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Detection of *Mycoplasma hyopneumoniae* and Porcine Reproductive and Respiratory Syndrome in Clinical Samples by Polymerase Chain Reaction

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Abstract

The aim of this study was to detect Swine Enzootic Pneumonia (SEP) and Porcine Reproductive and Respiratory Syndrome (PRRS) from clinical affected lung samples with PCR technique. In this technique three primers set used for *M. hyopneumoniae* detection were Cai (Forward: GAG CCT TCA AGC TTC ACC AAG A: Reverse: TGT GTT AGT GAC TTT TGC CAC C), Baumeister (Forward: TAG AAA TGA CTG GCA GAC AA: Reverse: GAG GCT TGA TTT TGG AGT C) and Caron (Forward; GAC CCG ATG AAA CCT ATT AAA ATA GAC: Reverse; GAA GCG AAA TTA AAT ATT TTT AAT TCG ATA CTG). For the detection of PRRS virus the primer sets used were Oleksiewicz (Forward: GAA CCT GCC CAI CAC G: Reverse: TAC CCC TAA TTG AAT AGG GGA) and Suárez (Forward: GGG AAT GGC CAG CCA GTC AAT CAA CTG T: Reverse: TGT AGA AGT CAC GCG AAT CAG GCG CAC T).Nine pigs aged between 6-10 weeks were collected from farms in Selangor, Malaysia. One healthy pig and 8 other pigs with clinical signs of respiratory distress problem were sacrificed and lung bronchoalveolar lavage samples were obtained. Healthy pigs were selected as negative control while samples were harvested from 4 pigs with suspected SEP and 4 with respiratory problems for *M. hyopneumoniae* and PRRS virus detection. Base on the result, the Caronand Caiprimer sets were able to detect SEP from the affected lungs. For PRRS virus, RNA was extracted using easy-BLUE™ Total RNA Extraction Kit and converted it to cDNA with Maxime RT PreMix Kit. The Oleksiewicz primer set was ideally suited for the detection of PRRS virus.

Keywords: Swine enzootic pneumonia, PRRS, PCR, primer

Introduction

Mycoplasma pneumonia, also known as Swine Enzootic Pneumonia (SEP) which cause by *M. hyopneumoniae,* is a highly contagious disease of pigs. The disease occurs worldwide and considers one of the most important diseases. It will disrupt the feed conversion ratio (FCR) and average daily weight gain of the affected pigs. In addition, about 40 to 80% of lung lesion at Malaysia abattoir were related to SEP infection. Swine Enzootic Pneumonia can be considered endemic in most of the farms in the country. The organism normally inhabits the respiratory tract of pigs and can be transmitted between pigs by direct contact. The severity of disease varies from farm to farm and this is influenced by factors such as husbandry, farm biosecurity, pig density and secondary bacterial infections. Porcine Reproductive and Respiratory Syndrome (PRRS) is an important viral disease in the pig industry worldwide. This disease, especially that caused by the highly pathogenic PRRS virus, has caused great losses to the pig production worldwide. For the diagnosis of the disease, bacterial culture and isolation are the most common used diagnostic methods; however this method is not ideal for *M. hyopneumonaie* because it is very fastidious and difficult to culture. With the PRRS viral pathogen, a series of viral passage is needed before it can be isolated and identified. Therefore, the objectives of this study were to select suitable a primer set using an optimized PCR technique as an alternative for traditional diagnostic procedure for SEP and PRRS.

Materials and Methods

Nine pigs aged between 6 to 10 weeks were collected around farms in Selangor. One healthy pig (as negative control) and 8 other pigs with clinical signs of respiratory distress problem were sacrificed and postmortem performed and lung samples bronchoalveolar lavage fluid collected for analyses. The DNA was extracted from the lung samples using i-genomic CTB DNA extraction Mini Kit (iNtRON Biotechnology) according to the method described by the manufacturer. RNA was extracted using easy-BLUE™ Total RNA Extraction Kit (iNtRON Biotechnology) according to the manufacturer protocol. cDNA was synthesis by using Maxime RT PreMix Kit, (iNtRON Biotechnology). In this technique three primers set used for *M. hyopneumoniae* detection were Cai (Forward: GAG CCT TCA AGC TTC ACC AAG A: Reverse: TGT GTT AGT GAC TTT TGC CAC C) (Cai *et al*., 2007), Baumeister (Forward: TAG AAA TGA CTG GCA GAC AA: Reverse: GAG GCT TGA TTT TGG AGT C) (Baumeister *et al*., 1998) and Caron (Forward; GAC CCG ATG AAA CCT ATT AAA ATA GAC: Reverse; GAA GCG AAA TTA AAT ATT TTT AAT TCG ATA CTG) (Caron *et al*., 2007). For the detection of PRRS virus the primer sets used were Oleksiewicz (Forward: GAA CCT GCC CAI CAC G: Reverse: TAC CCC TAA TTG AAT AGG GGA) (Oleksiewicz *et al*., 1998) and Suárez (Forward: GGG AAT GGC CAG CCA GTC AAT CAA CTG T: Reverse:

TGT AGA AGT CAC GCG AAT CAG GCG CAC T) (Suárez *et al*., 1993).The amplification was performed in the Swift™ Maxi Thermal Cyclers, (ESCO) and the samples were subjected to gel electrophoresis for band size detection.

