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Determination of serum cardiac biomarkers and plasma D-dimer levels in anemic sheep with babesiosis

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Abstract: In this study, the cardiac effects of anemia and venous thromboembolism (VTE), reportedly caused by the hemolyzed erythrocytes occurring in sheep with babesiosis, were investigated using cardiac markers and D-dimer (DD). The study included 34 sheep: 24 infected Akkaraman sheep (1–3 years old, diagnosed with babesiosis based on clinical and laboratory findings) and a control group of 10 noninfected healthy sheep of the same breed and age. Hematocrit (Hct) levels were measured in blood obtained from both groups. Cardiac troponin I (cTnI), creatine kinase-MB (CK-MB), and aspartate aminotransferase (AST) levels were measured in serum samples. In addition, the levels of DD were also measured in plasma samples. Sheep with babesiosis were subsequently divided into 3 subgroups according to their Hct levels, which ranged from 13.2% to 16.3% in the first group (n = 8), 20.1% to 25.6% in the second group (n = 8), and 27.4% to 30.3% in the third group (n = 8). Evaluations of the measurements of cTnI, CK-MB, and AST levels indicated statistically significant differences between infected and healthy sheep. Statistically significant differences were not found for DD levels between the 2 groups.

Key words: *Babesia*, cardiac biomarkers, D-dimer, hematocrit, sheep

1. Introduction

Ovine babesiosis, the most important hemoparasitic tick-borne disease of small ruminants, is caused by several species, including *Babesia ovis*, *B. motasi*, *B. crassa*, *B. taylori*, *B. foliata*, and the recently described *Babesia* sp. Xinjiang and *Babesia* sp. BQ1. Two species, *Babesia ovis* and *Babesia* sp. Xinjiang, are highly pathogenic, especially in sheep, and cause severe infections characterized by fever, anemia, icterus, and hemoglobinuria (1–4). *Babesia* species can cause severe economic losses in sheep and goats, particularly in tropical and subtropical climates. Clinically, babesiosis can be acute, subacute, or chronic. Uncomplicated babesiosis is further divided into mild, moderate, or severe disease, depending on the severity of anemia (5,6).

Babesia species cause hemolysis of erythrocytes, which leads to anemia symptoms (7). Intraerythrocytic parasites, such as *Babesia* and *Plasmodium*, are thought to produce hemolytic effects by oxidative stress and increased lipid peroxidation. Invasions of parasites demolish hematopoiesis and thus cause anemia (2,6), which is defined as a reduction in the number of erythrocytes and/or a subnormal concentration of hemoglobin per unit volume of blood. The decrease in red cell mass results in reduced oxygen transport and may lead to tissue hypoxia and hemodynamic and nonhemodynamic compensatory events. Anemia may have adverse effects on the heart and circulatory system in humans and animals (8).

Studies have yet to investigate myocardial damage in sheep with babesiosis using cardiac biomarkers. Heart lesions are a rare complication of babesiosis (9); nevertheless, postmortem examinations of dogs with babesiosis have reported foci of myocardial necrosis showing macrophage and neutrophil infiltration, as well as subepicardial and subendocardial hemorrhage. Dilated cardiac blood vessels containing considerable numbers of parasitized erythrocytes and free parasites have also been reported in canine babesiosis (9,10). Ecchymotic hemorrhages in the epicardium, endocardium, and myocardium have been reported in bovine theileriosis and equine babesiosis (11,12). These changes probably develop due to one of 2 mechanisms—severe inflammatory response and anemic hypoxia—believed to cause the observed tissue damage in babesiosis.

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Troponins and myoglobins are cardiac-originated proteins whose biochemical parameters are used to determine the existence and degree of myocardial injury. Creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) are also used for this purpose, although they lack sensitivity and specificity (10,13) The levels of these test substances may also be increased in liver, kidney, and muscle disorders. Hepatic and renal failure and rhabdomyolysis can also develop in babesiosis (10,11). Troponins consist of 3 distinct myofibrillar proteins (I, C, and T) that regulate the calcium-mediated interaction between actin and myosin in both cardiac and skeletal muscle. Cardiac troponin I (cTnI) is the only form that is uniquely expressed in the myocardium. Both cTnI and cardiac troponin T (cTnT) have been widely recognized as highly sensitive and specific blood markers for the noninvasive diagnosis of increased cardiomyocyte permeability. Blood concentrations appear to correlate with the extent of myocardial injury in both humans and animals. CK enzyme has 3 isozymes classified according to their origin. Those are CK-MB (heart), CK-MM (skeletal muscle), and CK-BB (brain). Cardiac-originated CK is among the diagnostic tests mostly used. However, the relatively short duration of increase in serum CK-MB level during cardiomyopathy and its existence in extracardiac muscle tissues limit its diagnostic value (13,14). The levels of cTnI and CK-MB are used as specific tests for assessment of acute myocardial damage (11,14).

