RESEARCH ARTICLE /SHORT COMMUNICATION

Theileria annulata Induced Secondary Hypoxia Causes Ischemic Myocarditis in Cattle Calves

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Abstract

Involvement of the myocardium in theileriosis is not a common phenomenon but is commonly ignored under field conditions due to lack of proper knowledge about the pathophysiology of the disease. In this study, the electrocardiographic (ECG) features and alteration in serum concentration of cardiac-specific biomarkers like cardiac troponin-I (cTnI) and creatinine kinase (CK-MB) were evaluated in healthy (n=10) and theileriosis affected (n=12) cattle calves. There were alterations in the ECG features and serum concentrations of cTnI and CK-MB in theileriosis-affected calves as compared to healthy ones. Electrocardiographic study in diseased animals revealed the presence of Sinus tachycardia, 1st degree AV block, Atrial premature complex, Left atrial hypertrophy, and Right atrial hypertrophy in 91.66%, 33.33%, 16.67, 50.00%, and 50.00% cases, respectively. Serum concentrations of cardiac troponin-I in healthy and diseased animals were 0.144±0.119 ng/mL and 1.271±0.342 ng/mL respectively, while serum concentrations of CK-MB in healthy and diseased animals were 34.039±2.983 ng/mL and 49.710±3.332 ng/mL, respectively. Results showed alteration in standard electrocardiographic features and change in normal serum concentration of cardiac biomarkers, both indicative of cardiac involvement in the clinical *Theileria annulata* infection.

Keywords: Arrhythmia, Cardiac troponin-I, CK-MB, Electrocardiography, Theileriosis.

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Introduction

Theileriosis is an important tick-borne disease, causing widespread alteration in physical, haemato-biochemical, electrocardiographic, and cardiac biomarkers parameters. Clinically, the disease is characterized by fever, anorexia, lymphadenopathy, pale mucous membrane, ocular and nasal secretions, exophthalmos, petechial hemorrhages on conjunctival mucous membrane along with melena and occasionally haemoglobinuria. In addition to typical signs of theileriosis, various cardiac lesions include excessive pericardial fluid, ecchymosis, and petechial hemorrhages on the epicardium, myocardium, and endocardium (Omer *et al.,* 2003), degeneration of myocardial fibers, and diffuse infiltration of inflammatory cells have been documented. Although the exact reason behind cardiac involvement is unclear, it is believed that anemic hypoxia and acid-base and electrolyte derangements may lead to disturbance in myocardial cell membrane integrity, which is clinically manifested as abnormal electrocardiography and alteration in cardiac-specific biomarkers. Alteration in bovine-specific cardiac biomarkers and electrocardiographic features reflect the involvement of myocardium in the pathophysiology of theileriosis. Cardiac troponin I (cTnI) and creatinine phosphokinase (CK-MB) are considered as bovine specific cardiac biomarkers, but the former has been considered as the more specific for the detection of cardiac myocytes damage, with virtually absolute myocardial tissue specificity and higher sensitivity (Babuin and Jaffe, 2005; Diniz *et al.,* 2008) than CK-MB. Therefore, this study has been aimed to evaluate ECG features and serum concentration of these cardiac biomarkers in *Theileria* infected and healthy calves.

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Materials and Methods

The work was carried out during the period of August-2016 to April-2017 at DUVASU, Mathura, Uttar Pradesh.

Screening of Animals

Screening of animals suspected for *Theileria* was done based on the presence or absence of classic clinical symptoms of theileriosis which include a significant increase in rectal

temperature (40–41.5°C), tachycardia (>80 beats/min), lymphadenopathy (prescapular or prefemoral), anaemia (Kachhawa *et al.,* 2016) and conjunctival petechiae (Qayyum *et al.,* 2010). Total seventy-one (71) calves were screened and further analyzed to confirm disease by optical microscopy of thin blood smears stained with Leishman's stain for piroplasm and molecular diagnosis such as polymerase chain reaction (PCR). About 2 mL and 5 mL of whole blood were collected from the jugular vein of the calves in screw-capped sterilized glass vials with and without ethylene diamine tetraacetic acid (EDTA) as an anticoagulant, respectively. DNA isolation and subsequent PCR analysis from whole blood were done by using a standard protocol. Serum samples were stored at – 20^0C till further use for assay of cardiac biomarkers. Microscopic examination, followed by PCR assay, confirmed a total of twelve (n=12) calves positive for theileriosis. These cases were further analyzed for electrocardiographic parameters and cardiac biomarker alteration. Ten apparently healthy calves (n=10) of the same age group were taken as a control to compare the results

Electrocardiographic and Cardiac Biomarker Studies

Electrocardiography of screened cases of theileriosis as well as the control group was done with RMS-Vesta301i-electrocardiograph. Serum samples were analyzed for bovine specific cardiac biomarkers (cardiac troponin-I and CK-MB) using commercial Blue Gene ELISA kits and results compared with control animals. Test performance parameters for biomarkers were calculated by online software (Guyatt and Rennie, 2002), and area under curve (AUC) was determined through online software (MedCalc, 2014).

