Antigenic and genetic characteristics of zoonotic influenza viruses and development of candidate vaccine viruses for pandemic preparedness

September 2015

The development of candidate influenza vaccine viruses (CVVs), coordinated by the World Health Organization (WHO), remains an essential component of the overall global strategy for pandemic preparedness.

Zoonotic influenza viruses continue to be identified and often evolve both genetically and antigenically, leading to the need for update of CVVs for pandemic preparedness purposes. Changes in the genetic and antigenic characteristics of these viruses relative to existing CVVs, and their potential risks to public health, justify the need to select and develop new CVVs.

Selection and development of CVVs are the first steps towards timely vaccine production and do not imply a recommendation for initiating manufacture. National authorities may consider the use of one or more of these CVVs for pilot lot vaccine production, clinical trials and other pandemic preparedness purposes based on their assessment of public health risk and need.

This document summarizes the genetic and antigenic characteristics of recent zoonotic influenza viruses and related viruses circulating in animals and updates the availability of CVVs. Institutions interested in receiving these CVVs should contact WHO at gisrs-whohq@who.int or the institutions listed in announcements published on the WHO website[1].

Influenza A(H5)

Since their re-emergence in 2003, highly pathogenic avian influenza (HPAI) A(H5N1) viruses of the A/goose/Guangdong/1/96 haemagglutinin (HA) lineage have become enzootic in some countries, have infected wild birds and continue to cause outbreaks in poultry and sporadic human infections. These A(H5N1) viruses have diversified genetically and antigenically, including the emergence of viruses with substitutions of the N1 gene for N2, N3, N6 or N8 genes, leading to the need for multiple CVVs. This summary provides updates on the characterization of A/goose/Guangdong/1/96-lineage A(H5) viruses and the current status of the development of influenza A(H5) CVVs.

Influenza A(H5) activity from 24 February 2015 to 21 September 2015

A(H5) human infections have been reported to the WHO by China (4 cases), Egypt (64 cases) and Indonesia (2 cases)[2]; in these countries A(H5) infections have also been detected in birds. Characterized cases in Egypt and Indonesia and three of the cases in China were caused by A(H5N1) viruses. One human infection in China was caused by an A(H5N6) virus. A(H5) viruses were detected in birds in Bangladesh, Bhutan, Bulgaria, Burkina Faso, Cambodia, Canada, China, China Hong Kong Special Administrative Region (SAR), Côte d'Ivoire, Egypt, Ghana, India, Indonesia, Islamic Republic of Iran, Israel, Kazakhstan, Myanmar, Niger, Nigeria, Republic of Korea, Romania, Russian Federation, Turkey, United States of America, Viet Nam, and West Bank and Gaza Strip (Table 1).

[1] http://www.who.int/influenza/vaccines/virus/en/
[2] WHO | Cumulative number of confirmed human cases of avian influenza A(H5N1) reported to WHO

Table 1. Recent A(H5) activity reported to international agencies

Country, area or territory	Host	Genetic clade
Bangladesh	Poultry	2.3.2.1a
Bhutan	Poultry	2.3.2.1a
Bulgaria	Wild birds	2.3.2.1c
Burkina Faso	Poultry	2.3.2.1c
Cambodia	Poultry	2.3.2.1c
Canada	Poultry	2.3.4.4 (H5N2)
China	Poultry/environmental	2.3.2.1c, 2.3.4.4 (H5N1/N2/N3/N6/N8/N9)
	Human (4)#	3 H5N1, 2.3.4.4 (H5N6)
China, Hong Kong SAR	Wild birds	2.3.4.4 (H5N6)
Côte d'Ivoire	Poultry	2.3.2.1c
Egypt	Poultry	2.2.1.2
	Human (64)	2.2.1.2
Ghana	Poultry	2.3.2.1c
India	Poultry/wild birds	2.3.2.1a/2.3.2.1c
Indonesia	Poultry	2.3.2.1c
	Human (2)	2.1.3.2a
Iran (Islamic Republic of)	Poultry	Unknown
Israel	Poultry	2.2.1.2
Kazakhstan	Wild bird	2.3.2.1c
Myanmar	Poultry	2.3.4.2
Niger	Poultry	2.3.2.1c
Nigeria	Poultry	2.3.2.1c
Republic of Korea	Poultry	2.3.4.4 (H5N8)
Romania	Wild birds	2.3.2.1c
Russian Federation	Wild birds	2.3.2.1c
Turkey	Poultry	2.3.2.1c
United States of America	Poultry/wild birds	2.3.4.4 (H5N1/N2/N8)
Viet Nam	Poultry	2.3.2.1c/2.3.4.4 (H5N1/N6)
West Bank and Gaza Strip	Poultry	2.2.1.2

