

## PATHOMORPHOLOGICAL STUDIES ON OVINE PARATUBERCULOSIS IN AN ORGANISED SHEEP FARM IN KARNATAKA

SRIKANTH, M.,<sup>1</sup> NARAYANASWAMY, H. D.,<sup>1</sup> SATYANARAYANA, M. L.,<sup>1</sup>  
SUGUNA RAO.,<sup>1</sup> RATHNAMMA, D.,<sup>2</sup> RANGANATH, L.,<sup>3</sup> MUKURTAL,  
S. Y.,<sup>2</sup> SARVESHA, K.<sup>1</sup> AND MANJUNATHA, S. S.<sup>1</sup>

<sup>1</sup>Department of Veterinary Pathology; <sup>2</sup>Department of Veterinary Microbiology; <sup>3</sup>Department of Veterinary Surgery and Radiology, Veterinary College, Hebbal, Bengaluru 560024.  
E. Mail: srikanthammaji@gmail.com Cell: 09964258029

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**Abstract:** Paratuberculosis (Johne's disease) is one of the major economically important disease of small ruminants caused by *Mycobacterium avium* subspecies paratuberculosis (MAP) – a slow growing acid fast bacterium. It causes granulomatous enterocolitis, lymphadenitis and lymphangitis in domestic and wild ruminants. The present study was conducted to record pathomorphological changes in naturally occurring ovine paratuberculosis in an organised sheep farm. The study included 36 paratuberculosis suspected morbid cases in an organised Bannur sheep farm in Mandya district, Karnataka, India. Clinical findings such as intermittent diarrhoea, jowl oedema, cachexia, anaemia, paleness of mucous membrane were recorded in paratuberculosis suspected cases. Out of 36 paratuberculosis suspected morbid cases, 12 showed gross lesions such as muscular atrophy, thickening and corrugation of intestines especially at ileocaecal junction, cording of mesenteric lymphatics with enlarged and oedematous lymph nodes. Gelatinous fat atrophy was very conspicuous in omental, mesenteric, pericardial and perirenal fat. Microscopically lesions were classified on the basis of their location in relation to intestinal lymphoid tissue, intensity, inflammatory cell types and numbers of mycobacteria. In four sheep, paucibacillary type of lesions were observed which were characterized by small granulomas in villi, lamina propria and lymphoid follicles. The granulomas contained few macrophages with clear and large nuclei and abundant cytoplasm with heavy infiltration of lymphocytes. Eight cases revealed multibacillary type of lesions with large granulomas characterized by extensive infiltration of macrophages and epithelioid cells along with acid fast organisms with few lymphocytes infiltration. In conclusion, ovine paratuberculosis was diagnosed based on classical gross and histopathological lesions were confirmed positive by IS900 specific PCR in an organised semi intensive sheep farm in Karnataka in Bannur sheep breed.

**Key words:** Paratuberculosis, Multibacillary, Paucibacillary, Bannur sheep,

### INTRODUCTION

Paratuberculosis (Johne's disease) is a chronic bacterial disease of global importance in mainly domestic and wild ruminants, caused by *Mycobac-*

*terium avium* subsp. paratuberculosis [1]. The infection in small ruminants is also considered of worldwide distribution, diagnosed in sheep and goats in many countries. Ovine paratuberculosis is distributed worldwide, these include Australia, New

Zealand and South Africa, Great Britain, Norway and Austria, para-mediterranean countries, e.g., Greece, Spain, Portugal, Morocco and Jordan [2-4]. Of continuing concern has also been the potential zoonotic link between paratuberculosis and human inflammatory bowel diseases, including Crohn's disease. With increasing certainty of a role for *M. avium subsp. paratuberculosis* in Crohn's disease, efforts to diminish or remove the organism from the human food chain should be encouraged [5].

Disease prevalence in India in sheep and goats was reported by various workers. Mathur et al. [6] detected MAP in 13.3% of 188 sheep by smear examination of ileo-caecal valve and mesenteric lymph nodes in Rajasthan. In a study reported prevalence of 0.77% in sheep and 0.4% in goats based on tissue smear examination [7]. Sheep from farms in Andhra Pradesh and Uttar Pradesh, revealed prevalence of 2.2% by faecal culture, 1.7% by AGID and 22.4% by ELISA [8]. Seroprevalence of 10.3% was reported by ELISA in sheep at CSWRI, Rajasthan [9]. Seroprevalence of 18.33% was reported in cattle in south-west Bengaluru, Karnataka by indirect ELISA [10].

