

Characteristics of the emergent influenza A (H1N1) viruses and recommendations for vaccine development

26 May 2009

The novel influenza A (H1N1) virus which is causing infection among humans, was first identified in the United States on 15 April 2009, and shown to be genetically related to recent swine influenza viruses, but to have a genetic make-up not previously detected among viruses infecting either swine or human populations¹. It was later shown that outbreaks of respiratory illness experienced in several parts of Mexico in March and April 2009 were due to these novel influenza A (H1N1) viruses². Subsequently the novel A (H1N1) virus has caused outbreaks of disease in several countries, initially in North America, and has now spread to 46 countries worldwide. The influenza A (H1N1) virus is thus considered to pose a pandemic threat and on 29 April 2009 WHO raised its influenza pandemic alert level to phase 5.

The emergence and spread of the novel influenza A (H1N1) virus has been of great concern globally. The selection, development and availability of A (H1N1) vaccine viruses are essential components of the global strategy for pandemic preparedness and response. This summary provides an update on the characterization of available novel influenza A (H1N1) viruses affecting humans and makes a recommendation on suitable viruses for development of vaccines against this emergent influenza A (H1N1) infection. WHO, through the Global Influenza Surveillance Network, will continue to monitor the evolution of the influenza A (H1N1) virus, and will update recommendations of vaccine viruses whenever needed.

This recommendation does not replace the annual WHO recommendations of vaccine viruses for seasonal influenza vaccines. WHO will update the recommendations of vaccine viruses for inclusion in seasonal influenza vaccines in September 2009 for the southern hemisphere and in February 2010 for the northern hemisphere, as usual.

Activity of the novel influenza A (H1N1), March–May 2009

Between March and 26 May 2009, novel influenza A (H1N1) virus infections have been reported in the Americas, Asia, Europe and Oceania. Over this period, nearly 13 000 cases have been reported in over 40 countries. Those countries reporting the largest number of confirmed cases include Canada, Japan, Mexico, Panama, Spain, the United Kingdom and the United States of America³.

Although disease caused by the novel influenza A (H1N1) virus has generally been mild, severe illnesses resulting in hospitalization and death have occurred in Canada, Costa Rica, Mexico and the United States of America (USA). It is too early to determine the impact of the

¹ Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. Emergence of a Novel Swine-Origin Influenza A (H1N1) Virus in Humans. *New England Journal Medicine* 2009;361:1-11

² Christophe Fraser et al. Pandemic Potential of a Strain of Influenza A (H1N1): Early Findings. *Science*. 2009 May 14. [Epub ahead of print] See: <http://www.sciencemag.org/cgi/rapidpdf/1176062v2.pdf>

³ <http://www.who.int/csr/disease/swineflu/en/index.html>

emergence of the influenza A (H1N1) virus, although large community-wide outbreaks and school outbreaks with high attack rates have been reported⁴.

Virus characterization

Antigenic and genetic analyses are used in combination with epidemiologic information to define emergent antigenic variants and their spread and to select the most appropriate viruses to recommend for inclusion in vaccines. Antigenic relationships are evaluated mainly by haemagglutination inhibition (HI) tests using post-infection ferret antisera against egg- and/or cell-culture grown viruses. Phylogenetic analyses of haemagglutinin (HA) and neuraminidase (NA) genes help to define the genetic relatedness of antigenic variants to their predecessors and to elucidate the molecular basis for antigenic drift. The incidence of antigenic variants associated with influenza outbreaks in different countries is also an important criterion for selection of epidemiologically relevant vaccine virus candidates.

Antigenic characteristics of the novel influenza A (H1N1) viruses

Haemagglutination inhibition (HI) tests using post-infection ferret antisera have indicated that available novel A (H1N1) viruses isolated in North America, Europe and Oceania are antigenically homogeneous and antigenically distinct from currently circulating seasonal influenza A (H1N1) viruses and are most closely related to A/California/7/2009(H1N1)v viruses (Table 1). The emergent viruses are antigenically similar to North American lineage triple-reassortant A (H1N1) swine influenza viruses, represented by A/Illinois/09/2007, that have circulated in pigs over the last 10 years in the USA, and which have occasionally infected humans during the same period⁵.