Results and Discussion

Electrocardiographic Findings

Various ECG parameters, recorded in animals of healthy and diseased groups, were amplitude and duration of P wave, PR interval, duration of QRS complex, R wave amplitude, QT interval, amplitude and duration of T wave, etc. Numerical values of all the parameters were tabulated for easy understanding (Table 1). The data showed no significant difference in any of the ECG parameters. Further, the individual ECG analysis revealed the presence of sinus tachycardia in 11 calves (91.66%), 1st degree AV block in 4 calves (33.33%), Atrial premature complex (APC) in 2 calves (16.67%), and left and right atrial hypertrophy each in 6 calves (50.00%) (Table 2; Fig. 1 & 2). Sinus tachycardia present in some of the theileriosis-affected calves may be due to pain, hyperthermia, anemia, electrolyte imbalance, or fall in the arterial blood pressure (Radostits *et al.,* 2007). Some of these changes are present in the affected calves and cause this abnormal finding. First-degree AV block occurs due to delay in conduction at the atrioventricular valve and may be associated with cardiomyopathy or electrolyte imbalance seen in some diseased calves (Radostits *et al.,* 2007). Various alterations in waveform and complexes suggested the involvement of the myocardium in the pathophysiology of theileriosis. Although the exact mechanism of myocardial involvement is not clear to date (Miranda *et al.,* 2006), reduced oxygen supply to the myocardium followed by anemia and secondary hypoxia combined with increased oxygen consumption due to long term tachycardia (Miranda *et al.,*

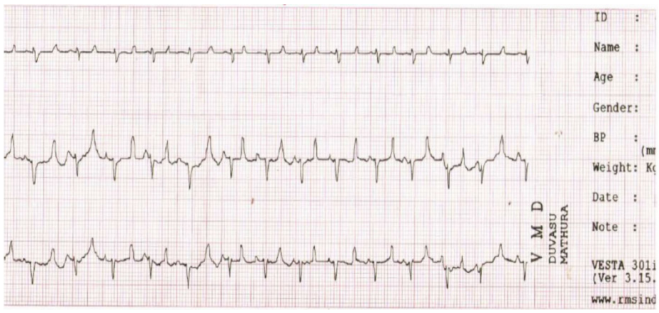

Fig. 1: ECG showing sinus tachycardia (ST) in theileriosis affected calf (ID-6748, Date: 31.12.2016)

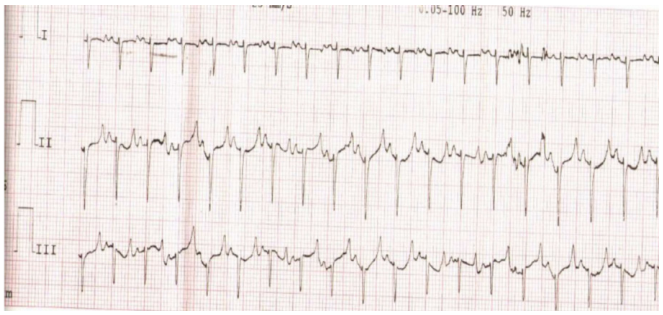

Fig. 2: ECG showing atrial premature complex (APC) in theileriosis affected calf (ID-3189, Date: 13.09.2016)

Table 1: Various ECG parameters of control and *Theileria* infected calves

S. No.	*Parameters*	*Control (n = 10)*	*Diseased (n = 12)*
1	P Duration (sec)	0.0380 ± 0.001^{NS}	0.040 ± 0.005^{NS}
2	P Amplitude (mv)	0.160 ± 0.014^{NS}	0.182 ± 0.015^{NS}
3	P-R interval (sec)	0.096 ± 0.006^{NS}	0.102 ± 0.010^{NS}
4	QRS duration (sec)	0.052 ± 0.004^{NS}	0.050 ± 0.004^{NS}
5	R amplitude (mv)	0.833 ± 0.062^{NS}	0.821 ± 0.077^{NS}
6	QT Interval (sec)	0.277 ± 0.022^{NS}	0.224 ± 0.018^{NS}
7	T amplitude (mv)	0.29 ± 0.03^{NS}	0.31 ± 0.021^{NS}
8	T duration (sec)	0.066 ± 0.012^{NS}	0.08 ± 0.011^{NS}

n= No. of animals, Different superscript differ significantly (P < 0.01), NS- non-significant

