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## Studies on comparative haematological parameters in *Theileria annulata* infected cattle

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### Abstract

Theileriosis is a group of disease caused by variety of tick species worldwide. *Theileria* is an obligate intracellular protozoan parasite that parasitizes host red blood cells (RBC) and white blood cells (WBC). Disease is mostly seen in cattle, sheep and goat as well as in wild and captive ungulates. Most important species of theileria infecting cattle is *T. annulata* and *T. parva*. *T. annulata* is the cause of Bovine Tropical Theileriosis which leads to heavy morbidity and death tolls around the globe. Tick species responsible for the disease transmission in India is *Hyalomma anatolicum*. Parasite is transmitted by the infected saliva of the tick during feeding. Reports on disease occurrence are more common in cross bred cattle and exotic breeds. Following study was conducted among 25 cross bred animals having a history of tick infestation with elevated body temperature, lymph node enlargement and anaemia as the main clinical signs. Blood sample collection was done by a jugular puncture in EDTA vials. Microscopic examination by Giemsa based thin blood smear was carried out for confirmatory diagnosis. Haematological parameters were studied and comparison between haematology and severity of infection was carried out. Treatment with Inj. Buparvaquone @2.5mg/kg single injection, Inj. Oxytetracyclines @10mg/kg for 3 days along with other Supportive therapy was adopted.

**Keywords:** theileriosis, dairy herd, haematological, buparvaquone

### 1. Introduction

Theileriosis is a disease caused by obligate intracellular protozoan parasite *T. annulata* which is transmitted by the bite of *Hyalomma anatolicum* ticks. Higher incidence rate has been recorded in exotic and crossbred cattle among all age groups, with general epidemiology of the disease in tropical and subtropical countries [6]. Disease caused by the parasite is very severe with estimated annual losses of about US\$ 384.3 million a in the Indian subcontinent [10]. Around 10 million cattle are at the risk for infection with tropical theileriosis.

Clinically affected animals show pyrexia up to 107° F with enlarged superficial lymph nodes, white mucous membranes, anaemia, anorexia, petechiae on conjunctiva and increased respiration rates which is further accompanied by dullness, salivation and lacrimation [3, 13]. According to Mudgal [11] young calves of less than one month of age are highly susceptible. Diagnosis of Theileriosis is mainly based on several clinical signs and is confirmed by microscopic examination of Giemsa stained thin blood or lymph node smears for the presence of piroplasms in red blood cells and macroschizonts in lymphocytes. Disease is more common following rains due to the increased tick population and stress among animals due to prevalence of hot and humid climatic conditions. Losses caused by *Theileria* include both direct and indirect losses. Direct losses are mainly due to disease development that causes death of the affected cattle whereas indirect losses occur after disease ceases and include various losses like production losses, fertility losses and losses due to cost of treatment undertaken.

### 2. Materials and Methods

#### 2.1 Blood sampling and physical examination

Study was conducted on 25 cross bred cattle including adult animals and young calves which were suspected for haemoprotozoan infection. Blood was collected in EDTA vials from dairy farms, quarantine sheds and polyclinic. Complete history of the animal including breed, age, sex and deworming status was gathered from the owner. Information regarding clinical symptoms/signs shown by the animal, present status of food and water intake, tick infestation if any was also recorded. Physical examination of the animals was done by recording the rectal

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temperature, pulse rate, colour of the conjunctiva mucous membrane and lymph node enlargement.

## 2.2 Microscopic examination

Thin blood smears were prepared and fixed with methanol for 5 minutes which were then stained with 5% Giemsa's stain solution for 30-35 minutes. Blood smears were carefully examined for the presence of *Theileria* piroplasms under oil immersion lens (100 x magnifications). Presence of small annular shape forms inside erythrocytes was considered positive. Infection severity was graded according to the number of piroplasms seen under one field. Samples having about  $\geq 10$ -12 piroplasms in one field were grouped as high severity followed by up to 5-8 as moderate and 1-3 as mildly infected. Samples having one or two parasite in different fields were classified as rare positive *Theileria*.

## 2.3 Haematological parameters

Samples found highly positive were further processed for haematological examination by digital haemo-analyzer. Haemoglobin, PCV, RBC, WBC and platelet counting was done.

For treatment of *T. annulata* positive cases, therapeutic regimen followed included Injection Buparvaquone (Butalex) @2.5 mg/kg by Intramuscular route and was repeated after 7 days, therapy was combined with supportive treatment using Injection Melonex @0.5mg/kg I/M, Inj.Dexona (5ml I/M), Injection Tribivet (10 ml I/M), along with fluid therapy and B-complex.

