HEMATOLOGICAL AND BIOCHEMICAL INVESTIGATION IN BOVINE BABESIOSIS AND THEILERIOSIS
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ABSTRACT
Fifteen out of 75 native cattle 5-7 years old from different localities at Sharkia Provence were used to study hematological changes in Babesia and Theileria infestation verified with the clinical signs during period from May 2009 to the end of April 2010. Animals were allotted into three groups (n=5/each), 1st group were healthy cattle free from blood parasite (control), 2nd group were suffering from babesiosis and 3rd group were suffering from theileriosis. Clinical examination included body temperature, rate of respiration and ruminal movement. Two blood samples were collected from each animals for determination of erythrogram and leukogram changes in whole blood and some biochemical parameters in separated sera. The results revealed that body temperature and respiration rate were significantly increased in cattle suffering from babesiosis and theileriosis while ruminal movement were significantly decreased. Hematological picture revealed a significant reduction in RBCs, HB and PCV in cattle infected with either babesiosis or theileriosis. Babesiosis induce significant reduction in MCV, MCH and MCHC (microcytic hypochromic anaemia) while theileriosis induced increase in MCV and decrease in MCH and MCHC (macrocytic hypo-chromic anaemia). Bovine infected with babesiosis and theileriosis revealed leukopenia, neutropenia and eosinopenia accompanied with lymphocytosis, monocytosis and basophilia compared to that of control group. The result of biochemicaly analysis showed that cattle infected with babesiosis and theileriosis revealed significant reduction in total protein, albumin, globulin, cholesterol, Triglycerides and glucose levels associated with significant increase in the activities of liver enzymes (AST, ALT, alkaline phosphatase) and total bilirubin. It could be concluded clinical signs namely red brown to coffee urine (in babesiosis), enlargement of superficial lymph nodes, corneal opacity and macrocytic hypochromic anaemia (in theileriosis) in addition to hematological picture (microcytic and macrocytic hypochromic anaemia in babiosis and theilorosis, respectively) could be useful for easily diagnosis of blood parasites

KEY WORDS: Biochemical, Blood Parasite, Hematological, Theileriosis

INTRODUCTION
Parasitic infection as blood parasite constitute a major problem in cattle breeding due to severe economic losses and lowered vitality which lead to decrease of animal production and increase the susceptibility to bacterial and viral infections [16]. The main clinical signs in the animals infected with blood parasite were fever (41.0–41.5°C), congested mucous membranes, anorexia, nasal discharge, lacrimation, haemoglobinuria, anemia, respiratory distress and eye lesions beside presence of ticks on the animal body [6]. Theileriosis and babesiosis cause severe economic losses and have effect on the immune status of the body [57]. Babesiosis is a tick-borne hemolytic disease of domestic and wild mammals caused by intraerythrocytic protozoan...
parasites of the genus Babesia [15]. Babesiosis is endemic in tropical and subtropical regions as Egypt [14]. Babesiosis is initiated by tick-borne transmission of the sporozoites, which subsequently invade host red blood cells in the infected animals [62]. It is mainly transovarian transmitted by ticks [34]. Theileriosis is an important parasitic disease of animal in Egypt which causes great economic losses in animals and their products. Theileria species is a protozoan parasite of cattle and domestic buffaloes, transmitted by ticks of genus Hyalomma and causes a disease named theileriosis [36]. Theileriosis have high morbidity and mortality rate in cattle [9]. Diagnosis of blood parasite in acute cases is mainly based on clinical signs of the infected animals which confirmed by microscopic examination of a thin blood film stained with Giemsa stain [53]. However, expertise in piroplasm microscopy is required in subclinical or chronic infections because parasitaemias are often extremely low and Theileria piroplasms may be difficult to be found in stained blood smears or otherwise may be missed [2]. For these reasons, the present study was planned to determine hematological picture and with protein profile, to confirm clinical signs for clinical diagnosis of babesiosis and theileriosis.

2. MATERIALS AND METHODS

2.1. Animals
The study was conducted on 75 native cattle of 5-7 years old from different localities at Sharkia Provence in the period from May 2009 to the end of April 2010.

2.2. Blood smears
Blood smears were prepared from ear vein, left to dry, fixed in methanol and stained by freshly prepared Geimsa stain for 45 minutes to look for parasites [53].

