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

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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.

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.

Zoonotic influenza viruses continue to be identified and often evolve both genetically and antigenically, leading to the need for addition 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.

This document summarizes the genetic and antigenic characteristics of recent zoonotic influenza viruses and related viruses circulating in animals[1] that are relevant to CVV updates. 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[2].

Influenza A(H5)

Since their re-emergence in 2003, highly pathogenic avian influenza (HPAI) A(H5) 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 viruses have diversified genetically and antigenically, including the emergence of viruses with substitutions of the N1 gene for N2, N3, N6, N8 or N9 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 22 September 2015 to 22 February 2016

A(H5) human infections have been reported to the WHO by Bangladesh (1 case) and China (6 cases) where A(H5) infections have also been detected in birds. The human infection in Bangladesh and one of those in China were caused by A(H5N1) viruses; the remainder of the infections were caused by A(H5N6) viruses. A(H5) viruses were detected in birds in Bangladesh, Cambodia, China, China Hong Kong Special Administrative Region (SAR), Côte d'Ivoire, Egypt, Ghana, India, Lao People's Democratic Republic, Nigeria, Republic of Korea and Viet Nam (Table 1).

[1] For information relevant to other notifiable influenza virus infections in animals refer to http://www.oie.int/wahis_2/public/wahid.php/Wahidhome/Home

[2] http://www.who.int/influenza/vaccines/virus/en/

Table 1. Recent H5 activity reported to international agencies

Country, area or territory	Host	Genetic clade
Bangladesh	Poultry	2.3.2.1a
	Human (1)[#]	2.3.2.1a
Cambodia	Poultry	2.3.2.1c
China	Poultry/environmental	2.3.2.1c, 2.3.4.4 (H5N1/N2/N6/N8)
	Human (6)	Unknown H5N1, 2.3.4.4 (H5N6)
China, Hong Kong SAR	Wild bird	2.3.4.4 (H5N6)
Côte d'Ivoire	Poultry	2.3.2.1c
Egypt	Poultry	2.2.1.2
Ghana	Poultry	2.3.2.1c
India	Poultry	2.3.2.1a
Lao People's Democratic Republic	Poultry	2.3.4.4 (H5N6)
Nigeria	Poultry	2.3.2.1c
Republic of Korea	Poultry	2.3.4.4 (H5N8)
Viet Nam	Poultry	2.3.2.1c, 2.3.4.4 (H5N6)

# denotes number of human cases reported to WHO within the 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 22 September 2015 to 22 February 2016 belonged to the following clades.

Clade 2.2.1.2 viruses were detected in poultry in Egypt. The viruses were genetically similar to viruses detected in previous periods. Antigenic data for these viruses is being generated.

Clade 2.3.2.1a viruses were detected in birds and a human in Bangladesh and in birds in India. The HA genes of the viruses from Bangladesh were similar to those previously characterized and the majority of the avian viruses reacted well with ferret antiserum raised against A/duck/Bangladesh/19097/2013 for which a CVV has been developed. No virus isolate is available from the human infection. The HA gene of the virus from India was genetically distinct; no antigenic data are available for this virus and the extent to which similar viruses circulate is unknown.

Clade 2.3.2.1c viruses were detected in birds in Cambodia, China, Côte d'Ivoire, Ghana, Nigeria and Viet Nam. The HA genes from these viruses were similar to those of viruses previously detected. The viruses from south-east Asia remained antigenically similar to A/duck/Viet Nam/NCVD-1584/2012 from which a CVV has been developed. The HA genes of the viruses from Africa and from viruses detected in wild birds in China have accumulated a number of amino acid substitutions relative to A/duck/Viet Nam/NCVD-1584/2012. Characterization of the African viruses is ongoing to determine if these genetic differences are associated with an antigenic change. No information was available for recent viruses from Indonesia.

Clade 2.3.4.4 viruses were detected in birds in Lao People's Democratic Republic, Republic of Korea and Viet Nam and in birds, environmental samples and humans in China. There was considerable genetic heterogeneity among the HA genes of these viruses (Figure 1) and some, including recent A(H5N6) viruses from Viet Nam, reacted poorly with post-infection ferret antiserum raised to the CVVs developed from A/Sichuan/26221/2014 (H5N6) and A/gyrfalcon/Washington/41088-6/2014 (H5N8) (Table 2).

