

# S-OtrH3N2 viruses: use of sequence data for description of the molecular characteristics of the viruses and their relatedness to previously circulating H3N2 human viruses

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## Citation style for this article:

Lina B, Bouscambert M, Enouf V, Rousset D, Valette M, van der Werf S. S-OtrH3N2 viruses: use of sequence data for description of the molecular characteristics of the viruses and their relatedness to previously circulating H3N2 human viruses. *Euro Surveill.* 2011;16(50):pii=20039. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20039>

Article published on 15 December 2011

**Emergence of influenza viruses from the animal reservoir is a permanent challenge. The rapid description and immediate sharing of information on these viruses is invaluable for influenza surveillance networks and for pandemic preparedness. With the help of data generated from the World Health Organization Collaborating Centre for Reference and Research on Influenza at the United States Centers for Disease Control and Prevention\*, we provide here information on the swine-origin triple reassortant influenza A(H3N2) viruses detected in human cases in the north-east of the United States.**

On 23 November 2011, the World Health Organization Collaborative Centre (WHOCC) for Reference and Research on Influenza at the United States (US) Centers for Disease Control and Prevention (CDC)\* reported three cases of documented infections with a triple reassortant influenza A(H3N2) virus of swine origin (S-OtrH3N2) that may have been acquired through human-to-human transmission [1]. In the last 10 years, at least 27 human cases of swine influenza virus infections had been observed in the US [2-4], all of which occurred after exposure to infected animals, and no human-to-human transmission had been reported so far. Noticeably, the 11 most recent cases recorded since August 2011 were due to viruses that had acquired the matrix (M) gene segment of the influenza A(H1N1) pdm09 virus through reassortment [1,5]. This may have resulted in enhanced transmission potential of the S-Otr H3N2 virus. Indeed, it has been reported that the acquisition by an S-Otr H1N1 virus of both the M and neuraminidase (NA) gene segments from the Eurasian swine lineage virus facilitated the emergence and the spread of the influenza A(H1N1) pdm09 virus [3]. In addition, epidemiological studies suggest that the four

last cases reported between the 23 November and 9 December were observed in patients with no direct or indirect contact with swine, implying that limited human-to-human transmission has occurred.

This event raises concerns about the potential of such swine-origin viruses to establish a sustained human-to-human transmission and about our ability to fight against this virus, should it become pandemic. Sharing molecular data at a very early stage of emergence facilitates *in silico* analysis and risk assessment. Thanks to the WHOCC at the CDC\*, the sequences of these viruses are available in the GISAID database (Table).

## Phylogenetic relationship

Based on the data available, it was possible to draw a phylogenetic tree comprising haemagglutinin (HA) sequences from both human and S-Otr influenza A(H3N2) isolates, to determine if there were common characteristics between these two groups of viruses. We performed this analysis for a 966 nt sequence (nt 72 to 1,038) of the HA1 regions of the HA genes of all human reference strains used in the influenza vaccines between 1972 and 2011, of six S-Otr H3N2 viruses isolated from human cases in 2011, and of two S-Otr H3N2 viruses detected in the swine population in 2010 and 2011 (Figure 1).

This analysis shows that the human HA1 phylogenetically most closely related to the S-OtrH3N2 viruses was the A/Wuhan/359/95(H3N2) virus. This is consistent with the timing of introduction of the human H3N2 viruses into the swine population in North America [4]. This closest homology was confirmed when the evolutionary distances were computed using the Tamura-Nei method [6]. In our analysis, the 966 nt HA fragment of

the S-Otr A/Iowa/08/2011(H3N2) virus showed 5.5% divergence from the A/Wuhan/359/95(H3N2), compared with 9.3% from the more recent A/Perth/16/2009(H3N2) virus. The evolutionary distances suggest a division of the human H3N2 viruses into two groups: one group of strains isolated between 1986 and 1999, which had the highest homology to S-Otr A/Iowa/08/2011(H3N2), and a second group comprising strains isolated before 1983 or after 1999, for which the divergence is larger than 8% and can reach as much as 11% (Figure 1).

