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Diversity of porcine reproductive and respiratory syndrome virus in Shandong, China

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Summary. – Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most economically significant pathogens in swine industry of China. To study infection and genetic variation of PRRSV, 637 tissue samples were collected from diseased pigs in Shandong, and then subjected to detection of PRRSV. The nsp2 and ORF5 genes were sequenced for investigation of variations and phylogenetic analysis. The results showed that positive rate of PRRSV was 9.58% in the collected samples. Phylogenetic analysis of GP5 showed that these strains were clustered into two lineages (1 and 8) indicating different genotypes of PRRSV were circulating in Shandong province. Meanwhile, sequence analysis Of nsp2 showed that the PRRSV strains with 30 amino acids deletions were dominant. Moreover, novel pattern of recombination/deletion and insertion in nsp2 was observed in these strains, indicating that novel PRRSV strains with different patterns of deletions or insertions in nsp2 are emerging in China. All the results suggested that continuous surveillance of PRRSV in China is warranted.

Keywords: PRRSV; GP5; nsp2; genetic analysis; Shandong

Porcine reproductive and respiratory syndrome (PRRS), one of the most important swine diseases, has caused enormous losses to the swine industry worldwide (Neumann *et al*., 2005). PRRS virus (PRRSV), the etiological agent of this disease, is an enveloped, single-stranded, RNA virus. GP5 of PRRSV is a major viral envelope protein encoded by ORF5 and is functionally important in viral infectivity, assembly, and the induction of viral neutralizing antibodies (Dea *et al*., 2000; Wissink *et al*., 2003). At the same time, it has been widely used for diagnostic identification and phylogenetic analysis due to its high degree of genetic diversity (Cha *et al*., 2004; Dee *et al*., 2001). The nonstructural protein (nsp2) coding region is recognized as the most variable gene, which undergoes significant variation associated with gene substitutions, deletions, and insertions (Kedkovid *et al*., 2010; Yoshii *et al*., 2008).

In 2006, a highly pathogenic PRRSV (HP-PRRSV) emerged in China and affected over 20 million pigs with about 400,000 fatal cases that had led to huge economic losses (Tian *et al*., 2007). The causative agent, HP-PRRSV has a unique discontinuous deletion of 30 amino acids in nsp2 (Tian *et al*., 2007; Tong *et al*., 2007). Shandong has the biggest swine industries in China, however, the prevalence and evolution of PRRSV circulating in swine in Shandong remains largely unclear. To investigate the epidemic situation of PRRSV in Shandong, a total of 637 tissue samples (spleens, lungs, and lymph nodes) were col-

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Abbreviations: GP5 = lycoprotein 5; HVR = hypervariable region; nsp2 = non-structural protein 2; PRRS = porcine reproductive and respiratory syndrome; PRRSV = porcine reproductive and respiratory syndrome virus

