Serum biochemical profile and protein fractions in cattle with Theileriosis

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SUMMARY

Theileriosis encountered frequently in Van region and causes huge economic losses in cattle, sheep and goats. This study was carried out to examine the serum biochemical profile and serum protein fractions, determined before and 7 days after buparvaquone treatment, in 28 cattle, diagnosed to be infected with T. annulata and in 15 healthy control animals from the same region. The ALT (alanine amino transferase) and ALP (alkaline phosphatase) activities and the concentrations of bilirubin and urea were markedly increased in sera from infected animals whereas the concentrations of glucose, minerals (Na, K and Ca), total proteins, albumin and of ß-globulins and the albumin/globulin (A/G) ratios were significantly depressed compared to the controls. In addition, buparvaquone treatment succeeded in alleviating the serum biochemical anomalies. These results show that liver and kidney failures occur during theileriosis leading to a global protein deficiency and that serum biochemistry and serum protein analysis may allow biochemical evaluation of treatment.

Keywords: cattle, Theileriosis, Theileria annulata, serum biochemistry, serum protein fractions, electrophoresis, albumin

Introduction

Theileriosis, caused by Theileria protozoa and infected by ticks, is a disease with high mortality rate. The most common species found in tropical and subtropical regions are T. annulata and T. parva. Theileriosis is often seen in cattle in the Eastern Anatolia Region, especially in the Van region. Clinical symptoms such as high fever, anaemia and haemoglobinuria are detected with this disease. Besides, the blood-shaped elements, especially the volume of red blood cells, are reduced. Diagnosis can be made accurately when blood protozoan is seen in erythrocyte in blood smear.

Agents joined the host system merge with the lymph circulation, reach to the regional lymph lumps, settle to lymphocytes and start to reproduce. T. annulata proliferates in the RES (Reticulo Endothelial System) cells and forms Koch corpuscles (the microshizonte stage of the parasite). Koch corpuscles are pathogen elements of Theileria. Agents adhere to red blood cells by proliférating in RES cells and move to the other lymphocytes. They grow in there slightly and enter in erythrocytes again, start dividing into two and four, having very small ring, rod, and commas forms [3, 17, 22, 28, 31, 32]. Important changes occur in the biochemistry and blood parameters of animals exposed to parasite invasion. Parasite diseases make animals vulnerable against trace element and vitamin deficiencies, and lead to anaemia [10, 11, 26, 28].

The blood biochemical profile should be determined in order to diagnose the disease, monitor the course of the disease, and follow up the response to therapy. Blood parameters, such as concentrations of glucose, urea, creatinine, cholesterol, triglycerides, albumin, globulins, total proteins, total lipids and bilirubin, electrolytes, such as Ca, Na, K, P, as well as the circulating activities of some enzymes such as amylase, AST (aspartate amino transferase), ALT (alanine amino transferase), ALP (alkaline phosphatase), and CK (creatine kinase) are among important biochemical parameters, such as amylase, AST (aspartate amino transferase), ALT (alanine amino transferase), ALP (alkaline phosphatase), and CK (creatine kinase) are among important biochemical parameters.
parameters in determining the biochemical profile. These parameters are very essential regarding to diagnosis, treatment and following up prognosis. Data regarding the pathogenesis and prognosis of the disease can be obtained from these parameters.

The distribution of the concentration of blood proteins or synthesis rates, destruction or disposals is affected by many diseases. Some patients with one or more of these factors can be examined. Abnormal serum protein profiles can be used in identifying the various types of disease. Modifications of total serum protein concentrations, the fractional distribution of blood proteins and Albumin/globulins (A/G) ratio are usually among the first signs of protein abnormality. The electrophoresis is an appropriate separation technique for monitoring the changes in the serum protein fractions [6, 21, 27]. In a normal serum electrophoresis, four main fractions, albumin, α, β and γ-globulins, are obtained. Albumin is the fastest migrating protein. It is seen as a very high peak. Behind it globulins (α1, α2, β1, β2, γ1 and γ2) are sorted [6, 21, 27]. Globulins are divided into sub-fractions as α1 and α2 in the cattle [35].

