Diagnostic and prognostic value of procalcitonin (PCT), C reactive protein (CRP), nitric oxide (NO) levels, and adenosine deaminase (ADA) activity in sheep with natural babesiosis before and after treatment

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Abstract: This study was carried out to reveal the importance of procalcitonin, C reactive protein, nitric oxide levels, and adenosine deaminase activity in the diagnosis and prognosis of the disease in naturally infected sheep with Babesia ovis. Thirty sheep diagnosed clinically and parasitologically as having Babesia ovis were allocated to 2 groups. The first group was treated only with imidocarb dipropionate and the second group with imidocarb dipropionate and flunixin meglumine. On the seventh day after treatment, blood samples were collected again from the sheep in the babesiosis-infected group and the treatment responses were assessed. Serum PCT (1.72 ± 0.34 ng/mL, P < 0.01), CRP (101.42 ± 11.73 µg/mL, P < 0.001), NO (15.77 ± 2.75 µmol/L, P < 0.01), and ADA (13.92 ± 0.88 IU/L, P < 0.01) were higher in sheep with babesiosis than in the healthy sheep (0.49 ± 0.04 ng/mL, 49.46 ± 4.57 µg/mL, 8.15 ± 0.63 µmol/L, 9.34 ± 1.19 IU/L, respectively). When PCT, CRP, NO, and ADA before treatment and after treatment in the infected sheep were compared, the levels of these parameters except for ADA in the second group were determined to have statistically decreased after the treatment. As a result, it has been concluded that the measurements of PCT, CRP, NO, and ADA in sheep with babesiosis may be useful for the diagnosis and prognosis of the disease when assessed in association with clinical examination.

Key words: Babesia ovis, PCT, CRP, NO, ADA

1. Introduction

Babesiosis is a hemoparasitic disease transmitted by ticks and it causes significant economic losses (1). Babesia ovis, B. motasi, B. crassa, and B. foliata are seen in sheep (2). Symptoms such as fever, anemia, hepatitis, and hemoglobinuria are observed in Babesia ovis infection, and it can result in death in certain cases (3).

Procalcitonin (PCT) is a prohormone of the hormone calcitonin. It is composed of 116 amino acids and lacks hormonal activity (4,5). It is ubiquitous and produced in the C cells (parafollicular cells) of the thyroid gland (6). PCT production is regulated physiologically and pathologically by the calcitonin-1 (CALC-1) gene located on chromosome 11 (7). PCT is increased in bacterial, parasitic, and fungal infections with severe systemic findings and sepsis, although it never increases in viral infections (4,8–10). In the presence of a microbial infection, CALC-1 gene expression is increased, which triggers PCT production in all parenchymal tissues (including liver, lung, kidney, adipocytes, and muscle) and differentiated cells in the body (11). Studies have shown that PCT levels are high in the patients with malaria (9,10). As with malaria caused by Plasmodium falciparum, babesiosis can also be classified as protozoal sepsis (12). In another study, significant differences in PCT levels were found between healthy dogs and dogs with babesiosis (13). It is stated that PCT has a diagnostic and prognostic value, and may also help to assess therapeutic efficacy, as it increases in accordance with the severity of the inflammatory response to the infection (4). It has been stated that the prognosis of patients whose PCT levels continuously increase is poor, and the prognosis of patients whose PCT levels decrease rapidly is good (14). CRP is an acute phase protein synthesized in the liver; it increases in a way similar to PCT in infections, and is a biomarker that is also used to monitor the progress of the disease. Unlike PCT, CRP levels can also increase in slight inflammatory reactions and viral infections (15).

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ADA is an enzyme that catalyzes the conversion of adenosine into inosine (16). ADA activity is elevated in many diseases that are stimulated by cellular immunity (17). NO, which is an important mediator of physiological and pathophysiological events, is produced mostly in macrophages, neutrophils, and mast cells (18).

LPS and IFN-γ-stimulated macrophages induce cytostatic and/or cytotoxic effects against bacteria, parasites, and tumor cells by producing a large amount of NO (19). Regarding babesiosis in bovines, NO, ADA, and TNF-α levels are reported to be significantly increased, and to be sensitive parameters in predicting the diagnosis and prognosis of the disease (17).