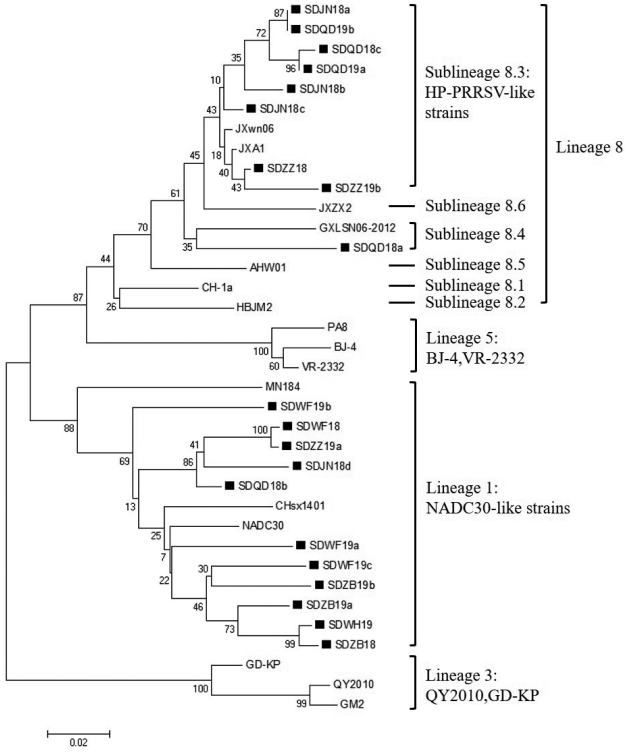

Fig. 1

Phylogenetic analysis based on amino acid sequences of PRRSV ORF5 gene

Phylogenetic analysis includes 20 sequences from this study and 16 reference ORF5 sequences of PRRSV in China and other countries. The tree was generated by neighbor-joining method and bootstrapped with 1,000 replicates using MEGA7 software. The black solid squares indicate the strains in this study.

lected from diseased pigs from June 2018 to June 2019 in Shandong. The 637 samples were homogenized in 1:5 ratio with phosphate-buffered saline (PBS), and centrifuged at 5,000 x *g* for 5 min. The supernatants were then collected for viral RNA extraction using TRIzol® reagent according to the manufacturer's instructions. Then PRRSV was detected by fluorescence quantitative RT-PCR using commercial kits (SENKANG, Beijing, China).

To analyze the genetic characteristics of PRRSV strains, nsp2 and GP5 genes were amplified and sequenced from the representative positive samples. Two pairs of specific primers targeting nsp2 and GP5 genes were designed as: nsp2-F: 5'-ATGGGCGACAATGTCCCTAAC-3' and nsp2-R: 5'-GAGCTGAGTATTTTGGGCGTG-3', with product size 380 bp; GP5-F:5'-TGTGAATTCATGTTGGGGAAATGCTTGACC-3' and GP5-R: 5'-CCACTCGAGCCTTTTGTGGAGCCGTGC TAT-3' with product size 603 bp. After viral RNA extraction, One-step RT-PCR was conducted in reaction mixtures according kit instructions (TaKaRa, Japan). The nsp2 and GP5 PCR products were purified and cloned into pMD®18-T vector. Nsp2 and GP5 genes were then sequenced by Sangon Biotech company.

Of the 637 samples collected from different fields of diseased pigs from June 2018 to June 2019 in Shandong province, 61 samples (9.58%) were positive for PRRSV, determined by fluorescence quantitative RT-PCR using commercial kits.

A phylogenetic tree was constructed based on the 20 deduced amino acid sequences of GP5 genes in this study together with 16 reference PRRSV sequences. As shown in Fig. 1, among the 20 strains, 11 strains were clustered into lineage 1, as represented by NADC30 strain. The other 9 strains belonged to lineage 8, with 1 strain being clustered into sub-lineage 8.4 and 8 strains being clustered into sub-lineage 8.3 with the representative strains JXA1 and JXwn06. The results indicated that different genotypes of PRRSV were circulating in Shandong province with dominant strain of lineage 1 represented by NADC30 strain.

To investigate the amino acid differences of nsp2, a 380 bp DNA fragments containing HVR (hypervariable region) from 11 positive samples was sequenced and analyzed. The amino acid sequences of 13 PRRSV strains including 2 reference strains VR2332 and JXA1 were aligned (Fig. 2). Nine out of 11 nsp2 HVR contained the same 30 amino acids deletions as JXA1. Compared with strain VR2332, strain SDQD18a had 1 amino acid deletion in its HVR. We also found that strain SDQD18c contained 21 amino acids deletions and 1 amino acid insertion compared with VR2332. The data revealed that nsp2 is highly variable and novel HP-PRRSV strains are emerging with amino acid deletions and insertions in nsp2.

