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CHAIN-BREAKING ANTIOXIDANTS, ASCORBIC ACID AND VITAMIN E, IN RESEPNSE TO CATTLE THEILERIOSIS (With 2 Tables and 2 Figures)

By

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**العلاقة بين مضادات الأكسدة المتمثلة في حامض الأسكوربيك
وفيتامين هـ ومرض الثيليريا في العجول**

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استهدفت الدراسة فحص تأثير مرض الثيليريا في العجول على مستوى مضادات الأكسدة مثل حامض الأسكوربيك وفيتامين هـ في مصل الحيوانات المصابة. تم إجراء الدراسة على عدد ١٨ بقرة هولشتاين من أحد المزارع الخاصة بمحافظة المنيا حيث تم فحص كل الحيوانات إكلينيكيًا وتم أخذ عينات دم وذلك لتشخيص الإصابة بطفيليات الدم وعمل اختبارات صورة الدم وكذلك قياس مستوى حامض الأسكوربيك وفيتامين هـ في الحيوانات محل الدراسة. أظهرت الدراسة وجود ثمانية حيوانات مصابة بالقراد وتظهر عليها أعراض الإصابة بطفيليات الدم والتي تم تأكيد الإصابة بها وذلك بتشخيص طفيل الدم الثيليريا في أفلام الدم الخاصة بها. وعلى الجانب الآخر أظهرت الدراسة خلو باقي الحيوانات من طفيليات الدم وأي أعراض مرضية أخرى وتم استخدامها كمجموعة ضابطة في الدراسة. أظهرت النتائج وجود انخفاض معنوي في مستوى مضادات الأكسدة حامض الأسكوربيك ($P < 0.01$) وفيتامين هـ ($P < 0.01$) في الحيوانات المصابة بالمقارنة بالحيوانات السليمة. هذا وقد أظهرت الدراسة زيادة التأثير السلبي للعوامل المؤكدة التي انعكست على الحيوانات المصابة في صورة الانخفاض المعنوي في مضادات الأكسدة وعلى ذلك توصي الدراسة باستخدام مصادر حامض الأسكوربيك وفيتامين هـ كإضافات علائق للحيوانات المصابة وذلك لتلافي التأثير السلبي للعوامل المؤكدة على الحيوانات المصابة.

SUMMARY

The goal of the present study was to investigate the effect of cattle theileriosis on blood serum ascorbic acid and vitamin E levels as a

model for studying the blood oxidative status in parasitized cattle. A total number of 18 Holstein cows were subjected to the study. All animals were examined clinically and blood samples were collected for evaluation of blood parasite, hemogram picture and vitamin E and ascorbic acid levels. Out of the examined number, 8 cattle were found infested with ticks and showed clinical manifestation of blood parasite infection, which were confirmed by the positive blood film for *Theileria* infection. The rest of the animals were proved healthy and used as control group for the study. The results of the evaluation study of the chain-breaking antioxidants, vitamin E and Ascorbic acid, in the diseased group as compared to the control group revealed significant decrease in blood serum ascorbic acid ($p < 0.01$) and vitamin E ($p < 0.01$) levels. The hematological picture revealed significant decrease in total erythrocytes count, hemoglobin concentration and packed cell volume %. The over all results suggest the presence of an increased oxidative stress in *Theileria*-infected cattle which was manifested by the reduction of the chain-breaking antioxidants levels, ascorbic acid and vitamin E in the blood of parasitized animals. The results of the study recommend the supplementation of *Theileria*-infected cattle with sources of vitamin C and vitamin E in addition to traditional treatment to overcome the oxidative stress associating the infection and to avoid the possible complications of secondary vitamin deficiency.

Key words: *Chain-breaking antioxidants, Vitamin C, Vitamin E, Theileriosis.*

INTRODUCTION

Important changes have been recorded in the biochemistry of hosts suffering from parasitic invasions that were based on the species of the parasites and the sites of the hosts they invade (Aksakal 1987).

Bovine theileriosis, is one of the most serious diseases in grazing cattle, the main symptoms of this disease is anemia (Abdel Rahman *et al*, 1989). Although the pathogenesis of this anemia is not clear, reports have showed that erythrocyte survival rate declines with the parasitemia and that these phenomena occurs in both parasitized and non parasitized erythrocytes (Yagi and Kunugiyama, 1991).

