

Clinico-Laboratory Biomarkers of Systemic Inflammatory Response Syndrome Associated with *Theileria annulata* Infection in Cattle and its Prognostic Significance

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ABSTRACT

Bovine tropical theileriosis is an infectious multisystemic disease characterized by variable clinical signs including fever, lymphadenopathy, anaemia and sometimes haemoglobinuria. Present study was designed to assess the clinico-laboratory markers of systemic inflammatory response syndrome (SIRS) associated with bovine tropical theileriosis and its impact on prognosis of disease. Twenty two clinical cases of theileriosis were investigated for clinical and laboratory biomarkers of systemic inflammation. Clinical analysis revealed 86.36% of infected animals showed SIRS. Laboratory analysis revealed elevation in Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) in 57.89% and 42.10% *Theileria annulata* infected cattle, respectively, while 31.57% animals showed both elevated NLR and PLR. Results of the study revealed the presence of systemic inflammation in majority of cases of bovine tropical theileriosis which exert effect on the prognosis of disease.

Keywords: Buparvaquone, Cattle, Clinico-Laboratory Biomarkers, Systemic inflammation, Theileriosis.

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INTRODUCTION

Bovine tropical theileriosis is a multisystemic, tick born parasitic disease caused by a haemoprotozoan *Theileria annulata* (Sivakumar *et al.*, 2014). Haematopoietic organs like bone marrow, spleen, lymph nodes and liver are major targets of pathogen which causes great morbidity and mortality (Flach and Ouhelli, 1992). Piroplasm and schizonts are major stages in life cycle regulating the pathophysiology of disease. Systemic inflammation by *Theileria annulata* can lead to release of various cytokines into systemic circulation which has detrimental effects on various body organs (Glass *et al.*, 2012). The exaggerated response of host immune cells to localize and eliminate pathogens or toxins may lead to development of systemic inflammatory response syndrome (SIRS) and disease progress (Chakraborty and Burns, 2023). Assessment of SIR could be of significance in understanding the pathophysiology of disease. Various systemic inflammatory response biomarkers have already been studied in animals affected with various infectious diseases. Neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR), lymphocyte-to-monocytes ratio (LMR) and derived NLR ratio (d-NLR, neutrophil count divided by the result of WBC count minus neutrophil count) are some of the important markers for SIR (Ying *et al.*, 2014). Neutrophil-lymphocyte ratio (NLR) (Balta *et al.*, 2013; Channappanavar and Perlman, 2017), and platelet-lymphocyte ratio (PLR) can be a good biomarker for systemic inflammation, severity and prognosis in many diseases (Aktas *et al.*, 2023). Since no study has been reported to assess the *T. annulata* induced SIRS based on clinical characteristics and laboratory parameters, present study was designed to assess systemic inflammation

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based on clinical and laboratory investigation in *Theileria annulata* infection in cattle.

MATERIALS AND METHODS

Animal Selection, Clinical Characteristics and Sampling

Twenty two *Theileria annulata* naturally infected female cattle with mean age of 4.9 years (22.73%, 63.64% and 13.64% cattle were in the age group of 1-3 year, 4-7 years and 8-10 years, respectively) were included under the study. Screening and confirmation of theileriosis was done based on classical

clinical signs and optical microscopy as described by Taylor *et al.* (2007) and Qayyum *et al.* (2010). All the animals clinically suspected for theileriosis were scrutinized for phenotypic estimates, vital parameter analysis and blood sampling. Individual cattle was ardently examined for various clinical manifestation like fever, lymphadenopathy, mucus membrane color, cardiac distress, respiratory distress, tick infestation, nasal discharge, conjunctival petechiae, lacrimation, diarrhea and recumbency etc. Percentage positivity for each clinical manifestation was calculated. Substantially, thin film blood smear, from left ear capillary, was prepared from each of the cattle presented for parasitological examination. Simultaneously, 2 mL whole blood sample was obtained by aseptic jugular venipuncture technique in a sterile vial containing anticoagulant (3.6 mg EDTA) for haematology. Animals with negative optical microscopy results along with normal haemato-biochemical parameters were encased as control (n=7).

Assessment of Systemic Inflammation (SIRS)

Systemic inflammation was assessed based on the presence of certain clinical characteristics like tachycardia (HR >80 bpm), hyper (T >106 °F) or hypothermia (T <98.6 °F), tachypnea (RR >30 bpm), leukocytosis (TLC >12000/μL) or leukopenia (TLC <4900/μL) (Radostits *et al.*, 2007). Animals which fulfilled two or more above mentioned criteria were categorized as SIRS positive. Besides, neutrophil to lymphocyte ratio (NLR) and platelets to lymphocyte ratio (PLR) were calculated for each of the presented case as per formula mentioned below-

NLR = Absolute neutrophils count / Absolute lymphocyte count

PLR = Absolute platelet count / Absolute lymphocyte count

RESULTS AND DISCUSSION

Clinical Investigation

Clinical disquisition revealed variable clinical manifestations of which tachycardia was the most consistent (95.45%) finding followed by anaemic mucus membrane (86.36%), fever (68.18%), recumbency (50%), tick infestation (41%) and nasal discharge (36.4%). Conjunctival petechiae, diarrhea was not reported in any case, while in two animals (9%) uncommon mammary gland affections were observed. Mean ±SE value of rectal temperature (°F), heart rate (bpm) and respiratory rate (per min) were 102.5±0.31, 104.59±3.43 and 39.54±2.7, respectively.

