Human Infections with Novel Reassortant Influenza A(H3N2)v Viruses, United States, 2011

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During July–December 2011, a variant virus, influenza A(H3N2)v, caused 12 human cases of influenza. The virus contained genes originating from swine, avian, and human viruses, including the M gene from influenza A(H1N1)pdm09 virus. Influenza A(H3N2)v viruses were antigenically distinct from seasonal influenza viruses and similar to proposed vaccine virus A/Minnesota/11/2010.

round the world, cases of human infection with Aswine-origin influenza viruses have been reported sporadically (1-5). From 1990 through 2010, a total of 27 cases of human infection with these viruses were confirmed by the US Centers for Disease Control and Prevention (CDC) (4,6). Of these cases, 21 were caused by triple-reassortant influenza A viruses (13 subtype H1N1, 1 subtype H1N2, and 7 subtype H3N2), which have inherited genes from classical swine, avian, and human influenza viruses. The 2009 influenza pandemic, caused by a variant triple reassortant influenza virus, influenza A(H1N1)pdm09 virus (7.8), proved that swine influenza viruses (SIVs) can cause widespread infection among humans and result in substantial economic costs. In 2010, an increase in the number of human cases of swine-origin influenza (H3N2) virus infection prompted selection of a candidate vaccine virus of swine origin, A/Minnesota/11/2010 (H3N2)v (9).

Systematic surveillance and characterization of novel viruses infecting humans and SIVs in swine are critical for early detection of viruses with pandemic potential. Since 2009, CDC has provided public health laboratories with a real-time reverse transcription PCR (rRT-PCR)–based Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (S. Lindstrom, R. Garten, A. Balish, B. Shu, S. Emery, L. Berman, K. Sleeman, L. Gubareva, J. Villanueva, A. Klimov); and Battelle, Atlanta (N. Barnes)

assay for diagnostic testing for influenza (*10*). This assay enables detection and discrimination of influenza A virus subtypes H1N1, H3N2, and H1N1pdm09 and preliminary identification of triple-reassortant viruses possessing the nucleoprotein gene originating from SIVs.

The Study

In 2011, public health laboratories in 5 states detected 12 cases of human infection with a novel variant of influenza virus, influenza A(H3N2)v virus, by using the CDC rRT-PCR protocol. Respiratory specimens from these patients were sent to CDC for virus confirmation. History of direct or indirect contact with swine was confirmed for 6 patients. However, swine contact could not be verified for the other 6, suggesting that these infections might have been contracted through limited person-to-person transmission (11-13) (Table 1). All 12 patients recovered fully from their illness (10-12).

Genetic sequence analysis of RNA isolated from clinical respiratory specimens (Table 1) revealed that these influenza A(H3N2)v viruses possessed a combination of gene segments not previously found in humans (Figure 1). Of the 8 gene segments, 7 (hemagglutinin, neuraminidase, polymerase basic proteins 1 and 2, polymerase acidic protein, nucleoprotein, and nonstructural protein) were similar to those of triple-reassortant SIV A(H3N2) currently circulating in North America and to those from human triple-reassortant influenza A(H3N2) viruses isolated in 2010 from Pennsylvania, Minnesota, and Wisconsin (4), including the proposed vaccine virus of swine origin, A/Minnesota/11/2010 (14) (Figure 2, panel A; online Technical Appendix Figure, wwwnc.cdc.gov/ EID/pdfs/11-1922-Techapp.pdf). However, the M genes of all 2011 influenza A(H3N2)v viruses were inherited from a pandemic (H1N1) 2009 virus (Figure 2, panel B). Although SIVs of subtypes A(H3N2) and A(H1N2) with the M gene of influenza A(H1N1)pdm09 virus have been detected in swine since 2009 (15), influenza A(H3N2)v virus possessing the M gene of influenza A(H1N1)pdm09 virus had not been detected in humans.

