Identification of Two Porcine Reproductive and Respiratory Syndrome Virus Variants Sharing High Genomic Homology but with Distinct Virulence: Summary by MSHMP

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- Two novel HP-PRRSV variants (XJ17-5 and JSTZ1712-12) that have the new genetic feature of 150-amino-acid deletion in *nsp2* were identified.
- Even though XJ17-5 and JSTZ1712-12 isolates share high genomic homology, they had distinct pathogenicity for piglets.
- Fragment comparisons identified 34 amino acid differences between the two isolates which might be related to distinct virulence.

In this study published in Viruses in 2019, authors describe the distinct virulence of two novel highly pathogenic PRRSV variants with high genomic homology found in China. In 2017 the two variants were isolated, one from lungs and sera of an outbreak in a 4,500-sow farm in the Xinjiang province. This severe outbreak was characterized by high fever, >1,000 abortions and high sow and pre-weaning mortality rate and severe abortions (XJ17-5). The second variant was obtained from a ~200 sow herd clinically healthy pigs from Jiangsu province (JSTZ1712-12) for routine epidemiological investigation. ORF5 sequencing showed all samples from Xinjiang province shared 100% nucleotide identity, as did all samples from Jiangsu province. However, ORF5 comparison of samples from Xinjiang and Jiangsu provinces revealed 99.83% nucleotide identity. In addition, *nsp2* sequencing showed that their *nsp2* shared 99.33% nucleotide identity.

Total RNA from cultures of a lung sample from Xinjiang province and a serum sample from Jiangsu province was extracted from the cell culture and the complete genomes were determined. The amplicons were purified and cloned into pEASY-T1 Vector. At least three recombinant clones for each fragment were sequenced. The complete genome and each fragment were aligned to determine the similarity between the isolates, with the addition of 50 other PRRSV genomes representative of Chinese isolates. Genomes of both variants share 99.45% nucleotide identity. Genomic comparison with other representative PRRSV strains showed that they share the highest nucleotide identity with the HP-PRRSV strain. Neither isolate was detected as a recombinant virus. In addition, each fragment alignment identified that both isolates have the discontinuous 30-amino-acid deletion at 481 and 533–561 positions of *nsp2*, which is the genetic hallmark of HP-PRRSV. Remarkably, they also have a continuous 120 amino-acid deletion at 628–747 positions of *nsp2*. The results indicated that these isolates are novel HP-PRRSV variants.

The authors also performed an animal challenge study to determine both isolates' virulence. Briefly, fifteen 4-week-old PRRSV-free piglets were randomly assigned to three groups (five piglets per group). Piglets in two groups were intranasally and intramuscularly inoculated with 2 mL $10^{5.0}$ median tissue culture infectious dose of each isolate, while piglets in the third group were inoculated with Minimum Essential Medium Eagle (MEM media) to serve as the negative control. XJ17-5-infected pigs showed clinical signs including dyspnea, anorexia and diarrhea, which was not present in the other groups. These pigs also had significantly lower body weight than mock-infected pigs at 11 dpi. Necropsy examination revealed lung consolidation and extensive hemorrhages in XJ17-5-infected pigs but not in JSTZ1712-12-infected or mock-infected pigs, and red blood cells and serous exudation at histopathological examination were observed only in XJ17-5-infected pigs. Deaths occurred at 11 dpi, 13 dpi and 14 dpi only among XJ17-5-infected pigs. PRRSV antigens could be detected in both JSTZ1712-12-infected and XJ17-5-infected pigs but not in mock-infected pigs in the immunohistochemical examination. A total of 34 amino acid differences between XJ17-5 and JSTZ1712-12 were identified, including 28 differences within nonstructural proteins (*nsp1α*, *nsp1β*, *nsp2*, *nsp9* and *nsp10*) and 6 differences within structural proteins (*GP3*, *GP4*, *GP5* and *N*). The amino acid changes are not related to in vitro adaptation; therefore, they are more likely correlated with virulence determinants. A majority of the mutations (21 out of 34) spread throughout the genome are unique for high virulent XJ17-5 isolate, and 19 out of the 21 mutations are identical in avirulent JSTZ1712-12 and 9 other high homologous HP-PRRSV variants identified by other research groups. Therefore, it is rational to speculate that these mutations have higher probability to associate with the virulence.

The authors conclude with a discussion that virulence determinants of PRRSV isolates are likely strain-specific and the virulence determinants for one parental virus might not be the same for the other genetically distinct parental virus. Thus, the identification of natural HP-PRRSV variants with high genomic similarity but distinct virulence provides ideal viruses to analyze the virulence determinants of these HP-PRRSV variants. Full research article is available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6783987/