Detection of *Theileria* spp. in blood samples and estimation of haematological and biochemical changes in sheep in Al-Diwaniya province

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Abstract:
The present study was carried out to detection the prevalence of *Theileria* spp. in sheep in Al-Diwaniya province and assess the hematological and biochemical changes occurred in sheep naturally infected with *Theileria* spp. Blood samples were collected from (132) sheep from five regions in Al-Diwaniya province. Result revealed that the overall prevalence was (26.51%). Ratios of prevalence were significant differences among different regions, the highest prevalence was (42.85%) in Al-Diwaniya while, the lowest (9.09%) in Sumar.

The clinical signs were recorded in *Theileria* -infected sheep were enlargement of prescapular lymph nodes, loss of appetite, pale mucous membranes, and congestion of mucous membranes, coughing and diarrhea. In addition, present of ticks on the various parts of infected sheep. Statistically significant increase (p<0.05) in the body temperature of diseased sheep compared with uninfected sheep. Haematological study revealed that statistically significant decreases(p<0.05) in red blood cell counts, haemoglobin concentration and packed cell volume with non-significant increase(p<0.05) in white blood cell counts and recorded significant increase(p<0.05) in the MCV and MCH values, while the MCHC value was non significant differences in the infected sheep when compared with uninfected sheep.

The biochemical study revealed that statistically significant decrease(p<0.05) were observed in serum total protein, albumin and non significant increase(p<0.05) were recorded in globulin level, alanine aminotransferase(ALT) and creatinine, with significantly increase(p<0.05) in the levels of aspartate aminotransferase(AST), cholesterol and urea in the infected sheep when compared with uninfected sheep.

Key words: *Theileria*; Sheep- protozoa; Haematological parameters; Biochemical parameters; Clinical signs; Prevalence; Al-Diwaniya province.
Theileria spp. is a tick-transmitted protozoan parasite belonging to phylum Apicomplexa (1). Theileriosis associated with Theileria spp in cattle, sheep and goats as well as in wild and captive ungulates (2). Theileria ovis was widely distributed in sheep and goats has with being present in Africa, Asia, India, and parts of Europe, caused the benign form of theileriosis (3).

Theileria separata is present only in Africa, Theileria hirici (synonym T. lestoquardi) is the more virulent form of malignant ovine theileriosis is endemic in Middle East and is probably present in regions of north Africa and parts of china(4).

In addition, a different species of Theileria (Theileria sp1.called T.luwenshuni and Theileria sp2. called T.uielenbergi) reported are a part of pathogenic parasites for small ruminants in China (5). In acute infections of malignant ovine theileriosis is characterized by fever, very high mortality in 3-6 days, anemia, jaundice, and enlargement of lymph nodes (2). Recovery form disease is often slow and subacute signs are recurrent fever and anemia (4).

Al-Abood et al. (6) recorded the infected rate of blood parasite (Theileria and Anaplasma) in sheep in Basrah was (17.5%). Theileria spp. was found in (18.29%) of sheep in the East and Southeast Anatolia in Turkey (7). The aim of the present study was to detection of Theileria spp. prevalence in sheep in the Al-Diwaniya province and assess the haematological and biochemical changes occurred in sheep naturally infected with Theileria spp. in Al-Diwaniya province.

Materials and Methods:
This study was carried out in Al-Diwaniya province during the period from January 2011 to October 2011. A total (132) of blood samples were collected from randomly selected sheep herds located in Al-Diwaniya province includes Al-Diwaniya, Afak, Al-Hamza, Al-Digara and sumar. The sheep (male and female the age range from 2-4 years old) were clinical examined for presence of superficial lymph nodes enlargement, change of appetite and examined of conjunctival and eyes mucus membranes, diarrhea, coughing and ticks present on different parts of body and rectal temperature was measuring by using a digital thermometer.
Blood samples obtained from jugular vein of sheep, about (10) ml of blood was taken from each animal, 3 ml was placed in a K2-EDTA tube used to evaluate some of blood parameters include Red blood cell count (RBC), White blood cell count(WBC), Packed cell volume(PCV), Haemoglobin concentration(Hb), indices( mean corpuscular volum(MCV), mean corpuscular haemoglobin(MCH)and mean corpuscular haemoglobin concentration MCHC) were estimated as described by (8,9) and for the preparation of blood films were fixed in absolute methyl alcohol (5min) and stained with 10% Giemsa (30min), microscopically examined under oil immersion lens (8).

Other 7ml was put into coagulant free tube to take the serum; sample was centrifuged at 3000 rpm for 10 min (9). The serum to estimated the biochemical parameters, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, were determined by Reflotron® Plus (Roche, Germany) . While other biochemical parameters include total protein, cholesterol, albumin and urea were determined by using spectrophotometer and using commercially diagnostic test kits supplied by (Spinreact, Spain, Cromatest, Spain, Fortress diagnostics, UK) following their user's manuals. Globulin was calculated by subtracting the serum albumin values from total protein values (10).

