

Original Research Article

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Comparative Efficiency of Whole blood and FTA Card for Diagnosis of Theileriosis in Buffaloes of Nagpur Region, India

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ABSTRACT

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Theileriosis is a tick borne haemoprotozoan disease caused by *Theileria annulata* and *Theileria orientalis*, mainly. The disease is known to be highly fatal and is also responsible for heavy economical loses in dairy industry. In the present study, blood samples were collected from the buffaloes suspected for theileriosis. A total of 50 blood samples from jugular vein of buffaloes were collected in EDTA tube and a drop of fresh blood was also taken on FTA card for DNA isolation. For Polymerase chain reaction, the primer sequences were used as per Kakati *et al.*, and Kolte *et al.*, The PCR assay employs primers specific for the gene encoding 30kDa major merozoite surface antigen of *Theileria annulata* and major piroplasm surface protein gene of *Theileria orientalis*. Out of total 50 blood samples, 13 were positive for *Theileria annulata* by both whole blood PCR and FTA card PCR whereas for *Theileria orientalis*, 23 were positive by whole blood PCR and 27 were found positive by FTA card PCR. It was found that diagnosis of Theileriosis by using FTA card is highly recommended as it is sensitive, less laborious, less chances of DNA degradation, easy and quickest method of diagnosis as compared to Whole blood.

Introduction

Livestock is one of the fastest growing fields of agriculture with the higher productivity from buffaloes. As the demand of food derived from animals is on rise that has led to the introduction of new technologies which included cross breeding programmes. Due to cross breeding, milk production has increased to an extent that according to 19th livestock census, India ranks first in buffalo population.

But on the other, it has led to introduction of new diseases in which theileriosis is one of the most economically important disease as it causes sudden reduction in milk yield and death in severe cases. Theileriosis, a tick borne haemoprotozoan disease of ruminants caused by parasite belonging to the genus *Theileria* under the sub-order Piroplasmorina (Demessie and Derso, 2015). *Theileria* is an obligate intracellular haemoprotozoan that infects both domestic and wild animals

especially animals of bovidae family (OIE, 2014). The disease is transmitted by ixodid ticks of the genera *Hyalomma*, *Haemaphysalis*, *Ambylomma* and *Rhipicephalus* (Mans *et al.*, 2015). The infected secretions remain in the salivary gland of ticks and while feeding on the host blood, the secretions are first vomited in the blood stream of the host and then the blood is sucked as a source of food by ticks.

The disease is characterised by clinical signs like sudden reduction in milk yield, enlarged lymph node, anorexia, emaciation, depression, lacrimation, salivation, corneal opacity, diarrhoea and death in non-treated cases. There are several number of species of *Theileria* that infect buffaloes but the most commonly encountered species in tropical areas are *Theileria annulata* and *Theileria orientalis*. Usually microscopic examination is the most common method of diagnosis but due to lack of sensitivity, new techniques were opted which included PCR, as it is reliable and species specific confirmatory diagnosis can be made easily even in low grade parasitemia.

Materials and Methods

Animals

The study was conducted on 50 buffaloes with the history of any of the following symptoms, which includes; high temperature (102-105°F), swollen lymph node, reduced milk yield, emaciation, depression, anorexia, lacrimation, salivation, diarrhoea, constipation and petechial haemorrhages in eye.

Blood sampling

Blood samples were collected individually in 2ml of EDTA coated vials for DNA isolation, labelled and brought to the lab. A drop of fresh blood collected from jugular vein was taken on FTA card (Himedia) and were

transported to lab in polythene zip lock bags as per the instructions given by manufacturer.

Isolation of genomic DNA

The DNA was isolated from whole blood and FTA card by using Favorgen Blood Genomic DNA Extraction Mini Kit and Himedia Insta DNA kit, respectively. The concentration of DNA was quantified by using Nano-drop spectrophotometer. The samples with ratio of O.D. 260/280 of 1.8 and above were selected for PCR whereas samples below the ratio 1.8 conc. were again subjected to DNA isolation. The assay employs primers specific for the gene encoding the 30-kDa major merozoite surface antigen of *Theileria annulata* (Kolte *et al.*, 2017) and Major merozoite surface protein gene of *Theileria orientalis* (Kakati *et al.*, 2015).

Polymerase Chain Reaction (PCR)

The assay employs primers specific (Table 1) for the gene encoding the 30-kDa major merozoite surface antigen of *Theileria annulata* (Kolte *et al.*, 2017) and Major piroplasm surface protein gene of *Theileria orientalis* (Kakati *et al.*, 2015).

