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Research Paper

Large Sequence Polymorphism-12 Based Faecal PCR for Detection of Johne's Disease in Domestic Ruminants

Medical Science

KEYWORDS : Faecal PCR, Johne's disease, large sequence polymorphisms, Mycobacterium avium subsp paratuberculosis, paratuberculosis.

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ABSTRACT

Mycobacterium avium subsp. paratuberculosis (MAP) causes paratuberculosis or Johne's disease in cattle, sheep and goats. There are many methods for diagnosis of Johne's disease and among these, cultural identification is the "gold standard" for diagnosis in spite of its long turn over time. Works on mycobacterial genomics revealed that genomic reduction through the loss of large sequence polymorphisms is the major contributor of genomic diversity in M avium subspecies. LSPP 12 is present in mbt A gene of mycobactin synthesis operon replacing LSP 4 in M avium and LSPP12 internal sequence PCR can be used for specific detection of MAP. LSPP12 faecal PCR gave similar results as that of IS 900 PCR and only MAP DNA gave specific amplification with LSPP12 PCR compared to mother mycobacteria used in this study.

INTRODUCTION

Johne's disease has been considered as a major disease of ruminant population for over a century. It is caused by Mycobacterium avium subsp paratuberculosis (MAP). The disease has an increasing economic impact worldwide and its zoonotic relevance in association with Crohn's disease in human. Disease is characterized by chronic progressive enteritis. The control of Johne's disease has been hampered by the difficulty to detect subclinical infection. Organisms may be present in insufficient numbers in faeces to be detected by current culture methods. At early stages of infection animals do not elicit an immune response that is detectable by current serological tests. Isolation of organism is considered as gold standard for the diagnosis but it will take more than 16 weeks. Currently IS900 based faecal PCR is used for rapid detection but it may give false positive reactions with other environmental mycobacteria. So a rapid detection strategy is to be developed and standardized for specific detection of MAP

The M. avium species consist of a group of organisms that are genetically related but phenotypically diverse. Mycobactin synthesis operon consists of ten genes namely mbt A-J (Li et al, 2005). LSPs are major genetic events that can be sources of the genetic variability found within M. avium complex (MAC) (Castellanosa et al, 2012). Semret and coworkers (Semret et al, 2004, 2005) hypothesized the use of LSPs for differentiating host associated species of M avium. Stratmann (Stratmann et al, 2004) found out that 17 LSP'S are there in MAP genome. A large sequence polymorphism, LSPA 4 between mbtA and mbtJ genes is present among M. avium isolates but it is missing in MAP. In MAP, LSPA4 is replaced by a different, 19-kb sequence called LSP^{P} 12 determined by PCR to be highly specific to MAP isolates and is reason for its mycobactin dependence. In this effort these polymorphisms for specific detection of MAP in clinical samples and to differentiate from various other mycobacterial species is studied.

MATERIALS AND METHODS

M phlei MTCC 1724, M bovis AN5, M avium D4, M avium 1723, M microti MTCC 1727, M tuberculosis H37Rv and M.avium subsp paratuberculosis ATCC 19698 cultures were obtained from Biological products division, Indian veterinary research institute, Bareilly for this study. Faecal samples from 20 cattle, 20 sheep and 28 goats were collected from slaughter house located at Thrissur, Kerala. From slaughter house of Bareilly, Uttar Pradesh faecal samples of 21 cattle, 10 sheep and 17 goats were also collected. All those animals had a history of diarrhea for a long period.

Extraction of genomic DNA from cultures was done by Cetyl Trimethyl Ammonium Bromide (CTAB) lysis method (Kauppinen et al, 1994). Isolation of genomic DNA from the faecal sample of animals was done using Qiagen QIAamp DNA stool mini kit. Genomic DNA isolated by Qiagen QIAamp DNA stool mini kit method from faecal samples yielded 100ng/µl DNA. Genomic DNA isolated from cultures by CTAB method yielded 90ng/µl DNA.

The primers used for specific amplification of LSP^{P} 12 were 5'-TCTGAACCCGGCTACACAC-3' (forward primer) and 5'-CATGCCGGTGTGAGTACAA- 3'(reverse primer) with an annealing temperature of 58° C. Simultaneously all samples were subjected to IS900 nested PCR (Doosti and Moshkelani, 2010) for confirmation.