Although many parameters are used in the diagnosis of babesiosis and in the prediction of its prognosis, no study has reported the importance of PCT as a biomarker in the prognosis and diagnosis of the pre- and post-treatment phases of naturally occurring babesiosis in sheep. The aims of this study were to determine the value of PCT as a biomarker in naturally occurring babesiosis in sheep and also to determine its relationship with other parameters (i.e., NO, ADA, CRP) used for this particular disease.

2. Materials and methods

2.1. Animals and treatment

The animal material of this study consisted of 45 Akkaraman breed sheep with an average of 30–40 kg live weight and 3–4 years of age in the Van Province region between June and July 2016. The diagnoses were made with weight and 3–4 years of age in the Van Province region of 468 bp belonging to the target gene region. The diagnosis of babesiosis was made after the May–June period and was confirmed by microscopic examination of Giemsa-stained smear slides and PCR analysis.

2.2. Diagnosis of babesiosis

The diagnosis of babesiosis was made after the May–June period using microscopic examination of Giemsa-stained smear slides and PCR analysis. The diagnosis of babesiosis was confirmed by detection of babesial parasites in the blood smear slides. The PCR analysis was performed as reported by Erster et al. (20). For the PCR phase, a BioRad T100 model thermal cycler was used. The amplification product obtained in the PCR was subjected to electrophoresis in an ethidium bromide-stained agarose gel to be controlled in terms of the region of 468 bp belonging to the target gene region.

2.3. Hematological analysis

Complete blood counts (MS4 Hematology, Melet Schloesing, Osny, France) were performed on the same day in anticoagulated blood samples (WBC: white blood cell; LYM: lymphocyte; MON: monocyte; NEU: neutrophil; EOS: eosinophil; RBC: red blood cell; MCV: mean corpuscular volume; PCV: packed cell volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red distribution width; HGB: hemoglobin).

2.4. Serum biochemical examinations

Blood samples without anticoagulant were centrifuged at 3000 rpm for 10 min (Rotofix 32, Hettich, Kirchlengern, Germany) to separate the sera. Serum samples were stored at −80 °C until all blood samples were collected. The blood urea nitrogen (BUN), creatine, creatine kinase (CK), total protein, albumin, glucose, total bilirubin (T-bilirubin), direct bilirubin (D-bilirubin), and alkaline phosphatase (ALP) levels were measured in serum samples using a biochemical analyzer device (BS-120, Mindray, Shenzhen, China) after all blood samples were collected. PCT, total NO, ADA (all 3, MyBioSource, San Diego, CA, USA), and CRP (Cusabio, Houston, TX, USA) levels were measured with a microplate spectrophotometer (Epoch, BioTek, Winooski, VT, USA) with sheep-specific ELISA test kits.

2.5. Statistical analyses

For the preparation of statistical data, the SPSS 24 statistical program pack was used. Parameters are given as mean ± SEM. A normality test was performed with a Kolmogorov–Smirnov test. Independent samples and paired-samples t-tests were used for normal distribution samples, and the Mann–Whitney U test and Wilcoxon test were used for those without normal distribution. The Mann–Whitney U test and independent-samples t-test were used to compare the control and babesiosis groups and the differences between G1 and G2 after the treatment. The paired-sample t-test and the Wilcoxon test were used to compare G1 and G2 before and after treatment. P < 0.05 was regarded as statistically significant. Receiver operating characteristic (ROC) curve analysis was used to determine the values of the diagnostic cutoff points of the parameters used in this study.
(ROC) curves were drawn for PCT, CRP, NO, and ADA as a measure of discriminating power between the control and BT babesiosis groups. The ROC curve shows the false-positive rate (1 – specificity) and true-positive rate (sensitivity) of a test. Diagnostic accuracy was assessed by calculating the areas under the ROC curves (AUC). Youden’s index (YI) (YI = sensitivity + specificity – 1) was used to choose the most appropriate cut-off points for PCT, CRP, NO, and ADA parameters.

3. Results

3.1. Clinical findings
The sheep included in the study showed one or more of the following clinical findings: depression (30/30), anorexia (30/30), pale mucous membranes (18/30), fever (25/30), and hemoglobinuria (25/30). There were no deaths among the sheep examined and all of them responded to the treatment positively.

