Genomic Sequence of a Swine Pasivirus Type 1 Strain Identified in U.S. Swine

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ABSTRACT We report for the first time in the United States the identification of a swine pasivirus (SPaV) strain with a genomic sequence identity of less than 80% to other SPaVs reported in Europe and China, using a next-generation sequencing (NGS) technique in sow tissues collected from an animal study conducted in 2001, suggesting virus circulation in domestic swine.

Swine pasivirus (SPaV) is a relatively new member of the family Picornaviridae. The Pasivirus genus was recently formed in group 4 of this family, with swine pasivirus type 1 (SPaV1) as the proposed type species, which is now Pasivirus A (1); SPaV1 was first identified in fecal samples of healthy piglets in France in 2012 (2). Subsequently, similar viruses were reportedly identified in China, Hungary, Germany, and Romania (3–5). Here, we report the identification of SPaV1 in U.S. swine.

In an effort to identify unknown viral agents in pig tissues derived from a previous animal study conducted in 2001 concerning porcine reproductive and neurological syndrome (6), next-generation sequencing (NGS) was attempted. Various tissues (lung, lymph node, spleen, and tonsil) from sows were processed to prepare tissue homogenates, which were then clarified by low-speed centrifugation and concentrated by ultracentrifugation for nucleic acid extraction for RNA viruses. The nucleic acid extracts were used to make NGS libraries according to the manufacturer’s instructions using the TruSeq stranded total RNA library prep kit (Illumina, Inc., San Diego, CA). Sequencing was performed on a MiSeq system (Illumina, Inc.), as previously described (7). Sequences obtained from the MiSeq run were analyzed using Kraken (8), and viral genome sequences of interest were de novo assembled by a pipeline developed in-house, as described previously (7).

Unexpectedly, an almost full-length pasivirus genome of 6,695 nucleotides was obtained from a sow tissue pool. The virus strain was designated as SPaV1/US/17-50816IA60467-1/2001 (here, US SPaV1). Analysis of the US SPaV1 sequence demonstrated that the virus has a genomic organization similar to that of prototype SPaV1 (GenBank access no. JQ316470), in which 6,412 nucleotides of the genome encode a polyprotein flanked by 5’- and 3’-noncoding sequences. A pairwise comparison of the polyprotein-coding sequences between US SPaV1 and other swine pasiviruses whose sequences were available in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) revealed a nucleotide identity from 76.6% to 81.0%, while the deduced amino acid sequences had 82.1% to 89.7% identity among them. VP1 shares 73.4 to 79.8% nucleotide (nt) identity and 76.4 to 90.9% amino acid (aa) identity among them. In a sequence comparison of the 5’-untranslated region (UTR), a putative internal ribosome entry site (IRES) located there showed 94.7% to 98.2% identity between the 5’-UTR of US SPaV1 and other pasivirus sequences.
The identification of SPaV1 in U.S. swine shows a broader global distribution of the virus. As the animal study (6) was actually conducted in 2001, SPaV1 may have circulated in swine populations much longer than previously believed. More research will be needed to elucidate its evolution, diversity, and health significance.

**Accession number(s).** The complete polyprotein-coding sequence of U.S. swine pasivirus type 1 (SPaV1/US/17-50816IA60467-1/2001) has been submitted to GenBank under the accession number MG674090.

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**REFERENCES**

1. Zell R, Delwart E, Gorbalenya AE, Hovi T, King AMQ, Knowles NJ, Lindberg AM, Pallansch MA, Palmenberg AC, Reuter G, Simmonds P, Skern T, Stanway G, Yamashita T. ICTV Report Consortium. 2017. ICTV virus taxonomy profile: *Picornaviridae*. J Gen Virol 98:2421–2422. [https://doi.org/10.1099/jgv.0.000911](https://doi.org/10.1099/jgv.0.000911).

2. Sauvage V, Ar Gouilh M, Cheval J, Muth E, Pariente K, Burguiere A, Caro V, Manuguerra JC, Eloit M. 2012. A member of a new *Picornaviridae* genus is shed in pig feces. J Virol 86:10036–10046. [https://doi.org/10.1128/JVI.00046-12](https://doi.org/10.1128/JVI.00046-12).

3. Hanke D, Pohlmann A, Sauter-Louis C, Höper D, Stadler J, Ritzmann M, Steinirgl A, Schwarz B-A, Akimkin V, Fux R, Blome S, Beer M. 2017. Porcine epidemic diarrhea in Europe: in-detail analyses of disease dynamics and molecular epidemiology. Viruses 9:177. [https://doi.org/10.3390/v9070177](https://doi.org/10.3390/v9070177).

4. Yu JM, Li XY, Ao YY, Li LL, Liu N, Li JS, Duan ZJ, Kapoor A. 2013. Identification of a novel picornavirus in healthy piglets and seroepidemiological evidence of its presence in humans. PLoS One 8:e70137. [https://doi.org/10.1371/journal.pone.0070137](https://doi.org/10.1371/journal.pone.0070137).

5. Zaulet M, Petrovan V, Birladeanu AM, Stoian AMM, Kevorkian SEM, Nichita C, Eloit M, Buburuzan L. 2017. Identification and prevalence of swine pasivirus 1 in eastern Romanian pig farms. J Vet Diagn Invest 29:305–311. [https://doi.org/10.1177/1040638717696044](https://doi.org/10.1177/1040638717696044).

6. Pogranichny RM, Schwartz KJ, Yoon KJ. 2008. Isolation of a novel viral agent associated with porcine reproductive and neurological syndrome and reproduction of the disease. Vet Microbiol 131:35–46. [https://doi.org/10.1016/j.vetmic.2008.02.026](https://doi.org/10.1016/j.vetmic.2008.02.026).

7. Zhang J, Zheng Y, Xia XQ, Chen Q, Bade SA, Yoon KJ, Harmon KM, Gauger PC, Main RG, Li G. 2017. High-throughput whole genome sequencing of porcine reproductive and respiratory syndrome virus from cell culture materials and clinical specimens using next-generation sequencing technology. J Vet Diagn Invest 29:41–50. [https://doi.org/10.1177/1040638716673404](https://doi.org/10.1177/1040638716673404).

8. Wood DE, Salzberg SL. 2014. Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol 15:R46. [https://doi.org/10.1186/gb-2014-15-3-r46](https://doi.org/10.1186/gb-2014-15-3-r46).