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Molecular identification of mycobacterium tuberculosis complex in bovine lymph nodes

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Abstract
Bovine tuberculosis (bTB), caused by members of the Mycobacterium tuberculosis complex (MTBC), poses a significant threat to both cattle and human health. This research employs a molecular approach to detect and identify MTBC isolates from bovine lymph nodes. A combination of culture-based isolation, biochemical tests, and polymerase chain reaction (PCR) analysis was used to enhance sensitivity and accuracy. The study identified MTBC isolates in lymph nodes of infected cattle. The findings underscore the importance of molecular methods in the surveillance and control of bTB.

Keywords: MTBC, lymph node, isolation, PCR

Introduction
Mycobacterium tuberculosis complex (MTBC) is a group of closely related pathogenic bacteria that includes the notorious human pathogen, Mycobacterium tuberculosis (Esteban and Munoz-Egea, 2016) [4]. While MTBC is primarily associated with human tuberculosis, its presence in animals, particularly cattle, holds significant implications for both veterinary and public health (Zhang *et al*., 2022) [15]. Cattle are a recognized reservoir for a subset of MTBC species, such as *Mycobacterium bovis*, which can cause bovine tuberculosis (bTB) (Awah-Ndukum *et al*. 2016) [2]. Importantly, these bacterial species have the potential to cross the species barrier, leading to zoonotic transmission to humans. (Anaelom *et al*., 2010) [1]. Therefore, the surveillance, detection, and identification of MTBC in cattle populations have become pivotal in understanding the dynamics of this pathogen, preventing disease spread, and safeguarding public health (Humblet *et al*., 2009) [6].

Bovine tuberculosis, caused by MTBC members including M. bovis, remains a serious concern for the global livestock industry due to its detrimental effects on cattle health and productivity (OIE, 2009) [7]. Additionally, the potential transmission of MTBC from cattle to humans underscores the importance of comprehensive studies aimed at identifying and characterizing MTBC isolates in bovine populations (Regassa *et al*., 2010; Tenguria *et al*., 2011) [9, 13].

Traditionally, the detection of MTBC in cattle relied on culture-based methods and biochemical tests, which are time-consuming and often lack the desired sensitivity and specificity (Roring *et al*., 2000) [10]. However, recent advances in molecular biology and microbiology have offered more rapid and precise techniques for the identification of MTBC isolates (Ramadan *et al*., 2012) [8].

Among these techniques, polymerase chain reaction (PCR)-based assays have emerged as valuable tools for the detection and molecular characterization of MTBC in various sample types, including lymph nodes -the primary sites of MTBC infection in cattle (Taylor *et al*., 2001; Wards *et al*., 1995) [12, 14].

This research study presents an in-depth exploration of the molecular identification of MTBC in bovine lymph nodes. We employ a combination of culture-based isolation, biochemical tests, and PCR analysis to enhance the sensitivity and accuracy of MTBC detection and species-level identification. By dissecting the genetic profiles of MTBC isolates from cattle lymph nodes, we aim to contribute to a better understanding of the epidemiology of bovine tuberculosis, its potential impact on human health, and the development of targeted control measures.

Materials and Methods

Sample Collection

A total of Fifty lymph node samples were collected from cattle exhibiting clinical signs suggestive of bTB. The samples included retropharyngeal, mediastinal, and mesenteric lymph nodes, which are known sites of MTBC infection. Samples were also selected based on palpability and, when necessary, aided by diagnostic imaging techniques.

Isolation and Culture

Lymph node samples were decontaminated with 0.75% HPC as per (Nasr *et al*., 2016) and homogenized, and cultured on Lowenstein-Jensen (LJ) medium. Cultures were incubated at 37 °C for eight weeks, with regular monitoring for growth.

Biochemical Tests

Colonies displaying characteristics suggestive of mycobacteria were subjected to biochemical tests, including niacin accumulation, nitrate reduction, and catalase activity, to confirm the presence of MTBC and were also subjected to acid fast (Ziehl-Neelson) staining.

DNA Extraction

DNA was extracted from confirmed MTBC isolates using HiPure DNA Purification kit (Hi media).

PCR Analysis

PCR was performed using specific primers, INS1 (forward) 5' CGTGAGGGCA TCGAGGTGGC 3' /INS2(reverse) 5' GCGTAGGCGTCGG A 3' targeting the insertion sequence IS6110 for 245 base pair fragments in MTC and PCR was performed as per (Filia *et al*., 2016) [5]. For amplification reaction volume of 25 µl was made containing 12.5 µl of Taq PCR Master Mix, 1 µl of forward primer (10 pmol/µl), 1 µl of reverse primer (10 pmol/µl), 5.5 µl nuclease free water and 5 µl of DNA template of the sample. PCR cycling conditions included an initial denaturation at 94 °C for 5 mins, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 62 °C for 1 min, extension at 72 °C for 1 mins and final extension at 72 °C for 7 mins. Polymerase chain reaction products were analysed by gel electrophoresis.