According to genetic analysis results, amino acid diversity among influenza A(H3N2)v hemagglutinins was low (0–3 aa) compared with that of influenza A/Minnesota/11/2010. In addition, there have been no conserved amino acid changes in the hemagglutinin when comparing 2011 influenza A(H3N2)v from humans with 2011 influenza A(H3N2) SIVs. In particular, the known receptor binding site of the hemagglutinin protein of influenza A(H3N2)v virus was typical of SIV A(H3N2) viruses recently isolated in North America.

Hemagglutinins of the influenza A(H3N2)v viruses differed substantially from the hemagglutinin of the 2011–12 human seasonal vaccine virus, A/Perth/16/2011

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Table 1. Results of analysis of viral RNA isolated from original clinical samples from persons with influenza A(H3N2)v virus infection, United States, 2011*

| 0 | | | | | |
|-------------------------------------|---------------------------|-------------------|-------------------|-------------------------|----------------------------|
| | Contact with | Specimen | Specimen | rRT-PCR–positive | |
| Influenza virus strain | swine (11–13) | collection date | type | results+ | Genes sequenced‡ |
| A/Indiana/08/2011 | No | Jul 24 | NPS | InfA, H3, pdmInfA | Full genome |
| A/Pennsylvania/09/2011 | Indirect | Aug 20 | NPS | InfA, H3, pdmInfA | Full PB2, PB1, HA, NP, NA, |
| - | | - | | | M, NS, partial PA |
| A/Pennsylvania/10/2011 | Direct | Aug 26 | NPS | InfA | Full NS, partial HA, M, NA |
| A/Pennsylvania/11/2011 | Indirect | Aug 25 | NPS | InfA, H3, pdmInfA | Full PA, NP, NA, M NS, |
| | | - | | | partial PB2, PB1, HA |
| A/Maine/06/2011 | Direct | Oct 10 | NPS | InfA, H3, pdmInfA | Full genome |
| A/Indiana/10/2011§ | Direct | Oct 22 | Cell culture | InfA, H3, pdmInfA | Full genome |
| A/Maine/07/2011 | Direct | Oct 24 | NPS | InfA | Partial HA, M, NS |
| A/Iowa/07/2011 | No | Nov 14 | NPW | InfA, H3, pdmInfA | Full PB2, PB1, PA, NP, NA, |
| | | | | | M, NS, partial HA |
| A/Iowa/08/2011 | No | Nov 14 | NS | InfA, H3, pdmInfA | Full genome |
| A/Iowa/09/2011 | No | Nov 14 | NS | InfA, H3, pdmInfA | Full genome |
| A/West Virginia/06/2011 | No | Nov 21 | NW | InfA, H3, pdmInfA | Full genome |
| A/West Virginia/07/2011 | No | Dec 07 | NPS | InfA | Partial HA, NA, M |
| *Influenza A/H3N2)v/ influenza viru | e variant identified in h | umane: rDT DCD re | al timo rovorco t | ranscription DCD: NDS r | acophanyngoal cwah: DB |

*Influenza A(H3N2)v, influenza virus variant identified in humans: rRT-PCR, real-time reverse transcription PCR; NPS, nasopharvngeal swab; PB, polymerase basic protein; HA, hemagglutinin; NP nucleoprotein; NA, neuraminidase; M, matrix protein; NS, nonstructural protein; PA, polymerase acidic protein; NPW, nasopharyngeal wash; NS, nasal swab.

†Results obtained by using the Centers for Disease Control and Prevention Human Influenza Virus Real-Time RT-PCR Diagnostic Panel. \$\$ Sequences available from GenBank and the online Technical Appendix Table (wwwnc.cdc.gov/EID/pdfs/11-1922-Techapp.pdf).

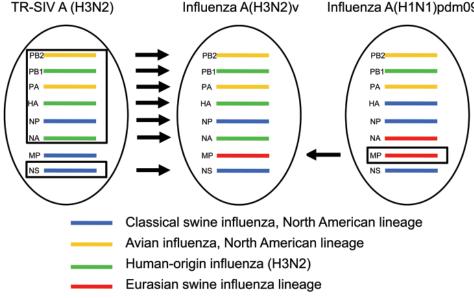
§This patient was >18 years of age; all others were <18.

