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Molecular detection of Mycobacterium tuberculosis complex in bovine blood samples#

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Abstract

Bovine tuberculosis (bTB), predominantly caused by *Mycobacterium bovis,* is one of the most neglected zoonotic diseases of cattle. The lack of documented information on bTB is one of the most important hurdles in controlling the disease. The present study was carried out to detect the presence of Mycobacterium tuberculosis complex (MTBC) from blood samples of cattle presented for slaughter, using polymerase chain reaction. The study revealed presence of MTBC in seven out of 50 animals (fourteen per cent) with symptoms suggestive of bTB. The detection of the organism emphasises the need of further prevalence studies in Kerala, to implement proper control strategies.

Keywords: *Bovine tuberculosis, MTBC,* IS*6110 PCR*

Bovine tuberculosis (bTB) is a chronic infectious disease of cattle which has high zoonotic impact. *Mycobacterium bovis* belonging to MTBC, is the primary etiological agent of the disease. However, other members of MTBC also cause disease in cattle, the symptoms of which are not substantially different from *M. bovis* infection (OIE, 2009). Increased detection of *M. tuberculosis* in cases of bTB have been reported (Srivastava *et al.,* 2008; Sweetline Anne *et*

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al., 2017). Similarly, tuberculosis in bovines caused by *Mycobacterium orygis* (*M. orygis*) and *Mycobacterium caprae* (*M. caprae*) of MTBC are also gaining importance (OIE, 2009; Refaya *et al.,* 2019).

Despite the high economic impact and zoonotic implications, bTB is one of the most neglected diseases. The lack of documented information about the disease is one of the major hurdles in the control of bTB. In Kerala, MTBC has not been reported till date from cattle. Hence, the present study was carried out to detect the presence of MTBC in blood samples of cattle presented for slaughter in Thrissur district.

Materials and methods

Samples

The blood samples were collected from 50 animals presented for slaughter at the Thrissur corporation slaughter house, Kuriachira, and showing symptoms suggestive of bovine tuberculosis *i.e*. emaciation with or without respiratory signs. The samples were collected a day prior to slaughter in BD vacutainer® EDTA tubes (BD, U.K.) and transported to laboratory in ice for further processing.

Polymerase chain reaction

The DNA extraction from blood and positive control was performed using DNeasy blood and tissue kit (Qiagen, Germany) as per the manufacturer's guidelines. Commercially available Onco-BCG-40 mg injection of Serum Institute of India Ltd. (SII), India was used as the positive control. The DNA pellet was reconstituted in 50 µL elution buffer. Primers used for the PCR were based on previously described sequences (Collins *et al.*, 1993), specific for an insertion element- IS*6110,* found exclusively within the members of *MTBC.*

The forward (5'-GACCAC GACCGAAGAATCCGCTG-3') and reverse (5'-CGGACAGGCCGAGTTTGGTCATC-3') primers were based on sequences at positions 252 to 274 and 674 to 696 of IS*6110*. The PCR reactions were performed using 2X Emerald Amp PCR master mix (Takara Bio, USA) as per the manufacturer's instructions. In brief, the reaction mix comprised of 12.5 µL of master mix, 1 µL each of forward and reverse primers (10 p*M*/µL), 1 µL of DNA template and nuclease free water to make a total volume of 25 µL. The reaction cycle involved initial denaturation of 96 °C for 10 min followed by 35 cycles of amplification consisting of denaturation at 96 °C for 1 minute, annealing at 58°C for 1 minute and extension at 72°C for 1 minute with a final extension at 72 °C for 5 minutes. The final amplified product was identified using submarine agarose gel electrophoresis system using two per cent agarose gel containing ethidium bromide, using Tris Borate EDTA buffer at a voltage of 70 V. The gel was visualised and documented using an automatic gel documentation system (Bio-Rad, USA).

Results and discussion

In the present study, out of 50 blood samples, seven were found to be positive and yielded amplicons of 445 bp, specific for IS*6110* (Fig 1). This is the first report of direct detection of MTBC from blood samples of cattle in Kerala.

