MOLECULAR IDENTIFICATION OF THEILERIOSIS IN GOATS OF KERALA
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Abstract: A PCR-RFLP protocol targeting 18S ribosomal RNA was utilised to identify Theileria/Babesia spp among naturally infected goats of Kerala. Among the apparently healthy adult animals subjected to the study, the protocol could detect theileriosis in 64 per cent goats while blood smear examination revealed piroplasm in only 18 per cent samples. None of the goats harboured Babesia spp. The results of the study highlighted the enhanced sensitivity of PCR-RFLP protocol for providing molecular evidence of subclinical theileriosis and stressed the need to investigate the species spectrum of Theileria and the host resistance status for the infection.

Keywords: Goats, Kerala, PCR-RFLP, Theileria spp.

Introduction

Theileriosis and babesiosis are the most economically important protozoan infection with a major impact on small ruminant production in tropical countries. Babesiosis in small ruminants is caused by Babesia ovis, B. motasi and B. crassa, whereas, theileriosis is caused by at least six different species of Theileria, of which T. lestoquardi (hirci) is highly pathogenic and T. ovis is mildly pathogenic (Aktas et al., 2005). Acute piroplasmosis in goats is often detected by microscopic examination of Giemsa-stained peripheral blood smears. Theileria spp. is a small, highly pleomorphic piroplasm, accurate detection of which demands considerable technical expertise, especially due to the small size of caprine RBC’s (Li et al., 2014). Babesia spp. also occurs in the erythrocytes and causes infection clinically similar to theileriosis. Moreover, routine microscopy fails to detect subclinical infections which develop after the acute phase. Hence, a sensitive and specific diagnostic tool for accurate detection and differentiation of piroplasm is required. Nested PCR (Atlay et al., 2005), reverse line blot hybridisation (Atlay et al., 2007), PCR (Durrani et al., 2011; Iqbalet al., 2011; Shahzad et al., 2013) and PCR-RFLP (Razmi and Yaghfoor, 2013; Hegab et al., 2016) were utilised for detection of ovine theileriosis in sheep in different countries. Reports from India, based on

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the results of blood smear examination, indicated that theileriosis was prevalent in small ruminants of different states including Karnataka (Harish et al., 2006), Kerala (Anumol et al., 2011), Arunachal Pradesh (Tayo et al., 2011), Tamil Nadu (Velusamy et al., 2015), Madhya Pradesh (Sahu et al., 2016) and Tripura (Das, 2017). However, the presence of tick transmitted intraerythrocytic piroplasms in goats of South India is neither identified nor characterised by molecular methods. A PCR protocol to detect and differentiate *Theileria* and *Babesia* spp. in goats would serve to detect the infection timely and to initiate accurate treatment protocols. Hence, with this objective the study was undertaken for standardising a molecular tool to detect theileriosis and babesiosis in Kerala, South India and to assess the carrier status for these pathogens among goats.

**Materials and methods**

**Sample collection:** Whole blood samples of goats (n=50) were collected from different places of Kerala viz., Thrissur, Ernakulam, Palakkad and Wayanad. The goats selected for the study were apparently healthy adult stock belonging to Malabari or Malabari cross breeds. Thin peripheral blood smears were also simultaneously collected and subjected to Giemsa’s staining and observed under the microscope for morphological identification.

**PCR protocol:** DNA isolation was done from whole blood using phenol- chloroform method (Sambrook and Russell, 2006). Specific primers targeting a region of 18S ribosomal RNA gene of the piroplasms (TF15′-GCATTGTATTTACTGTCAGGGG-3′ and TR15′ GATAAGGTTCACAAAACTTTCCCTAG-3′) were selected according to Jalali et al., (2013). Later, PCR was done in a 12µL reaction mix containing 6.5 µL of master mix (SRL, India), 12.5 pmol each of forward and reverse primers, 1 µL of template DNA and 4 µL of nuclease free water. The amplification was performed in a gradient thermocycler (Biorad, USA) with the following program: an initial denaturation step at 94°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 56-60°C for 60 s and extension at 72°C for 60 s with a final extension step of 72°C for 5 min. A non-template control was included in each run. The amplicons were electrophoresed on 1.5% agarose gel and viewed in gel doc (Biorad, USA). The PCR amplified DNA products were sequenced (SciGenom Labs Pvt Ltd, Kochi).

**RFLP protocol:** Restriction fragment length polymorphism (RFLP) of PCR products of the 18S rRNA gene was done with the enzyme HindIII, to detect the presence of restriction sites that would confirm the genus identity and differentiate between *Theileria* and *Babesia* spp. The enzymatic digestion was carried out in 20 µl reaction mixture consisting of 10 µl PCR amplicons, 2 µl of 10X corresponding buffer, and 0.1 µl (1 U) restriction enzyme and
nuclease free water. The digestion mixture was incubated at 37°C for one hour. Electrophoresis was carried out using 12 per cent polyacrylamide gel and analysed.

**Results and discussion**

Out of the total 50 samples subjected to the study, nine blood smears were positive for piroplams of *Theileria* spp. Thirty two samples revealed a PCR product of 872bp size (Fig 1) which was sequenced bidirectionally. The aligned sequence upon analysis by BLASTn tool ([https://blast.ncbi.nlm.nih.gov](https://blast.ncbi.nlm.nih.gov)) revealed 99% similarity to several published sequences of corresponding gene of *Theileria* spp. (KF597086.1, KF597085.1, KF597080.1, KF597079.1, AB602881.1, AB602878.1, AB602877.1, AB012201.1, AB012200.1, KF597087.1). Further, PCR- RFLP with *HindIII* yielded two bands of approximately 449bp and 423bp sizes (Fig 2), which proved that all positive samples were *Theileria* spp. as per Jalali *et al.*, (2013) and Zaeemi *et al.*, (2011).

It is noteworthy that while piroplasmosis could be detected in only18 percent goats by light microscopy, PCR-RFLP could identify the infection in 64 per cent animals suggesting the high sensitivity of the protocol. Lack of sensitivity of conventional microscopy in detection ovine theileriosis was reported by Atlay *et al.* (2005), Razmi *et al.* (2013), Shahzad *et al.* (2013) and Hegab *et al.* (2016). None of the goats were found infected with *Babesia* spp. Anumol (2011) could identify four cases of *Babesia* spp out of 250 anaemic goats in Kerala. The PCR-RFLP protocol proved to very accurate to detect *Theileria* species which otherwise could not be reliably detected and differentiated according to their morphology by microscopy, especially in subclinical infections.

A high prevalence of theileriosis as detected by PCR-RFLP in apparently healthy animals signals the presence of subclinical infection or the existence of non-pathogenic species which needs to be ruled out further. Studies are to be carried out to differentiate the various species of *Theileria* prevalent in goat population of the State. The possibility of the Malabari and Malabari cross breeds to withstand the clinical effects of theileriosis also warrants detailed investigation.

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References


**Fig 1:** PCR amplicons of *Theileria* spp  
Lanes: L1, L2, L3, L4, L5, L6- Amplicons of *Theileria* spp.
L7- No template control
M : 100 bp ladder

**Fig 2:** *Hind II* digested products of *Theileria* spp.
Lanes- L1, L2, L3, L4, L5 *Hind II* digested products
M- 100 bp ladder