Molecular Studies on Theileriosis and Identification of *Theileria Orientalis* in India Using PCR

R. Anupama, S.R. Srinivasan* and M. Parthiban
Department of Clinical Medicine, Ethics and Jurisprudence, Madras Veterinary College, Chennai 600 007.

(Received : 22-11-2013; Accepted : 27-03-2014)

Abstract

The study comprised of 12 clinical cases of Theileriosis in cattle and 30 apparently healthy cross bred cattle which were subjected to PCR screening for *Theileria annulata* and *Theileria orientalis*. The amplicons of *Theileria annulata* and *Theileria orientalis* were subjected to automated sequencing. The twelve clinical cases of Theileriosis which were initially confirmed by blood smear examination and later subjected to PCR revealed *T.orientalis* in 8 cases, *T.annulata* in 3 cases and combined infection in 1 case. All the blood smear samples of apparently healthy cattle were found negative for Theileriosis. However, PCR screening of the blood samples of these cattle revealed 11 positive for *Theileria* sp. infection comprising 5 *T. orientalis* 3 *T. annulata* and 3 combined *T.orientalis* and *T. annulata*. Theileriosis in India is mainly thought to be caused by *T. annulata*. However, in this study, *T.orientalis* was identified as the predominant causative agent of Theileriosis.

**Key words:** Theileriosis, PCR, Cattle

Theileriosis is of great economic importance in several countries of Asia and Africa, where millions of cattle are at risk of exposure to this disease. Studies on Theileriosis in India are concentrated on *T. annulata* which causes tropical theileriosis and very little study has been done in India on benign theileriosis. Estimating the carrier status of theileriosis in the population is an important epidemiological parameter. Recently PCR has been the most preferred method (Aktas et al., 2006) for detection of *Theileria* species in epidemiological studies, because the sensitivity of theileria infection in cattle was higher using PCR than microscopic examination of Giemsa stained blood smears. Hence, the highly sensitive PCR technique was used in this study for molecular identification of theileria to assess the carrier status in apparently healthy cross bred cattle.

**Materials and Methods**

The study included 30 apparently healthy cattle of university research farm, madhavaram, Chennai and 12 clinical cases of theileriosis attending madras veterinary hospital. The positive case samples were subjected to PCR using Theileria orientalis and Theileria annulata specific primers (Martin-Sanchez et al., 1999). The Genomic DNA of the parasite was extracted from whole blood by using DNA purification kit (Fermentas, Germany) as per the manufacturer’s instructions. The final DNA pellet was dissolved in 30 µl of nuclease free water and stored until use. The PCR master mix was prepared by using PCR red dye master mix (Genei Pvt. Ltd, India) 12.5 µl, sterile water 9.5 µl, Template DNA 1.0 µl, Forward primer 1.0 µl, Reverse primer 1.0 µl and final volume made up to 25 µl. The temperature profile of PCR condition was initially 5 min for denaturation at 94°C, denaturation for 1 min at 94°C, annealing for 1min at 60 °C, elongation for 1 min at 72°C and final extension for 10 min at 72 °C. The denaturation, annealing and elongation were repeated for 30 cycles in a PCR thermal cycler (Perkin-Elmer, USA). The PCR products were electrophoresed in 1.5 % agarose gel with ethidium bromide in 1 X Tris acetate EDTA buffer. The agarose gel was viewed under transilluminator (Fotodyne, USA).
Results and Discussion

Blood Smear Examination in clinical cases revealed that the percentage of parasitaemia was very low in majority of the cases. The average number of piroplasms per RBC was one and the piroplasms were predominantly rod shaped and exceptionally elongated in case of *Theileria orientalis* infections and annular in *Theileria annulata* infections. Schizonts could not be demonstrated in most of cases. Typical bar and veil structures characteristic of *Theileria orientalis* were present in few blood smears.

The major causative agent for Theileriosis was thought to be *Theileria annulata* in India. However, Harikrishnan et al. (2001) reported *T.orientalis* in a calf in Tamil Nadu based on morphology of the organisms. Tanaka et al. (1993) reported the detection of *Theileria sergenti* (syn. *Theileria orientalis*) from clinical cases of theileriosis. Kiltz (1986) reported that the percentage parasitaemia varied between 0.1 per cent to 16.5 per cent and 79 per cent of the animals had only less than 1 per cent parasitaemia and the organism was found to be *T.orientalis*. In this study, the average number of piroplasms per RBC was one.

Becerra et al. (1983) reported that the piroplasms were predominantly elongated which were exceptionally long, bar and veil structures in *Theileria orientalis* infection. In the present study, typical bar and veil structures characteristic of *Theileria orientalis* were also present in few blood smears.

A total of 42 genomic DNA from 30 apparently healthy cattle and 12 clinical cases of Theileriosis were amplified using *Theileria* genus specific primers and the expected amplicon size of 1098 bp was obtained in positive cases (Fig. 1A). The amplified product was used as template in the nested PCR for amplification of *T. annulata* and the expected amplicon size of 453 bp was observed in agarose gel (Fig. 1B).

The expected product size of 875 bp was obtained for *T.orientalis* using MPSP gene specific primers (Fig.1C). The sequencing data of our local *T. orientalis* showed 95 percent homology with sequences of other *T.orientalis* parasites available in the GenBank.

PCR and sequencing analysis of this study revealed that 12 clinical cases comprised of 3 *T.annulata*, 8 *T.orientalis* and 1 combined *T.annulata* and *T.orientalis* infections. Samples from 11 / 30 apparently healthy cattle revealed 3 *T.annulata*, 5 *T.orientalis* and 3 combined infection of *T.annulata* and *T.orientalis* infection.

Mimioglu et al., (1972) stated that piroplasms may be difficult to detect in blood smears, because of very low parasitaemia in carrier animals. The advent of the PCR has allowed the development of sensitive diagnostic assays for microbial infections. The PCR alone may not be sufficient to confirm the disease.

---

**Fig. 1** Agarose gel electrophoresis- PCR products

**Fig.1A**

*Theileria genus* (SSUrRNA gene)
Lane M – 100 bp DNA ladder
Lane 1, 2, 3–*Theileria* positive
Lane 4 – Negative control

**Fig.1B**

*T. annulata* (Tams1 gene)
Lane M – 100 bp ladder
Lane 1,2 – *T.annulata* positive

**Fig.1C**

*T.orientalis* (MPSP gene)
Lane M –100 bp ladder
Lane 1,2,3,4–*T.orientalis*
Lane 5,6–Negative controls
Hence, in this study PCR followed by sequencing was done and blast analysis showed 95% homology with the existing *T. orientalis* sequence available in the genbank.

**Summary**

Twelve clinical cases of Theileriosis by PCR revealed that the *T. orientalis* in 8 cases, *T. annulata* in 3 cases and combined infection in 1 case. All the blood smear samples of apparently healthy cattle were found negative for Theileriosis. However, PCR screening of the blood samples of these cattle revealed 11 positive for *Theileria* sp. infection comprising 5 *T. orientalis*, 3 *T. annulata* and 3 combined *T. orientalis* and *T. annulata*. Theileriosis in India is mainly thought to be caused by *T. annulata*. However, in this study, *T. orientalis* was identified as the predominant causative agent of Theileriosis in India.

**References**


