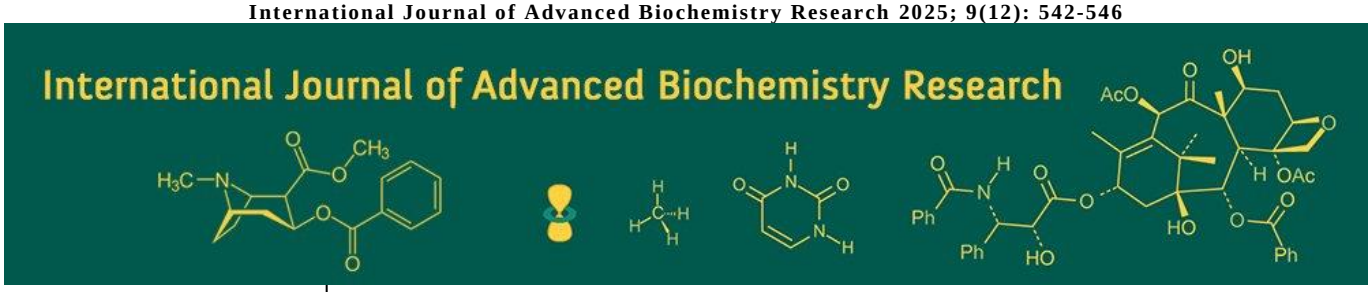

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Epidemiology and Haemato-biochemical studies in theileriosis infected cattle

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Abstract
Theileriosis is a tick-born haemoprotozoan disease caused by *Theileria* spp., which is one of the major constraints of cattle production in India. Exotic and crossbred cattle are more susceptible than the indigenous breeds of cattle. It is characterised by elevated rectal temperature, enlargement of prescapular lymphnodes, lacrimation, salivation, nasal discharge and reduced milk production. A total 135 suspected cattle were screened for theileriosis from Veterinary Clinical complex of the college, in anand, Animal Disease Investigation Laboratory, ARDA Division, Amul Dairy, Anand, and from surrounding villages. Amongst them 47.41% (64/135) cattle were found positive for theileriosis, diagnosed by microscopic examination. Highest incidence of theileriosis in cattle was found in age group 6-10 years (54.00%) and the lowest incidence was found in <6 month of age group (28.57%). The sex wise incidence was higher in female (49.59%) than in male (25%). The breed wise incidence found was higher in HF crosses (52.34%) than the indigenous breeds (28.57%). The haematological finding revealed that the values of haemoglobin, packed cell volume, total erythrocytes count, lymphocytes and mean corpuscular haemoglobin concentration were decreased and Neutrophils, total leucocytes count, monocytes, eosinophils, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were increased in theileriosis infected cattle as compared to healthy cattle. The serum biochemical examinations revealed that the values of total protein, albumin, total glucose was decreased in theileriosis infected cattle however, Gamma glutamyl transferase, aspartate aminotransferase, total bilirubin and alanine aminotransferase were increased in theileriosis infected cattle as compared to healthy cattle. Serum creatinine and blood urea nitrogen were showed non-significant difference in theileriosis infected cattle.

Keywords: Cattle, clinical signs, Giemsa staining, Haemato-biochemical assay, theileriosis

Introduction
Bovine Theileriosis is one of the most prevalent and economically significant diseases of cattle. Exotic and crossbred cattle are more susceptible than the indigenous breeds of cattle. *Theileria* spp. is an obligate intracellular protozoan parasites. Taxonomically they are placed under Phylum: *Apicomplexa*, Class: *Piroplasmasida*, Order: *piroplasmorida* and Genus: *Theileriae* under Family: *Theileridae.* It infects wild and domestic animals throughout the world. In life cycles of *Theileria* spp. both invertebrate and vertebrate hosts are involved and it is transmitted by vectors like ticks of family *Ixoxidae* (Perera *et al.*, 2013) [18]. *Theileria* spp. are small round, ovoid, irregular or bacilliform shaped parasites with an apical complex comprised only of rhoptrics. *Theileria* spp. can be found in both erythrocytes and lymphocytes of their host. the vector responsible for transmission is biting tick *Hyalomma anatolicum anatolicum*. (Neits, 1967) [15]. *Theileria* sporozoites are transmitted to susceptible animals via saliva of feeding ticks. The clinical signs of theileriosis in cattle include anorexia, enlargement of superficial lymphnode, drop in milk yield, nasal discharges, lacrimation, dyspnoea, anaemia and in later stage some time diarrhoea and dysentery. Haematological and sero-biochemical alterations are the indicators of severity of disease and are considered to be good tools for the diagnosis and prognosis for effective therapy (Nazifi *et al.*, 2010) [14].

