

EPIDEMIOLOGY, CLINICO-PATHOLOGY AND THERAPEUTIC MANAGEMENT OF BOVINE TROPICAL THEILERIOSIS

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Received: 04.10.2021; Accepted: 26.02.2022

ABSTRACT

The study addresses clinico-epidemiology, diagnosis, haemato-biochemical alterations, total antioxidant capacity and therapeutic management of bovine tropical theileriosis (BTT) in cattle. A total of 540 cattle formed the research material. The incidence of BTT recorded was 19.25% (104/540). Cattle aged 2-6 years (50.96%) with Holstein Friesian breed of cattle (91.56%) was found to be more predisposed to BTT. Female animals (84.61%) and monsoon season (48.07%) recorded higher number of cases of BTT. Study recorded elevated vitals in the detailed clinical examination and, microscopic presence of abundant intraerythrocytic piroplasms (83/104) and, Koch blue bodies (KBB) in the cytoplasm of the infected lymphocytes (21/104). Polymerase chain reaction (PCR) confirmed the presence of *Theileria annulata* as the etiological agent (104/104). Hematological analysis revealed anemia and, lymphocytosis with neutropenia ($p \leq 0.01-0.05$). Significant increase ($p \leq 0.05$) in the concentrations of blood urea nitrogen (BUN) and decrease ($p < 0.001$) of total antioxidant capacity (TAC) were observed in BTT. Electrocardiographic examination revealed sinus tachycardia. Treatment with buparvaquone, oxytetracycline and supportive drugs was found effective in resolution of clinical signs.

Keywords: Cattle, Lymphadenopathy, *Theileria annulata*, Total antioxidant capacity

How to cite: Rajgor, D.C., Devi, S., Raval, S.H., Patel, A.C. and Patel, R.M. (2022). Epidemiology, clinico-pathology and therapeutic management of bovine tropical theileriosis. *Haryana Vet.* 61(SI-2): 63-67.

Bovine tropical theileriosis (BTT) is one of the most prevalent haemoprotozoan disease caused by intracellular *Theileria annulata* transmitted by *Hyalomma anatolicum*. The economic losses due to tropical theileriosis are estimated to be Rs. 8426.7 crore/annum in India (Narladkar, 2018). The subclinical carrier infections induce subtle pathological changes impacting productivity of animals (Perera *et al.*, 2014). Cross-bred cattle were found to be more susceptible to theileriosis as compared to the zebu cattle (Rather *et al.*, 2015). High rise of body temperature up to 107°F and enlarged superficial lymph nodes are the most commonly reported clinical manifestations of theileriosis. In the upgradation of indigenous cattle population of India under 'Operation Flood', BTT had been once recognized as a serious constraint (Bhatia and Shah, 2001). Banaskantha district, milk-shed area of Gujarat state, faces the serious problems of BTT. The present communication aimed to study the epidemiology, clinico-haemato-biochemical alterations and therapeutic management of BTT.

MATERIALS AND METHODS

A total of 540 cattle (412 cross breed and 128 indigenous breed) aged between one month to six year old presented with the history of ectoparasitic infestation, inappetence, debilitation, loss of milk production, nasal discharge, lacrymation, respiratory distress and febrile episodes since last 10 days at Teaching Veterinary Clinical Complex, Veterinary College, Deesa, Gujarat were

screened for BTT. History taking and detailed clinical examination were the integral part of the screening to aid the diagnosis. Thin blood smear examination prepared from the marginal ear vein blood and stained with Giemsa stain was employed as the mainstream conventional diagnostic test. The parasite was identified according to the morphological characters described by Soulsby (1982). Also, lymph node aspirate smears (LNAS) stained with eosin methylene blue (EMB) stain were prepared from the enlarged prescapular lymphnodes for demonstration of Koch's blue bodies (KBB) in lymphocytes as described by Jain (1986).

About 5ml of blood sample was collected from the jugular vein of cattle aseptically, divided into K3 EDTA containing tubes for haematological examination and serum clot activator for biochemical examinations and total antioxidant assay (TAC). Hematological parameters *viz.* total erythrocyte count (TEC), hemoglobin concentration (Hb), packed cell volume (PCV), total leukocyte count (TLC), platelets (PLT), neutrophil, lymphocyte, monocyte and eosinophil were measured using automatic haematology analyser (Nihon Kohden, Japan). Biochemical parameters *i.e.* total protein (TP), albumin, creatinine, blood urea nitrogen (BUN), glucose, gamma-glutamyl transferase (GGT) were quantified using standard assay kits (Reactivos GPL, Barcelona) with the help of clinical chemistry analyser (Randox Rx-Monaco, United Kingdom). TAC assay was performed by using total antioxidant capacity assay ELISA kit manufactured

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by sigma Aldrich Pvt. Ltd. (Valko *et al.*, 2007) in accordance with the manufacturer's instructions.

