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Prognostic Value of Cardiac Biomarkers in Naturally Infected Anaplasmosis, Theileriosis and Babesiosis Associated Cardiac Damage in Dairy Cows

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ABSTRACT

Background: Treating severe forms of hemoprotozoan diseases are challenging. Myocardial damage in such parasitic diseases are often neglected during routine clinical practice. The present study was designed to assess the deleterious effects of naturally infected cattle with hemoprotozoa on the cardiovascular system and the consequent myocardial involvement.

Methods: The positive animals were subjected to clinical examination, haemato-biochemical analysis, estimation of cardiac biomarkers such as cardiac troponin I (cTnI), creatine kinase myocardial band (CK-MB), lactic dehydrogenase enzyme (LDH), ultrasonography and Base Apex Electrocardiography to find their prognostic significance in cattle with hemolytic anemia.

Result: A significant change in these biomarkers was noticed post treatment, indicating their utility as prognostic markers as well as to assess the efficacy of therapy.

Key words: Bovine, Cardiac troponin I, Hemoprotozoa, Myocardial injury.

INTRODUCTION

Hemoparasitism is a condition commonly found in animals and it exerts negatively on the health, production, reproduction and performance of the affected animals. This study is aimed at determining theeffect of different hemoparasites on the cardiovascular system and the consequent myocardial involvement in the affected cattle in Cauvery Delta of Tamil Nadu and their prognostic significance.

However, cTnI is now the preferred biochemical parameter in human medicine for assessing myocardial necrosis and myocyte damage, with virtually absolute myocardial tissue specificity as well as higher sensitivity than CK isoenzyme CK-MB, cardiac troponin C and cardiac troponin T. A remarkable homology exists in amino acid sequence between human and animals and tissue reactivity of cTnI of various species revealed that cTnI can be used in animals (Apoorva *et al.,* 2022). Elevated cTnI has been reported in calves and cattle indicating that elevations in the blood would serve as useful biomarkers of myocardial injury (Venkatesan *et al*., 2020). Hence the present study was designed to evaluate the cardiac biomarkers, lectrocardiography and ultrasonography in hemoprotozoa associated cardiac damage. cTnI and CK-MB can be used as myocardial markers in hemoprotozan affected animals.

MATERIALS AND METHODS

The total of 56 suspected cattle wasscreened for hemoparasites based on clinical signs and the confirmation of the infection was made through routine peripheral blood smear examination at Large Animal Cardiology Referral Clinics of Veterinary Clinical Complex, Orathanadu from August 2018 to August 2019 for a period of one year from different places of Cauvery delta region of Thanjavur district, Tamil Nadu. Leishman stained thin blood smears were prepared and examined from cattle suspected for hemoparasites. Clinical examination of animal revealed presence of fever, pale mucus membranes, strenuous breathing and decrease in appetite. Animals that were found positive, identified as infected while those who were found negative serve as control. A total of 32 infected and 10 healthy control were studied. For hematologic and biochemical analysis, approximately 10 mL blood was collected from jugular vein into EDTA for whole blood

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examination and serum activator tube for biochemical analysis and was used to quantify Cardiac Troponin I (cTnI), creatine kinase myocardial band (CK-MB) and lactic dehydrogenase enzyme (LDH). cTnI was assessed in serum samples using the Point-of Care immunoassay i-STAT analyzer, supplied by Abbott Healthcare Pvt. Ltd. (Fig 1)

Trans-thoracic ultrasonography of heart was performed with Esaote Lab versionultrasound machine. The ECG was recorded on a bipolar base apex lead system using limb lead I. Animal was kept in standing position on an insulating floor; no clipping or shaving was carried out for electrode attachments. The positive electrode of Lead I (left arm) was attached to the skin of the fifth intercostals space just caudal to the olecranon and the negative electrode (right arm) on the jugular furrow about lower 1/3rd of the left side of the neck (Yogeshpriya *et al*., 2021) (Fig 2). ECG was obtained with the paper speed of 50mm/sec and calibration of 10 mm equal to 1 mV.

On day 3rd and 7th of post treatment, blood collection, ultrasonogram and electrocardiography of 32 infected and 10 control animals were again made. Serum was separated from blood of all the animals both before and after therapy and stored in -20°C until further use. The data obtained were statistically analyzed using T-test with SPSS software.

RESULTS AND DISCUSSION

In the present study, a total of 54 highly suspected cases of hemoparasitic cattle blood smears were examined, out of which 32 animals were found to be positive for haemoprotozoa. Among 32 blood smear examined, 14 revealed *Anaplasma* sp, 10 smears showed *Babesia* sp and 8 had *Theileria* sp) (Fig 3-6). Since the animal shows clinical disease and confirmed for blood parasitic infection, then treatment was given as per standard protocol (Radostits *et al*., 2000).