Statistical analysis: Using (SPSS) version 14.0 data were analyzed using T-test analysis. Using Chi-square (X²) test .p<0.05 was accepted to be statistically significant.

Results:

In the present study showed the overall prevalence of Theileria spp .was (26.51%) in sheep from Al-Diwaniya province. Ratios of prevalence were differ with different regions, the were significant differences at level (p<0.05) among different regions in sheep infected with Theileria.spp. the highest prevalence was (42.85%) in Al-Diwaniya while the lowest (9.09%) in Sumar (Table 1).

The clinical signs of theileriosis in sheep were loss of appetite, gross enlargement of the prescapular lymph nodes, pale mucous membranes, and congestion of mucous membranes, diarrhea, coughing and ticks were found on various parts of the body (Table 2). Statistically significant increase (p<0.05) in body temperature of Theileria-infected sheep compared with control group (Table 3). Hematological examination revealed that statistically non-significant increase (p<0.05) in WBC counts between infected sheep compared with control group. There were significant decreased in RBC counts .Hb concentration and PCV percentage in the diseased sheep compared with control group, While The MCV and MCH values were revealed significant increased (P<0.05)in the infected sheep compared with the healthy sheep. The MCHC value was non-significant difference between diseased sheep compared with the control group (Table 3).

The statistical analysis revealed that the levels of total protein and albumin were significantly decreased (p<0.05) in the infected sheep compared with the control group, While non-significant increased (p<0.05) in the level of globulin, alanine aminotransferase (ALT) and creatinine in the infected sheep compared with control group. There were significant increases (p<0.05) in the levels of aspartate aminotransferase (AST), cholesterol and urea between infected sheep and control group (Table 4).
Table (1): Prevalence of *Thieleria* spp. infection in sheep according to five regions in Al-Diwaniya province.

<table>
<thead>
<tr>
<th>N</th>
<th>Regions</th>
<th>Total number of samples</th>
<th>Positive sample</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Al-Diwaniya</td>
<td>14</td>
<td>6</td>
<td>42.85</td>
</tr>
<tr>
<td>2</td>
<td>Afak</td>
<td>29</td>
<td>4</td>
<td>13.79</td>
</tr>
<tr>
<td>3</td>
<td>Al-Hamza</td>
<td>29</td>
<td>12</td>
<td>41.37</td>
</tr>
<tr>
<td>4</td>
<td>Al-Digara</td>
<td>38</td>
<td>11</td>
<td>28.94</td>
</tr>
<tr>
<td>5</td>
<td>Sumar</td>
<td>22</td>
<td>2</td>
<td>9.09</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>132</td>
<td>35</td>
<td>26.51</td>
</tr>
</tbody>
</table>

Calculated Chi square = 11.16, Tabulated $X^2(0.05) = 9.48773$
Degree of freedom=4, P-value: 0.02482348
Significantly different (p<0.05) between this regions.

Table (2): Percentage of clinical signs in *Theileria* spp. infected sheep were recorded from total diseased number (n=35)

<table>
<thead>
<tr>
<th>N</th>
<th>The clinical signs</th>
<th>Number of infected animals</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Enlarged of the prescapular lymph nodes</td>
<td>13</td>
<td>37.14</td>
</tr>
<tr>
<td>2</td>
<td>Loss of appetite (inappetence)</td>
<td>13</td>
<td>37.14</td>
</tr>
<tr>
<td>3</td>
<td>Pale of mucous membranes</td>
<td>12</td>
<td>34.28</td>
</tr>
<tr>
<td>4</td>
<td>Congestion of mucous membranes</td>
<td>6</td>
<td>17.14</td>
</tr>
<tr>
<td>5</td>
<td>Ticks detected on different parts of body</td>
<td>18</td>
<td>51.42</td>
</tr>
<tr>
<td>6</td>
<td>Diarrhea</td>
<td>3</td>
<td>8.57</td>
</tr>
<tr>
<td>7</td>
<td>Coughing</td>
<td>2</td>
<td>5.71</td>
</tr>
</tbody>
</table>

Table (3): The Effect of infection with *Theileria. spp.* on temperature and haematological parameters in sheep.