(58-60 aa), which resulted from divergent evolutionary paths for the H3 hemagglutinin in swine and human viruses. The effect of these substitutions on virus antigenicity was examined in the hemagglutinationinhibition assay by using a panel of reference ferret antiserum. Hemagglutination-inhibition analysis of 6 available influenza A(H3N2)v virus isolates revealed no measureable inhibition by antiserum against the current human seasonal influenza A(H3N2) vaccine virus, A/ Perth/16/2009 (Table 2), indicating that influenza A(H3N2)v virus is antigenically distinct from influenza A(H3N2) viruses currently circulating among humans.

All influenza A(H3N2)v viruses tested were antigenically similar, demonstrating hemagglutination-

inhibition titers with only a 2-fold difference from antiserum against other influenza A(H3N2)v viruses. These viruses were also antigenically closely related to earlier human triple-reassortant virus isolates that contained the M gene from classical SIVs (A/Wisconsin/12/2010, A/ Pennsylvania/14/2010, and A/Minnesota/11/2010). All influenza A(H3N2)v viruses tested were also antigenically closely related to the proposed vaccine reassortant X-203 (13) between triple-reassortant A/Minnesota/11/2010 (H3N2) and A/PR/8/34 (H1N1) (Table 2).

The level of cross-protective immunity against influenza A(H3N2)v in humans previously vaccinated and/or exposed to previously circulated seasonal influenza A(H3N2) viruses is unknown. The antigenic



Influenza A(H1N1)pdm09

Figure 1. Derivation of genes segments of novel influenza A(H3N2) viruses isolated from humans, United States, 1990-2011. TR-SIV, triple reassortment swine influenza virus.

DISPATCHES

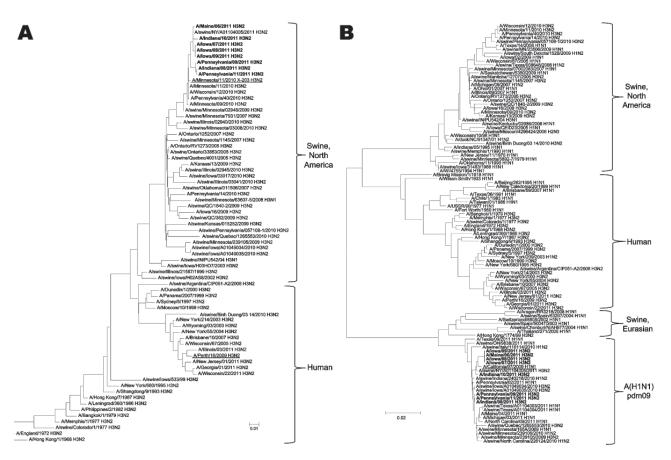


Figure 2. Phylogenetic analysis of the A) hemagglutinin and B) matrix genes of influenza A(H3N2)v viruses. Sequences obtained from human A(H3N2)v isolates in the United States during 2011 are shown in **boldface**; sequences of proposed vaccine virus are <u>underlined</u>. Scale bars indicate number of base substitutions per site.

characterization described here demonstrates that vaccination with the current trivalent influenza vaccine might not provide immune protection against influenza A(H3N2)v virus. A vaccine containing a contemporary influenza A(H3N2)v or an antigenically similar virus (such as A/Minnesota/11/2010) might be needed to elicit protective immunity.

