

Haematobiochemical alterations in crossbred cattle suffering from theileriosis

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Abstract

The present study was conducted to determine the alterations in haematological and biochemical parameters in cross bred cattle of two organized herds suffering from theileriosis. Thirty seven samples from Farm I and 29 blood samples from Farm II were collected randomly and screened for parasite microscopically. Cattle were classified into clinically affected animal and apparently healthy animals on the basis of clinical signs and microscopic examination of stained smears. Haematobiochemical values of clinically infected cattle (n=8 & 10) were compared with apparently healthy cattle (n=15 & 19) in Farm I & Farm II, respectively. Statistically significant reduction in mean values of Hb, RBC and PCV and non significant increase in total leukocyte count with variable changes in neutrophil and lymphocyte count was observed as major haematological alteration in infected cattle in both the farms. Biochemically, significant high activity of AST enzyme was observed in infected cattle indicative of changes associated with hepatic injury. Haematobiochemical alterations revealed anemia and impaired liver function in infected cattle. It can be concluded that along with specific therapy, supportive therapy including hematinics and hepatoprotectant agents should be used for treatment of theileriosis in cattle.

Keywords: Theileriosis, Cross bred cattle, Haematobiochemical changes, AST

Tropical theileriosis is a tick borne hemoprotozoan disease caused by *Theileria annulata*. Cross bred cattle and young calves are highly susceptible to the disease. In India, annual losses due to theileriosis are estimated to be Rs. 8426.7 crores (Narladkar, 2018). Disease inside the host starts with inoculation of sporozoites by ticks which transmutate into microschorizont in the lymph nodes (lymphocytes), the schizont undergoes further multiplication and differentiation to merozoites, which are released upon lysis of the infected lymphocytes. Merozoites affect the erythrocytes leading to development of piroplasms (Singh *et al.*, 2014). The disease is lymphoproliferative in its early phase resulting in enlargement of lymph nodes, later on enters lymphodestructive phase which is associated with a pronounced leucopenia (Khan *et al.*, 2011).

The main clinical signs shown by affected cattle are fever, pale mucous membrane and swollen lymph nodes which indicate about the disease. For confirmation of disease status, microscopic examination of Giemsa stained blood smear is considered as Gold standard. Presence of characteristic piroplasm either ring shaped, dot shaped or comma shaped in stained smear is considered as positive. Hematological and

sero-biochemical alterations are the indicators of severity of disease and are considered to be good tools for the diagnosis and prognosis for effective therapy (Nazifi *et al.*, 2010). Therefore, the present study was conducted to determine the impact of parasite on health status of cattle by determining haematological and biochemical parameters of cattle suffering from clinically theileriosis.

Material and Methods

Sample collection and area of sampling

For estimating the haematological and biochemical alterations in animals suffering from clinical theileriosis, 37 blood samples from Monu farm, Village Tokas, Hisar (Farm I) and 29 blood samples from Pehalwan Farm, Village Lahli, Rohtak were collected (farm II) and analyzed. Approximately 5 ml of blood samples, 2ml in vials coated with potassium EDTA and 3 ml in serum vials without anticoagulant was collected from all the animals. Clinical examination of animals viz. recording of rectal temperature, lymph node examination and mucous membrane examination was done to differentiate the clinically infected animals from non-clinical or apparently healthy animals correlating with microscopic examination findings of stained blood smears.

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Conventional parasitological examination (Giemsa Staining)

Paired thin blood smear prepared from collected samples were stained with Giemsa stain for 30 min (Benjamin, 1978). Blood smears were examined for intraerythrocytic forms (signet ring, dot, or comma shaped) of *T. annulata* piroplasms under 100 × objective magnifications. About 20 microscopic fields per slide were observed to view the parasite. The presence of single piroplasm was recorded as positive for particular parasite. Animals were classified to be suffering from theileriosis and apparently healthy as per the clinical signs shown by the animals.

Haematological examination

Complete hematological examination was done using fully automated haematology cell counter (MS4s, Melet Schloesing Laboratories, France). The parameters measured were haemoglobin (Hb) in g/dl, total leucocyte count (TLC) in $10^3/\text{mm}^3$, differential leukocyte count (DLC lymphocytes (L) in %, monocytes (M) in %, neutrophils (N) in %, eosinophils (E) in % and basophils (B) in %.

