

Prevalence of theileriosis in cross-bred cattle: its detection through blood smear examination and polymerase chain reaction in Dehradun district, Uttarakhand, India

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Abstract

Aim: Theileriosis is a major threat to dairy and related industries. Theileria is a protozoan parasite that causes theileriosis, a fatal disease. The aim of the present study was to assess the prevalence of theileriosis in crossbred cattle belonging to Dehradun district, Uttarakhand, India.

Materials and Methods: We screened 301 blood samples each month from apparently normal/healthy crossbred cattle from various locations of Dehradun district. Samples were tested using Giemsa's staining technique and specific PCR test.

Result: Microscopic examination of blood smears revealed 27.2% (82) overall prevalence of theileriosis. The highest prevalence was found in rainy season with a prevalence rate 45.4%. By Theileria genus specific PCR test, 98 samples (32.5 %) were found to be positive for theileriosis.

Conclusion: Our study suggests that theileriosis is spreading to even the temperate region in Uttarakhand and immediate screening norms/policies are needed to reduce the extent of spread. There is a need for further investigation using molecular technique.

Keywords: blood smear, PCR, seasonal prevalence, Theileria, Uttarakhand.

Introduction

Haemoprotozoan diseases cause devastating losses to the livestock industry and thus pose major constraints to the dairy industry throughout the world. In this regard, ticks are mostly related to initiation of many diseases. The hot and humid climate is highly favourable for the development and survival of ticks. In particular, ticks spread Theileria which pose a serious challenge to the exotic crossbred cattle population. *Theileria annulata* and *Theileria parva* are considered to be the most pathogenic species of Theileria. Tropical theileriosis is one of the most prevalent diseases of cattle caused by *T. annulata* [1] and is transmitted through Ixodid tick of genus *Hyalomma*. Theileriosis has serious economic impact in view of mortality and reduced milk yield. Most of the haemoprotozoan parasites are tick borne and are of great economic importance in Asia and have always been formidable barriers to the survival of crossbred cattle in India. In India the annual loss reported due to tropical theileriosis is approximately US\$ 800 million [2]. In India theileriosis has been reported from geographical regions such as Punjab, Haryana, and Gujarat. In Bangalore north, the occurrence of *T. annulata* among

crossbred cattle has been reported in 2009 [3]. In Northern Kerala 16 % positive cases of theileriosis has been reported in crossbred cattle [4]. 37% cattle reported positive for the haemoprotozoan infection in Kaira and Anand Districts of Gujrat [5]. Vahora et al [5] also reported that the higher incidence of Theileria during the monsoon season. A case of tropical theileriosis was also reported from West Bengal in 2012 [6]. An outbreak of theileriosis in cattle has been reported from Punjab with 4.86% mortality rate [7].

The present study was aimed to assess the spread of Theileria in Uttarakhand, this paper highlights the problem of theileriosis and its season wise prevalence rate in crossbred cattle in Dehradun district of Uttarakhand, India.

Materials and Methods

The study was conducted in the Dehradun district of Uttarakhand, India. Geographic location of Dehradun is in between 29°58' and 31°2'30" North and 77°34'45" and 78°18'30" East. The climate of Dehradun is moderate due to its location at the foot of the Himalayas. The clinical manifestation of theileriosis recorded in cross bred cattle during the summer and monsoon season are high temperature, swelling of lymph nodes, weakness, increased respiration and nasal discharge.

