Hemato-Biochemical Alterations in Water Buffaloes Clinically Infected with Bovine Theileriosis Before and After Treatment by Buparvaquone

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Abstract
Most of researches about theileriosis have been done about its effect in cows and derelict it in water buffaloes. So this investigation is aimed to study the hematological and serum constituents alterations in buffaloes affected with bovine theileriosis in Egypt. A total of thirty six buffaloes about 3-5 years old were examined in this study. Twenty six were naturally infected by theileria. Another ten buffaloes of the same age and apparent healthy were kept as control. The infected buffaloes were treated by buparvaquone (50 mg/20 kg B.W). Blood were taken from the healthy, infected non treated buffaloes and then post one and two weeks from buparvaquone administration. The affected buffaloes revealed high fever, corneal opacity, lacrimation, enlargement of the prescapular lymph node. Blood smears confirm presence of piroplasms of T. annulata in red blood corpuscles. Hematological analysis showed a significant reduction in erythrocytes count (RBCs), packed cell volume (PCV) and hemoglobin concentration (Hb) in non treated buffaloes infected by theileria. Normocytic normochromic anemia was reported rapidly with the first appearance of signs. Latterly, macrocytic hypochromic anemia. Total leucocyte count significantly increased soon after infection and persists until the first week post buparvaquone treatment. Theileria infected non treated buffaloes revealed an increase in the serum activities of aminotransferase (AST and ALT). Also serum levels of total bilirubin, unconjugated bilirubin (UB), conjugated bilirubin (CB), creatinine and blood urea nitrogen level were increased. In the opposite, side, the total serum proteins and albumin levels significantly decreased. Significant increase in malonyldialdehyde (MDA) activity was recorded with a significant decease in the catalase activity (CAT) in T. annulata clinically infected buffaloes. These alterations were improved by treatment of the diseased buffaloes by buparvaquone two weeks post administration.

Keywords: biochemistry, buffaloes, Buparvaquone, hematology, theileriosis

INTRODUCTION
Blood parasites represent a major health problem in cattle breeding due to severe economic losses. That, lead to a decrease of animal production and an increase both of susceptibility to other infections and mortalities (Miodrag et al., 2012). Theileriosis is transmitted by vector ticks of the genus Hyalomma, so it is one of a tick borne disease of cattle (Glass et al., 2003). It is called tropical theileriosis or Mediterranean or even Egyptian fever. It is caused by Theileria annulata (T. annulata). It spread from the Mediterranean and Middle East area, from Morocco to Western parts of India and China (Razmi et al., 2003). T. annulata is one of the most destructive blood parasite affecting cattle, buffaloes, and even sheep.

Early diagnosis of T. annulata infection in bovines is mostly based on clinical signs as well as the detection of piroplasms in red blood corpuscles in Giemsa stained blood smears (Kaufmann, 1996). Early therapeutic approach can prevent the high mortality rates (Modi and Bhadesiya, 2014).

Basic control of tropical theileriosis depends on acaricides and chemotherapy (Tait and Hall,
Buparvaquone is currently the most specific used chemotherapeutic treatment against theileriosis from 1980 till now (Müller et al., 2015). Many studies have been done about theileriosis in cows and derelict its effect in water buffaloes, so the present study is to estimate the effects of bovine theileriosis in buffaloes and the efficacy of buparvaquone as anti theilerial drug. The evaluation will be done by hematological, biochemical and antioxidant examination.

**MATERIALS AND METHODS**

**Animal**

The present study was carried out on twenty six clinically diseased buffaloes showed clinical symptoms of theileriosis and confirmed by detection of intraerythrocytic stages of the hemoparasite and another ten clinically have been used as apparent healthy buffaloes.

**Treatment of infected animals:**

The diseased buffaloes were treated with BUTAJECT, (Adwia Pharmaceuticals, Egypt 50 mg buparvaquone /ml) by recommended dose 50 mg/20 kg body weight by intramuscular in the neck region.

**Blood film:**

Thin blood smears from the ear vein of diseased buffaloes. The smears were dried off then fixed by methyl alcohol and stained with commercial Giemsa stain (Coles, 1986). They have been be examined under oil immersion lens of microscope at (100X) according to (Kaufmann, 1996). Examination of blood smear for theileria was done by cross-section method to give representative examination according to (Ali and Radwan, 2011).

