Biochemical, Hematological studies in cattle naturally infected with *Theileria annulata*

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**ABSTRACT**

The aim of the present study dealt with biochemical and hematological changes in cattle which presented to the Azad clinic of veterinary school in Urmia during the period between Dec 2008 to January 2012. A total of 985 cattle with positive theileriosis were examined. Data were collected on hematological conditions of the cattle. Ages of these slaughtered buffaloes ranged from 6 to 9 years, (79%) males and (21%) females. We observed a significant increase in all hematologic parameters including hemoglobin and hematocrit levels and WBC, platelet and RBC counts in newborns delivered spontaneously (p<0.001). The lymphocyte counts were similar in two groups. Male infants and newborns with higher gestational age showed a higher hemoglobin and hematocrit levels. Increased parity and gravidity correlated with a decrease in all hematological values of umbilical vein blood except of lymphocyte count (p<0.001). Prolonged duration of first and second stage was associated with higher mean leukocyte, neutrophil, platelet, hemoglobin and hematocrit values. Biochemical studies showed increase in activity of alanine amino transferase, aspartate amino transferase, total bilirubin, blood urea nitrogen and icterus index, with decrease in total serum protein.

Key words: Biochemical, Infected Cattle, Theileria, Annulata

**INTRODUCTION**

It is estimated that 250 million cattle in many countries including Iran, Turkey, India, and China are at a risk of the disease, which causes serious economic loss through bovine mortality and loses productivity [1, 2]. Because these diseases are most severe in recently introduced animals, they are a constraint on the importation of new breeds or improved stock. The two diseases with the greatest economic impact in cattle are East Coast fever (infection with *Theileria parva*) and tropical theileriosis (infection with *Theileria annulata*). *Theileria lestoquardi*, which causes a severe disease with a high morbidity and mortality rate, is the most important species in sheep and goats. Theileriosis results from infection with protozoa in the genus *Theileria* of the suborder Piroplasmorina. It can be associated with electrolyte imbalance, overdosing with calcium, digoxin, and cardiomyopathy. In buffaloes with theileriosis maybe there were these factors [3]. *Theileria* spp. are obligate intracellular parasites. The two most important species in cattle and water buffalo are *T. parva*, which causes East Coast fever, and *T. annulata*, which causes tropical theileriosis. A number of other *Theileria* species including *T. mutans, T. buffeli, T. velifera, T. taurotragi* and *T. sergenti* can also infect domesticated and wild ruminants. Many of these organisms are carried asymptptomatically, but some can cause anemia, and concurrent infections may increase the severity of East Coast fever or tropical theileriosis. *T. lestoquardi* (formerly *T. hirici*) is the most virulent species in sheep and goats. *T. separata* and the nonpathogenic species *T. ovis* also occur in small ruminants. Most of the previous studies on haematological parameters in *T. annulata* infection were carried out on experimentally infected calves [4, 5]. The present investigation was conducted to study haematological parameters in Friesian cattle naturally infected with *T. annulata*.

**MATERIAL AND METHODS**

This study was conducted during different seasons of the year; the samples were collected from animals which were presented to Urmia veterinary clinics and other farms with clinical signs include generalized lymphadenopathy, fever, anorexia and loss of condition with decreased milk yield. Petechiae and ecchymoses on the conjunctiva and oral mucous membranes. Lacrimation, nasal discharge. A total of 285 cattle, 2-5 years of age and from both sexes were examined and compare with 100 healthy animals. Careful clinical examination of all suspected animal were carried out. Blood was collected from...
jugular vein. Haematological examination was done including red blood cells count (RBCs) and white blood cells count (WBCs), hemoglobin (Hb), and packed cell volume (PCV) were done by (Automatic full digital cell counter, Beckman USA). In addition, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated mathematically, while differential leucocytic count was determined by four field meander method. Thin blood smears were taken from the vein of the ear and stained with Giemsa were used to identify the theileriosis and the percentage of parasitemia. Blood serum samples were tested for biochemical serum analysis of total proteins, albumin, glucose, ALT, AST, GGt, Iron, Copper, and total iron binding capacity (TIBC) were estimated spectrophotometry using commercial chemical kits supplied by Randox. The obtained data were statistically analyzed by means of computer based statistical program t test.

