



Haematological alterations and molecular detection of theileriosis in crossbred cattle

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ABSTRACT

Theileriosis is a fatal haemoprotozoan disease which is a major threat to dairy and related industries. This study was undertaken to ascertain haematological changes and molecular diagnosis of *Theileria annulata* in crossbred cows. The infected group showed significantly lowered mean values of TEC, Hb, PCV and thrombocytes. Also the red blood cell (RBC) indices, viz. MCV, MCH, and MCHC were significantly lower indicating hypochromic microcytic anaemia. Out of 652 blood samples analysed by Giemsa stain, the overall prevalence of theileriosis was 36.3% during July 2015 to June 2017. Highest positivity of Theileriosis was noticed in summer (40.1%) followed by Spring/autumn (38.3%), rainy (34.3%) and lowest in winter (31.5%). About 48% blood samples were positive for *Theileria annulata* by 18SrRNA and TASP gene based PCR.

Key words: Anaemia, Crossbred cattle, Haemoprotozoan, PCR, Theileriosis

Bovine tropical theileriosis, caused by intracellular blood protozoan *Theileria annulata* and transmitted by *Hyalomma* is considered to be a life threatening disease of crossbred cattle in many parts of the world including India (Uilenberg 1982). Major characteristics of disease are lymphadenopathy, splenomegaly, fever, anemia, weakness and loss of body weight (Omar *et al.* 2002, El Deeb *et al.* 2009). Theileriosis has significant economic impact as a result of mortality and reduced milk yield. Majority of the haemoprotozoan parasites are tick borne and are of considerable economic importance in Asia and have always been ominous obstacle to the survival of cattle in India (Soundrarajan 2006). Tropical theileriosis accounts for annual loss of approximately US\$ 800 million in India (Devendra 1995). Theileriosis has been observed in different geographical regions in India such as Punjab, Haryana and Gujarat. In Northern Kerala and Gujarat (16% and 37%), positive cases of theileriosis has been reported in cattle (Nair *et al.* 2011, Vahora *et al.* 2012). Much of the pathology in theileriosis is because of intra-lymphocytic schizogony (Morrison *et al.* 1989) and related alteration in biochemical and haematological parameters (Razmi *et al.* 2003, Radostits *et al.* 2007). The present study was aimed to determine haematological changes, molecular diagnosis and the seasonal prevalence of *Theileria* in Ambala district of Haryana, India.

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MATERIALS AND METHODS

Blood samples (652) were examined from cattle at Disease Investigation Laboratory from July 2015 to June 2017. Blood samples from cattle having complain of anorexia, pyrexia, decrease in milk yield, enlarged superficial lymph nodes, pale conjunctival mucous membrane, haemoglobinuria, nasal discharge, respiratory distress etc. were brought to the laboratory. Blood smears were prepared on clean glass slides and stained with Giemsa stain by the standard technique. The stained blood smears were examined for the presence of haemoprotozoan infections. The haemoprotozoan were identified to species level as per morphological characters described by Soulsby (1982). Haematological parameters, viz. haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC) and red blood cell indices like mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were analysed using blood cell counter (MS4Se, Melet Scholasing Lab). The differences of means of estimated parameters between *T. annulata* infected and healthy control groups were compared using Student's t-test (Snedecor and Cochran 1994). These blood samples were kept at 4°C and further analysed by PCR. DNA was extracted from the blood samples as per manufacturer's instructions (Qiagen). The DNA quality and integrity was checked using the Nanodrop instrument and subsequently by running the DNA samples on the 0.8% agarose gel. DNA samples were then stored at –80°C. *Theileria annulata* specific primers for the gene 18SrRNA and TASP (*Theileria annulata* surface protein gene) were used for diagnosis and amplification of the

125 bp and 504 bp gene product using the following primers (18SrRNA For Primer: ACGACTCCTTCAGCACCTTG and 18SrRNA Rev Primer: AAATTAAGCCGCAGCTCCAC; tasp For Primer: ATGAAATTCTTCTACCTTTT and tasp Rev Primer: CTTGGGGCGCTTATCATGATCG). The PCR conditions used for amplifying the 18SrRNA and TASP gene were 95°C for 3 min, followed by 35 cycles of 95°C (1 min), 61°C (20 sec) 18SrRNA/55°C (1 min) tasp, 72°C (1 min) and a final extension of 72°C (5 min). The genomic DNA isolated from the pre-established *in vitro* culture of *T. annulata* cell line was used as positive control for all the PCR reactions. A no template reaction was also used as a negative control for all the PCR reactions.