Table 1 Results of haemagglutination inhibition tests of influenza A(H1N1) viruses with post-infection ferret sera^A

Antigens	Ferret antisera to reference viruses (titres)				
	A/swine/Iowa/30	A/Illinois/9/2007 ^B	A/California/4/2009 ^C	A/California/7/2009 ^D	A/Brisbane/59/2007 ^E
Antigens					
A/swine/Iowa/30	320	5	5	5	<10
A/Illinois/09/2007	160	5120	2560	5120	<10
A/California/04/2009	20	1280	1280	1280	<10
A/California/07/2009	80	1280	1280	1280	<10
A/Brisbane/59/2007	20	ND ^F	<40	<40	160
Novel A (H1N1) isolates					
A/Texas/05/2009	160	2560	1280	1280	<10
A/Mexico/4108/2009	160	1280	1280	1280	<10
A/Mexico/4596/2009	160	2560	1280	1280	<10
A/Mexico/4646/2009	160	1280	1280	1280	<10
A/New York/18/2009	160	2560	2560	2560	<10
A/Washington/11/2009	20	2560	1280	1280	<10
A/New Mexico/04/2009	80	2560	2560	2560	<10
A/El Salvador/211/2009	40	2560	1280	2560	<10
A/El Salvador/213/2009	20	1280	640	1280	<10
A/Hawaii/09/2009	80	2560	2560	1280	<10
A/Costa Rica/4314/2009	80	2560	2560	2560	<10
A/Costa Rica/4857/2009	20	1280	640	1280	<10
A/England/195/2009	20	ND	1280	1280	<10
A/Israel/644/2009	20	ND	1280	1280	<10
A/Netherlands/602/2009	20	ND	1280	1280	<10
A/Auckland/1/2009	20	ND	1280	2560	<10
A/Auckland/3/2009	20	ND	1280	2560	<10

^A Data provided by WHO Collaborating Centres for influenza

^B Antisera raised against earlier human influenza A (H1N1) virus associated with swine infection

^C Antisera raised against the emergent novel human influenza A (H1N1) virus

^D Antisera raised against human seasonal influenza A (H1N1) virus

^E ND = not determined

⁴ http://www.who.int/csr/resources/publications/swineflu/technical_consultation_2009_05_06/en/index.html

Genetic and phylogenetic characterization

Phylogenetic analysis of the eight gene segments indicates that the novel influenza A (H1N1) virus is a reassortant of swine influenza A viruses from North American and Eurasian lineages (H1N1, H1N2 and/or H3N2) which has gene segments originating from swine, human and avian influenza A viruses. The sequences of the haemagglutinin (HA) and neuraminidase (NA) genes of the A (H1N1) viruses isolated in Australia, Canada, Denmark, Germany, Israel, Italy, Japan, Mexico, Netherlands, New Zealand, Portugal, Republic of Korea, Spain, Sweden, United Kingdom and the USA are available in GenBank⁶.

Phylogenetic trees of the HA and NA genes show that, so far, the sequences of the novel influenza A (H1N1) viruses are relatively homogeneous (Figure 1). The HA gene is most closely related to the HA genes of swine H1N1 and H1N2 viruses isolated from swine in North America and Asia. The NA gene is most closely related to the N1 genes of influenza A (H1N1) viruses isolated from pigs and birds in Europe and Asia (Eurasian-lineage swine influenza viruses) (Figure 2).

