Use of dual priming oligonucleotide system-based multiplex RT-PCR assay to detect five diarrhea viruses in pig herds in South China

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Abstract

In this study, a specific and simple method based on the dual priming oligonucleotide (DPO) system was developed to simultaneously detect transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV), porcine rotavirus A (PRV-A), porcine delta coronavirus (PDCoV), and swine acute diarrhea syndrome coronavirus (SADS-CoV), associated with the major enteric RNA viruses in pigs. The multiplex RT-PCR method based on the DPO system simplified the primer design and did not require optimization of the annealing temperature. Specificity analysis revealed that the method could specifically detect TGEV, PEDV, PRV-A, PDCoV, and SADS-CoV without any cross-amplification of other circulating swine viruses. The limit of detection of the method was as low as 103–104 copies/μL plasmid of each virus. The method also had good repeatability, and obvious results were seen in three repeat experiments with an interval of 45 days. This optimized multiplex RT-PCR method was used to evaluate 181 clinical swine samples that were collected from four provinces of China between September 2016 and August 2018. The results showed that the positive detection rates of PEDV, PDCoV, SADS-CoV, PRV-A, and TGEV were 30.94% (56/181), 17.67% (32/181), 11.6% (21/181), 9.39% (17/181), and 0.55% (1/181), respectively. Mixed infection of two or more viruses was also common. The DPO system-based multiplex RT-PCR could be a useful tool for detecting enteric virus infections. This method has the advantages of easy operation, low cost, high detection efficiency, and short running time for early diagnosis in clinical cases.

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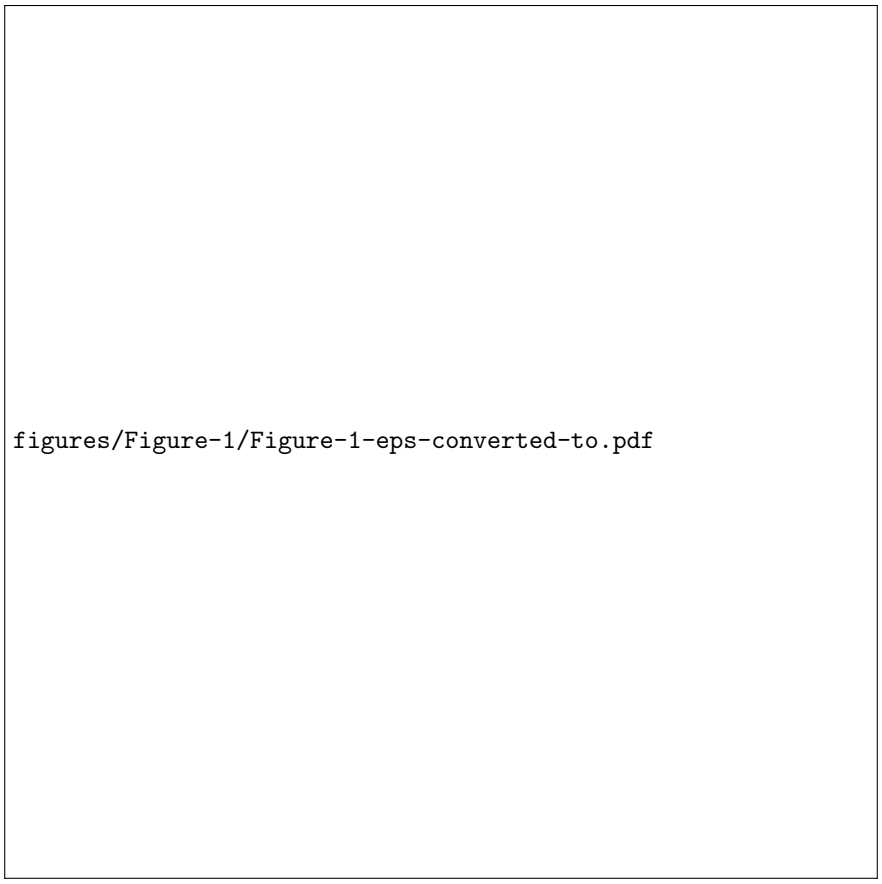