**Samples:**

Blood sample was collected from jugular vein of each buffaloo and divided into two portions. The first portion of the blood was collected in EDTA tubes for hematological parameters. The second was collected without anticoagulant for serum separation for biochemical parameters analysis. Samples were taken from the healthy, infected non treated buffaloes and then post one and two weeks from buparvaquone administration.

**Hematological studies:**

Blood samples for hematological examination were sent to laboratory within two hours for determination of (RBCs, Hb, PCV, MCV, MCHC, MCH and WBCs) using full automatic digital cell counter (Hospitex Hemascrreen 18, Italy).

**Serum biochemical studies:**

The serum was separated by centrifugation at 3000 rpm for 25 min and stored at −20°C until used, then have been tested spectrophotometrically for the biochemical parameters. Total proteins, albumin and globulins were measured (Doumas et al., 1981; Drupert, 1974; Coles, 1986) respectively. Activities of serum aminotransferases (ALT and AST) were determined (Reitman and Frankel, 1957), while serum creatinine and blood urea nitrogen levels were estimated; (Putton, 1977, Determination of serum blood urea nitrogen) (Putton and Crouch, 1977). Serum reduced GSH was determined according to the method of Beutler et al. (1963). While, malonyldialdehyde (MAD) was determined according to the method of Ohkawa et al. (1979). Catalase (CAT) was determined by the method of Aebi (1984).

**Statistical analysis:**

By using SPSS version 8 for windows, the obtained data were statistically analyzed. One way analysis of variance (ANOVA) have been applied (Tamhane and Dunlop, 2000). Means at the same rows followed by distinct letters were significantly distinct. The letter (a) was representing the highest value.

**RESULTS AND DISCUSSION**

Mediterranean coast fever or tropical theileriosis caused by T. annulata. It is one of the most important diseases of cattle in Egypt, which lead to severe economic losses among them (Abou-El-Naga et al., 2005). The most obvious clinical symptoms on theileria infected buffaloes were fever (>40°C), anorexia, enlargement of lymph nodes, corneal opacity and lacrimation (Fig. 1 and 2). These clinical signs are similar to those reported in theileria infected cows (AL-Hosary et al., 2015). Blood smears revealed presence of theileria trophonyte (Fig. 3) in side erythrocytes in the present study.

Results of erythrogram (Tab. 1) revealed that theileria infected non treated buffaloes showed a significant decrease in erythrocytes count, packed cell volume and hemoglobin when compared with the healthy control. This reduction may probably because of severe damage caused by the organisms inside the erythrocyte during their multiplication (Ganguly et al., 2015).
obtained data of the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) were classified the produced anemia as a normocytic normochromic in infected non treated buffaloes. Macrocytic hypochromic anemia was pronounced in the buparvaquone treated buffaloes one and two weeks post buparvaquone administration. This picture may be a signs of regenerative anemia (Coles, 1986). Mean corpuscular hemoglobin (MCH) revealed a significant increase in the theileria infected non treated buffaloes compared with the healthy control that confirm hemolytic condition (Feldman et al., 2000). The erythrogram returned toward the normal values two weeks post buparvaquone administration. This indicated that the treated buffaloes start the resumption to their normal status. The recuperation could be due to the helps of buparvaquone in elimination of both piroplasmic and lymphocytic stages of the protozoan parasites from both blood and lymph nodes (AL-Hosary et al., 2015).

Leucogram (Tab. 2) revealed leukocytosis in the theileria infected non treated buffaloes which is entirely due to lymphocytes proliferation in the lymphoid organs and granulocyte as a defensive response to invading protozoans (Modi et al., 2015). Also the lymphocytosis may be due to that the intra-lymphocytic theilerial parasites stimulate the host cells, leading to growth of lymphocytes (Yamaguchi et al., 2010). The same results were obtained by (Stockham et al., 2000).