In order to speculate on possible cross-protection, it is important to analyse differences in the antigenic sites. An alignment of the amino acid sequences of the HA1 subunit spanning the five antigenic sites of the HA protein [7] shows differences between the S-OtrH3N2 viruses and the human influenza A(H3N2) strains (Figure 2). Although we observed only few differences in the antigenic sites C, D and E, the differences in

antigenic sites A and B were more significant. Antigenic site B, closest to the receptor-binding site has been proposed to contribute most to the antigenic characteristics of the HA protein [8].

### Implications for diagnostics

The sequence analysis of the other gene segments like M, NP or NA also provided information on the capability of molecular diagnostic procedures to detect the S-Otr H3N2 virus. These viruses acquired the M gene segment from the human influenza A(H1N1)pdm09 virus. This implies that the generic detection through RT-PCR targeting the M gene will have very good sensitivity also for the S-Otr viruses. However, depending on possible mismatches in the primers and/or probes, the RT-PCR targeting the HA or NA gene segments may either be lacking sensitivity or possibly fail to detect the S-Otr H3N2 virus. Conversely, in terms of alertness and surveillance, the use of H3 and/or N2 subtyping

**TABLE**

S-Otr influenza A(H3N2) viruses isolated from swine and humans in 2010 and 2011 used for the phylogenetic analysis

Segment ID	Segment	Country	Collection date	Isolate name	Originating Laboratory	Submitting Laboratory	Authors
PI_ISL_83701	HA	United States	10 Jun 2011	A/Minnesota/11/2010	Minnesota Department of Health	WHO Collaborating Centre for Reference and Research on Influenza, Centers for Disease Control and Prevention, Atlanta	Shu B, Emery S, Garten R, Lindstrom S
EPI_ISL_99213	HA	United States	23 Nov 2011	A/Iowa/07/2011	Iowa State Hygienic Laboratory	WHO Collaborating Centre for Reference and Research on Influenza, Centers for Disease Control and Prevention, Atlanta	Emery S, Shu B, Garten R, Lindstrom S
EPI_ISL_99214	HA	United States	23 Nov 2011	A/Iowa/08/2011	Iowa State Hygienic Laboratory	WHO Collaborating Centre for Reference and Research on Influenza, Centers for Disease Control and Prevention, Atlanta	Emery S, Shu B, Garten R, Lindstrom S
EPI_ISL_99215	HA	United States	23 Nov 2011	A/Iowa/09/2011	Iowa State Hygienic Laboratory	WHO Collaborating Centre for Reference and Research on Influenza, Centers for Disease Control and Prevention, Atlanta	Emery S, Shu B, Garten R, Lindstrom S
EPI_ISL_99419	HA	United States	23 Nov 2011	A/Indiana/08/2011	Indiana State Department of Health Laboratories	WHO Collaborating Centre for Reference and Research on Influenza, Centers for Disease Control and Prevention, Atlanta	Shu B, Emery S, Garten R, Lindstrom S
EPI_ISL_99418	HA	United States	23 Nov 2011	A/Indiana/10/2011	Indiana State Department of Health Laboratories	WHO Collaborating Centre for Reference and Research on Influenza, Centers for Disease Control and Prevention, Atlanta	Shu B, Emery S, Garten R, Lindstrom S
EPI_ISL_97080	HA	United States	07 Sep 2011	Sw/Indiana/A01049653/2011	NA	NA	Nezami SG, Sun D, Zhang J, Stensland WR, Strait EL, Yoon K-J
EPI_ISL_93357	HA	United States	20 Jun 2011	Sw/Pennsylvania/A01049256/2010	NA	NA	Sun D, Nezami SG, Zhang J, Stensland WR, Strait EL, Yoon K-J

NA: not available.

We gratefully acknowledge the authors, originating and submitting laboratories of the sequences from GISAID's EpiFlu Database, on which this analysis is based.

