

HEMATO-BIOCHEMICAL PROFILING OF *THEILERIA*-INFECTED CATTLE AND BUFFALOES: AN IN-DEPTH INVESTIGATION INTO CLINICAL SIGNALMENT AND PATHOPHYSIOLOGICAL IMPLICATIONS

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ABSTRACT

Bovine theileriosis is considered as a major barrier to improving output from India's vast bovine population. A one-year study was conducted to determine the hemato-biochemical alterations and Clinical signs in *Theileria* infected cattle and buffaloes in Indore district from July 2022 to June 2023. In a group of 480 dairy animals, 72 tested positive, resulting in an incidence rate of 15%. The incidence of theileriosis was found to be significantly different ($p < 0.01$) between cattle (21.25%) and buffaloes (8.75%). *Theileria*-infected cattle showed significant declines ($p < 0.01$) in Hb, PCV, TEC, lymphocytes, and MCHC, while total leukocyte count increased ($p < 0.01$). Basophils and MCV did not change significantly. Infected buffaloes had increased TLC, basophils and decreased mean Hb, PCV and lymphocytes. No changes were found in eosinophils. Significant increases in ALT, AST, creatinine, bilirubin, and BUN were found ($p < 0.01$) in dairy animals. Predominant clinical signs observed in animals suffering from theileriosis were reduced milk yield (100%), swollen prescapular lymph node (83.33%), fever (88.89%), anorexia (58.33%), anaemia (58.33%), depression (51.39%), and respiratory distress (45.83%) recorded, respectively.

Keywords: *Theileria*, Hemato-Biochemical, Clinical signs, Cattle, Buffaloes.

INTRODUCTION

Bovine tropical theileriosis is a significant impediment to the dairy industry and livestock production in India. It is a tick-borne haemoprotozoan disease that affects cattle and buffaloes, caused by *Theileria annulata*. Collectively, various *Theileria* species affect cattle and buffaloes. The most pathogenic and economically important species are *Theileria annulata* (Bovine tropical theileriosis), *Theileria parva* (East Coast fever) and *Theileria orientalis* (Oriental theileriosis) are transmitted by *Ixodid* ticks of the genera *Hyalomma* and *Rhipicephalus* species. Theileriosis causes severe economic loss due to decreased weight gain, drop in milk yield, abortions, and in some cases leads to death in animals. Globally, the annual economic loss of US\$ 13.9 to US\$ 18.7 billion is due to tick-borne diseases as reported earlier (Atif *et al.*, 2012). The annual economic loss due to bovine tropical theileriosis in India has been estimated to the tune of US\$ 384.3 million per annum (Minjauw and McLeod, 2003). However in a recent study, these losses were calculated to the tune INR 8,427 crores annually (Narladkar, 2018). The prevalence of theileriosis in cattle and buffaloes is predicted to be 17% and 7%, respectively, based on peripheral blood smear examination (Singh *et al.*, 2022). The disease is usually characterized by high temperature, lymph node enlargement, and reduction in milk yield, progressive anaemia, lacrimation, hyper salivation, diarrhoea or constipation and in some cases dyspnea also (Sharma *et al.*, 2019). However *Theileria annulata* preferentially infects B-lymphocytes and cells of myeloid lineage, while *Theileria parva* infects T-lymphocytes (Swami, *et al.*, 2019). Laboratory diagnosis of haemoparasites in cattle is largely based on the microscopic examination of peripheral blood smears. It depends on various factors including level of parasitaemia, quality of blood smear, technical expertise and the staining method used (Samantaray *et al.*, 2008). Diagnosis of Theileriosis is mainly based on clinical symptoms and microscopic examination of stained blood and lymph node biopsy smears. But smear method is often associated with technical problems, wrong diagnosing and low sensitivity in diagnosing the carrier cows (Shayan and Rahbari 2005). Hemet-biochemical parameters also help in better understanding the level of disease, prognosis and diagnosis of the theileriosis.

MATERIALS AND METHODS

Collection of Data, Blood Samples and Screening for Theileriosis

Total 480 blood samples (192 cattle, 192 buffaloes and 48 cattle calves, 48 buffalo calves) were collected from Indore district of Madhya Pradesh, specifically in the tehsils of Depalpur, Sawer,

Mhow, and Indore was conducted for a period of one year, from July 2022 to June 2023 from dairy animals and calves with clinical signs similar to theileriosis were examined. The clinical signs, age, sex, breed, season etc. were recorded. Blood samples (5 ml) were collected aseptically from the jugular vein of cattle and buffaloes.