Functional neuraminidase inhibition assays indicated that 6 influenza A(H3N2)v virus isolates were sensitive to the neuraminidase inhibitors oseltamivir and zanamivir. No genetic markers known to decrease sensitivity to neuraminidase inhibitors were found in the neuraminidase genes of all 12 influenza A(H3N2)v viruses. Similar to pandemic (H1N1) 2009 viruses, influenza A(H3N2)v

| Table 2. Hemagglutinin-inhibition assay | y results, ir | ncluding t | he 6 avail | able influe | enza A(H3 | N2)v virus | es isolate | d in 2011 | , United States* |
|---|--------------------------------------|------------|------------|-------------|-----------|------------|------------|-----------|------------------|
| | Titers to reference ferret antiserum | | | | | | Specimen | | |
| Influenza virus strain (culture method) | PER/16 | KS/13 | WI/12 | PA/14 | MN/11 | X203 | IN/08 | IN/10 | collection date |
| A/Perth/16/2009 (egg)† | 1,280 | <10 | <10 | <10 | 20 | 20 | <10 | <10 | 2009 Apr 7 |
| A/Kansas/13/2009 (MDCK cells) | <10 | 640 | 80 | 160 | 40 | 40 | 40 | 80 | 2009 Jul 29 |
| A/Wisconsin/12/2010 (MDCK cells) | <10 | 40 | 1,280 | 320 | 640 | 320 | 640 | 1,280 | 2010 Sep 10 |
| A/Pennsylvania/14/2010 (egg) | <10 | 160 | 320 | 640 | 320 | 320 | 640 | 640 | 2010 Oct 26 |
| A/Minnesota/11/2010 (egg) | <10 | <10 | 320 | 160 | 640 | 1,280 | 320 | 160 | 2010 Nov 26 |
| A/Minnesota/11/2010 X-203 (egg)‡ | 10 | <10 | 80 | 40 | 320 | 640 | 160 | 80 | Not applicable |
| A/Indiana/08/2011 (MDCK cells)* | <10 | 10 | 1,280 | 640 | 640 | 320 | 1,280 | 1,280 | 2011 Jul 24 |
| A/Indiana/10/2011 (MDCK cells)* | <10 | 40 | 1,280 | 320 | 1,280 | 640 | 1,280 | 1,280 | 2011 Oct 22 |
| A/Indiana/10/2011 (egg)* | <10 | 10 | 1,280 | 320 | 640 | 320 | 1,280 | 1,280 | 2011 Oct 22 |
| A/lowa/07/2011 (MDCK cells)* | <10 | 10 | 1,280 | 640 | 1,280 | 640 | 1,280 | 2,560 | 2011 Nov 14 |
| A/Iowa/08/2011 (MDCK cells)* | <10 | 40 | 1,280 | 640 | 640 | 640 | 1,280 | 2,560 | 2011 Nov 14 |
| A/Iowa/09/2011 (MDCK cells)* | <10 | 40 | 1,280 | 640 | 1,280 | 640 | 2560 | 2,560 | 2011 Nov 14 |

*Influenza A(H3N2)v, virus variant identified in humans, United States, 2011. Gray shading indicates antigenically similar viruses.

†Current seasonal influenza A(H3N2) vaccine virus.

‡Reassortant virus possessing the hemagglutinin and neuraminidase genes of A/Minnesota/11/2010 and the remaining 6 genes of A/PR/8/34.

viruses have genetic markers (V27A, S31N) in the M2 protein that confer resistance to the antiviral medications amantadine and rimantadine.

Conclusions

The detection of multiple cases of human infection with influenza A(H3N2)v virus within a 5-month period in 5 US states, coupled with possible human-to-human transmission, underscores the need for continued influenza surveillance at the swine–human interface. Coordinated surveillance of human and animal influenza viruses enables rapid detection of human infections with novel influenza viruses and timely identification of new virus variants in swine. As was evident during the 2009 influenza pandemic, this information is vital for development of resources that might be needed to effectively respond to the emergence and spread of a novel influenza virus in humans.

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Dr Lindstrom is the team lead of the Diagnostics Development Team of the Virus Surveillance and Diagnosis Branch, Influenza Division, at the Centers for Disease Control and Prevention. His research interests are development and qualification of molecular diagnostic testing procedures for influenza viruses, confirmatory diagnostic testing and reporting of human cases of influenza, including infections potentially caused by novel influenza viruses.

