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SHORT COMMUNICATION

A Concurrent tuberculosis and paratuberculosis in a beetal goat

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ABSTRACT

A two year old female beetal goat was presented with history of diarrhea and progressive weight loss. On hematology, severe anaemia (Hb = 2.9g/dl) and neutrophilic leukocytosis were observed. Ante-mortem fecal examination revealed positive for acid fast bacilli. The animal succumbed to death. On postmortem examination, the carcass showed emaciation and diarrhoeic feces found to be adhered in the perineal and tail region. Grossly, lung revealed edema, congestion along with hard caseated nodules in focal areas and trachea was frothy. Intestine revealed catarrhal enteritis, and also showed thickening of the intestinal mucosa at the caeco-iliac junction. Mesenteric lymph nodes were swollen and caeseated. Impression smear from intestine and lung were positive for acid fast bacilli. Histopathologically lung exhibited focal to multifocal necrotizing lesion at the centre and surrounded by epitheloid cells and Langhan's giant cell, indicating typical TB granuloma. Further, immunohistochemistry and PCR using specific primers for *Mycobacterium tuberculosis* complex and *Mycobacterium avium* subsp paratuberculosis confirmed the presence of tuberculosis and paratuberculosis respectively.

Keywords: Acid fast bacilli, beetal goat, johne's disease, tuberculosis

Tuberculosis and paratuberculosis are mycobacterial diseases where in goat tuberculosis is caused by *Mycobacterium bovis* and *Mycobacterium caprae* and paratuberculosis is caused by *Mycobacterium avium* subsp paratuberculosis (MAP)[1]. Both diseases have serious impact on production and economic losses. Having a wide host range *M. bovis* is a huge concern as a zoonotic agent[2]. Tuberculosis transmission occurs primarily through inhalation but can also spread via ingestion of contaminated substances, secretions, and through the placenta to the fetus[3]. However, Paratuberculosis is mainly transmitted via the fecal-oral route, with animals getting infected by grazing on contaminated pasture or sucking contaminated teats[4]. Both the diseases are more associated with crowded population of animals or animals kept under stressful conditions. Tuberculosis causes emaciation, fever, inappetance, and chronic moist cough, while paratuberculosis features are persistent diarrhea, submandibular edema, and emaciation, both leading to significant economic losses to the farmers[5,6]. Mycobacterial diseases are diagnosed through clinical signs, gross observation, histopathology, microscopic examination on sputum, fecal sample or any other secretion with Z-N staining, and molecular assays like PCR, immunohistochemistry.

A two year old female beetle goat was presented to Guru Angad Dev Veterinary and Animal Sciences, Hospital with the history of diarrhea and progressive weight loss. Haematological and faecal smear examination using Ziehl-Neelsen staining was done. Animal was treated symptomatically but could not survive and after death, it was presented for post-mortem examination. Systemic necropsy examination was conducted and gross findings were recorded. Impression smear of the lungs and intestine were prepared and stained with Modified Ziehl-Neelsen staining for detection of acid fast bacilli. For histopathology, lungs, lymph nodes and intestine were collected in 10% neutral buffered formalin. The fixed tissue was embedded with paraffin and 4-µm thickness tissue sections were cut and then stained with routine hematoxylin and eosin (H&E). Stained sections were viewed under microscope (Olympus BX 61) and photomicrographs were taken. Immunohistochemical staining and PCR were done for additional confirmation.

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Hematological investigation revealed presence of severe anaemia (with Hb = 2.9g/dl) and neutrophilic leukocytosis with TLC = 15800/ul. Faecal sample stained with Ziehl-Neelsen stain showed positive result for acid fast bacilli. On external examination, the carcass showed emaciation (Fig. 1) and the anal area was soiled with faeces. Upon doing systemic necropsy examination, caseous nodules were observed on the lungs along with congestion and edema (Fig. 2). The trachea was filled with frothy exudates (Fig. 3). Intestine

