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MOLECULAR IDENTIFICATION OF CARRIER STATUS OF THEILERIOSIS AND BABESIOSIS IN DAIRY CATTLE OF KERALA

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Abstract: A total of 125 blood samples of disease suspected cattle were collected from household farmers of Palakkad, Perumbavoor areas and also from the University Livestock farms at Thumburmuzhi, Thiruvizhamkunnu and Pookode. Microscopic examination of the 125 Giemsa stained blood smears revealed *Theileria* spp. in 58 (46.4 %) samples and *Babesia* spp. in two (1.6%) samples whereas Polymerase chain reaction (PCR) of the same samples revealed 72 (57.6%) positive for *Theileria* spp. and two (1.6%) positive for *Babesia bigemina*. Higher prevalence rate of *Theileria* spp. in apparently healthy animals indicates its carrier status. However, the low incidence of *Babesia* spp. when compared to *Theileria* spp. needs further evaluation with regard to the vector distribution and host resistance.

Keywords: Theileriosis, Babesiosis, PCR.

Introduction

Haemoprotozoan diseases instigate overwhelming losses to the livestock industry throughout the world. Most of the haemoprotozoan parasites are tick borne and is of great economic importance in Asia and has always been an arduous barrier to the survival of ruminants in India. The most important and readily measurable direct effects of these diseases are manifested by losses in productivity including poor weight gain and feed conversion, reduced milk yield and reproductive capacity, decreased work capacity and anaemia leading to condemnation and even death. A recent estimate of US$ 498.7 million per annum was calculated as the cost of controlling ticks and tick borne diseases in India (Minjauw & McLeod, 2003). Animals recovered from these diseases may act as carriers creating a potential source of infection to the healthy susceptible population (Callow, 1984). Hence the early diagnosis of these diseases in carrier animals has got great significance as they are the sources of infection for the vectors.

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Morphological identification of the parasites in the blood and other tissues of the infected animals is the conventional method used for the diagnosis of these diseases. Sometimes in case of low parasitaemia, infected cells can remain undetected. Moreover, the identification of different species of haemoprotozoa such as *Theileria* and *Babesia* on the basis of morphology is not only difficult but also erroneous. New developments in molecular biology have generated exciting possibilities for improved diagnosis of parasitic diseases. Molecular diagnosis of haemoprotozoan diseases involves several PCR-based diagnostic procedures, which help in identification of the parasite upto the species or even strain level (Figueroa *et. al,* 1993). Hence the present study focuses on a molecular survey of haemoprotozoan organisms such as *Theileria* and *Babesia* prevalent in cattle of Kerala.

Materials and Methods

Blood sample collection

A total of 125 cattle blood samples were collected from household farmers of Palakkad, Perumbavoor areas and also from the University Livestock farms at Thumburmuzhi, Thiruvizhamkunnu and Pookode. Out of 125 samples, 35 samples were from clinically ill animals whereas 90 samples were from apparently healthy animals. Blood samples were collected in six ml K_2 EDTA coated BD vacutainer under aseptic conditions and maintained in ice packs until arrival at the laboratory. The samples were stored at −20◦C until tested.

Blood smear examination

Blood smears were prepared using the blood from peripheral circulation. Giemsa staining procedure was carried outand examined to identify the presence of haemoprotozoans.

Extraction of DNA from blood samples

DNA was extracted from the stored anticoagulant added whole blood with the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's protocol and subsequent steps were carried out according to the instructions.

Polymerase Chain Reaction (PCR) protocol

PCR reaction mixture and gradient cycling conditions were initially standardised using primer sets designed to amplify thespecific 18S rRNA gene sequences. DNA extracted from blood with heavy parasitaemia during microscopic examination of stained blood smears served as positive controls for the respective species. No template control (NTC) was included in each run.PCR was performed using the gotaqmastermix (Promega) with 25pmol of each primer and 2µL of template DNA. For *Theileria* spp. detection, primers Th.g-F and Th.g-R (F- 5' AGT TCT GAC CTA TCA G 3', R- 5' TTG CCT TAA ACT TCC TTG 3')

(Nair *et al*., 2011) which amplify a fragment of approximately 1098 bp of the small subunit of ribosomal DNA were employed. The primers B.bg-F and B.bg-R (F 5'CATCTAATTTCTCTCCATACCCCTCC3',R5'CCTCGGCTTCAACTCTGATGCCAAA G 3') (Nair *et al*., 2011) were employed to detect a 278 bp. *B.bigemina*. The reaction protocol involved an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 59°C for 30 s and extension at 72°C for 60 s; and a final elongation at 72°C for 5 min. The PCR amplicons were visualized by electrophoresis in 1.5% agarose gel stained with ethidium bromide (0.1 mg/ml) under UV transilluminater. Results were captured and recorded using a digital imaging system (gel Doc, bio-rad). Blood samples collected from apparently healthy as well as symptomatic dairy cattle were screened for the presence of *Theileria*spp., and *Babesia*spp. by the standardized PCR protocols.

