Malondialdehyde Status, Trace Minerals and Hematologic Results of anemic- *T. equi* infected Egyptian Horses

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**Article History:** Received: March 17, 2015 Revised: June 10, 2015 Accepted: June 21, 2015

**ABSTRACT**

Equine piroplasmosis is a clinically significant widespread tick borne disease affecting equine population. Thus, this study aims to describe the clinical signs, correlation between the developed anemias and mineral status along with the lipid peroxidation product Malondialdehyde (MDA) in naturally occurring *T. equi* infected horses in Egypt. Twenty-five horses of different age and sex were involved in this study; horses have signs compatible with babesial infection were examined. Fever, hemoglobinuria and icterus were the most consistent clinical signs recorded in this study. The hematology picture showed decrease in red cell parameters along with leucocytosis. Macrocytic hypochromic anemia was found in infected horses alongside relative increase in copper and relative decrease in ferrous and zinc. MDA showed very statistically significant difference when compared to control data. In conclusion, high level of MDA along with reduction in PCV, HB, and RBCs count is indicating the presence of oxidative stress and implicating the process as a cause of anemia in *T. equi* infection. The mineral status (Cu, Zn, Fe) appeared to be directly affected by the parasite and the mineral status influenced by the anemic syndrome associated with *T. equi*. Inversely correlation between zinc and MDA level might be used in planning the treatment strategy of *T. equi*. Including these minerals in treatment regimen of *T. equi* will help to counter the anemic nature of the disease.

**Key words:** *T. equi*, Trace Minerals, Malondialdehyde, Hematology, Equine

**INTRODUCTION**

In equine practice, equine piroplasmosis is a widespread tick borne malady affecting horses all over the world (Kuttler 1988; DeWaal 1992; Rothschild and Knowles 2007). The clinical presentation of the disease varies; nevertheless, it mainly consists of fever, anemia, hemoglobinuria and icterus (Camacho et al., 2005; Deger et al., 2009; Abedi et al., 2014; Hussain et al., 2014). Two agents, namely *T. equi*, *T. caballi*, have been incriminating; however, *T. equi* is known to be the cause of the virulent illness while the infection with *B. caballi* tends to be mild to unapparent (Friedhoff et al., 1990).

Anemia defined as decrease the ability of blood to carry oxygen, one of the most common causes of anemia in horses is the hemo-protozoan parasite called “Babesia” (Morris 2006). Anemia can develop due to blood loss, destruction or decrease bone marrow production of RBCs (Merck publishing and Merial 2007).

A great portion of horses infected with babesia regardless the clinical presentation of disease suffering from varying degree of anemia manifested as decrease in RBCs, PCV, and hemoglobin (Allen et al., 1977; Zobba et al., 2008; Wise et al., 2013), acute infection are also characterized by decrease serum iron levels (DeWaal et al., 1987).

Trace minerals are essential for wellbeing and growth of animals, Zinc is involved in plentiful biological operations; zinc plays an integral role in antioxidant enzyme "superoxide dismutase" (Mates 2000; Classen et al., 2011). Copper is an essential component required to prevent anemia development (Sullivan and Ochs 1987), copper can operate as antioxidant by neutralizing the free radicals, as well (Araya et al., 2006).

The wide-known function of iron is the contribution in heme molecule synthesis, copper is mandatory to iron uptake in hemoglobin synthesis operation (Deger et al., 2005). Iron deficiency may lead to hypochromic microcytic anemia (Bainton and Finch 1964; Bian et al., 2003).

In recent years, several researches pinpoint the role of highly reactive oxygen radicals in pathogenesis of equine

babsiosis (Deger et al., 2009), but still scarce papers discussing this topic.

Free oxygen radicals result in lipid peroxidation, which can be assessed by estimation of Malondialdehyde (MDA) levels (Esterbauer 1996). MDA is widely acceptable biomarker for lipid peroxidation estimation (Moore and Roberts 1998). RBCs membrane lysis causing RBCs fragility could be attributed to lipid peroxidation process takes place throughout T. equi infection (Ambawat et al., 1999).

The current study designed to investigate hematologic alterations, selected mineral status (Zn, Cu and Fe) along with MDA level in anemic horses naturally infected with babesiosis.

**MATERIALS AND METHODS**

Twenty-five horses (n=25) of different age and sex were involved in this study; each horse was subjected to comprehensive physical examination including pulse rate, respiratory rate, superficial lymph nodes, visible mucous membrane status, chest auscultation and fecal sample examination; the rectal temperature and clinical presentation were recorded, the existence of ticks on the coat was determined. Ticks were collected and identified based on morphological characteristics (Nuttal et al., 1998). All the infected cases were collected during August and September 2014 from Giza governorate, Egypt.

