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Epidemiological, clinical and haematobiochemical alteration in crossbred cattle infected with theileriosis in North Gujarat

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Abstract
Tropical theileriosis in crossbred cattle transmitted mainly by *Rhipicephalus, Hyalomma, Dermacentor* tick vectors in India now a days. Hot humid climate triggers the tick population responsible for most of the haemoprotozoan diseases in tropical countries. Affected animals exhibit a clinical signs like frequent pyrexia, lymphadenopathy and severe anemia. It results into death of an animal and loss of production. The present work was undertaken to study of epidemiology, evaluation of haemato-biochemical alteration in the crossbred cattle infected with theileriosis in North Gujarat.

Keywords: Biochemical, cattle, hematology, theileria

Introduction
Haemoprotozoan infections are widely spread in cattle and cause devastating losses to the livestock industry worldwide (Shahnawaz *et al.*, 2011) [1]. Theileriosis is a tick-born disease of domestic and wild animals and one of the most economically harmful livestock diseases in tropical and subtropical regions (Inci *et al.*, 2009; Gul *et al.*, 2015) [2, 3] caused by different spp. of Theileria. Among them, *Theileria annulata* is the most pathogenic spp. reported in India (Spickler, 2010) [4].

The prevalence depends upon the geographical region and several other factors like tick density, climatic conditions, age, gender, management practices, and immunity of particular animals (Gul *et al.*, 2015) [3]. Tropical theileriosis is more severe in exotic and cross-bred cattle (*Bos taurus*) than in indigenous cattle (*Bos indicus*). Clinical signs like fever, immune depression, anorexia, lymphadenopathy, and secondary bacterial respiratory infection manifest Theileriosis. Lacrimation, corneal opacity, nasal discharge, and diarrhea are also observed and can lead to heavy mortality in cattle if proper treatment is not given in time (Radostits *et al.*, 2007; Muhanguzi *et al.*, 2014) [5, 6]. Recently, pseudo-pericarditis has also been reported (Prajapati et al., 2019) [7]. The diagnosis of theileriosis is mainly based on clinical findings and microscopic examination (Aktas *et al.*, 2006) [8]. A molecular method like PCR is a good option for confirmation, particularly in low parasitemia (Collins *et al.*, 2002) [9]. Looking towards the above facts, the present study was conducted to know the epidemiology and clinico-hemato-biochemical variations in crossbred cattle with theileriosis reared in the North Gujarat region.

Material and Methods
A total of 524 crossbred cattle reported at the clinical complex Deesa and the surrounding area from 2018 to 2019 were scrutinized for Theileriosis. The prevalence was calculated based on age, sex, season, and housing pattern. All animals were clinically examined for various physiological parameters, *viz.* temperature, heart rate, respiration rate, the color of the conjunctiva and vaginal mucous membrane, and enlargement of superficial lymph nodes before sample collection. Approximately 5 ml of blood was collected in K_3EDTA from the jugular vein. A thin blood smear examination was conducted under a 100X oil immersion lens under a microscope to check for the presence of the theileria organism in red blood cells and Koch's blue body in lymphocytes according to the method described by Soulsby (1982) [10]. For confirmation, PCR was done. Primers used in the present study are listed below.

Primers used in the present study are listed below.

Theileria Genus	Forward- GTAACCTTTAAAAACGT Reverse- GTTACGAACATGGGTTT
Theileria annulata	Forward- AAT CCT GAC ACA GGG AGG TAG TGA C Reverse- CTA AGA ATT TCA CCT CTG ACA GT

Haematology of samples was performed on whole blood to estimate haematological parameters (Hb, RBCs, WBCs, DLC, Platelets, PCV, MCV, MCHC) using a haematology analyzer (Nihon kohden hematology analyzer) and serum biochemical parameters (TP, AST, ALT, Creatinine, BUN) were quantified using standard assay kits (Reactions GPL) with the help of clinical chemistry analyzer (Randox Monaco). Moreover, 10 clinically healthy cattle were selected from an organized farm and served as a control group. Data generated from the study were statistically analyzed in SPSS software (v. 20.0).

Result and Discussion

In the present study, 524 crossbred cattle were included from the clinical complex, Deesa, and areas of Deesa taluka of Banaskantha district in a year. Based on clinical symptoms, 212 cases were suspected of theileriosis. Out of them, 122 samples were found positive for theileriosis in blood smear examination. Further, 36 blood samples were confirmed by PCR method. The overall prevalence of theileriosis was reported to be 23.28% (122/524). Age-wise, sex-wise and season-wise, and housing patterns wise prevalence of theileriosis was illustrated in Table 1.