This study was planned to investigate effects of theileriosis which is prevalent in the region and cause economic losses, on serum protein fractions. Determining the abnormal situations occur here and the biochemical profile of the blood by electrophoretic methods could help to diagnose the disease, monitor its prognosis, and follow up the response to therapy. Whereas literature survey revealed the data of biochemical profile in animals experimentally infected with Theileria, this study was planned to obtain information on biochemical profile and serum protein fractions in naturally infected cattle and to help better understanding the pathogenesis of the disease.

Material and Method

ANIMALS

A total of 43 cattle, 1-7 years old and belonged to different breeds, from the Van region were included in this study. Animals brought to the Department of Internal Medicine Clinic, Faculty of Veterinary Medicine, Yüzüncü Yıl University, were examined for the presence of Theileria by microscopic examination and indirect fluorescent antibody test. Among them, 28 were diagnosed to be sick and 15 others living in the same conditions were found healthy after parasite examination and served as controls. Infected animals were treated by buparvaquone (2.5 mg/kg, Butalex “-Cevadif ™ / Turkey) only once by intramuscular way.

PARASITOLOGICAL ANALYSIS

Firstly, clinical examination was performed and disease-specific findings (high fever, anaemia, haemoglobinuria, lymph node enlargement) were recorded. Blood smears were prepared from blood samples taken from each animal’s ear vena before and after treatment for microscopic examination. Smears were fixed in 3 minutes with methyl alcohol after air-dried. Prepared Giemsa dye solution was poured onto the fixed blood smears and rested for 30 minutes. At the end of this period, smears were washed with distilled water and allowed to dry. Smears were investigated on the microscope (Ceti, Belgium) at x100 magnification with immersion oil. Piroplasme forms of T. annulata in the erythrocytes in smears were evaluated in smears.

SEROLOGICAL AND BIOCHEMICAL ANALYSES

Blood samples were taken into tubes without anticoagulant from V. jughularis of infected animals before and 7 days after treatment whereas blood samples were taken only once in healthy controls. After clotting for 2 hours at room temperature and centrifugation (1500g, 10 minutes, room temperature), sera were carefully harvested and stored at -20°C until analyzed.

Serological diagnosis was made using indirect fluorescent antibody test (IFAT) in Bornova Veterinary Control and Research Institute Laboratory of Parasitology. Serum antibody titres higher than 1:20 were assessed as positive.

In order to determine the serum biochemical profile (concentrations of glucose, urea, creatinine, albumin, globulins, total proteins, bilirubin, Ca, Na, K, P and activities of amylase, ALT (alanine amino transferase) and ALP (alkaline phosphatase) and the VetScan brand (ABAXIS USA) biochemistry analyzer and its kits were used.

Serum protein fractions were examined and quantified throughout electrophoresis (Titan III, serum protein electrophoresis 3023, Helena, Bioscience Europe, EC4000 Series 90 programmable Thermo, Thermo Electron Corp.) and obtained electrophoresis bands were assessed by the Platinum Scanning System software program.

STATISTICAL ANALYSIS

The variance analysis was performed for determining the differences between controls, infected animals before and after treatment with the SPSS 11.5 package program. Duncan’s test was applied for multiple comparisons. Differences were considered as significant when p values were less than 0.05.

Results

Serum biochemical profiles in healthy cattle and in cattle naturally infected with T. annulata were summarized in Table I. Whereas creatininemia and the amylase activity were not significantly altered in diseased animals, the bilirubin and urea concentrations as well as the serum ALT and ALP activities were markedly increased before treatment (p < 0.05) and in parallel, glycaemia and serum mineral Na, K and Ca concentrations were significantly depressed (p < 0.05). Nevertheless, the phosphataemia which also slightly
diminished has not significantly differed from the mean control value. It was also observed that 7 days after the buparvaquone treatment, the ALT activity was closely related to the control values while the bilirubin, urea and glucose concentrations as well as the ALP activity were remained slightly altered but not significantly compared to the control values. However, although they have significantly increased compared to the pre-treatment values ($p < 0.05$ for Na and K concentrations), the serum Na, K and Ca concentrations were still significantly decreased compared to the control values ($p < 0.05$).