Antioxidant vitamins such as E, C, and A protect the cells from damage by free oxygen radicals generated as a result of parasitism (Medzyavichyus *et al*, 1989 and Smith 1989). Vitamin C status of ruminants may be important in relation to their health and disease. Ascorbic acid plays an important role as an antioxidant (Kanter 1998). It

has also been suggested to be of value in alleviating both physiological and pathological stress (Hemingway 1991). Moreover, the interaction between antioxidant vitamin C and E was investigated and it was reported that there is a sparing effects of vitamin C on vitamin E (Miller *et al*, 1993).

In the present study it is hypothesized that the oxidative free radicals released from parasitized blood cells are responsible for the consumption of the antioxidant agents and hence the reduction of the chain-breaking antioxidant levels in parasitized cattle. To test this hypothesis, the current study were conducted to investigate the alteration of the antioxidant agents, vitamin E and ascorbic acid, in response to cattle theileriosis as an indicator of the blood oxidative status of parasitized cattle.

MATERIALS and METHODS

1 - Animals: A total number of 18 Holstein cows (5-7 years old) from a private farm in Samalot district, El-Menya governorate, Egypt were subjected to the study. The animals were divided into two groups; the first group used as a diseased group and composed of 8 animals that showed signs of blood parasite infection upon clinical and laboratory examinations. The second group was the control group and composed of 10 that were proved to be healthy after careful clinical and laboratory examinations.

2 - Samples and adopted methods

A. Whole blood samples: 5 ml whole blood samples were collected from the jugular vein in a clean, dry tubes containing disodium salt of EDTA as anticoagulant. Blood smear were done directly after collection, fixed in absolute methanol and kept in dry container. The remained blood samples were kept in cold container containing ice and used for hematological analysis (Coles 1986). Total red blood cell counts (T/l), hemoglobin concentration (Hb g/dl), packed cell volume (PCV, %), mean corpuscular volume (MCV fl), mean corpuscular hemoglobin (MCH pg) and mean corpuscular hemoglobin concentration (MCHC %) were determined by using blood cell counter (Cell-Dyn 1700). Blood smears stained with Giemsa stain and differential leucocytic count was carried out using Four Field-Meander method according to Coles (1986).

B. Blood serum samples: 10 ml whole blood samples without anticoagulant were collected in clean and dry tubes. These samples used for separation of serum according to Coles (1986). Blood serum levels of

ascorbic acid and vitamin E were determined according to Omaye *et al*, 1979 and Martinek 1964 respectively.

C. Statistical analysis: Statistical analysis of the obtained data was done by means of software computer program SPSSWin 1997.

RESULTS

Clinical findings: Diseased animals were found infested with ticks in the preneal, flank and neck regions. Upon clinical examination, they showed different degrees of emaciation, enlarged prescapular and/or prefemoral lymph nodes, anemic mucous membranes and lacrimation.

Laboratory findings: *Theileria* infected cattle were identified by examination of blood smears, which revealed the presence of intracellular signet ring *Theileria* species parasites. The hematological analysis, as shown in Table 1, revealed significant decreases in total erythrocytes count ($p < 0.01$), hemoglobin concentration and in PCV % ($p < 0.01$). Analysis of antioxidant vitamins as shown in Table 2 revealed significant decrease in blood serum vitamin C level ($p < 0.01$) as well as blood serum vitamin E level ($p < 0.01$).

Table 1: Values (Mean \pm standard deviation (X \pm SD) of erythrocytes picture in control and *Theileria*-infected cattle.

	Total RBCs count (T/l)	Hb Conc. (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (%)
Clinically healthy cattle (Control)	6.85 \pm 0.50 6.18 - 7.63	11.40 \pm 0.65 10.60 - 12.60	32.00 \pm 2.00 28.00 - 34.00	47.18 \pm 3.80 44.40 - 55.50	16.64 \pm 0.64 15.63 - 17.50	35.27 \pm 2.51 31.70 - 38.70
<i>Theileria</i> infected cattle	5.06 \pm 1.23** 3.30 - 6.50	6.88 \pm 0.89** 5.20 - 8.20	25.75 \pm 5.14** 20.00 - 35.00	53.01 \pm 7.35* 43.70 - 85.30	14.21 \pm 3.32* 9.07 - 19.40	27.37 \pm 5.38** 21.07 - 35.60

*: Significant ($p < 0.05$).

** : Highly significant ($p < 0.01$).

Table 2: Values (Mean \pm standard deviation (X \pm SD) of serum vitamin C and E levels in control and *theileria* infected cattle.