For hematology, blood was collected from jugular vein in EDTA-containing tubes; clinical hematology was done within 2 hours after the sample collection (Feldman et al., 2000). Giemsa-stained thin blood films were inspected under the microscope for direct recognition of intraerythrocytic stages of the hemoparasite (Brown 1993); only horses given positive results in the three blood films were involved in this study. Sera of infected animals were analyzed for Zn, Cu and Fe using specific test kits (Spectrum-Diagnostics, Egypt).

MDA was calorimetrically analyzed using respective chemical test kit (Biodiagnostic, Egypt) according to manufacturer’s instructions.

The hematological, minerals and MDA data of infected horses were compared with control data collected from the same locality. Statistical analysis was performed using Student’s t test (STATISTICA for Windows, version 5.1., StatSoft, Inc. 1984 -1996). P <0.05 was considered of statistical significance.

**RESULTS**

Pertinent signalmen of the affected horses were recorded, the highest infection rate was found in age group of 6-9 years (11/25, 44%), followed by age group above 9 years old (8/25, 32%) and the least affected group was those between 1-5 years (6/25, 24%). The stallions appeared to be almost equally infected (14, 56%) as mares (11, 44%). The collected ticks were identified based on their morphological characteristics as *Rhipicephalus spp.*

The most recorded clinical sign was fever (39.5 ± 0.194) presented in all infected animals. The other clinical signs recorded were inappetence, hemoglobinuria, and icterus and congested mucous membrane as well.

Three stained blood film of each animal were examined under oil-immersion lens, the examination reveals the presence of small pifiform, circular merozoite in RBCs, which is indicative for *T. equi.*

The mean hematologic values are shown in table 1. Significant decrease in RBCs, HB content and PCV percentage were recorded along with significant increase in WBCs count were observed. Red cell indices showed significant increase in MCV along with significant decrease in MCHC suggesting macrocytic hypochromic anemia. Significant decrease in platelet count was observed.

The mineral status of the infected animals are shown in table 2. A relatively high copper level along with relatively low zinc and ferrous levels were observed in infected horses compared to control data.

MDA levels are shown in table 2, result showed extremely statistically significant increase in MDA level in infected horses compared to the control data.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient data</th>
<th>Control data</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (<em>×10^6/µl</em>)</td>
<td>4.324±0.212*</td>
<td>6.18±0.149</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>9.134±0.749*</td>
<td>15.42±1.320</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>27.25±1.41*</td>
<td>34.40±0.93</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>64.019±1.744*</td>
<td>55.70±1.123</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>33.179±2.065*</td>
<td>44.800±1.125</td>
</tr>
<tr>
<td>WBCs (<em>×10^3/µl</em>)</td>
<td>13.504±0.980*</td>
<td>6.7200±0.333</td>
</tr>
<tr>
<td>Platelets (<em>×10^3/µl</em>)</td>
<td>113.45±10.65*</td>
<td>170.40±27.96</td>
</tr>
</tbody>
</table>

* Show statistically significant difference at P<0.05.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient data</th>
<th>Control data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper 22.48±1.823*</td>
<td>13.782±0.274</td>
<td></td>
</tr>
<tr>
<td>Zinc 14.95±1.002*</td>
<td>51.66±4.216</td>
<td></td>
</tr>
<tr>
<td>Iron 92.400±15.702*</td>
<td>177.98±18.242</td>
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<tr>
<td>MDA 6.1581±0.6496*</td>
<td>2.578±0.3123</td>
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* Show statistically significant difference at P<0.05.

**DISCUSSION**

Equine babesiosis is one of the most significant tick-borne sickness, with an economic global impact on the equine business (Zobba et al., 2008). The highest occurrence of the disease was at 6-9 years, previous reports dealt with the seropositivity showed higher affinity of *T. equi* to exist in older equids (Kouam et al., 2010; Salib et al., 2013); however, ‘Grandi et al., 2011 found no statistical association between the infection status and age.

The stallions appeared to be almost equally infected as mares; the gender appeared not to play a role in the infection rate (Asgarali et al., 2006; Karatepe et al., 2009; Kouam et al., 2010), however other contradicted reports showed higher infection rate in males than females (Salib et al., 2013), males were significantly less affected than mares (Shkap et al., 1998).

Higher infection rate was found in summer, the infection rate is strongly correlated with the tick population, climatic aspects, such as temperature and humidity have great influence on the habitat and dynamics of ticks’ population (Young and Leitch 1981; Chilton and Bull 1994; Leschnick et al., 2008). *Rhipicephalus spp.* was identified as the vector, the genus *Rhipicephalus* is
considered an African type tick with 63 spp confined to Afro-tropical territory only and one was thought to be restricted to African territory has recently gained access to Arabian peninsula (Walker et al., 2000; Olwugh et al., 2007).

