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Gross and histopathological characterization of *Mycobacterium avium* subsp. *paratuberculosis* infection in goats of the Malwa region

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Abstract

This study examined the prevalence, pathological characteristics, and molecular identification of *Mycobacterium avium* subspecies *paratuberculosis* (MAP), the causative agent of paratuberculosis (Johne's disease), in goats. A total of 250 goats presented for slaughter at the Cantonment Board Slaughterhouse, Mhow (Indore), were systematically examined to assess the occurrence of paratuberculosis. Multiple samples, including faecal matter, blood, intestinal tissue, and mesenteric lymph nodes, were collected and analyzed. The study revealed an overall prevalence rate of 38.80%, with 97 out of 250 goats testing positive for the disease based on faecal smear examination. Clinical observations recorded before slaughter showed several consistent signs among affected animals. These included chronic diarrhoea, the passage of soft or pasty faeces, progressive weight loss leading to emaciation, and a rough, unthrifty hair coat. Such symptoms are indicative of long-standing intestinal involvement and poor nutrient absorption. On post-mortem examination, characteristic gross lesions were observed. The intestinal mucosa appeared thickened and corrugated, often accompanied by visible congestion. Additionally, the mesenteric lymph nodes were found to be enlarged, oedematous, and corded, suggesting an ongoing inflammatory process. Microscopic examination using haematoxylin and eosin (H&E) staining revealed typical histopathological features of paratuberculosis. These included chronic granulomatous enteritis marked by mixed inflammatory cell infiltration, formation of microgranulomas, shortening and fusion of intestinal villi, presence of multinucleated giant cells, and areas of caseous necrosis in the lymph nodes. Further confirmation was achieved through Ziehl-Neelsen staining, which demonstrated clusters of acid-fast bacilli within the affected tissues, supporting the diagnosis of *Mycobacterium avium* subspecies *paratuberculosis* infection.

Keywords: *Mycobacterium avium* subspecies *paratuberculosis* (MAP), haematoxylin and eosin (H&E), Ziehl-Neelsen staining, granulomatous enteritis, multinucleated giant cells

Introduction

Small ruminants play an important role in the lives of poor people and in the rural economy in developing countries like India. *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the causative agent of Johne's disease in ruminants that causes chronic granulomatous enteritis leading to emaciation and occasional diarrhoea in sheep and goats (Gupta *et al.*, 2012 and Richards *et al.*, 2021) [6, 14]. In goats, thickening and folding of the intestinal wall, corrugation, granular mucosa, serous atrophy of fat and thickening of mesenteric lymphatic vessels were seen grossly. Oedematous enlargement and occasionally calcium deposits were seen in the mesenteric lymph nodes (Thakur *et al.*, 2019) [19]. Johne's disease (JD) is now worldwide in distribution due to the global trade of animals, incurring huge economic losses to the dairy industry. The disease has a wide host range and affects many ruminant species like cattle, sheep, goats, deer, llamas, bison, etc. (Harris and Barletta, 2001). Significantly high prevalence of MAP infection has been reported in sheep and goats (22.5%), and in many organized cattle and buffalo herds (29%) in India (Kumar *et al.*, 2007 and Singh *et al.*, 2008) [8, 16]. Paratuberculosis (Johne's disease), a chronic infectious disease of ruminants, has been recognized in the United States for about 75 years. Chaubey *et al.* (2017) [2] reported a bio-load of 23% in goats for MAP during the last 31 years in India. The Johne's disease is characterized by therapy-resistant diarrhoea, progressive emaciation, and a decrease in milk

production, finally leading to death. The clinical symptoms appear after an incubation period of up to several years in the final stage of the disease (Whitlock and Buergelt, 1996)^[21]. In small ruminants, softening of faeces is observed in individual animals, but severe watery diarrhoea, typical for cattle, occurs very rarely. There is evidence that goats are more susceptible to MAP than sheep and have a shorter clinically unapparent interval, faster disease progression compared to sheep and cattle (Stewart *et al.*, 2006)^[17] and often show bacterial shedding before the peripheral immune response is measurable (Pickrodt *et al.*, 2023)^[13]. Histopathology of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in goats reveals chronic granulomatous enteritis characterized by lymphocytic and macrophage infiltration in the lamina propria, villous atrophy, and fusion. The disease, known as Johne's disease, frequently shows diffuse granulomatous lesions, Langhans giant cells, and large numbers of acid-fast bacilli in macrophages within the ileum, mesenteric lymph nodes, and Peyers patches.