PCR reaction components for Tams1 and MPSP gene

The PCR reaction was set up into 25µl reaction containing 1µl of each Forward and Reverse primers, 12.5µl Mater Mix (dNTPs, MgCl₂, Dye and Taq DNA Polymerase), 9.5µl of nuclease free water and 1µl of DNA template.

The cycling conditions in master gradient cyler for Tams 1 and MPSP gene

The reaction condition for Tams1 was initial denaturation at 95.0°C for 5 minutes followed by 30 cycles each consisting of individual

cycle with 95.0°C (30 seconds), 55.0°C (30 seconds), 72.0°C (1 min 10 seconds) with the final extension at 72.0°C (1 cycle) for 5 minutes.

The reaction condition for MPSP was initial denaturation at 98.0°C for 10 seconds followed by 35 cycles each consisting of individual cycle with 98.0°C (10 seconds), 55.0°C for 45 seconds, 72.0°C (30 seconds) with the final extension of 72.0°C for 2 minutes.

The PCR amplicons were then subjected to 0.8% of agarose gel electrophoresis and gel was examined under Gel documentation System (Gel Pro Analyser).

For confirmatory diagnosis, 25µl of PCR product positive for *Theileria annulata* and *Theileria orientalis* and 10µl of Tams1 gene and MPSP gene targeting primers were first sent to Eurofins Genomics India Pvt. Ltd. Bengaluru, Karnataka, India for sequencing. The obtained sequences were trimmed, assembled and saved in Fasta format.

Results and Discussion

Out of 50 samples of buffaloes, PCR found 13 samples (26%) positive for *Theileria annulata* by both whole blood PCR and FTA card PCR while for *Theileria orientalis*, 23 (46%) were positive by whole blood PCR and 27 (54%) by FTA card (Table 2).

On comparison of both the methods, it was found that 36 (72%) by whole blood PCR and 40 (80%) were found positive by FTA card PCR (Table 3). The present study is new and no comparison between whole blood PCR and FTA card PCR in buffaloes has been studied yet. For confirmatory diagnosis of *Theileria spp.*, PCR product positive for *Theileria annulata* and *Theileria orientalis* were sent to Eurofins Genomics India Pvt. Ltd. Bengaluru,

Karnataka, India for sequencing. After BLAST analysis on National Centre for Biotechnology Information, the sequenced samples were found similar to *Theileria annulata* and *Theileria orientalis*, which confirmed the diagnosis.

The present study has revealed that PCR is most sensitive assay for diagnosis of *Theileria annulata* and *Theileria orientalis* due to its efficacy to detect infection in chronic and carrier stages and DNA isolation by using FTA card is highly recommended technique over whole blood for diagnosis of theileriosis as it can detect infection from a drop of fresh blood, reliable, less laborious, less amount of blood is required, less chances of DNA degradation and quick results are seen. The present study is in partial agreement with Durrani *et al.*, (2008). They found 53.3% of positivity in buffaloes for *Theileria annulata*.

Similarly, Hassan *et al.*, (2018) used FTA cards to determine the prevalence of *Theileria spp.* in Pakistan. Several workers like Mahmood *et al.*, (2010), Kolte *et al.*, (2017), Paliwal *et al.*, (2019), Kundave *et al.*, (2015) have performed PCR using whole blood and also reported that PCR was most sensitive tool for diagnosis of theileriosis. Partial similar observations were recorded earlier by Ali and Radwan (2011), Ghanem *et al.*, (2013), Chaisi *et al.*, (2013), Sudan *et al.*, (2015) and many other authors.

The primers specific for the gene i.e. Tams1 (*Theileria annulata* merozoite surface protein) and MPSP (Major piroplasm surface protein) were used to detect *Theileria annulata* and *Theileria orientalis* with the amplicon size of 846bp for *Theileria annulata* (Fig. 1) and 776bp for *Theileria orientalis* (Fig. 2) was detected in blood samples. Similar primers were also used by Kolte *et al.*, and Kakati *et al.*, for diagnosis of *Theileria annulata* and *Theileria orientalis*, respectively.