Genomic DNA was extracted from 100 µL of the whole blood from infected animals using QIAamp DNA Blood Mini Kit (Qiagen, Germany) following the manufacturer's protocol. The quality and quantity of DNA was evaluated before use by spectrophotometer. The DNA was stored in small aliquots at -20 till further use. *Theileria* genus specific 18s rRNA primers (forward-AAT CCT GAC ACA GGG AGG TAG TGA C and reverse-CTAAGAATT TCA CCT CTG ACA GT) amplifying a fragment of 390bp were used to screen the samples (d'Oliveira *et al.*, 1995). Similarly, *T. annulata* species specific (Tams 1-forward GTAACCTTTAAAACGT and Tams 1-reverse GTTACGAACATGGGTTT) primer amplifying a fragment of 721 bp was used to confirm the species (Mohammed-Ahmed *et al.*, 2018). The gel was viewed on a ultra violet (UV) transilluminator (Invitrogen Life Technology E-Gel Imaginig System).

The data obtained from haemato-biochemical parameters were subjected to statistical analysis using one way ANOVA test and Dunnett's multiple comparisons test, where $p < 0.05$ has been considered as statistically significant and $p < 0.001$ has been considered as statistically highly significant.

RESULTS AND DISCUSSION

On the basis of observed clinical signs and combined application of traditional and molecular techniques, the overall prevalence rate of BTT recorded was 19.44% (104/540) in dairy cattle in Banaskantha district. Expansion of vector population, changing climate and emerging current pandemic, all stress upon the need of continuous screening of those animals presented with ticks, for BTT, especially in developing countries like India, where livestock sector contributes to the sustainable income of small household farmers to a greater extent.

Concerning the age of the animals, comparatively higher prevalence was observed in cattle of 2-6 year age group (50.96%) which is in accordance with Farooq *et al.* (2019) who recorded a higher prevalence of *T. annulata* in older cattle. Different physiological states like oestrus, pregnancy and lactation leading to temporary immune suppression leads to the increased rate of disease occurrence in cattle (Durrani, 2007). A lower prevalence rate was observed in calves of 0-6 month age group (37.5%) and heifers of 6 month-2 year of age (11.53%). Colostrum from an immunized cow fed to calves reported presence of antibodies to the infective stages (sporozoites, schizonts and piroplasms) in their serum and might protected the calves against theileriosis (Morzaria *et al.*, 1988).

Furthermore, crossbred cattle (91.56%; 76/83) were found to be more susceptible to theileriosis as compared to the indigenous cattle (87.50%; 28/32) which is in agreement with Rather *et al.* (2015). Reason that could be posulated for such observed resistance is the probable lesser burden of vector infestations in indigenous breed of cattle owing to genetics. Higher preference is been given to rear crossbred cattle at Banaskantha, Gujarat due to their higher milk yielding capacity which in-turn fulfills the higher demand for milk as well as milk products and hence might be considered as one of the contributing factors to higher number of cases. Finding of female animals (84.61%) showing higher predisposition for BTT as compared to male animals was consistent with the previous study (Alim *et al.*, 2012). Most of the dairies/livestock owners in this particular study area used to cull male animals at an early age due to economic reasons, therefore, the number of blood samples collected from male cattle, were comparatively lesser than their counterparts. Besides, female animal were kept longer for breeding and milk production purpose and supplied insufficient input/feed against their high demand, inflict considerable production stress and suppression of body immunity, might be acting as a major contributing factor for higher prevalence of haemoprotozoan infections in such female animals (Kamani *et al.*, 2010). Seasonal incidence recorded highest number of cases of BTT during monsoon (48.07%) which is in allingment with Vahora *et al.* (2012). The animals undergoing stress during monsoon season relative to higher humidity in the tropical region along with higher activity of *H. anatolicum* during monsoon season is the contributing factor to the occurrence of BTT.