Result and Discussion

For SEP, all 3 set of primers used in the study were able to detect the positive control which was extracted from a commercial vaccine. At 5ºC above and below T_m of primer did not show any significant different in result. The Cai primer set targets the 16s rRNA gene in small 30S ribosomal subunit. Based on the result, only 50% of samplesfrom pigs with typical *M. hyopneumoniae* lesion were positive. The Baumeister primer was used to detect *M. hyopneumoniae* in the bronchoalveolar lavage fluid of pigs. However, none of the lung samples produced specific bands. The Caron primer set targets antigenic determinants of 36 gene from cytosolic protein. All 4 samples with typical lung lesions were positive for *M. hyopneumoniae* infection, producing the 948 bp band (Figure. 1). From the results, the Caronprimer set was able to detect all *M. hyopneumoniae*-positive clinical lung samples. However, the bands obtained were very weak suggesting that the PCR protocol need to be optimized further. Meanwhile, for PRRS virus, the Oleksiewicz primer set, which targets the ORF 7 gene, was able to detect positive controls using the vaccine as DNA template. While Suárez primer set, which targets ORF 7 encoding nucleocapsid protein was not suitable as the positive control. For Oleksiewicz primer set, 4 out of 5 lung samples from pigs with respiratory distress problems produced a 660 bp band and some non specific bands. This suggests that the Oleksiewicz primer set still needs to be optimized further to ensure reliability of results. The Suárez primer set was unable to form the positive control with extracted DNA from vaccine, suggesting that this primer set was not ideal for the detection of PRRS virus.

Conclusion

Based on the result, the Baumeister primer set was not an ideal for the detection of *M. hyopneumoniae* in clinical lung samples. Further study was needed to determine the effectiveness of this primer set for *M. hopneumoniae* detection in the porcine bronchoalveolar lavage fluids. This study also demonstrated that the Cai and Caron primer sets were able to detected SEP in clinical lung samples. The Baumeister primer set was more ideal for *M. hopneumoniae* detection in clinical lung samples. For PRRS, the Oleksiewicz primer set was more ideally suited for PRRS virus detection in lung samples, while the Suárez primer set did not produce any result when the PRRS vaccine was used as the DNA template.

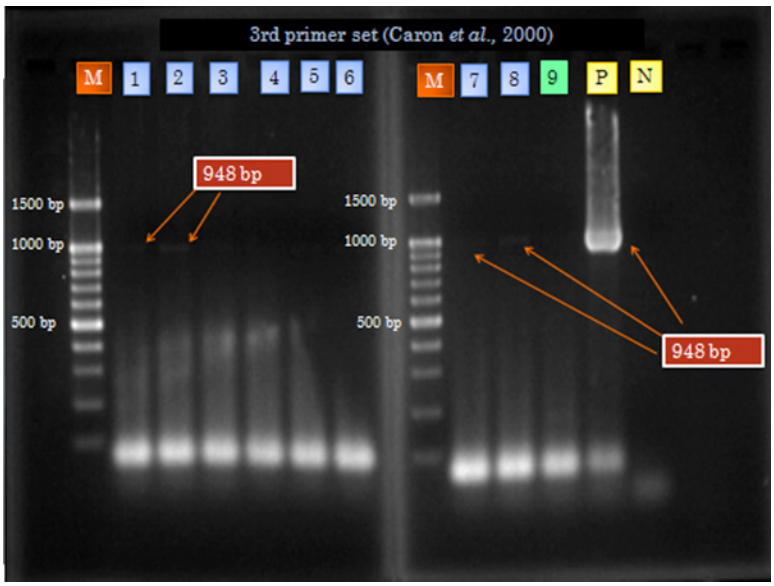

Figure 1. Electrophoresis of polymerase chain reaction product of porcine lungs samples. Primer set [Caron (Forward; GAC CCG ATG AAA CCT ATT AAA ATA GAC: Reverse; GAA GCG AAA TTA AAT ATT TTT AAT TCG ATA CTG)]. M is 100 bp BLUE extended DNA Ladder. Columns 1, 2, 7, and 8 were sample from pig withrespiratory distress and gross lung lesions. Columns 3, 4, 5 and 6 are samples from pigs with respiratory distress without clearly demarcated lung lesions. Column 9 is form from a clinical healthy pig. P = positive control with DNA from vaccine; N = negative control with distilled water. Columns 1, 2, 7, 8 were positive control showing the 948 bp band.

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