## 3. Results

The infection produced in cross bred cattle led to increase in body temperature (above 103°F), weakness, lymph node enlargement, anaemia, tachycardia and dyspnoea in later stages. Blood smear examination revealed the presence of piroplasm in the RBCs, upon laboratory examination the ticks

were identified as *Hyaloma anatolicum*.

Among the 25 animals studied, 19 animals showed infection ranging from severity high to low. Among these 5 were highly positive as seen by Giemsa stained thin blood smear examination. Both samples from calves were highly positive whereas samples from Tharparker were found negative. The details of breed-wise distribution are tabulated in the Table 1.

**Table 1:** Breed-wise distribution of cases

No.	Breed	Cases	% of cases
1	Holstein Friesian	18	72.2%
2	Tharparkar	5	20.8%
3	Cross bred calf	2	8%

In this study, 24 cases were from female animals (96%) and 1 case from male animal (4%). The age-wise distribution shows that, 23 cases (92%) were from the group above 2 years. Below 2 years represents 2 cases (8%). Disease is more severe in exotic cattle with age less than two years. In the present study number of cases reported from young calves was less (two out of total twenty five); however both cases were highly positive indicating more severity in young animals than adults.

Out of the total studied cases; 7 animals (28%) showed pyrexia with temperature above 103°F. Normal bovine body temperature ranged from 100.5°F to 102.5°F. In 2 cases (8%) tick infestation was reported and 3 (12%) cases showed marked enlargement of superficial lymph nodes. Pale conjunctival mucous membrane and anaemia was also reported. Blood smears showing annular or ring shaped piroplasm inside erythrocytes were considered positive for *Theileria annulata*. Samples found positive were further analysed haematologically. Details on haematological data are presented in the Table 2. Comparison between total parasitemia to that of infection severity was done, the results of which are depicted in the Table 3 (Fig 1).

**Table 2:** Haematological parameters of cases found positive

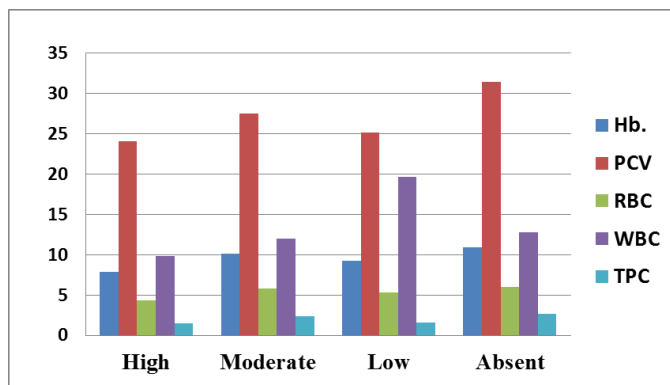
S. No.	Hb (g/dl)	PCV (%)	RBC ( $10^6/\text{mm}^3$ )	WBC ( $10^3/\text{mm}^3$ )	PLT (lakhs / $\text{mm}^3$ )
1	5.8	21	2.99	6.7	1.09
2	7	22	3.3	6.8	1.3
3	5.3	24.5	1.9	8.1	0.74
4	8.7	24.5	5.25	10.1	0.35
5	9.8	27.6	5.47	13.1	1.44
6	11.5	30.1	7	4.9	3.69
7	8.3	21.2	4.49	17.8	0.39
8	9.9	25.8	5.88	8.2	4.48
9	11.3	32.4	6.08	25.7	3.02
10	8.6	21.9	4.69	14.9	0.5
11	10.5	29.7	6.37	10.6	2.17
12	7.8	21.3	5.13	18.6	1.14
13	8.9	24.7	4.95	18	2.15
14	9.4	26.4	4.96	14.5	4.18
15	5.3	13	2.67	5.1	2.25
16	13	39	7.62	13.6	3.9
17	14	41.6	7.69	8.2	2.2
19	12.8	37	7.3	22.6	1.03
Avg - 9.33		26.87	5.21	12.64	2.00

**Table 3:** Comparison between various physical and blood parameter according to severity of infection in dairy herd (n=25)

Parameter → Severity ↓	Rectal Temperature (°F)	Hb. (gm /dl)	PCV (%)	Total RBC ( $10^6/\text{mm}^3$ )	Total WBC ( $10^3/\text{mm}^3$ )	Total platelet count
High (n=7)	103.5	7.9	24.1	4.3	9.8	1.5
Moderate (n=3)	102.4	10.1	27.5	5.8	12.0	2.4
Low (n=10)	101.8	9.2	25.2	5.3	19.7	1.6
Absent (n=5)	*	10.9	31.4	6.0	12.8	2.7

\*Temperature not recorded

All *T. annulata* infected cattle had lower RBC, Hb, PCV and PLT values compared to the normal range. Above table shows that infected animals showed positive correlation with increased temperature. Hb. and PCV values were decreased in severely infected cattle followed by increased levels which may be due to regenerative process. These results can be correlated with previous studies [7].