Fig 1: Point of care analysis-cardiac troponin analysis.

In the ultrasonographic examination the hyperechogenic pericardium and epicardium were separated by varying quantity of hypoechogenic exudate (Fig 7and 8). When the heart was ultrasonographically examined from the left side, the evenly spread hypoechogenic pericardial effusion could be seen in 6 of these 32 cows. Ultrasound guided pericardial fluid yielded aseptic pericardial effusion without any foreign body in such cases and no bacterial growth observed on culture.

In the current study, significantly elevated cTnI levels observed in naturally infected *Anaplasma*, *Theileria* and *Babesia* animals (Table 1) whereas, the mean cTnI concentration was 0.08±0.03 ng/ml in healthy control group. Among 32 animals, two of them showed very high cTnI value, in *Theileria* (34.98 ng/ml) and *Anaplasma* (24.16 ng/ml) affected cattle, respectively. The measured CK-MB in *Theileria*, *Babesia* and *Anaplasma* affected cattle were also above the normal range. There were significant differences in cTnI, CK-MB and LDH values between healthy and diseased groups.

Electrocardiographic assessment showed prolonged QTc and STc interval in the animals affected with hemoparasites. The prolonged QT interval (>0.40 seconds) and ST interval (>0.32 sec) in cows with hemoparasites confirmed the electrolyte imbalance or cardiac changes in 6 animals (Fig 9). The Corrected QT interval (QTc) and ST interval (STc) based on Bazett's formula showed >0.45 sec and >0.36 sec in all six cows (Fig 10) which indicated subclinical hypocalcemia in the hemoprotozoan affected animals. ECG interpretations showed that 18 infected cattle had sinus tachyarrthymia and 14 cattle had normal sinus rhythm. The affected animals don't have any bradycardia.

The animals positive for Anaplasmosis was treated with Inj. Oxytetracycline @ 20 mg/kg b.wt. in 500 ml normal saline i/v, Meloxicam @ 0.5 mg/kg b.wt. i/m, liver extract @ 12-15 ml i/m for consecutive five days along with supportive fluid therapy and oral iron supplements (Bol. Ferritas). Those animals positive for Babesiosis were treated with a single dose of Inj. Diminazene aceturate @ 5.0 mg/Kg b.wt. i/m and the Theileria positive cases were treated with Inj.



Fig 2: Electrocardiogram in a cattle-base apex limb lead system.

Buparvaquone @ 2.5 mg/kg b.wt. i/m (single dose) along with Inj. Oxytetracycline @10 mg/kg body weight for a period of 10 days followed by supportive therapy and oral hematinics.

The incidence of haemoprotozoan diseases varies on climatic conditions such as temperature, humidity, rainfall and geographical area (Radostits *et al.,* 2000). An increased number of *Anaplasmosis*, *Babesiosis* and *Theileriosis* were observed in this study might be due to the involvement of vector (tick) *Rhipicephalus sp* in the transmission of haemoprotozoan diseases, as it is the most common tick species found in cattle in the Cauvery delta region (Jayalakshmi *et al*., 2019). Measurement of cTnI is a very sensitive way of demonstrating myocardial cell damage in animals with clinically suspected myocarditis (Tunca *et al.,* 2008). Serum cardiac troponins are the earliest appearing biochemical markers during myocardial damage. During myocardial cell damage increased in cTnI proteins have a high sensitivity and absolute specificity; they are released even in micro injuries into the blood stream (Oyama and Sisson, 2004). These proteins are usually present in blood either at very low concentrations or below the limit of detection for most of the assays (Gunes *et al.,* 2008). Overall, the degenerative changes in the internal organs as indicated by cardiac biomarkers may be due to anemic hypoxia. Infiltration of circulating immune complexes may further complicate the problem (Aulakh *et al.,* 2005).

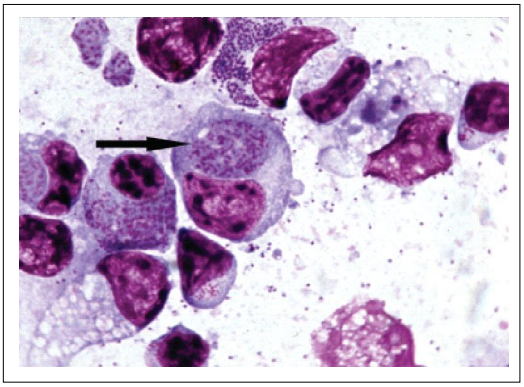


Fig 3: Koch's blue bodies (Giemsa staining 100x).

