AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Complete Genome Sequences of Two Novel Human-Like H3N2 Influenza A Viruses, A/swine/Oklahoma/65980/2017 (H3N2) and A/Swine/Oklahoma/65260/2017 (H3N2), Detected in Swine in the United States

Michael A. Zeller,[a,b] Ganwu Li,[a] Karen M. Harmon,[a] Jianqiang Zhang,[a] Amy L. Vincent,[b] Tavis K. Anderson,[b] Phillip C. Gauger[a]

[a]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa, USA

[b]Virus and Prion Research Unit, National Animal Disease Center, USDA-ARS, Ames, Iowa, USA

ABSTRACT Two novel human-like H3N2 influenza A virus strains, A/swine/Oklahoma/65980/2017 (H3N2) and A/swine/Oklahoma/65260/2017 (H3N2), were isolated from porcine samples submitted to the Iowa State University Veterinary Diagnostic Laboratory in the United States.

Influenza A virus (IAV) causes respiratory disease in swine and is a pathogen shared between humans and pigs. Genetic drift and spillover of human IAV with subsequent reassortment may result in human-like IAV strains novel to swine. We describe here two novel IAV strains detected in swine in 2017 that contain human seasonal influenza virus gene segments potentially transmitted through reverse zoonosis.

Nasal swabs originating from one swine production system, but separate locations in Oklahoma, were submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL) in 2017. An H3N2 virus with a novel human-like hemagglutinin (HA) sequence was detected using the VetMAX Gold swine influenza virus (SIV) subtyping real-time PCR (Thermo Fisher Scientific, Waltham, MA). Whole-genome sequencing was performed on strains A/swine/Oklahoma/65980/2017 (H3N2) and A/swine/Oklahoma/65260/2017 (H3N2), isolated in Madin-Darby canine kidney cells. Nucleic acids were extracted using the MagMAX pathogen RNA/DNA kit (catalog number 4462359) and a KingFisher Flex system (both Thermo Fisher Scientific) to construct sequencing libraries using TruSeq (Illumina, Inc., San Diego, CA). Sequencing was performed on a MiSeq system (Illumina, Inc.) following standard Illumina protocols at the ISU VDL (1, 2). Approximately 2,000,000 raw sequencing reads per sample were preprocessed using Trimmomatic version 0.36 and subjected to sequencing quality analysis with FastQC (3, 4). Quality-trimmed total reads were mapped against reference sequences downloaded from the NCBI Influenza Sequence Database (ftp://ftp.ncbi.nih.gov/genomes/INFLUENZA/) using BWA-MEM (5). Mapped reads were extracted using SAMtools (6) and used for *de novo* assembly. For each segment, contigs were assembled using ABySS (7) and SPAdes (8). The resulting contigs were manually curated in SeqMan Pro to remove contamination and trim chimeric contigs, thus generating a consensus sequence per segment.

A comparison of the nucleotide sequences of both strains demonstrated that the HA, NA, and M sequences were 99.5%, 99.9%, and 99.5% similar, respectively. The NS1 sequences were identical, and the PB1, PB2, and PA sequences had greater than 99.7% identity. The HA sequences of both isolates demonstrated 99% nucleotide (nt) identity to that of the human IAV strain A/Baltimore/0294/2017 (H3N2) (GenBank accession number KY949654). Each isolate had 10 amino acid substitutions in the HA compared

Received 29 August 2018 **Accepted** 24 October 2018 **Published** 21 November 2018

Citation Zeller MA, Li G, Harmon KM, Zhang J, Vincent AL, Anderson TK, Gauger PC. 2018. Complete genome sequences of two novel human-like H3N2 influenza A viruses, A/swine/Oklahoma/65980/2017 (H3N2) and A/swine/Oklahoma/65260/2017 (H3N2), detected in swine in the United States. Microbiol Resour Announc 7:e01203-18. https://doi.org/10.1128/MRA.01203-18.

