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Evaluation of Haematochemical Parameters in Crossbred Cattle Naturally Infected with *Theileria annulata* in Iran

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Abstract: The aim of this study was to investigate the variations of some blood biochemicals in crossbred cattle naturally infected with *Theileria annulata* in order to better understand of treating diseased animals. Serum biochemical parameters were studied in adult crossbred cattle naturally infected with *Theileria annulata* in Iran. One hundred cows affected with tropical theileriosis were studied and compared with 50 clinically healthy cows. Cattle clinically infected with *T. annulata* had significantly lower serum total protein, calcium, cholesterol and triglycerides concentrations and significantly higher alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity, phosphorus, sodium, potassium, bilirubin (direct and indirect) and Blood Urea Nitrogen (BUN) concentrations than the healthy cattle.

Key words: Haematochemical parameters, cattle, tropical theileriosis

INTRODUCTION

Tropical Theileriosis is one of the most prevalent and economically important fatal diseases of cattle in Iran (Hashemi-Fesharki, 1988; Mirzaei, 2007). According to the researches in Razi Institute of Iran, the principal causative agent of bovine theileriosis in this country is *Theileria annulata*, mainly transmitted by ticks of the genus *Hyalomma*. Every year theileriosis occurs when *Hyalomma* ticks are active in warm seasons. Tropical theileriosis is a progressive lymphoproliferative disease of cattle caused by the protozoan parasite, *T. annulata* (Taylor *et al.*, 1992; Omer *et al.*, 2003). The parasite acts as a serious problem to cattle production in endemic areas, causing lethal infections in exotic cattle and considerable mortality in indigenous and crossbred stocks (Forsyth *et al.*, 1997). The disease is observed in South Europe, North Africa, middle and South Asia and the Middle East and threatens approximately 250 million cattle (Young, 1981; Viseras *et al.*, 1997; Salih *et al.*, 2007).

Most of the previous studies on biochemical parameters in *T. annulata* infection have been carried out on experimentally infected calves (Laiblin *et al.*, 1978; Yadav and Sharma, 1986; Sandhu *et al.*, 1998; Singh *et al.*, 2001). The present research was undertaken to determine the incidence, distribution, epizootiology and pathogenicity of tropical theileriosis in Iran. The biochemical parameters in crossbred cattle naturally infected with *T. annulata* were studied as an aid to better understanding of the pathogenesis and supportive therapy of tropical theileriosis. Based on these aspects the present study was carried out to investigate the impacts of *Theileria annulata* on some serum components in crossbred cattle naturally infected with the parasite.

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MATERIALS AND METHODS

Animal Materials

In this study which was conducted from May to September 2006 blood biochemical parameters of 100 crossbred cows admitted to the veterinary hospital of Tabriz Azad University with clinical signs of theileriosis and 50 healthy cows from the same region was compared. All animal used in this study were aged between 1-4 years old and were of the same breed.

The infected animals were selected on the basis of clinical examination. Clinical symptoms of infected animals were including of swelling of lymph nodes, high temperature, lung oedema, paleness or petechiation of the mucous membranes. Blood smears were also prepared from an ear vein of each animal to confirm theileriosis microscopically. Giemsa staining of slides revealed piroplasms of *T. annulata* in the RBCs of infected group. Lymph node aspirates were taken from suspected cases to show Koch blue bodies.

Samples Collection

Venous blood samples for determination of biochemical parameters were collected into 5 mL tubes centrifuged at 3000 x g for 15 min and stored at -20°C for < 2 weeks before analysis.

Serum Analysis

Serum magnesium, phosphorus, calcium and bilirubin (direct and indirect), Blood Urea Nitrogen (BUN), total protein, cholesterol and triglycerides concentrations and activity of the serum enzymes alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate amino transferase (AST) were determined by spectrophotometry (Shimadzu Model AA 6200, Tokyo, Japan).

Sodium and potassium concentrations were determined by flame photometry (Digital Flame Analyzer Model 2655-00, Cole-palmer) Instrument Company, Chicago, IL, USA.

Statistical Analysis

Student's t-test was used for comparison of measured factors between two groups (controls and clinical cases).

All values were expressed as mean and SEM and $p < 0.05$ was considered as statistically significant.

RESULTS

In addition to the pyrexia and the swelling of superficial lymph nodes, which were used as indicators for clinical evaluation, inappetance tachycardia, dyspnoea and weakness were also recorded. Exophthalmia, lacrimation, salivation, bilirubinurea and mucohaemorrhagic diarrhoea were occasionally observed.

There were significant decreases in the serum total protein, calcium, cholesterol and triglycerides concentrations in the cattle infected with *T. annulata* compared with the controls.

There was, however, no significant difference in the concentration of magnesium between infected and the controls. The activity of the serum enzymes ALP, ALT, AST and the concentrations of phosphorus, sodium, potassium bilirubin (direct and indirect) and BUN in the affected cattle were significantly higher than the controls (Table 1).