Results

The lesions observed in the affected lungs or lymph nodes of the suspected carcass were the presence of circumscribed yellowish white lesions and in some miliary lesions were observed. Majority of lesions were found to be calcified. (Fig. 1)

Detection of MTBC Isolates

Of the 50 lymph node samplessubjected to isolation, 34 samples yielded positive cultures on LJ medium (Fig.3) indicating the presence of mycobacteria. Subsequent biochemical tests and Acid-fast staining (Fig.4) confirmed that these isolates were members of the Mycobacterium tuberculosis complex.

PCR Confirmation

PCR analysis using IS6110-specific primers provided further confirmation of MTBC presence in all 34 isolates. Amplification products were consistent with the expected size of 245 bp indicating the presence of MTBC DNA(Fig.5).

Discussion

The molecular approach employed in this study successfully identified and confirmed the presence of Mycobacterium tuberculosis complex isolates in bovine lymph nodes. This method, combining culture-based isolation, biochemical tests, and PCR analysis, enhanced the sensitivity and accuracy of MTBC detection, addressing the limitations of traditional diagnostic methods.

These findings highlight the significance of molecular techniques in the surveillance and control of bovine tuberculosis. Early and accurate detection is critical for implementing effective control measures, safeguarding cattle health, and reducing the risk of zoonotic transmission to humans (Bhembe *et al*., 2014) [3]. Additionally, the ability to identify MTBC species, such as *M. bovis*, is vital for understanding the epidemiology of bTB and tailoring control strategies accordingly (Taylor *et al*., 2007) [11].

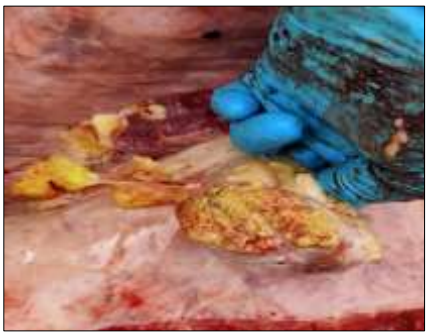


Fig 1: Mediastinal lymph node with Caseous severe granulomatous lymphadenitis

Fig 2: Enlarged lymph node

Fig 3: Growth of MTC cultures on LJ media

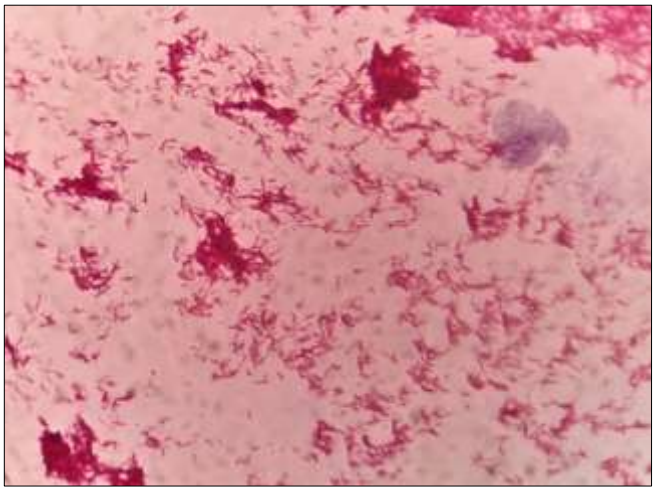
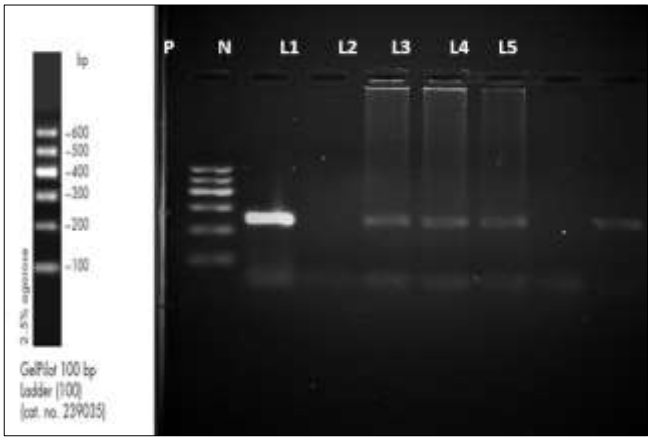


Fig 4: Clumps of acid-fast bacilli



L: DNA 100bp ladder
P: Positive control
N: Negative control
L1-L5: samples

Fig 6: Polymerase chain reaction based on DNA extraction from Lymph nodes

Conclusion

In conclusion, this research demonstrates the value of a molecular approach in the detection and identification of MTBC isolates from bovine lymph nodes. Such methods have the potential to revolutionize bTB diagnostics and contribute to the global effort to combat this complex disease. Future research should focus on further refining these molecular techniques and exploring their application in larger-scale surveillance efforts.

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