3.2. PCR results
In the PCR analysis, B. ovis was detected in all blood samples of the sheep with babesiosis.

3.3. PCT, NO, CRP, and ADA results
PCT, NO, and CRP levels and ADA activity were found to be higher in sheep with BT babesiosis than in healthy sheep, and this value was found to be statistically significant. (Table 1). BT PCT, NO, CRP, and ADA values of animals in G1 and G2 had decreased in AT, and all parameters in G1 and G2 were statistically significant except for ADA. No statistical significance was found between PCT, NO, CRP, and ADA in G1 and G2 comparisons AT (Table 2).

The AUC, cut-off values, sensitivity, specificity, and Youden’s index of the parameters were identified using ROC analysis for pretreatment of Babesia ovis-infected sheep (Table 3). ROC curve analysis showed that CRP had the highest AUC (AUC 0.86) compared with PCT (AUC 0.80), ADA (AUC 0.78), and NO (AUC 0.74) (Figure and Table 3).

3.4. Hematological findings
When blood parameters were examined, it was found that BT levels of WBC, LYM, RBC, PCV, and HGB were statistically significant when compared to healthy sheep, and that WBC and LYM were high and RBC, HGB, and PCV were low in sheep with babesiosis (Table 4). When comparing the BT and AT blood parameters, statistical significance was determined only in MCH in G1 animals, and in monocyte, neutrophil, RBC, PCV, and HGB levels in G2. When the AT results of G1 and G2 were compared, no statistical significance was determined for any blood parameters (Table 5).

### Table 1. PCT, CRP, NO levels, and ADA activity in the control group and all sheep having babesiosis for the BT period (mean ± SEM).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 15)</th>
<th>BT babesiosis (n = 30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT (ng/mL)</td>
<td>0.49 ± 0.04</td>
<td>1.72 ± 0.34</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>CRP (µg/mL)</td>
<td>49.46 ± 4.57</td>
<td>101.42 ± 11.73</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>NO (µmol/L)</td>
<td>8.15 ± 0.63</td>
<td>15.77 ± 2.75</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>ADA (IU/L)</td>
<td>9.34 ± 1.19</td>
<td>13.92 ± 0.88</td>
<td>P &lt; 0.01</td>
</tr>
</tbody>
</table>

The control group and all sheep having babesiosis were compared before treatment (BT). Statistical significance was accepted as P < 0.05.

### Table 2. Before-treatment (BT) and after-treatment (AT) PCT, CRP, and NO levels, and ADA activity of G1 and G2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G1</th>
<th>G2</th>
<th>AT G1–G2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT (ng/mL)</td>
<td>1.98 ± 0.55</td>
<td>0.53 ± 0.08*</td>
<td>1.45 ± 0.42</td>
</tr>
<tr>
<td>CRP (µg/mL)</td>
<td>101.91 ± 17.72</td>
<td>54.01 ± 6.48*</td>
<td>100.92 ± 16.01</td>
</tr>
<tr>
<td>NO (µmol/L)</td>
<td>17.58 ± 4.45</td>
<td>4.28 ± 0.89**</td>
<td>13.97 ± 3.31</td>
</tr>
<tr>
<td>ADA (IU/L)</td>
<td>16.01 ± 1.10</td>
<td>9.95 ± 1.40**</td>
<td>11.84 ± 1.16</td>
</tr>
</tbody>
</table>

G1 and G2 were compared BT and AT among themselves. For statistical significance, * P < 0.05, ** P < 0.01. In addition, P-values for comparing the AT values of G1 and G2 are given in the rightmost column of the table.
3.5. Biochemical findings
When biochemical data were evaluated, it was found that the differences between ALP, total bilirubin, albumin, and glucose levels of the sheep with babesiosis and the healthy sheep were statistically significant (Table 6). There was a decrease in ALP and albumin levels and an increase in T-bilirubin levels in sheep with babesiosis. In BT and AT comparisons, a statistically significant decrease was determined for albumin in the G1 group, and a significant increase in BUN in G2. When G1 and G2 were compared for AT, a statistically significant increase was determined for T-bilirubin and D-bilirubin in G2, and a decrease in total protein levels in the same group compared to G1 (Table 7).