# denotes number of human cases reported to WHO within reporting period

Antigenic and genetic characteristics of influenza A(H5) viruses

The nomenclature for phylogenetic relationships among the HA genes of A/goose/Guangdong/1/96-lineage A(H5) viruses is defined in consultation with representatives of the WHO, the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE) and academic institutions[3].

Viruses circulating and characterized from 24 February 2015 to 21 September 2015 belonged to the following clades.

Clade 2.1.3.2a viruses were detected in a father and son in Indonesia. The HA genes of these viruses were identical to each other and similar to that of the CVV derived from A/Indonesia/NIHRD11771/2011. No antigenic information is available.

Clade 2.2.1.2 viruses were detected in poultry in Israel and the West Bank and Gaza Strip and in poultry and humans in Egypt. The viruses were genetically similar to viruses detected in previous periods. Antigenically the viruses available for testing reacted well with ferret antiserum raised against A/Egypt/N04915/2014 for which a CVV is under development.

[3] http://onlinelibrary.wiley.com/doi/10.1111/irv.12324/epdf

Clade 2.3.2.1a viruses were detected in birds in Bangladesh, Bhutan and India. The HA genes of the viruses from Bangladesh and Bhutan and from one of the two viruses from India were similar to those of viruses detected previously. The majority of viruses from Bangladesh reacted well with ferret antiserum raised against A/duck/Bangladesh/19097/2013 for which a CVV has been developed. The HA gene of the second virus from India was genetically distinct; no antigenic data are available for this virus.

Clade 2.3.2.1c viruses were detected in birds in Bulgaria, Burkina Faso, China, Côte d'Ivoire, Ghana, India, Kazakhstan, Niger, Nigeria, Romania, Russian Federation, Turkey and Viet Nam. Increased genetic heterogeneity in HA gene sequences was observed within recent viruses from Africa and Europe within this clade (Figure 1), and some antigenic diversity was observed. Most isolates remained antigenically similar to the CVV derived from A/duck/Viet Nam/NCVD-1584/2012.

Clade 2.3.4.2 viruses were detected in poultry in Myanmar. These viruses were genetically similar to previously circulating viruses in Myanmar. The viruses reacted well with post-infection ferret antiserum raised against the CVV developed from the clade 2.3.4.2 virus A/chicken/Bangladesh/11RS1984-30/2011.

Clade 2.3.4.4 viruses were detected in birds in Canada, China Hong Kong SAR, Republic of Korea, United States of America and Viet Nam and in birds, environmental samples and a human in China. The HA genes of these viruses were similar to those of viruses isolated previously. While considerable genetic heterogeneity exists among viruses in this clade, the majority of recent viruses react well with post-infection ferret antiserum raised to A/Sichuan/26221/2014 (H5N6) or A/gyrfalcon/Washington/41088-6/2014 (H5N8)-like viruses. Due to the high incidence of poultry outbreaks in North America caused by A(H5N2) clade 2.3.4.4 viruses, analysis of additional A/gyrfalcon/Washington/41088-6/2014-like CVVs with an N2 neuraminidase is ongoing to optimize coverage of recent viruses in this group.