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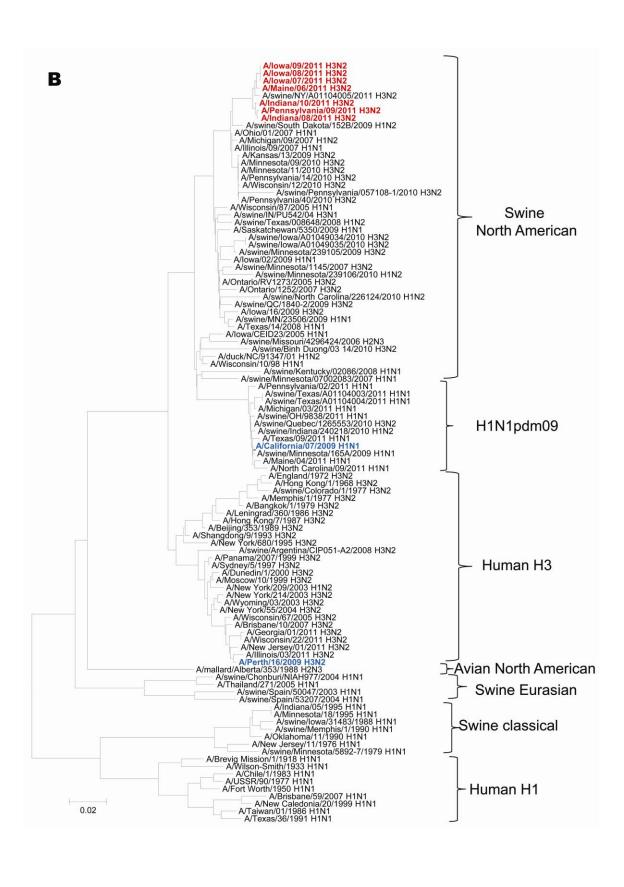
Address for correspondence: Alexander Klimov, Centers for Disease Control and Prevention, Mailstop G16, 1600 Clifton Road NE, Atlanta, GA 30333, USA: email: axk0@cdc.gov

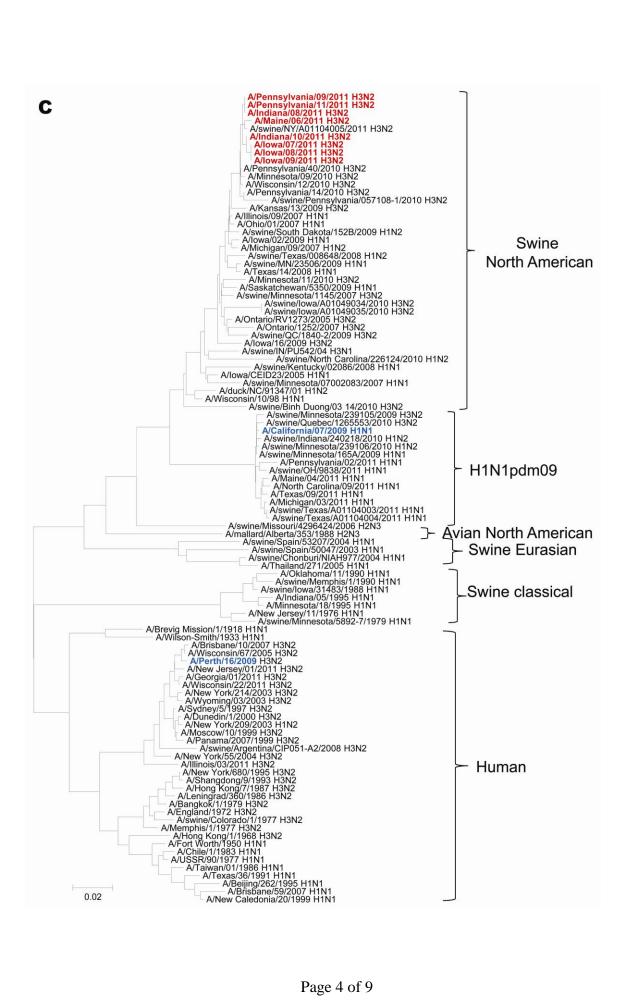
Human Infections with Novel Reassortant Influenza A(H3N2)v Viruses, United States, 2011