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

Table 2. Haemagglutination inhibition reactions of influenza A(H5N6) viruses

REFERENCE ANTIGENS	Subtype	AN/1	GZ/1 RG35	ck/ HK	SH/26221 RG42A	VN/ A324	gyr/WA RG43A
A/Anhui/1/2005	H5N1	**1280**	80	40	<#	<	<
A/Guizhou/1/2013 IDCDC-RG35	H5N1	160	**160**	20	<	<	<
A/chicken/Hong Kong/AP156/2008	H5N1	40	20	**80**	<	<	<
A/Sichuan/26221/2014 IDCDC-RG42A	H5N6	<	<	<	**320**	160	40
A/chicken/Viet Nam/NCVD-14-A324/2014	H5N6	<	<	<	160	**160**	<
A/gyrfalcon/Washington/41088-6/14 IDCDC-RG43A	H5N8	<	<	<	160	80	**640**
TEST ANTIGENS							
A/duck/Laos/XBY118/2015	H5N6	<	<	<	160	160	320
A/chicken/Viet Nam/NCVD-15A51/2015	H5N6	<	<	<	40	20	<
A/duck/Viet Nam/NCVD-15A52/2015	H5N6	<	<	<	10	<	<
A/duck/Viet Nam/NCVD-15A57/2015	H5N6	<	<	<	160	160	80
A/chicken/Viet Nam/NCVD-15A59/2015	H5N6	<	<	10	80	80	20
A/chicken/Viet Nam/NCVD-15A61/2015	H5N6	<	<	<	40	40	<
A/duck/Viet Nam/NCVD-15A74/2015	H5N6	<	<	<	20	20	<
A/chicken/Viet Nam/NCVD-15A122/2015	H5N6	<	<	<	20	20	<
A/chicken/Viet Nam/NCVD-15A129/2015	H5N6	<	<	<	20	20	<

represents a haemagglutination inhibition titre of <10

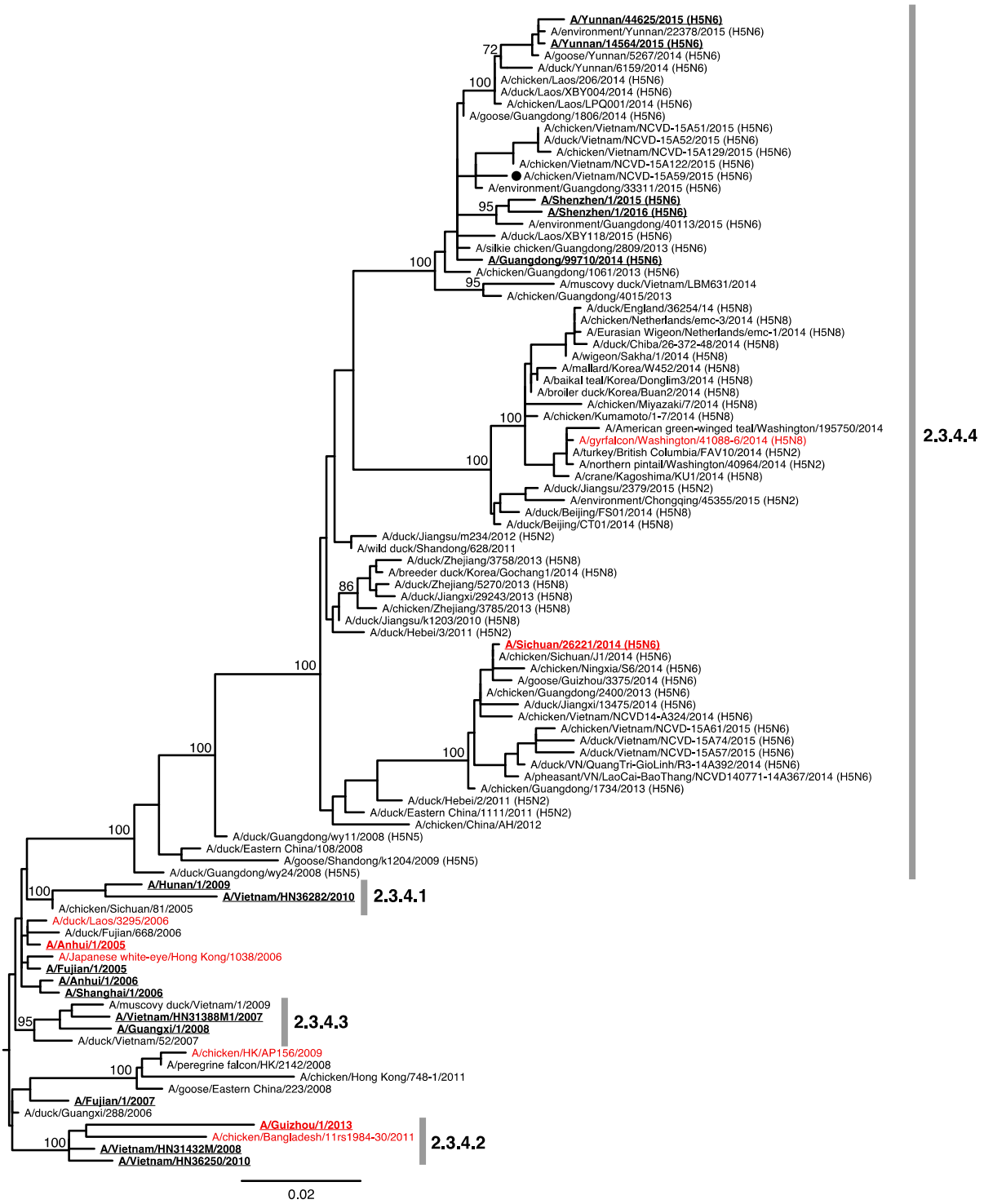

Figure 1. Phylogenetic relationships of A(H5) clade 2.3.4 HA genes. The available CVVs are in red. The proposed CVV is indicated by a dot (•). Human viruses are underlined and in bold font. The scale bar represents the number of substitutions per site. NA subtypes other than N1 are specified. Bootstrap supports of topology are shown above selected nodes.