2.3. Experimental Design:
Fifteen cattle out of seventy five native cattle which divided into three equal groups, 1st group healthy cattle as control, 2nd group cattle suffering from babesiosis and 3rd group cattle suffering from theileriosis. Clinical examination body temperature, rate of respiration and ruminal movement were done. Two blood samples were taken from each animal by jugular vein puncture, 1st sample was taken in tub contain EDTA for determination of erythrogram, leukogram and blood indices (Mean Corpuscular Volume, Mean corpuscular hemoglobin and Mean Corpuscular Haemoglobin Concentration) according to (26), meanwhile 2nd sample was taken in centrifuge tube to obtain clear serum for estimation of total proteins [12], albumin [13] and globulin mathematically, transaminases (AST, ALT) [47], alkaline phosphatase (AST), glucose [54], cholesterol [46] and triglycerides [48].

2.4. Statistical analysis:
The obtained data were statistically analyzed according to [42].

3. RESULTS AND DISCUSSION

Clinical investigations (table 1&2) revealed that cattle suffering from babesiosis were showed clinical symptoms, including fever, anorexia, congested mucous membranes, with presence of tick on the animal body and presence of hemoglobinuria in cattle suffering. Same clinical signs were reported previously [18] where cattle suffering from babesiosis show
fever, anorexia, congested mucous membranes and dark brown to coffee urine and this brown urine may be due to severe haemolytic process associated with the presence of Babesia within red blood cells. Cattle infected with theileriosis showed enlargement of the superficial lymph nodes beside anorexia, congested mucous membranes. Clinical observation of the cattle infected with theileria species were in agreement with the findings of other studies [40, 59] who stated that the main clinical signs in buffaloes infected with Theileria were pyrexia (40.5–41.5°C), slight nasal discharges, enlargement of superficial lymph nodes, salivation, decreased milk yield, and respiratory distress.

The results of the hematological investigation in cattle suffering from babesiosis and theileriosis revealed significant reduction in erythrocyte count, haemoglobin content, packed cell volume%. Microcytic hypochromic anaemia was occurred to significant reduction in MCV and MCHC in case of babesiosis, while in theileriosis there was macrocytic hypochromic anemia, increase in MCV and decrease in MCHC table (3). These results were identical to that recorded formerly [10] in cattle suffering from babesiosis. Changes in erythrocytegram picture might be due to destructive effect of the parasites on erythrocytes [44, 58] in cattle infected with babesiosis. Also, Homer et al. [24] mentioned that protozoan intraerythrocytic parasites induced lyses of the infected RBC, which resulting in severe clinical symptoms, such as anemia, fever, and hematuria. Microcytic hypochromic anaemia observed in cattle infected with babesiosis could be attributed to intravascular haemolysis of red blood cells in this field [41]. Besides, theileriosis induced general anemia with fall in total erythrocyte count, packed cell volume% and hemoglobin content [17, 49].

### Table 1 Incidence of Babesia and Theileria infection in cattle in different localities at Sharkia Province based on blood film examination.

<table>
<thead>
<tr>
<th>City</th>
<th>Total number</th>
<th>Healthy No</th>
<th>Healthy %</th>
<th>Diseased No</th>
<th>Diseased %</th>
<th>Babesiosis</th>
<th>Theileriosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abu-Kabeer</td>
<td>12</td>
<td>10</td>
<td>83.33</td>
<td>2</td>
<td>16.67</td>
<td>2</td>
<td>16.67</td>
</tr>
<tr>
<td>Kafr-Sakr</td>
<td>10</td>
<td>9</td>
<td>90</td>
<td>1</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Menia-Ikamh</td>
<td>13</td>
<td>9</td>
<td>69.23</td>
<td>4</td>
<td>30.77</td>
<td>1</td>
<td>7.69</td>
</tr>
<tr>
<td>Abo-Hamad</td>
<td>14</td>
<td>12</td>
<td>85.71</td>
<td>2</td>
<td>14.28</td>
<td>1</td>
<td>7.14</td>
</tr>
<tr>
<td>Ibrahimia</td>
<td>15</td>
<td>14</td>
<td>93.33</td>
<td>1</td>
<td>6.67</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Blibas</td>
<td>11</td>
<td>9</td>
<td>81.82</td>
<td>2</td>
<td>18.18</td>
<td>1</td>
<td>9.09</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>63</td>
<td>84%</td>
<td>12</td>
<td>16%</td>
<td>5</td>
<td>6.67</td>
</tr>
</tbody>
</table>

