

HAEMATO-BIOCHEMICAL ALTERATION WITH RESPECT TO THERAPY IN CROSSBRED CATTLE CALVES NATURALLY INFECTED WITH BOVINE TROPICAL THEILERIOSIS

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ABSTRACT

The present study was carried out to know the alteration in haemato-biochemical changes caused by *Theileria annulata* before and after the successful treatment of Bovine tropical theileriosis (BTT). A total of 100 crossbred cattle calves suspected for theileriosis by clinical signs (fever, inappetance, anaemia and respiratory distress, generalized lymphadenopathy, petechiae in conjunctiva, oral and nasal mucosa and unilateral or bilateral exophthalmia) were selected and studied in and around Mhow of Indore district, Madhya Pradesh, India from the period April, 2017 to March, 2018. Out of 100 Giemsa stained thin blood smear examined for *T. annulata*, 23 cross bred cattle calves were found positive. Positive calves were given treatment with two doses of buparvaquone @ 2.5 mg/kg intramuscularly at a interval of 72 hours with supportive treatment and haematobiochemical alteration were recorded at day 0 and day 7 post treatment. Mean±SE values of Hb, TEC and PCV at day 0 were estimated to be 7.18±0.14 mg/dl, 4.90±0.12×10⁶/μl and 22.49±0.32%, respectively. Mean±SE of Hb (11.03±0.31mg/dl), TEC (5.23±0.22×10⁶/μl), PCV (28.12±0.34%), increased significantly (P<0.01) as compared to infected group on day 7 post treatment. In infected calves, white blood cell count (10.12±0.25×10³/μl) increased significantly (P<0.01) than treated calves (8.23±0.86×10³/μl) which comprised of significant (P<0.01) lymphopenia (41.35**±0.28% Vs 58.93±1.23%) with neutrophilia (52.47**±0.81% Vs 33.86±1.35%) in infected calves. Moreover, increased significant (P<0.01) value of AST (126.54±0.56 IU/L) and ALT (44.36±0.34 IU/L) in infected animals as compared to post treatment value on day 7 of AST (95.01±3.63 IU/L) and ALT (31.30±1.62 IU/L) were reported. Reported haematobiochemical value on day '0' and after 7 days of treatment would be helpful to understand pathogenesis, prognosis and rational use of supportive therapy for treatment of BTT.

Keywords: Bovine tropical theileriosis, cattle calves, hematobiochemical alterations, treatment

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Bovine tropical theileriosis (BTT) caused by *Theileria annulata* and cyclically transmitted by *Hyalomma anatolicum anatolicum* is a fatal disease of exotic and cross bred cattle. BTT is the most prevalent disease among tick transmitted diseases in India (Velusamy *et al.*, 2014). Theileriosis is under reported due to low sensitivity of blood smear examination (Agrawal *et al.*, 2016). A total economic loss caused by BTT in India was INR 842.67 millions in 2017 (Narladkar, 2018). Similarly in various states of India like Gujarat, Karnataka, Kerala, Tamil Nadu and Uttarakhand the estimated economic losses caused by BTT were reported INR 30.96, 23.12, 8.61 34.30 and 9.13 million respectively (Narladkar, 2018). Severity of disease depends upon percentage of exotic blood in our indigenous cattle; therefore, *Bos taurus* are more susceptible than *Bos indicus* (Glass *et al.*, 2012). 80-85% calf mortality occurred in the first month of age and is major impediments for successful breeding programme in India (Singh *et al.*, 2009). The success of any commercial dairy farm depends upon success rate of calf but unfortunately calf morbidity and mortality is major concern faced by dairy farmers due to its irrefutable and irrevocable economic loss (Parvez *et al.*, 2020). Therefore, present study was carried out to know the calf morbidity/mortality caused by *T. annulata* and to record the haemato-biochemical alterations occurred before and

after the successful treatment of BTT.