The biochemical analysis of serum proteins determined by total protein concentrations and serum protein electrophoresis were presented in Table II and in figure 1. The total protein concentrations were significantly lowered in cattle naturally infected with *T. annulata* compared to the healthy controls ($p < 0.05$). Throughout the electrophoretic analysis of protein fractions, it was noted that the proportions of albumin and $\alpha_1$-globulin fractions were significantly depressed in diseased animals ($p < 0.05$) whereas those of the $\alpha_2$- and $\beta$-globulins were not significantly affected and the mean proportion of $\gamma$-globulins was significantly decreased in infected cattle ($p < 0.05$).

### Table I: Serum biochemical profiles in healthy cattle (n = 15) and in cattle naturally infected by *Theileria annulata* (n = 28) before and 7 days after treatment with buparvaquone. Results are expressed as mean ± standard error (SE).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls</th>
<th>Infected cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before T.</td>
<td>After T.</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>6.50 ± 0.51$^a$</td>
<td>27.87 ± 4.45$^b$</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.04 ± 0.17$^a$</td>
<td>4.10 ± 0.27$^b$</td>
</tr>
<tr>
<td>Urea</td>
<td>4.53 ± 0.83$^a$</td>
<td>8.26 ± 0.63$^b$</td>
</tr>
<tr>
<td>Creatinine</td>
<td>93.70 ± 9.72</td>
<td>104.31 ± 7.96</td>
</tr>
<tr>
<td>Na</td>
<td>173.37 ± 1.83$^a$</td>
<td>146.27 ± 3.99$^b$</td>
</tr>
<tr>
<td>K</td>
<td>6.90 ± 0.19$^a$</td>
<td>5.19 ± 0.17$^b$</td>
</tr>
<tr>
<td>Ca</td>
<td>2.52 ± 0.12$^a$</td>
<td>1.96 ± 0.14$^b$</td>
</tr>
<tr>
<td>P</td>
<td>1.97 ± 0.13</td>
<td>1.68 ± 0.12</td>
</tr>
<tr>
<td>Amylase</td>
<td>42.93 ± 5.20</td>
<td>49.04 ± 3.95</td>
</tr>
<tr>
<td>ALT</td>
<td>16.32 ± 1.57$^a$</td>
<td>22.93 ± 2.15$^b$</td>
</tr>
<tr>
<td>ALP</td>
<td>52.82 ± 6.31$^a$</td>
<td>111.40 ± 14.85$^b$</td>
</tr>
</tbody>
</table>

$^a,b,c$: Different superscripts indicate significant differences ($p < 0.05$ or more) between groups.