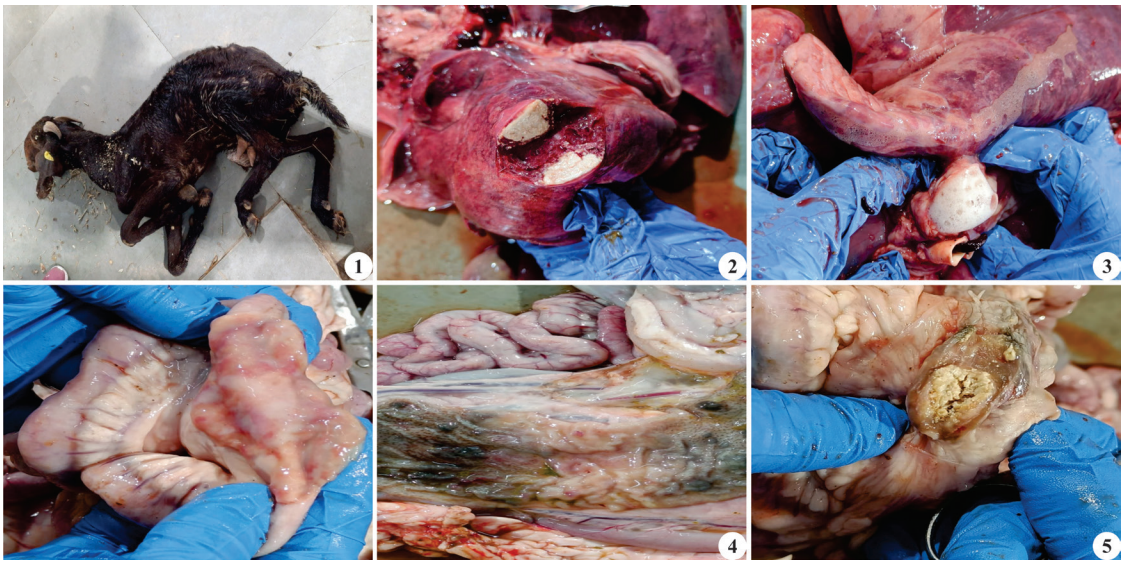

Fig. 1. Goat: Emaciated carcass of Beetal goat; **Fig. 2.** Lung showing caseous nodule; **Fig. 3.** Trachea filled with froth; **Fig. 4A & B.** Intestine with thickened and corrugated mucosa; **Fig. 5.** Mesenteric lymphnode showing caseation and mineralization.