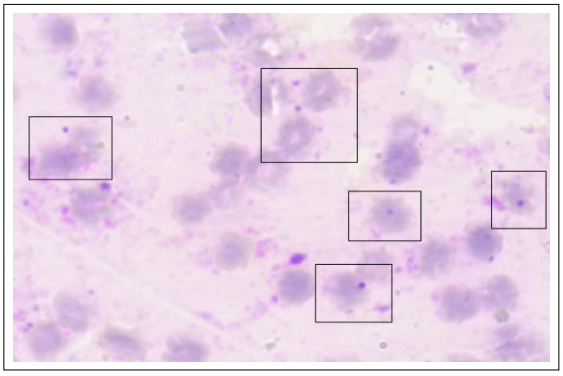


Fig 6: *Anaplasma* sp., in an affected cattle.

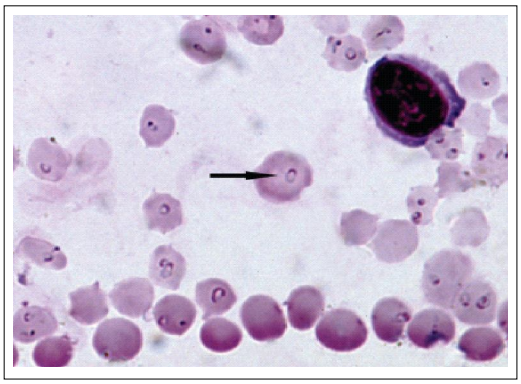


Fig 4: *Theileria annulata* (Giemsa staining (100x).

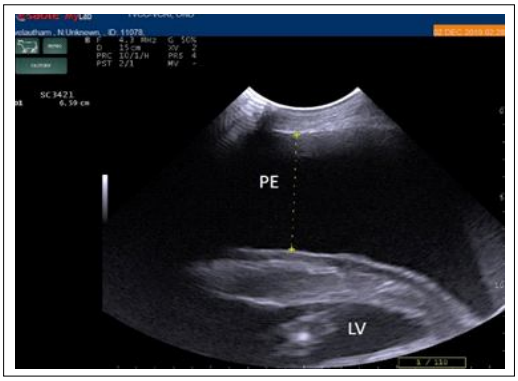


Fig 7: Pericardial effusion in *Anaplasma* sp. Affected cattle.

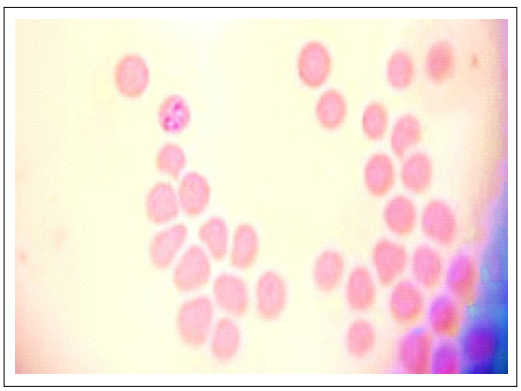


Fig 5: *Babesia bigemina* giemsa stained blood smear.

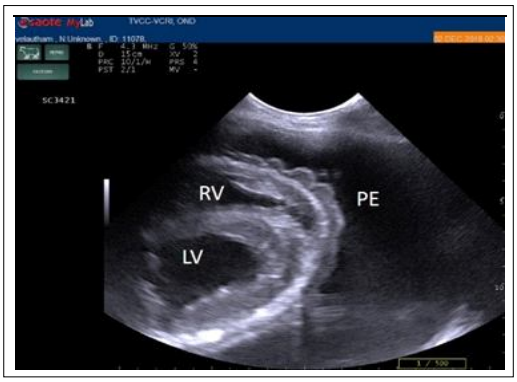


Fig 8: Pericardial effusion in cattle with babesiosis.

Myocardial effects are very rare in protozoan diseases. On the other hand, increasing serum cTnI levels have been reported in equine piroplasmosis, canine babesiosis, trypanosomiasis and bovine theileriosis. Cardiac troponin I is released from myocytes in both reversible and irreversible myocardial injury. The changes in myocyte membrane permeability resulting from the injury could be enough for the release of cardiac troponins from the free cytosolic pool of myocytes without structural damage. It may be speculated that increased oxygen consumption by the myocardium during a prolonged period of tachycardia is combined with a reduced oxygen supply to myocardium attributed the release of troponins from the heart secondary to anemia in cattle.

