

HEMATOLOGICAL AND BIOCHEMICAL STUDIES ON CATTLE THEILERIOSIS WITH SPECIAL REFERENCE TO ITS EFFECT ON SOME ANTIOXIDANT AGENTS

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ABSTRACT

The goal of the present study was to investigate the effect of cattle theileriosis on general health condition and some haematological and biochemical parameters specially Ascorbic acid and vitamin E as a model for studying the blood oxidative status in parasitized cattle . A total number 140 cows were subjected to the study their age ranged from 6 months to over 5 years belonged to some private farms in different localities at Kalubeia governorate ,all animals were examined clinically showed 46 suspected cases (30.7%) which had signs of theileria infection and the laboratory parasitological examination confirmed the infection with theileria annulata the rest of the animals were proved healthy and used as control .The affected cows had high body temperature (40-41C),enlarged superficial lymphnodes ,anorexia ,congestion of mucous membranes and increased respiratory rates .In advanced cases of the disease signs of anemia ,general weakness in addition to presence of ticks on animal body . The study cleared that animals at age of 6 months to one year have a higher rate of infection (33.3%) followed by the age of 1-3 years (30%),3-5 years (23.3%) and finally over 5 years (8%). Hematological investigation of the affected animals proved presence of anemia represented by significant decrease of total erythrocytic count ,haemoglobin concentration and packed cell volume .The leukogram showed that there was leukocytosis as a result of neutrophilia ,lymphocytosis and monocytosis . The result of evaluation study of chain -breaking antioxidants vitamin E and ascorbic acid revealed significant decrease of these vitamins in diseased animals compared with control . Concerning with biochemical changes the infected animals showed significant decrease in total protein, albumin and glucose level .Activities of serum transaminases (AST,ALT)total bilirubin ,urea and creatinine showed significant increase .

INTRODUCTION

Tropical theileriosis is tick borne disease of cattle caused by *Theileria annulata* infection appears to be endemic in Egypt consider one of the major health problems of live stock (*Shkap et.,al 2003 and Aktas et.,al 2004*). The disease is amajor programs in many parts of the middle east and Asia where about 200 millon cattle are said to be at risk of infection (*Bakheit and Latif 2002*).

Bovine theileriosis clinically manifested by marked fever ,swelling of superficial lymph nodes ,inappetance ,tachycardia ,dyspenia and general weakness (*Omer et.,al 2003*) the mains symptoms of this disease is anemia (*Abdel-Rahman et.,al 1989*).Studies of (*Forsyth et., al 1999*) had represented anew view on the destructive effect on the parasite on the host organs and tissue

Antioxidant vitamins such as E,Cand A protect the cell from damage by free oxygen radical generated as aresult of parasitism (*Medzyavichyus et.,al1989*) and (*Smith 1989*). Vitamin C status of ruminants may be important in relation to their health and disease. Ascorbic acid play an important role as an antioxidant (*Kanter 1998*). It has also been suggested to be of value in alleviating both physiological and pathological stress (*Hemingway1991*). More over ,the interaction between antioxidant vitamin Cand E was investigated and it was reported that there is asparing effects of vitamin C on vitamin .E (*Miller et., al 1993*) . The present study aimed to focus light on this disease ,its clinical signs ,age susceptibility ,the changes of some hematological blood biochemical component and alteration of antioxidant agents vitamin Eand ascorbic acid in response to cattle theileriosis.

MATERIALS AND METHODS

1-Animals:

The present study was carried out on 140 caws aged from 6 months to over 5 years old collected from different private farms in different localities at Kalubeia governemate during summer season .The animals were clinically examined for signs of health and disease ,special care to body temprature ,corneal opacity and wasting according to the method described by (*Radostits et., al 2002*). The animals divided into two groups ,the first consisted of 94 apparantly healthy animals (free from blood parasite after blood examination)and kept as control group .The second consisted of 46 animals suffered from signs of the disease during blood examination of these animals ,besides presence of ticks on their body .