Materials and Methods

The present investigation was carried out in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Mhow (M.P.). A total of 250 goats of different breeds, presented for slaughter at the Mhow Cantonment Board slaughterhouse, were included in the study. Comprehensive ante-mortem and post-mortem examinations were performed on all animals. The goats originated from various regions of the Malwa tract, including Mhow, Manpur, Dhar, Indore, and Maheshwar.

Tissue sample collection for histopathology

Goats after slaughter were examined grossly for any lesions associated with paratuberculosis and after the examination, the representative intestine and mesenteric lymph node tissue samples were collected in 10% neutral buffer formalin for further histopathological examination.

Processing of tissue samples

Tissue samples were trimmed to a smaller size (4 mm to 1 cm thickness), preserved in the 10% neutral buffered formalin for at least 24-48 hours. Further these tissues were processed for histopathological studies by the standard procedure described by Lillie and Fullmer (1976)^[10]. In this method, tissues were dehydrated in graded alcohol, cleared in xylene and embedded in paraffin. The samples of desired thickness were washed in running tap water overnight. The tissues were dehydrated in ascending grades of alcohol viz. 50%, 70% and 90%, three changes in absolute alcohol for one hour each, cleared in xylene-I, xylene-II for one hour each and embedded in paraffin wax having melting point more than 56 °C in four waxes (I, II, III and IV) for one hour in each wax. After 4 hours impregnation of tissue in paraffin wax the blocks were formed in L moulds using molten wax. Tissues were placed in moulds in such a way that the desired surface was remain downward. The blocks were cooled at room temperature. The tissue sections were sectioned at 4-5 micron thickness with the help of semi automatic microtome and sections were taken on egg albumin glycerol (50% egg albumin and 50% glycerol) with thymol crystals precoated slides.

Haematoxylin and eosin staining

These sections were processed by the conventional procedure of staining using routine haematoxylin and eosin for histopathological studies described by Lillie and Fullmer (1976)^[10]. Before staining, slides were slightly heated in oven to deparaffinize and then cleared by two changes of xylene for 10-15 minutes each. After clearing, the tissue sections were kept in changes of descending grades of alcohol, viz. 100%, 90%, 70% and 50% for two minutes each. After that, the slides were dipped in haematoxylin for 10-15 minutes followed by 4-5 dips in acid alcohol and ammonia water for 1 minute. Slides were stained in 2% aqueous Eosin for 2-5 minutes. After staining with eosin followed by changes of 50%, 70%, 90% and 100% ascending grades of alcohol followed by clearing of tissue section in xylene for 2 minutes. Mounting was done with coverslip using DPX. Slides were dried, cleaned and properly labelled and were examined for microscopic lesions.

Lesions of paratuberculosis in the tissues of intestine and mesenteric lymph nodes were categorized on the basis of peyer's patches and granuloma formation. The status of lesions were correlated with different diagnostic tests.

Special tissue staining

Staining of tissue sections by acid fast staining was performed according to the procedure described by Prophet *et al.* (1994). Tissue samples from representative intestine and mesenteric lymph node of goat were thoroughly examined for any apparent gross lesions and were stained with slightly modified Zeihl-Neelsen's acid fast staining method. In this method, in initial step, the tissue sections were deparaffinized on hot plate followed with dips in xylene for 10 minutes followed by dip in descending grades of alcohol, viz. 100%, 90%, 70% and 50% for 2 minutes each, then rehydrate with distilled water. After this, the tissue sections were flooded with carbol fuchsin and intermittent heating using spirit lamp for 30 minutes and washed in distilled water gently for 8 minutes. After that, sections were decolorized with the 1% acid alcohol reagents for 1-2 minutes and then followed by washing in distilled water. Methylene blue was used as a counterstain for 1-2 minutes on the sections followed by a gentle wash with distilled water, then dehydrate stained sections with two changes of absolute alcohol each for 1 minute followed by clearing in two changes of xylene for 2 minutes each and mounted with the DPX. After the staining procedure, the sections were examined under oil immersion to examine the presence of pink colored rod shaped acid-fast bacilli in positive tissue sections.

Results and Discussion

Gross lesions

All 250 slaughtered goats were carefully examined for lesions suggestive of paratuberculosis. Affected animals showed marked emaciation, weakness, significant weight loss, and a rough, dull hair coat (Fig. 1). Out of the goats aged 6 months to 4 years, 156 were suspected of MAP infection based on gross findings.