Systemic Inflammation Analysis

Out of total *T. annulata* affected cattle, 19 animals (86.36%) showed presence of SIRS (Table 1). Concurrent tachycardia and tachypnoea along with normal leukocyte count was the predominant (36.36%) feature of SIRS followed by concurrent tachycardia and tachypnoea along with leukocytosis (27.27%). Concurrent tachycardia and tachypnea complicated by leukopenia was present in 13.64% animals, while 9.1% animals had tachycardia and leukocytosis. Results indicate that vital parameters abnormality may or may not be complicated by abnormal leukocyte count while abnormal leukocyte count is always associated with either cardiac or respiratory distress. Electrolyte imbalance, inflammatory cells infiltrations and secondary anaemic hypoxia induced myocardial ischemia leads to disintegration of myocardial cell membrane and compromised cardiac functions (Gupta *et al.*, 2021) which clinically manifested as increased heart and respiration rate in order to maintain cardiac output and tissue oxygenation. Concurrent leukocytosis in some cases of clinical bovine tropical theileriosis represents secondary bacterial infection.

Table 1: Clinico-laboratory markers of systemic inflammation in *T. annulata* clinically affected cattle

Markers under study	Characteristics of SIRS	No of animals positive for given characteristic	Number of survivors	Mortality (%)
Clinical markers	Tachycardia+Tachypnoea	08 (36.36%)	08	0.00
	Tachycardia+Tachypnoea+Leukocytosis	06 (27.27%)	05	16.67
	Tachycardia+Tachypnoea+Leukopenia	03 (13.64%)	01	66.67
	Tachycardia+Leukocytosis	02 (9.1%)	02	0.00
Laboratory markers	Elevated Neutrophil to Lymphocyte ratio (NLR)	*17 (57.89%)	16	9.10
	Elevated Platelets to Lymphocyte ratio (PLR)	*08 (42.10%)	08	0.00
	Elevated NLR+PLR both	06 (31.57%)	04	33.33

*Based on cutoff value

Significant difference was observed in the mean value of NLR and PLR between healthy and diseased animals (Table 2). Significant increase of NLR in *T. annulata* infected animals has already been recorded (Aktas *et al.*, 2023) but no reports are available on PLR in literature. Based on cut off value (1.09), 17 (77.27%) animals showed elevation in NLR indicating presence of sepsis or systemic inflammation in majority of *Theileria annulata* infected cattle. Normal NLR with at least

one abnormal vital parameter (temperature, respiration or heart rate) was reported in 5 (22.73%) animals indicating non-immune inflammation. NLR reflects the severity of sepsis, stress and inflammation (Zahorec, 2021). Elevated level of NLR in present study indicates *T. annulata* induced sepsis. Host cell interaction of *T. annulata* leads to production of free radicals and biomembrane damage which provoke the immune system to produce inflammatory cells which in



turn lead to the neutrophilia, hence elevation of NLR. Platelet to lymphocyte ratio (PLR) is considered as potent systemic inflammatory biomarker with good prognostic value in case of many cardiovascular affections and neoplasm (Kurtul and Ornek, 2019). *Theileria annulata* induced ischemic myocarditis (Gupta *et al.*, 2021) and DNA damage (Radakovic *et al.*, 2016) has already been established hence elevation in PLR can be predicted in bovine tropical theileriosis. Based on cutoff value (119.23), 42.10% (8/19) animals showed elevated PLR with at least one vital parameter abnormality. Results indicate that elevated level of PLR is always associated with vital parameter abnormality, while elevation in NLR may or may not be associated with vital parameter abnormalities. Among laboratory parameters, increase in NLR is predominant

parameter to reflect the systemic inflammation induced by *T. annulata*. Six animals (31.57%) showed elevation in both NLR and PLR. Mortality rate of animals with systemic inflammation was 15.78% which shows that systemic inflammation compromise the survivability of animals. Maximum mortality (66.67%) was reported in animals with concurrent tachycardia, tachypnoea and leukopenia followed by 16.67% animals showing concurrent tachycardia, tachypnoea and leukocytosis. In reference to laboratory analysis it was found that higher mortality (33.33%) was associated with simultaneous elevation of NLR and PLR as compared to solitary elevation of NLR (9.1%). Elevation of PLR alone is not associated with any mortality.

Table 2: NLR and PLR values in healthy and *T. annulata* clinically affected animals (Mean \pm SE)

Estimates	Healthy	Diseased	P value
NLR	0.47 \pm 0.08 ^a (Range: 0.25-0.85)	2.15 \pm 0.26 ^b (Range: 5.45-5.71)	0.0014
PLR	90.49 \pm 3.9 ^a (Range: 77.54-107.57)	177.1 \pm 22.88 ^b (Range: 19.42-503)	0.045

*Different superscripts within the row are significantly different at $p < 0.05$.

Conclusively, it can be said that NLR is more sensitive and reliable marker to assess the systemic inflammation than PLR. Animals with tachycardia, tachypnea, leukopenia and increased NLR and PLR both have minimum chances of survival and must be taken under special consideration. This is a preliminary study and there is need to establish the SIRS criteria by more precise clinical studies with regulated environmental conditions of animals to provide new insight for understanding the pathophysiology and prognosis of disease.

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