### Table 3. AUC, sensitivity, specificity, cut-off values of PCT, CRP, NO, ADA for pretreatment Babesia ovis-infected sheep.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AUC 95% confidence interval</th>
<th>P</th>
<th>Cut-off value</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Youden's index</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT (ng/mL)</td>
<td>0.80 (0.65–0.94)</td>
<td>&lt;0.01</td>
<td>0.48</td>
<td>0.93</td>
<td>0.60</td>
<td>0.53</td>
</tr>
<tr>
<td>CRP (µg/mL)</td>
<td>0.86 (0.75–0.97)</td>
<td>&lt;0.01</td>
<td>71.0</td>
<td>0.63</td>
<td>0.93</td>
<td>0.57</td>
</tr>
<tr>
<td>NO (µmol/L)</td>
<td>0.74 (0.59–0.89)</td>
<td>&lt;0.001</td>
<td>9.93</td>
<td>0.87</td>
<td>0.67</td>
<td>0.53</td>
</tr>
<tr>
<td>ADA (IU/L)</td>
<td>0.78 (0.63–0.92)</td>
<td>&lt;0.01</td>
<td>13.47</td>
<td>0.70</td>
<td>0.87</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Youden's index was used to choose an appropriate cut-off value.

Figure. ROC curves of the diagnostic performance of PCT, CRP, NO, and ADA for pretreatment Babesia ovis-infected sheep.

4. Discussion
*B. ovis*, which is commonly found in Turkey, is highly pathogenic in sheep and is characterized by fever, anemia, icterus, and hemoglobinuria. In our study, the high fever, anorexia, icterus, and hemoglobinuria which were seen in sheep with babesiosis are similar to clinical findings in previous studies (2,21).

The fact that the levels of RBC, PCV, and HGB in infected animals are significantly reduced compared to healthy animals shows compatibility with previous studies (22–24). The decrease of these parameters reveals the presence of anemia, which occurs as the result of destruction caused by a parasite on RBC. Morphological classification of the anemia can be done according to MCV.
and MCHC (25). MCV and MCHC values in the control and the babesiosis group were close to each other and of the reference intervals (26). This situation can be considered as the type of normocytic-normochromic anemia in sheep with babesiosis. Normocytic-normochromic anemias are known as nonregenerative anemia (25). Animals with nonregenerative anemia have normal RDW values as long as they have no significant dyserthropoiesis (25).

The control group and all sheep having babesiosis at BT were compared. Statistical significance was accepted as P < 0.05.

Table 4. Hematological parameters in the control group and all sheep having babesiosis for the BT period (mean ± SEM).

<table>
<thead>
<tr>
<th>Hematologic parameters</th>
<th>Control (n = 15)</th>
<th>BT babesiosis (n = 30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (×10⁶/µL)</td>
<td>11.07 ± 0.62</td>
<td>18.54 ± 1.97</td>
<td>P &lt;0.01</td>
</tr>
<tr>
<td>LYM (×10³/µL)</td>
<td>3.46 ± 0.53</td>
<td>11.10 ± 2.15</td>
<td>P &lt;0.05</td>
</tr>
<tr>
<td>MON (×10³/µL)</td>
<td>0.63 ± 0.10</td>
<td>0.44 ± 0.07</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>NEU (×10³/µL)</td>
<td>6.68 ± 0.65</td>
<td>6.60 ± 0.77</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>EOS (×10³/µL)</td>
<td>0.27 ± 0.07</td>
<td>0.39 ± 0.08</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>RBC (×10⁶/µL)</td>
<td>9.95 ± 0.17</td>
<td>8.87 ± 0.32</td>
<td>P &lt;0.05</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>28.96 ± 0.43</td>
<td>28.39 ± 0.45</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>28.50 ± 0.42</td>
<td>24.86 ± 0.83</td>
<td>P &lt;0.01</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>10.24 ± 0.16</td>
<td>10.07 ± 0.14</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>35.56 ± 0.82</td>
<td>35.81 ± 0.54</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>14.79 ± 1.00</td>
<td>14.15 ± 0.67</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>10.22 ± 0.21</td>
<td>8.91 ± 0.31</td>
<td>P &lt;0.01</td>
</tr>
</tbody>
</table>

Table 5. BT and AT hematologic parameters of G1 and G2.