### Table II: Biochemical profiles of serum proteins in healthy cattle (n = 15) and in cattle naturally infected by *Theileria annulata* (n = 28) before and 7 days after treatment with buparvaquone. Results are expressed as mean ± standard error (SE).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls</th>
<th>Infected cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before T.</td>
<td>After T.</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>85.8 ± 5.1$^a$</td>
<td>71.9 ± 5.2$^b$</td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion (%)</td>
<td>36.72 ± 1.10$^a$</td>
<td>30.54 ± 1.48$^b$</td>
</tr>
<tr>
<td>Concentration (g/L)</td>
<td>31.51 ± 1.52$^a$</td>
<td>21.96 ± 1.12$^b$</td>
</tr>
<tr>
<td>$\alpha_1$-globulin</td>
<td>7.44 ± 1.97$^a$</td>
<td>5.57 ± 2.60$^b$</td>
</tr>
<tr>
<td>Concentration (g/L)</td>
<td>6.38 ± 1.08$^a$</td>
<td>4.00 ± 1.23$^b$</td>
</tr>
<tr>
<td>$\alpha_2$-globulin</td>
<td>14.21 ± 1.21</td>
<td>15.26 ± 2.15</td>
</tr>
<tr>
<td>Concentration (g/L)</td>
<td>12.19 ± 1.84</td>
<td>10.97 ± 0.58</td>
</tr>
<tr>
<td>$\beta$-globulin</td>
<td>11.21 ± 1.07</td>
<td>11.78 ± 0.82</td>
</tr>
<tr>
<td>Concentration (g/L)</td>
<td>9.62 ± 0.71$^a$</td>
<td>8.47 ± 0.89$^b$</td>
</tr>
<tr>
<td>$\gamma$-globulin</td>
<td>33.64 ± 2.80$^a$</td>
<td>36.92 ± 3.54$^b$</td>
</tr>
<tr>
<td>Concentration (g/L)</td>
<td>28.86 ± 2.03</td>
<td>26.54 ± 1.36</td>
</tr>
<tr>
<td>A/G</td>
<td>0.59 ± 0.03$^a$</td>
<td>0.44 ± 0.03$^b$</td>
</tr>
</tbody>
</table>

A/G: albumin/globulins.

Different superscripts in the same row indicate significant differences ($p < 0.05$ or more) between groups.
increased (p < 0.05) (Table II and figure 1). Taking into consideration the proteinemia, the calculated concentrations of albumin and α1-globulins also appeared greatly depressed in diseased animals (p < 0.05) while the concentrations of α2- and γ-globulins remained similar to the control values. Consequently, the A/G ratio was also dramatically decreased in infected animals (p < 0.05), but especially because of low albuminaemia. Nevertheless, after the treatment, the electrophoretic profiles and the quantitative analysis of the serum protein fractions were similar to those observed in healthy cattle (Table II, figure 1).

**Discussion**

Theileriosis cases are seen quite frequently in the summer in Turkey due to the fact that ticks are active in the summer. It affects cattle, sheep and goats and causes considerable economic losses. Biochemical profile of host animal provides information about pathogenesis and prognosis of the disease. The changes in enzymatic activities in blood are useful in the clinical diagnosis of many diseases. The distributions of the concentration of blood proteins or synthesis rates, their destruction or disposal are affected by many diseases. Significant changes occur in the blood biochemical parameters of the animals exposed to parasite invasion.

By digesting intracellular haemoglobin, parasites living in erythrocytes may induce massive haemolysis, leading to drop of haematocrite and haemoglobinemia and to increase in bilirubinemia [3, 17, 26]. Total bilirubin concentrations were strongly elevated in dogs [14] and cattle [1, 2] with babesiosis as well as in cattle [29, 31] and calves [33, 37] with Theileria infections. In agreement with that, bilirubinemia in the present study was dramatically increased in cattle naturally infected with *T. annulata* and remained elevated in some animals despite the buparvaquone treatment. In cases of babesiosis and theileriosis, high bilirubin concentrations were observed and may be related to liver failure during the disease development and to haemolytic anaemia. In the present study, serum urea concentrations but not creatininemia were significantly enhanced in *T. annulata*-infected calves [33, 37] and cattle [23, 31], probably because of renal damage associated to the increase in haemoglobin catabolism. By contrast, OMAR et al. [29] found relatively low creatinine concentrations in naturally infected cattle.

It has been reported in studies carried out with calves and cattle infected with *Theileria*, that serum glucose concentrations decreased significantly [20, 23, 37]. It has been considered that hypoglycaemia observed in theileriosis may result from persistent fever due to the disease and inappetence developed by the severity of disease and its duration [20]. In this study, hypoglycaemia was also evidenced in cattle naturally infected with *T. annulata* and partially recovered after treatment.

