



POST THERAPEUTIC CARDIOPULMONARY EVALUATION OF CATTLE WITH CHRONIC THEILERIOSIS

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Summary

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Bovine theileriosis is one of the economically important diseases caused by *Theileria annulata* in Andhra Pradesh, India. Very limited documentation is available on cardiopulmonary signs and its post therapeutic evaluation in cattle with theileriosis. Hence the present study was carried out on eight adult cattle with chronic theileriosis showing signs of respiratory distress and cardiovascular abnormalities. Confirmation of theileriosis was done by demonstration of organisms in the stained blood smears; the infected cattle were injected with buparvaquone along with symptomatic and supportive medications. On evaluation of cardiopulmonary function, tachypnea, dyspnea, tachycardia and muffled heart sounds were observed. Haemato-biochemical evaluation revealed the presence of anaemia, low serum albumin levels, elevated serum creatine kinase myocardial band isoenzyme levels. Electrocardiography abnormalities were observed on the day of presentation. Post therapeutic assessment revealed improvement in the clinical signs, but without reduction in the serum creatine kinase myocardial band isoenzyme levels after therapy which was indicative of myocardial inflammation. Electrocardiography divulged persistence of ventricular premature complex, atrial fibrillation even after therapy. There were no changes in the echocardiographic parameters viz. left ventricular internal dimension at end-diastole, left ventricular internal dimension at end-systole, interventricular septum thickness at end-diastole, interventricular septum thickness at end-systole, left ventricular posterior wall thickness at end-diastole, left ventricular posterior wall thickness at end-systole on day zero, 7th and 14th day of therapy. The presence of cardiac abnormalities usually causes reduction in the cardiac function, development of respiratory abnormalities which make the animals reluctant to feed intake and ultimately causes reduction in milk yield. The present research study concludes that the cardiopulmonary signs developed in cattle with theileriosis which might be due to anaemia and changes in the serum albumin levels which aggravated the myocardial tissue hypoxia.

Key words: cardiopulmonary function, cattle, evaluation, theileriosis

INTRODUCTION

Theileriosis is one of the most common vector borne protozoan diseases of dairy cattle in India. Theileriosis caused by

Theileria annulata is transmitted by ixodid ticks of the genus *Hyalomma* in Andhra Pradesh. It causes considerable

economic losses in the terms of decreased weight gain, drop in milk yield, abortions, and sudden death in animals. Diagnosis of theileriosis was carried out by identification of intra-erythrocytic forms i.e., signet ring, dot-, or comma-shaped *T. annulata* piroplasms in the red blood cells on stained blood smears (Tuli *et al.*, 2015). The main clinical signs of theileriosis are fever, slight nasal and ocular discharge, anorexia, salivation, enlargement of superficial lymph nodes, respiratory distress, acute anaemia, jaundice, and death due to asphyxia. It is a progressive lymphoproliferative disease (Fartashvand *et al.*, 2013). Theileriosis can lead to elevated risk of secondary infections. Haematological and biochemical changes observed in tropical theileriosis are influenced by the parasite's virulence, the infectious dose, the animal's breed, immunity, and local climate conditions (Mahmmud *et al.*, 2011). Without any heart abnormalities, pseudo-pericarditis was observed as symptom which was identical to pericarditis. The mechanism of pseudo-pericarditis formation is described as pressure on the vena cava caused by the enlargement of the mediastinal lymph nodes surrounding the cranial and caudal vena cava, which prevents blood flow and causes clinical symptoms like jugular enlargement and oedema. Haemolytic anemia, secondary hypoxia, and vasculitis are the three main pathognomic characteristics of tropical theileriosis (Fartashvand *et al.*, 2013). Even though the precise mechanism of myocardial involvement is still unclear, the hypothesised causes of myocardial damage in theileriosis are parasitised lymphoblast accumulation in visceral blood vessels including the heart followed by anaemia and secondary hypoxia i.e. decreased oxygen supply to the myocardium due to long-term tachycardia. Sivajothi &

Reddy (2022) stated that the severity of anaemia and parasitaemia might be contributing factors for pathophysiology of myocardial damage and also observed that theileriosis is a risk factor for elevation of cardiac biomarkers in bovines. Very limited research is available on cardio pulmonary signs and post therapeutic assessment of cardio pulmonary system in cattle.