CK-MB and LDH are cytoplasmic enzymes with a high activity in heart, skeletal muscle, liver, kidney and red blood cells. These enzymes are indicators of a higher level of cellular damage and their increased activity is a consequence of their increased release from the damaged cells and a reflection of metabolic changes in the inflamed tissues especially in the heart (Graeber *et al*., 1990). Higher levels of AST and CK-MB have been detected in cattle with babesiosis than in healthy cattle. In anaplasmosis, degenerative changes are observed in different organs due to hypoxic conditions created by anemia. Further changes may be due to immunological reactions produced by parasite. Very few published reports are available in literature on histopathological changes in bovine anaplasmosis.

Table 1: Cardiac biomarkers in hemoparasites affected cows.

Variables	CTnI ng/dl	CK-MB	LDH
Control groups	0.08±0.03	87.23±1.61	784±2.20
Theileria infected group	2.91±1.12**	342±3.4**	2212±0.13**
Babesia infected group	0.82±0.08*	254±2.22*	1854±0.02*
Anaplasma infected group	1.92±0.21**	312±0.31**	2012±1.08**

* Significant at p<0.05; ** Significant at p<0.01.

LDH activity rises slowly after myocardial infarction and becomes maximal after CK-MB elevations (Ohman *et al*., 1982). Determinations of LDH activity have been used diagnostically to determine whether acute myocardial infarction occurred in the days before a patient was evaluated (Adams *et al*., 1993). Measurement of cTnI is clearly more sensitive than the LDH cutoff value for retrospective diagnoses of acute myocardial injuries. Resolution of this problem has been advanced by the development of techniques that separate CK into its three isoenzymes containing MM, MB and BB (Van Der Veen and Willebrands, 1966). Separation and quantification of MB isoenzyme, which is found almost exclusively in heart muscle, provides a more specific indicator of acute myocardial infarction than total CK alone. CK-MB as a cardio specific enzymehas been introduced as a sensitive marker of myocardial injury (Roe *et al*., 1972). Recent studies report that although the sensitivity of cTnI is comparable to that of CK-MB, its specificity seems to be higher (Adams *et al*., 1994). In the diagnosis of acute myocardial infarction, the measurement of elevated levels of CK-MB and LDH are well known (Jaffe *et al*., 1984).

The changes in the length of the QT interval depend on the heart rate and various other factors. Shortening of the QT interval typically occurs in pathological conditions such as hypercalcemia and digitalis intoxication. QT interval prolongation can be caused by various factors, including imbalance of electrolytes such as hypokalemia and hypocalcemia (Koyama *et al*., 2004).

It was well established that cardiac troponins are cleared from blood faster than CK-MB, but concentrations stay



Fig 9: Prolonged QT interval in cattle affected with anaplasmosis (50mm paper speed).

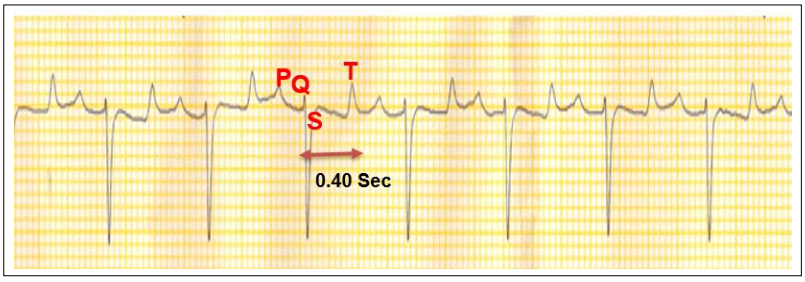


Fig 10: Prolonged QT interval in cattle affected with babesiosis (25 mm paper speed).

elevated longer. Because 94% of troponins are bound in the structure of myofibrilla and 6% are dissolved in cytosol, in cases of severe cell damage the proportion that is dissolved in the cytosol can immediately be released into blood by diffusion. In contrast, the major proportion of troponins can only be released after proteolytic disintegration. The animal with very high troponin values might be due to above said reason. This delayed release from the injured cell is the reason for the relatively long period during which increased troponin concentration is detectable in serum, although the fragments continue to be subjected to proteolytic disintegration within the blood stream. These findings indicate continued, delayed release of troponins from necrotic myocytes.

CONCLUSION

Cows with noncardiac diseases may have some degree of myocardial injury. The magnitude of cTnI increased may assist clinicians in evaluating the risk of an adverse outcome and help guide decision-making regarding treatment and prognosis. In conclusion, the results in this study demonstrated that serum CK-MB, troponin I and LDH concentrations along with electrocardiography could be used for evaluating myocardial injury in hemoparasites.

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Conflict of interest

All authors declare that they have no conflict of interest.

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