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Clinico-haemato-biochemical and electrocardiography alterations in buffaloes with theileriosis

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Abstract
Different studies on clinical theileriosis have been reported in cattle with scarce reports on buffaloes in India. Hence, the present study was designed to study the clinico-haematological and electrocardiography changes in buffaloes with theileriosis. Among the screened buffaloes, 16 adult buffaloes were identified as suffering with clinical theileriosis based on the microscopic examination of blood smears. Depressed demeanour, pyrexia, inappetence, cachexia, dyspnea, absence of rumination, lacrimation, tachycardia, tachypnea, scleral congestion, enlarged prescapular lymph nodes and sunken eyeballs were the most repeatedly noticed clinical signs in buffaloes diagnosed with theileriosis. The haemato-biochemical analysis showed marked anemia, leukocytosis, lymphocytopenia, and increased levels of AST, blood urea nitrogen, creatinine, and potassium along with reduced levels of albumin, total serum protein, and calcium. Electrocardiographic analysis revealed sinus tachycardia, ventricular extrasystole, atrial fibrillation, atrial premature complex, premature ventricular complex, ventricular tachycardia, and first-degree atrioventricular block. Therapy was carried out using oxytetracycline and buparvaquone along with supportive medications. Therapeutic response was favorable in early intervention of the disease but poor in the chronicity of the disease. The results of the study help guide the therapeutic approaches and assess the involvement of other organs in buffaloes with chronic theileriosis. Further studies are required to investigate the factors involved in the unfavorable therapeutic response to chronic theileriosis in buffaloes.

Keywords: Theileriosis, buffaloes, electrocardiography, haematology

Introduction
Tropical theileriosis is a tick-borne haemoprotozoan disease caused by *Theileria annulata* and transmitted by *Hyalomma* spp. Theileriosis has widely been reported in cattle, particularly in crossbred cattle from all over the world (Nalbantogiu, 2003; Syed *et al.*, 2014) [5, 13]. However, there are few published reports of theileriosis in buffaloes (Hasanpour *et al.*, 2008; Bhosale *et al.*, 2020) [4, 1]. During the course of the study, changes in hematological and serum biochemical parameters will help the clinician to formulate the best therapeutic regimen (Reddy and Sivajothi, 2016) [9]. Literature available on the electrocardiographic changes in buffaloes with theileriosis was meager in India. Hence, the present research communication documents the haemato-biochemical and electrocardiographic alterations in clinical theileriosis in buffaloes.

Materials and Methods
The present study was carried out at the College of Veterinary Science, Proddatur. During the study period, 16 adult buffaloes were identified as suffering from clinical theileriosis and selected for the study. Ten apparently healthy buffaloes were also selected as a control group. Infected buffaloes were diagnosed by observation of parasites in the stained peripheral blood smear and lymph node aspirations. The parasites were evident as schizonts, in circulating lymphocytes and aspirates from the enlarged lymph nodes stained with Giemsa. Peripheral blood smears were collected, air-dried, fixed with methanol, stained with Giemsa, and examined for the presence of piroplasm-infected erythrocytes and for the degree of infection (Figure.1). Blood was collected with an anticoagulant to measure the hemoglobin, PCV, RBC, WBC, and differential leukocyte count. Serum was separated from the blood and stored at -20 °C for estimation of biochemical parameters such as total protein, albumin, aspartate aminotransferase (AST), blood urea nitrogen (BUN), and creatinine levels. The differences in means of hematological and biochemical parameters between buffaloes with theileriosis and normal animals were compared using the Student's t-test (Table-1).

Electrocardiography was carried out as per standard procedure (Reddy *et al.*, 2015) [8]. All the cases were treated with an injection of oxytetracycline @ 20 mg/kg IV once daily for five days and a single dose of buparvaquone @ 2.5 mg/kg deep intramuscularly on the day of confirmation of disease (Reddy and Sivajothi, 2017) [10, 12]. Supportive treatment included Inj. Dextrose 20% @ 1-lit intravenously for 5 days, Inj. Vitamin B complex @ 10 ml intramuscularly for 10 days, injection Feritas @ 10 ml/day along with oral probiotics for 20 days.