MATERIALS AND METHODS

Present research study was carried out at Department of Veterinary Medicine, College of Veterinary Science, Proddatur, Andhra Pradesh, India from January 2022 to December 2023. During the study period eight adult cattle weighing from 300 to 420 kg body weight with chronic theileriosis having the signs of respiratory distress, cardiovascular abnormalities were selected. Cattle were privately owned, all the cattle had calved (from 3 to 6 months post calving) and were free from other specific diseases at the locality. Theileriosis was diagnosed based on the presence of intraerythrocytic forms of *Theileria annulata* piroplasms in the stained blood smears. All the cattle were injected intramuscularly with buparvaquone at 2.5 mg/kg body weight along with flunixin meglumine at 2 mg/kg, and 10 ml of a multivitamin injection (Tribivet). Orally, liver tonic fortified with yeast and vitamin (Brotone Vet) 50 g twice a day; and syrup 3D RED is a nutritional feed supplement – 30 mL twice daily orally as per the need of individual cases were administered (Reddy & Sivajothi, 2017). Assessment of post therapeutic cardiopulmonary function was carried on the day of presentation, 7th day (average) and 14th day (average) post therapy. The studied parameters were from the clinical examination, haematobiochemical analysis, electrocardiographic

and echocardiographic studies. Five mL of blood were collected into vacutainers containing EDTA as anticoagulant for haematology and 5 mL of blood in clot activator vacutainers to separate the serum for biochemical analysis.

The electrocardiography was carried out by base apex lead system in a single channel machine in lead I (Reddy *et al.*, 2015). M-mode echocardiography was carried out with Esaote My lab 40 VET ultrasonographic scanner with a phased array probe having variable frequency from 2 to 3.5 MHz at right parasternal long axis view (Khalphallah *et al.*, 2023). Ten apparently healthy privately owned cattle which had calved 3 to 6 months ago, in the present locality with similar environmental conditions, with body weight of 300 to 400 kg were chosen for blood and serum analysis, electrocardiography and echocardiography to obtain the normal values at the local geographical region.

The obtained results were subjected to statistical analysis using SPSS 23.00 version. The sample size was 8 in the disease group and 10 for the apparently healthy group. One way ANOVA was utilised to compare the values on the different day of examination.

RESULTS

Post therapeutic evaluation of cardiopulmonary function in cattle with theileriosis by clinical examination on 0th, 7th and 14th days of post therapy revealed tachypnea percentages of 87.50% (7/8), 62.5% (5/8), 25.0% (2/8); dyspnea 75.0% (6/8), 62.5% (5/8), 37.5% (3/8); tachycardia 75.0% (6/8), 50.0% (4/8), 25.0% (2/8); cardiac murmurs 50.0% (4/8), 50.0% (4/8), 37.5% (3/8); muffled heart sounds 50.0% (4/8), 50.0% (4/8), 37.5% (3/8); cardiac arrhythmias 62.5% (5/8), 62.5% (5/8), 50.0% (4/8).

Table 1. Post therapeutic assessment of haemato-biochemical findings in cattle with theileriosis

Parameters	Apparently healthy cattle (n=10)	Cattle with theileriosis (n=8) Day of examination			P value
		0 th day	7 th day	14 th day	
Haemoglobin (g/L)	108.3±6.9	78.2±7.7	89.7±3.4	92.4±3.8	0.025
Hematocrit (%)	32.01±1.02	23.12±0.18	16.08±0.62	29.08±0.91	0.033
Red blood cells (×10 ¹² /L)	5.77±0.27	3.98±0.36	4.47±0.52	4.66±0.62	0.037
Albumin (g/L)	29.2±0.26	18.3±0.12	22.1±0.43	28.1±0.38	0.029
LDH (U/L)	277.3±9.9	518.8±11.1	498.7±22.1	402.3±19.1	0.280
GGT (U/L)	16.69±0.12	34.54±7.08	42.09±3.02	26.06±4.01	0.120
CK-MB (U/L)	55.05±2.08	708.19±29.19	718.88±21.88	587.72±28.87	0.300

Legend: LDH: lactate dehydrogenase; GGT: gamma glutamyl transferase; CK-MB: creatine kinase myocardial band.

