

Complete Genome Sequence of Two Variant Porcine Reproductive and Respiratory Syndrome Viruses Isolated from Vaccinated Piglets

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Porcine reproductive and respiratory syndrome virus (PRRSV) continues to affect the Chinese swine industry. Since 2006, variant PRRSV strains sharing two unique discontinuous deletions of 30 amino acids in the nonstructural protein Nsp2 have become dominant in Chinese swine herds and have caused huge economic losses to the swine industry in China. Here we report the complete genome sequence of two novel PRRSV variants isolated from vaccinated piglets with additional amino acid deletions in Nsp2.

n recent years, porcine reproductive and respiratory syndrome virus (PRRSV) infections have caused great financial losses to the Chinese swine industry (11). PRRSV is a small, enveloped, single-stranded positive-sense RNA virus, which belongs to the family Arteriviridae in the order Nidovirales (3). With regard to genetic diversity, PRRSV is mainly classified into two types: European (type 1) and American (type 2). These two types share approximately 60% genome sequence homology with each other (8) and it is believed up to 20% genetic variation within each genotype (4). PRRSV was first isolated in China in 1996, and several variant isolates were later reported (1-2, 10, 13-14). These isolates were both genetically and pathologically heterogenic. PRRSV has shown remarkable genetic variation through mutation or recombination, resulting in the emergence of novel variants (4, 5, 7, 10). Here we report two new PRRSV variant strains with additional deletions in the nonstructural protein Nsp2.

PRRSV strains YD and DC were isolated from sera of vaccinated piglets from two different farms. To determine the complete genome sequence of these isolates, 16 pairs of primers were designed based on PRRSV strains VR-2332 and JXA1 to generate overlapping amplicons by reverse transcription-PCR as described previously (14). The PCR products were purified and cloned into pMD19-T vector (TaKaRa) and sequenced using an automated genome sequencer (ABI3730). The terminal sequences were obtained by using a kit for rapid amplification of cDNA ends (RACE kit; TaKaRa). DNAStar version 7.0 and ClustalX were applied to the genomic analysis. The complete genome sequence of YD and DC were 15,253 and 15,317 nucleotides in length, respectively, excluding the poly(A) tails. Comparative genomic analysis revealed that YD showed a higher similarity (97.9% to 98.5%) with Chinese PRRSV variants, including JXA1, Henan-1, HEB1, HUB1, HUN4, SY0608, TP, KP, and CG, while DC showed a lower identity (96.6% to 97.9%) with those isolates. Complete genome sequence alignment of YD and DC with JXA1, which was the representative isolate of the Chinese highly pathogenic PRRSV (HP-PRRSV), showed that, besides the two unique discontinuous deletions of 30 amino acids in Nsp2, additional deletions of 22 amino acids between positions 489 to 512 (P489 to K512) were found in the YD strain and a single amino acid deletion at position 153 in the DC strain. The Nsp2 deletions were a remarkable

feature of the highly virulent strains, such as MN184A and HP-PRRSVs, which appeared in North America in 2002 and mainland China in 2006, respectively. However, previous studies revealed that the deletions in Nsp2 were not related to the virulence of the virus (6, 9, 12). The unique deletions within Nsp2 found in the YD and DC strains are intriguing and still need further investigation. The genome data of two novel PRRSV variants isolated from vaccinated piglets reported in our study will be helpful for understanding the epidemiology and evolution of PRRSV.

Nucleotide sequence accession numbers. The virus genome sequence of strains YD and DC are available in GenBank under the accession numbers JF748717 and JF748718, respectively.

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