OXIDATIVE STRESS AND HEMATOLOGICAL PROFILE IN THEILERIA ANNULATA CLINICALLY INFECTED CATTLE BEFORE AND AFTER TREATMENT

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ABSTRACT

Bovine theileriosis is a destructive disease that affected cattle of all ages, breeds and sex and leads to severe losses in production and reproduction. To assess the antioxidant status and oxidative stress in bovine theileriosis due to Theileria annulata, blood samples were collected from 17 clinically infected cattle referred to the Veterinary Teaching Hospital, Assiut University. Complete blood picture, Nitric oxide, Malondialdehyde and total antioxidants capacity were determined and the results were compared with those of 10 healthy controls. The concentration of Nitric oxide (NO), Malondialdehyde (MDA) were significantly higher (P < 0.0001) and the total antioxidant capacity (TAC) was significantly lower in the infected cattle than in healthy ones (P < 0.001). Conventional and molecular techniques will help in early and accurate diagnosis and enables the effective treatment. The treatment with buparvaquone aims to eliminate the parasite from the blood and lymph node and consequently improvement in the clinical state without any adverse effect on the animal’s cells. After treatment noticeable improvement was observed in clinical signs and significant increase in total RBCs count (6.58 ± 0.98 x10^6/mm^3) and hemoglobin concentration (9.82 ± 0.98g/dl) compared with (4.37 ± 2.05 X10^6/mm^3 and 6.67 ± 2.76 g/dl) before treatment, respectively. Also, the oxidative stress was significantly altered and a significant increase of the (TAC), significant decrease of (NO) and (MDA) was noticed.

Key words: Bovine Theileriosis, Oxidative stress, Buparvaquone.

INTRODUCTION

Bovine theileriosis in cattle is a disease caused by protozoan parasite known as Theileria annulata, transmitted by ticks of genus Hyalomma. More than 250 million domestic cattle have been estimated to be at risk (Robinson, 1982). This disease leads to severe losses in production and reproduction of cattle (Al-Gaabary, 1995). Theileria annulata infection in cattle resulted in anemia and other adverse effects on the hematological profile, so, supplementation of the diseased animals with supportive treatment is recommended to help those animals to resume their normal productivity early (AbdEllah and AL-Hosary, 2011).

Conventional diagnosis of this disease depends on examination of Giemsa stained thin blood film and lymph smears. This method is limited to the acute stage of the disease where the parasitemia is high enough to be detected microscopically. During chronic and carrier stages the level of parasitemia usually below the microscopic detectable level. The application of genotypic assays for the diagnosis of bovine theileriosis has shown recent advances. Molecular identification provides two primary advantages to phenotypic identification; it is more rapid turnaround time, and improved accuracy of identification (Aktas et al., 2005). Polymerase chain reaction (PCR) offers important advantages such as the higher sensitivity and specificity over conventional techniques in detecting both piroplasm-infected and carrier animals. This has been verified in a number of studies performed on a wide range of animals. The PCR assay has its superiority in separating parasitic infection associated with clinical signs (clinical form) from that without clinical signs (sub-clinical form) (Aktas et al., 2005 and AL-Hosary et al., 2009, 2013).

Using 30KDa major merozoite surface antigen of T. annulata protozoan parasite (Tams-1 gene) is more appropriate. Tams-1 gene is the most abundant and immune-dominant antigen on the surface of merozoites and piroplasms of Theileria annulata. It is a molecule with a molecular mass of approximately 30 KDa (Altay et al., 2007; Murat et al., 2008 and AL-Hosary et al., 2009, 2013).
**Materials and Methods**

1. **Animals**

   A total number of 27 cattle aged from one to five years old were subjected to this study during the period from July to November, 2014; 10 parasitologically free healthy cattle were used as control group and 17 clinically infected cattle with *Theileria annulata*. Infected cattle were selected on the basis of clinical examination and positive blood and/or lymph node smears as well as Tams-1 target based PCR, examination was done before and after treatment.

2. **Clinical Examination and Conventional diagnosis**

   Clinical examination was performed for all animals. The clinical signs of *T. annulata* infection were observed and recorded. Thin blood smears were prepared from the ear veins of all cattle, after preparation stained by 8% Giemsa stain then examined on Olympus Microscope (Olympus, Japan) using oil immersion lens at X1000 magnification (Charles, 2002).

