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 this group known as the Mycobacterium tuberculosis Complex (MTC) comprising of M. caprae, M. africanum, M. pinnipedii, M. canetti, M. mungi, M. microti and M. oryogis 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 of hsp65 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. paratuberculosis 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 are reports 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 is almost 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, Ziehl 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 postmortem 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% H2SO4 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 incubated at 37°C for up to 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 IS6 110 region (Eisenach et al., 1990) [8] 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 Beetle 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’ type 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) [1, 11]. The tested lung sample confirmed presence of Mycobacterium pathogen showing amplification at 441 bp (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 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 (100bp plus 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

References