Despite the disadvantage of reduced specificity of IS*6110* PCR, several researchers have reported its application in the sensitive detection of MTBC (Collins *et al.,* 1993; Swift *et al.,* 2016; Sweetline Anne *et al.,* 2017).

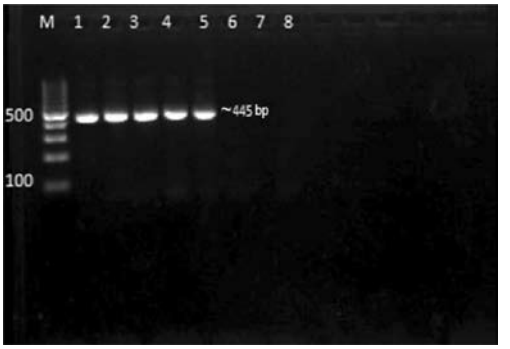

Fig. 1 Detection of MTBC using IS*6110* specific PCR (Representation)

Lane M- 100 bp Molecular marker
L 1: Positive control
L 2-5: Positive samples
Lane 6,7: Negative samples
Lane 8: no-template control

Mycobacterium have been more frequently identified in blood samples of cattle compared to milk and other samples. Cezar *et al.* (2016) reported detection of *M. bovis* from blood samples in two per cent of cattle maintained in 20 properties in Brazil. They suggested blood PCR as a highly sensitive method for detection of members of MTBC, compared to milk samples. Khan *et al.,* (2012) suggested the use of blood samples for molecular identification of *M. bovis* in a study which reported about 54 per cent of buffaloes positive compared to positivity of two per cent using tuberculin test.

In the present study, all the animals were examined post-slaughter and none showed gross macroscopic lesions. The sensitivity of identifying lesions specific for bTB in post mortem was found to be very low (Corner *et al.,* 2012). However, the reports of detection of viable bacteria in blood of animals without any macroscopic lesions (Swift *et al.,* 2016) emphasise the importance of the disease. Isolation of *M. bovis* and *M. tuberculosis* from blood of apparently healthy animals had also been reported (Srivastava *et al.,* 2008), suggesting a silent circulation of live organism in cattle, without any suggestive clinical signs.

Despite several reports of mycobacteremia in bovines (Srivastava *et al.,* 2008; Cezar *et al.,* 2016) and humans (Ahmed *et al.,*1998), the development of disseminated infection and bacteremia in bTB has been a less-studied aspect due to limitations in isolation of the organism by culture method. Though isolation of organism remains the gold standard test for definitive diagnosis of bTB (OIE, 2019), the long incubation period, requirement of ten to hundred organisms in the sample, cumbersome procedure, less sensitivity and requirement of stringent biosafety facilities often limited its use (Romero *et al.,* 1999). Hence, molecular detection techniques like PCR and real-time PCR gains importance owing to the rapidity and sensitivity of the tests to detect MTBC.

Though *M. bovis* had been reported to be the predominant cause of bTB, the presence of other organisms were not excluded, as *M. tuberculosis* (Srivastava *et al.,* 2008; Sweetline Anne *et al.,* 2017), *M. orygis* (Refaya *et al.,* 2019), *M. caprae* and *M. pinnepedi* (OIE, 2009) also had been reported to cause bTB. Hence the positive samples obtained in the present study have to be further analysed for species confirmation. Precise identification of the organism is imperative to obtain a comprehensive knowledge on the organisms causing bTB in Kerala.

Conclusion

Bovine tuberculosis is one of the most important zoonotic diseases in cattle. The present study detected MTBC in the blood samples of seven animals, out of 50 samples subjected to PCR specific for IS*6110*. The molecular detection yielded a per cent positivity of 14, circumventing the laborious pathogen isolation procedure. Though the study was carried out with samples collected from the abattoir, the results are indicative of a possible prevalence of bTB in dairy cattle also. The presence of the organism in blood, as demonstrated in this study, raises concern on zoonosis and risk to humans as well as animals and emphasises the need of large-scale studies on bTB diagnosis for control and eradication of the disease.

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Conflict of interest

The authors declare that they have no conflict of interest

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