<table>
<thead>
<tr>
<th>Hematologic parameters</th>
<th>G1</th>
<th>G2</th>
<th>AT G1–G2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BT (n = 15)</td>
<td>AT (n = 15)</td>
<td>BT (n = 15)</td>
</tr>
<tr>
<td>WBC (×10⁶/µL)</td>
<td>15.55 ± 2.21</td>
<td>14.66 ± 1.92</td>
<td>21.53 ± 3.14</td>
</tr>
<tr>
<td>LYM (×10³/µL)</td>
<td>8.04 ± 2.52</td>
<td>5.32 ± 1.25</td>
<td>14.16 ± 3.38</td>
</tr>
<tr>
<td>MON (×10³/µL)</td>
<td>0.59 ± 0.11</td>
<td>0.58 ± 0.08</td>
<td>0.28 ± 0.05</td>
</tr>
<tr>
<td>NEU (×10³/µL)</td>
<td>6.62 ± 0.89</td>
<td>8.16 ± 1.62</td>
<td>6.57 ± 1.30</td>
</tr>
<tr>
<td>EOS (×10³/µL)</td>
<td>0.30 ± 0.082</td>
<td>0.54 ± 0.15</td>
<td>0.49 ± 0.13</td>
</tr>
<tr>
<td>RBC (×10⁶/µL)</td>
<td>8.35 ± 0.49</td>
<td>8.41 ± 0.42</td>
<td>9.38 ± 0.39</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>29.13 ± 0.73</td>
<td>26.97 ± 1.87</td>
<td>27.65 ± 0.47</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>23.92 ± 1.33</td>
<td>24.10 ± 1.21</td>
<td>25.81 ± 0.99</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>10.41 ± 0.13</td>
<td>10.01 ± 0.18*</td>
<td>9.73 ± 0.22</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>36.16 ± 0.80</td>
<td>35.12 ± 0.57</td>
<td>35.45 ± 0.75</td>
</tr>
<tr>
<td>RDW (%)</td>
<td>15.06 ± 1.27</td>
<td>15.63 ± 1.32</td>
<td>13.25 ± 0.33</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>8.68 ± 0.52</td>
<td>8.45 ± 0.44</td>
<td>9.13 ± 0.35</td>
</tr>
</tbody>
</table>

G1 and G2 were compared as BT and AT among themselves. For statistical significance, *: P <0.05, **: P <0.01. In addition, P values for comparing the AT values of G1 and G2 are given in the rightmost column of the table.
The type of normocytic–normochromic anemia we have determined here is compatible with that in previous studies on cattle (27) and sheep (2) with babesiosis. The increase in the number of WBCs in animals with babesiosis is consistent with the findings of Esmaeilnejad et al. (22), while differing from the study by Rahbari et al. (23). The WBC increase can be attributed to lymphocytosis-induced immune enhancement. NSAIDs (nonsteroidal anti-inflammatory drugs) may lead to some hematologic side effects such as slowing of hemostasis, prolongation of bleeding, and rarely aplastic anemia, thrombocytopenia, agranulocytosis, and blood dyscrasias (28). The decrease in RBC, PCV, and HGB and increase in monocyte and neutrophil counts in G2 after treatment may be due to flunixin meglumine, an NSAID drug.

In sheep with babesiosis, the increase in T-bilirubin is consistent with findings in the study by Sevinç et al. (2), and the decrease in ALP and albumin are similar to those reported by Yeruham et al. (29). Elevated T-bilirubin may result from excessive erythrocyte degradation and

Table 6. Biochemical parameters in the control group and in all sheep having babesiosis for the BT period (mean ± SEM).

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Control (n =15)</th>
<th>BT babesiosis (n = 30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP (U/L)</td>
<td>82.59 ± 5.64</td>
<td>74.00 ± 9.48</td>
<td>P &lt;0.05</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>30.29 ± 7.12</td>
<td>30.22 ± 4.03</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.03 ± 0.01</td>
<td>0.16 ± 0.04</td>
<td>P ≤0.01</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dL)</td>
<td>0.02 ± 0.01</td>
<td>0.1 ± 0.03</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.61 ± 0.17</td>
<td>7.20 ± 0.22</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.48 ± 0.07</td>
<td>2.20 ± 0.05</td>
<td>P &lt;0.01</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>159.18 ± 43.66</td>
<td>268.92 ± 73.17</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>55.69 ± 3.60</td>
<td>45.19 ± 2.45</td>
<td>P ≤0.01</td>
</tr>
<tr>
<td>Creatine (mg/dL)</td>
<td>1.26 ± 0.12</td>
<td>1.11 ± 0.06</td>
<td>P &gt;0.05</td>
</tr>
</tbody>
</table>

The control group and all sheep having babesiosis BT were compared. Statistical significance was accepted as P <0.05.

