RESEARCH ARTICLE

Hemato-Biochemical Studies in Theileriosis Infected Gir Cows and Jaffrabadi Buffaloes

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Abstract

This study was conducted to assess the hemato-biochemical alterations in theileriosis infected Gir cows and Jaffrabadi buffaloes from the southwestern region of Gujarat, India. The prevalence of theileriosis in indigenous bovines was studied by screening 518 blood samples of clinically suspected animals during one year period. Among the screened samples, 85 were found to be *Theileria* spp. positive microscopically. Overall, significantly higher ($p < 0.001$) incidence of *Theileria* spp. infection was noted in Gir cattle (25.00%, n = 62) compared to Jaffrabadi buffaloes (8.51%, n = 23). Similarly, higher incidence of theileriosis was recorded in both cattle and buffaloes during rainy season (27.90, 10.20%) followed by summer (23.68, 9.80%) and winter (23.25, 4.16%, respectively). Age-wise, the frequency of theileriosis was more in animals >1 year compared to younger ones <6 months of age, while it was more common in females than males. However, the differences in incidences among different risk-factors were insignificant ($p > 0.05$) in both cattle and buffaloes. Hematological analysis revealed significant ($p < 0.05$) decrease in haemoglobin, hematocrit, red blood cells, lymphocytes, and significant ($p < 0.01$) increase in total leucocyte counts, mean corpuscular volume, mean corpuscular haemoglobin and neutrophil counts. Serum biochemistry revealed significant ($p < 0.05$) increase in AST, ALT, ALP, GGT, total bilirubin and blood urea nitrogen in *Theileria* infected bovines compared to the control.

Keywords: Gir cattle, Hematology, Jaffrabadi buffaloes, Prevalence, Seasonal influence, Serum biochemistry, Theileriosis.

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Introduction

In recent years, theileriosis has emerged as major fatal disease of bovines specially crossbred and exotic animals. Although indigenous breeds are quite resistant to infection, they act as potential reservoirs/carrier and are dangerous to other breeds of bovines (Acharya *et al.,* 2015). Bovine theileriosis is haemoprotozoan disease caused by *Theileria* spp. belonging to family Theileridae (Soulsby, 1982). *T. annulata* and *T. parva* are considered to be the most pathogenic species of *Theileria*. Tropical theileriosis is one of the most prevalent diseases of bovine in India, and it is mainly transmitted by *Hyalomma* ticks. The disease is characterized by lymphadenopathy, splenomegaly, fever, anaemia, weakness and loss of body weight (Maharana *et al*., 2016[a]). The infection is due to the presence and multiplication of the parasites in leucocytes and then erythrocytes resulting in progressive and severe anemia (Ayadi *et al.,* 2017).

The temperature of Junagadh district, South-western Gujarat, is favorable for ticks responsible for the transmission of theileriosis in bovines. Gir cattle and Jaffrabadi buffaloes are two important species of animals in this area because of dual-purpose, *i.e.,* the supply of draught power and milk. The summer season is very hot and dry, rainy is hot and humid, while winter is short and moderate cold with annual temperatures ranging from 6.5 °C to 44.7 °C. The average annual rainfall was 1492.2 mm during 2019 with only 55 rainy days (Chopada *et al*., 2020). Looking to the scanty information available on theileriosis in indigenous bovine, this study was planned to understand the potential problems related to tick-borne diseases in bovines through the investigation of prevalence of *Theileria* spp. infections in terms of season, age, sex, and hemato-biochemical changes associated with infections in indigenous Gir cattle and Jaffrabadi buffaloes.

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Materials and Methods

Randomly, a total of 518 blood samples from Gir cattle (248) and Jaffrabadi buffaloes (270) presented at Veterinary Clinical Complex (VCC) of Veterinary College and the surrounding areas of Junagadh were collected from jugular vein in sterile clot activators and K_3EDTA vacutainers for a period of one year between July-2019 to June 2020. While collecting the samples, history of the animal, like age, sex, breed, etc. were also recorded. The samples were brought to the Clinical diagnostic laboratory of VCC. To identify the *Theileria* infection, thin blood smears were prepared, air dried, fixed with methanol for 2-3 minutes, stained with 20 times diluted Giemsa's stain (Himedia, India) for 30 minutes, and examined for the presence of blood protozoan parasite under oil emersion objective (100x) of light compound microscope.

