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Complete Genome Sequence of Highly Virulent Porcine Reproductive and Respiratory Syndrome Virus Variants That Recently Emerged in the United States

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A recent outbreak of particularly virulent disease caused by porcine reproductive and respiratory syndrome virus has occurred in swine herds across the United States. We report here the complete genome sequence of eight viral isolates from four Nebraska herds experiencing an outbreak of severe disease in 2016.

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, positive-sense, single-stranded RNA virus that belongs to the family *Arteriviridae* in the order *Nidovirales* (1). Infection by PRRSV causes reproductive failure in sows and respiratory disease in young pigs (2, 3). Based on genetic and antigenic differences, PRRSV has been divided into two major genotypes: the European genotype (type 1) and the North American genotype (type 2) (4–6). Substantial genetic diversity exists both between and within genotypes, leading to a wide degree of clinical severity ranging from a lack of clinical signs to fatal disease (7).

Since 2014, there have been frequent outbreaks of unusually severe PRRS in the United States. The associated PRRSV isolates have been classified as type 1-7-4 according to a widely used approach for genetic classification based on restriction fragment length polymorphism (RFLP) analysis of the open reading frame 5 (ORF5) (8, 9). However, genetic relatedness/diversity between strains is not adequately described by this approach (10). Therefore, the objective of this work was to obtain the full genome sequence of PRRSV isolates associated with severe disease outbreaks to better understand the molecular characteristics of these newly emerging PRRSV variants.

Serum was collected from infected pigs on four Nebraska farms

experiencing virulent PRRSV outbreaks in 2016. From these samples, eight PRRSV variants were isolated using porcine alveolar macrophages (PAMs). Total RNA was purified from the first passage viral supernatant of infected cell cultures using Trizol LS (Life Technologies, Carlsbad, CA). Sequencing libraries were prepared using the Illumina TruSeq RNA Kit and sequenced with 2 × 300 paired end reads on the MiSeq platform (Illumina, San Diego, CA). Index adapters were removed from raw sequence reads using cutadapt (11) and trimmed reads were screened against the UniVec_Core database (NCBI) to remove contaminating vector sequences. Assembly of viral genomes was performed using template-assisted assembly, where trimmed reads were mapped to reference PRRSV genomes (MN184C accession no. EF488739 and NVSL 97-7,895 accession no. AY545985) using Geneious software (version 9.1.3, Biomatters, Auckland, New Zealand [12]). Reads that mapped to the reference genomes were then *de novo* assembled and annotated for each sample (Table 1).

The complete genome sequences share 99% nucleotide identity, although *in silico* RFLP analysis of the ORF5 sequence revealed predicted variation. Seven of the eight isolates sequenced here are predicted RFLP type 1-7-4; while the eighth isolate is type 1-7-2 as a result of a single nucleotide substitution in the second SacII restriction site (Table 1). Phylogenetic analysis of PRRSV

TABLE 1 *De novo* genome assemblies of eight virulent PRRSV isolates^a

| PRRSV isolate | Nebraska farm | Genome length (nt) | Mean coverage | RFLP | Accession no. |
|---------------|---------------|--------------------|---------------|-------|---|
| NCV-13 | 1 | 15,097 | 18,535 | 1-7-4 | KX192112 |
| NCV-16 | 2 | 15,101 | 13,659 | 1-7-4 | KX192113 |
| NCV-17 | 2 | 15,097 | 20,128 | 1-7-4 | KX192114 |
| NCV-21 | 3 | 15,098 | 12,327 | 1-7-2 | KX192115 |
| NCV-23 | 4 | 15,107 | 7,748 | 1-7-4 | KX192116 |
| NCV-24 | 4 | 15,097 | 9,685 | 1-7-4 | KX192117 |
| NCV-25 | 4 | 15,098 | 16,194 | 1-7-4 | KX192118 |
| NCV-26 | 4 | 15,097 | 6,160 | 1-7-4 | KX192119 |

^a nt, nucleotides; RFLP, restriction fragment length polymorphism (8, 9).

whole genome sequences reveals that this strain is clearly related to the other 1-7-4 viruses in this study, despite the difference in RFLP classification (not shown). Together, this set of complete genome sequences will further our understanding of PRRSV evolution and provide valuable information to more finely delineate the viral genomic sites associated with changes in viral virulence.