Table 2: Cardiac arrhythmia in *Theileria* infected calves

Abnormality	*Frequency (n = 12)*
Sinus tachycardia	91.66% (11/12)
1st degree AV block	33.33% (4/12)
Atrial premature complex	16.67 (2/12)
Left atrial hypertrophy	50.00% (6/12)
Right atrial hypertrophy	50.00% (6/12)

Table 3: Cardiac markers of control and *Theileria* infected calves

Parameters	*Control (n = 10)*	*Diseased (n = 12)*
cTnI (ng/mL)	0.144 ± 0.119^{b}	1.271 ± 0.342^{a}
CPK-MB (ng/mL)	34.039 ± 2.983^{b}	49.710 ± 3.332^{a}

Means with different superscripts within the row differ significantly ($p < 0.05$)

2006 and Zellweger *et al.,* 2003), accumulation of parasitized lymphoblast in blood vessels of viscera including heart (Radostitis *et al.,* 2007) may be the reasons for myocardial damage in theileriosis. Above ECG findings suggested that theileriosis-affected calve are predisposed to cardiac ischemia, necrosis, and general cardiac myocyte injury (Ralli *et al.,* 2005).

Bovine Specific Cardiac Biomarkers

Analysis of cardiac biomarkers in serum like cardiac troponin-I (cTnI) and creatinine kinase isozyme (CK-MB) revealed a significant rise in diseased calves compared to healthy control calves indicating the involvement of the heart in the pathogenesis of theileriosis. The mean value of cardiac troponin-I in healthy and diseased calves was 0.144±0.119 ng/mL and 1.271±0.342 ng/mL ($p < 0.05$), while that of creatinine kinase isozyme were 34.039±2.983 ng/mL and 49.710±3.332 ng/mL ($p < 0.05$), respectively. Troponins are complex of three regulatory proteins (I, C, and T), which play an essential role in the skeletal and cardiac muscle contractions by controlling the calcium mediating interaction between actin and myosin. Out of these three proteins, only troponin-I is uniquely expressed in the myocardium (Collinson and Gaze, 2007); hence, the serum concentration of cardiac troponin-I reflects the integrity of the myocardial cell membrane. Previously, cardiac troponin-T was considered as an essential biomarker of cardiac muscle damage (Falahati *et al.,* 1999), but recent studies suggested that cardiac troponin-I is a more sensitive and specific biomarker of cardiac muscle damage (Gunes *et al.,* 2005). In the present study, the increased level of cardiac troponin-I was indicative of disturbed myocardial cell membrane integrity leading to the leakage of cardiac troponin-I in plasma. This may occur due to the secondary hypoxia caused by anemia leading to vascular damage and ischemia of the myocardium (Razavi *et al.,* 2015). Another cardiac biomarker used for studying myocardial damage is creatinine kinase isozyme (CPK-MB) which is released in circulation due to cardiac and skeletal muscle damage (Adams *et al.,* 1994). Although CK-MB is more specific than total CK for cardiac muscle injury, it is a less cardiac-specific biomarker than troponins (Adams *et al.,* 1993). Increased level of CK-MB in theileriosis-affected calves may be attributed to myocardial muscle cell necrosis due to anemic anoxia occurring due to severe haemolytic anemia, hepatic damage, and secondary rhabdomyolysis to prolonged recumbency, and haemoglobinuria leading to ischemic and toxic nephrosis (Radostitis *et al.,* 2007). However, due to the presence of CK-MB in extracardiac tissues, it cannot be used as the sole cardiac-specific biomarker for cardiac muscle damage (Babuin and Jaffe, 2005).

Conclusion

From the study, it can be concluded that *Theileria annulata* infection in calves causes a change in the cell membrane integrity of myocardial muscle, leading to conduction abnormality and alteration in electrocardiographic parameters and an increase in serum level of cardiac biomarkers such as cardiac troponin-I and creatinine kinase isozyme (CK-MB). It can be used as important diagnostic tools for the effect of theileriosis on the myocardium.

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