### Table 2 Clinical examination of animals (Body temperature, respiratory rates & ruminal movement of healthy and diseased cattle (n= 5)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy cattle</th>
<th>Diseased Babesiosis</th>
<th>Diseased Thelariosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature (°C)</td>
<td>38.6±0.62</td>
<td>40.54±0.52*</td>
<td>40.90 ± 0.55*</td>
</tr>
<tr>
<td>Respiratory rate/min</td>
<td>22.17±1.93</td>
<td>32.20±2.04**</td>
<td>31.19±1.96**</td>
</tr>
<tr>
<td>Ruminal contraction/2min</td>
<td>4.39±0.25</td>
<td>2.52±0.41**</td>
<td>2.79±0.56*</td>
</tr>
</tbody>
</table>

* = P< 0.05 and ** = P < 0.01
Our observations were in accordance with those documented previously [1, 33, 45] stated that Theileria induce significant decrease in packed cell volume, total erythrocytic count and hemoglobin content. Besides, theileria in calves induced macrocytic hypochromic anaemia [52]. This might be attributed to the toxic metabolites of theileria species which have harmful effect on bone marrow and interfere with the process of erythropoiesis [21]. In the present study, it has been shown that bovine babesiosis and theileriosis evoked leukopenia, neturopenia and eosinopenia accompanied with lymphocytosis, monocytosis and basophilia compared to that of control group (Table, 4). Also, there was significant increase in monocytes in Babesia infection due to their role as active mediators in the innate immune response [11]. Formerly it was mentioned that babesia disintegrates the leukocytes resulting in leukopenia [29, 37]. Our results were in accordance with that recorded previously [15] mentioned that monocytosis in animals infected with babesiosis species occurred as a mean of body defense against infection. The increased number of lymphocytes in our result was previously reported [8]. Similar findings were reported formerly [38, 40] as a significant increase in lymphocyte counts was fund in buffaloes with theleriosis. In this field, former authors [35, 52] mentioned that theileria infection induced significant decrease in leukocytic count. This finding was supported by earlier observations [43, 51] found that leukopenia, neturopenia and eosinopenia accompanied with lymphocytosis, monocytosis and basophilia in cattle infected with Theileria. The obtained data were in accordance with those previously obtained [50] who mentioned that in cattle infected with theleriosis induced significant elevation in lymphocytes and decrease in total leukocytic count. Benjamine [8] postulated that the lymphocytosis was marked during the formation of antibodies in response to antigens and also during theleria infection.

The obtained of biochemical analysis reported in the table (5) showed significant decrease in serum total protein, albumin and globulin levels while A/G ratio was insignificantly decreased in cattle infected with babesiosis or theileriosis .These results were in consistence with that illustrated in buffaloes[3, 37]. Earlier studies [19, 31] stated that babesiosis in dogs induced hypoalbuminemia.

### Table 3 Erythrogram of healthy and diseased cattle (n=5)

<table>
<thead>
<tr>
<th>Groups</th>
<th>R.B.C.(10^6/mm³)</th>
<th>H.B (gm %)</th>
<th>P. C.V. (%)</th>
<th>MCV (fl)</th>
<th>MCH (Pg)</th>
<th>MCHC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.06± 0.89</td>
<td>13.22±1.18</td>
<td>34.72±113</td>
<td>43.08±0.44</td>
<td>16.40±0.66</td>
<td>38.0±0.42</td>
</tr>
<tr>
<td>Babesiosis</td>
<td>4.52± 0.92</td>
<td>9.09 ± 1.34</td>
<td>27.17±2.18</td>
<td>40.20±0.51**</td>
<td>13.47±0.59</td>
<td>33.51±0.83**</td>
</tr>
<tr>
<td>Theilariosis</td>
<td>5.47±0.61</td>
<td>10.18±1.22</td>
<td>28.61±0.33</td>
<td>56.16±2.94**</td>
<td>14.95±0.17</td>
<td>26.6±1.48**</td>
</tr>
</tbody>
</table>

* = P < 0.05 and ** = P < 0.01.