Hemato-Biochemical Studies

Blood and serum samples collected from the *Theileria* spp. positive animals (n = 85) were routinely examined for hematological and serum biochemical analysis. 12 apparently healthy animals from the institutional farm were periodically checked and used as control. Hematological analysis was done by using Automatic Whole Blood Analyzer (Mindray BC - 2800 Vet, China) and biochemical analysis of the serum samples was done by Serum Auto-analyzer (MicroLab-300, China).

Statistical Analysis

The incidence of *Theileria* spp. infection in bovines was calculated in percentage values and compared by Chi-square ($\chi 2$) test, where $p \leq 0.05$ was considered a significant difference. The data recorded on hemato-biochemical parameters were analyzed by the unpaired 't' test (Snedecor and Cochran, 1994).

Results and Discussion

Incidence of *Theileriosis*

Among the 518 blood samples collected from bovines of Junagadh district (248 from Gir cattle and 270 from Jaffrabadi buffaloes), total 85 animals were found positive for *Theileria* spp. Infections. Accordingly, the overall incidence of *Theileria* infection was 16.40 %, where significantly ($p < 0.001$) higher incidence was recorded in Gir cattle (25.00 %) compared to Jaffrabadi buffaloes (8.51%) (Table 1). Recently, a similar incidence of *Theileria* infection in Gir cattle (Swami *et al*., 2019) and indigenous bovine calves (Brahmbhatt *et al*., 2019) has

Table 1: Incidence of *Theileria* spp. infection in indigenous bovines

Overall incidence of Theileria infection in bovines						
Indigenous bovines	*Total B/S Examined*		*No. positive*		*% incidence*	
Gir cattle	248		62		25.00**	
Jaffrabadi buffaloes	270		23		8.51	
Total	518		85		16.40	
$\chi 2 = 25.5995$, p = 0.00001 **						
Age-wise incidence						
Age	*Total B/S examined*		*No. positive*		*% incidence*	
	Cattle	*Buffalo*	*Cattle*	*Buffalo*	*Cattle*	*Buffalo*
< 6 month	27	13	06	01	22.22	7.69
>1 Year	221	257	56	22	25.33	8.56
Cattle: $\chi 2 = 0.1247$. p = 0.724007; Buffalo: $\chi 2 = 0.1598$, p = 0.689308						
Sex-wise incidence						
	Total B/S examined		*No. positive*		*% prevalence*	
Sex	*Cattle*	*Buffalo*	*Cattle*	*Buffalo*	*Cattle*	*Buffalo*
Male	45	12	08	01	17.77	8.33
Female	203	258	54	22	26.60	8.52
Cattle: $\chi 2 = 1.5294$; p = 0.216209. Buffalo: $\chi 2 = 0.2555$. P = 0.613259						
Season-wise incidence						
	Total B/S examined		*No. positive*		*Percent prevalence*	
Season	*Cattle*	*Buffalo*	*Cattle*	*Buffalo*	*Cattle*	*Buffalo*
Monsoon	86	147	24	15	27.90	10.20
Summer	76	51	18	05	23.68	9.80
Winter	86	72	20	03	23.25	4.16
Cattle: $\chi 2 = 0.5973$; p = 0.741816 Buffalo: $\chi 2 = 2.3938$; p = 0.302123						

been reported. However, a lower incidence of *Theileria* spp. was reported in bovines as 7.08 % (Maharana *et al*., 2016[a]) and 2.3 % (Kumar *et al*., 2016) from same geographical area. This might be because of less number of samples included in later studies.

Concerning age and sex, among both the species (Cattle and Buffalo), the animals above 1 year of age were more susceptible (25.33%; 8.56%) than less than 6 months of the age (22.22%, 7.69%), while the incidence of the disease was higher in females (26.60%, 8.52%) than in males (17.77%, 8.33%, respectively). However, differences were insignificant ($p > 0.05$). These findings were in close association with those of Singh *et al.* (2017) and Khan *et al.* (2017), who reported higher infection in adult than young age. Though inverse age resistance is not a general phenomenon in theileriosis, as this region looks like *Theileria* endemic, stronger passively acquired immunity from the mother might contribute to the lower incidence of hemoprotozoan parasites in younger age group of animals. Moreover, Panda *et al*. (2011) and Maharana *et al.* (2016[b]) reported a higher prevalence of theileriosis in females than males due to less population of male animals and farmers are interested in raising female calves as replacement stock. As a result, more blood samples from female bovines were presented for diagnosis.