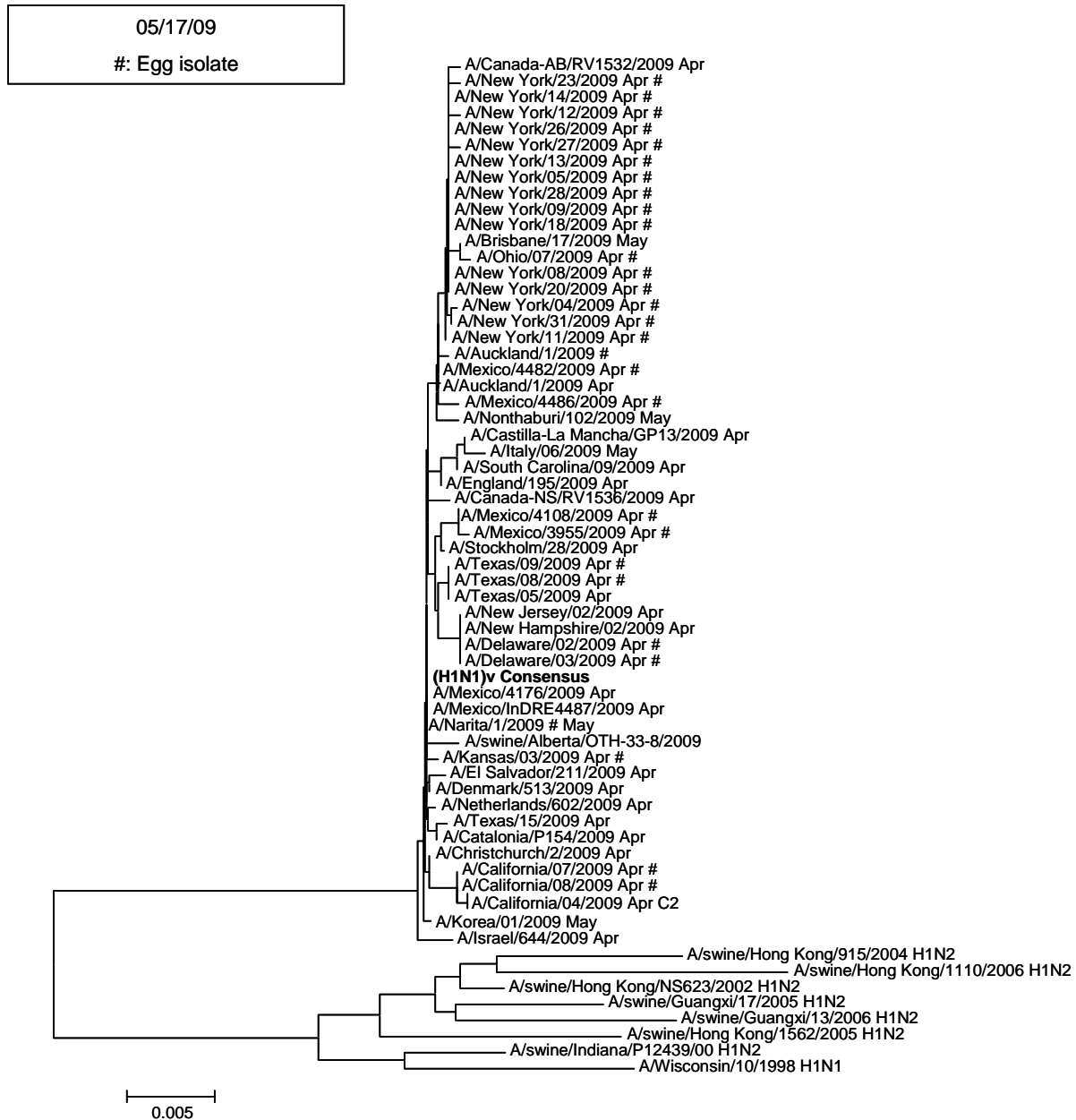
Mutations previously identified to confer resistance to oseltamivir or zanamivir have not been observed in the NA gene of the viruses characterized to date. The novel influenza A (H1N1) viruses tested so far in functional assays were sensitive to both these antiviral drugs.

An asparagine at position 31 of the M2 protein, associated with resistance to amantadine and rimantadine, has been observed in all the viruses analysed to date.

⁵ See footnote 1

⁶ <http://www.ncbi.nlm.nih.gov/Genbank>

Figure 1 Phylogenetic tree of classical swine-lineage H1 haemagglutinin including the novel A (H1N1) and nearest sequences available. The tree was constructed using the Neighbor-Joining method, Tamura-Nei nucleotide substitution model⁷. Information produced by the WHO Global Influenza Surveillance Network.



⁷ Tamura K et al. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 2007; 24:1596–9. See: <http://mbe.oxfordjournals.org/cgi/content/full/24/8/1596>

Figure 2 Phylogenetic tree of Eurasian swine-lineage N1 neuraminidase including the novel A (H1N1) and nearest sequences available. The tree was constructed using the Neighbor-Joining method, Tamura-Nei nucleotide substitution model⁷. Information produced by the WHO Global Influenza Surveillance Network.



Studies with inactivated seasonal influenza virus vaccines

Although cross-immunogenicity studies with seasonal influenza vaccines are still ongoing, preliminary data indicate that immunization with seasonal vaccines induces little or no cross-reactive antibody to the novel influenza A (H1N1) viruses.

Recommended virus for novel influenza A (H1N1) vaccines

The majority of the novel influenza A (H1N1) isolates are antigenically and genetically related to the A/California/7/2009 (H1N1)v virus. Should vaccines be prepared against the novel influenza A (H1N1) virus, it is therefore recommended that vaccines contain the following:

An A/California/7/2009 (H1N1)v -like virus

WHO Collaborating Centres (WHO CCs), Essential Regulatory Laboratories (ERLs) and other partners are developing high-growth reassortant A/California/7/2009 (H1N1)v -like viruses. WHO will announce on its website the availability of these reassortants as soon as they are available.

As with seasonal influenza vaccines, national control authorities should approve the specific vaccine viruses used in each country. National public health authorities are responsible for making recommendations regarding the use of the vaccine.