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Mycobacterium tuberculosis complex (MTC) detection in a beetal goat from Assam

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Abstract
In the genus of Mycobacterium which has recently been reclassified into four different genera, *Mycobacterium tuberculosis* is the best known pathogen known to cause Tuberculosis (TB) mainly in humans and also in animals. Zoonotic tuberculosis is primarily caused by Mycobacterium bovis while the genetically related other members of these group known as the *Mycobacterium tuberculosis* Complex (MTC) comprising of M. caprae, M. africanum, M. pinnipedii, M. canetti, M. mungi, M. microti and M. orygis causes TB in several other animal species and human. A carcass of 3 year old female goat was presented in our department which was subjected for post mortem examination. The carcass was in debilitated and cachectic condition. During necropsy the lungs revealed grayish white nodular growths of various sizes. Impression smears from the cut surfaces of the nodules revealed acid fast bacilli with Ziehl Neelsen staining. Histopathology of the lung showed typical tuberculous granulomatous lesion. The tissue was subjected to molecular analysis by amplification ofhsp65 gene (441 bp) for Mycobacterium Genus confirmation. Further, IS6110 region was targeted (123bp) for confirmation of the MTC group.

Keywords: Tuberculosis, *Mycobacterium* tuberculosis complex (MTC), Zoonosis, Goat, Tuberculous granuloma

Introduction
Pathogenic mycobacteria are known to cover a wide range of hosts. In animals, *M. bovis*, *M. avium* and *M. avium* subsp. *paratuberculosis* are the principle mycobacterial pathogens. *M. bovis* causes bovine tuberculosis and is known to be zoonotic in nature. *M. avium* and *M. partuberculosis* are classified under opportunistic pathogens and *M. avium* is often associated with diseases in poultry and pigs, while *M. paratuberculosis* leads to Johne's disease in cattle (Gavin *et al.*, 2018) [7]. The global incidence of Tuberculosis (TB), caused by *M. tuberculosis*, in 2017, stands at 10 million new cases per 100, 000 population and India holds the highest burden of the global TB cases with approximately 2.74 million cases and 0.4 million mortality (WHO, 2018) [15]. Tuberculosis is also an important zoonotic infection caused by members of the *Mycobacterium tuberculosis* complex which affects domestic as well as wild and captive animals (Gavier-Widen, 2016). Although eradication policy of zoonotic TB was started all over the world, TB still remains endemic in most of the countries (Zanardi *et al.*, 2018). According to the census of 2012, the goat population in India stands at over 135 million contributing to important source of protein and textile resource (www.nddb.coop).Infections by members of MTC such as *M.bovis* and *M. caprae* possess a growing threat to goat farming that produces severe exudative lesions with cavitation in the lungs with significant implication for public health causing a significant economic burden (Crawshaw *et al*., 2008., Zanardi *et al*., 2013; Pesciaroli *et al*., 2014) [2, 16, 9]. Being zoonotic in nature, transmission to humans mainly veterinarians, abattoir workers or livestock and crop farmers are also documented (Rodríguez *et al*., 2009) [10]. Although there arereports on *M. bovis* infection in goat (Domingo *et al*, 2009; Hiko *et al*., 2011; Romha *et al*., 2018) [4, 8, 11] documented literature of tuberculosis in goat isalmost nonexistent from Assam. Report of *M. tuberculosis* and other mycobacteria from bovine is available in the region which indicates the presence of these pathogens in livestock (Vise *et al*., 2017) [14]. The present study records a case of tuberculosis in a Beetle goat caused by *Mycobacterium tuberculosis* complex and was confirmed by path morphology, Zeihl Neelsen (ZN) staining, and molecular identification.

Materials and Methods

A three year old female Beetle goat was presented in the department of pathology, CVSc, AAU, Guwahati. A detailed post mortem examination was conducted. This involved visual inspection, palpation, incision of the carcass and internal organs. Samples of lungs, liver, kidneys, lymph nodes were examined. The organs were incised and inspected for the presence of abscess, cheesy masses and tubercles. Lung tissues suspected for TB were fixed in 10% buffered formalin for histopathology. Impression smears were taken from the incised nodule of the lungs. The heat-fixed smears were stained for observation of acid fast bacilli as per the standard protocol. For bacteriological culture confirmation, the suspected lung tissue was collected in sterile containers and processed as per earlier described (van Ingen *et al.,* 2010) [13]. Briefly, the surface of the excised lung sample was decontaminated in boiling water and triturated. The homogenized tissue was then decontaminated with 3 volume parts of 6% H_2SO_4 and centrifuged at 12000 rpm for 10 minutes. The sediment was inoculated separately in two Lowenstein Jensen (LJ) slants containing sodium pyruvate and glycerol respectively. The tubes were inoculated at 37°C for upto 8 weeks.