3. **Molecular diagnosis:**

   3.1. **Tams-1 target based PCR assay**: Genetic confirmation of *T. annulata* infection was carried out by using Tams-1 target based PCR.

   2.3.2 **DNA Extraction**:

      DNA extraction from whole blood was carried out according to Manufacturer's instructions of the commercial kits QIA amp blood kit, Qiagen, Ltd, UK, Cat No. 51104.

   3.3 **DNA amplification**: For the standard PCR, primer Tams1 F (5′ ATG CTA CAA ATG AGG AT) and Tspms1 R (5′GGA CTT ATG AGA AGA CGA TGA 6) Amplifying a (785 bp) fragment of the *Theileria annulata* 30 KDa major merozoite surface antigen gene, Tams1, was used (Kirvar et al., 2000 and AL-Hosary et al., 2009, 2013).

   3.4. **Cycling conditions**: PCR was performed by incubating the samples at three temperatures corresponding to three steps (Denaturation, Annealing, and Extension). 94°C for five minutes, followed by 37 cycles consisting of one minute at 94°C, one minute at 55°C, two minutes at 72°C and final extension step at 72°C for ten minutes longer then the samples were stored at 4 °C until use in the next step. The cycling condition carried out in Biometra thermocycler (Professional basic, Thermocycler, version 11/06 Biometra, An Analytik, Jena Company-Germany).

   In addition to the samples positive control sample contain "DNA from Theileria annulata infected lymph" and negative control sample that contain no DNA at all were included in the amplification step.

3.5. **Gel Electrophoresis:**

   The electrophoresis chamber was connected to 75 volt power supply for 1:30 hour, 10µl of each PCR product were separated by electrophoresis on 1.8% agarose gel (GX 040.90, Gen agarose, L.E., Standard DNA /RNA agarose, Molecular Biology Grade, Inno–Train Diagnostic, D–61476, Kronberg/Taunus) Containing Ethidium bromide as 1 µl /ml electrophoresis buffer. Using 100 bp DNA–ladder in (SCI–PLAS, HU 10, 5636, UK). Then the result obtained through High Performance Ultraviolet Transilluminator, (UV, INC, UK). The image of the PCR products containing the DNA sequence of 785 bp were amplified using DOC–It ®LS, Image acquisition–software, (UVP, INC, UK).

4. **Blood sampling and routine hematological examination**

   Whole blood samples were collected directly from jugular vein, in vacutainer tubes contained EDTA for routine hematological examination and into heparinized vacutainer tubes for measurement of total antioxidant capacity (TAC), Nitric oxide (NO) and Malondialdehyde (MDA). Hematological analysis was carried out using automatic blood cells counter (Medonic CA 620, Sweden).

5. **Biochemical assays and analysis**

   Total anti-oxidant capacity (TAC) was determined by using commercial kits supplied from Biodiagnostic Company for diagnostic reagents: Dokki, Giza, Egypt. The determination of the total anti-oxidant capacity was performed by the reaction of anti-oxidants in the sample with a definite amount of exogenously provide hydrogen peroxide (H₂O₂) according to the methods of (Koracevic et al., 2001). The anti-oxidants in the sample eliminate a certain amount of the provided H₂O₂, MDA and NO levels were estimated using commercially available test kits supplied by Biodiagnostic-Egypt, according to the...
methods described by (Okawa et al., 1979; Montgomery and Dymock 1961).

6. Treatment protocol
Buparvaquone (Bupaquone) (BVP Ltd. co. Kerry, IRLAND) was used in treatment of the clinically infected animals. Bupaquone was used at dose rate of 2.5 mg / kg body weight, second dose was required within 48:72 hours from the initial dose. Marbofloxacine (Marbocyl 10%) was used as antibiotic therapy to control the respiratory complications. It used at dose of 2 mg/1kg. (Marbocyl10% 1ml per 50 kg BW, intravenous or intramuscular, Intercova, Animal Health Products-Egypt) according to (AL-Hosary et al., 2010).

