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Short Communication

INCIDENCE OF BOVINE TROPICAL THEILERIOSIS IN CATTLE IN CENTRAL AND SOUTHERN REGIONS OF CHHATTISGARH, INDIA

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ABSTRAT: The present study investigated the incidence of tropical theileriosis in cattle caused by *Theileria annulata* in the central and southern parts of Chhattisgarh State, India. One hundred fifty-nine blood samples were collected randomly from apparently healthy indigenous and crossbred cattle and subjected to microscopy and cytochrome b (Cyto b) based PCR analysis. The overall incidence of tropical theileriosis in Chhattisgarh was found to be 37.1% and 56.6% by microscopy and PCR assay, respectively. The prevalence was higher in the Central region (37.61% and 60.55% by microscopy and PCR, respectively), compared to the Southern region of Chhattisgarh (36% and 48%, respectively, by microscopy and PCR).

Key words: Tropical theileriosis, Microscopy, PCR, Incidence, Cattle.

Bovine tropical theileriosis is caused by blood protozoa *Theileria annulata*. *Hyalomma anatolicum*, a multi-host tick, transmits the parasite (Bhattacharyulu *et al*. 1975). The infection poses a serious threat to the health and productivity of dairy cows. The disease is endemic in tropical and subtropical regions globally, including India (Uilenberg 1981, Dumanli *et al*. 2005). Theileriosis was one of India's top 10 cattle diseases in 2014-15 (Annual Report, PD-ADMAS 2014-15), costing the country US$ 239.5 million per year (Minjauw and Mcleod 2003). Exotic and crossbred cattle are particularly vulnerable to diseases, which can be lethal if left untreated. In endemic areas, subclinical *T. annulata* infection results in a persistent carrier condition in cattle, which acts as a source of infection for ticks (Maharana *et al*. 2016). The infection has been widely documented from all agro-climatic zones of India (Panda *et al*. 2011, Edith *et al*. 2018, Mohmad *et al*. 2021). The cattle population in Chhattisgarh, India, is estimated to be around 9.98 million (20th Livestock Census, India). Over the past ten years, crossbred cow breeding has expanded in Chhattisgarh, leading to higher milk output, but at the same time, the occurrence of tropical theileriosis also soared.

Macroschizont and piroplasm in Giemsa-stained smears prepared from a lymph node biopsy and blood, respectively, are used to identify the infection. However, this method fails to detect the organism in asymptomatic carrier animals (Gao *et al*. 2002). Furthermore, it is difficult to distinguish amongst *Theileria* species based on morphological characteristics (Liu *et al*. 2010). The microscopical detection approach is inadequate for comprehensive studies due to the impractical time required, especially for animals with low parasitaemia. As a result, molecular tools like PCR, which can identify extremely low levels of parasitemia, will be crucial for sensitive detection of the pathogen in carrier animals. Based on microscopy, only a solitary report is available on the prevalence of *T. annulata* infection in cattle in the Durg district of Chhattisgarh. Hence, the present study was carried out to investigate the incidence of tropical theileriosis in crossbred and indigenous cattle in the Central and Southern regions of Chhattisgarh using microscopy and PCR assays.

The study

Sample collection

One hundred fifty-nine blood samples were collected at random from indigenous and crossbred adult cattle in

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three districts of Chhattisgarh's Central region *viz*. Durg (20), Dhamtari (54) and Bilaspur (35) and two districts of the Chhattisgarh's Southern region *viz.* Jagdalpur (28) and Dantewada (22). Approximately two millilitre of blood for each sample was collected in an EDTA-coated vacutainer, and samples were brought to the laboratory using icepacks.

Blood sample investigation by microscopy

Giemsa-stained thin blood smears were subjected to microscopic analysis for the presence of *Theileria* parasites. Each slide was checked for the presence of parasites in approximately twenty microscopic fields. Even if one piroplasm was found, the sample was considered as positive.

Extraction of genomic DNA and PCR assay for amplification of cytochrome b gene of *T. annulata*

The GeneJET Genomic DNA Purification Kit (Thermo Scientific) was utilized to isolate DNA from 200 µl of blood samples. A pair of primers (CytobF – 5' ACTTTGGCCGTAATGTTAAAC 3'; CytobR – 5' CTCTGGACCAACTGTTTGG 3'), reported previously by Bilgic *et al*. (2013), specific for the *T. annulata* cytochrome b gene, was custom synthesized and used in the present investigation for molecular detection and identification of the organism. The PCR was carried out in a 25 µl reaction volume comprising 12.5 µl of 2x Dream *Taq* Green PCR master mix (Thermo Scientific), 10 pmol of each primer (CytobF and CytobR), 2 µl of template DNA and 8.5 µl of sterile water. PCR reaction was carried out in MJ Research Thermal Cycler (USA). PCR conditions described by Mohamd *et al.* (2021) were used to amplify the target gene. The PCR amplicons were electrophoresed on a 1.4% agarose gel stained with ethidium bromide, and the image was recorded in a gel documentation system.