Simultaneously, the lung sample was subjected to molecular analysis through PCR for mycobacterial detection. DNA was extracted from the triturated lung sample using column-based tissue DNA isolation kit (D Neasy, Qiagen, USA). Initial confirmation of *Mycobacterium* genus was done using the established primers for partial amplification of the *hsp65* gene (Telenti *et al*., 1993) [12]. Another PCR amplification was done for the *IS* 6110 region (Eisenach *et al*., 1990) [5] which is known to be specific for *Mycobacterium tuberculosis* complex (MTC). Controls such as *M. bovis*, *M. tuberculosis*, *M. vaccae*, were used for these two PCR.

Result and Discussion

The Beetal goats were procured from Punjab, most of which died due to haemonchosis and enterotoxaemia. This single goat presented symptoms of tuberculosis. The goats were transported from low humid (70-75%) to high humid areas (85-90%) which due to stress might have made them susceptible to various diseases. The macroscopic feature of granulomas was characterized by the presence of dry nodules in varying sizes ranging from 0.5 to 3 mm. All the nodules almost were similar in sizes, protruding from the lung parenchyma (Figure 1). The appearance of the nodules indicated milliary tuberculosis. The color of the nodules was grayish white and consistency was hard on palpation. Upon incision of the nodules, cheese like materials with gritty substances were observed. Staining of tissue smear from lung revealed presence of rod shaped, acid fast bacilli indicating the presence of *Mycobacterium* cells (Figure 2). Microscopically, the multiple tuberculosis nodules revealed foci of eosinophilia homogenous masses of caseation with dark blue colored areas of calcification (Figure 3). A zone of cellular reaction was observed around the caseo necrotic areas. Degenerated neutrophils, macrophages, epithelioid cells and lymphocytes were admixed in the areas. The typical Langhan' stype of giant cells were evident in the lesions (Figure 4). The complete granuloma was surrounded by fibrous connective tissue capsule. Sections of lungs also revealed highly dilated and congested blood vessels, edema and emphysema in the alveoli. Although culture was attempted, isolation was unsuccessful due to gross contamination.

Molecular investigation through PCR provided rapid confirmation of tuberculosis infection. The partial mycobacterial *hsp65* gene, also called the 'Telenti fragment' is conserved in nature and is widely used for successful mycobacterial identification (Devallois *et al*., 1997; Brown-Elliott *et al*., 2018) [3, 1]. The tested lung sample confirmed presence of *Mycobacterium* pathogen showing amplification at 441bp (Fig 5). To further ascertain the origin, insertional sequence *IS6 110* was used for the detection of MTC as *hsp 65* is unable to differentiate the members of MTC from other species of the Genus. Amplification of 123 bp (Fig 6) which confirmed the presence of MTC.

The subject of tuberculosis eradication could be compromised by non-reporting and weak surveillance of tuberculosis cases in other animals including goats. As goats are known to be susceptible to both *M.tuberculosis* and *M. bovis* alike, close observation may be necessary (Crawshaw *et al*., 2008, Rodríguez *et al*., 2011) [2]. Disease monitoring through immunological assays and molecular identifications are vital for understanding the epidemiology of the MTC members as the facilities for confirming through culture is not always available. Mandatory testing and slaughter policy in the country is lacking which adds to the threat of outbreak and spillovers. Strict quarantine practices along with extension activities to create of awareness remains some much needed interventions for effective prevention and control measures.

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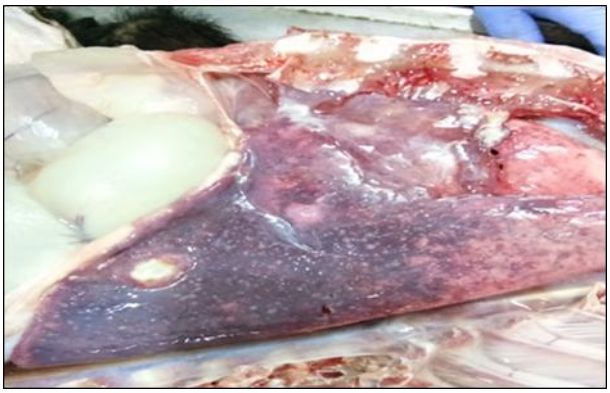

Fig 1: Greyish white nodules in the lung (.5-3mm)