Equine piroplasmosis causes different clinical picture depending on the severity of the infection. Fever, inappetence, hemoglobinuria, and icterus were the most consistent clinical signs recorded in this study; this clinical presentations agreed with previous reports (Camacho et al., 2005; Deger et al., 2009; Abedi et al., 2014; Hussain et al., 2014). However, the clinical picture has been described as changeable and often nonspecific (Bashiruddin et al., 1999). Marked hemolysis and consequential anemia were responsible for the clinical picture (Wise et al., 2013). In Mediterranean region in which piroplasmosis is an endemic disease, symptomatic illness can occur year round with specific or vague signs (Friedhoff et al., 1990; Zobba et al. 2008).

Small piriform, circular merozoite in RBCs, which is indicative for T. equi was found via microscopic examination, direct diagnosis includes demonstration of intraerythrocytic merozoites in Giemsa stained blood by molecular detection (Nagore et al., 2004; Alhassan et al., 2007).

Reduction in RBCs, HB content and PCV percentage along with significant increase in WBCs count were recorded in this study, piroplasmosis is known to induce anemia in most horses in spite of the clinical picture (Wise et al., 2013). The excessive intravascular hemolysis along with phagocytic role of neutrophils and monocytes in response to parasite antigen adhered to RBCs surface and toxic hemolytic factor released by the parasite itself might be contributed to the anemia (Ambawat et al., 1999; De Gopegui et al., 2007; Zygnier et al., 2007). The increased leukocyte count was observed, the elevation in infected RBCs along with stimulation of lymphoid system and bone marrow are directly correlated to elevated WBCs count (Sandhu 1996; Ike et al., 2005; Ibrahim et al., 2011).

The red cells indices showed increase in MCV (macrocytic) along with reduction in MCHC (hypochromic) anemia, red cell indices (MCV, MCH, and MCHC) have been reported to be variable (Ambawat et al., 1999). However, macrocytic hypochromic anemic pattern has been recorded in previous reports dealt with T. equi (Al-Saad 2009; Ibrahim et al., 2011). In recently published research, anemia was attributed to consequence of oxidative damage in RBCs (Nazifi et al., 2008) principally those correlated with SOD and GPx (Nazifi et al., 2009). Zinc-containing enzyme "Carbonic anhydrase" converts CO2 and without this enzyme, the conversion rate will be slower (Tripp et al., 2001; Kohen and Limbach 2001).

The mineral status of examined horses showed relatively high copper level accompanied by relatively low zinc and ferrous level, significant elevation in Cu along with significant reduction in Zn have been reported in anemic patients (Ece et al., 1997; Gürgöze et al., 2006; Turgut et al., 2007; Dede et al., 2008).

The protozoal agents is considered to increase the cytokines and effecter cells, zinc is believed to play a role in macrophage uptake and destroying the insulting agent (Wirth et al., 1989; Scott 2000). Wellinghamhausen et al., (1999) assumed the decrease in zinc level in patients with alveolar echinococcosis is correlated with increase the zinc utilization by the parasite, this assumption could be applied in equine piroplasmosis. Copper is directly proportional with humoral response to the infection (Dede et al., 2008); during inflammatory processes, increase the levels of ceruloplasmin, resulting in elevation of the copper levels (Healy and Tipton 2007). The significant decrease in serum iron can be contributed to anemia and excess utilization of iron in regulating hematopoietic process (Hussein et al., 1997).

MDA level showed extremely statistically significant increase in infected horses compared to control horses, MDA is the product of lipid peroxidation (Esterbauer, 1996). Nitric oxide production increased during equine babesiosis (Deger et al., 2009; Saleh 2009). There were several reports demonstrating the rise in MDA with other babesial agents (Kumar et al., 2006; Crongaj et al., 2010; Esmaeilnejad et al., 2012). This augment in lipid peroxidation in equine may be marker of oxidative stress and of erythrocyte injury caused by babesiosis (Deger et al., 2009). 'Murase et al., (1996) attributed the damage in non-parasitized erythrocytes to oxidative stress exposure during B. gibsoni infection. The inversely proportional between erythrocyte MDA and PCV percentages suggesting the augment in lipid peroxidation may be one of the factors implicated in the decrease of PCV values and consequently the anemia (Esmaeilnejad et al., 2012).

In conclusion, high level of MDA along with reduction in PCV, HB, and RBCs count is indicating the presence of oxidative stress and implicating the process as a cause of anemia in T. equi infection. The mineral status (Cu, Zn, Fe) appeared to be directly affected by the parasite and the mineral status influenced by the anemic syndrome associated with T. equi. Inversely correlation between zinc and MDA level might be used in planning the treatment strategy of T. equi.

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