Seasonal investigation on bovine theileriosis revealed an insignificant ($p > 0.05$) seasonal influence. The highest incidence of the disease was observed in both cattle and buffaloes during rainy season (27.90%, 10.20%) followed by summer season (23.68%, 9.80%) and winter season (23.25%, 4.16%, respectively). It might be due to the desired climate throughout the year for Hyalomma tick with optimum weather conditions during the rainy season. Velusamy *et al*. (2014) recorded similar significantly high prevalence of theileriosis during summer followed by moderate in monsoon and less in winter season. Our observations were, however, in agreement with those of Kohli *et al*. (2014), Vahora *et al*. (2012) and Bhatnagar *et al.* (2015), who reported that theileriosis was most prevalent in rainy season. These differences might be due to geographic location and climatic changes because high temperature and humidity are ideal for ticks' survival and breeding.

Hematological Alterations in *Theileria* Infection

The mean values for hematological parameters of indigenous bovines are presented in Table 2. The mean values of hemoglobin (Hb), red blood corpuscles (RBCs), packed cell volume (PCV) and lymphocytes were significantly ($p < 0.05$) reduced in infected bovines as compared to control group. This might be due to damage caused by the organisms inside the RBCs during their multiplication. This decline in Hb, PCV and RBC count levels is attributed to lysis of erythrocytes and erythrophagocytosis by piroplasms, which infect and replicate in it. The alterations in hematological indices observed during the infection concur with the findings of Modi *et al*. (2015) and Ganguli *et al*. (2017).

In contrast, Vahora *et al*. (2009) recorded normal PCV values and a decrease in RBC. Total leucocyte counts increased significantly ($p < 0.01$) in infected bovines as compared to control group, which was in accordance with the findings of Ugalmugle *et al*. (2010). Leucopenia is not a characteristic of the disease. There is progressive leucocytosis which is entirely due to lymphocytes. However, significant ($p < 0.01$) lymphopenia and neutrophilia were found in affected indigenous bovines. The mean values of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were significantly ($p < 0.01$) increased in the infected bovines compared to the control group. However, an insignificant ($p > 0.05$) change was recorded in mean corpuscular hemoglobin concentration

Table 2: Comparison of hematological values of *Theileria* infected and healthy control bovines

		Cattle		*Buffaloes*	
Sr. No.	*Parameter*	*Healthy control group (n = 6)*	*Infected group (n = 62)*	*Healthy control group (n = 6)*	*Infected group (n = 23)*
1	Hb (g/dL)	10.24 ± 0.13	7.67 ± 0.22**	14.32 ± 0.22	10.01 ± 0.30**
2	PCV (%)	28.88 ± 0.76	23.79 ± 0.25**	36.70 ± 0.98	26.80 ± 0.67**
3	TEC (10^6/µL)	6.63 ± 0.14	5.40 ± 0.16*	7.10 ± 0.33	5.41 ± 0.14*
4	TLC (10^3/µL)	8.96 ± 0.20	14.81 ± 0.35**	12.28 ± 0.51	15.19 ± 0.24**
5	Platelets (10^5/µL)	2.84 ± 0.12	2.77 ± 0.16	2.56 ± 0.26	1.92 ± 0.24
6	MCV (fl)	25.33 ± 1.42	66.76 ± 0.46**	49.17 ± 2.07	62.26 ± 0.79**
7	MCH (pg)	9.36 ± 0.38	17.79 ± 0.35**	13.07 ± 0.19	22.16 ± 0.32**
8	MCHC (g/dl)	36.46 ± 0.48	34.99 ± 0.28	35.00 ± 0.67	34.12 ± 0.43
9	Neutrophils (%)	34.33 ± 0.88	63.48 ± 0.94**	35.38 ± 1.12	62.09 ± 2.39**
10	Lymphocytes (%)	60.83 ± 0.98	32.83 ± 23.9**	58.70 ± 1.61	39.06 ± 1.11**
11	Monocytes (%)	4.33 ± 0.33	8.87 ± 0.15*	5.72 ± 0.40	6.18 ± 0.29
12	Eosinophils (%)	2.33 ± 0.80	2.74 ± 0.19	1.00 ± 0.00	2.19 ± 0.25
13	Basophils (%)	0.16 ± 0.16	0.47 ± 0.08	0.00 ± 0.00	0.56 ± 0.15

