

DETECTION OF *THEILERIA* PARASITE IN CATTLE OF TAMILNADU USING NESTED PCR

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ABSTRACT

Blood and ticks were collected from Theileria suspected cattle. DNA was extracted from the blood and ticks by standard DNA extraction procedure. The extracted DNA was then subjected to PCR using Theileria genus specific primers and then confirmed using Theileria annulata species specific primer. The PCR products were purified and subjected to sequencing. The Theileria sequences were analyzed using BLAST analysis.

Key words: *Theileria*, Nested PCR, Ticks, Blood

Theileriosis, caused by various intra erythrocytic protozoan parasites of the genus *Theileria*, is a tick – borne disease of domestic and wild animals. An estimated 250 million domestic cattle are at risk from the disease (Tait and Hall, 1990). It is one of the most economically devastating diseases of livestock all over the world. At least five species of *Theileria* (*T. parva*, *T. annulata*, *T. taurotragi*, *T. velifera* and members of the *T. sergenti* / *orientalis* / *buffeli* group) have been found to infect cattle. *Theileria* parasites enter the bovine host during tick feeding as sporozoites, which rapidly invade mononuclear leukocytes (Oliviera *et al.*, 1995).

Infection rates of *Theileria* in tick vectors are traditionally determined by staining dissected salivary glands with methyl-green pyronin (MGP) (Walker *et al.*, 1979). In order to complement the staining method of detection, Nested PCR is used

for diagnosis of theileriosis in cattle and sheep by Atlay *et al.*, 2005 and Martin Sanchez *et al.*, 1999 respectively. It is generally not possible to discriminate *T. annulata* from nonpathogenic *Theileria* species that may occur simultaneously within the same bovine host.

Hence, in the present study, the incidence of *Theileria* infections in Tamil Nadu, India was assessed from the suspected blood and tick samples using *Theileria* genus specific and *T. annulata* species specific primers by nested PCR assay.

MATERIALS AND METHODS

A total of 22 blood samples was collected in buffered citrate vacutainers (Precision glide), from cattle suspected for theileriosis and used for Giemsa staining and PCR. Similarly, ticks were collected for PCR assay.

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Giemsa Staining:

The blood smears examination and DNA isolation from blood by Saponin Lysis buffer method was done as per the standard procedure described by Oliviera *et al.*, (1995)

The ticks were isolated according to the procedure reported by Kok *et al.*, (1993). PCR was carried out by using following sets of primers as reported by Oliviera *et al.*, (1995). The first reaction was carried out using the *Theileria* genus specific primers for the SSU rRNA gene as given below.

Forward Primer 989:

5' AGTTTCTGACCTATCAG 3' - *Theileria* specific

Reverse Primer 990:

5' TTGCTTAAACTTCCTTG3' - *Theileria* specific

Then, nested PCR was carried out with the above forward primer, and *T. annulata* specific 5' TGCACAGACCCAGAGG 3' as the reverse primer.

For further confirmation the positive samples were analyzed using *T. annulata* species specific primers for another gene of the parasite, namely the 30-kDa gene,

For N516 5' GTAACCTTTAAAAACGT 3' and

Rev N517 5' GTTACGAACATGGGTTT 3'.

The obtained PCR products were purified and subjected to cycle sequencing (ABI Prism – 3730). The sequenced data were used for BLAST analysis to find the percentage homology.

RESULTS AND DISCUSSION

Out of 22 blood smear stained samples, only 3 samples showed the presence of *Theileria* parasite under 100X resolution by oil immersion. The DNA extracted from blood and ticks were amplified by nested PCR, in the first step the expected 1098-bp fragment using primers 989/990 (*Theileria* genus specific) was observed. The first step product was amplified using nested primers 989/1347 (*Theileria annulata* specific) and the expected 372bp fragment was observed in 1.5% agarose gel. (Fig 1). Out of 22 blood samples, 4 samples were positive by nested PCR. Similarly out of 15 tick samples, only 2 samples were positive by nested PCR.

The positive 4 blood and 2 tick samples were further confirmed using another primer set NS16 and NS17 and the *Theileria annulata* specific PCR product size of 721-bp (30 kDa gene product) fragments observed in 1.5% agarose gel (Fig 2).

All the four sequences were analyzed using BLAST (nucleotide-nucleotide) software (NCBI). Homology table was drawn to conclude that the sequence indeed is *Theileria annulata* and to find the homology between *Theileria annulata* strains in Tamil Nadu with those strains across the world. The 372-bp nested product produced 100% homology with strains from Ankara, Turkey and China. The 30kDa product yielded 99% homology with strains from Mauritania, Bahrain and Hissar.

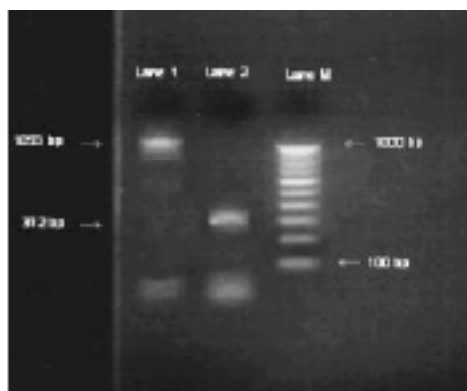
Amplification of parasite DNA is far more sensitive than parasite detection by conventional Giemsa stain. This is because sometimes the concentration of the parasite may be extremely low and cannot be detected by Giemsa staining method, but PCR sensitivity is extremely high which will amplify even a minute amount of *Theileria* parasite.

The mere appearance of a band of expected size does not always correlate with the presence of the virus genome. Hence confirmation of the PCR positivity needs to be performed by one of the several methods available such as nested PCR, southern blot, hybridization and sequencing. Further re amplification with the second set of internal primers served to verify the specificity of the first round product. With nested PCR, the transfer of reaction products from the first reaction effectively

serves to dilute out inhibitors that might be present in the sample initially. Therefore, a conventional PCR method that works poorly because of sub optimal conditions like unpurified samples can be salvaged, in many cases by the use of nested PCR.

Thus, the study helped in successful detection of species of *Theileria* parasite from the suspected cattle using PCR.

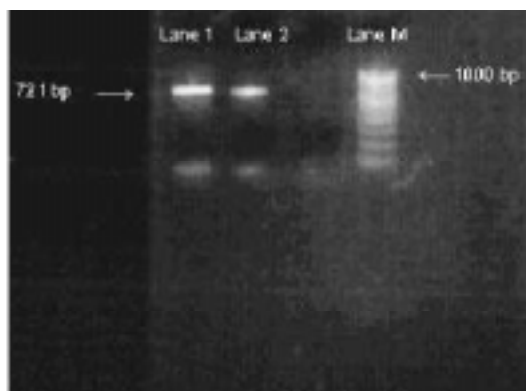
Fig. 1
Agarose gel electrophoresis of amplified DNA using nested PCR



Lane 1: 1098-bp amplified DNA fragment (*Theileria* specific);
Lane 2: 372-bp amplified DNA fragment (*T. annulata* specific);
Lane M: 100 bp DNA marker

Fig 2

Agarose gel electrophoresis of amplified DNA using primers for 30 kDa gene



Lanes 1 and 2: 721-bp amplified DNA fragment; Lane M: 100 bp DNA marker.

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