2- Samples :

Samples were taken from animals as follow:

- a- Thin blood smears were individually prepared from superficial ear vein ,fixed and stained with Giemsa stain for detection of blood parasites microscopically according to (*Feldman et.,al 2000*).
- b- Aspirated smears were also taken from superficial enlarged lymph nodes and examined according to (*Marquardt et.,al 2000*).
- c- Two blood samples were collected from jugular vein from each examined animals in dry sterile centrifuge tubes the first blood sample was collected with anticoagulant (Sodium salt of EDTA) and used for hematological studies according to (*Feldman et.,al 2000*), the second sample was collected in plain centrifuge tube for serum biochemical investigation .Serum total proteins (**Peters1968**) albumin (**Drupt 1974**) serum globulins were calculated ,glucose

(Trinder 1969) total bilirubin (Weigl 1975), aspartate aminotransferase (AST) alanine amino transferase (ALT) activities (Reitman and Frankel 1957), urea (Fossati et.,al 1980) as well as creatinine (Seeling and Wust 1969), blood serum levels of ascorbic acid and vitamin E were determined according to (Omaye et.,al 1979) and Martin 1964) respectively

d- All data were subjected to statistical analysis by stander T-test according to (Tamhane and Dunlop 2000).

RESULTS

1-Clinical finding:

Diseased animals were found invested with ticks in preneal ,flank and neck regions .Clinical examination revealed rise in body temperature ,enlargement of superficial lymph nodes ,weakness ,anorexia nasal discharge ,lacrimation which sometimes reach corneal opacity and tachycardia .

2-aboratory finding:

Theileria infected cattles were identified by examination of blood smear which revealed the presence of intracellular signet ring theileria species parasites .The results of the study dealing with parasitological and blood biochemical parameters were illustrated in tables as follow.

Table (1): The Prevalence of T. annulated infection among Cattle's in Kalubeia governarate.

| Examined animals | No | No of infected | Infection % |
|--------------------|-----|----------------|-------------|
| Apparently healthy | 94 | 0 | 0 % |
| Suspected disease | 46 | 43 | 93.4 |
| Total | 140 | 43 | 30.7 |

Table (2): The Prevalence of *T. annulatus* infection among Cattle Of different age.

| Age group Month | No of examined | No of infected | Infection % |
|-----------------|----------------|----------------|-------------|
| 6m – 1year | 45 | 15 | 33.3 |
| 1 – 3 year | 40 | 12 | 30 |
| 3- 5 year | 30 | 7 | 23.3 |
| Over 5 years | 25 | 2 | 8 |

Table (3): Effect of *T. annulatus* infection among Cattle On RBCs , Hb and PCV.

| Groups | RBCs mil/cm ³ | Hb g/dl | PCV% |
|--------------------------|--------------------------|----------|-----------|
| Control 2 | 8.55± | 11.26± | 33.49± |
| | 0.05 | 0.05 | 0.07 |
| Thileria infected cattle | 6.89±*** | 8.46±*** | 28.44±*** |
| | 0.12 | 0.14 | 0.028 |

* = significant at (p<0.05)

*** = significant at (p<0.001)

Table (4): Effect of *Thileria* infection on WBCs and differential leukocytic count on cattle.

| Parameters Groups | WBCs X 10 ³ /ml | Neutrophil | Lymphocyte | Monocyte | Eosino | basophil |
|--------------------------|-------------------------------|------------|------------|----------|---------|----------|
| Control | 9.55± | 3.06± | 5.40± | 0.58± | 0.55± | 0.17± |
| | 0.10 | 0.06 | 0.08 | 0.01 | 0.1 | 0.01 |
| Thileria infected cattle | 15.64± | 6.78± | 7.58± | 0.71± | 0.49± | 0.17± |
| | 0.88 *** | 0.57 *** | 0.33 *** | 0.01*** | 0.01*** | 0.01 *** |

* = significant at (p<0.05)

*** = significant at (p<0.001)

Table (5): Effect of Thileria infection On vitamin C and vitamin E levels on cattle.

