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## Alterations in serum profiles and cardiac status of cattle calves affected with theileriosis

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#### Abstract

The objective of the present study was to evaluate the changes in serum biochemical parameters in cattle calves below six month of age affected with theileria. The study was undertaken in calves at private dairy farms as well as animals of individual holdings in Bikaner district of Rajasthan. A total of 16 calves affected with theileria were selected, theileria in calves was confirmed on the basis of PCR for presence 30-kDa major *T. annulata* merozoite surface antigen; 721-bp gene fragment and eight clinically normal healthy calves were taken as control. Analysis of serum samples of the theileria affected calves revealed significant increase in A/G ratio, blood urea nitrogen, creatinine, ALP, AST, ALT, potassium, cTn-I and CK-MB while significant decrease in total protein, albumin, globulin, glucose, calcium and phosphorus. There was also decrease in serum sodium ion concentration as compared to control though it was not significant. Result suggests deviation of serum profile towards negative side along with hepatic and cardiac damage in calves.

**Keywords:** Theileriosis, *T. annulata*, cattle calves, serum biochemistry

#### Introduction

Bovine tropical theileriosis is one of the economically important haemoprotozoan diseases of large ruminants caused by protozoa of theileria genus transmitted by Ixodid ticks leading to heavy economic losses in terms of morbidity and mortality as well as expenses spent on prophylactic measures against disease and treatment (Durrani *et al.*, 2008) [7]. Weight loss, weakness, anorexia, pyrexia, conjunctival petechia, swollen lymph nodes, and anaemia are the most common symptoms. However, the later stages of theileriosis are associated with diarrhoea, dysentery and lateral recumbency (Stockham *et al.*, 2000; Constable *et al.*, 2017) [26, 4]. In addition to typical signs of disease, cardiac lesions, such as excessive pericardial fluid, ecchymosis and petechial haemorrhages on the epicardium, myocardium, and endocardium (Gill *et al.*, 1977; Omer *et al.*, 2003) [12, 18], degeneration of myocardial fibers and diffuse infiltration of inflammatory cells have been described. Although exact etiology is unknown, but due to anemic hypoxia and acid-base and electrolyte derangements, diffuse infiltration of inflammatory cells in some organs including heart may occur in theileriosis, so that myocardial cell membrane integrity is also compromised in theileriosis (Gill *et al.*, 1977) [12]. Cardiac troponin I (cTnI) control the calcium medium interactions between myofibrils, and its concentration is often correlated to myocardial damage (Gunes *et al.*, 2008) [13]. Alongside, creatinine phosphokinase myocardial band (CPK-MB) is also an indicator of acute myocardial infarction. Estimation of their level helps to assess cardiovascular damage caused by *T. annulata* (Gibson *et al.*, 2002) [11].

The serum biochemical alterations in calf affected with theileria are complex in nature comprising of serious imbalances of fluid, electrolyte, enzymes and cardiac biomarkers status which may be life threatening in the affected animals, so their values may be helpful during the treatment and knowing the prognosis. Keeping all these points in view, the present study was conducted to study alterations in serum biochemical minerals and enzymes along with cardiac status in positive cases of theileriosis.

#### Materials and Methods

##### Animals

Cattle calves showing classical clinical signs of theileriosis irrespective of sex and breed, below 6 months of age at private dairy farms as well as animals of individual holdings in Bikaner district of Rajasthan were the source of animals for the present study. A total of 16 cattle calves suffering from theileriosis which were confirmed on the basis of PCR for

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presence of 30-kDa major *T. annulata* merozoite surface antigen (721-bp gene fragment). Eight clinically healthy cattle calves were also randomly selected and served as control to study normal parameters.

### Serum Biochemical Profile

Serum biochemical parameters including glucose, total protein, albumin, globulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), serum creatinine (SCK) and blood urea nitrogen (BUN) were estimated by using the Turbochem 100 automated clinical chemistry analyzer by CPC diagnostics. Electrolyte estimation of serum samples for sodium and potassium were carried out by CKK lyte electrolyte analyzers (ARK diagnostics Bangalore pvt. Limited). Serum calcium and phosphorus were estimated by Inductively Coupled Argon Plasma Spectrometer (iCAP 7000 Series). Cardiac troponin-I and CK-MB status of serum samples were

estimated by using commercial GENLISA™ ELISA kit supplied by Krishgen BioSystem. The Principles, reagents required, procedure, calculation and precautions mentioned for each of them were followed as per operator's manual.