PRRS is one of the most important infectious diseases and leads to substantial economic losses to pig industry worldwide. Since its emergence in China in 1995, PRRSV has spread widely in pig farms and is continuously evolving. In 2006, the emergence of HP-PRRSV caused a large outbreak of porcine high fever syndrome (PHFD) characterized by high morbidity and high mortality (Tian *et al*., 2007). In our study, 61 out of 637 samples (9.58%) were positive for PRRSV, indicating that PRRSV infection rate is high in Shandong province in China. The phylogenetic trees based on amino acid sequences of GP5 gene showed that lineage 1 represented by NADC30 strain has become the dominant strain in Shandong. Meanwhile, the proportion of sub-lineage 8.3 with representative strains JXA1 and JXwn06 is also high, indicating that lineage 1 and sub-lineage 8.3 strains were the key points of prevention and control in Shandong province.

The nsp2 of PRRSV is a highly diverse protein. In addition, natural insertions and deletions have continued to occur in the HVR of nsp2, which led to genome size differences among PRRSV strains (Yoshii *et al*., 2008). In

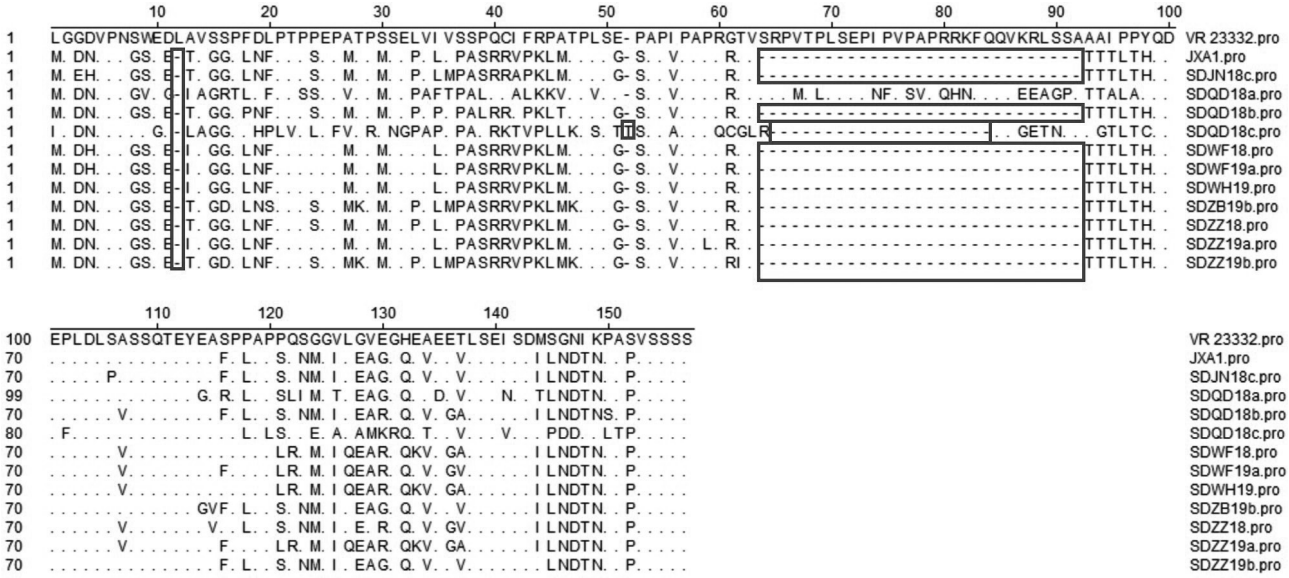

Fig. 2

High variable regions in nsp2 of PRRSV strains

The sequence of VR2332 is shown on the top; the consensus residues are hidden. The deleted or inserted amino acids are shown in the boxes.

this study, most of the strains have a discontinuous 30 amino acids deletions, suggesting that the strain with the 30 amino acids deletions in HVR of nsp2 is the dominant virus in Shandong province in China. It should be noted that the strain, SDQD18c, has one amino acid insertion at position 52 and continuous 20 amino acids deletion at position 65–84 compared to VR-2332, suggesting that strain with other type of amino acid deletions or insertion may have also been prevalent in Shandong province. Recent studies showed that PRRSV strains with deletions in nsp2 were more likely to be pathogenic (Brockmeier *et al*., 2012). The mechanism underlying spontaneous deletions in nsp2 during viral passages *in vivo* and their effect on viral pathogenicity remains a topic for further studies.