Paratuberculosis primarily involved the intestines and mesenteric lymph nodes. Intestinal examination revealed changes such as gelatinization of mesenteric fat and intestinal ballooning (Fig. 2), along with congestion, engorged blood vessels, and mild to moderate thickening of

the terminal ileum (Fig. 3). Additional findings included transverse corrugations, thickened intestinal walls, dilated lymphatics, and hyperemic mucosa with prominent Peyer's patches. In severe cases, nodular mucosal growths were observed (Fig. 4 and 5). Mesenteric lymph nodes were enlarged, edematous, and congested, often showing nodular surfaces and cording within the mesentery. On sectioning, they were approximately twice their normal size (Fig. 6). Present findings are consistent with the findings of Tafti and Rashidi (2000) ^[18], Tripathi *et al.* (2006) ^[20], Denaives *et al.* (2007) ^[4], Mir *et al.* (2009) ^[11], Chaturvedi *et al.* (2017) ^[1] and Derakhshandeh *et al.* (2018) ^[5].

Histopathology

H&E staining

Microscopic examination revealed granulomatous enteritis characterized by villous atrophy, epithelial desquamation, and necrosis. The lamina propria and submucosa showed infiltration of epithelioid cells, macrophages, plasma cells, lymphocytes and eosinophils, along with microgranuloma formation and occasional rupture of muscularis mucosa (Fig. 7 and 8).

Mesenteric lymph nodes showed capsular thickening, lymphoid hyperplasia, congestion, and in some cases, encapsulated caseo-necrotic masses and lymphoid depletion (Fig. 9). Results of the present study corroborate with the findings of Corpa *et al.* (2000) ^[3], Singh *et al.* (2013a), Mukartal *et al.* (2016), Chaturvedi *et al.* (2017) ^[1] and Tharwat *et al.* (2025).

Ziehl-Neelsen staining

Acid-fast staining demonstrated clumps of acid-fast bacilli within the intestinal lamina propria, along with inflammatory infiltration and tissue degeneration. The tissue sections displayed thickening of the submucosa and atrophy of the intestinal glands. In the lamina propria of the intestine, clumps of acid-fast organisms were identified alongside epithelioid cells and plasma cells (Fig. 10). Microscopically, there was moderate scattered infiltration of epithelioid cells, lymphocytes and plasma cells, with syncytial formations observed in the lamina propria. Some sections also revealed prominent germinal centers, lymphoid hyperplasia, necrotic granulomas, fibrosis and depletion of lymphoid cells, with heavy infiltration of plasma cells in the medullary sinus of the mesenteric lymph nodes. Acid-fast bacilli were demonstrated in only a few cases. Mesenteric lymph nodes showed plasma cell infiltration, fibrosis, and occasional acid-fast organisms. Hamid *et al.* (2018) reported that histopathological examination of intestinal tissues from suspected cases revealed infiltration of epithelioid cells, lymphocytes, macrophages, and giant cells, along with the presence of both free and phagocytosed acid-fast bacilli.

The present study highlights the importance of histopathological evaluation in the diagnosis of paratuberculosis in goats. Tissue samples, after detailed examination, were subjected to Ziehl-Neelsen staining to confirm the presence of acid-fast bacilli within the sections. The findings of this study are in agreement with earlier reports by Corpa *et al.* (2000) ^[3], Mir *et al.* (2009) ^[11], Chaturvedi *et al.* (2017) ^[1], and Idris *et al.* (2021) ^[9]. However, the results differ from those of Tripathi *et al.* (2006) ^[20], who documented multibacillary-type lesions with a high number of acid-fast organisms in tissue sections.



Fig 1: Photograph of goat showing signs of severe emaciation, weakness and significant weight loss.



Fig 2: Photograph showing gelatinization of mesenteric fat and intestinal ballooning (arrows).



Fig 3: Photograph showing gelatinization of mesenteric fat and congestion of intestine with enlarged oedematous mesenteric lymph node (arrows).



Fig 4: Photograph showing diffuse transverse corrugations and thickened intestinal mucosal walls of ileum.



Fig 5: Photograph showing light transverse corrugations and nodules (black arrow) in intestinal mucosa along with the oedematous mesenteric lymph node (blue arrow).



Fig 6: Photograph showing enlarged, oedematous and corded mesenteric lymph node.

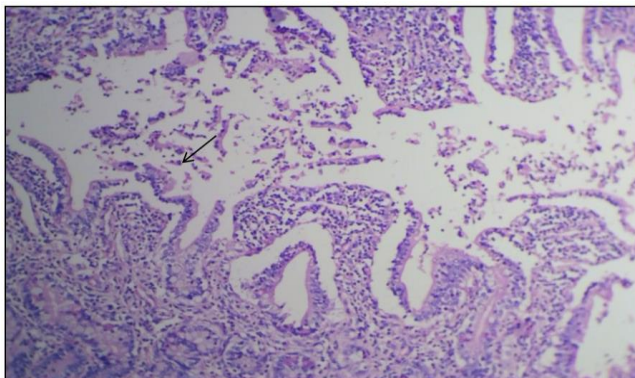


Fig 7: Photomicrograph showing shortened and broadened villi with infiltration of inflammatory cells (H & E stain, ×100) (arrow).