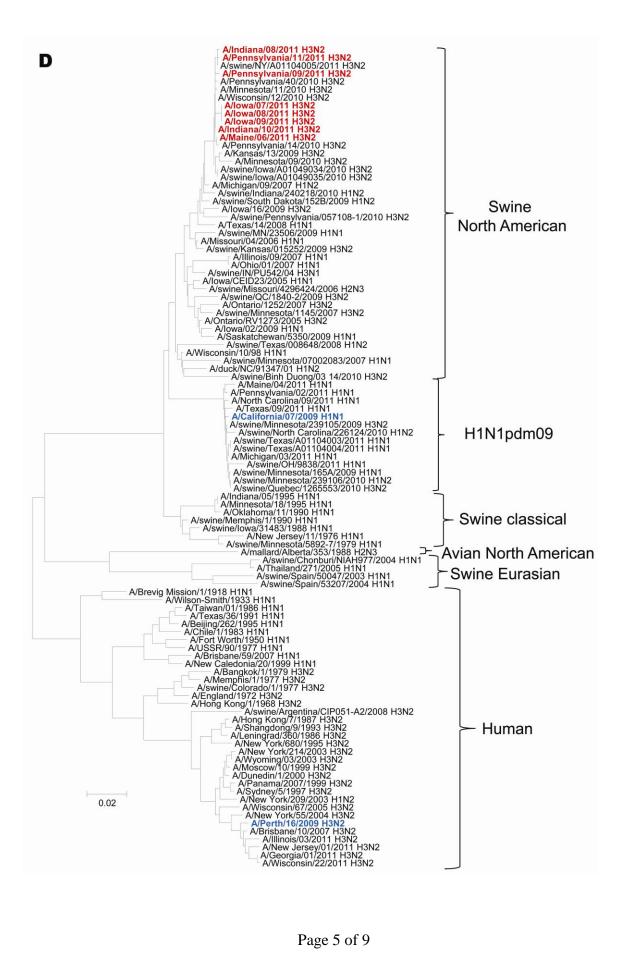
Technical Appendix

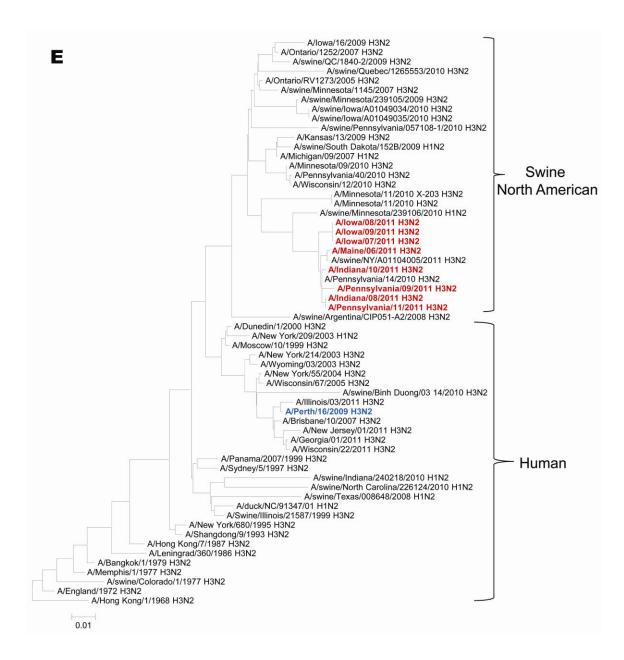
Technical Appendix Figure (following pages). Phylogenetic analyses of the A) polymerase basic (PB) protein 2, B) PB1 protein, C) polymerase acidic protein, D) nucleoprotein, E) neuraminidase, and F) nonstructural protein genes of influenza A(H3N2)v virus. Sequences obtained from human isolates in the United States during 2011 are shown in red; strains from 2009 are shown in blue. Scale bars indicate number of base substitutions per site.