Materials and Methods

The present investigation was undertaken from a period of January 2021 to June 2021. Total 135 samples were collected from cattle which were suspected for theileriosis presented at the Veterinary Clinical Complex (VCC), Veterinary College, Anand, and nearby villages of Anand district. The such suspected cattle were screened on the basis of clinical signs and blood smear examination (Giemsa's stain). The information on epidemiological parameter such as age, sex, breed, body condition score, types of floor, housing system and hygienic condition *etc*. were also collected in present study. All animals were grouped as per their age into following class < 6 months, 6 months to 3 years, 3 years to 6 years, 6 years to 10 years and > 10 years. Body condition score was classified in to three categories, *i.e.*, Good/Fair/Poor. Floor wise incidence was calculated according to types of floor, *i.e.*, kachha and pakka floor. Housing system wise incidence was calculated according to loose housing and conventional housing system and hygienic condition wise incidence was calculated based on three categories *i.e.*, Good/Fair/Poor. Blood samples (4 ml) were collected from jugular vein in sterile plastic K_3EDTA vials from all 6 healthy and 6 affected animals before the initiation of treatment for haematological analysis by Automatic Whole Blood Analyzer (Abacus Junior Vet-5) and 8 ml blood was collected in clot activator vials for serum. The serum biochemical parameters studied included, Total Protein g/dl, Total Bilirubin mg/dl, Total Glucose mg/dl, Aspartate aminotransferase (AST) U/L, Alanine aminotransferase (ALT) U/L, Gamma-Glutamyl Transferase (GGT) U/L, Serum Creatinine mg/dl, Blood Urea Nitrogen (BUN) mg/dl by using standard assay kits with the help of Clinical Serum Biochemistry Auto-analyser (CKK 300). The data was analyzed by using one way analysis of variance (ANOVA) by using standard statistical techniques described by Snedecor and Cochran (1994) [20].

Results and Discussion

In this study, a total number of 135 cattle suspected for theileriosis were screened on the basis of clinical signs and blood smear examination (Giemsa's stain). Out of 135 cattle 64 animals were diagnosed for theileriosis contributing overall incidence of 47.41 percent. The overall incidence of theileriosis is reported in Table1. The higher incidence rate of theileriosis has been reported (ranging between 25.00 to 50.00 percent) by Ananda *et al.* (2009) [4] and Durrani *et al.* (2010) [6]. At the time of sample collection age, sex, breed, body condition score, floor type, hosing system and hygienic condition *etc*. were collected. The clinical findings recorded in the affected animals were anorexia, dullness, depression, decreased milk production, weakness, salivation, enlarged prescapular lymphnodes, pale conjunctival mucous membrane, presence of ticks over the body, emaciation, nasal discharge, cessation of rumination, lacrimation, diarrhoea and sternal recumbency.

Age wise incidence of theileriosis was found to be the highest in age group 6-10 years (27/50, 54.00%), followed by 3-6 years of age group (23/45, 51.00%), 6 month-3 years of age group (11/30, 37.00%), >10 years of age group (1/3, 33.33%) and the lowest incidence was found in <6 month of age group (2/7, 28.57%). The detailed results are presented in Table 1. Similar findings was observed by Ananda *et al.* (2009) [4] and Brahma *et al.* (2018) [5]. The higher incidence of theileriosis in cattle above 3 years of age might be due to increased stress, increased number of lactation, increased production and weakening of body immune system. The sex wise incidence was higher in female (61/123, 49.59%) than in male (3/12, 25%). The detailed results are presented in Table 2. Similar findings was accordance with Panda *et al.* (2011). This type of observation might be due to interest in raising female calves by the farmers for milk production purpose. The breed wise incidence found was higher in HF crosses (56/107, 52.34%) than the indigenous breeds (8/28, 28.57%). The details of the results are presented Table 3. The present finding are in agreement with Farooq *et al.* (2019) [8]. This finding might be due to higher susceptibility of HF cross bred cattle to this disease than indigenous cattle breeds.