Editor Jelle Matthijnssens, KU Leuven, Department of Microbiology and Immunology, Rega Institute, Laboratory of Viral Metagenomics, Leuven, Belgium

Address correspondence to Phillip C. Gauger, pcgauger@iastate.edu.

to the most similar human strain. The NA sequence of A/swine/Oklahoma/65980/2017 was most similar (99% nt identity) to that of human IAV strain A/Tennessee/06/2017 (H3N2) (GenBank accession number CY226641), and the NA sequence of A/swine/Oklahoma/65260/2017 was most similar (99% nt identity) to that of human IAV strain A/Baltimore/0223/2017 (H3N2) (GenBank accession number KY950122). PB2, PB1, PA, NP, and NS genes were similar to swine-origin triple-reassortant IAV. Phylogenetic analysis indicates that the M gene was derived from the 2009 H1N1 pandemic matrix circulating in swine. The HA and NA were nested within a monophyletic clade of 2016 to 2017 human seasonal H3 IAV, suggesting novel human-to-swine transmission (9).

This study documents a human-to-swine spillover and the potential for human seasonal IAV to cross the species barrier and infect swine. The ISU VDL has detected 21 genetically similar human-like H3 strains since the fall of 2016 (http://influenza.cvm.iastate.edu/correlation.php). The viruses have acquired a swine internal gene constellation through reassortment (10, 11), with at least 10 amino acid mutations in the HA suggesting adaptation and transmission in swine. The USDA swine surveillance system also reported similar human seasonal IAV designated "human-like 2016" in the Influenza A Virus in Swine Surveillance report (http://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/downloads/fy2018quarter1swinereport.pdf).

Data availability. The complete genome sequence of A/swine/Oklahoma/65980/2017 (H3N2) has been deposited in GenBank under the accession numbers MG720213 through MG720220 and that of A/swine/Oklahoma/65260/2017 (H3N2) under the accession numbers MG720221 through MG720228.

ACKNOWLEDGMENTS

We thank the Iowa State University Veterinary Diagnostic Laboratory faculty and staff for assistance with sample processing and diagnostic testing. The USDA IAV surveillance program played a crucial role in the detection of emerging or novel IAV in swine, including human seasonal spillover.

This study was supported by the Iowa State University Presidential Interdisciplinary Research Initiative and the Iowa State University Veterinary Diagnostic Laboratory. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

M.A.Z. prepared the manuscript; G.L. performed next-generation sequencing; M.A.Z., A.L.V., and T.K.A. performed phylogenetic analysis; K.M.H., J.Z., and P.C.G. conducted diagnostic tests and hemagglutinin sequencing; and G.L., K.M.H., J.Z., A.L.V., T.K.A., and P.C.G. reviewed the manuscript.

REFERENCES

1. Zhang J, Zheng Y, Xia X-Q, Chen Q, Bade SA, Yoon K-J, 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.
2. Chen Q, Wang L, Zheng Y, Zhang J, Guo B, Yoon K-J, Gauger PC, Harmon KM, Main RG, Li G. 2018. Metagenomic analysis of the RNA fraction of the fecal virome indicates high diversity in pigs infected by porcine endemic diarrhea virus in the United States. Virol J 15:95. https://doi.org/10.1186/s12985-018-1001-z.
3. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
4. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.
5. Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25:1754–1760. https://doi.org/10.1093/bioinformatics/btp324.
6. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25:2078–2079. https://doi.org/10.1093/bioinformatics/btp352.
7. Simpson JT, Wong K, Jackman SD, Schein JE, Jones SJ, Birol I. 2009. ABySS: a parallel assembler for short read sequence data. Genome Res 19:1117–1123. https://doi.org/10.1101/gr.089532.108.
8. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
9. Price MN, Dehal PS, Arkin AP. 2010. FastTree 2–approximately maximum-likelihood trees for large alignments. PLoS One 5:e9490. https://doi.org/10.1371/journal.pone.0009490.
10. Gao S, Anderson TK, Walia RR, Dorman KS, Janas-Martindale A, Vincent AL. 2017. The genomic evolution of H1 influenza A viruses from swine detected in the United States between 2009 and 2016. J Gen Virol 98:2001–2010. https://doi.org/10.1099/jgv.0.000885.
11. Rajão DS, Walia R, Campbell B, Gauger PC, Janas-Martindale A, Killian ML, Vincent AL. 2016. Reassortment between swine H3N2 and 2009 pandemic H1N1 in the United States resulted in influenza A viruses with diverse genetic constellations with variable virulence in pigs. J Virol 91:e01763-16. https://doi.org/10.1128/JVI.01763-16.