Original Research Article

Serum Biochemical Study to Cattle-calves naturally infected with *Theileria annulata* Confirmed by Nested Polymerase Chain Reaction in Bikaner District of Rajasthan, India

Pavan Goyal[*], Anju Chahar, R.K. Tanwar, Fakhruddin and S.S. Soni

Department of Epidemiology and Preventive Veterinary Medicine, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner-334 001, Rajasthan, India

*Corresponding author

A B S T R A C T

A total of 100 cattle-calves irrespective of their age, sex and breed brought to Teaching Veterinary Clinical Complex of College of Veterinary and Animal science, Bikaner for treatment were screened for *Theileria annulata* infection. Blood samples were collected from jugular vein aseptically in vacutainers with and without anticoagulant for genomic DNA and serum isolation, respectively. The entire isolated DNA samples were subjected to nested polymerase chain reaction (nPCR), out of which 41 samples were found positive for *Theileria annulata* infection. All the serum samples were analyzed by using IDEXX vet test TM kit as per standard procedure. Serum biochemical analyses revealed statistically highly significant ($P<0.01$) decrease in Mean $\pm$ SE values of serum albumin and albumin-globulin ratio while non-significant ($P>0.05$) decrease in total protein and serum calcium; significant ($P<0.05$) decrease in serum glucose; significant ($P<0.05$) increase in serum alkaline phosphatase and SGOT while non-significant ($P>0.05$) increase in Blood urea nitrogen, serum creatinine, SGPT and serum globulin levels in nPCR positive cattle-calves as compared to nPCR negative cattle-calves.

Introduction

Bovine tropical theileriosis is a protozoan disease caused by blood protozoa *Theileria annulata* and it is transmitted by tick *Hyalomma anatolicum anatolicum.* It causes significant economic losses in large parts of Asia (Hasanpour *et al.,* 2013). It is mainly seen in cattle, sheep and goat as well as in wild and captive ungulates (Radostits *et al.,* 2007). This intracellular infection inflicts economic burden on cattle breeders in terms of mortality and morbidity as well as expenses spent on prophylactic measures against disease and treatment (Durrani *et al.,* 2008).

Theileria spp. infection can cause acute, subacute or chronic disease pathology (Gill *et al.,* 1977). In *T. annulata* infection, the most common clinical symptoms are weakness, weight loss, anorexia, high body temperature, petechia on the conjunctival mucosa, swollen lymph nodes, anaemia and cough. On later stages of theileriosis, infected animals cannot

stand up, their body temperatures are under normal values (< 38.5℃), icterus, dehydration and blood in faeces are the occasional clinical symptoms (Bakheit *et al.,* 2004; Radostits *et al.,* 2007). Calves (≤ 4 months of age) infected with *Theileria annulata* suffered from emaciation, anaemia, unilateral or bilateral exophthalmia, petechiae in conjunctiva, oral and nasal mucosa, and occasionally in the pinnae. Widespread subcutaneous nodules with 0.5 to 3.0 cm diameter are also detected, as well as enlarged superficial lymph nodes, particularly the submandibular, the retropharyngeal and sometimes the prescapular (Branco *et al.,* 2010).

Haemato-biochemical examination reveals significant (P≤0.05) decrease in total erythrocyte count, packed cell volume, haemoglobin, serum total proteins, albumin, globulins, glucose, calcium, phosphorus, cholesterol and triglycerides concentrations in cattle affected with bovine theileriosis compared with healthy controls, while significant (P≤0.05) increase in serum bilirubin and alanine aminotransferase of affected cattle compared with healthy controls (Khan *et al.,* 2011).

Materials and Methods

A total of 100 cattle-calves irrespective of their age, sex and breed brought to Teaching Veterinary Clinical Complex of College of Veterinary and Animal science, Bikaner for treatment were screened for *Theileria annulata* infection. Blood samples were collected from jugular vein aseptically in vacutainers with and without anticoagulant for genomic DNA and serum isolation, respectively. The entire isolated DNA samples were subjected to nested polymerase chain reaction (nPCR) using primers sets reported by D'Oliveira *et al.,* (1995). All the serum samples were analyzed by using IDEXX vet test TM kit as per standard procedure.

Results and Discussion

Mean ± SE values along with range of serum biochemical parameters of nPCR positive and negative cattle-calves are presented in Table 1 and Figure 1.