Biochemical examination

The serum samples were analyzed for estimation of biochemical test profile by using fully automated random access clinical chemistry analyzer (EM Destiny 180, Erba Diagnostics Mannheim GmbH-Germany). The serum biochemical parameters measured were SGOT in U/L, total protein in g/dl, albumin in g/dl, bilirubin

direct (BID) in mg/dl, bilirubin total (BIT), urea in mg/dl, creatinine in mg/dl, calcium (Ca) in mg/dl and phosphorus (P) in mg/dl.

Statistical analysis

For comparison of results, Independent T test was performed for determination of mean values and to compare positive sample values with negative samples values.

Results and Discussion

Results of haematological analysis of blood samples of Farm I and Farm II have been depicted in Table I. Mean haemoglobin values, mean packed cell volume and mean total erythrocyte count was found to be significantly lower ($P \leq 0.05$) in cattle suffering from theileriosis as compared to apparently healthy cattle in both the farms. Similar findings of anemia in cattle suffering from theileriosis have been observed by Khan *et al.*, 2011; Ganguly *et al.*, 2015; Neelam *et al.*, 2017; Somu *et al.*, 2017; Abdel-Hamied *et al.*, 2020. Many factors have been proposed to contribute for anemia observed in affected cattle including direct destructive effect of intraerythrocytic piroplasms, erythrophagocytosis of parasitized RBCs by macrophages, suppressed erythropoietic activity (Ugalmugle *et al.*, 2010), damaging effect of toxic metabolites of the protozoan, persistent blood loss caused by blood sucking-ticks (Geerts *et al.*, 2001) and suppressing effects of tumor necrotizing factor- α on erythropoiesis (Boulter and Hall, 2000).

Non-significant increase in total leukocyte count

Table I. Haematological alterations in cross bred cattle from Farm I and Farm II suffering from theileriosis

Animal Farm	Monu Farm, Village Tokas, Hisar (Farm I)		Pehalwan farm, Village Lahli, Rohtak (Farm II)	
	Infected cattle (n=8)	Apparently healthy cattle (n=15)	Infected cattle (n=10)	Apparently healthy cattle (n=19)
Hb (g/dl)	7.06±0.34 *	9.55±0.30	6.62±0.41*	7.98±0.29
PCV (%)	23.62±1.01*	31.3±0.84	26.80±1.49*	32.35±1.30
TEC (10^6 /dl)	5.38±0.24*	6.53±0.19	5.47±0.58*	6.94±0.27
TLC (10^3 /ml)	6.51±1.80	5.55±0.46	6.08±0.57	5.32±0.50
Neutrophil (%)	61.87±4.02	54.55±3.48	3.35±0.65	6.15±1.50
Lymphocyte (%)	27±3.58	32.39±1.97	93.59±1.03	89.95±2.24
Monocyte (%)	3.63±0.38	4.2±0.36	1.54±0.20	1.94±0.23
Basophil (%)	0.5±0.19	0.4±0.13	0.11±0.03	0.07±0.03
Eosinophil (%)	7.13±2.62	8.33±2.41	1.40±0.20	1.82±0.87

* indicates significant alteration in values ($p < 0.05$)

was observed in clinically infected cattle from both the farms. This is in accordance with the results of Ramin *et al.*, 2011, Ganguly *et al.*, 2015 and Somu *et al.*, 2017 whereas in contrast with the findings of Modi *et al.*, 2015 showing significant increase in TLC levels and Tuli *et al.*, 2015 showing statistical significant decrease in level of TLC. On differential leukocyte counting, lymphopenia was observed in samples from Farm I whereas lymphocytosis was observed in Farm II samples. With regard to differential leukocyte count, different researchers have shown increase in lymphocyte count in initial stage of infection (Tuli *et al.*, 2015; Somu *et al.*, 2017 and Abdeil-hamied *et al.*, 2020) and decrease in lymphocyte count (Ugalmugle *et al.*, 2010 and Modi *et al.*, 2015) in later stages. In present study, difference in lymphocyte count was observed as decrease in Farm I and increase in Farm II. This difference in lymphocyte count between two farms can be attributed to disease being present in different stages in animals of these farms. Leukocytosis with lymphocytosis is a feature of disease in initial stage and is mainly seen due to intra-lymphocytic theilerial parasites transforming the host cells, leading to clonal growth of lymphocytes (Yamaguchi *et al.*, 2010). Comparative lower lymphocyte values found in Farm I in present study indicates later stage of theileriosis being present in these animals. This can be due to large-scale destruction of leukocytes by the protozoans as reported by Abd El-Hamed *et al.*, 2016 coupled with the suppressed leukogenesis caused by the adverse effects of the protozoan toxic metabolites (Hussein *et al.*, 2007).