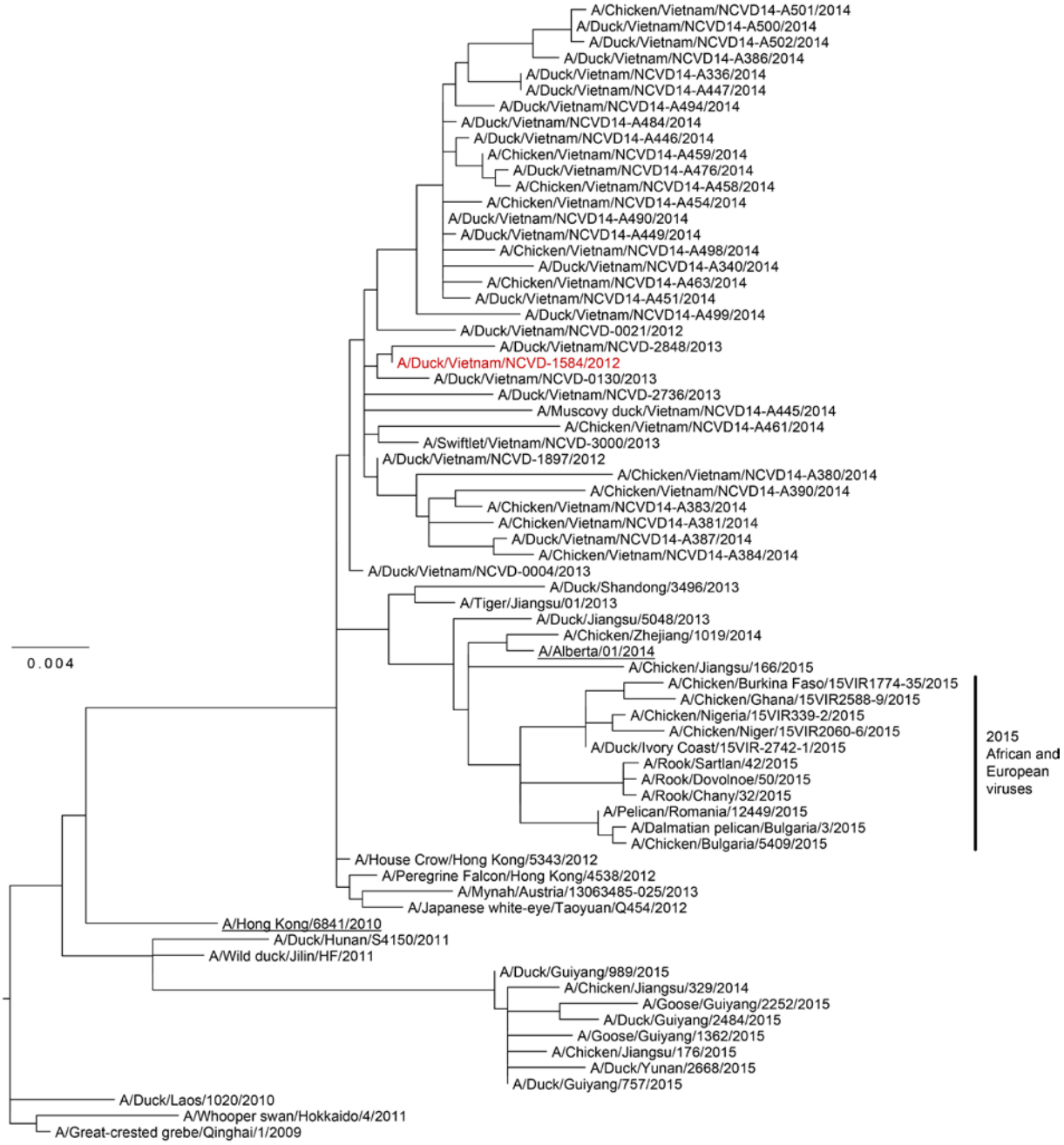

Figure 1. Phylogenetic relationships of A(H5N1) clade 2.3.2.1c HA genes. The available CVVs are in red. Human viruses are underlined. The scale bar represents the number of substitutions per site.

Influenza A(H5) candidate vaccine viruses

Based on the available antigenic, genetic and epidemiologic data, no new A(H5) CVVs are proposed. The available and pending A(H5) CVVs are listed in Table 2. National authorities may consider the use of one or more of these A(H5) CVVs for pilot lot vaccine production, clinical trials and other pandemic preparedness purposes based on their assessment of public health risk and need. As the viruses continue to evolve, new A(H5) CVVs may be developed.