Results and discussion

Microscopic examination of the 125 Giemsa stained blood smears revealed *Theileria* spp. in 58(46.4%) samples and *Babesia* spp. in two (1.6%) samples (Table:1). Piroplasms of *Theileria* spp. are characterized by various morphological appearances such as thin and thick rod shaped or annular with light staining trailing cytoplasm in lymphocytes, histiocytes and erythrocytes in Giemsa stained smears (Soulsby, 1982; Durrani *et al.,* 2008). All these forms could be detected in the Giemsa's stained smears in the study with the maximum of rod forms (Fig: 1). Lalchandani (2001) found a higher prevalence rate of *Theileria* spp. (58.82 %) in Kundhi buffaloes. However, there are reports of slightly lower prevalence rate of 42.28 per cent of *Theileria* spp. in cattle in Rajasthan (Bhatnagar *et al*., 2015) and a 40.66 per cent of the same in cattle of Northern Kerala (Nair *et al*., 2011). This clearly indicated that the prevalence of the pathogen has been unaltered since 2011 in the state despite the extensive treatment and control strategies adopted especially in dairy cattle sector.

Babesia organisms are round to pyriform, amoeboid form occurring in the erythrocytes (Soulsby, 1982). Pyriform shaped *Babesia* organisms could be detected in only two Giemsa's stained smears (Fig: 2). An occurrence of3.3 per cent babesiosis was reported by Chowdhury *et al.* (2006). A slightly higher prevalence rate of 2.6 per cent was reported by Nair *et al*.(2011) in cattle of Northern Kerala. These differences could be due to the geographical, seasonal and species variations (Bhatnagar *et al*., 2015).

The specific primers for *Theileria* spp. amplified the PCR product of 1098 bp (Fig: 3) and that of *B.bigemina* amplified the PCR product of 278 bp.(Fig:4) as expected. Out of the total 125 samples, 72 (57.6%) were found to be positive for *Theileria* spp.,and two (1.6%)

were positive for *Babesia bigemina* by PCR (Table:2). Higher prevalence of *Theileria* spp. was obtained by the PCR methods as compared to conventional examination of Giemsa stained blood smears. Even though the routine diagnosis of haemoprotozoans is based on light microscopy, this method is not sensitive enough or sufficiently specific to detect chronic carriers. Molecular techniques such as PCR enable sensitive and specific detection of parasites (Altay *et al*, 2008). Inspite of the reverse age resistance of *Babesia* spp, we have not identified a high prevalence of this infection in adult cross bred cattle. Higher prevalence rate of *Theileria* spp. in apparently healthy animals indicates its carrier status. However, the low incidence of *Babesia* spp. when compared to *Theileria* spp. needs further evaluation with regard to the vector distribution and host resistance. The sensitivity and specificity of the PCR was compared with conventional blood smear examination method. Sensitivity was found to be 93.54 per cent whereas the specificity was 77.77 per cent. The results of the present study highlight the need to rule out the existence of carrier status in dairy cattle before introducing any stressful conditions like transportation or vaccination. Appropriate tick control strategies are also to be adopted for containing the infection at reduced level in the herd in endemic areas.

Acknowledgement

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Blood smear examination

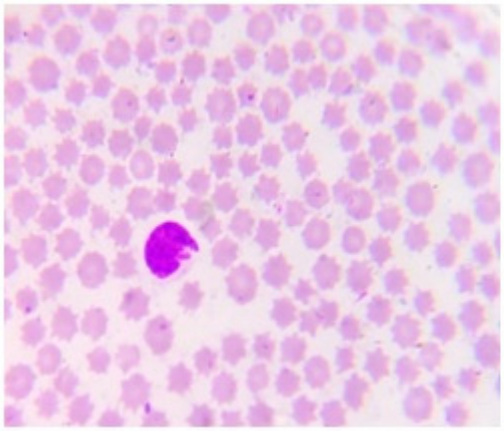

Fig. 1 *Theileria* spp

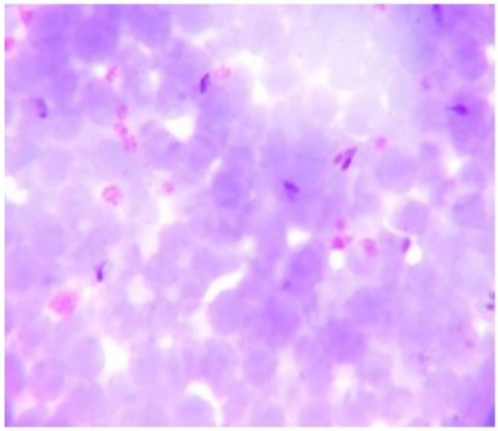

Fig. 2 *Babesia* spp

Agarose gel electrophoresis

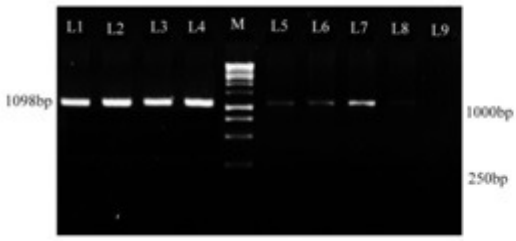

Fig. 3 *Theileria* spp

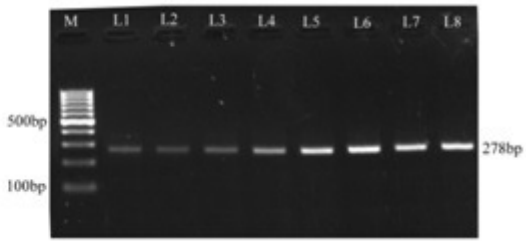

Fig.4 *B.bigemina*