Ethical approval: Permission was obtained from

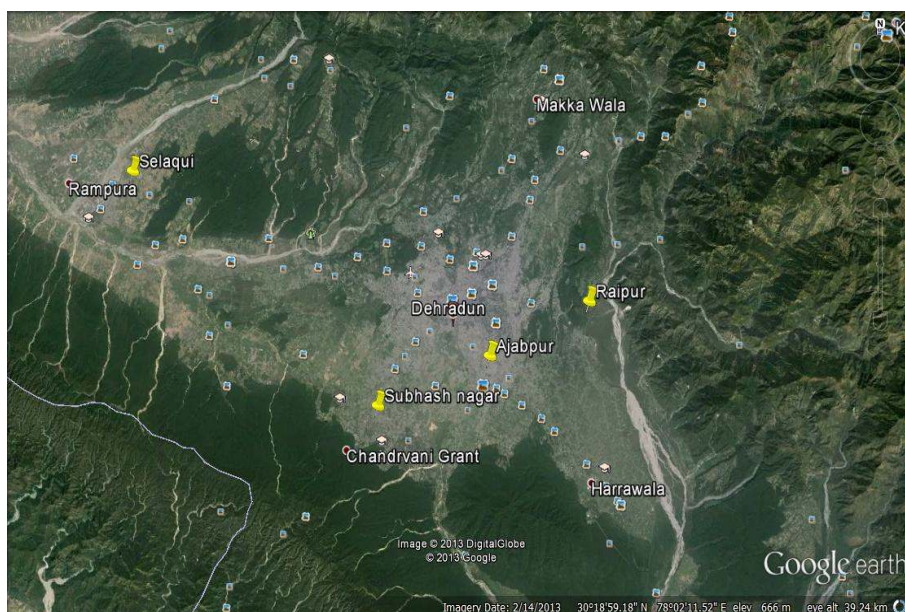


Figure-1. Map of study area: different areas of Dehradun (Raipur, Subhashnagar, Ajabpur and Selaqui region), Uttarakhand

Institutional Ethics Committee, Graphic Era University, Dehradun.

Collection of samples: A total number of 301 crossbred cattle were screened for haemoprotozoan parasite from different areas of Dehradun (Raipur, Subhashnagar, Ajabpur and Selaqui region) Uttarakhand, India (Fig.-1). Blood samples from ear veins of examined animals were collected for detection of *Theileria* piroplasms microscopically after staining with Giemsa's stain. Approximately 3ml blood sample was collected from jugular vein into tubes containing EDTA and stored at -20°C for DNA extraction. All the sampling was done as per ethical standards by a Veterinarian.

Microscopic examination (ME): Thin blood smears were prepared and fixed with methanol for 5 minutes. These were then stained with 5% Giemsa's solution and were examined under microscope after 30 minutes. Blood smears were carefully examined for *Theileria* under the oil immersion lens (100 x magnification). Even the presence of single piroplasms was considered positive for *Theileria*.

DNA extraction and polymerase chain reaction (PCR): DNA extraction was performed according to the standard Phenol-Chloroform protocol [8] and integrity of extracted DNA was checked by agarose gel electrophoresis. This purified DNA was used as template for the PCR. The *Theileria* genus specific primers for SSU rRNA gene used in the present study were 989(F) AGTTTCTGACCTATCAG and 990(R) TTGCCTTAAACTTCCTTG (9). Amplification was performed in a thermal cycler (Eppendorf AG, 5333) under the following conditions: 94°C for 3 min (initial denaturation) followed by 25 cycles of 94°C , 30 sec (denaturation), 52°C , 30 sec (annealing), 72°C 1.5 min (extension) and a final extension of 72°C for 5 min. A positive control sample representing *Theileria* sp. and

negative control were obtained from clinical case and healthy cattle diagnosed respectively at Indian Veterinary Research Institute (IVRI), Bareilly, India (from the lab in Division of Parasitology). Positive control and negative control was run along with the other samples. The amplification products were subjected to electrophoresis on 1% agarose gel. The length of amplified product was estimated using a base pair standard (1Kb, Merck) and the amplified products were visualized with an U.V. transilluminator.