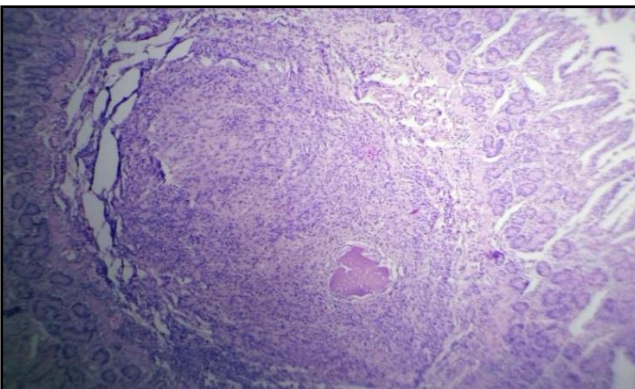


Fig 8: Photomicrograph showing granuloma with heavy infiltration of inflammatory cells in submucosa of intestine (H & E stain, ×400).

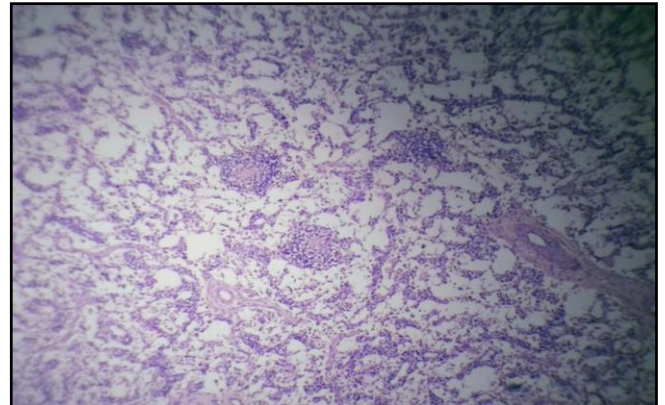


Fig 9: Photomicrograph of mesenteric lymph node showing granulomatous lesions with necrosis (H & E stain, ×400).

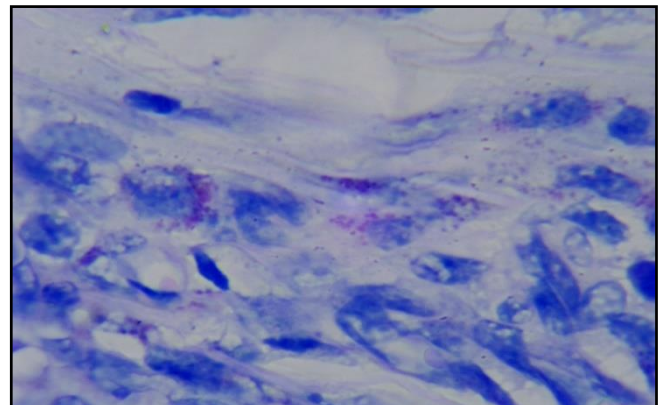


Fig 10: Photomicrograph showing MAP bacteria within macrophages of intestinal mucosa (ZN stain, ×1000).

Conclusion

In conclusion, the present study demonstrates that paratuberculosis is prevalent among goats slaughtered at the Mhow Cantonment Board, with a substantial proportion of animals showing characteristic gross and microscopic lesions. Clinically affected goats exhibited emaciation, weakness, weight loss, and poor coat condition, while post-mortem findings consistently revealed involvement of the intestines and mesenteric lymph nodes. Prominent gross lesions such as intestinal thickening, corrugation, congestion, and enlargement of mesenteric lymph nodes were indicative of chronic disease progression. Histopathological examination further confirmed the diagnosis, with typical features of granulomatous enteritis, including villous atrophy, epithelial damage, and infiltration of inflammatory cells such as epithelioid cells, macrophages, lymphocytes, and plasma cells. Lesions in the mesenteric lymph nodes, including lymphoid hyperplasia, caseo necrosis, and fibrosis, supported the systemic nature of the infection. Ziehl-Neelsen staining provided additional confirmation through the demonstration of acid-fast bacilli within intestinal and lymph node tissues, although their presence was limited in some cases, suggesting a paucibacillary form of the disease. Overall, the study underscores the diagnostic value of combining gross pathology, histopathology and special staining techniques for accurate detection of paratuberculosis, aligning well with previous research while highlighting some variation in bacillary load.

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