| Parameters Group | Vit. C (mg/dl) | Vit. E (mg/ dl) |
|--------------------------|------------------|-------------------|
| Control | 0.16 ± 0.001 | 5.91±0.06 |
| Thileria infected cattle | 0.10±0.03* | 3.54± 0.10* |

* = significant at (p< 0.05)

*** = significant at (p< 0.001)

Table (6): Effect of Thileria infection on total protein, albumin , globulin (gm/dl) and albumin/globulin ratio on cattle.

| PARAMETERS Group | Total protein | Albumin | Globulin | Albumin/globulin |
|--------------------------|---------------|---------|----------|------------------|
| Control | 7.60± | 4.23± | 3.37 ± | 1.26± |
| | 0.06 | 0.06 | 0.10 | 0.05 |
| Thileria infected cattle | 5.42± | 2.61± | 2.81± | 0.93± |
| | 0.12 | 0.11 | 0.10 | 0.06 |

Table (7): Effect of Thileria infection On liver function of cattle.

| PARAMETERS Group | Glucose mg/dl | Blood urea nitrogen mg/dl | Creatinine mg/dl | Total bilirubin mg/dl | ASTu/l | ALTu/l |
|--------------------------|---------------|---------------------------|------------------|-----------------------|--------|--------|
| Control | 60.70± | 30.52± | 1.37± | 0.98± | 57.50± | 20.60± |
| | 0.76 | 0.69 | 0.05 | 0.01 | 0.96 | 0.70 |
| Thileria infected cattle | *** | *** | *** | *** | *** | *** |
| | 51.70± | 40.50± | 1.96± | 1.31± | 71.60± | 34.90± |
| | 0.90 | 0.98 | 0.08 | 0.09 | 1.01 | 1.11 |

*** = significant at (p< 0.001)

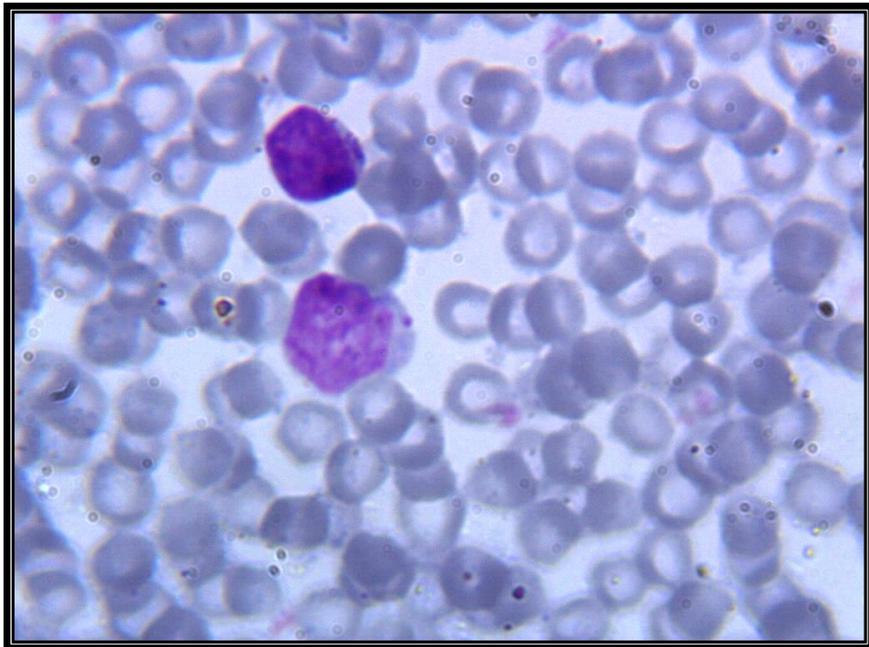
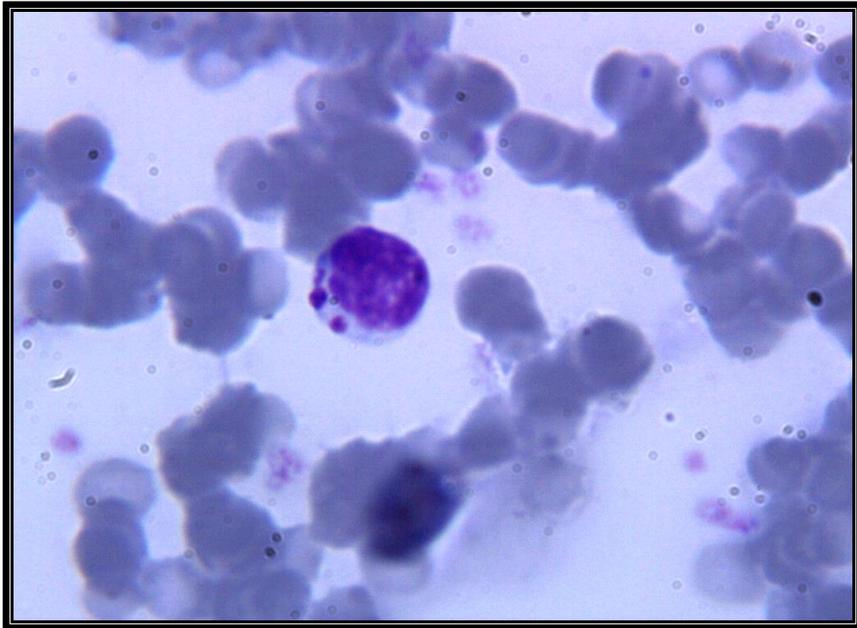