Table 1: Age-wise, sex-wise, season-wise, and housing pattern-wise prevalence of theileriosis in crossbred cattle

Sr. No.	Variables	Total cases (n=254)	Positive cases (n=122)	Prevalence (%)
a) Age-wise				
1	Calf (0 to 6 months)	173	42	24.27
2	Heifers (6 months to 3 years)	197	30	15.22
3	Adult (3 to 12 years)	133	48	36.09
4	Aged (> 12 years)	21	02	9.52
b) Sex-wise				
1	Male	178	23	12.92
2	Female	346	99	28.61
c) Season-wise				
1	Winter (November to February)	181	30	16.57
2	Summer (March to June)	185	27	14.59
3	Monsoon (July to October)	158	65	41.13
d) Housing pattern wise				
1	Pakka	231	30	12.98
2	Kachha	163	38	23.31
3	Open yard	130	54	41.53

Almost similar overall prevalence (ranging between 15 and 30 percent) of theileriosis has been reported earlier by various workers from India and abroad (Singh, 1991; Razmi *et al*., 2009; Modi *et al.*, 2015) [11, 12, 13]. A higher prevalence of 36.09% (48/133) was observed in the 3 to 12 years age group cattle, followed by 24.27% (42/173) in > 6 months of age and 15.22% (30/197) 6 months to 3 years of age. The highest prevalence of theileriosis age group of 3-12 years is similar to other researchers. (Dhar, 1991; Ananda *et al*., 2006; Rup Ram *et al*., 2006) [14, 15, 16]. In contrast to the present investigation, Grewal *et al.* (1997) [17], Palanivel *et al.* (2006) [18], and Masare *et al*. (2009) [19] observed a higher prevalence of theileriosis in crossbred calves below six months of age. The present study revealed that the highest prevalence, 28.61% (99/346), was found in female animals. A similar finding was reported by Durrani (2008) [20], Atif *et al*. (2012) [21], Saleem *et al.* (2014) [22], and Modi *et al.* (2015) [13]. In Gujarat, female cattle are more commonly reared than males due to reproduction and economic importance. This might be the reason for the higher prevalence of theileriosis in female cattle.

Moreover, the highest prevalence, 41.13% (65/158), was recorded in the monsoon season followed by the winter season, 16.57% (30/181), and the summer season, 14.59% (27/14.59). The high prevalence in monsoon season might be due to the high tick population due to the hot and humid environment. The incidence of theileriosis was not seasonal but occurred irregularly throughout the year due to the prevailing macroclimate, which is needed to spread the disease (Palanivel *et al*., 2006) [18]. Management plays a vital role in livestock farming. In the present study, the highest prevalence was found with open yard management at 41.53% (54/130), followed by kachha housing at 23.31% and pakka housing at 12.98%.

Theileriosis-affected crossbred cattle showed clinical signs like pale conjunctiva and vaginal mucus membrane, enlarged prescapular lymph nodes, lacrimation, and salivation, illustrated in Fig. 1.

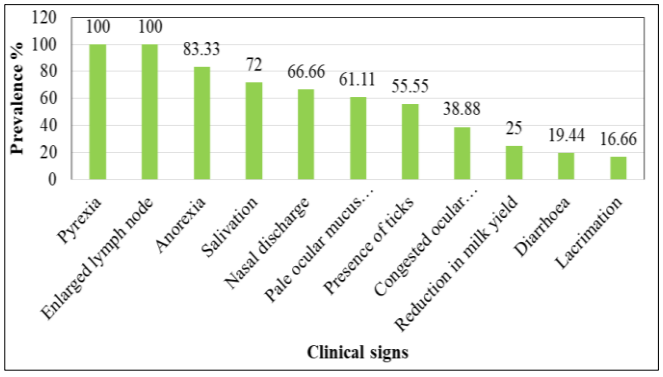

Fig 1: Percentage of different clinical signs in cattle affected with theileriosis (n=36)

Haematology and Biochemical analysis

The mean values for different haematological and biochemical parameters of infected and healthy crossbred cattle are presented in Table 2 and 3, respectively.

The alteration in haematological value observed during the infection was similar to those reported by earlier workers (Qayyum *et al*., 2010; Modi *et al.,* 2015) [23, 13]. Decreased values of Hb, RBC, and PCV were also reported by Muraleedharan *et al*. (2005) [24], Aulakh and Singla (2006) [25], and Masare *et al*. (2009) [19]. Vahora *et al*. (2009) [26] found a decline in RBC and Hb values, but PCV values were in the normal range. Low level of Hb, PCV, and TEC count was observed due to the lysis of erythrocytes by piroplasms which infects and replicate in it result into erythrophagocytosis (Preston *et al.,* 1992; Radostitis *et al*., 2007) [27, 5].