Overall, our results showed that PRRSV is widely distributed in Shandong province in China. GP5 based phylogenetic trees and sequence alignments showed that extensive genetic variability exists compared with the reference stains, which might allow these field strains to escape neutralization antibodies induced by current vaccines. In addition, novel patterns of recombination/deletion and insertion in nsp2 are emerging. All the results suggested that continuous surveillance of PRRSV in China is warranted.

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References

Brockmeier SL, Loving CL, Vorwald AC, Kehrli ME, Jr, Baker B, Nicholson L, Lager KM, Miller LC, Faaberg KS (2012): Genomic sequence and virulence comparison of four type 2 porcine reproductive and respiratory syndrome virus strains. Virus Res. 169, 212–221. https://doi.org/10.1016/j.virusres.2012.07.030

Cha SH, Chang CC, Yoon KJ (2004): Instability of the restriction fragment length polymorphism pattern of open reading frame 5 of porcine reproductive and respiratory syndrome virus during sequential pig-to-pig passages. J. Clin. Microb. 42, 4462–4467. https://doi.org/10.1128/JCM.42.10.4462-4467.2004

Dea S, Gagnon CA, Mardassi H, Pirzadeh B, Rogan D (2000): Current knowledge on the structural proteins of porcine reproductive and respiratory syndrome (PRRS) virus: comparison of the North American and European isolates. Arch. Virol. 145, 659–688. https://doi.org/10.1007/s007050050662

Dee SA, Torremorell M, Rossow K, Mahlum C, Otake S, Faaberg K (2001): Identification of genetically diverse sequences (ORF 5) of porcine reproductive and respiratory syndrome virus in a swine herd. Canad. J. Vet. Res. = Rev. Canad. Rech. Vet. 65, 254–260.

Kedkovid R, Nuntawan Na Ayudhya S, Amonsin A, Thanawongnuwech R (2010): NSP2 gene variation of the North American genotype of the Thai PRRSV in central Thailand. Virol. J. 7, 340. https://doi.org/10.1186/1743-422X-7-340

Neumann EJ, Kliebenstein JB, Johnson CD, Mabry JW, Bush EJ, Seitzinger AH, Green AL, Zimmerman JJ (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. https://doi.org/10.2460/javma.2005.227.385

Tian K, Yu X, Zhao T, Feng Y, Cao Z, Wang C, Hu Y, Chen X, Hu D, Tian X, Liu D, Zhang S, Deng X, Ding Y, Yang L, Zhang Y, Xiao H, Qiao M, Wang B, Hou L, Wang X, Yang X, Kang L, Sun M, Jin P, Wang S, Kitamura Y, Yan J, Gao GF (2007): Emergence of fatal PRRSV variants: unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark. PloS One 2, e526. https://doi.org/10.1371/journal.pone.0000526

Tong GZ, Zhou YJ, Hao XF, Tian ZJ, An TQ, Qiu HJ (2007): Highly pathogenic porcine reproductive and respiratory syndrome, China. Emerg. Infect. Dis. 13, 1434–1436. https://doi.org/10.3201/eid1309.070399

Wissink EH, van Wijk HA, Kroese MV, Weiland E, Meulenberg JJ, Rottier PJ, van Rijn PA (2003): The major envelope protein, GP5, of a European porcine reproductive and respiratory syndrome virus contains a neutralization epitope in its N-terminal ectodomain. J. Gen. Virol. 84, 1535–1543. https://doi.org/10.1099/vir.0.18957-0

Yoshii M, Okinaga T, Miyazaki A, Kato K, Ikeda H, Tsunemitsu H (2008): Genetic polymorphism of the nsp2 gene in North American type--porcine reproductive and respiratory syndrome virus. Arch. Virol. 153, 1323–1334. https://doi.org/10.1007/s00705-008-0098-6