Fig (1): Koch's blue bodies in Monocyte of infected cattle.

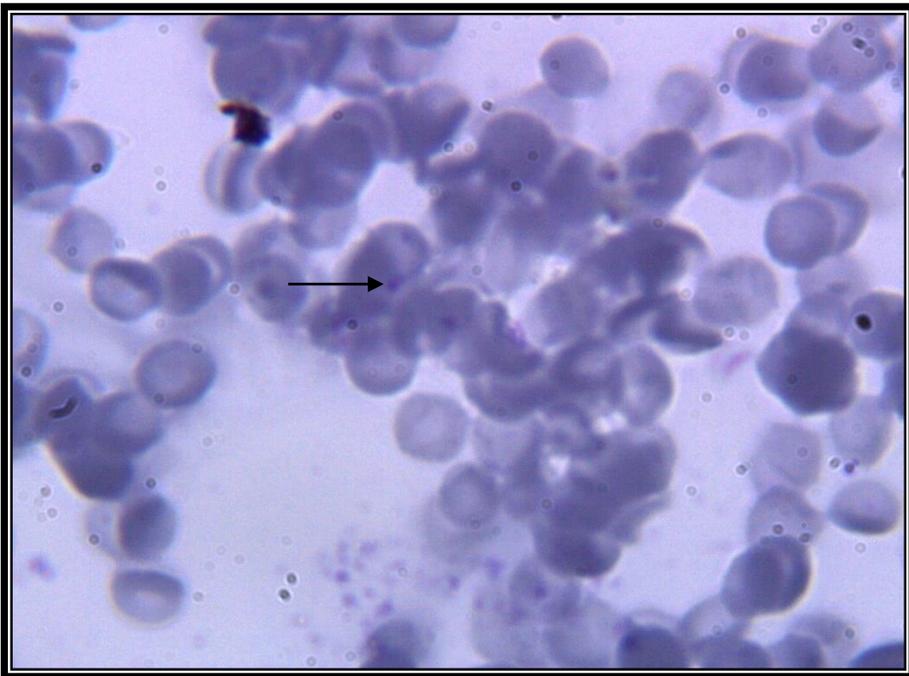
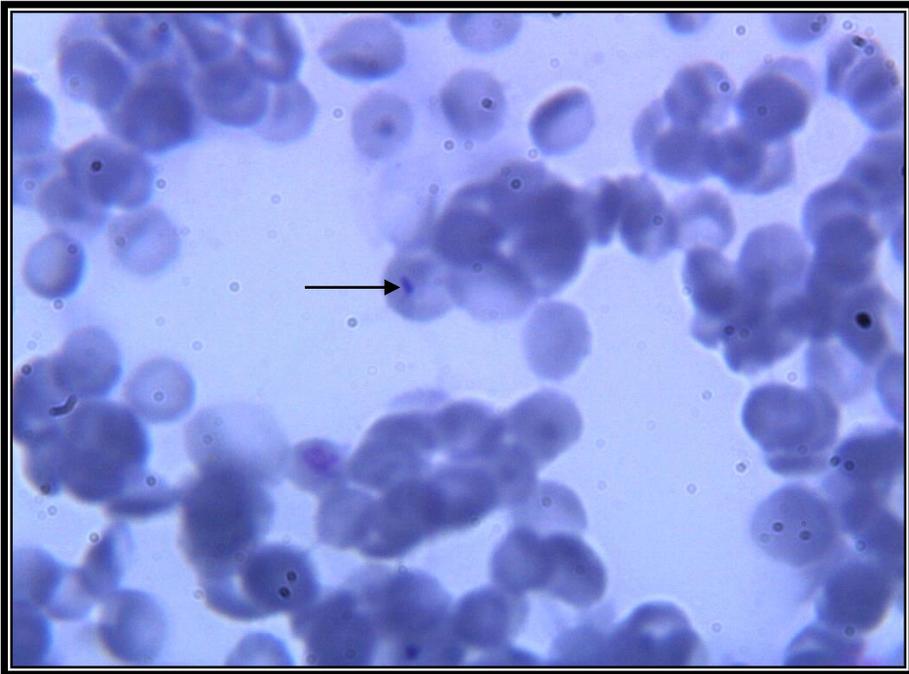