Table 2. Status of influenza A(H5) candidate vaccine virus development

Candidate vaccine viruses	Clade	Institution*	Available
A/Viet Nam/1203/2004 (CDC-RG; SJRG-161052)	1	CDC and SJCRH	Yes
A/Viet Nam/1194/2004 (NIBRG-14)	1	NIBSC	Yes
A/Cambodia/R0405050/2007 (NIBRG-88)	1.1	NIBSC	Yes
A/Cambodia/X0810301/2013 (IDCDC-RG34B)	1.1.2	CDC	Yes
A/duck/Hunan/795/2002 (SJRG-166614)	2.1.1	SJCRH/HKU	Yes
A/Indonesia/5/2005 (CDC-RG2)	2.1.3.2	CDC	Yes
A/Indonesia/NIHRD11771/2011 (NIIDRG-9)	2.1.3.2a	NIID	Yes
A/bar-headed goose/Qinghai/1A/2005 (SJRG-163222)	2.2	SJCRH/HKU	Yes
A/chicken/India/NIV33487/2006 (IBCDC-RG7)	2.2	CDC/NIV	Yes
A/whooper swan/Mongolia/244/2005 (SJRG-163243)	2.2	SJCRH	Yes
A/Egypt/2321-NAMRU3/2007 (IDCDC-RG11)	2.2.1	CDC	Yes
A/turkey/Turkey/1/2005 (NIBRG-23)	2.2.1	NIBSC	Yes
A/Egypt/N03072/2010 (IDCDC-RG29)	2.2.1	CDC	Yes
A/Egypt/3300-NAMRU3/2008 (IDCDC-RG13)	2.2.1.1	CDC	Yes
A/common magpie/Hong Kong/5052/2007 (SJRG-166615)	2.3.2.1	SJCRH/HKU	Yes
A/Hubei/1/2010 (IDCDC-RG30)	2.3.2.1a	CDC	Yes
A/duck/Bangladesh/19097/2013 (SJ007)	2.3.2.1a	SJCRH	Yes
A/barn swallow/Hong Kong/D10-1161/2010 (SJ-003)	2.3.2.1b	SJCRH/HKU	Yes
A/chicken/Hong Kong/AP156/2008 (SJ002)	2.3.4	SJCRH/HKU	Yes
A/Anhui/1/2005 (IBCDC-RG6)	2.3.4	CDC	Yes
A/duck/Laos/3295/2006 (CBER-RG1)	2.3.4	FDA	Yes
A/Japanese white eye/Hong Kong/1038/2006 (SJRG-164281)	2.3.4	SJCRH/HKU	Yes
A/chicken/Bangladesh/11rs1984-30/2011 (IDCDC-RG36)	2.3.4.2	CDC	Yes
A/Guizhou/1/2013 (IDCDC-RG35)	2.3.4.2	CDC/CCDC	Yes
A/goose/Guiyang/337/2006 (SJRG-165396)	4	SJCRH/HKU	Yes
A/chicken/Viet Nam/NCVD-016/2008 (IDCDC-RG12)	7.1	CDC	Yes
A/chicken/Viet Nam/NCDV-03/2008 (IDCDC-RG25A)	7.1	CDC	Yes
A/Sichuan/26221/2014 (IDCDC-RG42A)	2.3.4.4 (H5N6)	CDC/CCDC	Yes
A/gyrfalcon/Washington/41088-6/2014 (IDCDC-RG43A)	2.3.4.4 (H5N8)	CDC	Yes
Candidate vaccine viruses in preparation	**Clade**	**Institution**	**Availability**
A/duck/Viet Nam/NCVD-1584/2012-like	2.3.2.1c	NIBSC	Pending
A/environment/Hubei/950/2013-like	7.2	CDC/CCDC	Pending
A/Egypt/N04915/2014-like	2.2.1.2	NIBSC	Pending
A/northern pintail/Washington/40964/2014-like[#]	2.3.4.4 (H5N2)	CDC	Pending

