

Original Research Article

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Pathological and Molecular Diagnosis of Paratuberculosis in Sheep and Goats

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ABSTRACT

Keywords

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Johne's disease (JD) is caused by *Mycobacterium avium* Subsp. *Paratuberculosis* (MAP) and the disease is characterized by chronic diarrhea, progressive weight loss and emaciation. The present study was carried out to find out the MAP infection status by postmortem and molecular diagnosis in sheep and goats suspected for JD. Postmortem examination of six small ruminants (One sheep and five goats) revealed the pathognomonic necrotic findings such as characterised hypertrophy of mucosal surfaces of small intestine (terminal ileum) and cord like thickening of mesenteric lymphnodes. Acid fast staining of cytological smears prepared from the intestinal scrapings and mesenteric lymphnode of all animals revealed presence of clusters of acid fast bacilli. Mesenteric lymphnode and intestine were collected from during postmortem examination and subjected to polymerase chain reaction (PCR) using specific primers targeting IS 900 gene sequence of MAP which revealed positivity to MAP infection. The study concluded that postmortem examination combined with polymerase chain reaction is highly useful in accurate diagnosis of JD in small ruminants.

Introduction

Johne's disease is a chronic granulomatous bacterial disease of livestock caused by *Mycobacterium avium* Subsp. *Paratuberculosis* (MAP) characterized by progressive weight loss, chronic enteritis and poor response to treatment (Moges *et al.*, 2016). It mainly affects dairy cattle, sheep and goats and has also been reported in horses, pigs, deer, rabbits (Greig *et al.*, 1999). The first reported case of JD in sheep was diagnosed in 1967 - 1968 in Zaanen breed of goats (Zoi Dimareli- Malli, 2010). It has

recently emerged as one of the most widespread devastating infectious diseases of livestock around the world (Singh *et al.*, 2013). A wide array of procedures and laboratory tests ranging from conventional methods like skin sensitivity test, Ziehl-Neelsen (ZN) or acid fast staining (Buergelt and Ginn, 2000). Diagnosis of JD can also be based on clinical signs, postmortem lesions, and laboratory confirmation characteristics including direct test e.g. fecal smears, polymerase chain reaction (PCR) and indirect tests e.g., delayed-type hypersensitivity (DTH), interferon Assay, Enzyme linked

immune-sorbent assay (ELISA), Agar gel immunodiffusion (AGID) and complement fixation test (CFT) (Munir *et al.*, 2014). The insertion element IS 900 PCR has been routinely used to detect the presence of MAP as it was shown to be specific for MAP (Collins *et al.*, 1989). The present study describes the pathological and molecular diagnosis of JD in small ruminants.

Materials and Methods

Mesenteric lymph node and intestine from one sheep and five goat carcasses were collected during post mortem examination at Veterinary College and Research Institute (VC&RI), Namakkal, Tamil Nadu and postmortem lesions noticed were observed and recorded. The samples were collected by aseptic manner in a sterile container and stored at -20°C until further processing. Cytology smears were prepared from intestinal scrapings and mesenteric lymphnode and stained by acid fast staining technique as per the protocol recommended by Singh *et al.*, (2014). By using Qiagen stool mini DNA kit (Qiagen, USA) DNA was extracted from mesenteric lymph node and intestine as per the protocol described by Mobius *et al.*, (2007). Polymerase chain reaction was performed as per the method described by Millar *et al.*, (1995) using the primer set of forward: 5'GAA GGG TGT TCG GGG CCG TCG CTT AGG -3' and reverse: 5'-GGC GTT GAG GTC GAT CGC CCA CGT GAC-3' targeting IS 900 gene

sequence of MAP. The amplified PCR products were electrophoresed in 1.8 % agarose gel and positivity to MAP infection was detected by visualization of amplified PCR product size of 413 bp.

Results and Discussion

Postmortem examination of six small ruminants (One sheep and five goats) which showed the significant gross lesions like enlargement of mesenteric lymph nodes (Fig. 1) and hypertrophy of intestinal mucosal folds exhibiting typical “corrugation” (Fig. 2). The observed findings of the study are in correspondence with the reports of Sharif *et al.*, (2013) who reported that the enlargement of mesenteric lymphnodes and thickening of small intestinal mucosal folds with typical corrugations is pathognomic to JD. Hence based on necropsy findings the disease was diagnosed as JD.

Acid fast staining of cytological smears made from the intestinal scrapings and mesenteric lymph nodes of the animals showed the presence of acid fast bacilli. Visualization of groups of brightly pink coloured bacilli in acid fast stained smears or sections of tissues (intestines near ileo- cecal valve or mesenteric lymph nodes) is highly suggestive of paratuberculosis (Singh *et al.*, 2014). Based on the findings of above author the results of the present study revealed the positivity of the animals to MAP infection.



Fig.1 Cord like thickening mesenteric lymphnodes in Johne's disease



Fig.2 Corrugation of small intestinal mucosa in Johne's disease

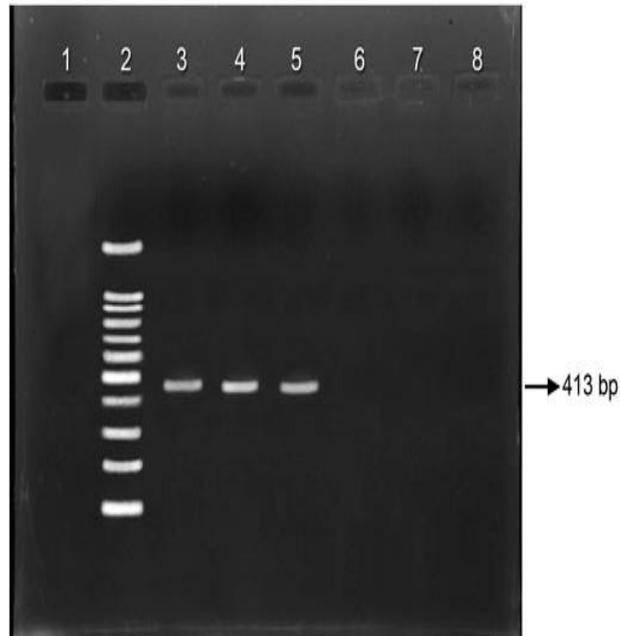


Fig.3 IS 900 gene specific PCR for *Mycobacterium avium subsp. Paratuberculosis* - Mesenteric lymph node and intestine

Cappuccino and Sherman (2008) reported that acid fast staining of intestinal impression smears and mesenteric lymphnode was highly useful in diagnosis of MAP infection. Sikandar *et al.*, (2013) reported that superiority of intestinal samples over mesenteric lymph node for the diagnosis of paratuberculosis with ZN staining technique. The results of the present study are in correlation with the findings of above authors.

Polymerase chain reaction confirmed the MAP infection status of the all animals. Tissue DNA extracted from intestine and mesenteric lymph node of all animals (one sheep and five goats) were subjected to IS 900 gene specific PCR for MAP which revealed positivity by showing 413 bp of PCR product (Fig. 3). Singh *et al.*, (2007) used IS 900 PCR for detection of MAP in intestine and mesenteric lymph nodes of ovines in India. Bauerfeind *et al.*, (1996) reported that the IS 900 provides a valuable tool in epidemiological studies of paratuberculosis in sheep and it is highly useful for the etiological diagnosis of JD in farm animals. It is

concluded that necropsy and PCR can be used together for accurate diagnosis of JD in small ruminants

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