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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 imidocarp dipropionate and the second group with imidocarp dipropionate and flunixine meglumin. 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 clinical and laboratory findings; 30 were naturally infected with *Babesia ovis* and 15 were healthy. The babesiosis-diagnosed sheeps were divided into 2 groups according to the treatment administered. Imidocarb dipropionate (1.2 mg/kg/bw) treatment was administered to the first group (G1), and imidocarb dipropionate (1.2 mg/kg/bw, IM) and flunixin meglumine (2.2 mg/kg/bw, IM, 2× with a 24 h interval) combined treatment was administered to the second group. Blood samples were taken from the *V. jugularis* of the infected sheep in the usual manner before the treatment (BT) and on day 7 after the treatment (AT) for laboratory analysis, using the required method. The control group consisted of healthy sheep which were found to be negative according to the results of the laboratory evaluations in terms of other blood parasites, and which had no disease history or clinical findings specific to babesiosis or other diseases, and which had the same regional and rearing conditions. All the animals used were kept in their normal environment during the study.

2.2. Diagnosis of babesiosis

The diagnosis of babesiosis was made after the May–Grünwald/Giemsa staining, with the detection of parasites in the blood smears. Blood samples brought to the laboratory in K_2EDTA plastic tubes were stored at –80 °C after immediate centrifugation at 3000 rpm for 15 min. DNA extraction from blood samples was performed using a commercially available kit, in accordance with the instructions for use. A primer couple was used; the forward primer specific for *B. ovis* was 5′-ATGTTGGCCAAGTATCTTGCC-3′ and the reverse primer was 5′-CTACGTCAATTTGGCCTTGAACTC-3′. 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-bilurubin), direct bilurubin (D-bilurubin), 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) 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).

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

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.

Parameters	G1		G2		AT G1–G2
	BT (n = 15)	AT (n = 15)	BT (n = 15)	AT (n = 15)	P
PCT (ng/mL)	1.98 ± 0.55	0.53 ± 0.08*	1.45 ± 0.42	0.57 ± 0.11*	P > 0.05
CRP (µg/mL)	101.91 ± 17.72	54.01 ± 6.48*	100.92 ± 16.01	53.66 ± 3.37**	P > 0.05
NO (µmol/L)	17.58 ± 4.45	4.28 ± 0.89**	13.97 ± 3.31	2.19 ± 0.20**	P > 0.05
ADA (IU/L)	16.01 ± 1.10	9.95 ± 1.40**	11.84 ± 1.16	8.16 ± 1.47	P > 0.05

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.

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

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

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

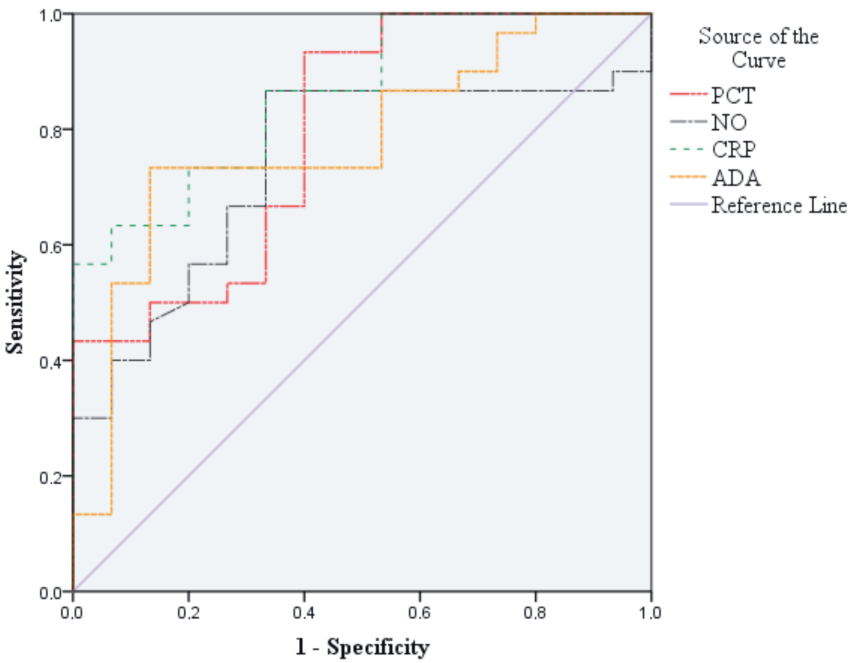

Figure. ROC curves of the diagnostic performance of PCT, CRP, NO, and ADA for pretreatment *Babesia ovis*-infected sheep.