Unlike the clinical signs in cattle, which are readily recognized as profuse watery diarrhoea and weight loss, the disease is more insidious in small ruminant flocks/herds, with affected animals eventually displaying progressive weight loss and exercise intolerance termed as 'an increase in the tail to the mob', with soft faeces in some animals [1]. Two main pathological forms of disease were described: paucibacillary form, in which the inflammatory infiltrate was composed of lymphocytes with some macrophages but few, if any, mycobacteria; and multibacillary form, in which macrophages filled with numerous mycobacteria were the main inflammatory cells [11]. In sheep or goats with no clinical signs or gross lesions, "focal" intestinal lesions, formed by small, well-demarcated granulomas located exclusively in the intestinal lymphoid tissue, were also described by Clarke [11] and Corpa et al. [12]. The present study reports the diagnosis of ovine paratuberculosis in an organized farm based on clinical symptoms, gross and histopathology, demonstration of acid-fast bacteria in tissue sections and confirmation by IS900 PCR in naturally occurring cases of paratuberculosis in Bannur sheep.

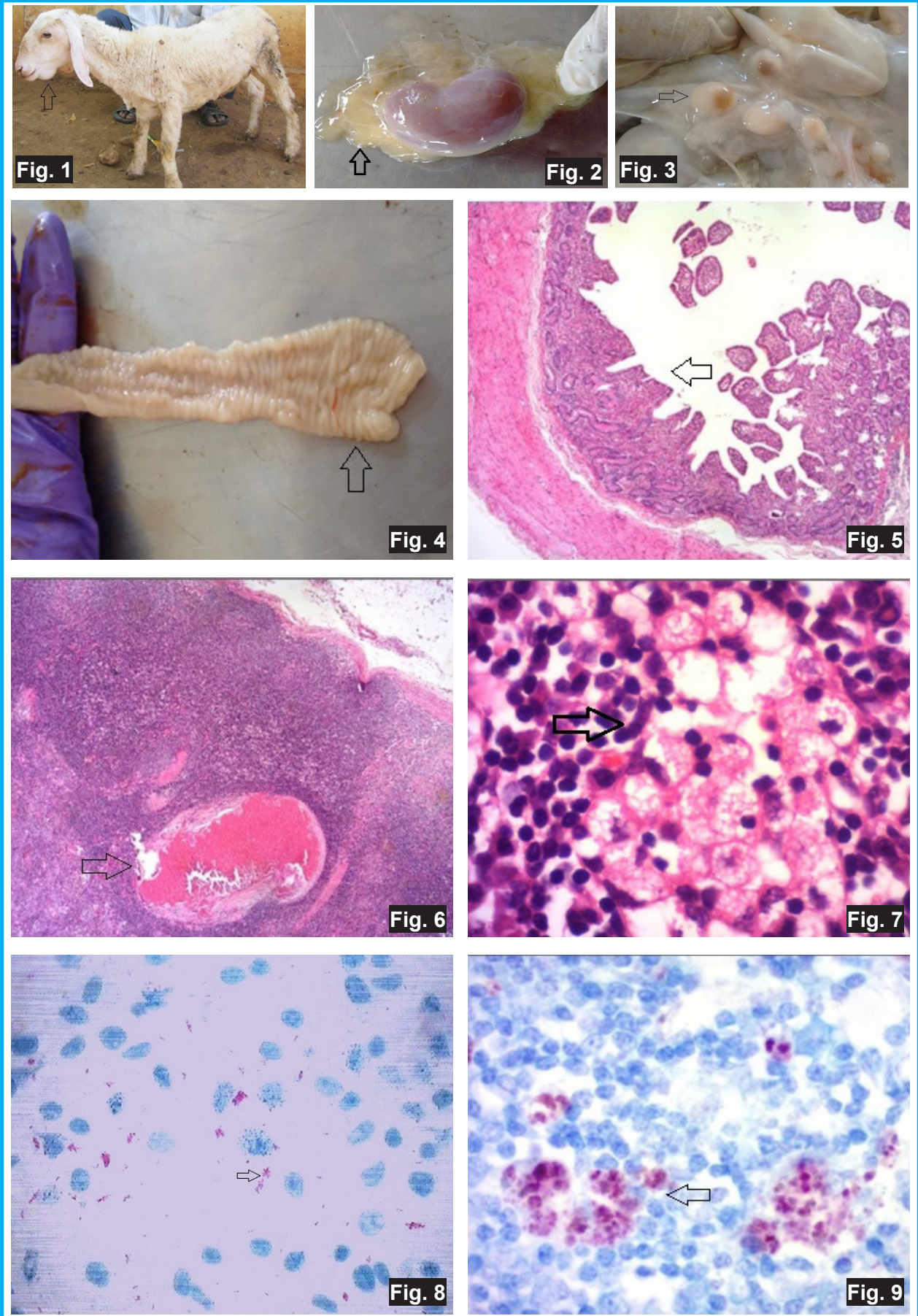
## MATERIALS AND METHODS

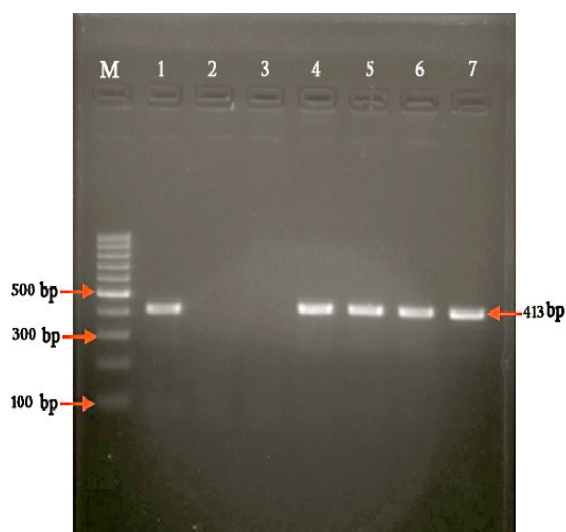
The study included 36 paratuberculosis suspected morbid cases in an organised sheep farm in Mandya district, Karnataka, India. The clinical symptoms of paratuberculosis were recorded in paratuberculosis suspected Bannur sheep breed. The carcasses were subjected to detailed post-mortem examination and the gross morphological lesions were recorded. Impression smears from ileocaecal junction and mesenteric lymphnodes were prepared and stained using ZN/Acid Fast Stains. The pieces of intestines from different portions, ileocaecal junction and mesenteric lymphnodes were collected in 10% buffered formalin. The tissues after proper fixation were processed to obtain haematoxylin and eosin (H&E) stained sections. Samples from the intestine and mesenteric lymph nodes with histopathological lesions were additionally stained by the ZN method.