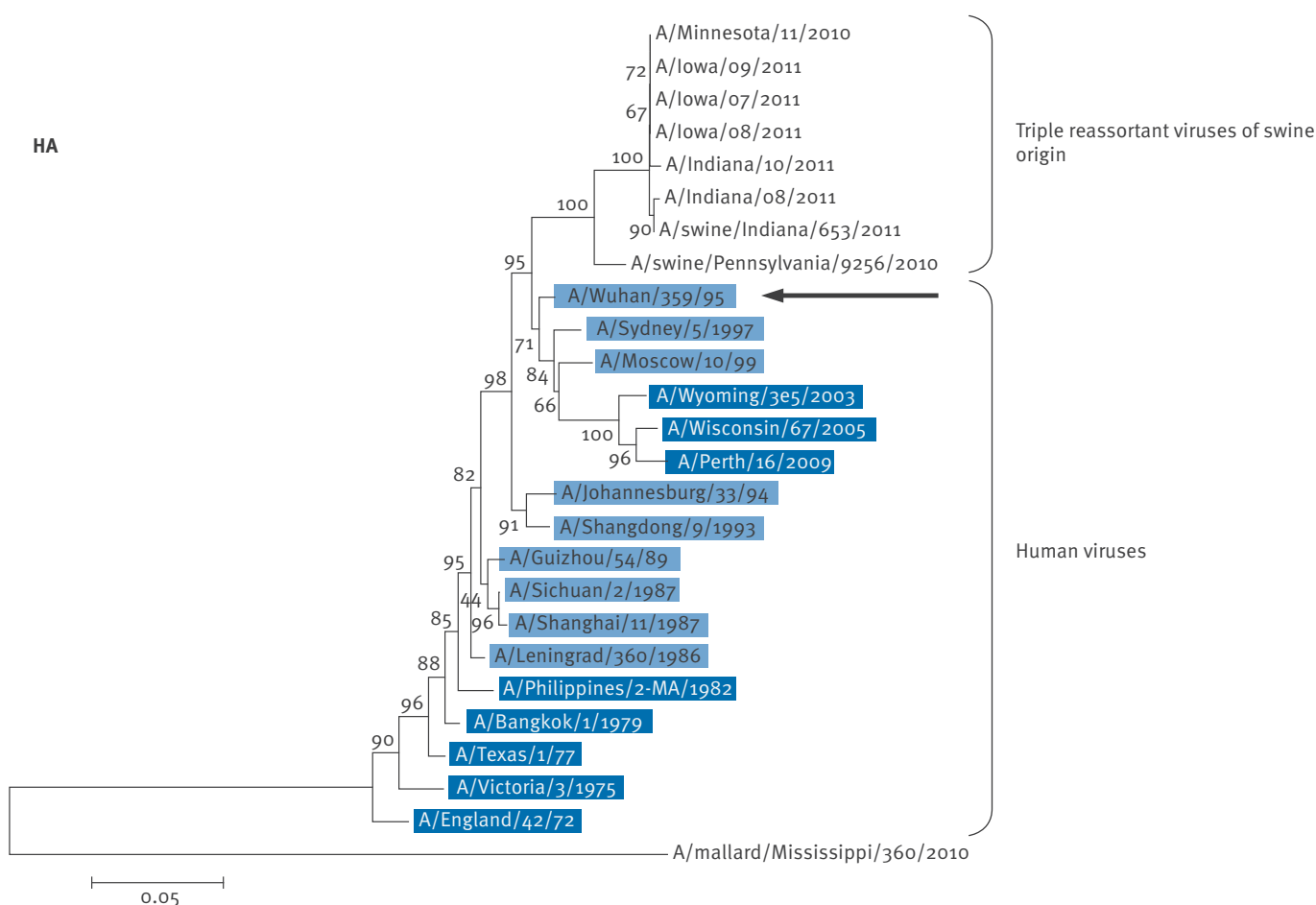
RT-PCRs that would have equal sensitivity for seasonal human and swine-origin H<sub>3</sub>N<sub>2</sub> viruses, should not be promoted at this stage because these procedures may fail to recognise cases of S-Otr H<sub>3</sub>N<sub>2</sub> virus infection. Hence, for accurate detection and surveillance, specific RT-PCR methods should be developed, or alternatively, predefined algorithms with already existing discriminating molecular tools need to be implemented [9]. Lastly, the NA and M2 sequences available from the recent isolates suggest that, as for the influenza A(H1N1)pdm09 virus, antiviral drugs that block the M2 ion channel will not be effective because the M2 sequence carries the S31N mutation associated with resistance. No known genetic markers for resistance to NA inhibitors have been detected in these new strains so far. This should be confirmed by phenotypic assays.

