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PCR based detection of paratuberculosis infection in a cattle: A case report

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Abstract
A crossbred jersey cow presented with the history of chronic watery diarrhoea and was not responding to the treatment. Clinical examination revealed that the rectal mucosa was thickened. Differential diagnosis were a parasitic infection and Johne's disease. Laboratory investigation revealed the presence of acid-fast organism in the rectal pinch sample. Further, the DNA extracted from the faecal sample and PCR targeting IS900 region of MAP was carried out to confirm the diagnosis.

Keywords: Johne's disease; IS900; PCR, MAP

Introduction
Paratuberculosis also known as Johne's disease is a chronic granulomatous disease affecting gastrointestinal tract of ruminants, caused by an obligate intracellular pathogen, *Mycobacterium avium* subspecies *paratuberculosis* (MAP) [1-4]. The disease is prevalent in most of the countries including India and is common in animals reared intensively. The infected animal remains asymptomatic in most cases and only 10-15% of the animals develop clinical signs. Transmission occurs by ingestion of the organism from the contaminated environment. Typically infected cattle present with a history of chronic recurrent diarrhea, weight loss and emaciation. The disease is mainly observed in adult animals as the incubation period is usually long. High numbers of organisms are shed in the faeces and in the milk of the infected cattle and spread the infection to young calves. The disease can be diagnosed by acid-fast staining of rectal pinch or faecal samples, culturing of the organism and by Polymerase chain reaction (PCR) based methods. The PCR targeting IS900 region of MAP is commonly used in the diagnosis of the cases [5, 6]. Serological methods like ELISA, AGID and CFT can also be used in screening of animals in large-scale [7].

Case report
A crossbred Jersey cow was presented to the Veterinary Clinical Complex (VCC), Veterinary College and Research Institute, Tirunelveli with the history of foul-smelling watery and mucoid diarrhoea for the past six months. Treatment was given by local veterinarian for past two weeks and the animal responded well to the treatment, but after 2 or 3 days, animal developed diarrhea again. On physical and clinical examination, the animal was having rectal temperature 102.7 F, heart rate 81 beats/min, respiration rate 25/min, visible mucous membrane appeared congested, skin turgor more than 4 sec, emaciated body, dull and depressed, rumen appeared doughy and motility was 5 per 5 min. On rectal examination, the wall of rectum appeared to be thickened with foul-smelling grey colored diarrhea. Clinical samples like rectal pinch, dung sample, were collected and subjected to laboratory investigation to rule out internal parasite and *Mycobacterium avium* subspecies *paratuberculosis* [1-2] infection. The animal was treated with fluids 10-20 ml/kg for correcting dehydration, sulphadimidine 100 mg/kg as gut acting antibiotic, tribivet 10 mg/kg as supportive therapy, melonex 0.1 mg/kg for reducing inflammation.

Laboratory investigation, result and discussion
Parasitic infection was ruled out by subjecting the faecal sample to parasitic examination. Later, the faecal sample and rectal pinch smear were subjected to acid-fast staining using staining kit (HiMedia) and examined by oil immersion microscopy wherein pink colored acid-

fast organisms occurring clumps as well as individually scattered on a blue background were observed. This tentatively indicated the presence of MAP infection as reported by Taylor *et al.* [8]. But as the sensitivity and specificity of the acid-fast staining technique are low [9], a confirmatory diagnostic test was performed. For this, the genomic DNA was extracted from the faecal sample using HiPura Multi-sample DNA purification kit (HiMedia) as per manufacturer's recommendations. Polymerase chain reaction (PCR) targeting IS900 region of MAP was set up in 25 µl reaction volume with primers described by Pillai and Jayarao [10] and amplified in a PCR thermal cycler. About 8 µl of the amplified product was electrophoresed in a 1% agarose gel in 1 X TAE buffer along with 100 bp DNA ladder, positive control, and non-template control samples. Presence of IS900 specific 229 bp unique amplicon in the sample and positive control sample was documented using gel documentation system while no amplification was observed for the non-template control sample (Fig 1). With regard to faecal sample screening for MAP by acid fast organism, Doyle [11] opined that 25-30% of the samples only would be positive and Thoresen *et al.* [12] reported that 10^6 bacteria/gm of faeces might be necessary to detect the organism by light microscopy. However, in the present case the rectal pinch sample collected turned out to be positive for acid fast organism which helped us to target the organism by PCR. Thus, in the present study, a PCR based diagnosis of Johne's disease in a cattle was carried out and this technique could be used to generate epidemiological data on the prevalence of MAP infection among cattle population. On the basis of case history, clinical findings, and basic laboratory investigation, a tentative diagnosis was arrived and the diagnosis was confirmed using PCR technique.

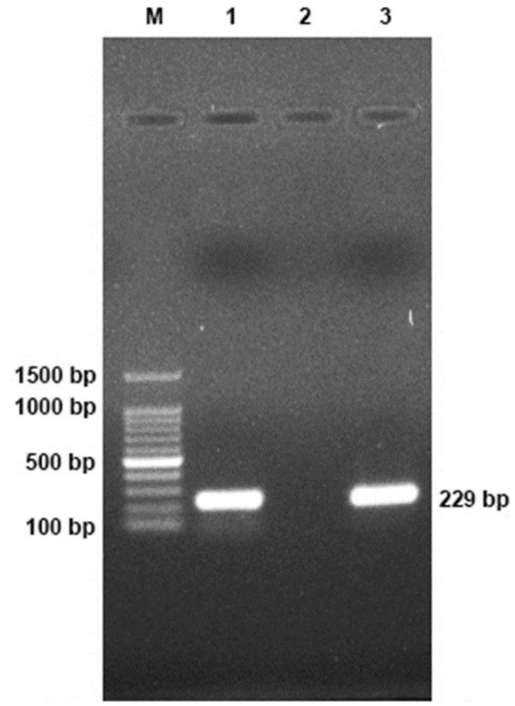

Fig 1: Agarose gel showing PCR products

Lane M: 100 bp DNA ladder
Lane 1: Positive control
Lane 2: Non template control
Lane 3: Test sample

Conclusion

In the present case, specific and early diagnosis could be obtained using the PCR assay which helps in identifying and culling of the infected animal thereby, preventing its further spread and environmental contamination in farm.

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