Table 7. BT and AT biochemical parameters of G1 and G2.

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>G1 (BT n = 15)</th>
<th>G1 (AT n = 15)</th>
<th>G2 (BT n = 15)</th>
<th>G2 (AT n = 15)</th>
<th>AT G1–G2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP (U/L)</td>
<td>82.00 ± 13.54</td>
<td>72.36 ± 11.19</td>
<td>65.33 ± 13.36</td>
<td>72.94 ± 11.56</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>34.22 ± 6.12</td>
<td>36.65 ± 9.06</td>
<td>25.88 ± 5.12</td>
<td>47.66 ± 8.63*</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.17 ± 0.05</td>
<td>0.09 ± 0.05</td>
<td>0.16 ± 0.05</td>
<td>0.27 ± 0.08</td>
<td>P &lt;0.01</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dL)</td>
<td>0.14 ± 0.06</td>
<td>0.05 ± 0.01</td>
<td>0.06 ± 0.02</td>
<td>0.14 ± 0.04</td>
<td>P &lt;0.05</td>
</tr>
<tr>
<td>Total protein (g/dL)</td>
<td>7.11 ± 0.23</td>
<td>7.53 ± 0.18</td>
<td>7.29 ± 0.40</td>
<td>6.88 ± 0.22</td>
<td>P &lt;0.05</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.13 ± 0.07</td>
<td>2.05 ± 0.09*</td>
<td>2.28 ± 0.08</td>
<td>2.11 ± 0.07</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>185.70 ± 64.35</td>
<td>565.82 ± 266.46</td>
<td>359.08 ± 134.23</td>
<td>782.84 ± 300.61</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>48.00 ± 2.18</td>
<td>45.06 ± 4.51</td>
<td>42.15 ± 4.47</td>
<td>57.46 ± 4.38</td>
<td>P &gt;0.05</td>
</tr>
<tr>
<td>Creatine (mg/dL)</td>
<td>1.02 ± 0.10</td>
<td>0.88 ± 0.08</td>
<td>1.20 ± 0.07</td>
<td>0.99 ± 0.08</td>
<td>P &gt;0.05</td>
</tr>
</tbody>
</table>

G1 and G2 were compared as BT and AT among themselves. For statistical significance, *: P <0.05, **: P <0.01. In addition, P values for comparing the AT values of G1 and G2 are given in the rightmost column of the table.

The type of normocytic–normochromic anemia we have determined here is compatible with that in previous studies on cattle (27) and sheep (2) with babesiosis. The increase in the number of WBCs in animals with babesiosis is consistent with the findings of Esmaeilnejad et al. (22), while differing from the study by Rahbari et al. (23). The WBC increase can be attributed to lymphocytosis-induced immune enhancement. NSAIDs (nonsteroidal anti-inflammatory drugs) may lead to some hematologic side effects such as slowing of hemostasis, prolongation of
liver damage (2), and decreased albumin level can be
due to liver function disorders, renal insufficiency, and
anorexia resulting from high body temperature (22). High
T-bilirubin levels observed in our study may be related
to erythrocyte degradation and decreased albumin; high
glucose levels, to anorexia. Low ALP levels have been
reported to have an association with severe anemia,
deficiency of vitamin C, B12, zinc, iron, or magnesium,
malnutrition, and hypothyroidism (30,31). In our study,
we think that low ALP levels were caused by the anemia.
In a study conducted on goats (32), it was determined that
flunixin meglumine caused an increase in BUN, creatine,
ALP, GGT, and AST levels. The increase in BUN level in G2
after treatment and the decrease in total protein level and
increases in T-bilirubin and D-bilirubin after treatment
when G1 and G2 were compared may be due to the effect
of flunixin meglumine, as mentioned by Safarchi et al.
(32). Flunixin meglumine has been reported to increase
CK activity in a single intramuscular dose application (33)
because it is a highly irritating agent. The increase in CK
in G2 after treatment, although not statistically significant,
may be caused by flunixin meglumine administered IM.