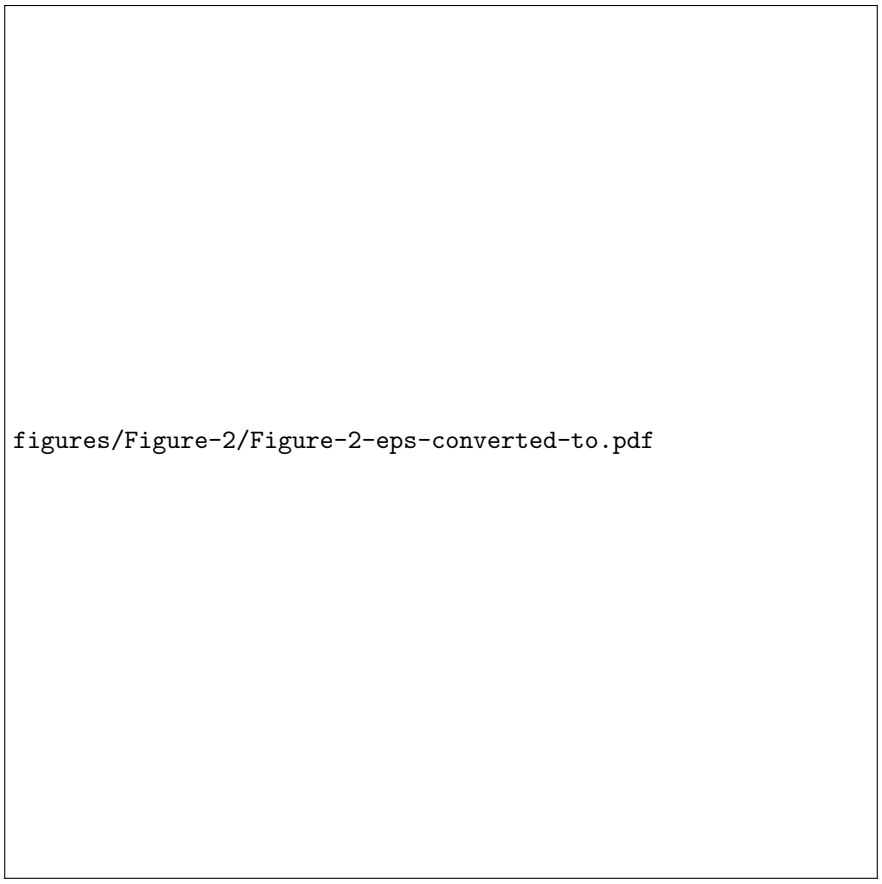

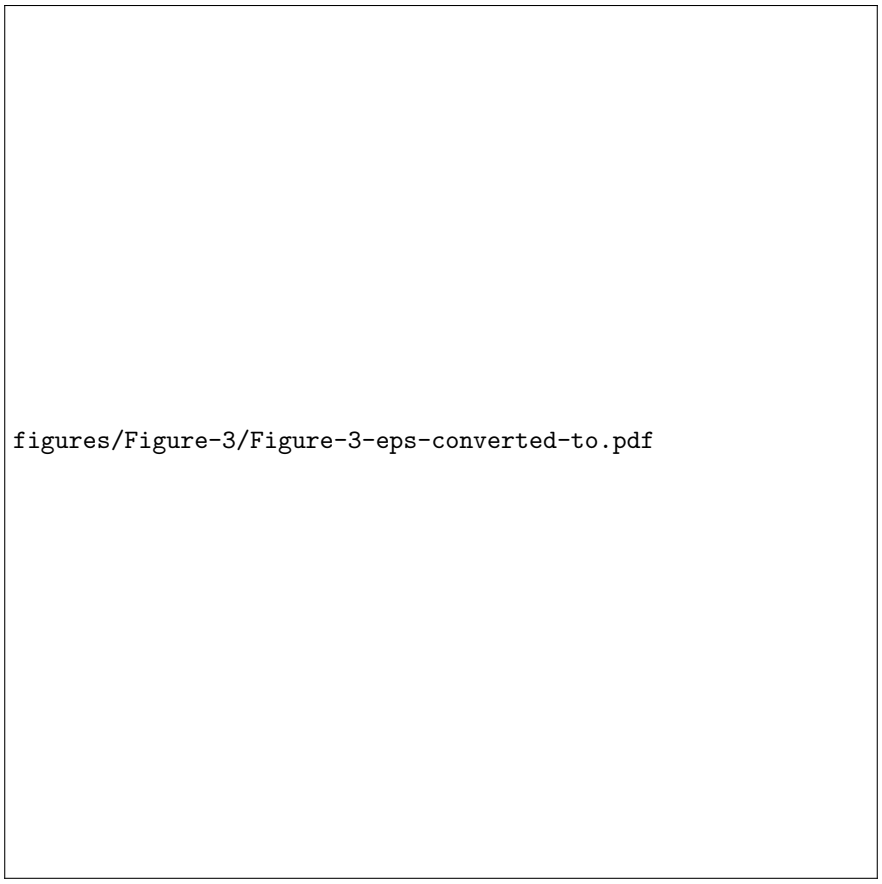

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