**DNA isolation and IS900 PCR:** Representative samples of intestines and lymph nodes were subjected to PCR analysis to confirm acid-fast bacteria. Tissue samples were processed for DNA isolation as per Van Embden et al. [13] technique. Samples were screened for the presence of MAP in tissue samples using IS900 PCR in Bannur sheep. DNA samples were amplified using specific IS900 (P90 and P91) primers published by Millar et al. [14]. Briefly, in a volume of 12.5 µl of 2X master mix, 0.5 µl forward primer (10 pmole/µl) and 0.5µl reverse primer (10 pmole/µl), 9.5 µl of nuclease free water and 2 µl of template DNA was added (total volume 25 µl). Total of thirty seven cycles were performed in a thermal cycler for complete amplification reaction. Thermal cycling conditions were initial denaturation at 94°C for 5 min (1 cycle), followed by 37 cycles of denaturation at 94°C for 30 s, annealing at 64 °C for 30 s, extension at 72 °C for 30 s and final extension at 72 °C for 7 min. The specific amplicon of 413bp product was analyzed by 1.5% agarose ethidium bromide gel electrophoresis (Fig. 10).

## RESULTS

**Clinical symptoms:** Clinical symptoms recorded in paratuberculosis affected sheep were intermittent diarrhoea, emaciation was noticed in spite of normal appetite, cachexia, anaemia, jowl oedema (Fig. 1), paleness of mucous membrane and rough





**Fig.10:** MAP specific amplicons (413 bp) by PCR using IS900 specific primers. lane M: 100 bp ladder; lane 1: Positive control (MAP DNA); lane 2 and 3: Negative control and no template control; lane 4-7: DNA samples isolated from intestine and lymph node tissues.

hair coat. It is difficult to identify early signs of paratuberculosis since animals generally remain bright and alert with good appetite. As the disease progresses, weight loss becomes evident.

**Gross pathology:** The gross and morphological lesions recorded were muscular atrophy, cachexia and hidebound condition, paleness of mucus membranes and internal organs, very less or no subcutaneous, mesenteric and omental fat, gelatinization of pericardial, perirenal (Fig. 2) and omental fat, accumulation of non inflammatory serous fluid in peritoneum and pericardial sac, cording and knotting of lymphatics with oedematous and enlarged mesenteric lymphnodes (Fig. 3), thickening of intestines specially at ileocecal junction (Fig. 4) and corrugations were recorded. In few cases caseous necrosis and calcification of mesenteric lymph nodes were recorded.