CRP increases after trauma, inflammation, and tissue
damage, especially in bacterial infections (34). CRP is a
nonspecific biochemical marker, although very useful in
inflammation (35). CRP has been reported to increase in
dogs naturally infected with Babesia canis (36,37). In our
study, we believe that the increase in the levels of CRP in
sheep with babesiosis sheep and their return to normal after
 treatment may be important in monitoring the disease.

PCT is used in the diagnosis of sepsis in human
medicine; it has been expressed that PCT is a good
predictor of inflammatory response parameters such as
body temperature, CRP, and leukocyte count. In addition,
PCT is used in the diagnosis of inflammatory diseases, and
in the prognosis and monitoring of response to treatment.
Although PCT is identified as a marker of bacterial
infections, it is also increased in acute malaria and fungal
infections (15). Babesiosis is similar in many respects to
human falciparum malaria (38). Babesiosis is characterized
by malaria-like fever, hemolysis, and hemoglobinuria (39).
Increased serum PCT concentrations have been reported in
patients with Plasmodium falciparum malaria (10,40,41). In
a study on dogs infected with Babesia canis, it was stated that
the PCT level was significantly increased in diseased animals
compared to healthy animals (13). In our study, it was also
found that in sheep with babesiosis, the PCT level was
significantly increased when compared to that of the healthy
sheep. There was a significant difference between BT and AT
PCT levels in both G1 and G2, and PCT levels appeared to
be decreased in treated animals. This can provide us with
important data in assessing the prognosis of the disease. It
has been reported that PCT production is not significantly
attenuated by steroidal and nonsteroidal antiinflammatory
drugs (5). In our study, the AT values of flunixin meglumine,
a nonsteroidal antiinflammatory drug used in G2, do not
seem to have significant differences when compared to the
values of G1.

The enzyme ADA increases due to stimulation of
cellular immunity (16). Its most important physiological
role is related to the differentiation and proliferation of
lymphocytes (42). Increased serum ADA activity has been
reported in various diseases such as leukemia (43), hepatic
diseases (44), thileriasis (16), and babesiosis (17,45) in
cattle. In our study, it was determined that ADA activity was
increased in sheep with babesiosis in the BT period, and
decreased to normal levels in the AT period. The increase in
ADA in sheep with babesiosis during the BT period may be
attributed to stimulation of lymphocyte-mediated immunity,
the erythrocytic destruction caused by the parasite, and/or
phagocytic activity of macrophages.

LPS and IFN-γ-stimulated macrophages produce
cytostatic and/or cytotoxic effects against bacteria, parasites,
and tumor cells by producing a large amount of NO (19).
It has been suggested during in vitro experiments that NO
reduces B. Bovis’s viability and B. bovis merozoites stimulate
NO production through monocytes/macrophages in the
presence of IFN-γ and TNF-α (46). Babesia ovis increases
the level of nitrite/nitrate, which is the oxidation product of NO,
in sheep (47) and goats (48). In our study, it was determined
that NO levels increased in sheep having babesiosis during
the BT period and decreased in the AT period. This increase,
seen in sheep having babesiosis during the BT period, may
be due to Babesia agents stimulating NO production in sheep
macrophages and increasing NO release. The reduction of
NO levels in the AT period may also be attributed to the
reduction of NO stimulation of sheep macrophages by
parasitic agents as a result of the efficacy of the treatment.

When the ROC curve characteristics of CRP and PCT
were compared to those of ADA and NO, the AUC of CRP
appeared superior to that of PCT, whereas the AUC of ADA
and NO appeared inferior to that of PCT, suggesting that
CRP provided the most accurate diagnostic performance for
pretreatment Babesia ovis-infected sheep. In one study (49),
the AUC value of PCT for severe P. Falciparum malaria was
found to be 0.78, and the AUC value we obtained is close to
this value.

In conclusion, the PCT, CRP, and NO levels and ADA
activity in sheep with babesiosis are useful parameters
to be measured and evaluated together with the clinical
examination for the diagnosis and prognosis of the disease.

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References


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