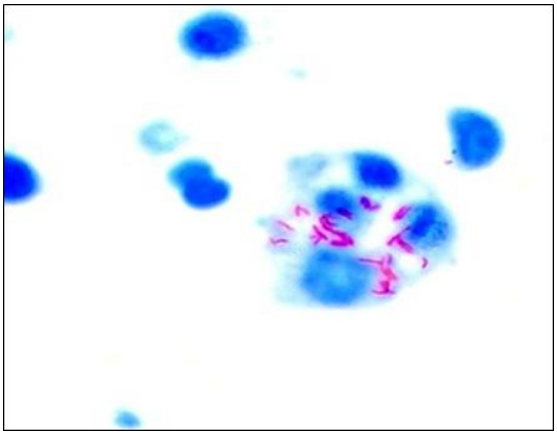

Fig 2: Demonstration of acid fast organisms in impression smear (Z-N stain)

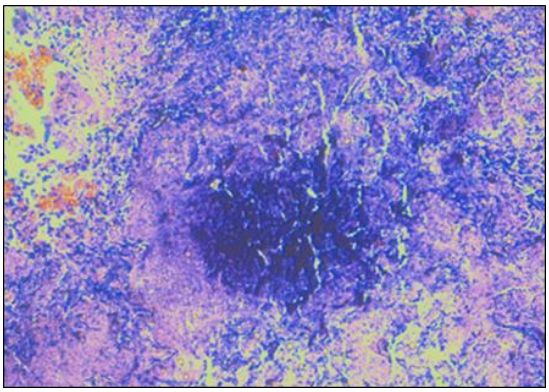

Fig 3: Lung section showing a typical granuloma with central area of calcification, H&E 20X

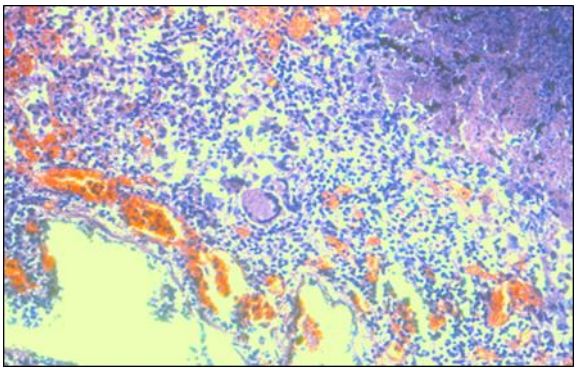

Fig 4: Lung section showing typical Langham's giant cells, H&E 40X

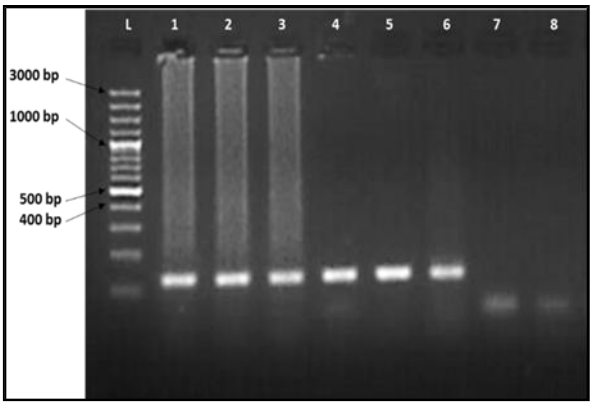

Fig 5: Genus confirmation by *hsp65* gene amplification (441bp). Lane L- Ladder (100plus bp marker). Lanes 1 to 3-Caprine lung sample; Lane 4- *M. bovis*; Lane 5&6- *M. tuberculosis*; Lane 7- *M. vaccae*; Lane 8- Negative control; Lane 9- NTC.

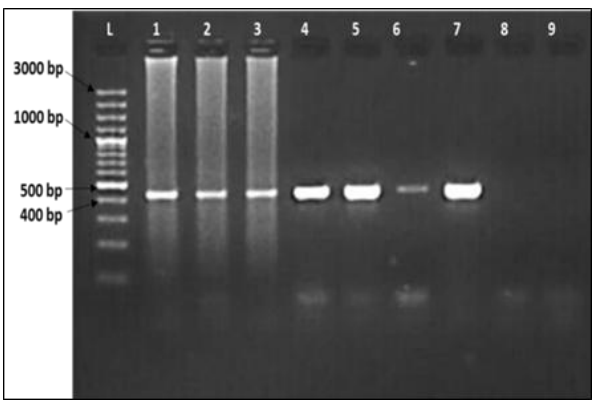

Fig 6: *Mycobacterium tuberculosis* complex (MTC) detection by IS6110 region amplification (123bp). Lane L-Ladder (100bp plus marker). Lane 1 to 3-Caprine lung sample; Lane 4- *M. bovis*; Lane 5&6- *M. Tuberculosis*; Lane 7- *M. vaccae*; Lane 8- Negative control

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