The obtained data of haemoprotozoan infections was pooled season-wise winter, viz. (December, January, February), summer (April, May, June), rainy (July, August, September) and spring/autumn (October, November, March).

RESULTS AND DISCUSSION

The infected group revealed significantly lower mean TEC ($4.78 \pm 0.21 \times 10^6/\mu\text{l}$), Hb (6.51 ± 0.29 gm/dl), PCV ($21.11 \pm 0.89\%$) and thrombocytes ($215.1 \pm 22.49 \times 10^3/\mu\text{l}$) than healthy control animals (Table 1). There were significantly lower values of red blood cell (RBC) indices, viz. MCV, MCH, and MCHC (Table 1) indicating hypochromic microcytic anaemia. However, the type of anaemia largely depends on the disease severity and accordingly various types of anaemia in anaplasmosis, babesiosis and theileriosis has been reported (Ashuma *et al.* 2013, Ganguly *et al.* 2015, Ganguly *et al.* 2017).

The distinguished decrease in the RBC count causing anaemia, might be because of intra-erythrocytic piroplasms (Preston *et al.* 1992). Even though it may also be attributed to an erythro-phagocytosis in place of a parasite-induced

Table 1. Mean values[#] of haematological parameters of crossbred cattle infected with *Theileria annulata*

Parameter	Cattle infected with <i>Theileria annulata</i> (20)	Healthy cattle (10)	P value
Hb (g/dl)	$6.51 \pm 0.29^{**}$	12.67 ± 0.14	<0.00001
PCV (%)	$21.11 \pm 0.89^{**}$	38.4 ± 0.67	<0.00001
TEC ($\times 10^6/\mu\text{l}$)	$4.78 \pm 0.21^{**}$	6.80 ± 0.104	<0.00001
TLC ($\times 10^3/\mu\text{l}$)	$6.11 \pm 0.96^{\text{NS}}$	6.21 ± 3.22	0.992
Lymphocyte (%)	$62.68 \pm 1.86^{**}$	51.6 ± 1.65	0.000142
Neutrophil (%)	$31.04 \pm 1.48^{**}$	46.9 ± 1.90	<0.00001
MCV (fl)	$44.75 \pm 1.56^{a**}$	55.64 ± 1.02	0.000072
MCH (pg)	$13.9 \pm 1.27^{**}$	18.37 ± 0.33	<0.00001
MCHC (gm/dl)	$30.59 \pm 0.54^*$	33.05 ± 0.50	0.0284
THR ($\times 10^3/\mu\text{l}$)	$215.1 \pm 22.49^*$	333.2 ± 10.05	0.001324

[#]Mean $\pm$ SE; *Infected and healthy cattle differed significantly at $P < 0.05$; **Significant at $P < 0.001$. Hb, Haemoglobin; PCV, packed cell volume; TEC, total erythrocyte count; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; TLC, total leukocyte count; THR, thrombocytes.

Table 2. Seasonal prevalence of *Theileria* in cattle in Ambala district (Haryana) from July 2015 to June 2017

Season	Total no. of sample tested	Theileria positive	% Positive
Rainy	282	97	34.3
Spring/Autumn	107	41	38.3
Winter	76	24	31.5
Summer	187	75	40.1
Total	652	237	36.3

lysis (Boulter and Hall 2000). The present study elucidated anaemia in animals infected by *T. annulata*. Infected cells released by TNF α cause suppression of haematopoietic progenitors and decrease in red blood cell production (Tizard 1992). It also minimize life span of RBC leading to anaemia. In theileriosis, the thrombocytopenia was probably been caused by increased destruction, consumption and degranulation of platelets in the peripheral blood, and attributable to suppressing of the release of platelets from the bone marrow into the blood stream by the parasite and its products (Yagi *et al.* 2002).