### Table 4 Erythrogram of healthy and diseased cattle (n=5)

<table>
<thead>
<tr>
<th>Groups</th>
<th>R.B.C.(10^6/mm³)</th>
<th>H.B (gm %)</th>
<th>P. C.V. (%)</th>
<th>MCV (fl)</th>
<th>MCH (Pg)</th>
<th>MCHC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.27±0.17</td>
<td>3.12±0.22</td>
<td>3.23±0.21</td>
<td>1.32±0.10</td>
<td>1.65±0.15</td>
<td>0.95±0.12</td>
</tr>
<tr>
<td>Babesiosis</td>
<td>9.75±0.16</td>
<td>1.93±0.35</td>
<td>3.88±0.19</td>
<td>1.80±0.17**</td>
<td>0.90±0.26*</td>
<td>1.24±0.03*</td>
</tr>
<tr>
<td>Theilariosis</td>
<td>9.70±0.19</td>
<td>1.59±0.42**</td>
<td>3.90±0.20**</td>
<td>1.64±0.12*</td>
<td>1.19±0.13*</td>
<td>1.33±0.10*</td>
</tr>
</tbody>
</table>

* = P < 0.05 and ** = P < 0.01.
The changes in the protein picture in animals suffering from babesiosis and theileriosis could be due to decrease protein production as a result of deprivation of diet protein resulting from anorexia and fever accompanied Babesia species and Theileria species infection [28]. The decrease in serum total protein in cattle infected with Babesia species or Theileria species might be due to decrease in albumin and globulin as a result of liver insufficiency [30]. Similarly, former studies [22, 39] recorded that Babesiosis and theileriosis has harmful effects on the hepatocytes leading to disturbed hepatic functions as decreased synthesis of albumin accompanied by an increase in albumin catabolism [38]. Also, decrease in protein picture might be due to distracted RBCs and its excretion in urine as albuminuria occurs during disease [23]. Our results revealed significant increase in the liver enzymes (AST–ALT and alkaline phosphates) and total bilirubin in cattle suffering from babesiosis or thalariosis as presented in table (6). These results were supported by that obtained before [5, 61, 56]. Our Also, babesiosis in dogs was found to induce a significant increase in serum activity of AST and ALT [19]. Also, Singh et al. [55] mentioned that liver enzymes (AST, ALT and alkaline phosphates) and total bilirubin were significantly increased in calves infected with theileriosis. On other hand [7] stated that the elevation in liver enzymes in babesiosis may be due to the hepatic damage and lesions induced by the parasite during its multiplication in the blood followed by disturbed liver function. Elevated liver enzymes could be due to necrosis and lysis of the erythrocytes or hyperbilirubinemia during Babesia infection [31, 32]. The obtained results showed significant decrease in cholesterol, triglyceride and glucose in cattle suffering from babesiosis and theileriosis (Table, 7). Calve and adult infected with Theileria species had a lower levels of glucose, triglyceride and cholesterol [20, 25, 60], which might be attributed to partial anorexia and metabolic disturbances of the diseased animals [41]. It was concluded that diagnosis of both babesiosis and theileriosis could be easily reached as babesiosis associated with red brown to coffee urine and microcytic hypochromic anaemia, while theileriosis is associated with enlargement of the superficial lymph nodes, corneal opacity and macrocytic hypochromic anemia.

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Proteins profile and Liver enzymes of healthy and diseased cattle (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Total proteins (g/ml)</td>
</tr>
<tr>
<td>Control</td>
<td>7.64±0.51</td>
</tr>
<tr>
<td>Babesiosis</td>
<td>5.82±0.24&quot;</td>
</tr>
<tr>
<td>Theileriosis</td>
<td>5.58±0.33&quot;</td>
</tr>
</tbody>
</table>

P < 0.05 and "P < 0.01

<table>
<thead>
<tr>
<th>Table 6</th>
<th>Liver function of healthy and diseased cattle (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>ALP (U/L)</td>
</tr>
<tr>
<td>Control</td>
<td>61.32±3.14</td>
</tr>
<tr>
<td>Babesiosis</td>
<td>76.13±4.81&quot;</td>
</tr>
<tr>
<td>Theileriosis</td>
<td>73.71±3.54&quot;</td>
</tr>
</tbody>
</table>

