

Identification and differentiation of *Haemophilus parasuis* sero-nontypeable strains using a species-specific PCR and the digestion of PCR products with Hind III endonuclease

Bob Chang Lin, DVM, MS, PhD
MVP Laboratories, Inc., Ralston, Nebraska

Abstract

Fifty-three sero-nontypeable strains, thirty-two serotypeable, and seven standard strains of *Haemophilus parasuis* had been examined with a species-specific PCR to confirm the identifications. The PCR amplicon of each strain was further digested with an endonuclease (Hind III) to produce DNA fragments, which allowed the fifty-three sero-nontypeable strains to be divided into 8 distinct restriction fragment length polymorphism (RFLP) patterns. The PCR-RFLP combination adopted in this study provided us with a molecular approach to the identification and differentiation of *H. parasuis* sero-nontypeable field strains.

Brief communications

Haemophilus parasuis has emerged as one of the most important pathogens in isolated and immune-naïve high health status pig herds during the past decade. So far, 15 serovars are described, and the most prevalent serovars in the United States are 2,4,5,12,13 and 14¹. A rather high percentage of nontypeable serovars was also noted^{1,2}. However, very little has been known about the virulence factors of *H. parasuis* field strains isolated from sick pigs. According to some previous studies, the virulence and antigenicity of *H. parasuis* field isolates might vary between different serovars and even between different strains of the same serovar². For this reason, fully relying on serotyping may not be adequate in an attempt to design appropriate immunoprophylactic measures.

Identification and confirmation of *H. parasuis* field strains can be difficult and cumbersome if the field isolates cannot be serotyped and further biochemical tests are needed. Recently, a species-specific PCR test for detection of *H. parasuis* has been described by Oliverella et al³, which will greatly improve the diagnosis of *H. parasuis* sero-nontypeable strains.

In this study, 53 sero-nontypeable strains, 32 serotypeable strains, and 7 standard strains of *H. parasuis* were examined with the species-specific PCR assay to confirm their identification. This PCR assay allowed the amplification of an 821 bp product, which was further digested with Hind III endonuclease to produce a DNA fingerprint. The reference strains were seven *H. parasuis* isolates of known serovars that were obtained from Dr. Rapp-Gabrielson. The field strains were isolated from pigs with systemic infection and sent to MVP Laboratories for diagnosis. All field strains were serotyped as described^{1,4}.

DNA extraction: Briefly, *H. parasuis* strains were grown on Frey chocolate agar for an appropriate time to ensure purity. Single colonies were boiled in 25 ul of sterile PBS (pH 7.2) in a screw capped tube for 10 minutes and then placed at -20°C for 10 minutes. The tube is then centrifuged at 14,000 × g for 3 minutes and the supernatant is removed and used as the DNA template.

PCR assay: The oligonucleotide primers that were used in this study are listed in Table 1. The PCR reaction mix for one test sample contains 5 ul of 10× PCR buffer, 4.8 ul of dNTP (each at a concentration of 2.5 mM), 1.0 ul of forward and reverse primers (each at a concentration of 40 uM), 4.0 ul of 25 mM magnesium chloride, 0.5 ul of Taq DNA polymerase (5U/ul), 29.7 ul of deionized water, and 5.0 ul of DNA template.

The PCR conditions are as follows: One cycle of 5 minutes at 94°C, followed by 30 cycles of 0.5 minute at 94°C, 0.5 minute at 59°C, and two minutes at 72°C. Then one cycle of 5 minutes at 72°C. Amplified products are analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide and recorded by using UV transillumination and Polaroid film.

DNA fingerprinting: Purification of the PCR product is performed using Wizard PCR Preps DNA

Table 1: Species-specific primers used to amplify DNA from *Haemophilus parasuis*

HPS-forward 5'-GTG ATG AGG AAG GGT GGT GT-3'
HPS-reverse 5'-GGC TTC GTC ACC CTC TGT-3'

Figure 1: The PCR results using DNA extracted from the culture of 6 sero-nontypeable *H. parasuis* strains. The expected 821 bp fragment of *H. parasuis* species-specific gene was amplified in all of the 6 strubs.

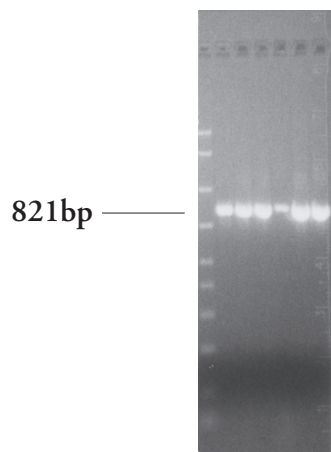
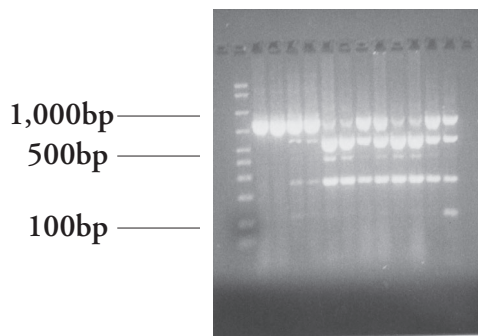


Figure 2: Agarose gel electrophoresis of fragments produced by Hind III digestion of PCR-amplified DNA. Lane 1 and lane 15, empty; lane 2, DNA ladder (50-2,000bp); lane 3, nontypeable, RFLP type 1A; lane 4, nontypeable, RFLP type 1A; lane 5, nontypeable, RFLP type 1B; lane 6, nontypeable, RFLP type 1B; lane 7, nontypeable, RFLP type 2B; lane 8, nontypeable, RFLP type 2B; lane 9, nontypeable, RFLP type 3A; lane 10, nontypeable, RFLP type 3A; lane 11, nontypeable, RFLP type 2B; lane 12, nontypeable, RFLP type 2B; lane 13, nontypeable, RFLP 3A; lane 14, nontypeable, RFLP type 4.