Results and Discussion

Depressed demeanour (16/16), pyrexia (16/16), inappetence (16/16), tachycardia (16/16), tachypnea (16/16), cachexia (15/16), dyspnea (14/16), absence of rumination (14/16), lacrimation (13/16), scleral congestion (13/16) and enlargement of prescapular lymph nodes (10/16), sunken eyeballs (9/16) and corneal opacity (2/16) were the most repeatedly noticed clinical signs in buffaloes diagnosed with theileriosis. Reported clinical signs were in association with previous reports of theileriosis in buffaloes (Hasanpour *et al.*, 2008 [4]; Bhosale *et al.*, 2020 [1]) [4, 1].

Infection is initiated by the transformation of macroschizont-infected cells in the lymph nodes draining the site of inoculation of sporozoites by ticks. The buffalo infective form of the parasite is the sporozoite transforming into schizonts in WBC of the mononuclear lineage. The schizont undergoes further differentiation to merozoites, which are released upon lysis of the infected cells. Once released from host cells, the merozoites enter erythrocytes. This is followed by the development of piroplasms in erythrocytes and the parasite becomes infective to the vector (Nalbantogiu *et al.*, 2003) [5]. During the disease process, *T. annulata* spreads through the lymphoid system and other organs rapidly and induces the production of TNF-α and IFN-γ these cytokines disrupt the physiological integrity of the bovines and are responsible for the exhibition of different variety of clinical symptoms including depression, pyrexia, anorexia, cachexia, and disseminated hemorrhages. Present findings were in agreement with other reports (Osman *et al.*, 2007; Hasanpour *et al.*, 2008) [6, 4].

Decrease in hemoglobin, packed cell volume and total erythrocyte count, leukocytosis, and lymphocytopenia were noticed. Increased AST values observed in affected buffaloes were attributed to hepatic injury resulting from anemic anoxia in buffaloes. The elevated level of BUN and creatinine was in agreement with Tuli *et al.*, (2015) [14] which might be attributed to increased turnover of proteins. On the contrary, some workers have reported a significant decrease in total leukocyte count in *Theileria annulata* infected buffaloes (Singh *et al.*, 2001) [11]. Reduction in serum calcium and elevated serum potassium was noticed in the present study. Development of anemia due to erythrocyte destruction by erythrophagocytosis due to an immune-mediated mechanism. Removal of the piroplasm-infected erythrocytes by macrophages in the organs of the reticuloendothelial system has been suggested as a cause of anemia. In addition, pro-inflammatory cytokines, particularly TNFα, have been implicated in mediating anemia associated with tropical theileriosis (Graham *et al.*, 2001) [3]. The decrease in RBC could be due to increased levels of activated complement products. Additionally, since oxidized erythrocytes may be destroyed easily by erythropagocytosis, oxygen radicals may also be involved in the pathogenesis of anemia (Bhosale *et al.*, 2020) [1]. In a few studies, it was noticed that leucocyte count increased immediately following *Theileria* infection and then significantly decreased within several days. *T. annulata*-induced leucopenia is mainly mediated by TNF-α. This decrease is related to the destruction of lymphocytes in lymphoid organs and the infiltration of these cells into various organs (Ghanem *et al.*, 2013) [3].

In theileriosis buffaloes, sinus tachycardia (9/16), sinus arrhythmia (5/16), ventricular extra systole (4/16), atrial fibrillation (4/16), atrial premature complex (3/16), premature ventricular complex (3/16), ventricular tachycardia (3/16) and first degree of the atrioventricular block (2/16). Recorded electrocardiographic findings might be due to variations in the rhythm and rate of the heart in animals by strong and varying autonomic influence and a reflection of primary myocardial disease and by acid-base imbalance. The reported electrocardiographic parameters were sinus tachycardia, atrial fibrillation, sinus arrhythmia, and first-degree atrioventricular block. Sinus tachycardia may be caused by pain, hyperthermia, anemia, electrolyte imbalance, and a fall in arterial blood pressure. Atrial fibrillation is associated with gastrointestinal, metabolic, and hemolytic diseases (Radostits *et al.*, 2010) [7]. Sinus arrhythmia is a normal physiological arrhythmia that occurs at slow resting heart rates and is associated with variations in the rate of discharge from the sinoatrial node and electrolyte imbalance. The first degree of atrioventricular block occurs when conduction is delayed at the atrioventricular node. It can be associated with electrolyte imbalance, overdosing with calcium, digoxin, and cardiomyopathy. According to the kinds of arrhythmias and electrolyte imbalance, it is concluded that arrhythmias in these buffaloes may be physiologic, and treatment, as well as balancing electrolytes, will remove arrhythmias. Based on these observations, it can be concluded that severe *T. annulata* infection is associated with profound changes in hematological and biochemical profiles and electrocardiography parameters (Reddy and Sivajothi *et al.*, 2017) [10]. Similar findings were previously recorded in buffaloes with clinical trypanosomosis (Sivajothi and Reddy, 2017) [12].