*P < 0.05 and "P < 0.01
Table 7 Lipogram and glucose of healthy and diseased cattle (n=5)

<table>
<thead>
<tr>
<th>Group</th>
<th>Cholesterol (mg/dl)</th>
<th>Triacylglycerid (mg/dl)</th>
<th>Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>119.84±1.42</td>
<td>63.12 ± 2.67</td>
<td>65.08±3.04</td>
</tr>
<tr>
<td>Babesiosis</td>
<td>104.34±0194**</td>
<td>47.39 ± 1.92**</td>
<td>48.53±1.92**</td>
</tr>
<tr>
<td>Theileriosis</td>
<td>107.40 ±1.09**</td>
<td>51.45 ±2.81*</td>
<td>50.17±1.83**</td>
</tr>
</tbody>
</table>

*P < 0.05 and **P < 0.01

4. REFERENCES

Investigations of bovin babesiosis and theileriosis

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Investigations of bovine babesiosis and theileriosis

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المتخصصة العربي

استهدف هذه الدراسة محولة تشخيص البابيزيوس والثاليزيوس من خلال الأعراض الظاهرة، صورة الدم، صورة البروتينات، الكلى، الزال، الجلوبويون، ووظائف الكبد في الابقار المصابة (عدد 75 بقرة) في امكاني مطرح مراقبة التربة للمعالجة في العلاج المبكر. تم أخذ عينات الدم من وريد الادن من كل الحيوانات على خلاص طيفي لل chíن الديدن الديدن المصبوغ بديسما. إضافة إلى قياس البروتينات، الكلي، الزال، الجلوبويون، ووظائف الكبد في مصل الدم. تم تحديد 15 بقرة منها عشر بقرات مصابة بطفيميات الدم تراوح أعمارها من 5-7 سنوات قسمت إلى ثلاث مجموعات متساوية كل منها تضم خمس بقرات. المجموعة الأولى أيفر استخدام من بقايا مصابة بالبابيزيوس (كانت جزءًا من اعراض مرتبة تتمثل في فقدان الشهيه وارتفاع درجة الحرارة واحتقان الأعوجل الخارجية للعين ورود بول من معدة). المجموعة الثانية أيفر مصابة بالثاليزيوس (كانت جزءًا من اعراض مرتبة تتمثل في ارتفاع درجة الحرارة وفروق انفية وعيناً والأنف واحتقان الرئة وحالات أخرى كانت عبارة عن شحوب وانتشار الأعوجل الخارجية للعين) للمعالجة. أشار النتائج إلى أن الإصابة بالبابيزيوس والثاليزيوس في الابقار أدت إلى حدوث نقص معنوي في عدد كرات الدم الحمراء، تركز في خلايا الدم في الحيوانات. المجموعة الثالثة أيفر مصابة بالثاليزيوس (كانت جزءًا من أعراض مرتبة تتمثل في ارتفاع درجة الحرارة وفروق انفية وعيناً والأنف واحتقان الرئة) للمعالجة. رغم أن الإصابة بالبابيزيوس والثاليزيوس تسبب في حدوث نقص معنوي في عدد كرات الدم الحمراء، إلا أن الالتباس بالخلايا المعدة العلاجية المختلفة بما حدث زيادة معنوية في الخلايا الحمراء في كل الحيوانات. النتائج بعد التعافي من الإصابة بالبابيزيوس والثاليزيوس أظهرت نقص معنوي ملحوظ في قيم كل من البروتينات، الكلي، الزال، الجلوبويون، الكلي، على الرغم من أن النتائج بعد التعافي من الإصابة بالبابيزيوس والثاليزيوس أظهرت نقص معنوي ملحوظ في قيم كل من البروتينات، الكلي، الزال، الجلوبويون، الكلي، الحمضية والكقلسيما. زيادة معنوية في انزيمات الكبد (أسيتيلامينوتراز، فيازز، اللانين، امينوتراز، فيازز، والفوسيات فازز) في الابقار المصابين بالبابيزيوس والثاليزيوس. النتائج تؤكد أن هذه الدراسة أن التغييرات في صورة الدم جنب الإعراض المرضي في الابقار قد يكون وسيلة مساعدة ومفيدة لتشخيص وتفريق بين الإصابة بطفيميات الدم (البابيزيوس والثاليزيوس).