7. Statistical analysis
Statistical analysis was conducted using SPSS version 16.0 for windows (SPSS, Chicago, USA). The results were analyzed using one way analysis of variance (ANOVA). Data were expressed as mean ± SD.

RESULTS

1. Clinical and Hematological Examination
The statistics of the measured parameters in healthy and clinically infected cattle before and after treatment are presented in Table 1. Present data showed that, the clinical signs of theileriosis were fever (>40°C), corneal opacity (Fig.1), enlargement of superficial lymph node (Fig.2), lacrimation, respiratory manifestations, nasal discharge (Fig.1), anorexia, paleness of mucous membranes and various degrees of ticks infestation. Treatment with Bupaquone was effective in the improvement in clinical state and in elimination of the protozoan parasites from the blood and lymph nodes.

The infection was confirmed conventionally by examination of the Giemsa stained thin blood film for detection of the intra-erythrocytic (signet ring) stage of the protozoan parasite *Theileria annulata* (Fig. 3). Also, all samples were subjected to Tams-1 target based PCR for confirmation of *T. annulata* infection. The results revealed that all examined animals were infected and the positive result was indicated by specific band at 785 bp (Fig. 4).

Hematological examination (Table. 1) revealed significant increase P (<0.01) of MCV, MCH and RDWₐ values and significant decrease of total erythrocyte count, PCV and hemoglobin in the diseased cattle compared to the control ones which indicated severe anemia. After treatment, the result revealed significant decrease of MCV and RDWₐ values and significant increase of total erythrocyte count, PCV and hemoglobin.
DISCUSSION

Bovine theileriosis is one of the infectious diseases which lead to severe economic losses in cattle, the goal of this study mainly was to investigate the therapeutic impact of treatment on the clinical signs, hematological profile and oxidative stress biomarkers in *Theileria annulata* naturally infected cattle. Our data showed that, the clinical signs of theileriosis were fever (>40°C), corneal opacity, enlargement of superficial lymph node, lacrimation, respiratory manifestations, nasal discharge, anorexia, paleness of mucous membranes and various degrees of ticks infestation. These clinical signs are in agreement with those obtained by (Radostits et al., 2000 and A.L-Hosary 2009, 2013). Considerable variation was noticed in the clinical signs of the infected animals after treatment. Recorded results revealed that early treatment with Bupaquone was highly efficient in improvement of clinical signs and also it helps in elimination of both piroplasmic and lymphocytic stages of the protozoan parasites from both blood and lymph nodes within 3–4 days post-treatment. Similar results were reported in cattle by Muraguri et al. (2006). All samples were subjected to Tams-1 target based PCR for confirmation of *T. annulata* infection. All examined animals were infected and the positive result was indicated by specific band at 785 bp. Hematological examination revealed significant increase of MCV, MCH and RDWα values and significant decrease of total erythrocyte count, PCV and hemoglobin concentration in the diseased cattle compared to the control ones which indicated severe anemia. This is in agreement with (Omer et al., 2002). The type of anemia usually classified as normocytic normochromic anemia, it was previously reported by (Sandhu et al., 1998 and Abd- Ellah and
AL–Hosary, 2011). After treatment, the result revealed significant decrease of MCV and RDWa values and significant increase of total erythrocyte count, PCV and hemoglobin. This indicated that the treated animals start the resumption of their normal status.

Free radicals and antioxidants may play integral roles in different aspects of pathogenesis of *Theileria annulata* infection. The results revealed a significant increase \( P (<0.01) \) in the levels of NO and MDA and a significant reduction \( P (<0.01) \) in the levels of TAC in *T. annulata* clinically infected cattle compared with healthy one. Reduction of TAC level may be attributed to the reduction in antioxidant enzymes as they are consumed by excessive free radicals in the infected animals this was in agreement with (Hassanpour et al., 2013). After treatment the finding were completely changed, the levels of NO and MDA have been significantly decreased and a significant increase in the TAC was noticed.