Fig. (2): Merozoite schizont of theileria in RBCs of infected cattle.

DISCUSSION

Diagnosis of clinical cases of acute theileriosis is fairly simple fever accompanied by enlargement of superficial lymph nodes during tick season is strongly suggestive of the disease (*Radostit et.,al 2002*). Blood films and lymph node smears revealed the presence of different forms of intra-erythrocytic trophozoites (fig 2) schizont(Kochs blue bodies) were observed in lymph nodes and monocytes (fig 1)these finding parallel to those described previously by (*Rodostits et.,al 2002*) and (*Ramazan and Uslu 2006*). Examination of suspected infectious animals revealed that 30.7%out of 140animals were infected with theileriosis (table 1)this result was similar to (*Abd-El-Raof et.,al 2000*), (*El-masry et.,al 2006*) they recorded that the incidence of theileriosis among cattle were 33.66%,29.37%respectively. However higher prevalence rates were reported by (*Song and Sang 2003*), (*Inoue et.,al 2001*) they detected theileriosis among cattle were 50.4% ,67.8% respectively. This difference may be attributed to the difference in localities and species of examined animals . Concerning the suseptability of age to theileriosis present data revealed that calves with age of 6 months to 1 yearwere highly susptible to theileriosis 33.3%which is nearly equal to that from (1-3) years 30%they decreased at age from (3-5) years 23.3%to reach the lowest infection rate at animals over 5years is 8%(table 2) which may be attributed to age resistance or previous infection and presence of a sort of immunity nearly similar results were obtained by (*El-masry et. al 2006*). Around this point (*Abd-El-Raof et. al ,2000*) reported that buffaloes with age of 6 months to 5 years were highly susptible to theileriosis 54.91%followed by those over 5 years old (15.79%), this variation may be attributed to the species of examined animals .Concering the changes in haemogram parameters ,in theileria annulata infected caws (table 3) showed that there was anemia as a result of significant decrease in erythrocytic count, Hb and PCVwhen compared with control group this result came in accordance with those of (*Omer et al,2002*).

