ISSN: 2574-1241
DOI: 10.26717/BJSTR.2018.11.002145
Xiangmei Zhou. Biomed J Sci & Tech Res

BIOMEDICAL Journal of Scientific & Technical Research

Mini Review Open Access

PCR Methods for Detecting Bovine Respiratory Pathogens

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Received: October 30, 2018; **Published:** December 05, 2018

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Abstract

Bovine Respiratory Disease (BRD) is an important disease in cattle production, BRD may be associated with one or more pathogens, of which Mycobacterium bovis, Mycoplasma bovis, and Klebsiella pneumoniae are three important pathogens. Fast and accurate detection methods are important for preventing and controlling BRD. This review focuses on the PCR detection methods for the above three pathogens in recent years.

Introduction

Bovine Respiratory Disease (BRD) is an important disease in cattle production, causing serious economic losses world widely [1]. The occurrence of BRD is a combination of multiple factors and may be associated with one or more pathogens [2]. Among them, *Mycobacterium bovis*, *Mycoplasma bovis*, and Klebsiella pneumoniae are three important pathogens. *Mycobacterium bovis* can infect many kinds of animals. Besides cattle, there are 50 kinds of vertebrates such as humans. Sick animals showed a gradual loss of body weight, anemia and cough. Cattle with active tuberculosis are the main source of infection. Their respiratory tract carrys bacteria, which are excreted from coughing and sneezing [3]. *Mycoplasma bovis* is one of the main pathogens involved in cattle pneumonia. It was found that 5.5% of the nasal swabs from cattle with respiratory symptoms were positive for *Mycoplasma bovis* [4]. Klebsiella pneumoniae, an important conditional pathogen, mainly exists in the intestine, respiratory tract and urogenital tract [5]. The incidence of respiratory and urinary tract is the highest. Aslan et al. isolated bacteria from bovine upper respiratory tract infections and found that Klebsiella pneumoniae accounted for 20% [6].

PCR Detection

Polymerase Chain Reaction

PCR technology is a molecular biotechnology in which DNA of pathogenic microorganisms is expanded to conventional detectable levels *in vitro*. Quan Z et al. designed a multiplex PCR with primers targeting the 16S rRNA, Rv3873 and a 12.7-kb fragment in the genomes of a Mycobacterium tuberculosis complex to differentiate *Mycobacterium bovis* from Mycobacterium tuberculosis and NTM species [7]. Gioia et al. developed and validated a multitarget PCR assay that can discriminate between Acholeplasma and Mycoplasma and identify Mycoplasma bovis [8]. Turton et al. identified and typed Klebsiella pneumoniae by PCR using capsular type-specific, variable number tandem repeat and virulence gene targets [9]. Fonseca et al. established a one-step multiplex PCR to identify klebsiella pneumoniae, klebsiella variicola and klebsiella quasipneumoniae in the clinical routine [10].

Quantitative Real-Time PCR

Quantitative Real-time PCR technology can achieve quantitative analysis, and it is more specific and sensitive than conventional PCR. Choi Y et al. developed a real-time PCR targeting 16S ribosomal RNA for the detection of Mycobacterium tuberculosis complex [11]. Sales et al. developed and validated two real-time PCRs targeting the PE-PGRS 20 gene and the region of difference 4 (RD 4) for the characterization of *Mycobacterium bovis* isolates. The qPCR for PE-PGRS 20 had 91% efficiency and a detection limit of 0.32 ng. The qPCR for RD4 had 100% efficiency, and a detection limit of 4 pg [12]. Cezar et al. developed a qPCR targeting the region of RD4, which showed that 0.25% milk and 2% blood samples were positive for *Mycobacterium bovis* [13]. Fu-Xiang et al. developed a TaqMan real-time PCR for detection of Klebsiella pneumoniae, which could be applied for early diagnosis of Klebsiella pneumoniae infection [14]. We developed a TaqMan-based multiplex real-time PCR assay primer and TaqMan probes were designed based on the specific 229 bp sequence of Mycobacterium bovis, the uvrC gene

of *Mycoplasma bovis* and the khe gene of Klebsiella pneumoniae. The assay sensitivity was 10 copies/μL. 37 bovine nasal swabs collected from cattle were identified, of which 21.62% (8/37) was Mycoplasma bovis-positive, 18.92% (7/37) was Klebsiella pneumoniae-positive, none (0/37) was Mycobacterium bovis-positive. However, *Mycobacterium bovis* was detected in nasal swabs of cattle with symptoms of respiratory disease.

Summary

PCR detection technology is a sensitive, specific and fast method for the detection of BRD. We believe that the establishment of a TaqMan-based multiplex real-time PCR for the simultaneous detection of *Mycobacterium bovis*, *Mycoplasma bovis*, and Klebsiella pneumoniae can contribute to the early diagnosis and control of BRD.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2018.11.002145

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