

Complete Genome Sequence of a Novel Variant Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Strain: Evidence for Recombination between Vaccine and Wild-Type PRRSV Strains

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Porcine reproductive and respiratory syndrome virus (PRRSV) is the etiologic agent of porcine reproductive and respiratory syndrome (PRRS), which can evolve continuously by random mutation or intragenic recombination. Here we report the complete genomic sequence of a PRRSV variant with nucleotide acid deletions and insertions in the nonstructural protein 2 (nsp2) gene and a possible recombination event between a modified live virus (MLV) vaccine strain and a prototype Chinese field strain.

Porcine reproductive and respiratory syndrome (PRRS) outbreaks occur worldwide and cause significant economic losses in the swine industry (6, 7). Even though modified live virus (MLV) vaccines were shown to provide solid protection against PRRSV infection, there were substantial barriers to the use of MLV vaccines in the field (2, 5). Considering the extensive use of MLV vaccines for preventing and controlling PRRS in mainland China, the appearance of a natural recombination event between a vaccine strain and a field strain is noteworthy.

Serum and tissue samples were collected from suspected growing pigs in Guangdong Province in 2011. Infected pigs were about 10 weeks old and displayed symptoms similar to those of original PRRS, including severe respiratory problems, diarrhea, and poor growth. In addition, this disease resulted in a \sim 2.2% mortality rate on this farm. A porcine reproductive and respiratory syndrome virus (PRRSV) field isolate, named GM2, was propagated on Marc-145 cells, and total viral RNA was extracted from the serum and infected cell culture and then used separately for the amplification of the genome. Basically, 16 primer pairs were used to generate overlapping amplicons by reverse transcription-PCR (RT-PCR) as reported previously (8), and 3'-terminal sequences of GM2 were obtained by a 3' rapid amplification of cDNA ends (RACE kit; TaKaRa). The PCR products were cloned into pMD19-T vector (TaKaRa), sequenced three times using an ABI 3730 Sanger-based genetic analyzer, and assembled using DNAStar version 7.0 to obtain the complete genome sequence. The completed sequence showed that, excluding the poly(A) tail, the genomic sequence of GM2 was 15,513 nucleotides in length. Genetic analysis demonstrated that GM2 shared 90.2% genome similarity with VR-2332, but a lower degree of genetic homology (87.7%) was observed than with the representative highly pathogenic PRRSV JXA1 strain. Compared with the VR-2332 strain, 36-amino-acid insertions and two continuous amino acid deletions in the nonstructural protein 2 (nsp2) region of GM2 were found. However, GM2 shared a very low identity with the SP strain (68.8%) and the Japanese strain EDRD-1 (70.5%), which was characterized by similar insertions within nsp2.

Recombination events were detected using both Simplot version 3.5.1 (3) and the recombination detection program version 4.1.3 (RDP4) (4). The results indicated that GM2 was a potential recombinant between the MLV RespPRRS/Repro vaccine strain and a recently emerging prototype Chinese field strain, named QYYZ. Putative recombination breakpoints were identified at positions 7547 (be-

ginning) and 11214 (end) of GM2, which are located in the nsp7 and nsp11 encoding regions, respectively. The recombinant fragment contained the whole nsp9 and nsp10 encoding regions, which encode the RNA-dependent RNA polymerase (RdRp) and helicase, respectively (1). The present finding will help us to understand the epidemiology and evolution of PRRSV, as well as how to control PRRS disease.

Nucleotide sequence accession numbers. The virus genome sequences of strains GM2 and QYYZ are available in GenBank under accession numbers JN662424 and JQ308798, respectively.

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REFERENCES

- 1. Fang Y, Snijder EJ. 2010. The PRRSV replicase: exploring the multifunctionality of an intriguing set of nonstructural proteins. Virus Res. 154:61–76.
- Kimman TG, Cornelissen LA, Moormann RJ, Rebel JM, Stockhofe-Zurwieden N. 2009. Challenges for porcine reproductive and respiratory syndrome virus (PRRSV) vaccinology. Vaccine 27:3704–3718.
- Lole KS, et al. 1999. Full-length human immunodeficiency virus type 1 genomes from subtype C-infected seroconverters in India, with evidence of intersubtype recombination. J. Virol. 73:152–160.
- Martin DP, et al. 2010. RDP3: a flexible and fast computer program for analyzing recombination. Bioinformatics 26:2462–2463.
- Murtaugh MP, Genzow M. 2011. Immunological solutions for treatment and prevention of porcine reproductive and respiratory syndrome (PRRS). Vaccine 29:8192–8204.
- Neumann EJ, et al. 2005. Assessment of the economic impact of porcine reproductive and respiratory syndrome on swine production in the United States. J. Am. Vet. Med. Assoc. 227:385–392.
- Zhou L, Yang H. 2010. Porcine reproductive and respiratory syndrome in China. Virus Res. 154:31–37.
- Zhu L, et al. 2011. Complete genomic characterization of a Chinese isolate of porcine reproductive and respiratory syndrome virus. Vet. Microbiol. 147:274–282.

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