Table 2. Electrocardiographic abnormalities in cattle with theileriosis

Types of abnormalities		Day of examination		
		0 th day	7 th day	14 th day
P wave abnormalities	P. mitrale, P. pulmonale, wandering pacemaker, atrial flutter	75.00%	62.50%	37.50%
QRS wave abnormalities	Wide QRS, low QRS voltage	62.50%	50.00%	50.00%
T wave abnormalities	Tall T wave, Short T wave, wide T wave, biphasic T wave	50.00%	50.00%	37.50%
ST segment abnormalities	ST elevation	50.00%	37.50%	25.00%
AV blocks	Primary and secondary AV block	62.50%	50.00%	37.50%

Table 3. Post therapeutic assessment of cardiac function by echocardiography in cattle with theileriosis

Parameters	Apparently healthy cattle (n=10)	Cattle with theileriosis (n=8) Day of examination			P value
		0 th day	7 th day	14 th day	
LVIDd (cm)	6.11 ± 0.18	6.37±0.19	6.34±0.34	6.31±0.28	0.07
LVIDs (cm)	4.17 ± 0.21	4.51±0.29	4.44±0.32	4.62±0.21	0.09
IVSd (cm)	2.31 ± 0.09	2.54±0.23	2.51±0.14	2.64±0.34	0.09
IVSs (cm)	2.81 ± 0.51	3.11±0.28	3.09±0.31	3.06±0.24	0.06
LVPWd (cm)	2.20 ± 0.18	2.33±0.19	2.27±0.38	2.31±0.17	0.12
LVPWs (cm)	2.89 ± 0.38	2.97±0.29	2.91±0.37	2.95±0.48	0.08

Legend: LVIDd: left ventricular internal dimension at end-diastole ; LVIDs: left ventricular internal dimension at end-systole ; IVSd: interventricular septum thickness at end-diastole ; IVSs: interventricular septum thickness at end-systole; LVPWd: Left ventricular posterior wall thickness at end-diastole; LVPWs: Left ventricular posterior wall thickness at end-systole

The results from haemato-biochemical analysis are documented in Table 1. There was a significant improvement in the levels of haemoglobin, serum albumin from 0th day to 14th day post therapy. Elevated levels of serum LDH, GGT and CK-MB remained constant during the therapy without statistical significance related to the day of examination.

The number of cattle with electrocardiography abnormalities assessed on the 0th, 7th and 14th evaluation day was present

as percentage (Table 2). Variations in the ECG parameters were analysed based on the morphological variations in the individual component of the electrocardiogram in comparison with the healthy cattle. P wave abnormalities were 75.00% (6/8), 62.5% (5/8) and 37.50% (3/8); QRS abnormalities – 62.50%, (5/8), 50.00% (4/8), 50.00% (4/8); T wave abnormalities: 50.00% (4/8), 50.00% (4/8), 37.50% (3/8); ST segment abnormalities: 50.00% (4/8), 37.50% (3/8), 25.00% (2/8); AV

block: 62.50% (5/8), 50.00% (4/8), 37.50% (3/8).

Post therapeutic evaluation of cardiac function by echocardiography (Table 3) noticed lack of statistical significant differences between evaluation days.

DISCUSSION

In the present study, the followed therapeutic regimen was in accordance with the previous studies. Syed *et al.* (2014) and Bhosale *et al.* (2020) utilised 2.5 mg/kg buparvaquone intramuscularly for treatment of theileriosis in bovines. In the present study, post therapeutic assessment recorded the remarkably haemato-biochemical changes which are specific for the study i.e. anaemia, low albumin levels and elevated serum cardiac enzymes; electrocardiography and echocardiography. Gradual reduction in the percentage of clinical signs were noticed in cattle with theileriosis during the course of study. Post therapeutic haemato-biochemical examination revealed significant improvement in the haemoglobin, packed cell volume, total erythrocyte count; serum albumin levels by the 7th and 14th day of therapy without significant changes in the serum LDH, GGT and CK-MB levels on the 0th day and 14th day of examination indicating constant elevation of cardiac muscle enzymes during the study period. Post therapeutic echocardiography revealed no significant change in between the echocardiographic parameters LVIDd, LVIDs, IVSd, IVSs, LVPWd and LVPWs on 0th day, 7th and 14th day respectively. Based on the present study findings, post therapeutic cardiac function was very essential to understand the clinical recovery in cattle with cardiac function abnormalities. No previous information was available on the assessment of prognostic factors in