<table>
<thead>
<tr>
<th>N</th>
<th>Groups Parameters</th>
<th>Infected group n=35</th>
<th>Control group n=97</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Temperature °C</td>
<td>39.422±0.084 B</td>
<td>38.804±0.047 A</td>
</tr>
<tr>
<td>2</td>
<td>WBC $\times 10^9$/µL</td>
<td>9.830±0.511</td>
<td>8.909±0.226</td>
</tr>
<tr>
<td>3</td>
<td>RBC $\times 10^9$/µL</td>
<td>7.181±0.227 B</td>
<td>8.986±0.136 A</td>
</tr>
<tr>
<td>4</td>
<td>HB g/dL</td>
<td>8.851±0.247 B</td>
<td>10.688±0.175 A</td>
</tr>
<tr>
<td>5</td>
<td>PCV%</td>
<td>31.825±1.789 B</td>
<td>35.945±0.583 A</td>
</tr>
<tr>
<td>6</td>
<td>MCV fl</td>
<td>45.477±3.270 B</td>
<td>40.177±0.554 A</td>
</tr>
<tr>
<td>7</td>
<td>MCH pg</td>
<td>12.405±0.233 B</td>
<td>11.923±0.096 A</td>
</tr>
<tr>
<td>8</td>
<td>MCHC g/dL</td>
<td>29.402±0.967</td>
<td>29.873±0.305</td>
</tr>
</tbody>
</table>

Values represent mean ±SE
Means within row with different letter differ significantly (p<0.05) between groups.
Table (4): The Effect of infection with *Theileria Spp.* on biochemical parameters in sheep.

<table>
<thead>
<tr>
<th>N</th>
<th>Groups</th>
<th>Infected group n=35</th>
<th>Control group n=97</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total protein g/dL</td>
<td>5.301±0.299 B</td>
<td>5.902±0.132 A</td>
</tr>
<tr>
<td>2</td>
<td>Albumin g/dL</td>
<td>3.178±0.161 B</td>
<td>3.561±0.068 A</td>
</tr>
<tr>
<td>3</td>
<td>Globulin g/dL</td>
<td>2.668±0.211</td>
<td>2.412±0.117</td>
</tr>
<tr>
<td>4</td>
<td>AST U/L</td>
<td>25.375±1.914</td>
<td>125.970±3.117 A</td>
</tr>
<tr>
<td>5</td>
<td>ALT U/L</td>
<td>0.722±0.041</td>
<td>0.656±0.029</td>
</tr>
<tr>
<td>6</td>
<td>Cholesterol mg/dL</td>
<td>143.360±8.195 B</td>
<td>111.429±5.976 B</td>
</tr>
<tr>
<td>7</td>
<td>Creatinine mg/dL</td>
<td>28.676±0.437 A</td>
<td>28.676±0.437 A</td>
</tr>
<tr>
<td>8</td>
<td>Urea mg/dL</td>
<td>42.281±2.894 B</td>
<td>42.281±2.894 B</td>
</tr>
</tbody>
</table>

Values represent mean ±SE

Means within row with different letter differ significantly (p<0.05) between groups.

**Discussion:**

The prevalence of *Theileria spp.* in sheep from Al-Diwaniya province was (26.51%). While, the infected rate of blood parasite in sheep in Basrah was (17.5%) (6). *Theileria spp.* was found in (11.9%) of sheep in south khorasan province in Iran and (18.40%) of sheep in around Kayseri (11, 12) Irshad et al. (13) reported (7.36%) prevalence of theileriosis in sheep in Pakistan.

In the five investigated areas in Al-Diwaniya province, the were significant differences among different regions in sheep infected with *Theileria spp.* the highest prevalence was (42.85%) in Al-Diwaniya while the lowest (9.09%) in Sumar. These results are in agreement with Al-Saeed et al. (14) was reported significant difference between the governorates of Kurdistan region in the *Thieleria*- infected cattle. *Thieleria spp.* can be transmitted by vector ticks (2). *Hyalomma a. anatolicum* is the most important vector for malignant ovine theileriosis (15). Dumanli et al. (16) showed the higher prevalence of tropical theileriosis in towns was found vector ticks indicate the positive correlation between the prevalence of disease and distribution of vector ticks. AL-Mahnaa (17) has shown the highest prevalence of ticks infected identified in ruminant in Al-Diwaniya city compared with other regions involved with study in Al-Diwaniya province. Al-Saeed et al. (14) was related the influenced the intensity of tropical theileriosis and infestation levels in ticks by seasonal variation, breeding and management system in any region.

The clinical signs of theileriosis in sheep were enlargement of prescapular lymph nodes, loss of appetite, pale mucous membranes, coughing, diarrhea and ticks were found on different parts of infected sheep. These clinical signs were in agreement with those obtained by (18, 19). Statistically significant increase in body temperature of *Theileria*-infected sheep compared with control group the result was in agreement with (18).

Hematological examination revealed non-significant increase (p<0.05) in WBCs between infected sheep compared with control group. Sandhu et al. (20) showed an initial non-significant leukocytosis in calves experimentally infected with *T.annulata* due to proliferation of lymphocytes in the lymphoid organs as defensive response to invading parasite. However, Al-Amiry(21) and Al-Obaidi and AlSaad (18) were showed significant increase in WBCs between *Theileria*-infected sheep compared with control group.