**Fig. 1.** Water buffalo showing enlargement of prescapular lymph node  
**Fig. 2.** Water buffalo showing corneal opacity and watery lacrimation  
**Fig. 3.** Showing intra erythrocytic trophozoite of *Theileria annulata*. 
On the contrary, (Omer et al., 2002) who reported a pan leucopenia in pure bred cattle infected with theileriosis in Saudi Arabia. Similar findings were obtained by (Ghanem et al., 2013) who reported that Egyptian water buffaloes clinically diagnosed as theileriosis showed a reduction in the total leucocyte counts. This variance in the leucogram may be due to the different infection stages in the different investigations, since within an acute phase of infection; there is a wave of leucocytic response, which gradually drop as the infection becomes chronic.

Disturbances in the liver and kidneys functions (Tab. 3 and 4) demonstrated a significant increase in the serum activities of aminotransferases (AST and ALT) in the theileria infected non treated buffaloes.

**Tab. 1.** Erythrogram in the normal buffaloes and clinically infected with *T. annulata* (mean values ± SE).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy buffaloes</td>
<td>Theileria infected buffaloes</td>
</tr>
<tr>
<td></td>
<td>Non treated</td>
<td>1 week post buparvaquone administration</td>
</tr>
<tr>
<td>RBCs (x 10^6/µl)</td>
<td>8.15±0.11</td>
<td>6.44±0.05</td>
</tr>
<tr>
<td>PCV %</td>
<td>40.04±0.49</td>
<td>32.04c±0.30</td>
</tr>
<tr>
<td>Hb gm/dl</td>
<td>13.56±0.02</td>
<td>11.76±0.10</td>
</tr>
<tr>
<td>MCV %</td>
<td>49.12±0.19</td>
<td>49.75±0.16</td>
</tr>
<tr>
<td>MCHC %</td>
<td>33.92±0.45</td>
<td>33.87±0.34</td>
</tr>
<tr>
<td>MCH pg</td>
<td>16.66±0.24</td>
<td>18.24±0.24</td>
</tr>
</tbody>
</table>

Means at the same row followed by distinct letters were significantly distinct and the letter *a* was representing to the highest value.

**Highly significant at P≤0.01
*significant at P≤0.05
RBC = red blood corpuscles
Hb = hemoglobin
PCV = packed cell volume
MCH = mean corpuscular hemoglobin
MCV = mean corpuscular volume
MCHC = mean corpuscular hemoglobin concentration

**Tab. 2.** Leucogram in the normal buffaloes and clinically infected with *T. annulata* (mean values ± SE).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy buffaloes</td>
<td>Theileria infected buffaloes</td>
</tr>
<tr>
<td></td>
<td>Non treated</td>
<td>1 week post buparvaquone administration</td>
</tr>
<tr>
<td>WBC (x 10^3/µl)</td>
<td>8.44±0.05</td>
<td>10.02±0.18</td>
</tr>
<tr>
<td>LYM (x 10^3/µl)</td>
<td>5.00±0.16</td>
<td>5.66±0.20</td>
</tr>
<tr>
<td>MID (x 10^3/µl)</td>
<td>1.94±0.02</td>
<td>1.04±0.03</td>
</tr>
<tr>
<td>GRA (x10^3/µl)</td>
<td>1.50±0.13</td>
<td>3.30±0.11</td>
</tr>
</tbody>
</table>

Means at the same row followed by distinct letters were significantly distinct and the letter *a* was representing to the highest value.

**Highly significant at P≤0.01
*significant at P≤0.05
WBC = White blood cells
LYM = lymphocytes
GRA = neutrophils, eosinophil's and basophil's
MID = monocytes and some eosinophil's
Also, serum levels of total bilirubin, unconjugated bilirubin (UB), conjugated bilirubin (CB), creatinine and blood urea nitrogen level showed a significant increase. In contrary, total serum proteins and albumin levels showed significant decrease. These changes probably indicated an inflammatory changes in hepatic and glomerular cells that in turn affected their functions (Abou-El-Naga et al., 2005). These results are in agreement with Sandhu et al. (1998) who reported a hepatic and renal damages in liver and kidneys of calves infected T. annulata that appeared in macroscopic and microscopic evaluation. These results were returned to the normal level two weeks post buparvaquone administration compared to the apparent healthy values.