**$p < 0.01$, *$p < 0.05$ between healthy and infected groups.

Table 3: Biochemical values of *Theileria* infected and non-infected bovines (Mean ± SE)

		Cattle		*Buffaloes*	
Sr. No.	*Parameter*	*Healthy Control (n = 6)*	*Infected group (n = 62)*	*Healthy Control (n = 6)*	*Infected Group (n = 23)*
1	ALT (IU/L)	30.41 ± 0.42	64.11 ± 1.32**	20.73 ± 0.81	60.00 ± 1.30**
2	AST (IU/L)	102.20 ± 1.52	312.30 ± 12.6**	121.00 ± 3.23	154.70 ± 5.34**
3	ALP (IU/L)	106.10 ± 8.71	153.10 ± 2.28**	178.60 ± 19.61	188.30 ± 5.36
4	GGT (IU/L)	9.17 ± 0.32	27.21 ± 0.60**	20.60 ± 1.66	58.45 ± 1.40**
5	TB (mg/dL)	0.47 ± 0.16	0.81 ± 0.04*	0.25 ± 0.01	2.21 ± 0.47**
6	TP (g/dL)	6.82 ± 0.06	5.83 ± 0.13*	6.98 ± 0.17	5.25 ± 0.15*
7	Albumin (g/dL)	3.45 ± 0.05	3.42 ± 0.32	3.45 ± 0.08	3.03 ± 0.11
8	Globulin (g/dL)	3.36 ± 0.09	2.42 ± 0.15	3.53 ± 0.51	2.22 ± 0.18*
9	A/G Ratio	1:0.97	1:0.745	1:1.02	1:0.72
10	BUN (mg/dl)	27.03 ± 1.91	35.20 ± 0.51**	26.04 ± 1.28	45.89 ± 1.30**
11	Creatinine (mg/dL)	1.23 ± 0.10	1.31 ± 0.08	1.53 ± 0.11	1.98 ± 0.15

**p < 0.01 & *p < 0.05, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phophatase, GGT = Gamma-glutamyl transferase, TB = Total billirubin, TP = Total protein, BUN = Blood urea nitrogen.

(MCHC) in infected animals as compared to control group (Ayadi *et al.,* 2017).

Biochemical Alterations in *Theileria* Infection

The mean values for serum biochemical parameters of infected indigenous bovines are presented in Table 3. The differences between mean values of serum creatinine, A:G ratio and albumin were non-significant, while the mean value of blood urea nitrogen (BUN) was significantly higher in theileriosis infected bovines as compared to normal control groups in both cattle and buffaloes. The serum globulin concentration was also reduced in infected animals in both the species but differed significantly only in buffaloes. Similar findings were also observed by Aulakh and Singla (2006). Levels of liver-specific enzymes AST and ALT were increased highly significantly ($p < 0.01$) in infected animals as compared to control group in both the species, which concurred well with previous reports of Saber *et al*. (2008) and Ugalmugle *et al*. (2010). Elevated ALT and AST indicate liver cell injury or damage such as hepatitis, cirrhosis, mononucleosis or other liver diseases. Higher AST levels can also indicate heart problem or pancreatitis. Significantly ($p < 0.05$) higher ALP was recorded in infected cattle as compared to healthy control, but in buffaloes, the difference was found non-significant. The total serum protein levels decreased significantly ($p < 0.01$) in infected cattle and buffaloes as compared to control groups. Similar observations were recorded by Ugalmugle *et al*. (2010). The low serum total protein concentration in cattle naturally infected with *T. annulata* was possibly due to hypoalbuminemia and hypoglobulinemia arising from liver failure (Omer *et al*., 2003).

Conclusions

The study revealed overall higher incidence of *Theileria* spp. in the indigenous bovines of Junagarh region (16.40%), where prevalence was significantly higher in Gir cattle (25.00%) compared to Jaffrabadi buffaloes (8.51%). In both the species, it was non-significantly higher in animals >1 year of age, in females and during monsoon season. The disease also significantly altered the hemato-biochemical parameters as compared to healthy animals. The information will be quite important in planning national strategy towards control of this disease in dairy bovines of the region.

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