Table 1: The effect of clinical infection with *Theileria annulata* on serum biochemical parameters (Mean±SEM) in crossbred cattle in Iran

Blood parameters	Controls	Infected cattle
ALP (IU L ⁻¹)	151.50±2.48	295.28±12.14 ^b
ALT (IU L ⁻¹)	18.40±2.48	26.88±0.880 ^b
AST (IU L ⁻¹)	60.05±2.16	150.30±11.06 ^b
Ca (mg dL ⁻¹)	10.76±0.16	8.28±0.280 ^b
Mg (mg dL ⁻¹)	2.21±0.30	2.28±0.250 ^{NS}
P (mg dL ⁻¹)	2.82±0.02	1.02±0.560 ^a
Na (meq L ⁻¹)	119.15±1.32	130.42±1.020 ^b
K (meq L ⁻¹)	7.05±1.16	8.36±0.270 ^b
Direct bilirubin (mg dL ⁻¹)	0.14±0.01	0.50±0.020 ^b
Indirect bilirubin (mg dL ⁻¹)	0.32±0.02	0.93±0.200 ^b
Total protein (g L ⁻¹)	6.43±0.16	5.67±0.090 ^b
Cholesterol (mg dL ⁻¹)	167.65±5.04	153.12±4.160 ^a
Triglyceride (mg dL ⁻¹)	84.65±1.62	72.64±3.130 ^a
BUN (mg dL ⁻¹)	15.74±2.18	24.63±2.070 ^b

Statistically significance between control and theileriosis groups: ^ap<0.05, ^bp<0.001, NS: Not significant

DISCUSSION

Laiblin *et al.* (1978) reported an increase in AST and no change in the ALT level during *Theileria annulata* infection. Yadav and Sharma (1986), Sharma *et al.* (1987) and Sandhu *et al.* (1998) reported a significant increase in the level of ALP. In the present study a significant increases in ALP, ALT and AST was also observed. These results agree with those of Sandhu *et al.* (1998) and Col and Uslu (2007). AST and ALT are involved in amino acid and carbohydrate metabolism. These enzymes are present in high concentrations in the muscles and liver. Elevation of these enzymes in the blood is indicator of organ necrosis (Murray *et al.*, 1996). *Theileria annulata* infection causes hepatic tissue damage that includes coagulative necrosis, distortion of hepatic cords and heavy infiltration of lymphocytes in the periportal areas, indicating sever damage to the hepatobiliary system due to hypoxia resulting from anaemia and jaundice (Sandhu *et al.*, 1998). In the present study, decreased serum calcium concentration in infected cattle could be attributed to the hypoalbuminaemia and kidney damage (Burtis and Ashwood, 1996).

Normal calcium levels were reported in previous experimental studies (Dhar and Gautam, 1977), but the present findings support those of Yadav and Sharma (1986) and Singh *et al.* (2001), who showed a decrease in blood calcium.

Omer *et al.* (2003) reported decrease in serum magnesium concentration in Friesian cattle naturally infected with *T. annulata*. However, the serum magnesium concentration was not significantly affected in the present study. The significant increase in the concentration of phosphorus in *T. annulata*-infected cattle could be attributed to the haemolytic anaemia caused by an immune-mediated haemolysis, resulting in an auto-immune reaction of affected erythrocytes (Hooshmand-Rad, 1976; Jain, 1993). The increased potassium and sodium concentrations in the *T. annulata*-infected cattle could be due to dehydration and kidney damage. The significant increase in concentration of bilirubin (direct and indirect) in the *T. annulata*-infected cattle may be related to the hepatic dysfunction and the, presumably, haemolytic anaemia. Similar results have been obtained by others (Hooshmand Rad, 1976; Yadav and Sharma, 1986; Sandhu *et al.*, 1998; Singh *et al.*, 2001; Omer *et al.*, 2003). The low Serum total protein concentration in cattle naturally infected with *T. annulata* was possibly due to hypoalbuminaemia and hypoglobulinaemia arising from liver failure. Singh *et al.* (2001) also reported significant decreases in the serum total protein, albumin and globulin concentrations and in the albumin:globulin ratio in crossbred calves experimentally infected with *T. annulata*. Biochemical analysis in the present study showed a significant decrease in serum cholesterol and triglycerides which is in line with the finding of Sharma *et al.* (1987) and Singh *et al.* (2001). However, Yadav and Sharma

(1986) reported an increase in the cholesterol concentration during the course of infection. The significant decrease in cholesterol and triglycerides in this study may be attributed to anorexia associated with the high rise of temperature and diarrhoea. A significant increase in BUN that was seen in this study could be due to the kidney damage. Sandhu *et al.* (1998) showed an increase in BUN and uric acid due to the kidney damage, since earlier investigation showed focal to diffuse coagulative histopathological changes necrosis, sever damage to collecting tubules, haemorrhages and lymphocytic aggregations in interstitial spaces. It has also been shown that parasitized lymphoid cells can infect non-lymphoid organs such as liver and kidney and inducing tissue damage (Forsyth *et al.*, 1999).

It was inferred from this study that supportive therapy for *T. annulata* infections in calves should include blood transfusions and suitable measures for correcting the defective liver and kidney functions in calves.

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