* **Institutions distributing the candidate vaccine viruses:**
CDC - Centers for Disease Control and Prevention, USA
CDC/NIV - Centers for Disease Control and Prevention, USA/National Institute of Virology, India
CDC/CCDC - Centers for Disease Control and Prevention, USA/China Center for Disease Control and Prevention
FDA - Food and Drug Administration, USA
HKU – University of Hong Kong, Hong Kong Special Administrative Region, China.
NIBSC - National Institute for Biological Standards and Control, a centre of the Medicines and Healthcare products Regulatory Agency (MHRA), UK
NIID - National Institute of Infectious Diseases, Japan
SJCRH - St Jude Children's Research Hospital, USA

A/northern pintail/Washington/40964/2014 (H5N2) is a A/gyrfalcon/Washington/41088-6/2014-like virus

Influenza A(H7N9)

Influenza A(H7) viruses have been detected in poultry populations worldwide with the associated disease ranging from mild to severe. Human infections with avian influenza A(H7N9) viruses were first reported to WHO on 31 March 2013.

Influenza A(H7N9) activity from 24 February 2015 to 21 September 2015
During this period, 105 human cases of avian influenza A(H7N9) virus infection were reported to WHO, all from China, bringing the total number of cases to 667 with 275 deaths reported. Recent A(H7N9) viruses were genetically similar to those detected previously. No new antigenic information is available.

Influenza A(H7N9) candidate vaccine viruses
Based on the current epidemiologic and virologic data, no new A(H7N9) CVVs have been proposed. Available A(H7N9) CVVs are shown in Table 3. National authorities may consider the use of one or more of these A(H7N9) CVVs for pilot lot vaccine production, clinical trials and other pandemic preparedness purposes based on their assessment of public health risk and need. As the viruses continue to evolve, new A(H7N9) CVVs may be developed.

Table 3. Status of influenza A(H7N9) candidate vaccine virus development

Candidate vaccine virus	Type	Institution*	Available
A/Anhui/1/2013 (H7N9) IDCDC-RG33A	Reverse Genetics	CDC	Yes
A/Anhui/1/2013 (H7N9) NIBRG-268	Reverse Genetics	NIBSC	Yes
A/Anhui/1/2013 (H7N9) NIIDRG-10.1	Reverse Genetics	NIID	Yes
A/Anhui/1/2013 (H7N9) SJ005	Reverse Genetics	SJCRH	Yes
A/Shanghai/2/2013 (H7N9) NIBRG-267	Reverse Genetics	NIBSC	Yes
A/Shanghai/2/2013 (H7N9) CBER-RG4A	Reverse Genetics	FDA	Yes
A/Shanghai/2/2013 (H7N9) IDCDC-RG32A	Reverse Genetics	CDC	Yes
A/Shanghai/2/2013 (H7N9) IDCDC-RG32A.3	Reverse Genetics	CDC	Yes

* **Institutions distributing the candidate vaccine viruses:**
CDC - Centers for Disease Control and Prevention, USA
FDA - Food and Drug Administration, USA
NIBSC - National Institute for Biological Standards and Control, a centre of the Medicines and Healthcare products Regulatory Agency (MHRA), UK
NIID - National Institute of Infectious Diseases, Japan
SJCRH - St Jude Children's Research Hospital, USA

Influenza A(H9N2)

Influenza A(H9N2) viruses are enzootic in poultry populations in parts of Africa, Asia and the Middle East. The majority of viruses that have been sequenced belong to the A/quail/Hong Kong/G1/97 (G1), A/chicken/Beijing/1/94 (Y280/G9), or Eurasian clades. Since 1998, when the first human infection was detected, the isolation of A(H9N2) viruses from humans and swine has been reported infrequently. In all human cases the associated disease symptoms have been mild and there has been no evidence of human-to-human transmission.