Increased levels of D-dimer (DD) occur under a variety of conditions where the coagulation system is activated. Surgery, trauma, infection, inflammation, pregnancy, disseminated intravascular coagulation (DIC), venous thromboemboli (VTE), ischemic cardiopathy, and thrombosis can all cause an increased level of DD. Clinically, DD is used in VTE and DIC diagnosis (15,16). Babesiosis infection is characterized by the development of intravascular hemolysis with rising parasitemia, resulting in profound anemia; the hematocrit level may fall to 20% (17). Intravascular coagulation may also occur, and devastated erythrocytes can block the vessels of internal organs (18).

The aim of this study was to investigate the potential cardiac damage that may develop as a result of the hemodynamic and nonhemodynamic events occurring in ovines naturally infected with *Babesia* species. For this purpose, measurements were made of the total erythrocyte counts, hemoglobin and hematocrit levels, and levels of cardiac markers (cTnI, CK-MB, and AST) and of the marker of the DIC condition (DD) in naturally infected ovines.

2. Materials and methods

2.1. Animals and parasitological examination

Twenty-four Akkaraman sheep aged from 1 to 3 years and showing typical symptoms of babesiosis, such as fever, anemia, hemoglobinuria, and icterus, were chosen for this study. Fixed blood smears from the ear tips of the sheep were prepared in methanol and stained with Giemsa stain in order to confirm the presence of *Babesia* parasites. Morphological and biometrical parameters, such as shape and site location of the parasite in any infected erythrocyte, were considered for differential diagnosis. The identification of *Babesia* sp. was confirmed according to several studies (1,5,19). Species identification was not performed in this study. All animals were presented to the Veterinary Teaching Hospital, Veterinary Faculty, Yüzüncü Yıl University, Van, Turkey, from June 2013 to September 2014. A total of 10 clinically healthy Akkaraman sheep from tick-free farms were used as the control group. Infected animals were divided into 3 subgroups (n = 8 in each) according to their hematocrit readings, which ranged from 13.2% to 16.3% in the first, 20.1% to 25.6% in the second, and 27.4% to 30.3% in the third subgroup. The subgroups were classified as mild, moderate, and severe anemia according to the hematocrit range.

2.2. Samples

Hematological, biochemical, and DD analysis was conducted on approximately 15-mL blood samples collected from the jugular vein into vacutainers containing EDTA and sodium citrate as an anticoagulant or into anticoagulant-free serum tubes (for biochemical analysis). Hematological parameters were determined immediately. Platelet-poor plasma was obtained by centrifugation at 1600 × *g* for 20 min at 4 °C and was analyzed immediately within 1 h. Serum was obtained by centrifugation at 3000 × *g* for 10 min. Serum samples were preserved at –20 °C until analysis.

2.3. Hematologic and D-dimer examinations

The global count of erythrocytes and hemoglobin and hematocrit levels were determined by automated cell counts (ACT-8 Coulter Miami-EUA). The plasma DD value was determined calorimetrically using commercial test kits (DD Kit Albio-Stago) by STA Compact.

2.4. Serum biochemical examinations

Serum cardiac troponin I (cTnI) values were determined calorimetrically using commercial test kits (Troponin I kit-DRG Diagnostic) on an ELISA reader (ELISA Reader-DAS for cTnI). Serum AST and CK-MB levels were measured spectrophotometrically (Photometer 5010 Boehringer Mannheim) using commercial test kits (Randox-UK) as instructed by the manufacturers.

2.5. Statistical analysis

The statistical evaluation of data was performed with the Mann–Whitney test using SPSS.

3. Results

Fever above 40 °C, hemoglobinuria, anemic conjunctiva, and increases in respiratory rate and heart pulse were detected during the examination of the infected animals. The measured values of hematocrit, cTnI, AST, and CK-MB in healthy and infected animals are shown in Table 1. Hematocrit values were statistically significant ($P < 0.002$) between heathy and infected animals. cTnI concentrations were between 0.00 and 0.35 ng/mL in control animals and between 0.010 and 1.3 ng/mL in infected animals, which showed significant increase in sick animals ($P < 0.001$). The AST and CK-MB values were also significantly higher in the infected animals compared to the control animals ($P < 0.01$ and $P < 0.05$, respectively). No statistically significant differences were found in DD levels between the 2 groups ($P > 0.1$). The hematological investigations revealed varying degrees of anemia in all sheep with babesiosis. In this study, increases in cTnI levels were detected that were directly proportional to increases in the intensity of anemia, and these results are shown in Table 2.

4. Discussion

Babesia is a blood-tissue parasite that is transmitted by hard ticks from the family Ixodidae (20). Ovine babesiosis is the most important seasonal sheep disease and has been observed in all geographical regions of Turkey (7,21). *B. ovis* was determined in sheep with microscopic, serological, and molecular studies in various regions of Turkey (2–4,7,20–24). In addition, Taşcı (25) reported the presence of *B. motasi* with a microscopic study that was conducted in Van. Species identification was not performed in this study.