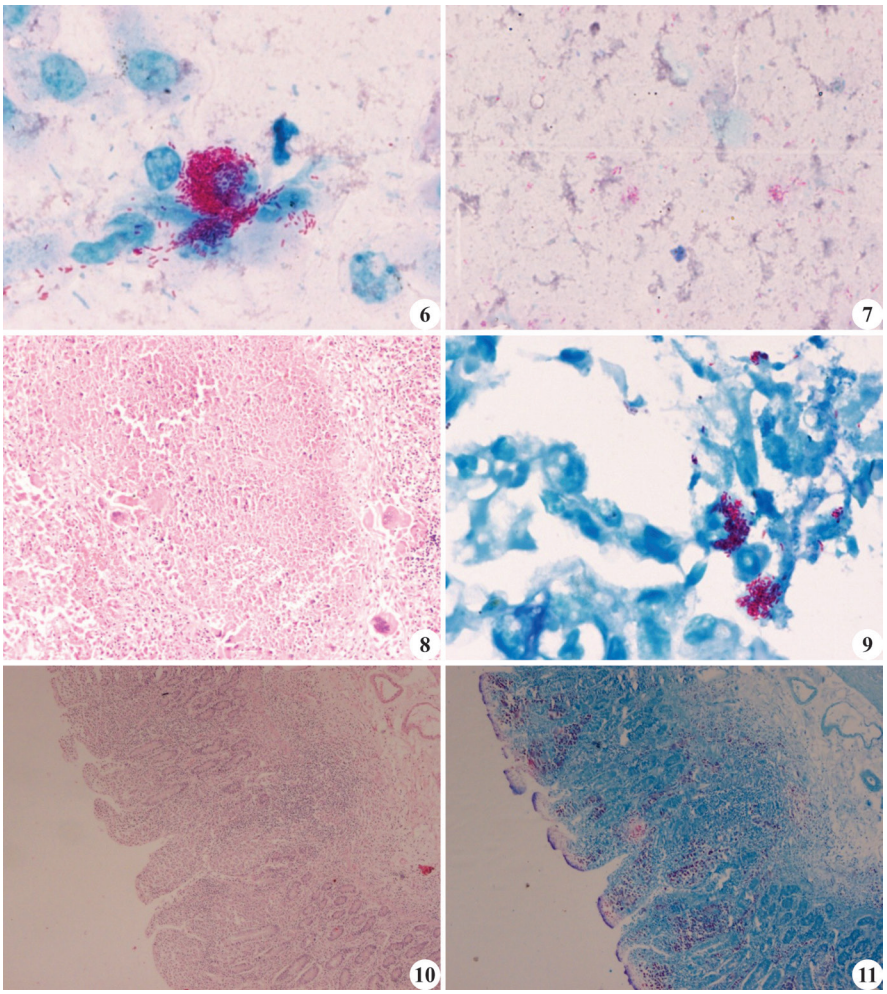

Fig. 6. Intestine: Impression smear showing acid fast bacilli with Ziehl-Neelsen stain; **Fig. 7.** Lungs: Impression smear showing acid fast bacilli within the inflammatory cell with Ziehl-Neelsen stain; **Fig. 8.** Lungs: Granuloma showing caseous necrotic area surrounded by inflammatory cells and giant cells (H&E x200); **Fig. 9.** Lungs: Acid fast bacilli within the inflammatory cells (ZN stain x1000); **Fig. 10.** Intestine: Thickened intestinal mucosa with mononuclear cells infiltration within the mucosa and sub-mucosa (H&E x100); **Fig. 11.** Intestine: Acid-Fast bacilli in the mucosa as well as within the mononuclear infiltrating cells (ZN x100).

showed generalized congestion and the mucosa of the intestine was thickened and corrugations at the ileo-caecal junction were observed (Fig. 4). Mesenteric lymph nodes were diffusely swollen and had pale and thick capsule and it had firm consistency along with caseous necrosis and mineralized matrix (Fig. 5). Microscopically, examination of impression smear prepared from intestine and lung showed positive for acid fast bacilli when stained with Ziehl-Neelsen stain (Fig. 6, 7). Histopathologically, lung showed typical tuberculous granuloma with central caseous necrosis surrounded by inflammatory cells and Langhan's giant cells (Fig. 8). Acid fast bacilli were present within the inflammatory cells and necrotic area (Fig. 9). In intestine the thickened intestine mucosa and sub-mucosa had diffused infiltration of mononuclear cells along with congestion (Fig. 10). Upon doing Z-N staining, acid fast bacilli were also appreciated within mononuclear cells of the intestinal mucosa (Fig. 11). In lymph node, there was caseous necrosis with infiltration of giant cells and mononuclear cells (Fig. 12). Similar to the intestine in the lymph node acid fast bacilli were observed within mononuclear cells and necrosed area (Fig. 13).

Additionally, DNA was extracted from tissue samples using a commercial kit (Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit) for amplification of IS*6110* PCR for detection of *Mycobacterium tuberculosis complex* (MTC) and IS900 PCR for detection of *Mycobacterium avium* subsp. paratuberculosis (MAP) (Table 1). PCR was performed for these 2 genes with cycling parameter of 94°C for 5 min (initial denaturation) followed by 30 cycles of 1 min at 94°C, 1 min at 60°C and 1 min at 72°C followed by final extension of 7 min at 72°C. Thermal cycling was performed in Gradient

