SHORT COMMUNICATION

Molecular Detection of Tropical Theileriosis in Cattle of Chhattisgarh Plains, India

Sonu Ram Rawte, Kashee Ram Baghel*, Savita Bisen, Susanta Pal, Lourde Raj

Abstract

Theileriosis, a tick borne protozoan disease, drastically affects the productivity of bovine with massive economic repercussion. The current investigation was conducted to determine the molecular prevalence of *T. annulata* employing cytochrome b gene based PCR assay. Investigation was conducted on 150 blood samples of cattle obtained from three districts, *viz.* Rajnandgaon (50), Balod (50) and Kabirdham (50) of Chhattisgarh state. Initially microscopic examinations of smears were done followed by PCR analysis of DNA samples. The overall prevalence of tropical theileriosis in plain region of Chhattisgarh was found to be 27.3% (41/150) and 54.0% (81/150) by microscopy and PCR assay, respectively. Among districts, highest prevalence 32% and 60% was recorded in Kabirdham followed by 22% and 54% at Balod and 28% and 50% at Rajnandgaon, by microscopy and PCR assay, respectively. Observations showed that, more number of infected animals were from unorganized farm (62.6%) than organized farm (45%). Present study revealed crossbred cattle more susceptible to *T. annulata* infection than indigenous animals.

Key words: Cattle, Cytochrome b, *Theileria annulata*, Theileriosis.

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Introduction

Tropical theileriosis, a significant haemoparasitic disease, poses serious impediments to the dairy cow's health and productivity. The causative agent, an intracellular protozoon, *Theileria annulata,* is transmitted among cattle by biting of a multi-host tick *Hyalomma anatolicum* (Durrani *et al*., 2008). This disease is widely prevalent in tropical and sub-tropical parts of the world (Morrison and McKeever, 2006). Clinical cases of theileriosis have been documented from all agro-climatic zones of India (Bansal, 2005; Baghel *et al*., 2021; Mohmad *et al*., 2022). The cattle of exotic breeds are more vulnerable to *T. annulata* infection and fatality may occur delaying on therapy. Native cattle are resistant to this infection, but harbour low parasitaemia and act as potential source of infection for ticks and crossbreds. Recently, milk production has increased due to cross-breeding programme in Chhattisgarh and the cases of theileriosis are also on increasing trend due to susceptibility of crossbreds and presence of potential tick vectors.

Traditionally, the infection is diagnosed by microscopic detection of macroschizont and piroplasm in Giemsa stained smear prepared from lymph node biopsy and blood, respectively. Asymptomatic carrier animals remain undetected by traditional method; therefore need to be detected employing molecular method to represent the clear picture on occurrence of *T. annulata* infection for epidemiological study. PCR assay is sensitive to detect carrier animals than microscopic test and gives accurate data on prevalence of theileriosis (Bilgic *et al*., 2013; Mohmad *et al*., 2022). However, only a few reports are on record on the prevalence of theileriosis from Chhattisgarh state. The objective of the present investigation was to study the prevalence of *T. annulata* in plain region of Chhattisgarh using microscopy and cytochrome b gene based assay PCR.

Department of Veterinary Parasitology, College of Veterinary Science and Animal Husbandry, Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya, Anjora, Durg-491001 (Chhattisgarh), India

Corresponding Author: Kashee Ram Baghel, Department of Veterinary Parasitology, College of Veterinary Science and Animal Husbandry, Dau Shri Vasudev Chandrakar Kamdhenu Vishwavidyalaya, Anjora, Durg-491001 (Chhattisgarh), India. e-mail: drkrbaghel76@gmail.com

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Materials and Methods

Blood Sample Collection and Examination

Cattle populations from three districts, *viz.,* Balod, Rajnandgaon and Kabirdham, which are located at plane region of Chhattisgarh, India, were included in the present study. A total of 150 blood samples, 50 from each district were collected at random from cattle suspected for theileriosis during March 2022 to August 2022. About 2 mL of blood was collected from native and crossbred cattle in EDTA coated

vacutainer and samples were brought to laboratory in ice pack for further processing.

Thin blood smears were prepared and stained with Giemsa stain as per standard protocol. Stained smears were examined for the presence of *Theileria* parasite. At least 20 fields per slide were keenly observed to conclude the result.