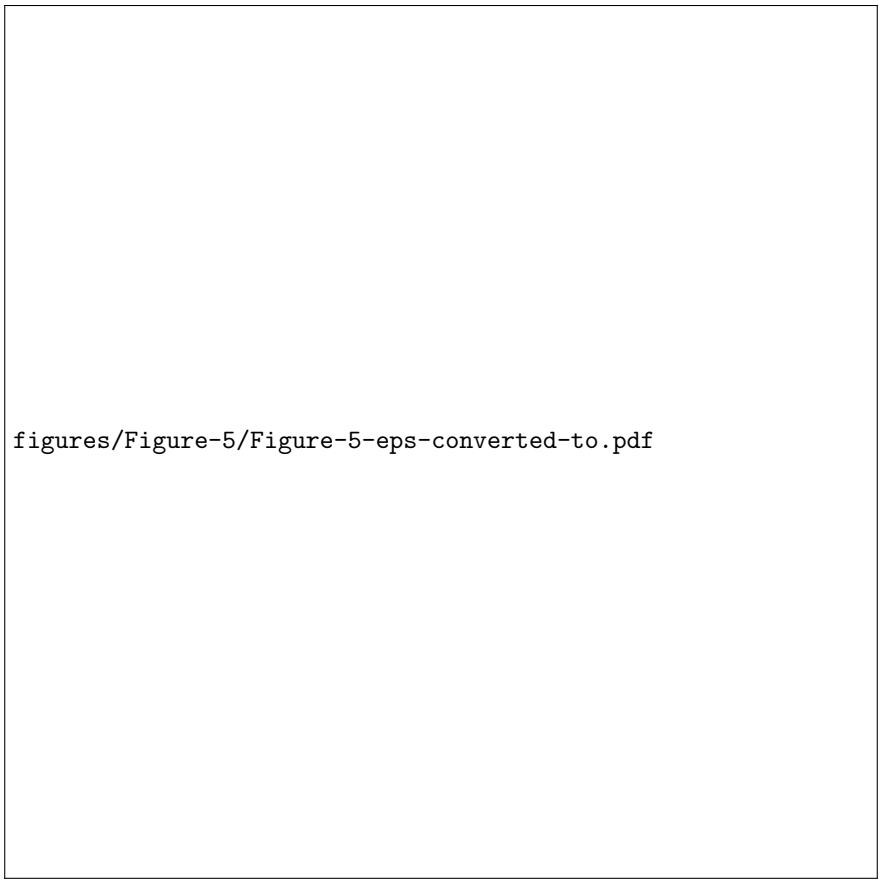

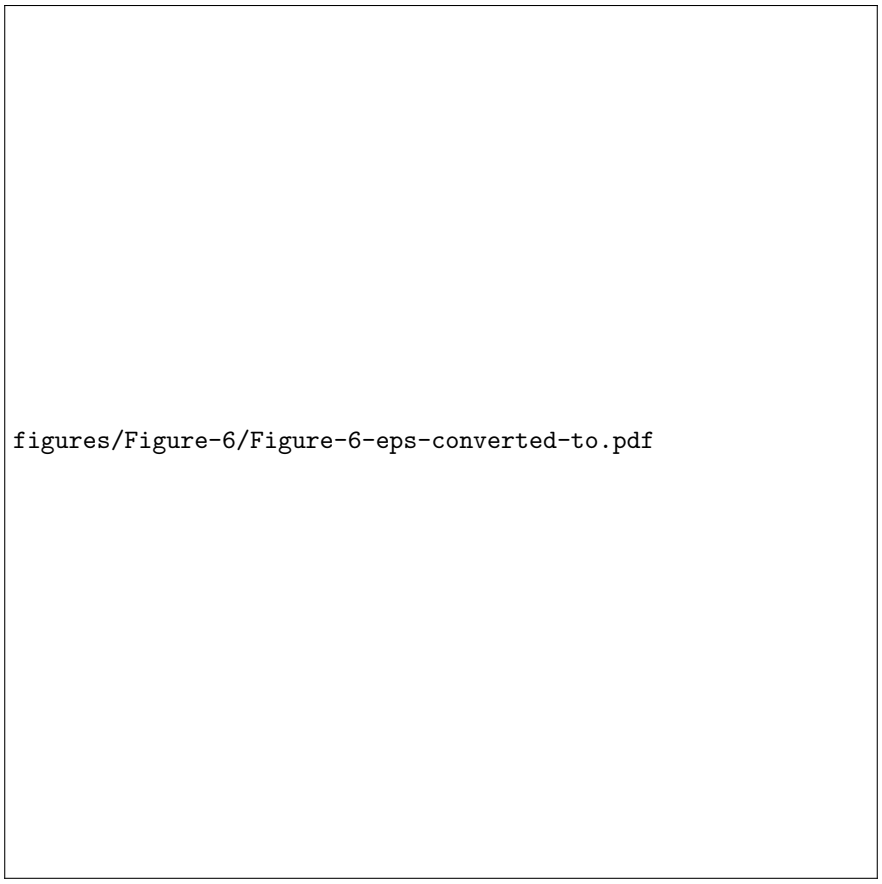

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