Influenza A(H9N2) activity from 24 February 2015 to 21 September 2015
Four human cases of A(H9N2) infection have been reported in this period with no fatalities. One A(H9N2) virus was isolated from a case in China. Genetically and antigenically this virus was similar to Y280-lineage A(H9N2) viruses known to circulate in birds in China. One G1-lineage A(H9N2) virus was isolated from a child in Bangladesh. This virus and those collected from poultry were genetically and antigenically similar to A/Bangladesh/0994/2011 for which a CVV has been produced. Two cases of A(H9N2) infection were reported in Egypt. Sequence data from one Egyptian case indicated the virus was genetically similar to previous G1-lineage A(H9N2) poultry viruses detected in Egypt. No antigenic

information is available for the human virus but recent A(H9N2) viruses from poultry in Egypt remain antigenically similar to existing CVVs.

Influenza A(H9N2) candidate vaccine viruses

Based on the current antigenic, genetic and epidemiologic data, no new CVVs are proposed. The available A(H9N2) CVVs are listed in Table 4. National authorities may consider the use of one or more of these A(H9N2) CVVs for pilot lot vaccine production, clinical trials and other pandemic preparedness purposes based on their assessment of public health risk and need. As the viruses continue to evolve, new A(H9N2) CVVs may be developed.

Table 4. Status of influenza A(H9N2) candidate vaccine virus development

Candidate vaccine viruses	Type	Clade	Institution*	Available
A/Hong Kong/1073/1999	Wild type	G1	NIBSC	Yes
A/chicken/Hong Kong/G9/1997 (NIBRG-91)	Reverse genetics	Y280/G9	NIBSC	Yes
A/chicken/Hong Kong/G9/1997 (IBCDC-2)	Conventional	Y280/G9	CDC	Yes
A/Hong Kong/33982/2009 (IDCDC-RG26)	Reverse genetics	G1	CDC	Yes
A/Bangladesh/994/2011 (IDCDC-RG31)	Reverse genetics	G1	CDC	Yes
Candidate vaccine viruses in preparation				
A/Hong Kong/308/2014-like	Reverse genetics	Y280/G9	SJCRH	Pending

* **Institutions distributing the candidate vaccine viruses:**
CDC - Centers for Disease Control and Prevention, USA
NIBSC - National Institute for Biological Standards and Control, a centre of the Medicines and Healthcare products Regulatory Agency (MHRA), UK
SJCRH - St Jude Children's Research Hospital, USA

Influenza A(H1N1) and A(H1N2) variants (v)[4]

Influenza A(H1N1) and A(H1N2) viruses circulate in swine populations in many regions of the world. Depending on geographic location, the genetic characteristics of these viruses differ. Human infections with swine A(H1) viruses have been documented for many years.

Influenza A(H1N1)v and A(H1N2)v activity from 24 February 2015 to 21 September 2015

Two cases of A(H1N1)v were identified in the United States during this reporting period. A fatal case was detected in Ohio during April in a person with potential occupational exposure to swine. A second severe case in Iowa was hospitalized in August. Direct contact with swine was reported. The HA genes of both viruses belonged to the classical swine gamma lineage[5] but were genetically distant to the A(H1N1)pdm09 vaccine virus, A/California/7/2009 (Figure 2).

Haemagglutination inhibition (HI) testing with ferret antisera raised against A/California/7/2009 and recent A(H1N1)v viruses showed significantly reduced titers to A/Ohio/9/2015 (Table 5). Likewise, ferret antisera raised to A/Ohio/9/2015 did not inhibit A/California/7/2009 or several other A(H1N1)v viruses well. Inhibition of A/Ohio/9/2015 by pooled human sera collected post-vaccination with the 2013-2014 seasonal influenza vaccine was also reduced compared to other viruses. These antigenic properties are likely due to specific amino acid residues identified in A/Ohio/9/2015 and found in a proportion of circulating swine A(H1) viruses in the United States.

[4] http://www.who.int/influenza/gisrs_laboratory/terminology_variant/en/
[5] http://onlinelibrary.wiley.com/doi/10.1111/zph.12049/epdf

Table 5. Haemagglutination inhibition reactions of influenza A(H1N1)v viruses.