In the present study, marked increases in serum ALP and ALT activities were evidenced in cattle naturally infected with *T. annulata* compared to the healthy controls. Several studies have also previously reported significant increases in both ALP and ALT activities in blood of dogs [15, 39] and cattle [1, 2] with babesiosis and in calves infected with *T. annulata* [22, 33]. Increased blood ALP activity was also recorded in *T. annulata* infected cattle [37] whereas decrease in this enzyme activity was noticed in sheep with acute babesiosis in only one study [38]. Increased blood ALT activity was previously found in cattle [20] and sheep [38] with babesiosis and in cattle [4, 29, 31] and buffalos [18] infected with *T. annulata*. *Theileria annulata* infection has been considered to be a cause of coagulation necrosis in the liver and to dense lymphocyte infiltration in periportal areas. Hypoxia, due to anaemia and to inflammatory infiltrates may cause damage in the hepatobiliary system, leading to increases in the serum activities of the hepatic enzymes [29, 33]. It was reported that acute pancreatitis developed in the dogs with babesiosis leading to significant increase in serum amylase activity [25]. The activity of this enzyme was also reported to be increased in animals infected with various endoparasites (*Fasciola spp.*, *Dicrocoelium dendriticum*, hydatid cysts, *Trichostongyliidae* and *Protostrongyliidae* etc.) [7]. However, in this study, there is no statistically significant difference in serum amylase activity between the control group and the *T. annulata* infected group during pre-treatment and post-treatment phases. Taken together, the serum biochemical

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**Figure 1:** Examples of electrophoretic profiles showing the densities of serum protein fractions in healthy cattle (control) and in cattle naturally infected by *Theileria annulata* (*n* = 28) before (pre-treatment) and 7 days after buparvaquone treatment (post-treatment).
analysis shows that theileriosis has a devastating effect on the liver functions in cattle and laboratory alterations may be caused by the toxic effects of the metabolites from Theileria sp. or from haemoglobin (such as bilirubin) [20] leading to hepatopathy and kidney failure but also by starvation [39].

Various studies have been carried out regarding mineral concentrations in animals with theileriosis. In one of these studies, statistically not significant reduction in serum calcium concentrations was reported in calves with experimental theileriosis [33] whereas significant decrease was found in buffaloes [18] cattle [29] and calves [22, 34, 37] naturally infected with *Theileria annulata* as well as in some acute systemic diseases (acute enteritis, acute septicemia etc.) [23]. In the present study, the serum Ca concentrations were also found to be dramatically and persistently depressed in naturally infected cattle despite the buparvaquone treatment. In parallel, sodium and potassium concentrations were also significantly lowered in diseased cattle before and after treatment and these results were in agreement with previous reports in cattle [23, 29] and buffaloes [18] with theileriosis. However, YADAV and SHARMA [37] reported unchanged natremia in calves infected with *T. annulata* but confirmed significant decrease in K concentrations. Significant reductions in blood P concentrations were also evidenced in animals infected with various endoparasites [7]. Phosphorus concentrations were significantly decreased in naturally infected cattle [29] and calves [37] and in buffaloes [18]. In calves with experimentally induced theileriosis, phosphorus concentrations significantly declined in the last weeks of the disease [33]. In this study, a slight decrease in P concentrations was also obtained in infected animals but the difference with the healthy controls was not statistically significant.

On the other hand, in this study, severe decrease in proteinemia was detected in the infected cattle and confirmed previous observations [20, 23, 34]. Nevertheless, OZAN *et al.* [31] and SANDHU *et al.* [33] reported that serum total protein concentrations did not change significantly in cattle and calves with natural or experimental theileriosis. OMAR *et al.* [29] reported that hypoproteinemia was likely due to hypoalbuminaemia and hypoglobulinaemia probably induced by liver failure.