The clinical vital parameters recorded in all the affected animals (n=104) were persistent fever ($103.03 \pm 0.12^{\circ}\text{F}$), tachycardia (97.48 ± 0.75 beats/min), increased respiratory rate (39.75 ± 0.42 breaths/min) and reduced ruminal motility (1.34 ± 0.07 per 2 min) which is in accordance with Neelam *et al.* (2017). Detailed clinical examination of the cow revealed lusterless dull hair coat infested with the developmental instars of ticks, including adult *H. anatolicum* over dewlap, axilla, ventral abdomen, udder and under the base of the tail region. Genesis of altered vitals and clinical signs in affected animals in the present study might be due to cytokines like, tumor necrosis factor- α (TNF- α) and interleukins (IL-1 and IL-6) produced by infected mononuclear cells (Glass *et al.*, 2003). Fever (upto 107°F), lymphadenopathy, emaciation, lachrymation, pallor eye and vaginal mucous membrane, depression and dyspnea are the common clinical findings pivotal to theileriosis (Afifi *et al.*, 2014). Enlarged superficial

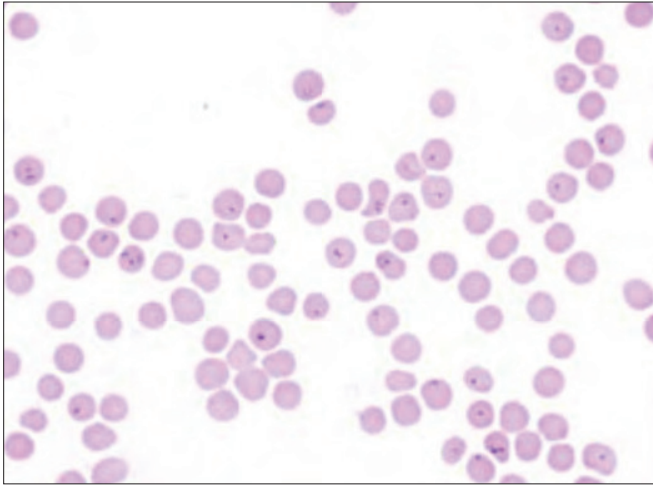


Fig. 1. *Theileria annulata* piroplasms in the RBC's of a cow

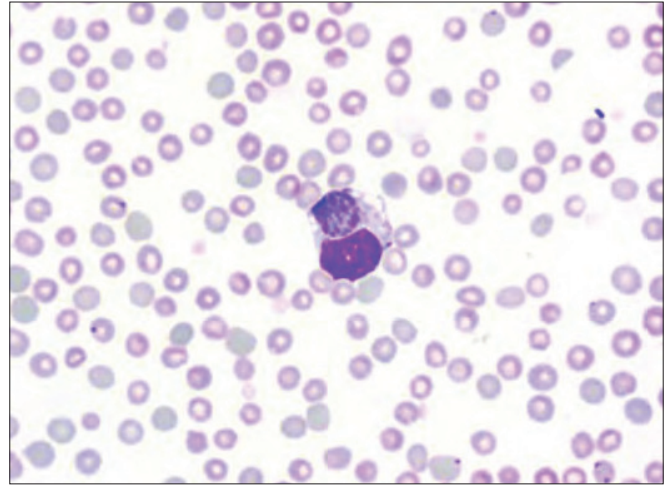


Fig. 2. Koch blue body in the lymphocyte of *T. annulata* affected cow

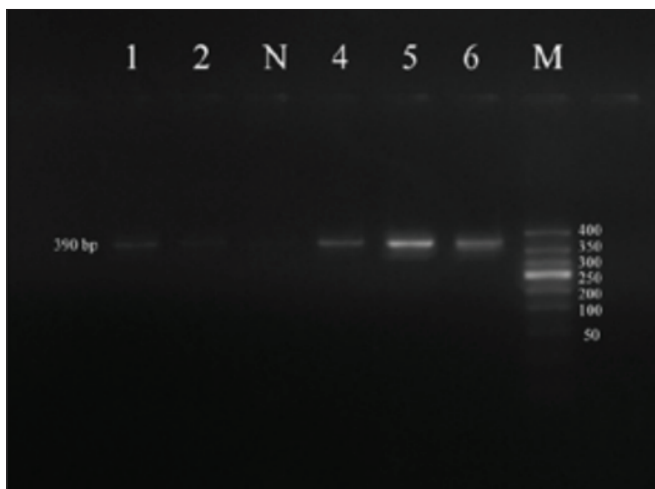


Fig. 3. PCR amplification specific to *Theileria* genus amplified target of 390 bp. Lanes with sample number 1 – 2, 4-6 are positive samples. LM: 50 bp DNA Ladder marker, N: Negative control

