



RESEARCH ARTICLE

Hemato-Biochemical Alterations in Cross Bred Cattle Affected with Bovine Theileriosis in Semi Arid Zone

Imtiaz Ahmad Khan*, Ahrar Khan¹, Azhar Hussain², Asif Riaz³ and Asma Aziz²

Department of Pathobiology, PMAS-Arid Agriculture University, Rawalpindi; ¹Department of Pathology, University of Agriculture, Faisalabad; ²Veterinary Research Institute, Zarar Shaheed Road, Lahore; ³Department of Veterinary Basic Sciences, PMAS-Arid Agriculture University, Rawalpindi, Pakistan

*Corresponding author: imtiazarana@hotmail.com

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ABSTRACT

This study was carried out to ascertain the changes in hematology and serum constituents in cross bred cattle affected with bovine theileriosis in semi arid zone of Pakistan. A total of 50 cross bred cows of age 2-5 years were included in the study. Twenty animals of same age were kept as healthy controls. The affected animals showed signs and symptoms of bovine theileriosis i.e., high rise in body temperature, general debility, enlarged prescapular lymph nodes, mucosal hemorrhages, conjunctivitis, etc. These animals were subjected to examination of peripheral blood smears for the presence of *Theileria annulata* schizonts in infected mononuclear cells and piroplasms in red blood cells. Significant ($P \leq 0.05$) decrease was observed in total erythrocyte counts, packed cell volume, hemoglobin, serum total proteins, albumin, globulins, glucose, calcium, phosphorus, cholesterol and triglycerides concentrations in cattle affected with bovine theileriosis compared with healthy controls, while significant ($P \leq 0.05$) increase was observed in serum bilirubin and alanine transaminase of affected cattle compared with healthy controls. Non significant differences were observed in serum magnesium and uric acid concentration in both affected and healthy controls. These observations revealed that bovine theileriosis caused by *T. annulata* in cross bred cattle in semi arid zone is associated with hemato-biochemical alterations.

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INTRODUCTION

Pakistan is predominantly an agricultural country with a semi-arid continental subtropical climate. It is located between latitude 23° and 36°N and longitude 60° and 75°E. Livestock production is being hampered due to number of diseases and management issues including lack of proper tick control in the country (Saddiqi *et al.*, 2010). Among the diseases, tick born infections are of great importance. Bovine theileriosis is a tick-borne hemoprotozoan disease caused by *Theileria annulata* which is transmitted by *Hyalomma*, tick in tropics (Mirzaei, 2007). *T. annulata* is one of the most devastating blood parasite affecting cattle, buffalo, and sheep. The disease causes serious economic losses through mortality and loss of productivity (Glass *et al.*, 2003). The disease has devastating impact on small farmers which represent the majority of livestock owners in endemic areas. The methods currently used to protect

against tropical theileriosis are expensive and all have serious limitations in efficacy and sustainability (Hasanpour *et al.*, 2008). The prevalence, morbidity and mortality of theileriosis are considerably high (Aktas *et al.*, 2004).

Theileria enters the cattle host body during tick feeding in sporozoitic form, which instantly enters in leukocytes of mononuclear lineage, where they got mature into macroschizont form. Macroschizonts develop further into microschantons and ultimately into merozoites, which are liberated from the mononuclear cells. These merozoites invade erythrocytes and develop into piroplasms. The main symptoms shown by affected cattle are high fever and long lasting anemia, due to intra-erythrocytic parasitism by piroplasms (Chae *et al.*, 1996; Shiono *et al.*, 2001; Nazifi *et al.*, 2010a). The disease is lymphoproliferative in its early phases resulting enlargement of lymph nodes, later on enters lymphodestructive phase which is associated with a

pronounced leukopenia. In the piroplasms phase in erythrocytes, the parasite becomes infective for the tick. General debility, weight loss, anorexia, high body temperature, petechial hemorrhages on conjunctival mucosa, enlarged lymph nodes, anemia and cough are the predominant clinical symptoms (El-Deeb and Younis, 2009). Hematological and sero-biochemical alterations are the indicators of severity of disease and are considered to be good tools for the diagnosis, prognosis for effective therapy (Col and Uslu, 2007; Nazifi *et al.*, 2010b). Therefore, the present study was designed and executed to determine the changes in affected cross bred cattle with bovine theileriosis in semi arid zone in Punjab, Pakistan.

MATERIALS AND METHODS

Experimental animals and study zone

The study was conducted (July 2008 to June 2009) in semi arid zone in two districts (Jhelum and Mandi Baha ud Din) of Punjab, Pakistan. A total of 50 cross bred (Friesian X Sahiwal) cattle suffering from bovine theileriosis were selected for study. All animals used in this study were female ranged from 2 to 5 years of age. These animals were reared and maintained under the optimal field management conditions. The affected cattle were selected on the basis of clinical signs, treatment history and presence of piroplasms in the thin blood films and ticks on their bodies. A total of 20 clinically healthy (Friesian X Sahiwal) cattle of almost same age from tick-free farms were used as a control.