### Results and Discussion

Average values (Mean  $\pm$  SE) of total serum protein (g/dl) in healthy control and theileria affected calves were  $7.00 \pm 0.16$  and  $4.64 \pm 0.13$  respectively. There was significant decrease in the total serum protein in theileria affected calves. Concentration of serum albumin (g/dl) and globulin (g/dl) in theileria affected calves ( $2.40 \pm 0.10$  and  $2.24 \pm 0.06$ ) were also significantly lower in comparison with healthy calves ( $3.15 \pm 0.10$  and  $3.85 \pm 0.08$ ). There was significant increase in the A/G ratio in theileria affected calves. The mean values of serum glucose (mg/dl) in healthy and theileriosis calves were  $54.15 \pm 2.15$  and  $35.77 \pm 1.32$ , respectively which were significantly lower (Table 1).

**Table 1:** Serum biochemical and enzymes (Mean  $\pm$  SE) of control and theileria affected calves

S. No.	Parameters	Healthy calves (n=8)	Theileriosis calves (n=16)	Statistical analysis (T test)
1.	Protein (g/dl)	$7.00 \pm 0.16$	$4.64 \pm 0.13$	**
2.	Albumin (g/dl)	$3.15 \pm 0.10$	$2.40 \pm 0.10$	**
3.	Globulin (g/dl)	$3.85 \pm 0.08$	$2.24 \pm 0.06$	*
4.	A:G ratio	$0.82 \pm 0.02$	$1.07 \pm 0.05$	*
5.	Glucose (mg/dl)	$54.15 \pm 2.15$	$35.77 \pm 1.32$	**
6.	BUN (mg/dl)	$20.13 \pm 1.02$	$34.47 \pm 1.20$	**
7.	Creatinine (mg/dl)	$0.69 \pm 0.04$	$1.68 \pm 0.08$	**
8.	ALP (U/L)	$72.25 \pm 1.54$	$83.63 \pm 1.69$	*
9.	ALT (U/L)	$28.27 \pm 1.99$	$62.67 \pm 5.19$	**
10.	AST (U/L)	$52.23 \pm 2.01$	$137.34 \pm 6.98$	**

\*\*= $p < .01$  \*= $p < .05$

Modi *et al.* (2015) [17], Kachhawa *et al.* (2016) [16], Singh *et al.* (2017) [24], Somu *et al.* (2017) [25], Degirmencay *et al.* (2021) [6], Ram *et al.* (2021) [20] and Ram (2022) [19] also reported similar findings in calves affected with theileriosis. The harmful effect of toxic metabolites of theileria and liver failure may also be the reason of hypoproteinaemia and hypoalbuminemia in theileria affected cattle (Singh *et al.*, 2001; Saber *et al.*, 2008; Al-Emarah *et al.*, 2012) [23, 21, 1]. *T. annulata* infection cause hepatic tissue damage that includes coagulative necrosis, distortion of hepatic cords and heavy infiltration of lymphocytes in the periportal areas (Sandhu *et al.*, 1998) [22], which is responsible for hepatic dysfunction. Anorexia and fever may also contribute to hypoproteinaemia (Tehrani *et al.*, 2013) [28]. The decreased serum glucose concentration may be due to the abnormalities in liver functions and abnormalities in metabolism and anorexic state of theileria affected animals (Sandhu *et al.*, 1998; Hussein *et al.*, 2007; El-Deeb *et al.*, 2009) [22, 15, 8]. The utilization of glucose by theileria parasites, hepatic dysfunction in infected calves and metabolic disturbances due to stable fever could be reasons behind the decreased glucose levels (Col and Uslu, 2006) [3].

Mean values of ALT (U/L) and AST (U/L) in healthy calves were  $28.27 \pm 1.99$  and  $52.23 \pm 2.01$ , whereas, in theileriosis affected calves were  $62.67 \pm 5.19$  and  $137.34 \pm 6.98$ . There was significant increase in the mean values of ALT and AST in theileriosis as compared to healthy control group. The findings of higher values of AST and ALT in the present study were in accordance with Modi *et al.* (2015) [17], Kachhawa *et al.* (2016) [16], Debarma *et al.* (2020) [5] and Ram *et al.* (2021) [20]. AST and ALT are involved in amino

acid and carbohydrate metabolism. These enzymes are present in high concentration in the muscles and liver. The elevations of these enzymes in the blood are indicator of organ necrosis (Forsyth *et al.*, 1999) [10]. *Theileria annulata* infection that caused hepatic tissue damage that includes coagulative necrosis, distraction of hepatic cords and heavy infiltration of lymphocytes in periportal area, indicating severe damage to hepatobiliary system due to hypoxia resulting from anaemia and hepatitis (Sandhu *et al.*, 1998; Stockham *et al.*, 2000) [22, 26]. This significant increase in serum AST and ALT might be due to hepatic dysfunction.