Antioxidants play complementary roles in many aspects of pathogenesis of T. annulata infection. The results presented in Tab. 5 revealed a significant increase in malonyldialdehyde (MDA) activity and a significant decrease in catalase activity (CAT) in theileria infected non treated buffaloes compared with healthy one. Reduction of CAT activity may be attributed to the reduction of antioxidant enzymes as they are used by excessive free radicals in the infected buffaloes (Hassanpour et al., 2013). The data obtained in the present study, regarding MDA levels were correlated with

### Table 3

**Total proteins, albumin and globulin levels in the normal buffaloes and clinically infected with T. annulata (mean values ± SE).**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Healthy buffaloes</th>
<th>Theileria infected buffaloes</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non treated</td>
<td>1 week post buparvaquone administration</td>
<td>2 week post buparvaquone administration</td>
</tr>
<tr>
<td>Total proteins (g/dl)</td>
<td></td>
<td>7.02 ± 0.05</td>
<td>6.07 ± 0.13</td>
<td>6.39 ± 0.11</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td></td>
<td>3.80 ± 0.10</td>
<td>2.97 ± 0.03</td>
<td>3.18 ± 0.10</td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td></td>
<td>3.22 ± 0.08</td>
<td>3.10 ± 0.09</td>
<td>3.21 ± 0.05</td>
</tr>
</tbody>
</table>

Means at the same row followed by distinct letters were significantly distinct and the letter a was representing to the highest value.

** Highly significant at P≤0.01

NS = non significant

### Table 4

**Some biochemical parameters in the normal buffaloes and clinically infected with T. annulata (mean values ± SE).**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Healthy buffaloes</th>
<th>Theileria infected buffaloes</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non treated</td>
<td>1 week post buparvaquone administration</td>
<td>2 week post buparvaquone administration</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td></td>
<td>41.81 ± 0.63</td>
<td>85.79 ± 1.23</td>
<td>74.33 ± 1.59</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td></td>
<td>17.25 ± 0.80</td>
<td>57.24 ± 1.04</td>
<td>47.14 ± 1.96</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td></td>
<td>0.37 ± 0.05</td>
<td>1.06 ± 0.06</td>
<td>0.87 ± 0.08</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dl)</td>
<td></td>
<td>0.15 ± 0.02</td>
<td>0.36 ± 0.01</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td>Indirect bilirubin (mg/dl)</td>
<td></td>
<td>0.22 ± 0.07</td>
<td>0.70 ± 0.06</td>
<td>0.57 ± 0.03</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td></td>
<td>0.53 ± 0.02</td>
<td>1.49 ± 0.09</td>
<td>1.46 ± 0.06</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td></td>
<td>16.2 ± 1.04</td>
<td>20.23 ± 0.63</td>
<td>20.11 ± 0.68</td>
</tr>
</tbody>
</table>

Means at the same row followed by distinct letters were significantly distinct and the letter a was representing to the highest value.

** Highly significant at P≤0.01 probabiliites

ALT = alanine aminotransferase

AST = aspartate aminotransferase
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Tab. 5. Catalase and malondialdehyde levels in the normal buffaloes and clinically infected with *T. annulata* (mean values ± SE).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy buffaloes</th>
<th>Theileria infected buffaloes</th>
<th>F test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non treated</td>
<td>1 week post buparvaquone administration</td>
<td>2 week post buparvaquone administration</td>
</tr>
<tr>
<td>Catalase (U/L)</td>
<td>2.61±0.07</td>
<td>1.49±0.11</td>
<td>1.79±0.07</td>
</tr>
<tr>
<td>Malondialdehyde (nmol/ml)</td>
<td>0.87±0.06</td>
<td>1.46±0.17</td>
<td>1.29±0.12</td>
</tr>
</tbody>
</table>

Means at the same row followed by distinct letters were significantly distinct and the letter ** was representing to the highest value.

****: Highly significant at 0.01 probabilities

those presented by Grewal et al. (2005). MDA level indicated that lipid peroxidation in erythrocytes of affected buffaloes was significantly higher than those of apparent healthy. After treatment by buparvaquone, these results were completely varied, the level of MDA has been significantly decreased and a significant increase in the activity of CAT has been recorded.

**CONCLUSION**

It could be concluded that *T. annulata* infected non treated buffaloes showed adverse alterations on the hematological profile, biochemical parameters of liver, kidneys and oxidative stress. These alterations were significantly improved two weeks after buparvaquone administration.

**CONFLICT OF INTEREST:** not exist.

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**REFERENCES**