cattle with theileriosis secondary to abnormal cardiac pulmonary function. Rasoulzadeh *et al.* (2021) recorded the cardiovascular biomarkers and histopathological alterations in cattle with babesiosis and reported these findings as helpful for the assessment of prognosis during the process. In the present study, the M mode echocardiography was utilized for measurements of cardiac chamber size and function. Tissue Doppler facilities are not available to carry on further studies to detect the more precise structural changes of heart for better understanding of prognosis.

The recorded electrocardiography data showed that theileriosis is more likely to induce cardiac ischemia, necrosis, and overall cardiac myocyte damage. Creatine kinase isozyme is released into the bloodstream as a result of damage to the heart and skeletal muscles, but it is less cardiac-specific biomarker than troponins. Myocardial muscle cell necrosis from anaemic anoxia brought on by severe haemolytic anaemia, hepatic damage, secondary rhabdomyolysis from prolonged recumbency, and haemoglobinuria resulting in ischemic and toxic nephrosis may all be responsible for the elevated level of CK-MB. However, CK-MB cannot be used as the only cardiac-specific biomarker for cardiac muscle damage because it is also present in extracardiac tissues.

Table 4 sums up the comparative studies' findings in bovines with theileriosis causing cardiac injury. Razavi *et al.* (2015) reported the effects on the cardiovascular system in cattle with theileriosis based on the basis of serum analysis of total homocysteine, cardiac troponin I, aspartate aminotransferase, alanine aminotransferase, creatine kinase and lactate dehydrogenase. Anaemia and consequently the hypoxia might have a role in

the parasite-induced myocardial injury in the diseased animals. Gupta *et al.* (2021) reported the electrocardiographic alterations in calves with theileriosis and documented the cardiac changes by serum cardiac troponin I and creatinine phosphokinase-myocardial band concentrations. In the present study, two cattle suffering with theileriosis showing signs of respiratory distress and developed brisket oedema have died. The observed prognostic factors from the two cases were presence of positive jugular pulsation and atrial fibrillation during the assessment

period; reduction of haemoglobin by 60%, 3 to 4 times elevation of serum LDH, GGT and 21 times elevation of serum CK-MB levels compared with the cattle that recovered from theileriosis; presence of pericardial fluid and increased thickness of post ventricular wall thickness. These factors will help the clinician to assess the therapeutic response and analyse the clinical outcome before initiation of therapy.

Ma *et al.* (2020) carried out a study on theileriosis in cattle and reported fever, enlargement of superficial lymph nodes, anaemia, and respiratory distress that

Table 4. Previous research findings on theileriosis on cardiac function in cattle

