

Case Study

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Johne's Disease (Paratuberculosis) in a Crossbred Cow: A Case Report

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ABSTRACT

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Johne's disease (Paratuberculosis), a debilitating chronic granulomatous enteritis of domesticated and wild ruminants that causes huge economic and production losses to the dairy farmers. A six-year-old Jersey crossbred cow was presented with a history of chronic diarrhoea, gradual weight loss, reduction in milk yield and poor response to therapy. Based on history, clinical examination, Ziehl-Neelsen staining and IS900 polymerase chain reaction of the faeces the case was diagnosed as Johne's disease. It warrants strict implementation of control measures to put off further spread Johne's disease in dairy animals.

Introduction

Johne's disease (Paratuberculosis) is chronic infectious granulomatous enteritis of domestic as well as wild ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (Windsor and Whittington, 2010; Sweeney, 2011; Momotani *et al.*, 2012). It is characterized by a long incubation

period followed by persistent diarrhoea, progressive emaciation, decreased milk production, oedema, anaemia and death (Harris and Barletta, 2001). Johne's disease is still incurable due to poor response of MAP organisms to anti-mycobacterial therapy as well as other treatments (Sharif *et al.*, 2013). The present case deals with a paratuberculosis (Johne's disease) in a crossbred cow.

Case history and Clinical examination

A six-year-old Jersey crossbred cow from private dairy farm, Madhavaram reported to Department of Veterinary Public Health and Epidemiology, Madras Veterinary College, Chennai with the history of gradual weight loss and persistent diarrhoea for the past one month that is neither offensive nor blood stained. The animal became progressively weaker and emaciated despite having good appetite and feeding. The milk yield also reduced from eight litres/day to two litres/day. The animal purchased four months back from the local sandy and maintained under the semi-intensive system of management. The animal treated by a practising veterinarian and non-responsive to the antimicrobial and anthelmintic therapy.

Clinical examination revealed emaciation with the evidence of watery diarrhoea in the animal (Figure 1). A large sized oedema also observed in the sub-mandibular region. The cardinal signs such as temperature, heart rate and respiration rate were all within normal range. Rectal palpation showed that animal had soft watery, light brown faeces and mild corrugation of the rectal wall also felt. Dung and rectal pinch samples were collected and transported to the laboratory under refrigerated conditions.

Laboratory diagnosis

About 1.5 gm of dung sample was mixed thoroughly with 10 ml of 0.90% sterile saline in a sterile tube and the tube was shaken vigorously to mix the suspension. Then suspension was allowed to settle for 30 minutes. All supernatant solution was transferred to another sterile tube and centrifuged at 3200 rpm for 30 min at room temperature. Then supernatant was discarded without dislodging the pellet, and the pellet was processed for smear preparation and

DNA isolation (Whipple *et al.*, 1991). Furthermore, faecal sample also subjected to centrifugal sedimentation to rule out the presence of parasitic ova/ oocyst.

The smears made from sediment pellet and rectal pinch were stained with commercial Ziehl–Neelsen staining kit (Hi-Media, Mumbai) as per the manufacturer’s instructions. The extracted DNA was amplified by Polymerase Chain reaction (PCR) using specific IS900 primers (Forward: 5’- CGT CGT TAA TAA CAA TGC AG - 3’and Reverse: 5’- GGC CGT GCG TTA GGC TTC GA -3’) (Giese and Ahrens, 2000). Presence specific PCR amplicons at 279 bp considered as positive for IS900 segment of MAP, which indicates Johne’s disease infection.

Results and Discussion

Examination of Ziehl-Neelsen stained rectal pinch and faecal smears revealed the clusters of acid-fast rods morphologically resembling *M. avium* subsp. *paratuberculosis* (Figure 2). Further examination of dung samples does not revealed the presence of any parasitic ova/cyst. The PCR showed an amplification product of 279 bp which corresponds to *M. avium* subsp. *paratuberculosis* (Figure 3). Based on history, clinical symptoms and laboratory investigation the case was diagnosed as Johne’s disease (paratuberculosis).

Johne’s disease (paratuberculosis) is a chronic wasting disease of dairy cattle that causes huge economic losses globally among dairy farmers primarily via reduction in milk production, infertility, increased incidence of mastitis and shorter life expectancy of animals (Ott *et al.*, 1999; Lombard, 2011). A wide array of diagnostic tests including single intradermal test, Ziehl-Neelsen staining, Cultural isolation, ELISA and Polymerase

chain reaction were used for the diagnosis of Johne's disease in dairy animals. As none of the test is 100% sensitive, a battery of diagnostic tests has been recommended to increase the accuracy of MAP diagnosis

(Stevenson, 2010). Further, treatment against paratuberculosis requires prolonged medication as a palliative therapy and recurrence of the disease may occur in the farm (St-Jean, 1996; Sharif *et al.*, 2013).

Fig.1 Emaciation and external appearance of cow



Fig.2 Clusters of MAP bacilli in dung smear

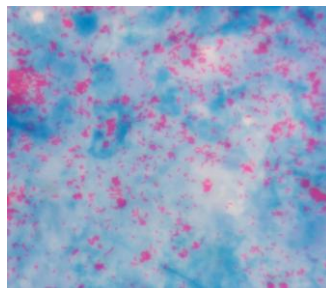
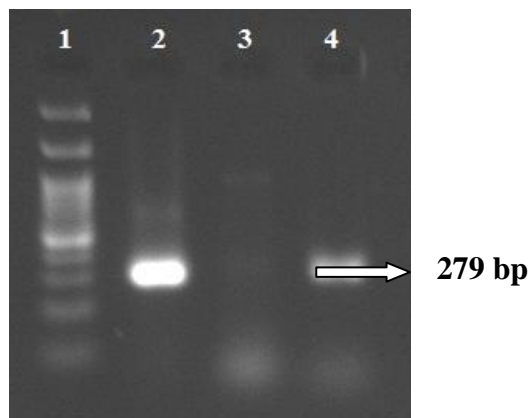


Fig.3 Agarose gel showing amplicons for IS900 PCR of MAP in suspected dung sample (Lane 1: DNA Marker (100bp), Lane 2: Positive control, Lane 3: Negative control, Lane 4: Suspected sample)



The present case revealed the presence of paratuberculosis in the dairy population of Tamil Nadu. It warrants comprehensive epidemiological studies and strict implementation of suitable control measures to combat against the scourge of paratuberculosis in animals.

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