RESULT

The haematological examination (Table 1) revealed significant decreases (p ≤ 0.05) in the Hb content, PCV%, RBCs, MCHC, basophils, lymphocytes counts in sick cattl, compared to the control ones. Neutropenia, eosinopenia, lymphopenia, monocytopenia with a significant increase (p ≤ 0.05) in the numbers of thrompocytes were recorded. The infection caused significant increases in total urea and total nitrogen in infected cattles compared to the control (P<0.05). Depressed phosphor, calcium, sodium, chloride and iron concentrations were observed when compared to the control (P<0.05). No significant differences were observed in manganese and zinc and glucose value.

Table 1: Comparison of normal values of blood parameters of normal with infected cattle with theileriosis. *indicates P≤0.05

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bas %</th>
<th>Eos %</th>
<th>Mon %</th>
<th>Lym %</th>
<th>Band %</th>
<th>Neut %</th>
<th>PMN %</th>
<th>RBC 10^9/µl</th>
<th>Fib /µl</th>
<th>TTP /µl</th>
<th>MCHC %</th>
<th>MCV fl</th>
<th>Hb /g</th>
<th>Pcv %</th>
<th>WBC 10^9/µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.9</td>
<td>0.7</td>
<td>2.0</td>
<td>0.5</td>
<td>0.7</td>
<td>0.3</td>
<td>0.7</td>
<td>28</td>
<td>0.5</td>
<td>0.3</td>
<td>41</td>
<td>40</td>
<td>0.8</td>
<td>12</td>
<td>0.5</td>
</tr>
<tr>
<td>Infected animals</td>
<td>*0</td>
<td>*1</td>
<td>0.7</td>
<td>*12</td>
<td>36</td>
<td>*5/58</td>
<td>0.7</td>
<td>6/5</td>
<td>*30/5</td>
<td>*8/1</td>
<td>*30/5</td>
<td>*46</td>
<td>*8/6</td>
<td>*28</td>
<td>12/5</td>
</tr>
</tbody>
</table>

Table 2: Comparison of normal values of biochemical parameters of normal with infected cattle with theileriosis. *indicates P≤0.05

<table>
<thead>
<tr>
<th>Groups</th>
<th>NPN meq/l</th>
<th>Zn ppm</th>
<th>Megnesium meq/l</th>
<th>Fe meq/l</th>
<th>Color Minol/l</th>
<th>Potassium meq/l</th>
<th>Sodium meq/l</th>
<th>Calcium meq/l</th>
<th>Phosphorus meq/l</th>
<th>Glucose meq/l</th>
<th>Urea meq/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15/51</td>
<td>0/74</td>
<td>2/73</td>
<td>99</td>
<td>108</td>
<td>5/5</td>
<td>145</td>
<td>9/8</td>
<td>4/9</td>
<td>58/6</td>
<td>33/2</td>
</tr>
<tr>
<td>Infected animals</td>
<td>*20/3</td>
<td>0/71</td>
<td>*2/63</td>
<td>*71</td>
<td>*68</td>
<td>*82</td>
<td>*115</td>
<td>*7/4</td>
<td>*3/1</td>
<td>57/7</td>
<td>*52/6</td>
</tr>
</tbody>
</table>