MATERIALS AND METHODS

A total of 100 crossbred cattle calves suspected for theileriosis on the basis of clinical signs *viz.* fever, inappetance, anaemia and respiratory distress, generalized lymphadenopathy, petechiae in conjunctiva, oral and nasal mucosa and unilateral or bilateral exophthalmia were selected and studied in and around Mhow of Indore district, Madhya Pradesh, India during field treatment of sick calves from the period April 2017 to March 2018. Aseptically 3 ml blood sample were collected through jugular vein from each animal on day 0 and day 7 of treatment, out of which 1 ml was kept in tube containing anticoagulant (EDTA) and 2 ml was kept in another tube which were not having any anticoagulant for the evaluation of serum biochemical parameters. Thin blood smears were prepared from animal blood at the site of collection and immediately fixed it with methanol. Blood sample were brought to Department of Veterinary Parasitology, College of Veterinary Science and A.H, Mhow and blood smears were stained by standard protocol for Giemsa staining and examined under microscope. Screening of blood smears was done under oil immersion lens (1000x) of a compound research microscope and examined minimum 100 microscopic fields to detect *Theileria* spp. Total erythrocyte count (TEC), hemoglobin (Hb), packed

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cell volume (PCV), total leucocyte count (TLC), differential leukocyte count including lymphocytes and neutrophils were also estimated by manual method (Schalm *et al.*, 1975). Serum total proteins, blood urea nitrogen, serum creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated using semiautomatic blood analyzer, as per the manufacturer's instructions and using standard kits. Paired "t" test was performed to study statistical analysis of haematological and biochemical values by online software www.graphpad.com/quickcalcs (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

Out of 100 animal's blood samples, 23 cross bred cattle calves were found positive for *T. annulata* after thorough examination of blood smear (Fig. 1). Among positive samples 60.87% (14/23) had high level of parasitemia (2-4 piroplasm in a microscopic field) while rest 39.13% (9/23) found with low level of parasitemia (0-1 piroplasm in a microscopic field). All the 23 positive animals were showing varying degree of clinical symptoms *viz.* high rectal temperature [20/23 (86.95%)] ranging from 104–106 °F, enlargement of superficial lymph nodes [17/23 (73.91%)], pallor mucous membrane [19/23 (82.60%)], respiratory distress [18/23 (78.26%)] inappetance [14/23 (60.86%) and exophthalmia [3/23 (13.04%)]. Animals were infested with ticks and ticks were identified as *Hyalomma a. anatolicum* on laboratory examination. Positive calves were given treatment with buparvaquone @ 2.5 mg/kg intramuscularly with supportive treatment for 5 days with oxytetracycline @10 mg/kg bw im, meloxicam @ 0.3 mg/kg im; 5 ml of single multivitamin injection containing vitamin A 2.5 lac IU, vitamin D3 25,000 IU, vitamin E 100 IU, and biotin 12.4 mcg/ml was also given intramuscularly and rumenotronics bolus 1 BD for 5 days treatment. The animals again screened for *T. annulata* after 72 hours post buparvaquone by Giemsa staining of thin blood smear and found positive. Thus a second dose of buparvaquone at same dose rate and route was given to all infected animals. Topical application of deltamethrin (1.25%) was used as per the recommendation of manufactures. From fifth day post-therapy, a remarkable improvement in the clinical condition was noticed. Appetites of calves were resumed completely after 7 days post treatment.

On day 0 and 7 days after treatment, hematological and biochemical parameters were presented (Table 1). Blood smears of all treated calves were found negative on day 5 after thorough examination of Giemsa stained blood smear. After the days 7 post treatment, mean value of red blood corpuscles (RBC), packed cell volume (PCV) and hemoglobin (Hb) increased significantly ($P<0.01$) as compared to mean values on day 0. Haematological values of Hb, PCV, and TEC in infected calves were lower as

Table 1
Hematobiochemical alterations in cattle calves infected with *T. annulata* on day '0' and day 7 post treatment

Parameters (units)	Mean ± S.E. (Day 0) (n=23)	Mean ± S.E. Day 7 post treatment (n=23)
Hematological parameters		
Hb (g/dl)	7.18** ±0.14	11.03±0.31
TEC ×10 ⁶ /μl	4.90** ±0.12	5.23±0.22
PCV (%)	22.49** ±0.32	28.12±0.34
TLC ×10 ³ /μl	10.12** ±0.25	8.23±0.86
Lymphocytes %	41.35** ±0.28	58.93±1.23
Neutrophils %	52.47** ±0.81	33.86±1.35
PLT ×10 ³ /μl	179.20±12.43	180.10±23.34
Serum biochemical parameters		
TP (g/dl)	4.35** ±0.12	6.70±0.17
Creatinine (mg/dl)	1.54±0.08	1.49±0.12
BUN (mg/dl)	10.80±0.31	9.82±0.56
AST (IU/L)	126.54** ±0.56	95.01±3.63
ALT (IU/L)	44.36** ±0.34	31.30±1.62