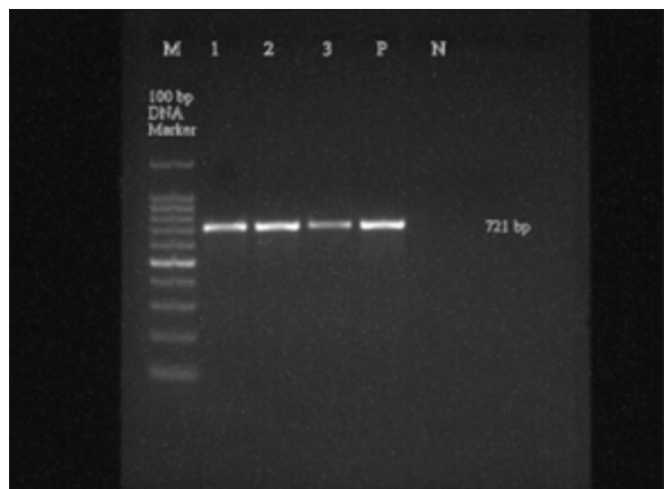


Fig. 4. PCR amplification of Tams 1 gene of *T. annulata* showing an amplified target of 721 bp. Lanes with sample number 1 – 3 are positive samples. LM: 100 bp DNA Ladder marker, N: Negative control, and P: Positive control

lymph node is the result of lymphocytic proliferation of schizonts causing inflammatory reaction and lymphoid hyperplasia (Ahmed *et al.*, 2008).

Microscopically, Giemsa's stained thin blood smear evidenced intraerythrocytic abundance of piroplasms (round, oval, coma and of signet ring shape) (Fig. 1) in 83 blood smears out of 104 confirmed cases. Lymph node aspirate smear (LNAS) examination showed Koch blue bodies (KBB) (Fig. 2) in the cytoplasm of the infected lymphocytes (21/104). Although, the study didn't aimed towards comparison between techniques, microscopy-based detection methods are still the cheapest, fastest and field oriented techniques to diagnose hemoprotozoan disorders with some limitations. More number of animals confirmed positive by PCR (n=104) including those confirmed with stained blood smears approves its efficacy in detection of *T. annulata* with greater specificity and sensitivity. Moreover, further application of PCR was done

for confirmation up to species level, to rule out the probable false-positive and or false-negative visualization of *T. annulata* during direct microscopy, and not to miss out chronic carrier animals. PCR reactions amplifying a band of 390 bp were considered positive for the *Theileria* genus (Fig. 3) and a band of 721 bp were considered positive for the *Theileria annulata* (Fig. 4). Accuracy and sensitivity of PCR for the detection of *Theileria* infection in cattle even at low parasitemia is well known (Charaya *et al.*, 2021). Decline in the sensitivity of traditional methods owes to long standing carrier animals, lack of differentiation of *Theileria* piroplasms and enhancement of the cattle immune system by further infections/ sensitized immune system of cattle to further infections during the tick season (Ayadi *et al.*, 2016).

Laboratory investigation of the peripheral blood revealed significantly ($p < 0.01$) suppressed Hb and PCV values and, altered lymphocyte and neutrophil counts

Table 1. Results (Mean±SE) of hematological parameters in apparently healthy and *T. annulata* infected cows

Parameters	Healthy (n=10)	Infected (n=104)	P-value
TLC (10 ³ /μl)	10.43±0.66	10.92±0.76	0.94
TEC (10 ⁶ /μl)	5.92±0.20	4.76±0.29	0.2
Hb (g/dl)	9.85±0.019	6.64±0.34***	0.0006
PCV (%)	29.5±0.66	19.42±1.02***	0.0007
PLT (10 ³ /μl)	267.90±9.67	290.14±22.86	0.9
Lymphocytes (%)	44.10±2.09	57.88±3.06*	0.034
Neutrophils (%)	49.37±2.36	34.76±3.50*	0.043

TEC: Total erythrocyte count, TLC: Total leukocyte count, Hb: Hemoglobin, , PCV: Packed cell volume, PLT: Platelet, *P(<0.05) and ** P(<0.001)

Table 2. Results (Mean±SE) of biochemical parameters in apparently healthy and *T. annulata* infected cows