For biochemical analysis, 2 ml of blood were kept in vials with EDTA anticoagulants and 3 ml of blood were kept in vials without anticoagulants. Blood samples were transported in iceboxes to the Department of Veterinary Parasitology, College of Veterinary Science and A.H., N.D.V.S.U., Mhow (M.P.). The blood samples were screened for theileriosis by examination of Giemsa stained blood smears. A total of 480 blood sample were screened and 72 were found positive. The positive blood samples were further put to haematological examination and 72 serum samples were collected for biochemical studies.

Hemato-Biochemical and Microscopic Examination

Thin blood smear on slide were prepared immediately as quick as possible after collection of blood. A procedure described by Rathore and Sengar (2005) were followed with some modifications. Thin blood smears were prepared and fixed with methanol for 30 seconds, allowed to dry and stained in a 9:1 dilution of Giemsa's stain for 40 min. Subsequently, it were washed with distilled water and allowed to dry. The stained slide was examined under compound microscope in oil immersion lens at 1000X magnification. Blood samples for haematology were examined for estimation of Hb - (gm/dl), MCV - (fL), MCHC - (gm/dl), TEC - (Millions/cu mm), PCV - (Percentage), TLC - (Thousand/cu mm) and DLC (Percentage) etc. Blood samples were analysed for various biochemical parameters including Alanine amino transferase (IU/L), Aspartate amino transferase (IU/L), Serum creatinine (mg/dl), Serum bilirubin (mg/dl), and Blood urea nitrogen (mg/dl). The analysis was conducted using an auto-analyzer (Jain, 1986) and biochemical analyzer with the use of commercial kits (Brar *et al.*, 2002).

Statistical Analysis

The average of hemato-biochemical values in theileriosis positive dairy animals were compared with that from apparently healthy dairy animals. The apparently dairy animals were randomly selected from field. The comparison was done to observe the effect of theileriosis on the haematobiochemical parameters in dairy animals. Effect of theileriosis on haematological and biochemical parameters in dairy animals was done using one way ANOVA (Snedecor and Cochran, 1994) by using SPSS software, and expressed as mean \pm SE, with $P < 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Haematological Alterations in *Theileria* Infected Cattle and Buffaloes

The cattle infected with theileriosis in the study exhibited a statistically significant decrease ($p < 0.01$) in the mean levels of Hb (10.26 ± 0.21 vs 7.38 ± 0.16), PCV (29.24 ± 0.35 vs 22.30 ± 0.49), TEC (4.71 ± 0.14 vs 3.62 ± 0.08), lymphocytes (62.6 ± 1.44 vs 31.57 ± 0.24), a ⁷⁴ MCHC (35.1 ± 0.54 vs 33.12 ± 0.22), while there was a statistically significant increase ($p < 0.01$) in the total leukocyte count (7.76 ± 0.25 vs 13.1 ± 0.12), monocytes (4.4 ± 0.37 vs 7.02 ± 0.12), agranulocytes (30.31 ± 1.92 vs 59.2 ± 0.31), and eosinophils (2.1 ± 0.28 vs 1.57 ± 0.10) in infected cattle compared to non-infected cattle. Negligible disparities were observed in basophils and MCV with values of 0.60 ± 0.27 compared to 0.65 ± 0.10 , and 62.41 ± 1.23 compared to 61.85 ± 0.72 , respectively. The study revealed significant haematological changes in buffaloes infected with theileriosis. There was a significant decrease in mean Hb ($13.210.22$ vs. $10.20.20$), PCV ($36.470.62$ vs. $28.650.29$), TEC (5.84 ± 0.14 vs 4.35 ± 0.07), and lymphocytes ($60.40.67$ vs. $38.10.81$), with a corresponding significant increase in TLC ($11.980.29$ vs. $14.010.14$), AGR (31.81 vs. $54.670.80$), and basophils ($0.80.25$ vs. $0.240.10$) in the infected buffaloes compared to the non-infected ones ($p < 0.01$). There were no statistically significant differences observed in the levels of eosinophils (1.80 ± 0.25 vs. 1.57 ± 0.16), MCHC (36.24 ± 0.38 vs. 35.54 ± 0.43), monocytes (5.70 ± 0.47 vs. 5.43 ± 0.30), and MCV (62.53 ± 0.56 vs. 65.94 ± 0.63). The data is presented in Table 1.