There was significant (P<0.05) decreased in serum glucose level in nPCR positive cattle-calves as compared to nPCR negative cattle-calves. Similar findings have been reported by Yadav and Sharma (1986), Col and Uslu (2007), Hussein *et al.,* (2007), Kizil *et al.,* (2007), Khan *et al.,* (2011) and Kumar (2012). There was non-significant (P>0.05) increased in blood urea nitrogen and serum creatinine in nPCR positive cattle-calves as compared to nPCR negative cattle-calves. Similar findings have also been reported by Omer *et al.,* (2003), Ugalmugle *et al.,* (2010) and Modi *et al.,* (2015).

There was significant (P<0.05) increased in SGOT while non-significant (P>0.05) increased in SGPT levels in nPCR positive cattle-calves as compared to nPCR negative cattle-calves. Similar findings have also been reported by Laiblin *et al.,* (1978) and Col and Uslu (2007).

There was significant (P<0.05) increased in serum alkaline phosphatase level in nPCR positive cattle-calves as compared to nPCR negative cattle-calves. Similar findings were also reported by Yadav and Sharma (1986), Sharma *et al.,* (1987), Sandhu *et al.,* (1998), Singh *et al.,* (2001), Alam and Nasr (2011) and Dede *et al.,* (2014).

There was non-significant (P>0.05) decreased in serum total protein and non-significant increased in serum globulin while highly significant (P<0.01) decrease in serum albumin and albumin-globulin ratio in nPCR positive cattle-calves as compared to nPCR negative cattle-calves.

Table.1 Mean ± SE values along with range of serum biochemical parameters of nPCR positive and negative cattle-calves
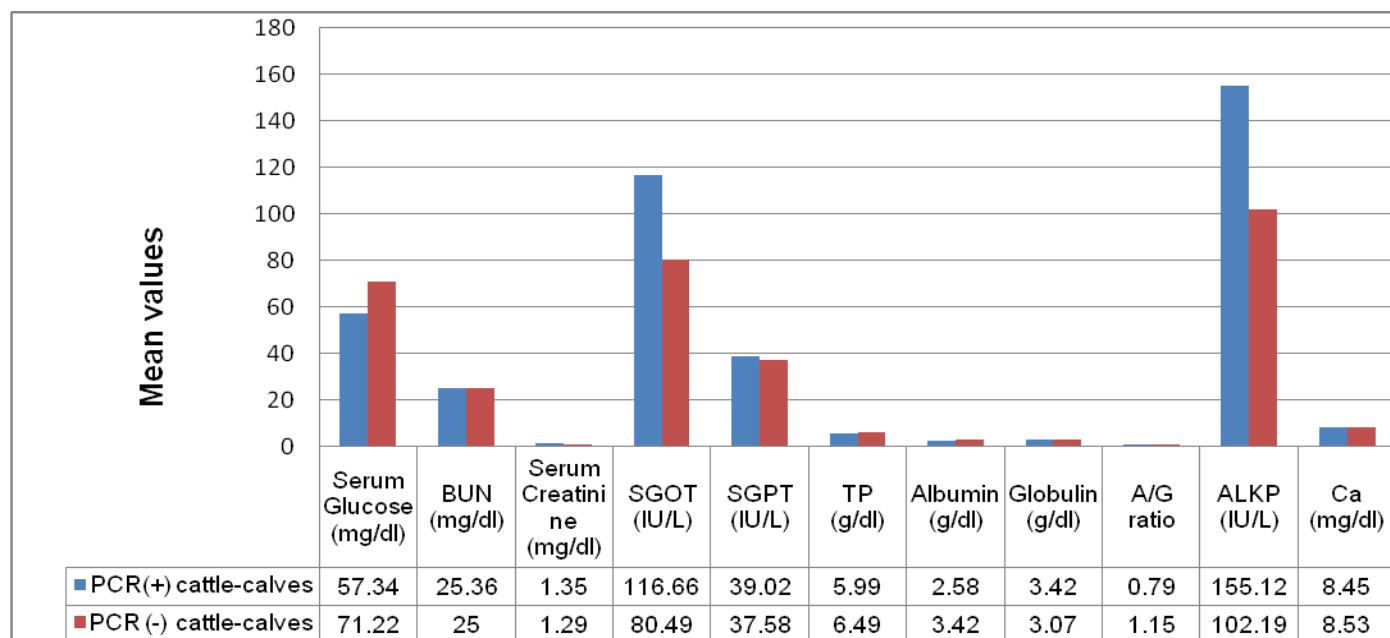
S. No.	Parameters	Mean ± SE		Range	
		nPCR positive cattle-calves	nPCR negative cattle-calves	nPCR positive cattle-calves	nPCR negative cattle-calves
1	Serum Glucose* (mg/dl)	57.34 ± 0.97^a	71.22 ± 0.86^b	15—151	20—160
2	BUN (mg/dl) (ns)	25.36 ± 0.71	25 ± 0.55	2—123	3—58
3	Serum Creatinine (mg/dl) (ns)	1.35 ± 0.18	1.29 ± 0.14	0.4—8.2	0.2—3.7
4	SGOT* (IU/L)	116.66 ± 1.47^b	80.49 ± 0.92^a	18—328	25—194
5	SGPT (IU/L) (ns)	39.02 ± 0.64	37.58 ± 0.65	10—100	10—77
6	TP (g/dl) (ns)	5.99 ± 0.19	6.49 ± 0.20	3—12	3.3—9.4
7	Albumin** (g/dl)	2.58 ± 0.14^a	3.42 ± 0.15^b	1.3—6	1.7—5
8	Globulin (g/dl) (ns)	3.42 ± 0.15	3.07 ± 0.15	1.1—6	1.5—4.9
9	A/G ratio**	0.79 ± 0.08^a	1.15 ± 0.09^b	0.43—1.82	0.69—2.39
10	ALKP* (IU/L)	155.12 ± 1.91^b	102.19 ± 1.51^a	10—554	13—453
11	Ca (mg/dl) (ns)	8.45 ± 0.24	8.53 ± 0.21	3—13	3.9—12.1