Accession number(s). The sequences are deposited in DDBJ/ENA/GenBank under the accession numbers listed in [Table 1](#).

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The use of product and company names is necessary to accurately report the methods and results; however, the United States Department of Agriculture (USDA) neither guarantees nor warrants the standard of the products, and the use of names by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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REFERENCES

1. Snijder EJ, Kikkert M, Fang Y. 2013. Arterivirus molecular biology and pathogenesis. *J Gen Virol* 94:2141–2163. <http://dx.doi.org/10.1099/vir.0.056341-0>.
2. Collins JE, Benfield DA, Christianson WT, Harris L, Hennings JC, Shaw DP, Goyal SM, McCullough S, Morrison RB, Joo HS, Gorcyca D, Chladek D. 1992. Isolation of swine infertility and respiratory syndrome virus (isolate ATCC VR-2332) in North America and experimental reproduction of the disease in gnotobiotic pigs. *J Vet Diagn Invest* 4:117–126. <http://dx.doi.org/10.1177/104063879200400201>.
3. Wensvoort G, Terpstra C, Pol JM, ter Laak EA, Bloemraad M, de Kluyver EP, Kragten C, van Buiten L, den Besten A, Wagenaar F, Broekhuijsen JM, Moonen PLJM, Zetstra T, de Boer EA, Tibben HJ, de Jong MF, van 't Veld P, Greenland GJR, van Gennep JA, Voets MT, Verheijden JHM, Braamskamp J. 1991. Mystery swine disease in the Netherlands: the isolation of Lelystad virus. *Vet Q* 13:121–130. <http://dx.doi.org/10.1080/01652176.1991.9694296>.
4. Nelsen CJ, Murtaugh MP, Faaberg KS. 1999. Porcine reproductive and respiratory syndrome virus comparison: divergent evolution on two continents. *J Virol* 73:270–280.
5. Kapur V, Elam MR, Pawlovich TM, Murtaugh MP. 1996. Genetic variation in porcine reproductive and respiratory syndrome virus isolates in the midwestern United States. *J Gen Virol* 77:1271–1276. <http://dx.doi.org/10.1099/0022-1317-77-6-1271>.
6. Murtaugh MP, Elam MR, Kakach LT. 1995. Comparison of the structural protein coding sequences of the VR-2332 and Lelystad virus strains of the PRRS virus. *Arch Virol* 140:1451–1460. <http://dx.doi.org/10.1007/BF01322671>.
7. Meng XJ. 2000. Heterogeneity of porcine reproductive and respiratory syndrome virus: implications for current vaccine efficacy and future vaccine development. *Vet Microbiol* 74:309–329. [http://dx.doi.org/10.1016/S0378-1135\(00\)00196-6](http://dx.doi.org/10.1016/S0378-1135(00)00196-6).
8. Rosendal T, Dewey C, Young B, Carman S, Ge L, Poljak Z. 2010. Distribution of genotypes of porcine reproductive and respiratory syndrome virus in Ontario during 2004–2007 and the association between genotype and clinical signs of disease. *Can J Vet Res* 74:118–123.
9. Wesley RD, Mengeling WL, Lager KM, Clouser DF, Landgraf JG, Frey ML. 1998. Differentiation of a porcine reproductive and respiratory syndrome virus vaccine strain from North American field strains by restriction fragment length polymorphism analysis of ORF 5. *J Vet Diagn Invest* 10:140–144. <http://dx.doi.org/10.1177/104063879801000204>.
10. Wang X, Marthaler D, Rovira A, Rossow S, Murtaugh MP. 2015. Emergence of a virulent porcine reproductive and respiratory syndrome virus in vaccinated herds in the United States. *Virus Res* 210:34–41. <http://dx.doi.org/10.1016/j.virusres.2015.07.004>.
11. Marcel M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnetJ* 17:10–12.
12. Kears M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. <http://dx.doi.org/10.1093/bioinformatics/bts199>.