Babesia species cause anemia due to hemolysis of erythrocytes (7,8,21). Animals with babesiosis were previously reported as showing hematocrit values that were evaluated as severely anemic at <15%, moderately anemic at 15%–30%, and nonanemic at >30% (9,10). In the present study, varying degrees of anemia were detected in infected sheep, where the hematocrit values ranged from 13.2% to 30.3%. The infected sheep also showed a decrease in their red blood cell (RBC) and hemoglobin values together with anemia, as shown in Table 2. Similar results have been reported previously (6,26,27).

Anemia may trigger cardiac ischemia and necrosis, resulting in myocardial damage (8,10,11). A similar mechanism may underlie the myocardial damage seen in sheep with babesiosis. The findings of our study support the idea that the severity of the increase in anemia parallels the increase in serum levels of cTnI; thus, the hypoxia that develops as a result of anemia could cause myocarditis and ultimately lead to cardiac damage in sheep with babesiosis.

cTnI is released by myocytes in both reversible and irreversible myocardial disorders. Analysis of serum cTnI is a highly sensitive and accurate method for the investigation of myocardial abnormalities. Karapınar et al. (28) reported that the serum cTnI biomarker is an important criterion in the diagnosis of myocardial damage, and that human cTnI measurement can be helpful

Table 1. Hematologic and biochemical parameters in clinically healthy and infected sheep with *Babesia* spp.

	Hct (%)	cTnI (ng/mL)	AST (U/L)	CK-MB (U/L)	DD (µg/mL)
Control group, n = 10	28.90 ± 2.08**	0.010 ± 0.031***	103 ± 23**	141 ± 80*	0.40 ± 0.22
Infected group, n = 24	21.92 ± 5.96	0.49 ± 0.43	198 ± 97	256 ± 152	0.39 ± 0.28

Statistical significance between the control group and the infected group: *P < 0.05, **P < 0.01, *** P < 0.001.

Table 2. Mean values ± SD of hematocrit, erythrocytes, hemoglobin, and troponin I in groups with mild, moderate, and severe anemia and the control group.

	Hct (%)	RBC ($\times 10^3$)	Hg (g/dL)	cTnI (ng/mL)
Group 1, n = 8 (mild anemia)	28.9 ± 1.04	3.72 ± 0.26	9.65 ± 0.32	0.021 ± 0.012
Group 2, n = 8 (moderate anemia)	23.3 ± 1.92**	2.99 ± 0.37**	7.9 ± 0.83**	0.039 ± 0.017**
Group 3, n = 8 (severe anemia)	15.1 ± 0.94***	1.98 ± 0.52***	5.02 ± 0.35***	0.98 ± 0.21***
Control group, n = 10	28.90 ± 2.08	4.74 ± 0.76	10.29 ± 0.62	0.010 ± 0.031

Statistical significance compared to the control group: **P < 0.01, *** P < 0.001.

in the diagnosis of myocarditis in lambs. In the present study, serum cTnI levels were measured using human cTnI kits. Statistically significant differences were determined between infected and healthy sheep ($P < 0.001$).

Myocardial effects are very rare in protozoan diseases. On the other hand, increasing serum cTnI levels have been reported in equine piroplasmosis, canine babesiosis, trypanosomiasis, and bovine theileriosis (5,8–12). AST and CK-MB are important biomarkers used in diagnoses of heart, liver, kidney, and muscle diseases. Higher levels of AST and CK-MB have been detected in cattle with babesiosis than in healthy cattle (11,27). Reyers et al. (29) reported that the values for hematocrit ($P = 0.037$), cTnI ($P = 0.002$), CK-MB ($P = 0.001$), and AST ($P = 0.003$) showed statistically significant differences between dogs with babesiosis and healthy dogs. In the present study, the levels of cTnI, AST, and CK-MB were higher in the infected sheep than in the healthy animals (Table 1).

Sheep that are naturally infected with babesiosis can show prolonged PT and APTT parameters (26), which may indicate DIC. The PT and APTT levels were not measured in the present study, although DD levels, an important biomarker used in diagnoses of DIC and VTE, were measured. However, no statistically significant differences were detected in DD levels between the infected and healthy sheep ($P > 0.1$).

In conclusion, sheep with babesiosis showed statistically significant differences compared to healthy sheep in all parameters except DD. Evaluation of the results leads to the suggestion that severe anemia may cause cardiac damage in sheep with babesiosis. Consequently, the investigation of cardiac markers would be a useful way to follow the prognosis of the disease, as well as to provide supportive therapy to reduce potential cardiac effects. In addition, cTnI, CK-MB, and DD levels have not been measured previously in sheep with babesiosis.

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