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-bilurubin levels in sheep with bebesiosis. 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-bilurubin and D-bilurubin in G2, and a decrease in total protein levels in the same group compared to G1 (Table 7).

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 bebesiosis 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

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

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

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

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

Hematologic parameters	G1		G2		AT G1–G2
	BT (n = 15)	AT (n = 15)	BT (n = 15)	AT (n = 15)	P
WBC (×10³/μL)	15.55 ± 2.21	14.66 ± 1.92	21.53 ± 3.14	17.33 ± 1.98	P >0.05
LYM (×10³/μL)	8.04 ± 2.52	5.32 ± 1.25	14.16 ± 3.38	5.48 ± 1.16	P >0.05
MON (×10³/μL)	0.59 ± 0.11	0.58 ± 0.08	0.28 ± 0.05	0.48 ± 0.08*	P >0.05
NEU (×10³/μL)	6.62 ± 0.89	8.16 ± 1.62	6.57 ± 1.30	10.96 ± 2.00*	P >0.05
EOS (×10³/μL)	0.30 ± 0.082	0.54 ± 0.15	0.49 ± 0.13	0.36 ± 0.066	P >0.05
RBC (×10⁶/μL)	8.35 ± 0.49	8.41 ± 0.42	9.38 ± 0.39	7.90 ± 0.46*	P >0.05
MCV (fl)	29.13 ± 0.73	26.97 ± 1.87	27.65 ± 0.47	29.02 ± 0.61	P >0.05
PCV (%)	23.92 ± 1.33	24.10 ± 1.21	25.81 ± 0.99	22.54 ± 1.18*	P >0.05
MCH (pg)	10.41 ± 0.13	10.01 ± 0.18*	9.73 ± 0.22	9.87 ± 0.21	P >0.05
MCHC (g/dL)	36.16 ± 0.80	35.12 ± 0.57	35.45 ± 0.75	34.33 ± 0.72	P >0.05
RDW (%)	15.06 ± 1.27	15.63 ± 1.32	13.25 ± 0.33	13.39 ± 0.45	P >0.05
HGB (g/dL)	8.68 ± 0.52	8.45 ± 0.44	9.13 ± 0.35	7.79 ± 0.49*	P >0.05

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.

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 bebesiosis. Normocytic–normochromic anemias are known as nonregenerative anemia (25). Animals with nonregenerative anemia have normal RDW values as long as they have no significant dyserethropoiesis (25).

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

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

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.

Biochemical parameters	G1		G2		AT G1–G2
	BT (n = 15)	AT (n = 15)	BT (n = 15)	AT (n = 15)	P
ALP (U/L)	82.00 ± 13.54	72.36 ± 11.19	65.33 ± 13.36	72.94 ± 11.56	P >0.05
BUN (mg/dL)	34.22 ± 6.12	36.65 ± 9.06	25.88 ± 5.12	47.66 ± 8.63*	P >0.05
Total bilurubin (mg/d)	0.17 ± 0.05	0.09 ± 0.05	0.16 ± 0.05	0.27 ± 0.08	P <0.01
Direct bilurubin (mg/d)	0.14 ± .06	0.05 ± 0.01	0.06 ± 0.02	0.14 ± 0.04	P <0.05
Total protein (g/dL)	7.11 ± 0.23	7.53 ± 0.18	7.29 ± 0.40	6.88 ± 0.22	P <0.05
Albumin (g/dL)	2.13 ± 0.07	2.05 ± 0.09*	2.28 ± 0.08	2.11 ± 0.07	P >0.05
CK (U/L)	185.70 ± 64.35	565.82 ± 266.46	359.08 ± 134.23	782.84 ± 300.61	P >0.05
Glucose (mg/dL)	48.00 ± 2.18	45.06 ± 4.51	42.15 ± 4.47	57.46 ± 4.38	P >0.05
Creatine (mg/dL)	1.02 ± 0.10	0.88 ± 0.08	1.20 ± 0.07	0.99 ± 0.08	P >0.05

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-inflamatory 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-bilurubin may result from excessive erythrocyte degradation and

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-bilurubin 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 assocciation 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 megluminine, as mentioned by Safarchi et al. (32). Flunixin meglumin 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 meglumin 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 leukosis (43), hepatic diseases (44), theileriasis (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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