Purification System (PROMEGA, Madison, WI), as described by the manufacturer. About 15ul of the purified PCR product was incubated with 1.5ul of Hind III (15U/ul) at 37°C for two hours. The digested products were analyzed by electrophoresis in 2% agarose gel as described.

All of the 85 field strains and 7 standard strains were found to have the expected 821 bp fragment of the *H. parasuis* species-specific gene (Figure 1). The pattern of the DNA fragments produced by the digestion of purified DNA amplicon with Hind III (Takara Shuzo, Shiga, Japan) allowed the 53 sero-nontypeable strains to be divided into 8 different restriction fragment length polymorphism (RFLP) patterns. They are composed of one to 4 major bands, with sizes between 260 bp and 821 bp (Figure 2 and 3, Table 2). The 32 serotypeable strains and 7 standard strains were divided into 7 RFLP patterns (Table 3).

The test results in Table 2 indicate that the PCR-RFLP can be used as a useful method for identification and differentiation among the *H. parasuis* seronontypeable strains, while the present serotyping protocol can not give a confirmation diagnosis. The test results in Table 3 also indicate that the PCR-RFLP can be used as an alternative method for studying the genetic relatedness

Figure 3: Agarose gel electrophoresis of DNA fragments produced by Hind III digestion of PCR-amplified DNA. Lane 1 and 11, empty; lane 2, DNA ladder (50-2,000 bp); lane 3, nontypeable, RFLP type 1A; lane 4, serotype 4, RFLP type 1A; lane 5, serotype 4, RFLP type 1B; lane 6, serotype 4, RFLP type 2A; lane 7, serotype 4, RFLP type 2B; lane 8, serotype 4, RFLP type 2B; lane 9, serotype 4, RFLP type 3A; lane 10, serotype 13, RFLP type 3B.

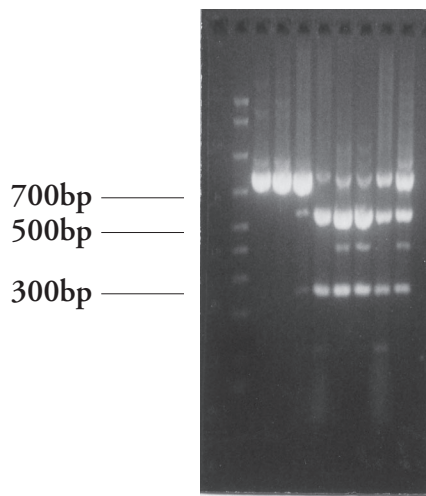


Table 2: PCR-RFLP patterns of 53 sero-nontypeable strains of *H. parasuis*

| PCR-RFLP pattern | Serotype | Number of field strains |
|------------------|----------|-------------------------|
| 1A | NT | 20 |
| 1B | NT | 3 |
| 2A | NT | 4 |
| 2B | NT | 6 |
| 2C | NT | 1 |
| 3A | NT | 8 |
| 3B | NT | 9 |
| 4 | NT | 2 |

NT: nontypeable

Table 3: PCR-RFLP patterns of 32 sero-typeable strains and 7 standard strains of *H. parasuis*

| PCR-RFLP | Serotype | Number of field strains |
|----------|-------------|-------------------------|
| 1A | 2 | 2 |
| 1A | 4 | 6 |
| 1A | 7 | 1 |
| 1A | 12 | 1 |
| 1B | 4 | 1 |
| 1B | 7 | 1 |
| 1B | 12 | 1 |
| 2A | 4 | 1 |
| 2B | 4 | 4 |
| 2B | 12 | 1 |
| 2B | 13 | 2 |
| 3A | 4 | 1 |
| 3A | 7 | 3 |
| 3A | 12 | 1 |
| 3B | 4 | 1 |
| 3B | 13 | 1 |
| 4 | 4 | 1 |
| 4 | 7 | 2 |
| 4 | 12 | 1 |
| 1B | Standard 2 | 1 |
| 2A | Standard 4 | 1 |
| 2A | Standard 5 | 1 |
| 2A | Standard 7 | 1 |
| 2A | Standard 12 | 1 |
| 1B | Standard 13 | 1 |
| 3A | Standard 14 | 1 |

among the strains of the same serotypes. In conclusion, the PCR-RFLP combination we adopted in this study provided us with a molecular approach to the differentiation of sero-nontypeable strains of *H. parasuis*.

References

1. Rapp-Gabrielson, V.J., and Gabrielson, D.A. 1992. Prevalence of *Haemophilus parasuis* serovars among isolates from swine. *Am J Vet Res* 53:659-664.
2. Rapp-Gabrielson, V.J., Kocur, G.J., Clark, J.T., and Muir, S.K. 1997. *Haemophilus parasuis*; Immunity in swine following vaccination. *Vet Med* 92:83-90.
3. Oliveira, S., Galina, L., and Pijoan, C. 2001. Development of a PCR test to diagnose *Haemophilus parasuis* infections. *J Vet Diagn Invest* 13:495-501.
4. Lin, B.C., and Cobb, S. 1994. A fuzzy ELISA for serotyping *Haemophilus parasuis* using heat-stable antigen extracts. *13th Int. Pig Vet. Soc. Cong.* 156.