Molecular Detection of *T. annulata*

DNA was extracted from 200 µL of whole blood using Gene JET whole blood genomic DNA purification mini kit (Thermo Scientific) following manufacturer's protocol. A previously published primer set specific to cytochrome b gene (F- 5' ACT TTG GCC GTA ATG TTA AAC 3'; R- 5' CTC TGG ACC AAC TGT TTG G 3') was custom synthesized to amplify 312 bp PCR product of *T. annulata* (Bilgic *et al*., 2013). The PCR mixture consisted of 12.5 µL of 2x Dream Taq Green PCR master mix (Thermo Scientific), 10 pmol of each primer (F & R), 2 µL of template DNA and nuclease free water up to 25 µL volume. The PCR was performed in thermal cycler as per cycling condition described by Bilgic *et al*. (2013). Initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 95°C for 50 s, primer annealing at 50°C for 50 s, and extension at 72°C for 1 min and final extension at 72°C for 10 min was performed. Known genomic DNA of *T. annulata* was used as positive control and nuclease free water was used as negative control. PCR product were resolved in 1.5% agarose gel containing 10 µg/ µL ethidium bromide in Tris-aceted-EDTA (TAE) buffer at 80V for 1 h and image was saved in gel documentation system.

Results and Discussion

Microscopic examination of 150 blood smears revealed intra-erythrocyte piroplasm of *T. annulata* in 41 samples (27.3%). PCR analysis revealed amplicons of 312 bp (Fig. 1), which confirmed the cytochrome b gene of *T. annulata* in 54% (81/150) of the samples (Table 1). The microscopic observation of present study corroborated with the finding of Naik *et al*. (2016), who reported 23.33% (35/150) prevalence of tropical theileriosis in Durg district of Chhattisgarh. Previous investigators (Mohmad *et al*., 2022; Baghel *et al*., 2021) also recorded high prevalence rate of *T. annulata* by cytochrome b gene based PCR compared to microscopy and explained that PCR is a sensitive test which can detect even carrier animals having low parasitaemia that remains undetected by microscopy. The target cytochrome b selected in present study for diagnosis of *T. annulata* is a multiple copy gene further improves the sensitivity of the PCR assay (Criado *et al*., 2006; Bilgic *et al*., 2013).

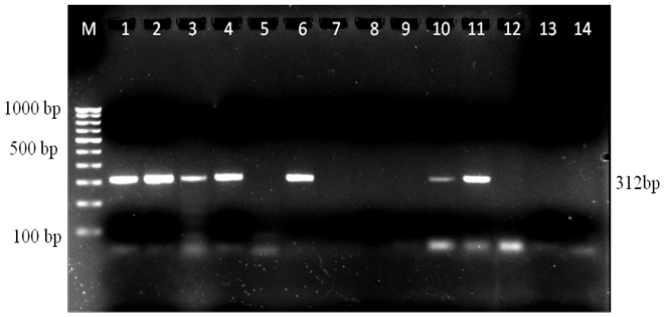

Fig. 1: Screening of field samples for detection of *T. annulata* using cytochrome b primers. Lane M: 100 bp plus DNA ladder, Lane 1: Positive control, Lane 2-13: Field samples, Labe 14: Negative control.

Molecular analysis of samples from different districts showed high prevalence rate of theileriosis in plain region of Chhattisgarh with highest at Kabirdham (60%), followed by Balod (52%) and Rajnandgaon (50%). The infection of *T. annulata* in organized and unorganized farm was found to be 36% and 64% at Rajnandgaon, 44% and 60% at Balod and 56% and 64% at Kabirdham district. Comparative findings between farms clearly indicated that high prevalence rate on unorganized farm (62.6%, 47/75) than organized farm (45%, 34/75). Similar observation on unorganized and organized farm was reported by Ghosh *et al*. (2020). The livestock farm management practices adopted at organized farm which includes well furnished sheds and regular spraying of insecticides to prevent ticks infestation on cattle may be attributed for low prevalence, while less attention of farmers on cattle of unorganized farms/household may be the reason for high prevalence of theileriosis.

Among breeds, 55.9% (52/93) of crossbred cattle and 50.8% (29/57) of indigenous cattle were found positive for *T. annulata* infection. The prevalence observed in crossbred cattle was significantly ($p<0.01$) higher than indigenous cattle. The high prevalence of *T. annulata* infection in crossbred cattle than indigenous breeds reconfirmed the earlier reports (Velusamy *et al*., 2014; Ghosh *et al*., 2020). It may be due to breed susceptibility to theileriosis, which was supported by Velusamy *et al*. (2014) and Ghosh *et al*. (2020), who found similar result in their studies.

In general, the result of present investigation on prevalence of *T. annulata* may be helpful to devise a suitable control strategy for the profitable production of livestock.

Table 1: Prevalence of *T. annulata* using microscopy and PCR in different districts of Chattisgarh

Districts	Rajnandgaon	Balod	Kabirdham	Total	Prevalence %
Sample	50	50	50	150	_
Microscopy	14	11	16	41	27.3
PCR	25	26	30	81	54.0

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