Total WBC (7.53±0.62) $\times 10^3$µl was increased non-significantly in affected crossbred cattle compared to the control group. A similar finding was also reported by Omer *et al.* (2002) [28], Qayyum *et al*. (2010) [23], and Modi *et al.* (2015) [13]. Sandhu *et al.* (1998) [29] demonstrated leukocytosis followed by leukopenia in theileria infections. However, significant (p<0.05) lymphopenia and neutrophilia were observed in crossbred cattle affected by theileriosis, which corroborated with findings reported by Aulakh and Singla (2006) [25] and Ugalmugle *et al*. (2010) [30]. In contrast to the present study, Omer *et al*. (2002) [28] found lymphocytopenia with neutropenia.

Biochemical alteration

Serum TP, AST, ALT, Creatinine, and BUN levels in healthy crossbred cattle were 6.32±0.18 g/dl, 70.56±3.56 IU/L, 23.55±0.69 IU/L, 1.27±0.09 mg/dl, and 13.85±0.88 mg/dl, respectively. While 5.27±0.22 g/dl, 102.54±8.57 IU/L, 36.68±3.43 IU/L, 1.52±0.09 mg/dl, and 19.02±1.18 mg/dl were found affected group, respectively. In our study, BUN was increased significantly (p<0.05) in an infected group compared to the control group. Similar findings were also observed by Sandhu *et al*. (1998) [29]. Singh *et al*. (2001) [35] demonstrated a significant rise in BUN and creatinine values. In contrast to the present study, BUN and creatinine levels did not show any deviation from the reference value (Ugalmugle *et al*., 2010) [30]. In the current study, serum creatinine level was slightly increased due to kidney damage because of trapping of agglutinated damaged infected erythrocytes and lymphocytes in glomeruli, resulting in glomerulonephritis. Increased BUN levels were exhibited due to hyperthermia, anemia, and increased protein catabolism.

AST and ALT values increased significantly (p<0.05) in the infected group compared to the control group. Similar observations were noticed by Sandhu *et al*. (1998) [29], Col and Uslu (2007) [31], Saber *et al*. (2008) [32], and Ugalmugle *et al*. (2010) [30]. An increase in AST level indicates liver damage, viz. primary or secondary liver and muscle necrosis (Benjamin, 2001) [33]. An increase in AST level in affected animals indicates hepatic tissue damage that occurs due to coagulative necrosis, distortion of hepatic cords with heavy infiltration of lymphocytes in the peri-portal areas results in jaundice (Modi *et al*., 2015) [13]. ALT levels also increased in the affected animal group, indicating either hepatic necrosis, an alteration in cell membrane permeability, or leakage of these cytoplasmic enzymes in the blood (Benjamin, 2001; Modi *et al*., 2015) [33, 13]. Total protein decreased significantly (p<0.05) in the infected group compared to the control group. Similar observations were found by Aulakh and Singla (2006) [25], Col and Uslu (2007) [31], Saber *et al*. (2008) [32], and Ugalmugle *et al*. (2010) [30]. Omer *et al*. (2003) [34] stated that low TP concentration in naturally infected cattle with *Theileria annulata* was possibly due to hypoalbuminemia and hypoglobulinemia arising from liver failure.

Table 2: Haematological alterations in theileriosis affected crossbred cattle (Mean±S.E.)

Parameter (units)	Control healthy (n=10)	Infected animals (n=36)
WBC (103/µl)	6.58±0.37	7.53±0.62
RBC (106/µl)	5.92±0.19	4.49±0.24*
HB (g/dl)	9.85±0.18	7.37±0.32*
PCV (%)	29.05±0.66	22.13±0.98*
MCV (fl)	47.57±1.35	50.70±0.98
MCHC (g/dl)	34.08±0.30	33.30±0.03
PLT (103/µl)	267.9±9.671	222.0±13.27
Lymphocytes (%)	57.87±3.05	42.19±1.87*
Neutrophils (%)	34.763±3.49	49.52±2.20*

Note: Means in different columns differ significantly (*p<0.05)

Table 3: Biochemical alteration in theileriosis affected crossbred cattle (Mean±SE)

Parameters (units)	Control healthy (n=10)	Affected animals (n=36)
TP (g/dl)	6.32±0.18	5.27±0.22*
AST (IU/L)	70.56±3.56	102.54±8.57*
ALT (IU/L)	23.55±0.69	36.68±3.43*
Creatinine (mg/dl)	1.27±0.09	1.52±0.09
BUN (mg/dl)	13.85±0.88	19.02±1.18*

Note: Means in different columns differ significantly (*p<0.05)

Conclusion

Enlargement of superficial lymph node with pyrexia is specific clinical sign of tropical theileriosis. Hematological and biochemical alterations in *Theileria annulata* infected crossbred cattle also helpful for the diagnosis and initiation of appropriate therapeutic regimen for a favorable outcome of the disease. PCR is the confirmatory diagnostic technic for the theileriosis.

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