REFERENCE ANTIGENS	Lineage	CA/7	IA/1	WI/28	MO/12	MN/33	OH/9	Post-Vacc Sera*
A/California/7/2009	pdmH1N1	**5120**	2560	5120	2560	2560	160	1280
A/Iowa/1/2006	H1N1v	20	**640**	20	1280	80	2560	20
A/Wisconsin/28/2011	H1N1v	5120	2560	**2560**	5120	2560	320	160
A/Missouri/12/2012	H1N1v	5120	5120	5120	**5120**	2560	1280	1280
A/Minnesota/33/2014	H1N1v	5120	5120	2560	5120	**5120**	160	640
A/Ohio/9/2015	H1N1v	<	80	20	20	80	**5120**	20
TEST ANTIGENS								
A/Iowa/39/2015	H1N1v	160	80	80	40	1280	640	320

* 2013-2014 Post-vaccine immune serum pool collected from adults 19-49 years of age

Influenza A(H1)v candidate vaccine viruses

Based on the available antigenic, genetic and epidemiologic data, an A/Ohio/9/2015-like CVV is proposed (Table 6). National authorities may consider the use of this A(H1)v CVV for pilot lot vaccine production, clinical trials and other pandemic preparedness purposes based on their assessment of public health risk and need. As the viruses continue to evolve, new A(H1)v CVVs may be developed.

Table 6. Status of A(H1N1)v candidate vaccine virus development.

Candidate vaccine viruses in preparation	**Type**	**Institution***
A/Ohio/9/2015	Reverse genetics	CDC