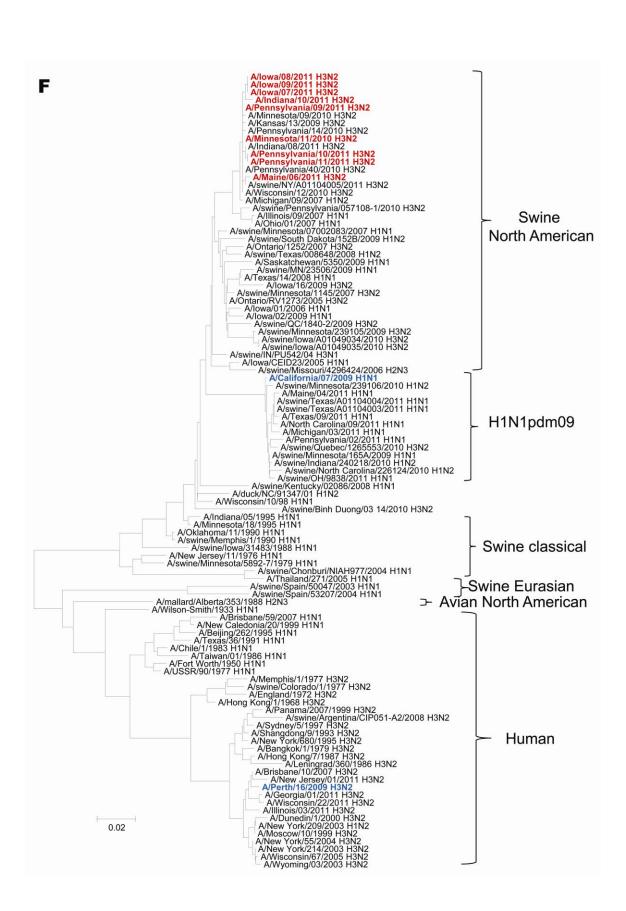












| Influenza Data | | | | |
|------------------------|------------|----------------------------|--|--|
| Segment ID | Segment | Collection date | Isolate name | Originating laboratory† |
| EPI344405 | HA | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI335629 | HA | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI333152 | HA | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI344400 | М | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI335632 | М | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI333148 | М | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI344404 | NA | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI335631 | NA | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI333151 | NA | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI344398 | NP | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI335630 | NP | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI333146 | NP | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI344399 | NS | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI335633 | NS | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI333147 | NS | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI344401 | PA | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI335628 | PA | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI333149 | PA | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI344403 | PB1 | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI335627 | PB1 | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI333150 | PB1 | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI344402 | PB2 | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI335626 | PB2 | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI333194 | PB2 | 2011 Jul 27 | A/Indiana/08/2011 | Indiana State Department of Health Laboratories |
| EPI344397 | HA | 2011 Oct 22 | A/Indiana/10/2011 | Indiana State Department of Health Laboratories |
| EPI344389 | HA | 2011 Oct 22 | A/Indiana/10/2011 | Indiana State Department of Health Laboratories |
| EPI340984 | HA | 2011 Oct 22 | A/Indiana/10/2011 | Indiana State Department of Health Laboratories |
| EPI344392 | M | 2011 Oct 22 | A/Indiana/10/2011 | Indiana State Department of Health Laboratories |
| EPI344385 | M | 2011 Oct 22 | A/Indiana/10/2011 | Indiana State Department of Health Laboratories |
| EPI340979 | M | 2011 Oct 22 | A/Indiana/10/2011 | Indiana State Department of Health Laboratories |
| EPI344396 | NA | 2011 Oct 22 | A/Indiana/10/2011 | Indiana State Department of Health Laboratories |
| EPI344388 | NA | 2011 Oct 22 | A/Indiana/10/2011 | Indiana State Department of Health Laboratories |
| EPI340983 | NA | 2011 Oct 22 | A/Indiana/10/2011 | Indiana State Department of Health Laboratories |