The blood cellular changes displayed a significant ($P < 0.001$) decrease in mean percent concentration of neutrophils ($31.04 \pm 1.48\%$) and increase in mean percent concentration of lymphocytes ($62.68 \pm 1.86\%$) in infected animals in comparison to healthy group, though the non-significant decline was observed in total leucocyte count. Host cells transformation by intra-lymphocytic theilerial parasites may be the cause of lymphocytosis, leading to clonal growth of lymphocytes (Yamaguchi *et al.* 2010).

The overall prevalence during July 2015 to June 2017 of *Theileria* in Ambala district was 36.3% by microscopic investigation of Giemsa stained blood smears. An expected amplified product of 125 bp and 504 bp was obtained from 120 out of 250 blood samples indicating the presence of *Theileria* parasite by 18SrRNA and TASP gene based PCR, respectively. The identification of theileriosis in acute cases mainly depends upon clinical signs and microscopic study of stained thin blood smears. Molecular and microscopic techniques for detection of *T. annulata* infection in cattle have been compared by Chauhan *et al.* (2015) in which

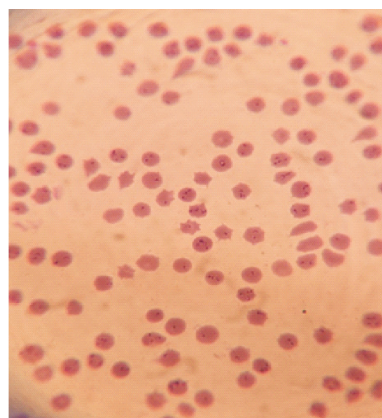


Fig. 1. Piroplasm of *Theileria* spp.

32.69% animals were found positive for theileriosis by microscopy and 46.15% by PCR assay. Charaya *et al.* (2016) also compared PCR assay with microscopy for detection of theileriosis in crossbred cattle population and recorded 62.06% *Theileria* sp. infection by PCR and only 34.48% by microscopy. Incidence of *T. annulata* infection is increasing, hence for the timely and sensitive detection of *T. annulata* infection in animal, PCR assay could be used as adjunct test along with microscopic examination. It will be helpful in devising more effective preventive and control strategies against theileriosis.

The prevalence of theileriosis differ in different regions and numerous factors regulate the occurrence of the tick-borne diseases such as sex, age, breed, season, tick density, geographical area and management (Kivaria *et al.* 2012, Magona *et al.* 2011). The present study was conducted from July 2015 to June 2017 to determine the seasonal prevalence of theileriosis in Ambala district (Haryana). Out of 652 blood samples analysed by Giemsa stain, 34.3% were positive in rainy season, 38.3% in spring/autumn, 31.5% in winter and 40.1% in summer season for theileriosis (Table 2). Highest positivity of Theileriosis was noticed in summer followed by spring/autumn and lowest in winter. Theileriosis is a fatal parasitic disease in India, and has been reported from several parts of the country with incidence of 21.1% in Tamil Nadu (Anandan *et al.* 1989), 16% in Northern Kerala (Nair *et al.* 2011), 17.7% in Karnataka (Muraleedharan *et al.* 1994) and 45.4% in Dehradun, Uttarakhand (Kohli *et al.* 2014). These dissimilarity noted in the prevalence may be because of the distinct geographical locations, time periods and various methods of sample analysis. Velusamy *et al.* (2014) also reported theileriosis to be significantly high during summer (14.4%), followed by moderate in monsoon (13.8%) and less in fair seasons (11.5%). The results of the present study were in accordance with report of Chakraborty (1993), from Ranchi, Bihar recording a high prevalence of theileriosis during summer (17.64%), followed by rainy (7.32%) and less in winter (5%). In contradiction to our findings, there are few reports (Radostits *et al.* 1994, Vahora *et al.* 2012, Roy *et al.* 2004) of higher prevalence of theileriosis during monsoon. Ganguly *et al.* (2017) also reported significantly higher prevalence of *T. annulata* in rainy season (37.26%) and summer (32.49%), and less in winter (26.61%). High prevalence can be correlated to the high activity of the tick vectors during summer and rainy season (Sangwan *et al.* 1995, Anandan *et al.* 2009, Vohra *et al.* 2012). The marked seasonal variation regarding the incidence of the theileriosis may be because of changes in macroclimate that is requisite for breeding of ticks.

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