Decrease in albumin concentrations was reported in various parasite diseases (*Babesia spp*, *Fasciola spp*, *Dicrocoelium dendriticum*, hydatid cysts, *Trichostrongylidae* and *Protostrongylidae* etc.) [7, 38, 39] and was associated to economic losses, weakness in immune system and muscles and the reduction in the quality of wool [7]. In *Theileria* infected calves [34] and cattle [20, 23, 29, 37], albuminemia was found considerably lower than in healthy animals. Similar results were obtained here but OZAN *et al.* [31] and SANDHU *et al.* [33] reported no changes in albumin concentrations in calves and cattle with theileriosis. Hypoalbuminaemia may result from increase in albumin excretion (dense albuminuria) due to renal failure, synthesis impairment and/or extensive protein degradation during prolonged fever [8, 9, 12, 35].

As far as serum globulins were concerned, the total concentration was usually determined in animals infected with various parasites: serum globulin concentrations were found to be increased in endoparasite infected sheep [7] and in *Theileria* and *Babesia*-infected cattle [21]. These alterations may be caused by the immune response against the disease [20]. However, decrease in the serum globulin concentrations was also observed in cattle and calves with theileriosis [29, 34, 37]. In addition, SINGH *et al.* [34] reported that serum immunoglobulin concentrations decreased in calves infected with *Theileria annulata*.

By contrast, globulin fractions in animals with theileriosis were rarely analysed. In sheep infected with *Babesia ovis*, APAYDIN and DEDE [5] found a significant increase in the α1-globulins and also a marked and persistent decrease in γ-globulins despite treatment leading to significant enhancement of the A/G ratio compared to the healthy controls. Some positive acute-phase proteins (APP) that play important roles in the natural defence mechanisms [13, 19] migrate in these electrophoretic zones. It has been reported that their circulating concentrations increased during parasite diseases [14, 16, 25] and in animals experimentally infected with *T. annulata* [19]. However, in the present study, the serum concentrations of α1-, α2- and β-globulins appeared slightly decreased in cattle naturally infected with *T. annulata* but significantly for α1-globulins. It is quite possible that the synthesis of positive APPs mainly by the liver during parasite infection occurred but remained too moderate for counteracting a global protein synthesis defect in this organ.

In cases of chronic infections [8, 24, 36], it was recorded an increased production of all immunoglobulins including IgA and IgM extending to the β-zone and called β-γ bridge [30]. Because of hypoalbuminaemia in acute and chronic liver diseases, the proportions of γ-globulins often appeared as elevated [8, 24, 36] but were not necessary coupled to the increase in the corresponding concentrations as observed in the present study in which the proportions of the γ-globulins but not their circulating concentrations were significantly enhanced in infected cattle compared to the healthy controls. In the present study, the A/G ratio was markedly decreased in cattle naturally infected with *T. annulata* as previously reported by SINGH *et al.* [34] in calves with *Theileria annulata*. In agreement with HUSSEIN *et al.* [20] who found that decreases in the A/G ratios depended from hypoproteinemia and hypoalbuminaemia in cattle with theileriosis, changes in this parameter observed in diseased animals may be mainly due to hypoalbuminaemia and secondary to global impairment in liver protein synthesis.

Additionally it was observed here that the buparvaquone treatment succeeded in correcting the electrophoretic profile of serum proteins, suggesting that a partial recovery in liver function.

Revue Méd. Vét., 2014, 165, 5-6, 137-143
As a conclusion, serum biochemistry and serum protein analysis have evidenced liver and renal damage coupled to alterations in mineral metabolism in cattle naturally infected with *T. annulata* and 7 days after buparvaquone treatment, animals have recovered at least partially. It is considered that blood serum protein fractions and changes in biochemical profile of cattle with theileriosis and rapid destruction of proteins with regard to the degree of haemolysis in erythrocytes might be connected with the high fever during the illness and the liver and kidney damage developing later.

Acknowledgement

This study was supported by Yüzyüce Yil University Scientific Research Projects Funding. Project no: 2007-VF-B15.

References


28. DEDE (S.) AND COLLABORATORS