Parameters	Healthy (n=10)	Infected (n=104)	P-value
TP (g/dl)	6.34±0.18	6.32±0.17	0.99
Alb (g/dl)	2.31±0.07	2.28±0.06	0.97
Creatinine (mg/dl)	1.27±0.9	1.57±0.14	0.59
BUN (mg/dl)	13.85±0.88	36.07±3.48*	0.033
Glucose (mg/dl)	36.10±2	61.03±4.57	0.34
GGT (U/l)	15.79±0.46	22.64±2.61	0.45

TP=Total protein, Alb=Albumin, BUN= Blood urea nitrogen, GGT=Gamma-glutamyl transferase and *P(<0.05)

(Table 1). The hematological parameters of cattle affected clinically with tropical theileriosis showed anemia, lymphocytosis with neutropenia (Charaya *et al.*, 2021). Alterations in the key anti-oxidant system of red cells owing to presence of hemo- protozoan inside RBCs hence, subjecting them to oxidant mediators assault and speeding up of RBCs removal via phagocytosis eventually leads to hemolysis and thus anemia (Razavi *et al.*, 2012) and relative decrease in haemoglobin concentration as well as total erythrocytes count. Hemopoietic organs especially bone marrow facing persistent detrimental effects of toxic metabolites of *Theileria*, interfering with the process of leukogenesis might be the reason for alterations in the leukogram (Ganguly *et al.*, 2015). The values of eosinophil and monocyte in the present study was undetectable by the automatic analyzer machine, hence could not be presented and or discussed.

Biochemical examination detected significantly ($p \leq 0.05$) higher levels of blood urea nitrogen (BUN) and, considerably higher gamma-glutamyl transferase (GGT) and glucose in the serum of affected animals (Table 2) which is almost in line with by Tehrani *et al.* (2013). Probable reason attributed to this is damage of liver and kidney tissues from infected lymphoid cells (Forsyth *et al.*,

1999) emphasizing the ability of the organism to damage different organ systems of animal body's resulting into elevation of different enzyme systems. Hepatic tissue damage caused by *T. annulata* infection includes coagulative necrosis, distortion of hepatic cords and heavy infiltration of lymphocytes in the periportal areas (Sandhu *et al.*, 1998). Hyperglycemia recorded in the present study may attributed to the destruction of liver cells, resulting into the release of glucose to the circulation (Abd Ellah, 2015).

The total antioxidant capacity (TAC) was found to be significantly decreased (0.305 ± 0.006) ($p < 0.01$) in theileriosis infected dairy animals in the present study as compared to healthy animals (1.434 ± 0.009) (El-Deeb and Iacob, 2012). Reduction of TAC level might be attributed to the reduction in antioxidant enzymes as they being utilized by excessive free radicals in the infected animals (Hassanpour *et al.*, 2013).

The mainstay of therapy was buparvaquone @2.5 mg/kg body weight as a single deep intramuscular dose along with oxytetracycline @10 mg/kg body weight intravenously for 5 days as an adjunct therapy in all the positive cases. Supportive therapy involved dextrose normal saline @ 4 liters IV, meloxicam @ 0.3 mg/kg IM. and chlorpheniramine maleate @ 0.5 mg/kg I.M., for three days. Oral supplementation with multivitamin (Vitamin A, D, E and K) @ 10 ml PO BID and liver supplement was advised to continue for two months. From day second post-therapy a remarkable improvement in the clinical condition was noticed. The owner reported, animals regained their normal appetite within 7 days post-therapy. Therapeutic regimen followed in the present study is in line with Mehesare *et al.* (2012) who used similar line of treatment and reported improvement. Supportive therapy was provided in order to revitalize the body during convalescence. Criteria for evaluating the efficacy of treatment were improvement in clinical signs seven day post treatment. Combination therapy involving buparvaquone with oxytetracycline was found effective against theileriosis with oxytetracycline specifically holding the ability to cure concurrent hemaoprotozoal and rickettsial infection in cattle (Muhammad *et al.*, 1999), thus reducing the stress factor. An irreversible impact of oxytetracycline on schizont development resulting in the failure of parasites to establish within host cells noticed if schizonts are detected earlier in *T. parva* infection (Spooner, 1990), a quiet important fact to be investigated for *T. annulata* infections. Buparvaquone @ 2.5 mg/kg IM as a single dose is a drug of choice for the treatment of theileriosis in cattle (Salama and Gaabarya, 2007). Increased cost for individual treatment of affected animals supports adjunct therapy with antibiotics.

In conclusion, it is worth mentioning here that BTT is a disease with massive potency to damage different animal body's system. Also, buparvaquone in combination with oxytetracycline is effective in clinical cases with better resolution of clinical signs.

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