Biochemical alterations observed in cattle suffering from theileriosis in Farm I and Farm II has been summarized in Table 2. Statistical analysis of serum parameters of infected cattle in both the farms revealed significant increase in aspartate aminotransferase level (SGOT) as compared to values in non-infected cattle within same herd. Regarding liver enzymes, significant high ($p < 0.05$) AST activities were observed in the sera of diseased cows. Similar results were recorded by Singh *et al.* (2001), Omer *et al.* (2003) and Kachhawa *et al.* (2016) who attributed the increased AST activity to the hepatic injury occurred during the disease. AST also exists in RBCs; therefore, the increased RBCs lysis occurred during the disease could be an important cause of elevated serum AST activity (Latimer, 2011).

Mean total protein level was found to be significantly decreased in infected cattle as compared to non-infected cattle in Farm I whereas protein level in Farm II was found to be far below the normal range and non-significant difference was found between infected and non-infected cattle. Regarding protein picture, the results showed a decrease in the serum total protein ($p > 0.05$) accompanied with highly significant ($p < 0.001$) decrease in the serum albumin in diseased cattle as compared to control which is in conjunction with the results of Kachhawa *et al.*, 2016; Devadevi *et al.*, 2018 and Abubakar *et al.*, 2019. This could be attributed to the decreased albumin synthesis caused by decreased feed intake and the impaired liver function in diseased cases. *T. annulata* has harmful effects on hepatocytes

Table II. Biochemical Alterations in cross bred cattle from Farm I and Farm II suffering from theileriosis

Animal Farm	Monu Farm, Village Tokas Hisar (Farm I)		Pehalwan Farm, Village Lahli Rohtak (Farm II)	
	Infected cattle (n=8)	Non infected cattle (n=15)	Infected cattle (n=10)	Non infected cattle (n=19)
SGOT (U/L)	76.94±9.00*	58.40±3.25	92.36±11.88*	57.57±2.45
Phosphorous (mg%)	5.56±0.58	5.83±0.35	6.318±0.35	6.435±0.47
Total Bilirubin (mg%)	0.16±0.02	0.15±0.01	0.11±0.01	0.09±0.05
Direct Bilirubin (mg%)	0.15±0.05	0.11±0.01	0.12±0.02	0.12±0.01
Urea (mg%)	14.34±2.10	9.87±1.72	49.34±4.34	45.47±6.39
Creatinine (mg%)	0.30±0.02	0.36±0.05	1.48±0.12	1.70±0.23
Calcium (mg%)	12.11±0.59	11.94±0.34	9.38±0.49	9.05±0.52
Protein (g%)	6.50±0.22	8.43±0.30	3.82±0.32	3.94±0.35
Albumin (g%)	2.46±0.17	2.43±0.11	2.93±0.09*	3.23±0.08
Globulin (g%)	4.04±0.05	6.00±0.19	0.89±0.23	0.71±0.27

* indicates significant alteration in values ($p < 0.05$)

of infected animals resulting in hepatic damage in the form of coagulative necrosis, hepatic cords distortion and heavy lymphocytic infiltration in the periportal areas as was explained by Sandhu *et al.* (1998) who reported macroscopic and microscopic damage in hepatic tissues of calves naturally infected with *T. annulata*. The liver damage associated with bovine theileriosis could be referred to the harmful effects of the toxic metabolites of the protozoan on the liver (Hussein *et al.*, 2007), inflammatory changes in hepatic cells as a result of trapping of the damaged infected erythrocytes, and lymphocytes (Abubakar *et al.*, 2019) and degenerative changes in liver cells caused by anemic hypoxia.

. Non-significant increase in total bilirubin, direct bilirubin, urea and calcium level and a non-significant decrease in phosphorous and creatinine was observed in infected cattle as compared to non-infected cattle in Monu Farm, Hisar. Concerning kidney functions, the obtained results in the present study revealed no significant changes in serum urea and creatinine levels in diseased animals. These findings are in accordance with those of Devadevi *et al.* (2018) and Abubakar *et al.* (2019) but disagree with the results of Ganguly *et al.* (2015) and Somu *et al.* (2017) who reported significant elevation of serum urea and creatinine concentrations in cattle with theileriosis. On the basis of present study results, it can be concluded that *T.annulata* greatly influence the health of cattle leading to anaemia and impaired liver function. Therefore, it is advised to add hematinics and hepatoprotectant agents along with specific therapy.

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