Means±SE in different columns differ significantly (**= $P<0.01$)

compared to mean value on day 7. The results were found in agreement with Aziz *et al.*, 2018. This may be probably due to various factors like severe damage caused by organisms inside the erythrocyte during their multiplication (Ganguly *et al.*, 2015); destruction of erythrocyte infected with *Theileria* schizonts owing to immune mediated mechanism like erythrophagocytosis; removal of piroplasm infected erythrocytes by reticuloendothelial system; oxidized erythrocyte are destroyed by oxygen free radicals responsible for anaemia (Khan *et al.*, 2011) and increased level of activated complement products (El-Deeb *et al.*, 2012). To mitigate the effect of oxidative stress induced by *T. annulata* on erythrocytes, vitamins A, D3 E and H have been injected as antioxidant potential of these vitamins have been documented by Singh *et al.*, 2012. Two doses of buparvaquinone was also used by previous workers (Verma and Singh, 2016; Pal *et al.*, 2020) In infected calves, white blood cell count increased significantly ($P<0.01$) than treated calves which comprised of significant ($P<0.01$) lymphopenia with neutrophilia in infected calves. These finding were in corroboration with the findings of Ugalmugle *et al.* (2010). There were no significant differences of platelet value with respect to therapy. Significant ($P<0.01$) decreased value of serum total protein might be due to diseased lymph node resulting in to extra-vascular proteinaceous fluid in body cavities in addition to liver failure (Yogeshpriya *et al.*, 2017). Raised non significant value of creatinine suggested muscle damage due to anemic conditions (Swami *et al.*, 2019). There was a non-significant decrease in BUN level on day 7 post treatment. The increased BUN level on day "0"

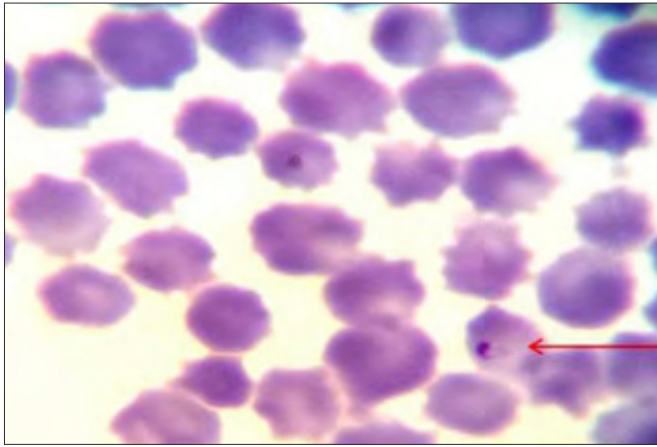


Fig. 1. Blood smear showing Intra erythrocytic *Theileria annulata* piroplasm (Red arrow) at 1000X

indicating the kidney damage which might be occurred due to haemorrhage and infiltration of lymphocytes in interstitial spaces (Swami *et al.*, 2019). Increased significant ($P < 0.01$) value of AST and ALT in infected animals might be due to combination of coagulation necrosis in hepatic tissue, distortion of hepatic cords, filling of periportal area with lymphocytes and anaemia induced hypoxia resulting in to destruction of hepatobiliary system (Akhter *et al.*, 2017; Rakha *et al.*, 2017). In conclusion the present study provided the valuable information on calf morbidity caused by *T. annulata*. Reported hematobiochemical value on day '0' and after 7 days of treatment would be helpful to understand pathogenesis, prognosis and rational use of supportive therapy for treatment of BTT particularly suitable measures for correcting the defective liver and kidney functions in calves. Further research is needed to find out the risk factors associated with it so that implementation of various mitigation factors to check the calf morbidity could be applied.

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