A significant difference was noticed in the RBC between the infected group and control groups. Similar alterations in animals naturally infected with *T. annulata* infection were also observed by Omer *et al.* (2002) and Ellah and AL-Hosary (2011). Acute *T. annulata* infections have been linked by multiple researchers to a decline in red blood cells counts, PCV and haemoglobin levels (Rayula and Hafeez 1995). The observed decline in RBC count leading to the development of anaemia could potentially be related to the presence of intra-erythrocytic piroplasms (Preston *et al.*, 1992) and/or an autoimmune response (Hooshmand-Rad, 1976). However, it is also plausible that this decrease is caused by erythro-phagocytosis rather than a lysis triggered by parasites (Boulter and Hall, 2000). The present observation has revealed the occurrence of anaemia in animals that have been infected with *T. annulata*. The release of TNF α by infected cells has been found to be accountable for the inhibition of haematopoietic progenitors and the subsequent decrease in red blood cell production (Tizard, 1992).

Additionally, it has been observed that this phenomenon leads to a decrease in the lifespan of red blood cells, ultimately resulting in the development of anaemia.

The present investigation unveiled the existence of two distinct forms of anaemia, namely microcytic hypochromic anaemia reported in infected cattle and macrocytic hypochromic anaemia in buffaloes. Holstein cattle and crossbred cattle have been found to exhibit a macrocytic hypochromic anaemia, as described by Omer *et al.* (2002), Durrani (2008), and Modi *et al.* (2015). In their study, Ganguly *et al.* (2015) documented the presence of normocytic hypochromic anaemia in crossbred cattle infected with *T. annulata*. The occurrence of anaemia may be attributed to both an immunological response (Hooshmand-Rad, 1976) and the effect of intraerythrocytic piroplasms (Preston *et al.*, 1992). Omer *et al.* (2002) also reported a statistically significant reduction in lymphocyte count in the infected group compared to the control group. This decrease could perhaps be attributed to the destruction of lymphocytes in lymphoid organs and their migration into different organs

Table 01 Haematological Alterations in *Theileria* Infected Cattle and Buffaloes

Haematological parameters	Healthycattle	Infectedcattle	Healthy buffaloes	Infected buffaloes
	n=10	n=51	n=10	n=21
Hb(gm/dl)	10.26±0.21	7.38±0.16**	13.21±0.22	10.2±0.20**
PCV (%)	29.24±0.35	22.3±0.49**	36.47±0.62	28.65±0.29**
TEC(10 ⁶ /cumm)	4.71±0.14	3.62±0.08**	5.84±0.14	4.35±0.07*
TLC(×10 ³ /cu mm)	7.76±0.25	13.1±0.12**	11.98±0.29	14.01±0.14**
LYM(%)	62.6±1.44	31.57±0.24**	60.4±0.67	38.1±0.81**
MON(%)	4.4±0.37	7.02±0.12**	5.7±0.47	5.43±0.30
GRA(%)	30.3±1.92	59.2±0.31**	31.8±0.81	54.67±0.80**
Eosinophils	2.1±0.28	1.57±0.10*	1.8±0.25	1.57±0.16
Basophils	0.6±0.27	0.65±0.10	0.8±0.25	0.24±0.10*
MCHC(gm/dl)	35.1±0.54	33.12±0.22**	36.24±0.38	35.54±0.43
MCV(fl)	62.41±1.23	61.85±0.72	62.53±0.56	65.94±0.63

NS=Non Significant, **p<0.01, *p<0.05

Biochemical alterations in *Theileria* infected cattle and buffaloes

In cattle infected with theileriosis, there was a statistically significant increase (p<0.01) in the

mean values of ALT (31.06±0.41 vs. 63.25±0.20), AST (100.98±0.56 vs. 153.15±0.70), serum creatinine (1.02±0.07 vs. 1.17±0.01), serum bilirubin (0.45±0.03 vs. 0.77±0.01), and blood urea nitrogen (25.93±0.51 vs. 32.61±0.23). In a comparable manner, a statistically significant difference ($p < 0.01$) was noted in the mean values of ALT (23.68 ± 0.81 vs 63.60 ± 0.86), AST (117.68 ± 1.30 vs 154.71 ± 1.45), serum creatinine (1.49 ± 0.02 vs 1.85 ± 0.03), serum bilirubin (0.25 ± 0.01 vs 2.24 ± 0.01), and blood urea nitrogen (26.69 ± 0.88 vs 46.22 ± 0.61) in buffaloes infected with theileriosis. The data are presented in Table 2.

Elevated serum activities of AST and ALT have been found to be strongly correlated with hepatic injury induced by the protozoa (Forsyth *et al.*, 1999). Moreover, it is worth noting that an observable rise in serum AST and ALT activities could potentially be attributed to muscle trauma resulting from extended periods of clinical recumbency in the context of theileriosis (Sandhu *et al.*, 1998). The observed significant increase in the levels of serum bilirubin can be attributed to two potential mechanisms: (1) impaired liver function resulting in a significant increase in serum bilirubin concentration, and (2) hemolytic anaemia (Hooshmand-Rad, 1976; Yadav and Sharma, 1986; Sandhu *et al.* (1998), Singh *et al.* (2001), and Omer *et al.* (2003).