***Institution distributing the candidate vaccine virus:**
CDC – Centers for Disease Control and Prevention, USA

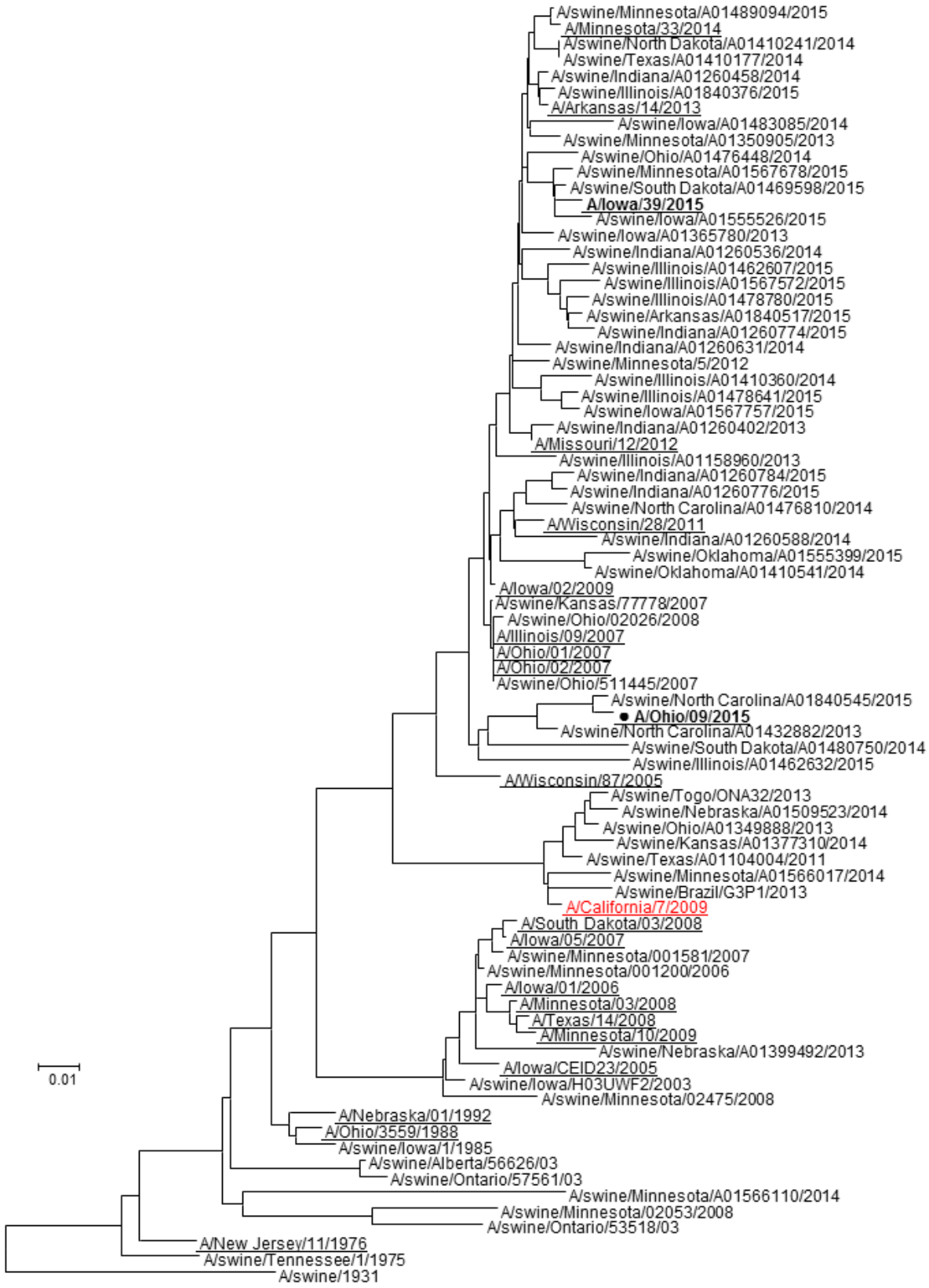

Figure 2. Phylogenetic relationships of A(H1N1)v HA genes. The available CVV is in red. The proposed CVV is indicated by a circle. All human viruses are underlined and 2015 human viruses are shown in bold font. The scale bar represents the number of substitutions per site.

Influenza A(H3N2)v

Influenza A(H3N2) viruses are enzootic in swine populations in most regions of the world. Depending on geographic location, the genetic and antigenic characteristics of these viruses differ. Human infections with swine A(H3N2) viruses have been documented in Asia, Europe and North America[6].

Influenza A(H3N2)v activity from 24 February 2015 to 21 September 2015

Two cases of A(H3N2)v were identified in the United States during this reporting period. Direct swine contact was reported in both instances. One patient from Michigan developed illness in June and recovered following oseltamivir treatment. In July, an immunocompromised person from Minnesota developed an acute respiratory illness and tested positive for A(H3N2)v. Virus isolates from each patient belonged to separate phylogenetic groups of the A(H3N2)v haemagglutinin tree. A/Michigan/39/2015 belonged to cluster IV-A[7], whereas A/Minnesota/38/2015 belonged to cluster IV-B. Genetically related swine viruses circulating in the United States during 2014-2015 were identified in both groups.

Despite some genetic diversity between these two viruses and the closest A(H3N2)v CVV, A/Minnesota/11/2010, HI tests indicated that these viruses were well-inhibited by ferret antisera raised to the A(H3N2)v CVVs, A/Minnesota/11/2010 and A/Indiana/10/2011. In addition, HI reactivity of both viruses to pooled human sera collected post-vaccination with the 2013-2014 seasonal influenza vaccine was comparable to other A(H3N2)v viruses and recent seasonal A(H3N2) vaccine viruses.

Influenza A(H3N2)v candidate vaccine viruses

Based on the available antigenic, genetic and epidemiologic data, no new A(H3N2)v CVVs are proposed. The available A(H3N2)v CVVs are listed in Table 7. National authorities may consider the use of one or more of these A(H3N2)v CVVs for pilot lot vaccine production, clinical trials and other pandemic preparedness purposes based on their assessment of public health risk and need. As the viruses continue to evolve, new A(H3N2)v CVVs may be developed.

Table 7. Status of A(H3N2)v candidate vaccine virus development

Candidate vaccine viruses	Type	Institution*
A/Minnesota/11/2010 (NYMC X-203)	Conventional reassortant	CDC
A/Indiana/10/2011 (NYMC X-213)	Conventional reassortant	CDC

*** Institution distributing the candidate vaccine viruses:**
CDC - Centers for Disease Control and Prevention, USA

[6] Myers, KP. et al. Cases of Swine Influenza in Humans: A Review of the Literature. Clin Infect Dis. 44:1084. 2007
[7] http://onlinelibrary.wiley.com/doi/10.1111/zph.12049/epdf