Table.1 Primers of different genes of *Theileria*

Gene	Primer	Product size (bp)	Reference
Tams 1	F- 5'TACTTGAAGCTTCCATGTTGTCCAGGACCAC 3' R-5'ATCTTGCTCGAGAAGGAAGTAAAGGACTGATGA 3'	846	Kolte <i>et al.</i> , 2017
MPS P	F- 5'CTTTCCTAGGATACTTCCT 3' R- 5'ACGGCAAGTGGTGAGAACT 3'	776	Kakati <i>et al.</i> , 2015

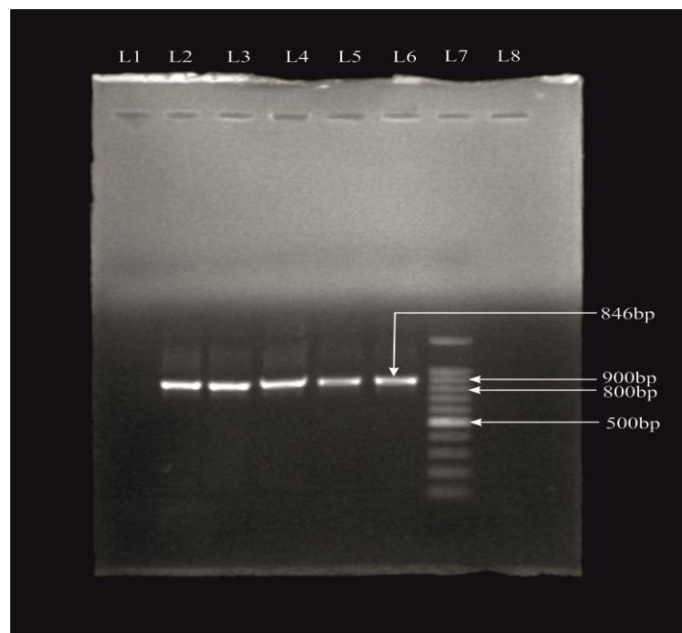
Table.2 Comparative efficiency of whole blood PCR and FTA card PCR positive for *Theileria annulata* and *Theileria orientalis*

S.No.	Test	<i>T.annulata</i>	<i>T.orientalis</i>
1	Whole blood PCR	26%	46%
2	FTA card PCR	26%	54%

Table.3 Percent prevalence of theileriosis by Whole blood PCR and FTA card PCR in buffaloes

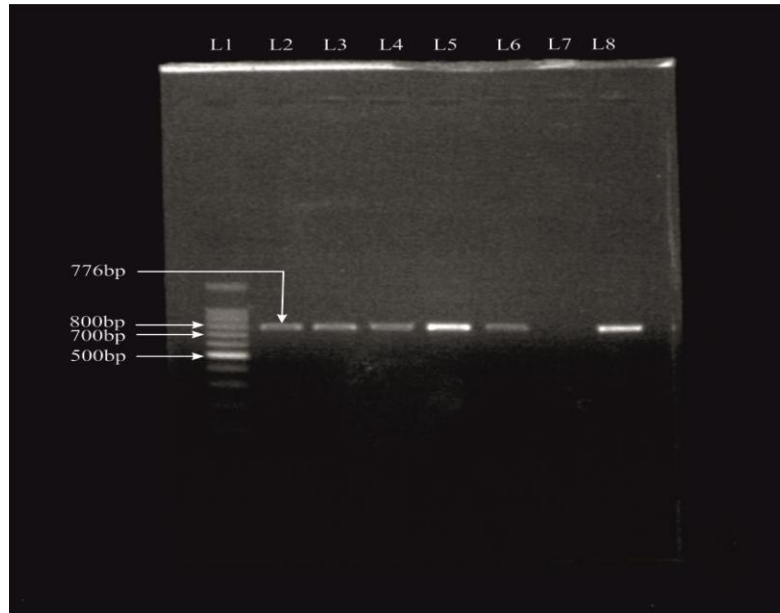
S.No.	Test	Buffaloes
1	Whole blood PCR	72%
2	FTA card PCR	80%

Fig.1 Agarose gel electrophoresis of amplified *T. annulata* DNA



L1: Negative control, L2: Positive control, L3-L4: FTA card DNA, L5-L6: Whole blood DNA, L7: 100 bp DNA ladder

Fig.2 Agarose gel electrophoresis of amplified *T. orientalis* DNA



L1: DNA ladder 100 bp, L2-L4: FTA card DNA, L5-L6: Whole blood DNA, L7: Negative control, L8: Positive control

PCR is recommended as best tool for confirmatory diagnosis of *Theileria annulata* and *Theileria orientalis* as it can detect infection even in low parasitemias. DNA isolation by using FTA card is recommended over whole blood as the chances of DNA degradation is less, less laborious and diagnosis can be made quickly as compared to whole blood.

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