The primary goal of therapy in theileriosis-affected animals is to eliminate the parasite and reverse the life-threatening anemia. Buparvaquone and oxytetracycline are the most widely used drugs for the treatment of theileriosis (Syed *et al.*, 2014 [13]). Two buffaloes with abnormal electrocardiography findings with a chronic history of diseases not responded to the treatment. In the present study, supportive therapy was carried out with dextrose to correct hypoglycemia resulting from anorexia, B complex to stimulate appetite and metabolism, and iron preparations to stimulate erythropoiesis. Buparvaquone was found highly effective in the early stage of buffaloes with theileriosis but it failed to improve the clinical condition of the animal in the chronic stages of the disease. It is opinioned that further studies are required to address the specific alterations in vital organs which cause mortality after treatment also. The results of the study help in formulating therapeutic approaches and assessing the involvement of other vital organs in buffaloes with theileriosis.

It can be concluded that *Theileria annulata* infection in buffaloes can cause a change in the cell membrane integrity of myocardial muscle and serum electrolyte changes leading to conduction abnormality and alteration in electrocardiographic parameters.

Table 1: Haematological and serum biochemical changes in buffaloes infected with theileriosis (Mean±S.E.)

Parameters	Apparently healthy buffaloes (n=10)	Buffaloes with theileriosis (n=16)	P - Value
Rectal temperature (OF)	100.23±0.06	102.19±0.22	0.001**
Heart rate (Per minute)	56.65±0.92	82.19±0.21	0.001**
Respiratory rate (Per minute)	16.88±0.61	24.28±0.41	0.001**
Haemoglobin (g/dl)	11.27±0.13	8.78±0.31	0.001**
PCV (%)	36.50±1.1	24.82±1.98	0.001**
TEC x10^6/cumm	7.45±0.09	4.54±0.94	0.001**
TLC /cumm	8378.0±236.1	9123.2±255.9	0.084 ^{NS}
Neutrophils /cumm	2555.6±101.22	3481.5±63.6	0.072 ^{NS}
Lymphocytes /cumm	5227.87±201.21	4269.4±107.8	0.045*
Monocytes /cumm	293.3±19.3	668.23±67.6	0.001**
Eosinophils /cumm	192.70±9.16	387.9±12.66	0.001*
Total protein (g/dL)	6.98±1.96	4.74±1.21	0.024*
Serum albumin (g/dL)	3.46±0.77	1.21±0.02	0.028*
AST (IU/L)	56.34±5.45	108.23±16.11	0.001**
BUN (mg/dL)	28.42±4.19	58.12±3.82	0.001**
Creatinine (mg/dL)	1.08±0.29	1.92±0.19	0.038*
Sodium (mEq/L)	134.4±2.09	130±3.01	0.082 ^{NS}
Potassium (mEq/L)	4.6±0.41	6.2±0.63	0.047*
Calcium (mEq/L)	10.5±0.92	8.6±0.88	0.032*

NS: Not Significant (p>0.05); *: Significant (p≤0.05); **: Highly Significant (p≤0.01)

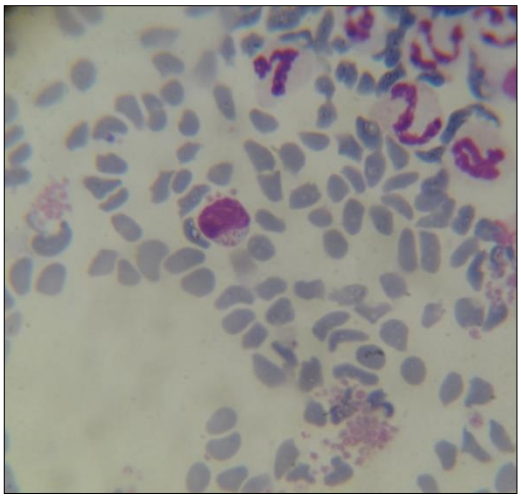

Fig 1: Blood smear stained with Giemsa showing the macroschizont of *T annulata* in lymphocyte and intra erythrocytic piroplasms of *T annulata* (Magnification 1000X).

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