In the present study, we aimed to provide information on the serum content of sialic acids (TSA, LBSA, and PBSA), total antioxidant capacity (TAC), and adenosine deaminase (ADA) activity in cattle affected with naturally acquired theileriosis and anaplasmosis. A total of 55 Holstein cattle, comprising of 15 clinically healthy control animals, 20 cattle with theileriosis, and 20 with anaplasmosis, were used. Diagnosis was based on clinical signs, Giemsa stained blood or lymph node aspirate, and PCR assay. For the PCR assay, Tams 1 primers were used. The obtained results suggested that the concentration of sialic acids and ADA activity were significantly higher; and TAC were significantly lower in the theileriosis and anaplasmosis groups in contrast to the control group. In conclusion, the increased level of sialic acids and ADA in theileriosis and anaplasmosis may be attributable to the stimulation of the host immune response. In contrast, the reduced level of TAC may reflect a decrease in the antioxidant capacity.

Key words: cattle, sialic acids, antioxidant capacity, adenosine deaminase, theileriosis, anaplasmosis.

Theileriosis and anaplasmosis are economically one of the most important and frequently fatal tick-borne diseases in cattle in tropical and subtropical regions of the world, including Turkey (6, 14). Since *Theileria annulata* and *Anaplasm marginale* are intraerythrocytic parasites, the detection of oxidative stress/antioxidant status and inflammatory parameters are important biomarkers for the host-parasite interactions. Although there are several biochemical studies on the blood parasites in cattle (2, 4, 10, 16), few reports are available regarding the host immune response and antioxidant status during natural infections of theileriosis and anaplasmosis.

Sialic acid (SA), an acetylated derivative of neuraminic acid, is widely distributed in mammal tissues and body fluids. The majority of sialic acids are found in either protein (PBSA) or lipid-bounded (LBSA) forms, while little amount is in the free forms. In addition, sialic acid is localised at the end chain of many acute phase proteins (3, 8, 24). Therefore, the detection of SAs may be a valuable indicator for diagnosis and prognosis of inflammatory diseases (20). Serum SAs values were analysed in many inflammatory and infectious diseases in cattle, such as pneumonia (11), theileriosis, anaplasmosis (4, 10), leptospirosis (13), traumatic reticulo-peritonitis (1), and keratoconjunctivitis (7).

Adenosine deaminase (ADA) is an enzyme that is present in all cells. ADA activity is elevated in many diseases where cellular immunity is stimulated (25). ADA activity was significantly increased with hepatic diseases, haematological malignancies, and infectious diseases. Measurement of ADA is used for the diagnosis and monitoring of autoimmune and inflammatory diseases because of its easy identification, high sensitivity, and low cost (19).

Oxidative stress may result from an imbalance between reactive oxygen species (ROS) and antioxidants levels (18). It is well known that ROS are produced by several pathological conditions and cause cellular damages such as lipid peroxidation and protein oxidation. The biological oxidative effects of free radicals on lipids and proteins are controlled by a spectrum of antioxidants (22). The antioxidant status of tissues can be described by the analysis of single components in the defence systems against ROS, as well as by the determination of total antioxidant capacity (TAC). In contrary, the TAC measurement does not represent the sum of activities of antioxidants; it could be used for clinical diagnosis, as it is an easy and less time-consuming procedure (9).
The aim of the present study was to evaluate the host immune response and antioxidant capacity by the use of serum total sialic acid (TSA), LBSA, PBSA, ADA, and TAC levels in cattle naturally affected with theileriosis and anaplasmosis.

**Material and Methods**

**Animals and sampling.** A total of 55 Holstein cattle, comprising of 35 females and 20 males, at the age of one to eight years, from the HATAY province of south Turkey, were used. The cattle were divided into three groups according to the clinical and parasitological examinations. Fifteen clinically healthy cattle were selected as the control (group I). Forty cattle with theileriosis and anaplasmosis were used in group II (n=20) and group III (n=20), respectively. Mixed infections were eliminated according to the microscopic examination. Ten millilitres of blood were collected from the jugular vein into the test tubes with silicon and EDTA for serum analyses and PCR examination, respectively. Serum samples were separated by centrifugation at 3 000 rpm for 10 min at room temperature and stored at -20°C until biochemical analyses. Bloods with EDTA were also stored at -20°C until DNA extractions.