Clinical examination

All the animals were subjected to examination of mucousal membranes (conjunctival, nasal and oral) for hemorrhages and prescapular lymph nodes for enlargement. Rectal temperature of each animal suffering from bovine theileriosis was recorded. Thin blood smears were prepared from the ear tip of each animal showing rise in body temperature (>102 °F), enlarged lymph nodes, cachexia and poor general health. The smears were air-dried, fixed with methanol, stained with 10% Giemsa stain and examined under oil immersion objective of microscope for piroplasms in red blood cells and infected leukocytes for schizonts of *T. annulata* (Soulsby, 1982).

Collection of ticks and blood samples

Ticks were collected manually from the animals under study. These were identified for their morphology from various body regions in separate containers having lid according to standard criteria (Walker *et al.*, 2003).

About 10ml blood was directly drawn from jugular vein in clean glass test tube. Half of the blood was shifted to an EDTA containing test tube from each animal for hematological studies. Hematological parameters included were total erythrocyte count, hemoglobin concentration and packed cell volume. Serum from each blood sample was separated and stored at -20°C until further use. Sera were analyzed for total proteins, albumin, globulins, bilirubin, cholesterol, glucose, triglycerides, uric acid, calcium, magnesium, phosphorus and alanine transaminase concentrations using commercial kits by colorimetric methods using spectrophotometer.

Statistical analysis

The data thus generated were subjected to un-paired t-test to compare the difference of hematological and biochemical parameters at $P\leq 0.05$ using computer software Minitab 13. Chi-square test was applied to know the statistical difference in cured and died animals.

RESULTS

In the present study, 50 cross bred cattle showing clinical signs of theileriosis were included and confirmed by smear test for the presence of schizonts of *T. annulata* in blood cells. Blood sucking ticks were collected from different parts of the body and were identified as *Hyalomma* spp. The clinical findings including high rise in rectal temperature (103-106 °F), general debility, enlargement of prescapular lymph nodes, hemorrhages on mucosal membranes (conjunctival, nasal, and oral), cachexia, dyspnoea, lacrimation, conjunctivitis and eye ball protrusion were recorded. These animals were treated with Buparvaquone (ButalexTM 50mg/ml injection ICI, Pakistan Limited) @ 1ml for 20 kg BW and was repeated after 48 hours. The ratio of 44 (88.0%) cured animals was significantly higher than those (12.0%) died ($\chi^2= 20.574$; $df=1$, $P\text{-Value} = 0.001$).

Significantly ($P\leq 0.05$) decreased total erythrocyte counts, packed cell volume and haemoglobin concentration were observed in infected cattle as compared to non infected controls (Table 1). In infected cattle significantly ($P\leq 0.05$) decreased serum total proteins, albumins, globulins, glucose, calcium, phosphorus, cholesterol and triglycerides concentrations compared with negative controls were recorded. Significant ($P\leq 0.05$) increase was observed in serum bilirubin and alanine transaminase concentrations in infected cattle compared with non infected control group. Non-significant difference was observed in serum magnesium and uric acid concentrations compared with non infected control group (Table 1).

Table 1: Different hematological and biochemical parameters (Mean \pm SD) in cross bred cattle affected with bovine theileriosis in semi arid zone

Parameters	Units	Group	
		Infected	Non-infected
Hematological parameters			
Total Erythrocyte Counts	$10^{12}/\text{L}$	4.97 \pm 0.61*	5.72 \pm 0.61
Packed Cell Volume	%	24.06 \pm 2.20*	29.65 \pm 2.82
Hemoglobin Conc.	g/dl	7.56 \pm 0.68*	9.36 \pm 0.79
Serum biochemical parameters			
Total Proteins	g/dl	7.28 \pm 0.91*	9.56 \pm 0.72
Albumin	g/dl	2.92 \pm 0.52*	3.62 \pm 0.55
Globulins	g/dl	4.36 \pm 0.85*	6.01 \pm 0.62
Bilirubin	g/dl	0.706 \pm 0.14*	0.313 \pm 0.09
Alanine Transaminase	U/L	101.16 \pm 11.21*	65.52 \pm 12.24
Glucose	mg/dl	24.69 \pm 6.33*	37.56 \pm 12.03
Calcium	mg/dl	7.17 \pm 0.81*	9.50 \pm 0.83
Phosphorus	mg/dl	3.822 \pm 0.54*	5.821 \pm 0.74
Magnesium	mg/dl	2.568 \pm 0.29	2.495 \pm 0.18
Uric Acid	mg/dl	4.062 \pm 0.52	4.06 \pm 0.59
Cholesterol	mg/dl	101.08 \pm 17.43*	146.05 \pm 23.61
Triglycerides	mg/dl	11.035 \pm 3.85*	17.605 \pm 4.14

Values with asterisk in a row differ significantly ($P\leq 0.05$) from that on non-infected group.

DISCUSSION

Hemoprotozoa like *Theileria*, *Babesia* and *Anaplasma* are considered to be the most important blood parasites of cattle and buffaloes in Pakistan (Khan *et al.*, 2004). Bovine theileriosis of cattle is a major constraint in the improvement of the livestock industry in larger parts of the World (El-Deeb and Younis, 2009). Theileriosis caused by *T. annulata* is a serious hemoprotozoan disease of cattle in tropics and sub-tropics. *T. annulata* is transmitted by ticks of the genus *Hyalomma* (Mirzaei, 2007).