(Means with different superscripted letters in the same row differ significantly)
ns (P>0.05) = non-significant *(P<0.05) = statistically significant,
** (P<0.01) = highly significant

Fig.1 Bar diagram showing mean values of serum biochemical parameters of nPCR positive and negative cattle-calves

	Serum Glucose (mg/dl)	BUN (mg/dl)	Serum Creatinine (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	TP (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	ALKP (IU/L)	Ca (mg/dl)
PCR(+) cattle-calves	57.34	25.36	1.35	116.66	39.02	5.99	2.58	3.42	0.79	155.12	8.45
PCR (-) cattle-calves	71.22	25	1.29	80.49	37.58	6.49	3.42	3.07	1.15	102.19	8.53

Similar findings were also reported by Dimopoullos (1970), Coles (1986), Behnke *et al.,* (1992), Turgut (2000), Singh *et al.,* (2001), Col and Uslu (2007) and Hussein *et al.,* (2007).

There was non-significant (P>0.05) decreased in serum calcium level in nPCR positive cattle-calves as compared to nPCR negative cattle-calves. Similar findings were also reported by Dhar and Gautam (1977) and Sandhu *et al.,* (1998).

Decrease in serum glucose level may be due to utilization of glucose by *Theileria* in the blood and hepatic dysfunction as a result of *Theileria* infection (Col and Uslu, 2007) or may result from persistent fever due to the disease and inappetence developed by the severity of disease (Yadav and Sharma, 1986; Hussein *et al.,* 2007 and Kizil *et al.,* 2007).

Increase in SGOT level may be due to hepatic tissue damage that includes coagulation necrosis, distortion of hepatic cords with heavy infiltration of lymphocytes in the periportal areas indicating severe damage to hepato-biliary system due to hypoxia resulting from anaemia and jaundice (Col and Uslu, 2007).

Increase in serum ALKP activity in infected animals indicates hepatic tissue damage including coagulative necrosis, distortion of hepatic cords and heavy infiltration of the hepatic tissues with lymphocytes. This further indicated the consequences of hypoxia resulting from anaemia and jaundice (Sandhu *et al.,* 1998).

Hypoalbuminemia may result from increase in albumin excretion (dense albuminuria) due to renal failure, synthesis impairment and/or extensive protein degradation during prolonged fever (Dimopoullos, 1970) or may be due to liver failure (Singh *et al.,* 2001) or

due to extravascular accumulation of proteinaceous fluids, resulting from diseased lymph nodes, and thus explained the oedema and body cavity effusions (Col and Uslu, 2007) or due to harmful effect of toxic metabolites of *Theileria* spp. on liver cells (Hussein *et al.,* 2007).

Decrease in serum calcium level may be due to the fact that animals are young and feed on whole milk, even in a state of recumbency (Sandhu *et al.,* 1998).

Bovine tropical theileriosis is a burning veterinary problem of the rural livestock oriented communities. It has profound effect on serum biochemical parameters and causes huge morbidity and mortality in cattle population, which reflects economic losses and elevates the poverty level.

Acknowledgements

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