**Clinical examination.** All the cattle included in this study were subjected to clinical and parasitological examinations. The infected animals showed clinical symptoms such as fever (>40.0°C), lymph nodes enlargement, inappetence, pale mucous membrane, petechiae in the conjunctiva, increased lacrimation, nasal discharge, and dyspnoea. Haemolytic anaemia and icterus were evident in advanced stages of the infections.

**Microscopic examination.** Smears were prepared with peripheral blood withdrawn from the ear tip and aspirated from enlarged lymph nodes. The smears were then fixed with methanol for 5 min and stained with 5% Giemsa solution for 30 min, and then examined under microscope. The degree of parasitaemia was recorded as the percentage of infected red blood cells in 1 000 red blood cells counted.

**PCR examination for *T. annulata***. DNA was extracted from 200 µl of infected blood sample by the use of commercial kits according to the manufacturer’s instructions (FERMENTAS). The amplification of *Theileria annulata* major surface 1 gene (Tams 1) of *Theileria* sp. by PCR was conducted using Tams 1 forward 5’-ATGCTGCAAATGAGGAT-3’ and reverse 5’-GGACGTGATGAGAAGACCGATGAG-3’ specific primers amplifying a 785 bp fragment. Following the sample preparation, PCR assays were carried out for 35 cycles on 94°C for 1 min, 45°C for 45 s, and 72°C for 35 s. Reaction products were electrophoresed in ethidium bromide containing 2% agarose gel in TAE buffer and visualised by UV gel documentation system (15).

**Biochemical analyses.** TSA analyses in serum samples were carried out according to the method reported previously by Sydows (23). Briefly, 400 µl of serum were treated with 3 ml of 5% perchloric acid for 5 min at 100°C and centrifuged at 1 400 g for 4 min. The supernatant (2 ml) was mixed with 400 µl of Ehrlich reagent (5 g pdimethylaminobenzaldehyde/50 ml HCl/50 ml distilled water). After incubation at 100°C for 15 min, a spectrophotometer (UV-1201, Shimadzu, Japan), was used to read the optical density at 525 nm. LBSA concentrations were measured colourimetrically on a spectrophotometer, as described by Katopodis et al. (12). PBSA values were calculated by subtracting of LBSA from TSA levels.

**Serum ADA activity** was determined by the use of the Giusti method (5), a colourimetric method based on the principle of measuring absorbance of the coloured indophenole complex at 628 nm.

**Total serum antioxidant activity** was determined by the method of Koracevic et al. (17). The assay measures the capacity of the samples to inhibit the production of thiobarbituric acid reactive substances (TBARS) from sodium benzoate under the influence of the free oxygen radicals derived from Fenton's reaction. This reaction can be measured spectrophotometrically and the inhibition of the development of colour may be defined as the TAC. A solution of 1 mmol/L uric acid was used as standard.

**Statistical analysis.** The results were analysed by a one-way analysis of the variance (ANOVA) followed by the Duncan test for multiple comparisons using computer software, SPSS Version 13.0 for Windows.

**Results**

Smears prepared from blood and lymph nodes of infected cattle revealed the presence of *T. annulata* or *A. marginale* in the red blood cells. The parasitaemia ranged from 2% to 45%. The presence of schizont infected cells was also observed in smears from the lymph nodes (>5%). *T. annulata* infection was confirmed both with Giemsa stained blood smears and by the PCR (Fig. 1). In contrast, all control animals were free of those pathogens.