Treatment of the infected animals for controlling the respiratory complication revealed a significant changes in the hematological profile as well as the oxidative stress and this could be contributed to the elimination of the protozoan parasite because the used drugs was directed to eliminates the parasites and thus prevents the transmission of signals necessary for induction of genes coding for growth factor and for the receptors involved in signal transduction (Mchardy, 1985; Jabbar et al., 1992). Marbofloxacine is a new third generation fluoroquinolone and used for control of the respiratory complication. It has broad spectrum bactericidal activity against Gram negative bacteria, including *Mannheimia haemolytica* and *Haemophilus* species, Gram positive bacteria and Mycoplasma species (Thomas et al., 2001). This allows animals to resume their normal state.

The same findings had been reported by Shiono et al. (2003) who reported that the level of MDA began to increase remarkably in *T. annulata* clinically infected animals. MDA evaluation indicated that lipid peroxidation in erythrocytes of affected cattle was significantly more than those of healthy cattle. In accordance with the findings from other studies (Saluja et al., 1999; Grewal et al., 2005), our results indicated that the lipid peroxidation in erythrocytes of affected cattle increases MDA production. Increased MDA concentration in erythrocytes of affected cattle may be an indication of elevated oxidative stress in theileriosis. Oxidative stress results when the production of the free radicals and reactive metabolites of oxygen exceeds their safe disposal by antioxidant mechanisms. Free oxygen radicals cause lipid peroxidation and the end product of lipidperoxidation is MDA. Determination of MDA allows detection of the degree of lipid peroxidation and level of free oxygen radicals indirectly (Yagi, 1998; Owen, 1996 and Hanan et al., 2013). The erythrocytes membrane is rich in polyunsaturated fatty acids, a primary target for reactions involving free radicals, and is very susceptible to lipid peroxidation (May et al., 1998; Devasena et al., 2001).

**CONCLUSION**

Bovine theileriosis causes adverse effect on the hematological profile. In addition to oxidative stress which is characterized by significant decrease in the TAC accompanied by increase in both NO and MDA. After treatment of the diseased animals with the specific drugs most of clinical signs were returned to their normal function, blood picture significantly improved and the oxidative stress was significantly declined. Tick eradication programs for animals and surrounding environment must be taken in consideration during treatment of diseased animals.

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ال всшиهضاغطه وصوره الدم في الابقار المصابه اكلينيكي باثيليريا الحلقيه قبل وبعد العلاج

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يعتبر مرض ثيلريا الماشيو من الامراض المدمره من جميع السلالات والاعمار ويؤدي إلى خسائر فادحة. هدف هذه الدراسة هو تقييم صوره الدم الكاملة للاكسدة والإكسدة الضاغطه في حالات الأمراض الماشيو بالاضافه الي اجمنلي القدره المضاده للأكسدة والاكسدة الضاغطه في حالات الأمراض الماشيو. وكذلك دور العلاج في تحسين هذه الاعدادات وقد تم تجميع سبع عشر عينة من عينات الدم من الابقار المصابه بالممرض والتي كانت تترده على المستشفى البيطرى التعليمي بجامعة أسوان. تم دراسة الأعراض الإكلينيكية الخاصة بالممرض على الحالات المحتمل اصابتها بهذا المرض. تم قياس كل من الامالونديالدهيد وكسيد النيتريلك كمؤشرات للأكسدة الضاغطه وكذلك اجمنلي القدره المضاده للأكسدة وايضا تم اجراء صوره دم كامله لكل حيوان ومن ثم مقارنة هذه النتائج بين الابقار سلعة اكلينيكيه. ووفقا للنتائج كانت معدلات الامالونديالدهيد وكسيد النيتريلك أعلى في الحيوانات المرضيه مقارنة بتلك الحالة من الأمراض. ومتتابعه الحيوانات بعد العلاج لاحظنا اختفاء الكثير من الأعراض التي كان يعاني منها الحيوان من ارتفاع في الجرباره وإعراض تنفسه وعامة في العين وعده الغدد اللطبيه لحجمها الطبيعي وكذلك تقليل ارتفاع في اجمنلي القدره المضاده للأكسدة وانخفاض في مؤشرات الأكسدة الضاغطه وبالتالي لصوره الدم فقد ارتفع العدد الإجمالى لكريات الدم الحمراء وكذلك تركز الهيموجلوبين عن تلك النتائج التي تم قياسها قبل تطبيق العلاج.