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Evaluation of a nested polymerase chain reaction assay to differentiate between two genotypes of *Porcine circovirus-2*

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Introduction

Porcine circovirus-2 (PCV-2) is associated with several diseases in pigs, including postweaning multisystemic wasting syndrome (PMWS). A new genotype of PCV-2 has been isolated from swine farms with and without clinical PMWS in North America. The purpose of the current study was to develop and evaluate a nPCR assay to detect and differentiate between the 2 genotypes PCV-2a and PCV-2b

Materials and Methods

Primer sets that could detect the 2 genotypes of PCV-2 were designed with GenBank data using the Primer3 program. For the specificity evaluation, the viral DNA of reference PCV-2 isolates was reacted with first round primer set and then the PCR products were performed with 2 differential primer sets. In two different tubes, the first round PCR product of PCV-2a and -2b virus were separately reacted with PCV-2a and -2b genotype-specific primer set. The sensitivity of the nPCR was examined in comparison with conventional 1-step PCR. The reference strains ($10^{4.0}$ TCID₅₀/ml) were serially diluted 10 fold up to 10^{-10} . A total of 80 serum samples from pigs between 8 and 14 weeks old from 6 different swine farms were randomly selected from a serum bank.

Results

The primer pairs designed to differentiate between 2 genotypes of PCV-2 specifically amplified each PCV-2 genotype without any nonspecific reaction. Four MN PCV-2a isolates and 8 MN PCV-2b isolates were detected by the genotype specific nPCR. The analytical sensitivity of the nPCR was examined by comparing the results by 1-step and nPCR assays using viral DNA extracts from 10-fold serially diluted samples of the reference PCV-2a and PCV-2b (Fig. 1). 12 sera from 2 different farms with known clinical history were confirmed by nPCR, but only 5 of the 12 sera were positive in the 1-step PCR (Fig. 2).

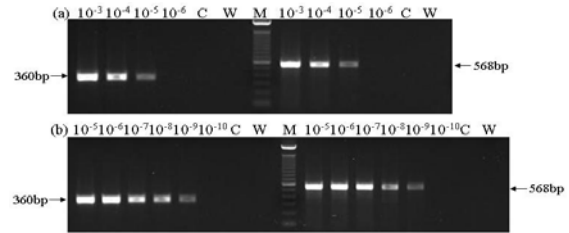


Figure 3. Sensitivity test. For (a) 1-step PCR and (b) Nested PCR

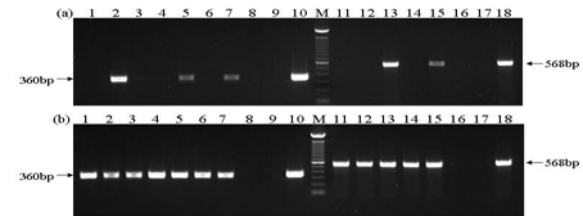


Figure 6. Comparison of PCV-2 detection by (a) 1-step and (b) nested PCR in serum samples.

Pig farms	Clinical PMWS	Year sampling	No. of sera	PCV-2 genotype		
				PCV-2a	PCV-2b	2a+2b
A	Yes	2006	30	0	23	0
B	Yes	2006	10	0	10	0
C	Yes	2006	10	6	10	6
D	Yes	2006	10	0	6	0
E	No	2004	10	5	0	0
F	No	2004	10	6	0	0

Table 1. Differentiation between PCV-2 genotypes 2a and 2b by n-PCR in serum samples collected from swine farms with a known clinical history of PMWS.

Discussion

The nPCR assay in the present study was highly sensitive, detecting up to 10^4 times more than conventional 1-step PCR. One great advantage of the nPCR described in the present study is that it can not only differentiate between the genotypes but can also detect dual infection of both genotypes. It is interesting that, PCV-2b was detected in the sera from swine farms that were diagnosed with PMWS in 2006, while PCV-2a was mainly detected in samples from the swine farms without PMWS in 2004.