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| EPI344395 | PB1 | 2011 Oct 22 | A/Indiana/10/2011 A/Indiana/10/2011 | Indiana State Department of Health Laboratories |
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| | | | | |
| EPI340982 EPI344394 | PB1 PB2 | 2011 Oct 22 2011 Oct 22 | A/Indiana/10/2011 A/Indiana/10/2011 | Indiana State Department of Health Laboratories Indiana State Department of Health Laboratories |
| EPI344394 EPI345358 | PB2 PB2 | 2011 Oct 22 2011 Oct 22 | A/Indiana/10/2011 A/Indiana/10/2011 | Indiana State Department of Health Laboratories |
| EPI340981 | PB2 PB2 | 2011 Oct 22 2011 Oct 22 | A/Indiana/10/2011 A/Indiana/10/2011 | Indiana State Department of Health Laboratories |
| EPI340961 EPI342688 | HA | 2011 Nov 14 | A/Indiana/10/2011 A/Iowa/07/2011 | Indiana State Department of Health Laboratories |
| EPI342683 | M | 2011 Nov 14 | A/Iowa/07/2011 A/Iowa/07/2011 | Iowa State Hygienic Laboratory |
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| EPI342681 | NP | 2011 Nov 14 | A/Iowa/07/2011 | Iowa State Hygienic Laboratory Iowa State Hygienic Laboratory |
| | | | | Iowa State Hygienic Laboratory |
| EPI342682 | NS | 2011 Nov 14 | A/Iowa/07/2011 | ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,, |
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| EPI342685 | PB2 | 2011 Nov 14 | A/Iowa/07/2011 | Iowa State Hygienic Laboratory |
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| EPI342694 | PB1 | 2011 Nov 14 | A/Iowa/08/2011 | Iowa State Hygienic Laboratory |

Technical Appendix Table. Gene sequence accession numbers for influenza A (H3N2)v, from The Global Initiative on Sharing All Influenza Data

| Segment ID Segment Collection date Isolate name Originating laboratory† EPI342639 PB2 2011 Nov 14 A/lowa/08/2011 Iowa State Hygienic Laboratory EPI342699 M 2011 Nov 14 A/lowa/09/2011 Iowa State Hygienic Laboratory EPI342697 NP 2011 Nov 14 A/lowa/09/2011 Iowa State Hygienic Laboratory EPI342697 NP 2011 Nov 14 A/lowa/09/2011 Iowa State Hygienic Laboratory EPI342700 PA 2011 Nov 14 A/lowa/09/2011 Iowa State Hygienic Laboratory EPI342700 PA 2011 Nov 14 A/lowa/09/2011 Iowa State Hygienic Laboratory EPI342701 PB2 2011 Nov 14 A/lowa/09/2011 Iowa State Hygienic Laboratory EPI339170 NA 2011 Oct 10 A/Maine/06/2011 Maine Health and Environmental Testing Laboratory EPI339164 NP 2011 Oct 10 A/Maine/06/2011 Maine Health and Environmental Testing Laboratory EPI339167 NA 2011 Oct 10 A/Maine/06/2011 Maine Health and Environmental Testing Laboratory EPI339167 PA | |
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*HA, hemagglutinin; M, matrix protein; NA, neuraminidase; NP, nucleocapsid protein; NS, nonstructural protein; PA, polymerase acidic protein; PB, polymerase basic protein. †Submitting laboratory for all samples was the Centers for Disease Control and Prevention.