Blood smears revealed abnormal erythrocyte morphology along with presence of theileria schizonts in infected mononuclear cells and erythrocytes in the present study. The infected erythrocytes appeared as echinocytes thorn-like protrusions on the surface. These variations in erythrocytic morphology are mainly due to theileria schizonts, erythrocytic oxidation, intravascular thrombi, and immune-mediated processes (Stockham *et al.*, 2000). In the present study, total erythrocyte counts, packed cell volume percentage and hemoglobin concentrations were significantly lower in infected animals as compared with non-infected cattle. Such findings have already been reported by Col and Uslu (2006) and Hasanpour *et al.* (2008). The decrease in erythrocyte count, packed cell volume and hemoglobin concentration resulted anemia in infected group. It has been reported that anemia occurs in lateral stages of theileriosis following parasitaemia (Mbassa *et al.*, 1994). Immune-mediated mechanism like erythrophagocytosis might be responsible for the destruction of erythrocytes infected with theileria schizonts (Uilenberg, 1981). Removal of piroplasm infected erythrocytes by macrophages in the reticuloendothelial system has been suggested as a cause of anemia (Campbell and Spooner, 1999). The decreased erythrocyte counts could be attributed to increased levels of activated complement products (Omer *et al.*, 2002). Moreover, oxidized erythrocytes are destroyed by erythrophagocytosis, oxygen free radicals may also be responsible for anemia (Mbassa *et al.*, 1994).

In the present study serum total protein, albumin and globulin concentrations in infected cattle were decreased. The findings were in line with the findings of Col and Uslu (2007) who reported hypoproteinemia and hypoalbuminemia. The results were in agreement with the findings of Yadav and Sharma (1986) and Singh *et al.* (2001). The decreased serum proteins concentrations may be attributed to extra-vascular proteinaceous fluid in body cavities due to diseased lymph nodes resulting edema (Stockham *et al.*, 2000). The decrease in total serum proteins was attributed to low albumin and globulin concentrations as a result of liver failure (Omer *et al.*, 2003). In the present study serum bilirubin concentration was increased in cattle infected with *T. annulata* compared with healthy controls. The findings were in accordance with the findings of Col and Uslu (2007) who also reported an increased serum bilirubin concentration. The increased serum bilirubin concentration may be attributed to hemolysis of parasitized erythrocytes in reticuloendothelial system and lymph nodes (Sandhu *et al.*, 1998). Further more, it has been reported that the increase in serum bilirubin concentration may be due to

hemolytic anemia and hepatic dysfunction (Omer *et al.*, 2003). Serum AST and ALT concentrations are the indicators of hepatic function (Forsyth *et al.*, 1999). In present study an increase in ALT concentration was observed in infected cattle compared with healthy controls which represent hepatic dysfunction. Similar findings were observed by Col and Uslu (2007) who also reported an increase in ALT concentration. The rise in serum ALT concentration may be due to muscular trauma as a result of prolonged recumbency due to bovine theileriosis (Col and Uslu, 2007).

In the present study, there was a decrease in serum glucose concentrations in infected cattle with *T. annulata* compared with healthy controls. The findings were in line with the findings of Col and Uslu (2007), they reported that, the decreased glucose serum concentration could be due to utilization of glucose by theileria in the blood and hepatic dysfunction as a result of theileria infection. The findings were contrary with the findings of Sandhu *et al.* (1998) who reported a non significant decrease in serum glucose in calves affected with theileriosis. There were decreased serum calcium and phosphorus concentrations in cattle infected with *T. annulata* in the present study which was also decreased in the previous studies (Omer *et al.*, 2003). Col and Uslu (2007) reported that hypocalcaemia in their study could be due to hypoproteinemia, decreased dietary intake, intestinal malfunction and kidney damage and the decreased phosphorus concentration due to diarrhea and renal wasting. Non significant differences were observed in serum uric acid and magnesium concentrations in infected and healthy controls, while previous studies revealed increased urea level in infected cattle with *T. annulata* (Sandhu *et al.*, 1998). There were significant decreases in serum cholesterol and triglycerides concentrations in theileria infected cattle as in previous findings reported by Singh *et al.* (2001). These decreases in cholesterol and triglyceride levels may be ascribed to anorexia and diarrhea (Col and Uslu, 2007). The findings were otherwise than the findings of Yadav and Sharma (1986) who recorded a marked increase in serum cholesterol concentration in experimentally *T. annulata* infected cattle from day 0 to day 15, followed by a sudden fall, reaching values below the pre-infection level by day 40. They reported that, this could be due to liver damage that results in a concurrent increase in the level of fats with the reduction of sugar and protein.

One the basis of present study's findings, it was concluded that bovine theileriosis caused by *T. annulata* in cross bred cattle in semi arid zone greatly influenced the hemato-biochemical constituents. These changes may provide help to understand the disease pathogenesis and could be used as a tool for diagnosis, prognosis and evaluation of the treatment given.

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