	Vitamin C (mg/dl)	Vitamin E (μ g/ml)
Clinically healthy cattle (Control)	0.17 \pm 0.067 0.12 - 0.31	6.02 \pm 0.93 4.27 - 7.46
<i>Theileria</i> infected cattle	0.064 \pm 0.031** 0.02 - 0.11	3.63 \pm 0.65** 2.95 - 5.01

** : Highly significant ($p < 0.01$).

Figure 1: Mean values of serum vitamin C level in control and theileria infested cattle

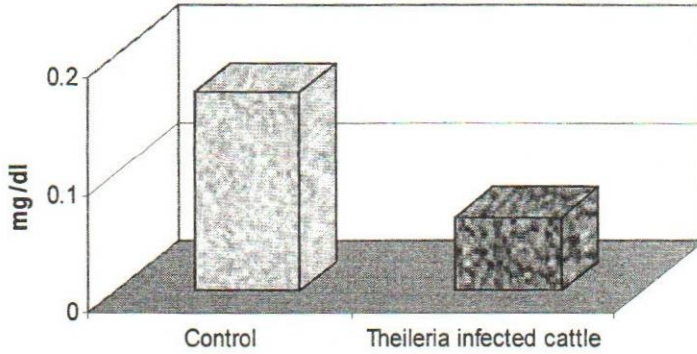
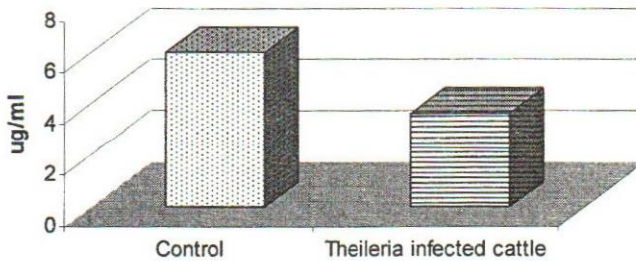


Figure 2: Mean values of serum vitamin E level in control and diseased cattle.



DISCUSSION

The tick-born protozoan disease, theileriosis, poses important problem for the health and management of domestic cattle in the tropics and subtropics. In Egypt, bovine theileriosis represents an important obstacle for the development of the livestock industry through the losses of milk and meat production (Abdel Rahman 1989).

The significant decrease in total erythrocytic counts and hemoglobin concentration can be attributed to the intracellular parasitism. Intra-erythrocytic parasites metabolize hemoglobin and produce O_2 , which causes oxidative stress, as a result, the free O_2 and H_2O_2 increase lipid peroxidation and causes oxidation of the sulfhydryl groups of the globin chains and damage of the erythrocyte cell membrane with the subsequent removal of the affected erythrocytes by active macrophages (Mishra *et al*, 1994 and Stocker *et al*, 1986). This was emphasized by a number of studies, which demonstrated that the amount of reactive oxygen radicals which cause lipid peroxidation are increased in the cells of hosts infected with different species of parasites, thereby causing cell and tissue damages (Smith 1989 and Sarin *et al*, 1993). The decreased PCV % ($p < 0.01$) occurred secondary to reduced total erythrocytic counts.

The interactions between antioxidant vitamin C and E was investigated and a sparing effects of vitamin C on vitamin E as well of vitamin E on vitamin C were recorded (Tanaka *et al*, 1997). The significant decrease in serum vitamin levels in the present study is attributed to the assimilation of these vitamins by the continuous release of reactive oxygen species. This was in accordance with previous study (Miller *et al*, 1993), which declared that stress, disease and induction of immune response increase requirement for nutrients, including vitamin E, A and C and essential trace elements. Vitamin C and vitamin E are chain-breaking antioxidants that protect the cells from oxidative damage. Vitamin E is very important for the synthesis of vitamin C (potent antioxidant vitamin) and synthesis of coenzyme Q-a compound that participates in the respiratory mechanism of the cell.

Results of the present study clearly demonstrate the importance of the additional supplementation of *Theileria*-infected animals with sources of vitamins along with the traditional systemic treatment to overcome the oxidative stress and to avoid the possible complications of vitamin E and C deficiencies. This is because vitamin E and vitamin C play an important role in the immune response and the reproduction of the animals. Previous studies have shown that low level of α -tocopherol (chemical, active form of vitamin E) represent a significant risk factor for clinical mastitis and other types of infection of affected animals (Weimann 1991).

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