Results

The study was conducted for 10 months from March to December 2012 to identify the seasonal prevalence of *Theileria* in Dehradun district. From a total number of 301 cattle analysed by Giemsa's stained blood smear (Fig.-2), 9.0% cows were found positive for *Theileria* as per blood smear in spring season, 19.6% were found positive in summer season, 45.4% were found positive in rainy season and 8.8% were found positive in winter season (Table-1). Highest numbers of positive cases were obtained in rainy season which corresponds to months between July and October (Fig.-3). Similar observations were observed in previous reports (3, 10). Microscopic examination of blood smears revealed 27.2% overall prevalence of theileriosis.

PCR reactions amplifying a band of 1098bp were considered positive for the infection (Fig.-4). Besides 82 positive samples that were identified using staining procedure, 16 other samples were also detected as positive for the disease using PCR. These cattle were categorized as "carrier cattle". Thus, *Theileria* genus specific PCR could detect 98 samples (32.5%) as positive. Along all the PCR a theileria positive sample (obtained/confirmed from IVRI, Bareilly) was run. Positive control sample always showed the requisite band of size 1098 bp.

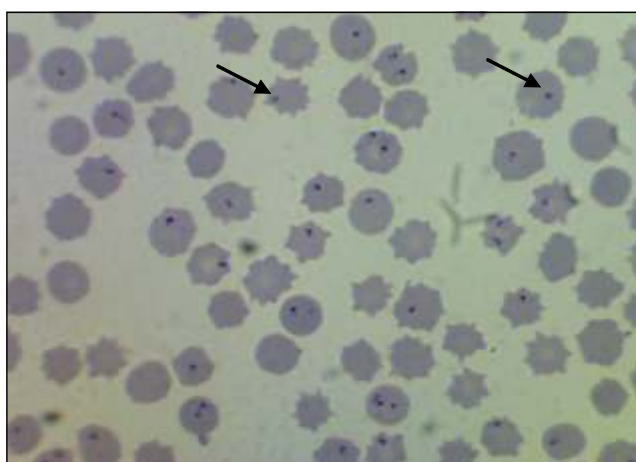


Figure-2. Microscopic Examination of Giemsa stained blood smear showing piroplasm of *Theileria* parasite (arrow shows the piroplasm stage of *Theileria* in erythrocyte)

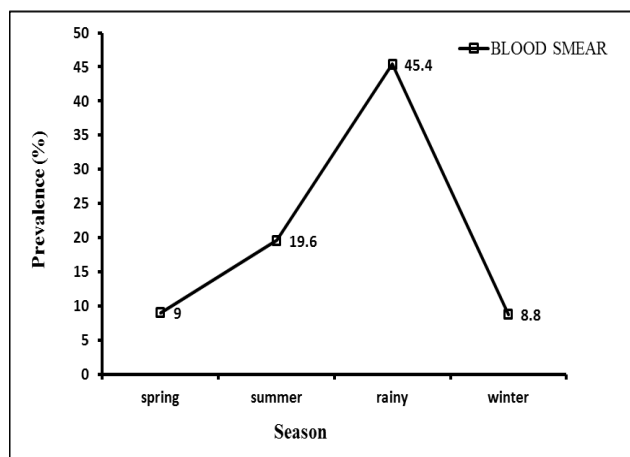


Figure-3. Graph showing the prevalence of theileriosis in different seasons- Spring (March, April), Summer (May, June, July), Rainy (August, September, October) and Winter (November, December)

Table-1. Season-wise incidence of *Theileria* detected in crossbred cattle during March-December 2012

Season	Subhashnagar	Raipur	Selaqui	Ajabpur	Private herds	Total observed	Total affected	Prevalence (%)
Spring (March, April)	15	7	18	10	5	55	5	9
Summer (May, June, July)	25	41	11	25	-	102	20	19.6
Rainy (August, September, October)	26	17	32	14	10	99	45	45.4
Winter (November, December)	16	7	11	9	2	45	4	8.8