![Fig. 1. Agar gel electrophoresis of PCR amplified DNA from *T. annulata*. Line 1 DNA Marker (50 bp DNA ladder-FERMENTAS). Lines 2-8: positive samples for *T. annulata* (785 bp). Line 9 - negative control.](image-url)

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*Note:* All the cattle included in this study were subjected to clinical and parasitological examinations. The infected animals showed clinical symptoms such as fever (>40.0°C), lymph nodes enlargement, inappetence, pale mucous membrane, petechiae in the conjunctiva, increased lacrimation, nasal discharge, and dyspnoea. Haemolytic anaemia and icterus were evident in advanced stages of the infections.
Table 1

<table>
<thead>
<tr>
<th></th>
<th>Group I (control, n=15)</th>
<th>Group II (theileriosis, n=20)</th>
<th>Group III (anaplasmosis, n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSA (mg/dL)</td>
<td>59.82±2.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.48±2.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>107.08±3.93&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>LBSA (mg/dL)</td>
<td>17.27±0.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.01±2.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.69±1.23&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PBSA (mg/dL)</td>
<td>42.55±2.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.47±2.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84.20±3.38&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ADA (U/L)</td>
<td>7.01±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.41±2.55&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.81±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>TAC (mmol/L)</td>
<td>0.69±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup>: differences are statistically significant among groups marked with different letters on the same line (P<0.05)

TSA, LBSA, PBSA, and TAC levels and ADA activity in controls as well as cattle with theileriosis and anaplasmosis were summarised in Table 1. Mean serum TSA, LBSA, and PBSA concentrations and ADA activity were significantly higher in the cattle with theileriosis or anaplasmosis in contrast to the control animals. However, TSA and PBSA levels were higher in the anaplasmosis group as compared to the theileriosis group. In contrast, the ADA level was higher in the theileriosis group. TAC values were significantly lower in cattle with theileriosis and anaplasmosis in contrast to the control group; however, there was no significant difference between the infected groups.

Discussion

As it was demonstrated, serum TSA, LBSA, and PBSA concentrations in cattle with theileriosis or anaplasmosis were higher than those of healthy cattle. The results of the present study are similar to the previous reports (4, 10). The increase in PBSA levels may be attributable to elevated serum acute phase proteins during inflammation. It is demonstrated that SA concentration increases rapidly following the inflammatory and injury process (1). The elevation of serum sialic acid concentrations in cattle with theileriosis or anaplasmosis may represent the host immune response to the parasite. The increased level of sialic acid may alter receptor-ligand interactions, which are known to play an important role in inflammation and immune response (10). In addition, the release of sialic acid from the glycolipids or glycoproteins of the lysed cell-membrane surfaces may result in the elevation of serum LBSA and TSA levels. Higher LBSA levels in theileriosis or anaplasmosis groups may be the result of sialic acids released from the cell membrane surface due to the erythrocyte lysis.

ADA levels may be found increased in many diseases due to the stimulation of cellular immunity. In this study, serum ADA was significantly higher (P<0.05) in the infected groups in contrast to the control group. The increase in serum ADA levels may result from the phagocytic activity of macrophages and/or erythrocyte damage caused by the parasites (25). We were unaware of finding documented reports regarding ADA activity values in cattle with theileriosis and anaplasmosis. A previous study performed by Kontas and Salmanoglu (16) reported increased serum ADA activity in another tick born parasitic disease, namely babesiosis.

In the present study, a significant decrease (P<0.001) in TAC levels was detected in cattle with theileriosis and anaplasmosis in contrast to the healthy control animals. In cattle infected with T. sergenti, the antioxidant levels of RBC decreased during the progression of anaemia (21). The findings in this study may suggest the alterations in antioxidative and oxidative balance due to the oxidative stress and ROS generation in the course of theileriosis and anaplasmosis. This alteration may be due to a decrease in the levels of enzymatic and non-enzymatic antioxidants, which are the component of antioxidant-defence system. Furthermore, results of the present study display significant negative correlations between TAC and TSA levels in cattle with theileriosis and anaplasmosis. ROS lead to both lipid and protein oxidation and liberate non-reducing terminal sialic acid residues, which are one of the most susceptible targets. In contrast, serum TSA is related to markers of lipid and protein oxidation and antioxidant parameters as well (22).

In conclusion, increased levels of TSA, LBSA, PBSA, and ADA in theileriosis and anaplasmosis may mimic induced host immune response. In addition, reduced level of TAC may indicate a decreased host antioxidant capacity.

References