Mean values of blood urea nitrogen (mg/dl) and serum creatinine (SC) (mg/dl) in healthy calves were  $20.13 \pm 1.02$  and  $0.69 \pm 0.04$ , whereas, in theileriosis were  $34.47 \pm 1.20$  and  $1.68 \pm 0.08$ . There was significant increase in the mean values of BUN and SC in theileriosis as compared to healthy control group. The elevated level of BUN and creatinine observed in the present study was in agreement with Tuli *et al.* (2015) [29] which might be attributed to increased turnover of proteins and may be increased because of inadequate renal perfusion (Constable *et al.*, 2017) [7].

The mean value of serum sodium (mmol/L) in healthy calves and theileriosis were  $130.88 \pm 0.76$  and  $128.71 \pm 0.30$  respectively. Though the decrease was statistically non-significant in affected calves as compared to healthy control. Hyponatraemia occurs as a result of excessive secretion of the sodium ions by intestinal villus cells which are lost through the intestinal tract of animals affected with diarrhoea (Constable *et al.*, 2017) [7].

The mean  $\pm$  SE values of serum potassium (mmol/L) in theileriosis ( $6.65 \pm 0.05$ ) were significantly increased as

compared with healthy calves (5.20±0.03). Our findings of hyperkalaemia might be due to increased retention of K<sup>+</sup> ion by kidney and increased tubular reabsorption of K<sup>+</sup> ion in response to acidosis and also due to cellular damage. In addition, movement of K<sup>+</sup> ion from intracellular to extracellular fluid plays as contributory factor for hyperkalaemia (Tasker, 1991) [27]. The mean ± SE values of serum calcium (mEq/L) in control

and theileriosis were 10.88±0.16 and 8.71±0.30 (Table 2). Findings of the present investigation revealed that there was significant decrease in the serum calcium concentration in suffering as compared to healthy calves. In theileriosis, hypocalcaemia was probably due to the hypoproteinemia, decreased dietary intake, intestinal malfunction, and kidney damage (Hasanpour *et al.*, 2008) [14].

**Table 2:** Mean ± SE values of serum electrolyte parameters of control and theileria affected calves

S. No.	Parameters	Healthy calves (n=8)	Theileria affected calves (n=16)	Statistical analysis (T test)
1.	Serum sodium (mmol/L)	130.88±0.76	128.71±0.30	NS
2.	Serum potassium (mmol/L)	5.20±0.03	6.65±0.05	*
3.	Serum calcium (mEq/L)	10.88±0.16	8.71±0.30	*
4.	Serum phosphorus (mg/dl)	5.21±0.06	4.56±0.05	*

\*\*=*p*<.01 \*=*p*<.05 NS=Nonsignificant (*p*>.05)

The values of serum phosphorus (mg/dl) in control and theileriosis were 5.21±0.06 and 4.56±0.05. There was significant decrease in the serum phosphorus concentration in theileriosis than healthy calves. The decreased serum phosphorus concentrations in cattle with theileriosis are results of diarrhoea and renal wasting (Hasanpour *et al.*, 2008) [14].

Mean values of cardiac troponin-I (ng/ml) and CK-MB (ng/ml) in healthy calves were 0.124±0.020 and 34.00±2.276 whereas; in theileria affected calves were 1.747±0.214 and 53.125±2.601, respectively. There was significant increase in the cTn-I and CK-MB values in theileria affected calves (Table 3).

**Table 3:** Mean ± SE values of cardiac biomarkers related parameters (cTn-I and CK-MB) of theileria affected and control calves

S. No.	Parameters	Healthy calves (n=8)	theileriosis calves (n=16)	Statistical analysis (T test)
1.	cTn-I (ng/ml)	0.124±0.020	1.747±0.214	**
2.	CK-MB (ng/ml)	34.00±2.276	53.125±2.601	**

\*\*=*p*<.01 \*=*p*<.05

Significant increase serum cTn-I in cattle with theileriosis compared to healthy animals may be due to the occurrence of tachycardia secondary to anaemia. Higher incidence of myocyte damages can occur in theileria infected cattle with anaemia (Fartashvand *et al.*, 2013) [9]. Increased level of CK-MB in theileria affected calves may be attributed to myocardial muscle cell necrosis due to anaemic anoxia occurring due to severe haemolytic anemia, hepatic damage, and secondary rhabdomyolysis to prolonged recumbency and haemoglobinuria leading to ischemic and toxic nephrosis (Constable *et al.*, 2017) [7]. 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 (Babuín and Jaffe, 2005) [2].

## Conclusion

Research conclude with findings of deviation in serum profile towards negative side along with marked hepatic and cardiac damage in theileriosis affected calves.

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