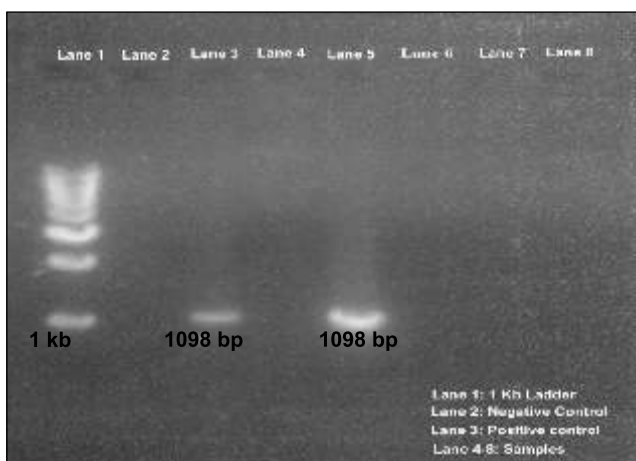


Figure-4. Agarose gel electrophoresis of amplified DNA (1098 bp) by using *Theileria* specific primer set 989/990. Lane1: 1Kb DNA marker; Lane 2: Negative control; Lane 3: Positive control (obtained from IVRI, Bareilly); Lane 4-8: Samples. Lane 5 shows *Theileria* positive sample while Lane 4, 6, 7 and 8 shows *Theileria* negative samples.

Discussion

The demand for milk is increasing in Dehradun, capital of Uttarakhand, India. Eventually, dairy with Holstein cows has become a big enterprise but theileriosis and babesiosis have extensive prevalence and high mortality rates due to these cause huge economic losses, as in several countries [11]. Theileriosis is a fatal parasitic disease which is seasonal - starts from the month of May, and its occurrence increases gradually till rainy season. The highest abundance of the ticks was reported in the month of July [12] whereas *Hyalomma* sp. of ticks is most abundant in June [13]. Carrier cattle (infected but not showing symptoms) are usually the chronic carriers of piroplasm. They become the source of infection when carrier cattle are reared together with healthy cattle. Ticks from carrier cattle act as vectors and transmit the disease to healthy cows (cross bred cows, like Holstein Friesian, are more

susceptible to infection).

The present study is focused on blood protozoan disease theileriosis and its prevalence as well as identifying the “carriers”. This study was carried out for ten months (March to December) and the cases positive for theileriosis showed that there was a high prevalence of these haemoprotozoans, especially in the rainy season, in Dehradun district. Age, sex, breed, tick density and season also affect the prevalence rate [14, 15]. With the availability of sequenced parasite genes and PCR, it is possible to detect parasites within blood samples [16]. PCR has allowed the development of sensitive and specific diagnostic assays for *Theileria* [16]. It is also reflected from our study that the entire positively stained samples were confirmed positive by PCR but an additional 16 samples were also detected as positive (“carriers”). As Dehradun is a hilly region, it was previously thought that the occurrence of these

haemoprotozoan is very less because of the temperate climate. But our study suggests that this problem is spreading in Dehradun area and also in areas located in middle Himalaya region (especially the valleys in central Himalaya) where farmers have introduced exotic cattle breed in an attempt to increase their dairy yields. So it is suggested that essential screening should be done before introduction of the cross bred cows to the areas where the chances of occurrence of such disease is very low.

The infected animals were treated with Buparvaquone but developed swelling on dewlap region. Theileria is a great threat to cross bred cows of American and European region. Therefore, prevention of small holder dairy farms from tick-borne pathogens would be of crucial importance to reduce poverty and to boost the productivity of the dairy industry.

Conclusion

It was concluded that theileriosis is prevalent in Dehradun district, Uttarakhand, India. There is a need for further investigations using molecular techniques for the identification of the carriers of the theileriosis.

Authors' contributions

UKA, AT and SK designed the experiment, sample collection. Experiment was performed by SK under supervision of UKA and AT. Manuscript preparation was supervised, reviewed and edited by UKA and AT. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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