RESULT AND DISCUSSION

Among the entire DNA isolated from different mycobacterial species only MAP ATCC19698 DNA showed amplification with LSP^{P} 12 sequence targeted primers yielding products of 430 bp (figure 1). All other mycobacterial species failed to produce any amplification with LSP^{P} 12 specific primers, indicating high specificity. Among DNA isolated from faecal samples, 31 samples showed positive with LSP^{P} 12, which included 5 cattle, 6 sheep and 20 goats (figure2). Similar result was obtained by IS 900 nested PCR. Hence PCR diagnosis using LSP^{P} 12 I primers can be used as an alternative method for specific detection of MAP in faeces.

Amplification of target genes with LSP^{P} 12 primers (MAP 2182 C and MAP 2188 C) was restricted to MAP strains only and resulting in a calculated specificity of 100% (Stratmann et al., 2004; Semret et al., 2005). Castellanosa et al. (2012) found out that LSP^{P}12 was 100% specific for MAP. Paustin et al. (2005) reported that difference in M avium subspecies is mainly due to LSPs.

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Figure 1. Agarose gel analysis of LSP^{P} 12 PCR products for detection of MAP among different Mycobacterial species- Lane 1- Mycobacterium tuberculosis H37Rv, Lane 2- M bovis AN-5, Lane 3- M avium subsp paratuberculosis ATCC19698, Lane 4- 100 bp DNA ladder, Lane 5- M microti 1727, Lane 6- M avium 1723, Lane 7- M avium D4, Lane 8- M phlei MTCC 1724.

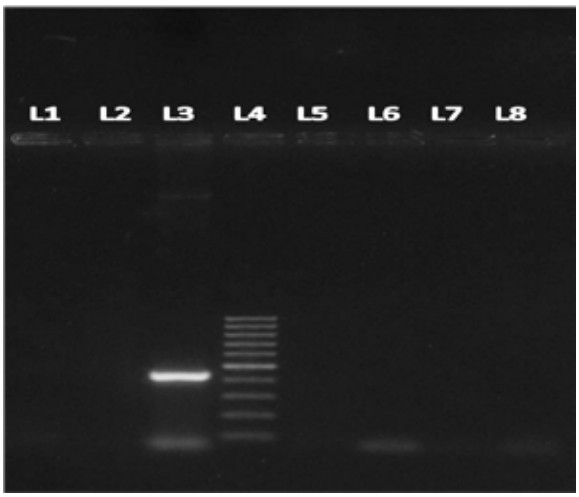
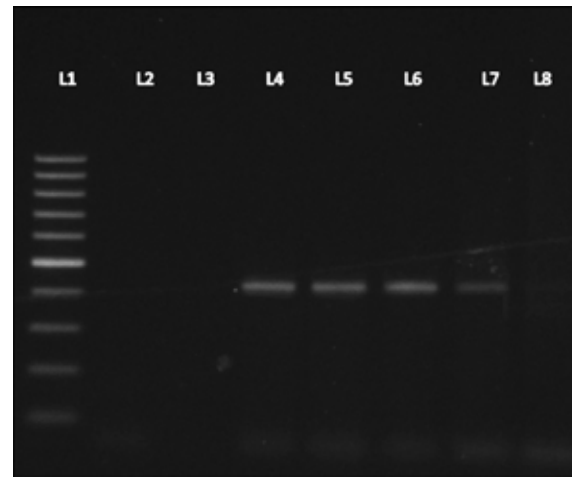


Figure 1- Agarose gel analysis of LSP^{P} 12 PCR products for detection of MAP in faecal sample of different animals. Lane 1- 100bp DNA ladder, Lane 2- faecal sample of cattle with no amplification of LSP^{P} 12, Lane 3- faecal sample of sheep with no amplification of LSP^{P} 12, Lane 4- faecal sample of cattle showing amplification of LSP^{P} 12, Lane 5- faecal sample of sheep showing amplification of LSP^{P} 12, Lane 6 and 7- faecal sample of goats showing amplification of LSP^{P} 12, Lane 8- faecal sample of goat with no amplification of LSP^{P} 12



CONCLUSIONS

LSP^{P} 12 PCR gave similar results as IS 900 nested PCR and specifically detects MAP from other Mycobacterial species. LSP^{P}12 can be used as an alternative method for detection of Johne's disease and can be used as a confirmatory test for MAP detection.

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