**Fig 1:** Graph showing comparison between severity and blood parameters

#### 4. Discussion

Tropical Theileriosis is a tick borne protozoan disease caused by *T. annulata*. Disease causes high mortality and morbidity among cross bred cattle worldwide. It is transmitted by bite of *Hyalomma anatolicum* ticks in India. Disease is characterized by high fever (rectal temperature > 39°C), Enlargement of superficial lymph nodes, anaemia, jaundice, drop in milk production. In severe cases there is laboured breathing, with rapid and shallow breaths followed by increased heart rates [5]. In the present study conducted total out of 25 cases, 7 animals were highly infected with rectal temperature with an average of 103.5° F. Out of these 7 highly positive cases 2 were calves below three months of age that showed increased severity of infection with the presence of ticks. Members of genus *Theileria* readily parasitize RBC resulting in progressive anaemia. Animals that have survived infections remain sub-clinically infected. Naturally occurring cases of in cattle were manifested by a wide variety of non-specific clinical signs. In this study all animals were having pyrexia and anorexia. Other symptoms shown by animals include lymphadenopathy, pale mucosa, lethargy and anaemia. The progressive increase in the percentage parasitaemia was associated with a progressive rise in body temperature and mortality in the calves [8]. The significant decrease in Hb. concentration, PCV and RBC count was seen [2]. Sharma *et al.* (1979) and Mehta and colleagues (1988) [14, 9] also reported progressive decrease in these parameters in *T. annulata* infections. Haemoglobinuria was not observed in any of the cases under study, as reported by Neitz (1957) and Gautam and colleagues (1970) [12, 4]. Leukopenia in the terminal stages resulted from large-scale destruction of lymphocytes by schizogony in lymphoid organs followed by infiltration and dissemination of these infected cells into various organs, resulting in a decreased count in the peripheral circulation as reported earlier [1, 15]. However, Mehta and colleagues [9] reported a leukocytosis in Theileriosis. Only a non-significant leukocytosis in the early stages of disease was observed in the present study which resulted from the proliferation of lymphocytes in the lymphoid organs as a defence mechanism. *Theileria annulata* is the cause of Bovine Tropical

Theileriosis in cross bred cattle worldwide. It is transmitted by bite of *Hyalomma anatolicum* ticks and mostly occurs in India, Pakistan, Korea and European countries. Parasite is present in lymphocytes, erythrocytes and histiocytes of host. Piroplasms stage present inside RBCs are pleomorphic and annular shaped and can be detected by blood smear examination. Most predominant signs of infection were increasing body temperature followed by lymphadenopathy. Presence of ticks on the body of an animal is also indicative of haemoprotozoan infections. There is a marked decrease in haemoglobin, PCV, RBC counts; this is due to anaemia leading to white mucous membranes. As the disease progress animal become dull, anorectic, rapid decrease in body weight and production status. In severe cases haemorrhagic diarrhoea is seen, this is due to ulcer formation in the gastrointestinal tracts leading to blood mixed faeces. Disease can be treated with Buparvaquonone @ 2.5mg/kg body weight which is also the drug of choice. Along with this supportive therapy is required. Oxytetracycline @10mg/kg is also beneficial in early stages of disease development before schizogony occurs in lymphocytes. Proper tick control is essential to decrease disease incidence.

#### 5. Conclusion

Bovine tropical theileriosis, a tick borne haemoprotozoan disease is the cause of heavy mortality and disease outcome in India. Disease occurs more commonly following rains due to increase in tick populations that transmit theileria protozoa through bite. Various clinical signs associated with disease are high fever, swollen lymph nodes, pale mucous membranes, anorexia, weakness and anaemia. In severe cases haemorrhagic diarrhoea has been reported. In the present study affected animals showed high fever, swollen lymph nodes with a history of tick infestation. On haematological and microscopic examination decrease in blood parameters and presence of theileria organism was confirmed in RBC's and lymphocytes. Effective treatment with the drug of choice buparvaquone along with supportive therapy was helpful to reverse the ill effects of the disease. However, in severely infected animals blood transfusion along with treatment is essential to safeguard animal's life. Other strategies like Integrated pest control measures to reduce tick population should be adopted for effective control of ticks to reduce the infection rate.

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