.05$ ).

2002) and erythrophagocytosis (Yagi et al., 2002). In addition, pro-inflammatory cytokines, particularly TNF- $\alpha$ , have been implicated in mediating anemia associated with tropical theileriosis (Graham et al., 2001).

In this study, significant decrease ( $p \leq 0.05$ ) was recorded in neutrophil, eosinophil, and lymphocyte counts in *T. annulata* infected buffaloes compared to those in the control ones. Similar findings were reported by Omer et al. (2002) in cattle and Osman and AL-Gaabary (2007) in buffaloes. This decrease is related to the destruction of lymphocytes in lymphoid organs and infiltration of these cells into various organs (Sandhu et al., 1998). However, no significant difference in absolute basophile and monocyte counts between healthy and infected cattle was recorded by Omer et al. (2002). This variation could be attributed to differences in the stage and severity of the disease.

The results revealed significant increase ( $p \leq 0.05$ ) in the levels of MDA in *T. annulata* infected water buffaloes compared with healthy buffaloes. In contrast, there were significant reduction ( $p \leq 0.05$ ) in the levels of NO, R.GSH, SOD, CAT, and TAC in *T. annulata* infected buffaloes compared with healthy buffaloes (Table 3). According to authors knowledge there is no available data concerning these levels in Egyptian water buffaloes. But some of these levels were estimated in cattle by Rezaei and Dalir-Naghadeh (2006).

Similar finding had been reported by Shiono et al. (2003a) who reported that the levels of MDA began to increase remarkably in proportion to the decrease of packed cell volume and increase of parasitemia in *T. sergenti*-infected cattle during the onset of anemia. During the serious stage of anemia, this oxidative index reached to its maximum value. They concluded that oxidative damage to the RBCs might play an important role in the pathogenesis of anemia in bovine theileriosis. Moreover similar picture was previously reported in cattle by Rezaei and Dalir-Naghadeh (2006). Based on our results, same pathogenic mechanism may also be involved in case of tropical theileriosis in Egyptian buffaloes.

The results revealed significant decrease ( $p \leq 0.05$ ) in the levels of glucose and G6PD in *T. annulata* infected buffaloes compared to clinically healthy buffaloes. Moreover, significant increase ( $p \leq 0.05$ ) in the levels of NEFA and BHBA in *T. annulata* infected buffaloes in comparison with healthy buffaloes indicates the ketotic state of these cases (Table 4).

The significant decrease in the activity of G6PD in infected buffaloes suffering from severe anemia is an

**Table 3**  
Levels of oxidants and antioxidants in *T. annulata* free and infected buffaloes.

Variable	Parasitologically healthy buffaloes (no. = 25)	Infected buffaloes (no. = 68)
MDA (nmol/g Hb)	24.68 ± 0.19	104.45 ± 2.16*
NO (mmol/mL)	25.8 ± 0.24	18.78 ± 0.21*
R.GSH (mmol/L)	7.23 ± 0.21	2.85 ± 0.23*
SOD (U/g Hb)	9.24 ± 0.1	6.37 ± 0.07*
CAT (U/ml)	2.69 ± 0.02	0.96 ± 0.03*
TAC (mmol/L)	1.46 ± 0.011	0.62 ± 0.03*

\* Means are significantly different at the level ( $p \leq 0.05$ ).

**Table 4**

Levels of glucose, BHBA, NEFA and G6PD in *T. annulata* free and infected buffaloes.

Variable	Control (no. = 25)	Infected buffaloes (no. = 68)
Glucose (mg/dL)	63.0 ± 0.6	37.2 ± 2.1*
BHBA (mmol/L)	1.08 ± 0.03	1.9 ± 0.01*
NEFA (mmol/L)	363.6 ± 5.5	536 ± 10.8*
G6PD (IU/g Hb)	22.45 ± 0.15	17.28 ± 0.29*

\* Means are significantly different at the level ( $p \leq 0.05$ ).

indicator of a metabolic disturbance in the erythrocytes and associated with increased RBCs hemolysis in theileriosis (Singari et al., 1991) and increased oxidative stress in endothelial cells (Leopold et al., 2003).

The obtained results were different from that reported by Grewal et al. (2005) who reported significant increase in the activity of G6PD in cattle naturally infected with *T. annulata*. The variation of G6PD activities might be related to the severity of the anemia. In concurrence with Agar and Board (1983) who found a direct relationship between erythrocyte G6PD activity and the activities of R.GSH and SOD in infected cattle. On the other hand, Grewal et al. (2005) reported significant rise in the activity GSH-Px in infected cattle.

According to the results of this study, catalase levels were significantly decreased ( $p \leq 0.05$ ) in affected buffaloes. It has been reported that catalase is of equal importance to GSH-Px in the defense of human erythrocytes against  $H_2O_2$  generating reactions (Harvey, 1989).

Evaluation of SOD levels in affected buffaloes showed significant ( $p \leq 0.05$ ) reduction compared to control ones. Reduced SOD levels were accompanied by decreased G6PD activity in infected erythrocytes. It appears that, during theileriosis, SOD similar to GSH, plays an important role in protection of erythrocytes against oxidative stress. Similar findings were reported in other parasitic infections. It has been reported that Plasmodium infected erythrocytes show decreased capacity of their antioxidant enzymes, including superoxide dismutase (Erel et al., 1997), catalase, glutathione peroxidase (Greve et al., 1999), G6PD (Roth et al., 1988), methemoglobin reductase (Stocker et al., 1985) and antioxidant substances such as Vitamin E (Griffiths et al., 2001). Moreover there was significant ( $p \leq 0.05$ ) reduction in the levels of TAC in infected buffaloes. This reduction may be attributed to the reduction in antioxidant enzymes as they are consumed by excessive free radicals in the infected animals.

According to our knowledge there is no available data concerning the correlation between *T. annulata* and ketosis (Table 4). These results may be attributed to anorexic state; hormonal changes and impaired liver function during the febrile stage of the disease.

It could be concluded that *T. annulata* plays an imperative task as an anemic, oxidative and ketotic stressor on the Egyptian water buffaloes.

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