Authors/Study	Pathological findings
Fartashvand <i>et al.</i> (2013) Elevated serum cardiac troponin I in cattle with theileriosis.	Severity of anaemia and parasitaemia might contribute to the pathophysiology of myocardial damage in cattle with theileriosis. The prognostic significance of increased serum cardiac troponin I concentrations in cattle with haemolytic anaemia merits further investigations.
Razavi <i>et al.</i> (2015) Bovine tropical theileriosis: effects on the cardiovascular system on the basis of serum analysis	Total homocysteine, cardiac troponin I, aspartate aminotransferase, alanine aminotransferase, creatine kinase and lactate dehydrogenase showed remarkable increases in infected cattle with theileriosis proportional to the parasitaemia rate. <i>Theileria annulata</i> in cattle can induce significant myocardial tissue damage. In addition, anaemia and consequently hypoxia might have a role in the parasite-induced myocardial injury in the diseased animals.
Kilinc <i>et al.</i> (2018) Relationship between cardiac injury, selected biochemical parameters, DIC, and hemogram levels in cattle with theileriosis	Significant increase in levels of cardiac troponin I cTnI, creatine kinase – myocardial band, creatine kinase and aspartate aminotransferase, together with anaemia, are important in the diagnosis of cardiac injury in cattle with theileriosis. Hypoxia due to infection could cause cardiac injury.
Ma <i>et al.</i> (2020) Cattle experimentally infected with <i>Theileria annulata</i> in China	Petechial and ecchymotic haemorrhages on the mucosa and serosal surface, severe jaundice, pulmonary oedema and emphysema were present. The pericardium was slightly enlarged and haemorrhagic and the coronal groove was severely jaundiced
Gupta <i>et al.</i> (2021) <i>Theileria annulata</i> induced secondary hypoxia causes ischemic myocarditis in cattle calves	Increased serum concentrations of cardiac troponin-I and creatine kinase – myocardial band levels in calves with theileriosis was reported. <i>Theileria annulata</i> infection in calves changes the cell membrane integrity of myocardial muscle, leading to conduction abnormality and alteration in electrocardiographic parameters and an increase in serum level of studied cardiac biomarkers.

might be due to development of pulmonary oedema and emphysema. Prajapati *et al.* (2022) reported the most common clinical findings of theileriosis-infected cattle: anorexia, emaciation, nasal discharge; anaemia, decreased total protein and albumin, increased gamma-glutamyl transferase. Palavesam *et al.* (2021) diagnosed theileriosis by peripheral blood smear examination and reported decreased levels of Hb, RBC, WBC and PCV and reduced serum total protein and albumin. Assessment of serum CK-MB, AST, and LDH enzyme considered as cardiac biomarkers in bovines with traumatic pericarditis and traumatic reticuloperitonitis reported elevation of CK-MB and LDH enzymes in myocardial damage (Nasr *et al.*, 2024). Meenal *et al.* (2023) documented increased A/G ratio and serum CK-MB levels in calves affected with theileriosis. Kundave *et al.* (2015) diagnosed the theileriosis in buffaloes and cattle by Giemsa staining blood smears and PCR. Recently, Castaneda-Salazar & Oliver-Espinosa (2024) reported the first case of theileriosis in Holstein and Simmental cows in Colombia with increased GGT levels.

In the present study, prognostic factors in cattle with chronic theileriosis were presence of jugular pulsation on clinical examination, atrial fibrillation on electrocardiography, and pericardial fluid accumulation on echocardiography. Identification of the findings are of help for the early diagnosis of complications during chronic theileriosis and for correction of therapeutic regimen with suitable therapeutic agents including diuretics, cardiac protectants and stimulants.

Cattle with theileriosis showed cardiac lesions like pericardial fluid accumulation, ecchymosis and petechial haemorrhages on the epicardium, myocardium, and endocardium along with degeneration of

myocardial fibers. These notifiable changes might be due to anaemic hypoxia and acid-base and electrolyte disbalance, diffuse infiltration of inflammatory cells in some organs including the heart, so that myocardial cell membrane integrity is also compromised. Cattle with theileriosis created a novel viewpoint on CK-MB and LDH as biomarkers. Creatine kinase (CK) is an intracellular enzyme present in greatest amounts in skeletal muscle, myocardium, and brain; smaller amounts occur in other visceral tissues. During the disruption of cell membranes due to hypoxia or other injury CK is released from the cellular cytosol into the circulation. On this basis, elevated serum levels of CK have been used as a sensitive but nonspecific test for myocardial infarction. Further future studies are advised on high-sensitivity troponin assays and advanced echocardiographic studies to record the more specific myocardial injury during the disease process.

The present research provides the salient clinical features and parameters to for the assessment of the prognosis while carrying out the therapy and to record the status of cardiopulmonary function in cattle with theileriosis.

A limitation of the study was that was conducted on eight infected cattle only due to the available resources. A larger sample size which will provide more precise data about the individual parameters under study. Further study also advised long-term follow up of clinical cases to ascertain the prognosis. Another limitation of the study includes lack of Doppler studies.

Future research studies on the effectiveness of cardioprotective drugs in cattle with theileriosis will be carried out.

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