## Implications for immunological cross-protection

In case of the emergence of a zoonotic virus with an HA derived from previously circulating human viruses, it needs to be established whether or not infections with human influenza viruses in the past seasons or vaccinations confer cross-protection against the new viruses. Indeed, during the recent pandemic in 2009, it was observed that upon infection or vaccination of elderly people previously exposed to influenza A(H1N1) viruses that shared common epitopes with the emerging pandemic virus, efficient cross-protection was induced through memory immune cells [10]. The comparison of the five antigenic domains of past influenza A(H<sub>3</sub>N<sub>2</sub>) human viruses with those of the S-Otr viruses showed similarities and differences. Hence, it is impossible to predict if pre-existing immunity will be efficient against this virus, even if it seems likely that some cross-protection will exist; seroepidemiological surveys should be carried out to support or disprove this

**FIGURE 1**

Phylogenetic analysis of the haemagglutinin genes (nt 72-1,038) of 26 influenza A(H<sub>3</sub>N<sub>2</sub>) viruses (vaccine strains and S-Otr viruses)



The evolutionary history and divergence were inferred using the neighbour-joining method. They were computed in MEGA5 (version 5.0), using the Tamura-Nei method and are in the units of the number of base substitutions per site. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. The analysis involved 26 nucleotide sequences and a total of 966 positions in the final dataset. The molecular data set was collected from GISAID.

The arrow shows the human strain with the closest homology (5.5% of divergence). The strains in light blue have less than 8% divergence with the S-Otr viruses, those in dark blue have more than 8% divergence.

hypothesis. One must also keep in mind that if there is pre-existing immunity to this virus, it may occur in different age groups than observed with the influenza A(H1N1)pdm09 virus.

## Conclusion

Overall, even if neither the evolution of these S-Otr H3N2 viruses nor their putative impact in the general population can be predicted enhanced surveillance with adapted diagnostic procedures will become necessary if these sporadic cases turn into sustained dissemination. According to the similarities observed between the sequences of the S-Otr and human H3 influenza viruses (especially those circulating before 1995), the likelihood of cross-protection is high, but should be confirmed with seroepidemiological studies.

## Acknowledgments

The authors acknowledge that this analysis has been possible only with the viruses and the molecular data provided in GISAID by the Indiana State Department of Health Laboratories, the Iowa State Hygienic Laboratory, the Minnesota Department of Health and the WHO Collaborative Centre for Reference and Research on Influenza at the Centers for Disease Control and Prevention.\*

This work was supported in part by the European Union Seventh Framework Programme [FP7/2007-2013] under the project PREDEMICS, EC [grant agreement number 278433].

