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Research article

Direct DNA extraction to detect *Mycobacterium bovis* from the lungs of buffaloes positive to intradermal tuberculin testing

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Abstract

Bovine tuberculosis (bovine TB), caused by *Mycobacterium bovis*, is an important significant zoonotic disease. The infection course is usually chronic, which directly affects animal health and production. Bacterial culture, which is time-consuming, is the gold standard of diagnosis. Nowadays, rapid molecular techniques such as polymerase chain reaction (PCR) are used for the rapid identification of bovine TB. However, a convenient and rapid DNA extraction method to detect mycobacterial organisms directly from tissue samples is understudied. In this study, tissue samples were collected from buffaloes that were positive to a single intradermal test. Three different direct DNA extraction methods were undertaken to find a simple procedure for rapid detection of *M. bovis* from tissue: 1) a tissue genomic extraction kit; 2) a combination of enzymatic (lysozyme) extraction and the tissue genomic extraction kit; and 3) boiling the tissue for 30 min before using a combination of lysozyme extraction and the tissue genomic extraction kit. The DNA samples were then used to identify *M. bovis* using PCR. The greatest yield of DNA concentration was obtained from the combination of enzymatic extraction and the tissue genomic extraction kit. In addition, this method also provided the highest percentage of positive results for *M. bovis*.

Introduction

Tuberculosis, commonly known as TB, is one of the most life-threatening infectious diseases and is distributed worldwide, especially in developing countries (World Health Organization, 2018). Bovine TB is considered as an important zoonotic disease transmitted from an infected cow to humans through inhalation and ingestion of contaminated or unpasteurized milk or milk products (The Center for Food Security & Public Health, 2009). The most common cause of TB in humans is *Mycobacterium tuberculosis*; however, *M. bovis* is a main cause of bovine TB (World Health Organization, 2018). These two mycobacterial species are members of the *M. tuberculosis* complex (MTC), for which infection can occur in a wide range of domestic animals, wildlife animals and humans (Wayne and Kubica, 1986). A routine technique that has been used to diagnose TB in cattle is a single intradermal tuberculin test (IDTT). Although the specificity and sensitivity of the IDTT is generally moderate to high (Francis et al., 1978; Wood et al., 1991), a false-positive reaction and a time-consuming retest within 60–90 d are two disadvantages of the IDTT. The interferon gamma (IFN-γ) assay is another ante-mortem diagnostic procedure to confirm bovine TB (Liébana et al., 1995). However, the gold standard method to diagnose bovine TB infection is a culture of causative mycobacterium on a specific medium. This subculture technique requires 8–12 wk for the growth of the isolates and an additional 2–3 wk for biochemical testing to identify the isolates. Moreover, the sensitivity of culture is not absolutely correct and false negative culture results may occur because it requires viable organisms. Unsuitable handling of tissues will cause some

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