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Detection of Glasser's Disease in Clinical Samples using Polymerase Chain Reaction

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Abstract

Glasser's Disease is one of the porcine common respiratory problems in our country. Glasser's disease is caused by *Haemophilus parasuis* (HP). The HP has a wide range of antibiotics sensitivity and can be treated during early infection; therefore early diagnosis is very important. In the past, diagnosis was based on history, clinical signs and postmortem lesions, then confirm by bacteria isolation and identification. These traditional microbiology methods are time-consuming and labourious. Polymerase chain reaction (PCR) is one of the methods that provide rapid and accurate diagnosis. The procedure can be completed within 1 to 2 days and only require one trained personal to perform.Currently, there is limited publish articles on PCR test for the diagnosis of Glasser's Disease in Malaysia. For the analysis 2 pairs of primers set for HP were selected; HPS1-forward (5' AGT ATG AGG AAG GGT GGT GT 3') and HPS1-reverse (5' CGT TTC GTC ACC CTC TGT GT 3') and HPS2-forward (5' TAG AAA AAA TCT TTA ATT G 3') and HPS2-reverse (5' CAC CAT AGA AAC TTC TTT TC 3'). Lung tissues, pericardial swabs and thoracic swabs samples were collected from farms in Sepang, Selangor, Malaysia. The PCR test was carried after some modifications and optimisation. Both HP primers able to detect positive clinical lung samples and can be further developed as PCR diagnostic tool in our country.

Keywords: Glasser's Disease, Polymerase Chain Reaction

Introduction

H. parasuis infection is costly to the pig industry because of it causes high mortality (Maclnnes *et al*., 2008). The most characteristic clinical signs of the infection are fibrinous polyserositis, polyarthritis and meningitis. The affected pigs are the 4 to 6 week age group in the nursery, and they die at 2 days post-infection with sign

of acute septicaemia (Rapp and Gabrielson, 1992).The problem of *H. parasuis* infection affects many countries including China, Japan, USA, Germany, Spain and Australia. Therefore, there is a need for correct diagnosis of the disease to reduce losses through the application of appropriate control measures (Oliveira and Pijoan, 2004). Early diagnosis of the disease is important because the causative agents are sensitive to a wide range of antibiotics. If treatment is given at early stage, the disease is curable. The conventional method of diagnosis of this disease is isolation of the causative agent, but this has been always difficult time-consuming because of the fastidious nature of the organisms. Polymerase chain reaction offers and advantage over the convention methods because of its rapidity and accuracy in the diagnosis diseases. In this study, the main objective was to determine suitable PCR primers set for the detection of HP in swine samples from farms in Malaysia.

Materials and Methods

Clinical samples

A total of nine lung samples were collected from postmortem piglets aged from 5 to 10 weeks old. One clinically healthy pig with no abnormal lung lesion was sacrificed as negative control. Lung tissues, pericardial and thoracic swabs samples were collected from farms in Sepang, Selangor, Malaysia. Two grams of lung tissues, which were obtained from naturally infected pigs were macerated with scissors then grinded by using mortar and liquid nitrogen. Then, 25 mg of ground tissues were transferred into 1.5 mL tube with spatula. The tissue samples were process according to manufacturer protocol (i-genome CTB DNA Extraction Mini Kit, Intron Biotechnology).

PCR Primers

Two set of primers were selected for this study from published articles. The first set of primers chosen is HPS1-forward (5' AGT ATG AGG AAG GGT GGT GT 3') and HPS1-reverse (5' CGT TTC GTC ACC CTC TGT GT 3'), by Oliveira *et al*. (2001).This primer set is widely used. The predicted PCR product using this primer set is 821bp. This set primer targets *H. parasuis* species-specific regions 16S small subunit ribosomal RNA gene sequence. The second set of primers is HPS2-forward (5' TAG AAA AAA TCT TTA ATT G 3') and HPS2-reverse (5' CAC CAT AGA AAC TTC TTT TC 3') (Lu *et al*., 2010). These primers target the ompA gene of the outer membrane of HP. The predicted PCR product using this primer is 1104bp in size. This is the latest primers set, which is created for genotyping purpose.

PCR Conditions

The PCR was carried out using the manufacturer commercial kit (i-Taq ™ DNA Polymerase, Intron). For the both set of primers, 25 μL reaction mixture containing 1 μL of extracted DNA templates, 0.5 μL of each 20 μM of primer, 0.5 μL of i Polymerase (5 U/μL), 2.5 μL 10x PCR buffer, 2.5 μLdNTP mixture (2.5 mM each) and 17.5 μL of double distilled water. The PCR was performed for 35 cycles consisting of denaturation for 1 min at 94°C, annealing for 1 min at 59°C, and extension for 1 min at 72°C using a thermal cycle (Swift maxi®, ESCO), lastly final extension for 5 min at 72°C.