Table 1: Age wise incidence of theileriosis in cattle

Age group	Total No. of suspected animals screened for theileriosis	No. of animals positive for theileriosis	Incidence (%)
< 6 month	7	2	28.57
6 months-3 years	30	11	37.00
3-6 years	45	23	51.11
6-10 years	50	27	54.00
> 10 years	3	1	33.33

Table 2: Sex wise incidence of theileriosis in cattle

Sex	Total No. of suspected animals screened for theileriosis	No. of animals positive for theileriosis	Incidence (%)
Female	123	61	49.59
Male	12	3	25.00

Table 3: Breed wise incidence of theileriosis in cattle

Breed	Total No. of suspected animals screened for theileriosis	No. of animals positive for theileriosis	Incidence (%)
HF Cross	107	56	52.34
Indigenous	28	8	28.57

The body condition score wise, the incidence of theileriosis was found highest 65.21% (30/46) in animals marked as poor body condition score, followed by 49.23% (32/65) in those marked as fair body condition score and the lowest 8.3% (2/24) in those marked as good body condition score. The detailed results are presented in Table 4. The floor type wise incidence was found higher in animals kept on kachha (46/90, 51.11%) than pakka (18/45, 40.00%) floor. These findings might be due to the hygienic condition that may not be maintained in kachha floor. The detailed results are presented in Table 5. Housing system wise incidence was higher in loose housing (43/72, 59.72%) than conventional

housing (21/42, 50.00%). The animals kept in a loose housing are more prone to the infection than conventional housing, because in loose housing system movement of animals is more so there are more chances to get the infection. The detailed results are presented in Table 5. The hygienic condition wise incidence was higher (35/55, 63.64%) in poor hygienic condition, followed by fair hygienic condition (24/59, 40.68%) and in good hygienic condition (5/21, 23.81%). The detailed results are presented in Table 6.

Table 4: Body condition score wise incidence of theileriosis in cattle

Body condition score	Total No. of suspected animals screened for theileriosis	No. of animals positive for theileriosis	Incidence (%)
Good	24	2	8.33
Fair	65	32	49.23
Poor	46	30	65.21

Table 5: Types of floor wise incidence of theileriosis in cattle

Types of floor	Total No of suspected animals screened for theileriosis	No of animals positive for theileriosis	Incidence (%)
Kachha	90	46	51.11
Pakka	45	18	40.00

Table 6: Housing system wise incidence of theileriosis in cattle

Housing system	Total No. of suspected animals screened for theileriosis	No. of animals positive for theileriosis	Incidence (%)
Loose housing	72	43	59.72
Conventional housing	42	21	50.00

Table 7: Hygienic condition wise incidence of theileriosis in cattle

Hygienic condition	Total No. of suspected animals screened for theileriosis	No. of animals positive for theileriosis	Incidence (%)
Good	21	5	23.81
Fair	59	24	40.68
Poor	55	35	63.64

Total 135 Geimsa's stained thin blood smears were examined from suspected cattle. Out of them, 64 cattle were found positive for piroplasm of *Theileria* spp. in erythrocytes. Out of 64 cattle, lymphnode fluid was collected from 12 cattle and at the time of lymphnode smear examination 8 cattle showed presence of schizont of *Theileria* spp. in lymphocytes. different shape of *Theileria* piroplasm observed in thin blood smear examination were round, oval, comma, signet ring and half moon in erythrocytes. These shapes of piroplasm are presented in Plate 4.3. These observations are in accordance with those reported by Nagar *et al.* (2019) [13] and Agina *et al.* (2020) [3]. Two forms of schizonts were recognized. Those which contained large chromatin granules were referred as macroschizonts, while those with small chromatin granules as microschizonts. The lymphocytes with macroschizonts are commonly known as Koch's blue bodies. These findings occurred well with the earlier observation of Ugalmugal *et al.* (2010) [23] and Modi *et al.* (2015) [12].