Table 2: Biochemical Alterations in *Theileria* Infected Cattle and Buffaloes

Biochemical parameters	Healthy Cattle	Infected Cattle	Healthy Buffaloes	Infected Buffaloes
	n=10	n=51	n=10	n=21
Alanine amino Transferase (IU/L)	31.06±0.41	63.25±0.20**	23.68±0.81	63.60±0.86**
Aspartate amino Transferase (IU/L)	100.98±0.56	153.15±0.70**	117.68±1.30	154.7±1.45**
Serum Creatinine (mg/dl)	1.02±0.07	1.17±0.01**	1.49±0.02	1.85±0.03**
Serumbilirubin (mg/dl)	0.45±0.03	0.77±0.01**	0.25±0.01	2.24±0.01**
Blood Urea Nitrogen(mg/dl)	25.93±0.51	32.61±0.23**	26.69±0.88	46.22±0.61**

NS=NonSignificant,** $p < 0.01$,* $p < 0.05$

Clinical Signs

In *Theileria* positive animals different clinical signs in high frequency group (>60%) were reduced milk yield 13/13(100%), tick infestation 67/72(93.06%) swollen pre scapular lymph node 60/72(83.33%), fever 64/72(88.89%) and animals having moderated frequency of clinical

signs were anorexia 42/72(58.33%), anaemia 42/72(58.33%) depression 37/72(51.39%), respiratory distress 33/72(45.83%), nasal discharge 32/72(31.94%) and animals having lowest frequency of clinical signs were diarrhoea 20/72(27.78%), emaciation 19/72(26.39%), constipation 10/72(13.89%), conjunctival haemorrhage 5/72(6.94%), lacrimation 4/72(5.56%) and salivation 3/72 (4.17%). Therefore, in present study it has been found that predominant clinical signs observed in animals suffering from theileriosis were tick infestation (*Hyalomma spp.*), reduced milk yield, swollen pre scapular lymph node, fever and anorexia (Table 3).

The current investigation revealed the primary clinical manifestations observed in animals infected with theileriosis, namely a decrease in milk production in all cases, swelling of the pre-scapular lymph node in 83.33% of cases, fever in 88.89% of cases, anorexia in 58.33% of cases, and anaemia in 58.33% of cases. Additionally, all animals exhibited tick infestation. The results of Omer *et al.* (2003), and Durrani *et al.* (2006), are in line with this. Tropical theileriosis is characterized by a significant increase in body temperature, ranging from 40-42°C, which is subsequently accompanied by symptoms such as lethargy, lacrimation, nasal discharge, swelling of superficial lymph nodes, and anaemia (Fartashvand *et al.*, 2013 and Singh *et al.*, 2013).

Table 3: Clinical Signs in Dairy Animals Suffering from Theileriosis

S.No.	Clinical Signs	No. of Animals Revealed	No. of Infected Animals	% Frequency	
1	Tick Infestation	67	72	93.06	} Frequency more than 60%
2	Reduced Milk Yield	13	13	100.00	
3	Swollen Pre-scapular lymph node	60	72	83.33	
4	Fever	64	72	88.89	
5	Anorexia	42	72	58.33	} Frequency between 30 to 60%
6	Anaemia	42	72	58.33	
7	Depression	37	72	51.39	
8	Respiratory Distress	33	72	45.83	
9	Nasal Discharge	23	72	31.94	} Frequency less than 30%
10	Diarrhoea	20	72	27.78	
11	Emaciation	19	72	26.39	
12	Constipation	10	72	13.89	
13	Conjunctival haemorrhage	5	72	6.94	
14	Lacrimation	4	72	5.56	
15	Salivation	3	72	4.17	

CONCLUSION

A highly significant difference ($p < 0.01$) in the incidence of theileriosis was seen between clinically suspected and non-clinical (apparently healthy) dairy animals in the present study. The mean levels of Hb, TEC, lymphocytes, and MCHC were significantly decreases ($p < 0.01$) in *Theileria*-infected cattle, while there was a significantly increase ($p < 0.01$) in the total leukocyte count, monocytes, agranulocytes, and eosinophils in infected cattle as compared to non-infected cattle. Predominant clinical signs observed in animals suffering from theileriosis were reduced milk yield (100%), swollen prescapular lymph node (83.33%), fever (88.89%), anorexia (58.33%), anaemia (58.33%), depression (51.39%), and respiratory distress (45.83%) recorded, respectively.

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