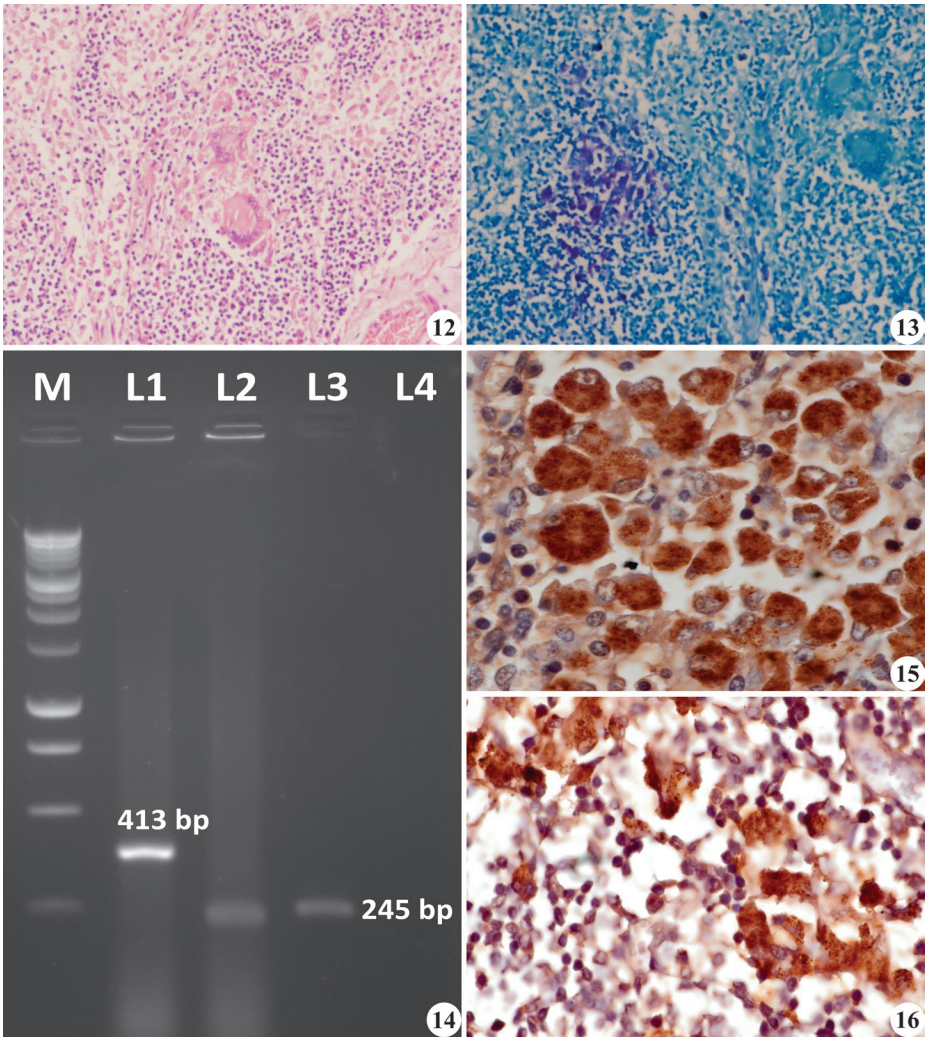

Fig. 12. Lymph node showing giant cells and lymphocytes (H&E 20X); **Fig. 13.** Acid-Fast bacilli within the mononuclear cells in lymph node (ZN x200); **Fig. 14.** Gel electrophoresis of PCR product showing 413 bp for MAP in intestine sample and 245 bp for MTC in lung sample. M = DNA ladder (1Kb); L1 = Intestine sample; L2, 3 = Lung sample; **Fig. 15.** Immunolocalization of *Mycobacterium* antigen within the mononuclear cells (IHC, DAB, x1000); **Fig. 16.** Immunolocalization of *Mycobacterium* antigen within the mononuclear cells in lymph node (IHC, DAB, x1000).

Table 1.

Organism	Primer	Sequence	Gene	Product size	Reference
Mycobacterium tuberculosis complex	INS1 (forward)	5'-CGT GAG GGC ATC GAG GTG GC-3'	IS6110	245 bp	Filia *et al.*[9]
(MTC)	INS2 (reverse)	5'-GCG TAG GCG TCG GTG ACA AA-3'			
Mycobacterium avium subspecies	P90B (forward)	5'-GAA GGG TGT TCG GGG CCG TCG CTT AGG-3'	IS900	413 bp	Millar *et al.*[10]
paratuberculosis (MAP)	P91B (reverse)	5'-GGC GTT GAG GTC GAT CGC CCA CGT GAC-3'			

Thermocycler (Thermo Scientific). The presence of amplified DNA was visualized by agarose gel electrophoresis. Gel electrophoresis of PCR product showing 413 bp for MAP in intestine sample and 245 bp for MTC in lung sample (Fig. 14). The animal was found to be positive for both the diseases. Immunohistochemical characterization of the acid fast bacteria was done in the lungs, intestine, lymphnode sample using *Mycobacterium bovis* antigen and positive immunolocalization of *Mycobacterium bovis* antigen found within the mononuclear cells (Fig. 15, 16). Thus, based on gross, cytology, histopathology, IHC and PCR, the case was confirmed as concurrent tuberculosis and paratuberculosis infection.

Mycobacterial diseases are chronic diseases and they have long asymptomatic period and even before manifesting clinical signs they start shedding organism, making it difficult to eliminate the disease[7]. Animals kept in crowded space with poor ventilation are more prone. Tuberculosis commonly presents with emaciation, fever, inappetance, and a chronic moist cough, while paratuberculosis is marked by persistent diarrhea, protein loss, sub-mandibular edema, and emaciation[5,6]. In advanced cases, affected animals may become reluctant to move and may die. Both diseases cause significant economic losses due to reduced weight gain, lower milk production, decreased fertility, and they also costs associated with diagnosis and treatment. Tuberculosis and paratuberculosis are very common diseases; however, concurrent infection of both diseases is rare. In India, goats are reared by poor people who usually are nomads and due to negligence the disease is often not properly reported and diagnosed so making it difficult to eradicate. Diagnosis of mycobacterial diseases can be made by observing clinical signs, gross observation and histopathology. Also, the same can be done by performing microscopic examination on sputum, fecal sample or any other relevant secretion using Z-N staining procedure and molecular assay like PCR which increases the sensitivity for diagnosis of the disease[8]. Mycobacterial disease are highly infectious so while diagnosing screening should be done for whole herd. Animals in crowded, poorly ventilated spaces are more susceptible, so proper hygiene, ventilation, and biosecurity measures are crucial. Sick animals should be isolated, and farmers, especially nomadic goat herders, need education on disease signs and reporting. Early detection tests are essential to control mycobacterial diseases effectively.

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