Electrophoresis and Imaging

Agarose gel (1.5%) was made by mixing 1.5 g of agarose gel powder (Agarose D-1, Low Electeoendomosis, Pronadisa, CONDA) in a 100 mL bijour bottle with 1% Tris-acetate-EDTA (TAE) to 100 mL. For primers HPS2, 2% agarose gel is used. Agarose gel is placed in the gel holder tank and submerged with 1% TAE buffer. Seven microlitres of 100bp marker (100 bp BLUE extender DNA ladder, Bioron) is loaded into the first well, while for HPS2, 1 kbp marker was used (1k bp ready-to-use ladder, Bioron). Two microliters loading dye (6x DNA loading dye, Fermentas) were mixed well with 8 μL of PCR product. The PCR products were run for 1 h at 66V. Subsequently, the gel was stained with ethidium bromide (BOP BASIC) solution for 30 min. The gel was placed under a UV gel imaging capturing machine (U: Genius, Syngene) to visualize the desire band size. The images were captured and recorded.

Results

With the two primers of HPS1-forward and HPS1-reverse, amplification of 16s rRNA *H. parasuis* species-specific gene sequence from tissue sample (Ts)1, 3, 5 and 8 produced a band at 821 bp (Figure 1).Ts 1 and 3 showed more prominent bands while bands of Ts 5 and 7 were slightly degraded.

The HPS2 primers set were designed to amplify o*mpA* gene of *H. parasuis* reference strains with specific fragment at about 1104 bp. The PCR results of HPS2 revealed that only Ts 1 and 3 were positive for *H. parasuis* (Figure 2), with Ts 1 showing prominent band and Ts 3 showing weak band.

For comparison, Ts 1, 3, 5 and 8 were amplified with HPS1 and HPS2, and then loaded into one gel. From the results, HPS2 was only able to detect 50% of the positive samples detected by the HPS1 primer set (Figure 3).

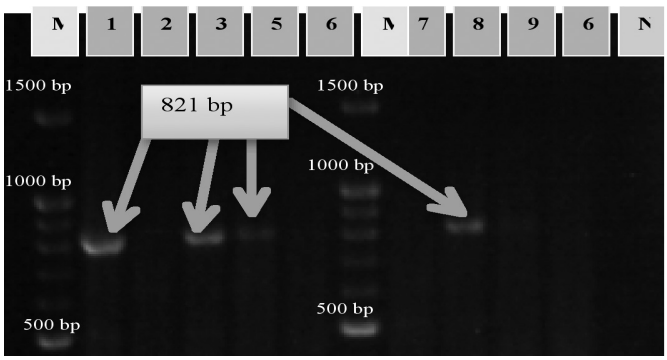

Figure 1. The PCR results of HPS1 from all lung tissue samples. M, 100 bp molecular marker. N=negative control with double distilled water.

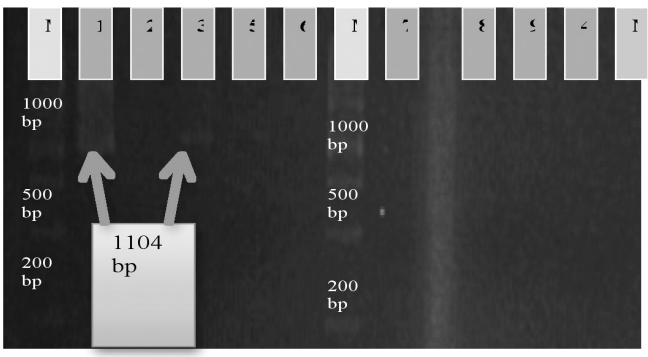

Figure 2. The PCR results of HPS2 from all lung tissue samples. M, 100 bp molecular marker. N= negative control with double distilled water.

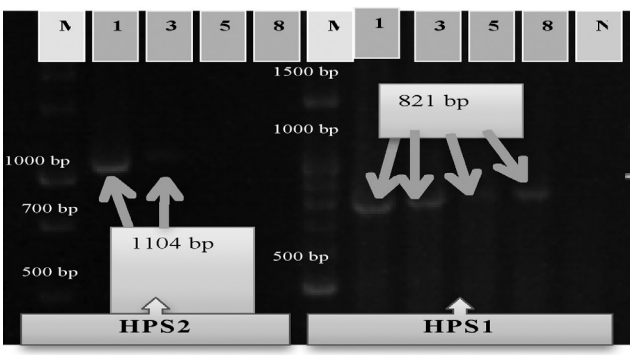

Figure 3. Ts 1, 3, 5 & 8 were amplified by HPS2 (lanes 2-5) and HPS1 (lanes 7-10). M for HPS2, 1k bp molecular marker.M for HPS1, 100 bp molecular marker. N= negative control with double distilled water.

Discussion

According to Oliveira *et al*. (2001), HPS1 could detect a minimum concentration of 1 x 102 cfu/mL of *H. parasuis* and 0.69 pg of pathogen DNA. This indicates that the HPS1 primer set is sensitive in detecting *H. parsuis* in pure culture and clinical samples. Our study suggests that the HPS2 primer setis less sensitive than the HPS1 primer set in the detection of *H. parasuis* samples. This is shown by the fact that HPS1 could detect Ts 5 and 7 while HPS2 could not. From the results, both HPS1 and HPS2 were able to detect H. parasuis in clinical samples and they did not amplify DNA from healthy pig and negative controls.

Conclusion

The results suggest that HPS1 and HPS2 primers are both suitable to be used as diagnostic tool in the detection of *H. parasuis* in clinical samples of pigs that show signs and lesions of the infection. The HPS1 primers are more sensitive and useful than the HPS2 primers for screening of Glasser's Disease.

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