(*Emam and Al-Kabbany 2004*) and (*El-masry et al 2006*) such changes could be due to the destructive effect of parasites on erythrocytes and failure of bone marrow to produce enough erythrocytes in response to the toxic material to compensate such losses (*Radostitis et.al,2002*), (*Shiono et. al,2004*) and (*Ramazan and Uslu 2006*).

Further,more intraerythrocytic parasites metabolize hemoglobin and produce O₂ which cause oxidative stress as a result, the free O₂ and H₂O₂ increase lipid peroxidation cause oxidation of sulfhydryl group of the globin chain and damage of erythrocyte cell membrane with the subsequent removal of affected erythrocytes by active macrophages (*Stocker et.al, 1986*) and (*Attia 2001*). 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 there by causing cell and tissue damages (*Smith 1989*) and (*Sarin et.al,1993*). The leukogram in the present study (table 4) illustrated that there was a leukocytosis as a result of neutrophilia, lymphocytosis and monocytosis in the infected cows in comparison with control, our results agreed with those documented by (*Stockham and Scott 2002*), (*Selim and Saadia 2007*). On the other hand our results were partially in contrary with the result obtained by (*Stochham et.al,2000*) who found only lymphocytosis. (*Omran and EL-Kholany 2003*) documented picture of stress leukogram. The interaction between antioxidant vitamin C and E was investigated (table 5) and asparing effects of vitamin C on vitamin E as well of vitamin E on vitamin C were recorded (*Tanaka et.al,1979*). The significant decrease in serum vitamin levels agree with (*Abdellah et.al,2006*) these 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 vitamins E,A and C, where vitamin C and E are chain – breaking antioxidants that protect the cells from oxidative damage . On the other hand additional supplementation of theileria infected animals with source of vitamins to over come the oxidative stress and to avoid possible complication of vitamin E and C deficiencies .Previous studies have shown that low level of 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*).

Dealing with changes in total protein in present study (table 6) indicated a high significant decrease in albumin level in infected caws. (*Sandhu et.al,1998, El Swalhy 1999 and Salem and Mohran 2003*) attributed marked reduction in albumin level to the harmful effect of the parasite on the liver and consequently reduction of albumin synthesis in the same time our result revealed hypoproteinemia agreed with (*Omer et.al,2003*). Anoxia as well as intestinal troubles and diarrhea may be responsible such reduction (*Kaneko 1997*) and (*Thomas,2000*). Serum glucose in caws infected with theileria annulata indicated high significant decrease when compared with control group this result supported by, *Selim and Saadia, 2007 and El masry et.al, 2006*). This can be attributed to the effect of the parasite on the liver (*Smith 1996*) which may inhibit gluconeogenesis or glycogen storage which are essential for the maintenance of blood glucose (*Tenant 1997*). Similarly serum AST and ALT enzyme besides total bilirubin values in this study revealed significant elevation in infected caws when compared with healthy control similar results were obtained by (*Omran and EL-Kholany 2003*) (*Salem and Mahran 2003*), (*Omer et.al,2003b*) and (*ELmasry et.al, 2006*) refered such increase to the degenerative changes of the liver in addition heamolysis of erythrocytes during thieleria infection. However

increase serum urea and creatinine levels explained by *Stockhan and Scott 2002* who attributed hemolytic disorder characterized by proximal tubular degeneration leading to renal insufficiency. However alteration on liver and kidney function obtained in present study were relevant with *Abou-ELhasan 1997, Singh et.al, 2001* they showed several and different macroscopic and microscopic lesions in liver and kidney of theileria infected calves resulting in hepatic and renal damage due to excessive production of cytokines.

In conclusion, the present study proved that ticks are very dangerous in animal house and must be eradicated specially during summer season also cattle theileriasis was accompanied by blood picture changes, impairment of liver and kidney function beside the reduction of chain breaking antioxidants levels ascorbic acid and vitamin E. We advise application of specific antiprotozoal drugs with source of vitamin C and E to overcome the oxidative stress associating the infection and to avoid the possible complications of secondary vitamin deficiency. Finally periodic examination of cattle for control and prevent the adverse effect of the parasites.

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