Haematological Findings

The haematological examination revealed that the mean±SE values of haemoglobin, packed cell volume, total erythrocytes count and lymphocytes were significantly decreased ($p<0.05$) in theileriosis infected cattle as compared to healthy cattle. Similar findings reported by Durrani *et al.* (2010) [6], Tehrani *et al.* (2013) [21] and Kachhawa *et al.* (2016) [10]. Neutrophils were significantly increased ($p<0.05$) and total leucocytes count, monocytes and eosinophils were non-significantly increased in theileriosis infected cattle as compared to healthy cattle. Similar observation reported by Tuli *et al.* (2015) [22] and Pandey *et al.* (2017) [17]. However, there was significant ($p<0.05$) increase in mean corpuscular haemoglobin and non-significant increase in mean corpuscular volume, while mean corpuscular haemoglobin concentration was non-significantly decreased in theileriosis infected cattle as compared to healthy cattle. Similar findings reported by Emarah *et al.* (2012) [7] and Goyal (2018) [9]. The results of haematological values presented in Table 4.

Table 8: Haematological findings of healthy and theileriosis infected cattle (Mean±SE)

Haematological Parameters	Healthy group (n = 6)	Theileriosis infected group (n = 6)
Hb (g/dl)	10.73^{b}±0.39	5.95^{a}±0.64
PCV (%)	32.34^{b}±0.69	20.19^{a}±2.39
TEC (10^{6}/µl)	7.21^{b}±0.09	4.59^{a}±0.66
TLC (10^{3}/µl)	7.20±1.01	9.79±1.44
L (%)	60.10^{b}±2.50	46.98^{a}±4.57
M (%)	1.95±0.35	3.22±0.65
N (%)	35.08^{a}±1.82	46.67^{b}±5.08
E (%)	1.15±0.37	3.08±0.82
B (%)	0.05±0.03	0.05±0.05
MCV (fl)	47.83±0.48	52.00±1.03
MCH (pg)	14.87^{a}±0.44	18.30^{b}±0.69
MCHC (g/dl)	33.28±1.52	29.53±0.55

Means with uncommon superscripts within the column (a,b) differ significantly ($p<0.05$)

Biochemical Findings

The serum biochemical examinations revealed that the mean±SE values of total protein and albumin were decreased non-significantly, while total glucose was decreased significantly ($p<0.05$) in theileriosis infected cattle as compared to healthy group. Similar findings reported by Sandhu *et al.* (1998) [19], Khan *et al.* (2011) [11] and Kachhawa *et al.* (2016) [10]. Gamma glutamyl transferase and aspartate aminotransferase were increased significantly ($p<0.05$), but total bilirubin and alanine aminotransferase were increased non-significantly in

theileriosis infected cattle as compared to healthy cattle. Similar findings noted by Acharya *et al.* (2017) [2] and Abubakar *et al.* (2019) [1]. Serum creatinine and blood urea nitrogen were showed non-significant difference in theileriosis infected cattle.

Table 4: Serum biochemical findings of healthy and theileriosis infected cattle (Mean±SE)

Serum biochemical parameter	Healthy group (n = 6)	Theileriosis infected group (n = 6)
Total Protein (g/dl)	7.05^{b}±0.38	5.48^{a}±0.44
Albumin (g/dl)	3.63±0.28	2.81±0.31
Total Bilirubin (mg/dl)	0.34±0.07	0.97±0.12
Total Glucose (mg/dl)	54.17^{b}±2.09	39.83^{a}±3.06
AST (U/L)	85.14^{a}±2.48	122.02^{b}±8.73
ALT (U/L)	28.54±2.07	38.39±4.58
GGT (U/L)	22.28^{a}±1.91	53.80^{b}±3.76
Serum Creatinine (mg/dl)	1.15±0.14	1.13±0.16
BUN (mg/dl)	12.11±0.52	11.76±0.49

Means with uncommon superscripts within the row (a,b) differ significantly ($p<0.05$).

Conclusion

The overall incidence of theileriosis in cattle was 47.41% among suspected cattle. Age wise incidence was found higher in 6-10 years of age group (54.00%). The HF crossbred cattle were found most susceptible as compared to indigenous breeds. The incidence of theileriosis was found highest in poor body condition score, animal kept on kaccha floor and loose housing system.

Theileriosis infected cattle causes significant decreased values of haemoglobin, packed cell volume, total erythrocytes count, lymphocytes, MCHC with neutrophilia, eosinophilia, and increased values of MCV and MCH as compared to healthy animals. The macrocytic hypochromic anaemia was found in theileriosis cattle. Biochemical observations revealed decreased values of total protein and albumin, while increased values of total bilirubin, AST, ALT and GGT compared to healthy animals.

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