**Histopathology:** Microscopically, lesions associated with paratuberculosis infection were classified on the basis of their location in relation to intestinal lymphoid tissue, intensity, inflammatory cell types and numbers of mycobacteria. In four sheep paucibacillary type of lesions were observed which were characterized by small granulomas in villi, lamina propria and lymphoid follicles. The granulomas contained few macrophages with clear and large nuclei and abundant cytoplasm with heavy infiltration of lymphocytes. Eight cases revealed multibacillary type of lesions with large granulomas were characterized by extensive infiltration of macrophages and epitheloid cells along with acid fast organisms with few lymphocytes infiltration (Fig. 7). Extensive caseation and calcification in mesenteric lymphnodes (Fig. 6) in few cases were observed. Thickening of mucosa with fibrous tissue was more prominent in multibacillary than the paucibacillary type. The mesenteric lymphnodes also revealed the same changes as that of intestine. A large number of acid-fast bacteria typical of *Mycobacterium avium subsp. paratuberculosis* were seen within the macrophages/epitheloid cells or scattered in clumps in necrosed areas of mucosa and submucosa of the intestine and lymphnodes were demonstrated by ZN staining of impression smears (Fig. 8) and tissue sections (Fig. 9).

**PCR:** Tissue samples were diagnosed as paratuberculosis based on classical gross and histopathological changes were further confirmed positive by IS900 specific PCR amplified at 413bp which is specific for Map (Fig. 10).

## DISCUSSION

Johne's disease causes serious economic losses to sheep farmers [15]. In the multiple stages of MAP

### Explanation of Figures

**Fig. 1:** Sheep showing clinical signs such as diarrhoea, emaciation, weakness, jowl oedema (arrow), paleness of mucous membrane.

**Fig. 2:** Kidney - showing gelatinous appearance of perirenal fat.

**Fig. 3:** Mesentery - showing swollen, oedematous lymph nodes (pallor cortex) with cording and knotting of mesentric lymphatics.

**Fig.4:** Intestine - showing thickening and corrugation (arrow) of mucosa.

**Fig. 5:** Intestine - showing atrophy, fusion and blunting of villi with infiltration of mononuclear cells in lamina propria. H&E x50.

**Fig. 6:** Mesenteric lymph node showing caseative necrosis surrounded by infiltration of mononuclear cells. H&E x50.

**Fig. 7:** Mesenteric lymph node - Macrophages exhibiting foamy cytoplasm. H&E x1000.

**Fig. 8:** Intestine - showing clusters of pink acid-fast bacilli (arrow). ZN x1000.

**Fig. 9:** Mesenteric lymph node showing clusters of pink acid-fast bacilli within the granuloma. ZN x1000.

infections, variable clinical signs were observed in different ruminant species, diarrhoea being the prominent clinical sign of the disease in cattle whereas it may not be present in majority of cases in sheep [16, 17].

In the present study, clinical signs exhibited by ailing animals such as intermittent diarrhoea and emaciation/cachexia, anaemia, jowl oedema, paleness of mucous membrane, rough hair coat which were suggestive that the animals were suffering from a debilitating disease. However, the disease was diagnosed as ovine paratuberculosis by gross, histopathological lesions and PCR studies. Also two forms of lesions such as multibacillary and paucibacillary type were also recorded. Clinical signs which were recorded in the present study are in line with the findings of Clarke. [11], Windsor, [1] and Gilmour, [16].

The characteristic lesions of paratuberculosis in sheep were located mainly in the intestines (Fig. 5) and mesenteric lymph nodes and these findings coincide with the reports of previous workers Kurade, [18], Tripathi, [19] and Reddy et al. [20]. Caseous necrosis and calcification of mesenteric lymph nodes was recorded (Fig. 6), which is one of the distinctive feature observed in paratuberculosis in small ruminants compared to other ruminants [21].

Multibacillary and paucibacillary lesions which were recorded in the present study were in accordance with Clarke and Little [22], Reddy et al. [20]. The multibacillary lesions of chronic granulomatous enteritis and lymphadenitis (particularly involving the mesenteric lymph nodes) in paratuberculosis is characterised by accumulation of epithelioid macrophages (Fig. 7) containing numerous *M. avium subsp. paratuberculosis* in the lamina propria and submucosa of the intestine. The paucibacillary lesions are typically more lymphocytic in nature with *M. avium subsp. paratuberculosis* being far less numerous [1].

### CONCLUSION

In conclusion, diagnosis of paratuberculosis was carried out based on pathological lesions and by demonstration of acid-fast bacilli. Acid-fast bacilli were further confirmed as *M. avium*

*subsp. paratuberculosis* based on PCR. Also, the two pathological forms of the disease such as multibacillary and paucibacillary were recorded in an organised semi intensive Bannur sheep farm.

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