Analysis of the results

Microscopic examination of blood smear revealed

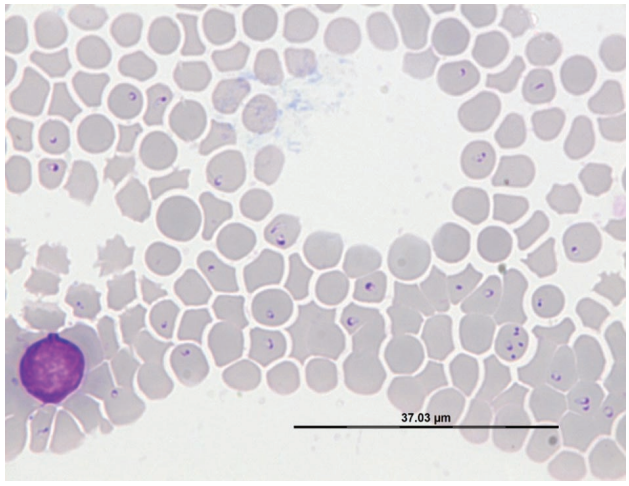

Fig. 1. Giemsa-stained blood smear showing *T. annulata* piroplasms in the erythrocytes.

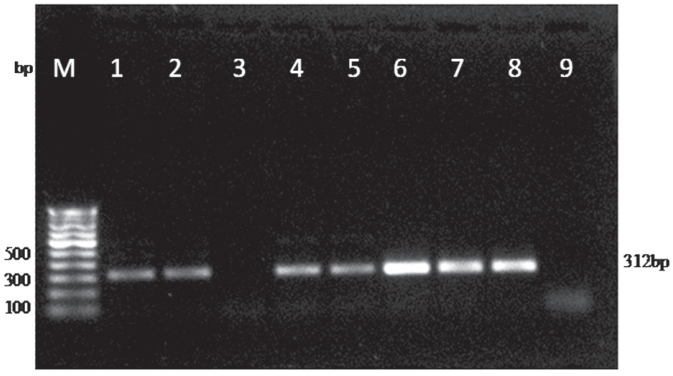

Fig. 2. 1.4% Agarose gel showing the PCR amplicons of cytochrome b gene specific to *T. annulata* in field samples. (Lane M: 100 bp DNA ladder, Lane: 1-7 field samples, Lane 8: Positive control, Lane 9: Negative control).

intra-erythrocyte piroplasm of *T. annulata* in 59 out of 159 (37.1%) samples (Fig. 1), while cyto b gene-based PCR (Fig. 2) detected *T. annulata* in 56.6% (90/159) samples (Table 1). The cyto b gene-specific primers were shown to be extremely sensitive in detecting tropical theileriosis. The laboratory standardized cyto b gene-based PCR assay can identify even very low levels of *T. annulata* infection. Abundant mitochondria and higher copy numbers of the gene may account for the

Table 1. Incidence of *T. annulata* using microscopy and PCR.

Chhattisgarh								
	Central region				Southern region			Incidence
Districts	**Durg**	**Dhamtari**	**Bilaspur**	**Sub-Total**	**Jagdalpur**	**Dantewada**	**Sub-Total**	**Total**
Samples	20	54	35	109	28	22	50	159
Microscopy	16	15	10	41 (37.61%)	12	6	18 (36%)	59 (37.1%)
PCR	19	26	21	66 (60.55%)	15	9	24 (48%)	90 (56.60%)

greater sensitivity in identifying parasitaemia (Mohmad *et al*. 2021). In addition, cytochrome b gene-directed PCR has been found to be 20% more sensitive than ribosomal DNA for detecting *T. annulata* infection in animals (Lau 2009). The high incidence of theileriosis found in this study could be ascribed to the high abundance of tick vectors, as the hot and humid climate is ideal for ticks and, as a result, piroplasm survival (Chauhan *et al*. 2015). Previous studies also showed that PCR has a higher sensitivity and specificity than traditional microscopic testing for diagnosing tropical theileriosis (Charaya *et al*. 2016, Edith *et al*. 2018, Mohmad *et al*. 2021).

In the current study, the incidence of *T. annulata* infection in cattle was found to be 37.61% and 60.55% by microscopy and PCR, respectively, in the Central region of Chhattisgarh, while the incidence of infection in the Southern part of Chhattisgarh was 36% and 48%, respectively, by microscopy and PCR. The high incidence of tropical theileriosis in the current investigation was substantiated by the high prevalence of *H. anatolicum* ticks (68.38%) infesting cattle in the state (Jadhao *et al.* 2020). Earlier, Naik *et al*. (2016) reported 35/150 (23.33%) prevalence of tropical theileriosis in cattle in the Durg region of Chhattisgarh, India. The low incidence of *T. annulata* infection in the southern part of Chhattisgarh (Jagdalpur and Dantewada districts) may be due to better farm management practices and fewer vector ticks (*H. anatolicum*) in that region.

This is the first report on molecular detection of *Theileria annulata* in cattle in Chhattisgarh, India. Based on comparative analysis, cytochrome b gene-based PCR assay is a suitable method for detecting carrier cattle, and the result obtained would help to design a strategy for effective control of the *T. annulata* infection and tick vector in the Chhattisgarh state.

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