There were significant decreased in RBC counts, Hb concentration and PCV percentage in the diseased sheep compared with control group which confirmed
anemia in the infected sheep. These results were in agreement with other reports (6, 22, 23) Bell- Sakyi et al. (24) showed the significant lower of PCV of sheep carrying *Anplasma* and ort/Thileria than of sheep without parasite. In addition, Al-Sultan et al. (25) and Karawan (26) reported that decreased in RBC counts, Hb concentration and PCV percentage in *Theileria*-infected cattle. The pathogenesis of the anemia is not clear but occurs reasons hemolytic factor reported in the serum of acute affected cattle and may macrophages can damage red blood cells (2). In addition Nazifi et al. (23) showed that severe progressive anemia in ovine malignant theileriosis indicate increase exposure of RBCs to oxidative damage. Grewal et al. (27) indicated that *Theileria* infection led to increased oxidative stress to the animals and even increased activities of antioxidant enzymes could not decrease this oxidative stress this may be cause of elevated erythrocyte fragility due to membrane lysis and lower haemoglobin concentration.

The MCV value was revealed significant increase in infected sheep compared with the healthy sheep. Indicating macrocytic anemia. Razavi et al. (22) and Omer et al. (28) reported a significant increase in MCV value in malignant ovine theileriosis and in *T. annulata* infected cattle.

The diagnostic clinical chemistry findings are the levels of total protein and albumin were significantly decreased in the infected sheep compared with the control group. This observation is in agreement with studies were reported that a significant decrease in the levels of total protein and albumin in cattle infected with *Thieleria annulata* (29, 30). On other hand, Baghshani et al. (31) who revealed a significant decrease in albumin and non-significant decrease in total protein in sheep infected with *Theileria*. In addition, AlSaad et al. (32) who recorded a significant decrease in total protein in goat infected with *Theileria*. Singh et al. (33) attributed the decrease in total protein content of serum due to hypoalbuminaemia may be to reduced synthesis because of the effect on the liver. A similar report was showed decrease of serum total protein this may be due to decrease dietary intake, diarrhea and it decrease synthesis in the liver caused by direct or indirect effect of parasite on the liver (18). While statistically non-significant increased (p<0.05) in the level of globulin in the infected sheep compared with control group. Binta et al. (34) demonstrated remarkable hyperglobulinaemia was evident in cattle infected with *Thileria taurotragi* and *Thileria mutans* , but contradicts to the result showed significantly lower serum globulin in cattle naturally infected with *Thieleria annulata* (35).

Increased synthesis of APPs (acute phase proteins) may contribute to hyperglobulinemia that occurs in association with inflammatory responses to tissue injury and/or foreign antigens (10). In studies that described significant increase in APPs (haptoglobin, serum amyloid A, ceruloplasmin and fibrinogen) with an increase in the parasitaemia rate in malignant ovine thileriosis and in bovine tropical thileriosis and showed highest sensitivity and specificity of serum amyloid A was a suitable indicator of inflammatory reactions in the both diseases (22, 36).

There were significant increases in the levels of aspartate aminotransferase and non-significant increase in the levels alanine aminotransferase. This observation is in agreement with that of Baghshani et al. (31) showed the levels of AST and ALT were increased in diseased sheep with thileriosis although the only significant elevated of AST activity. On the other hand, Al-Obaidi and AlSaad (18) showed significant increase ALT and AST in sheep infected with *Thieleria hirci*. Shahnavaz et al. (37) who detected elevated in AST and ALT levels in cattle.
infected with *Theileria annulata* indicate hepatic dysfunction. Lotfollahzadeh *et al.* (38) who reported the increased activities of serum enzyme indicated the hepatic injuries associated with *Theileria annulata* and *Babesia bigemina*.

In the present study, infected caused significant increase cholesterol. This observation is in agreement with study was reported increased in cholesterol levels in cattle infected with *Theileria annulata* (37). The elevated of cholesterol attributed to liver damage result in a concurrent elevated of fats level with reduction of sugar and protein (39). However, Baghshani *et al.* (31) recorded non-significant increase of cholesterol levels in sheep naturally infected with theileriosis.

The non-significant increase in creatinine level was observed in the present study. However, Col and Uslu (29) who reported an increased in the creatinine levels in cattle naturally infected with *Theileria annulata*, but contradicts to the result showed non-significant decrease in creatinine concentration in the infected sheep with theileriosis (31).

The significant increase in urea level between infected sheep and control group. These agree with previous studies that were showed increase in urea levels in sheep infected with *Theileria* (18, 31). An increased in urea concentrations may be related to kidney damage (29).

**References:**