DISCUSSION

Laboratory tests are tools helpful in evaluating the health status of an individual. It is important to realize that laboratory results may be outside of the so-called "normal range" for many reasons. These variations may be due to such things as race, dietetic preference, age, sex, menstrual cycle, degree of physical activity, problems with collection and/or handling of the specimen, non-prescription drugs (aspirin, cold medications, vitamins, etc.), prescription drugs, alcohol intake and a number of non-illness-related factors. Any unusual or abnormal results should be analyzed with different variants. Potassium is controlled very carefully by the kidneys. It is important for the proper functioning of the nerves and muscles, particularly the heart. Any value outside the expected range, high or low, requires medical evaluation. Sodium is also regulated by the kidneys and adrenal glands. There are numerous causes of high and low sodium levels, but the most common causes of low sodium are diuretic usage, One hundred animals each from on the basis of clinical signs (emaciation, anemia, difficulty in walking, shrinking of neck muscles, difficulty in rising up, sunken eyes, infertility, skin eczema) & the presence of ticks on the body. The clinical picture of diseased animals was not exactly the same as mentioned by Mahmmod [6]. According to them fever and swelling of lymph nodes are the main clinical findings in bovine theileriosis. Omer also mentioned similar findings [7]. Zoli et al [8] studied bovine theileriosis as under recognized disease in cattle. Confirmation of positive samples were carried out after blood examination. A total of 600 samples of blood were taken from during the months of July 2003 to September 2003 out of which 107 samples of blood proved to be positive by staining with Giemsa stain method. The overall incidence
The observed clinical findings in cattle with theileriosis were such as anorexia, corneal opacity, enlarged superficial lymph nodes. These findings were in agreement of Sandhu et al. [4] and Radostits et al. [9]. Anorexia could be attributed to persistent fever; moreover the enlargement of superficial lymph nodes could be explained by lymphoid hyperplasia in early stage of the disease. The corneal opacity was explained by Irvin and Mawmachi [10] as a result of white blood cells infiltration. Comparison of overall mean values of PCV, Total erythrocyte count and hemoglobin concentration showed that the values greatly reduced in the infected animals as compared to normal values, the lower RBC, HGB, and HCT values in animals with clinical signs were not a surprise because anemia is one of the pathognomonic signs of the disease. The type of anemia present requires proper attention as a lack of corresponding low values for erythrocyte indices suggests a benign macrocytic and hyperchromic anemia. The same phenomenon was observed by two studies. [11,12]. According to them the extensive hemorrhages, abdominal ulcers and persistence of parasitic stages in erythrocytes lead to lower hematological values. Madzingira studied protein antigen of *Theileria parva* macro-Schizont, immune precipitation with blood picture of diseased cattle [13]. According to them the total erythrocyte count markedly reduced in the animals having the infection. Marcotty studied immunization of tropical theileriosis by using infected and experimental methods [14]. They observed that the animals suffered from theileriosis showed anemia and schizogony. The ALT, AST, GGT, and BUN values showed no increase, in contrast with what had been reported by other researchers in severe infection. Therefore, the low BUN, Mg, AST, and GGT levels found in class 2 may be aspecific and related to the underlying conditions of fever, anorexia, weakness, and inactivity. Multiple factors may account for these findings: (1) smaller high-quality protein intake results in decreased BUN levels because nearly all the ingested proteins are used for protein synthesis (2) magnesium is an essential dietary element for animals, and green plants are an excellent source of Mg because it is contained in chlorophyll; (3) reduced exercise caused by bodily discomfort may explain the low AST and GGT values in class 2, because AST and GGT are usually considered to be markers of greater physical activity; (4) homeostatic mechanisms are efficient in maintaining plasma calcium levels within the reference range so low Ca levels are accompanied by low ALB values, given the interdependency of calcium and albumin metabolisms. The does not seem to be a particularly relevant parameter; similarly to our findings, Hasanpour found no changes in total WBC count in the sample they studied [15]. The only point of interest is the different behavior of the N and L. The fact that infected cattle without clinical signs had significantly lower N and higher L levels than did the animals in may be explained by the inversion generally occurring in the N/L ratio during the evolution from acute to subacute disease. The increase in neutrophils, which are the chemical mediators of acute inflammation, is followed by a predominance of lymphocytes, which are typical of a more specific immune response. Macrophage activation is known to occur during and a protective role has been documented for macrophages during infection with several theileriosis Macrophage-derived soluble factors have been postulated to be responsible for killing these parasites. The role of T cells and cytokines in fatal and resolving experimental babesiosis was demonstrated in mice. Hemoparasites were found in the red cells of 7% of the subjects with no clinical signs. The presence of parasites in healthy subjects triggers interleukin-12 (IL-12) induction, which then stimulates interferon gamma (IFN-γ) production by natural killer cells. Protection during acute infection results from IFN-γ-mediated macrophage activation and production of metabolites that are toxic to the pathogens.

**REFERENCES**

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