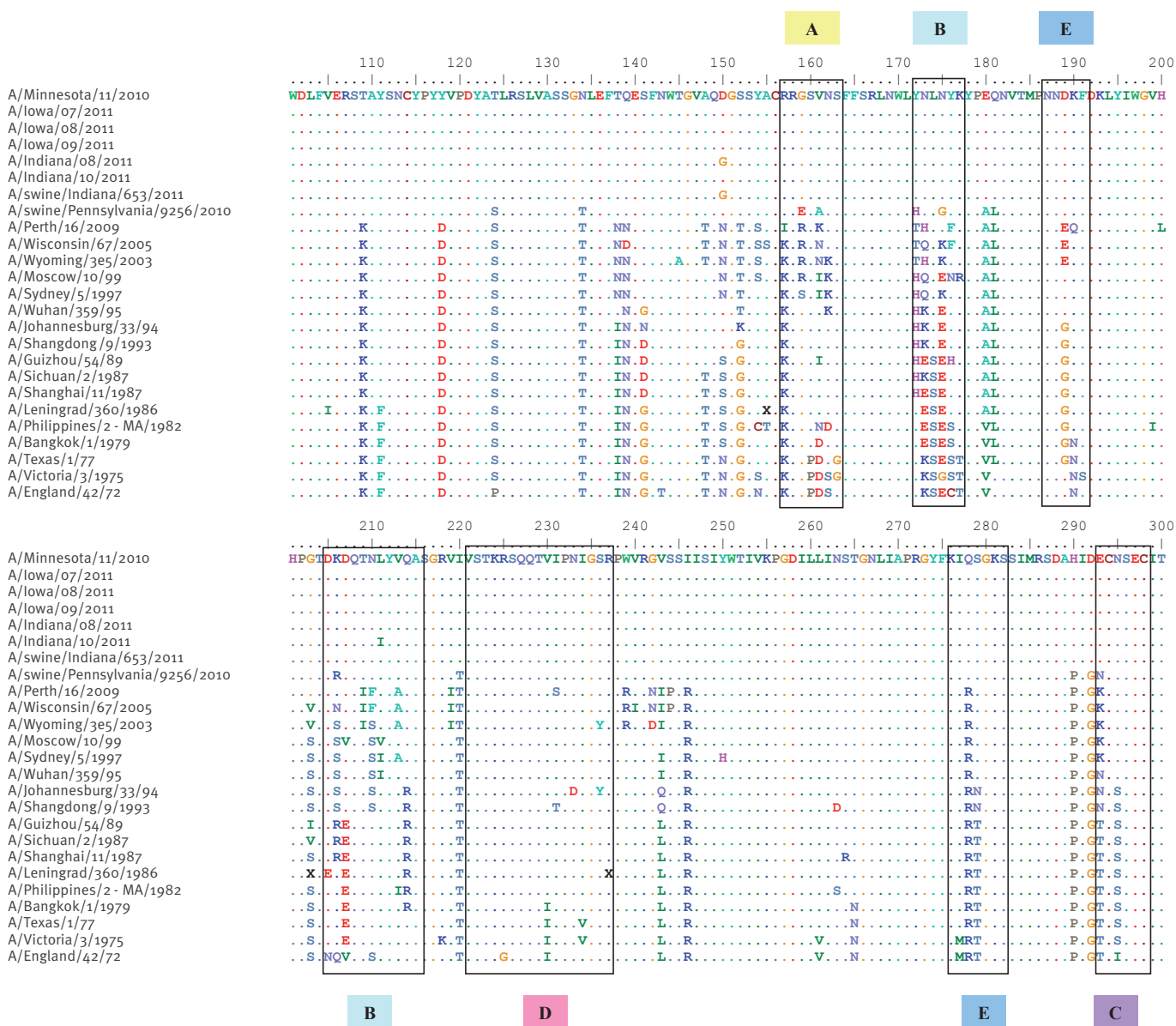
## \*Correction

On request of the authors, the following changes were made in this article on 23 December 2011:

The Table was replaced. The text “the United States Centers for Disease Control and Prevention” was replaced with “the

**FIGURE 2**

Amino acid sequence alignment of the haemagglutinin protein of 25 influenza A(H3N2) viruses (vaccine strains and S-Otr viruses) with antigenic sites A–E



WHO Collaborating Centre for Reference and Research on Influenza at the United States Centers for Disease Control and Prevention” in the Abstract, the Introduction and the paragraph before the Table. An acknowledgement was added.

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