Influenza A(H5) candidate vaccine viruses

Based on the available antigenic, genetic and epidemiologic data, a new A/chicken/Viet Nam/NCVD-15A59/2015-like CVV is proposed. The available and pending A(H5) CVVs are listed in Table 3. As the viruses continue to evolve, new A(H5) CVVs may be developed.

Table 3. 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	2.3.2.1c	NIBSC	Pending
A/environment/Hubei/950/2013	7.2	CDC/CCDC	Pending
A/Egypt/N04915/2014-like	2.2.1.2	NIBSC	Pending
A/chicken/Viet Nam/NCVD-15A59/2015-like	2.3.4.4	SJCRH	Pending

* **Institutions developing and/or distributing the candidate vaccine viruses:**

CDC - Centers for Disease Control and Prevention, USA
NIV - National Institute of Virology, India
CCDC - Chinese 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

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 22 September 2015 to 22 February 2016

During this period, 44 human cases of avian influenza A(H7N9) virus infection were reported to WHO, all from China, bringing the total number of cases to 721 with 286 deaths[4] reported. Recent A(H7N9) viruses were genetically similar to those detected previously. Comparison of human and avian viruses using haemagglutination inhibition (HI) assays showed that the majority tested remained antigenically similar to the CVVs derived from A/Anhui/1/2013-like and A/Shanghai/2/2013-like viruses.

Influenza A(H7N9) candidate vaccine viruses

Based on the current epidemiologic and virologic data, no new A(H7N9) CVVs are proposed. Available A(H7N9) CVVs are shown in Table 4. As the viruses continue to evolve, new A(H7N9) CVVs may be developed.

Table 4. 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

[4] Communication from WHO Collaborating Center, Beijing.

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 but one human case the associated disease symptoms have been mild and there has been no evidence of human-to-human transmission.

Influenza A(H9N2) activity from 22 September 2015 to 22 February 2016

Six human cases of A(H9N2) infection have been reported in this period with no fatalities. Five A(H9N2) viruses were isolated from humans in China. One of these viruses was associated with severe disease although underlying conditions likely contributed to this. Genetically and antigenically the tested viruses were similar to Y280-lineage A(H9N2) viruses known to circulate in birds in China. One A(H9N2) human case was detected in Bangladesh. Although no virus was isolated from this individual, sequencing of clinical material showed that the HA gene of the infecting virus was similar to A/Bangladesh/994/2011 for which a CVV has been produced.

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 5. As the viruses continue to evolve, new A(H9N2) CVVs may be developed.

Table 5. 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
A/Hong Kong/308/2014 (SJ008)	Reverse genetics	Y280/G9	SJCRH	Yes

* **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) variants (v)[5]

Influenza A(H1N1) 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 activity from 22 September 2015 to 22 February 2016
A non-fatal A(H1N1)v human case was detected in the United States of America during October in a patient with reported exposure to swine. Virus was not recovered from this case. Genomic sequences obtained from clinical specimen confirmed that the HA gene belonged to the classical swine gamma lineage[6] but was genetically distant from the A(H1N1)pdm09 vaccine virus, A/California/7/2009, and the A(H1N1)v CVV, A/Ohio/9/2015.

Two cases of Eurasian (EA) lineage avian-like A(H1N1)v viruses were identified in China during this reporting period. These cases were detected in different cities in Yunnan Province during November 2015. The HA genes of viruses recovered, A/Yunnan-Longyang/SWL1982/2015 and A/Yunnan-Wuhua/SWL1869/2015, grouped with those of swine influenza viruses circulating in China and a previously reported A(H1N1)v detected in China, A/Hunan/42443/2015 (Figure 2). Haemagglutination inhibition (HI) testing of these viruses is not yet available. Due to the lack of an existing CVV representing these EA lineage viruses a new CVV is proposed.

Influenza A(H1)v candidate vaccine viruses
Based on the available genetic and epidemiologic data, an A/Hunan/42443/2015-like CVV is proposed (Table 6). 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	Type	Institution*
A/Ohio/9/2015	Reverse genetics	CDC

Candidate vaccine viruses in preparation	Type	Institution
A/Hunan/42443/2015-like	Conventional reassortant and Reverse genetics	CCDC and NIBSC

***Institution distributing the candidate vaccine virus:**
CDC - Centers for Disease Control and Prevention, USA
CCDC - Chinese Center for Disease Control and Prevention, China
NIBSC - National Institute for Biological Standards and Control, a centre of the Medicines and Healthcare products Regulatory Agency (MHRA), United Kingdom

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

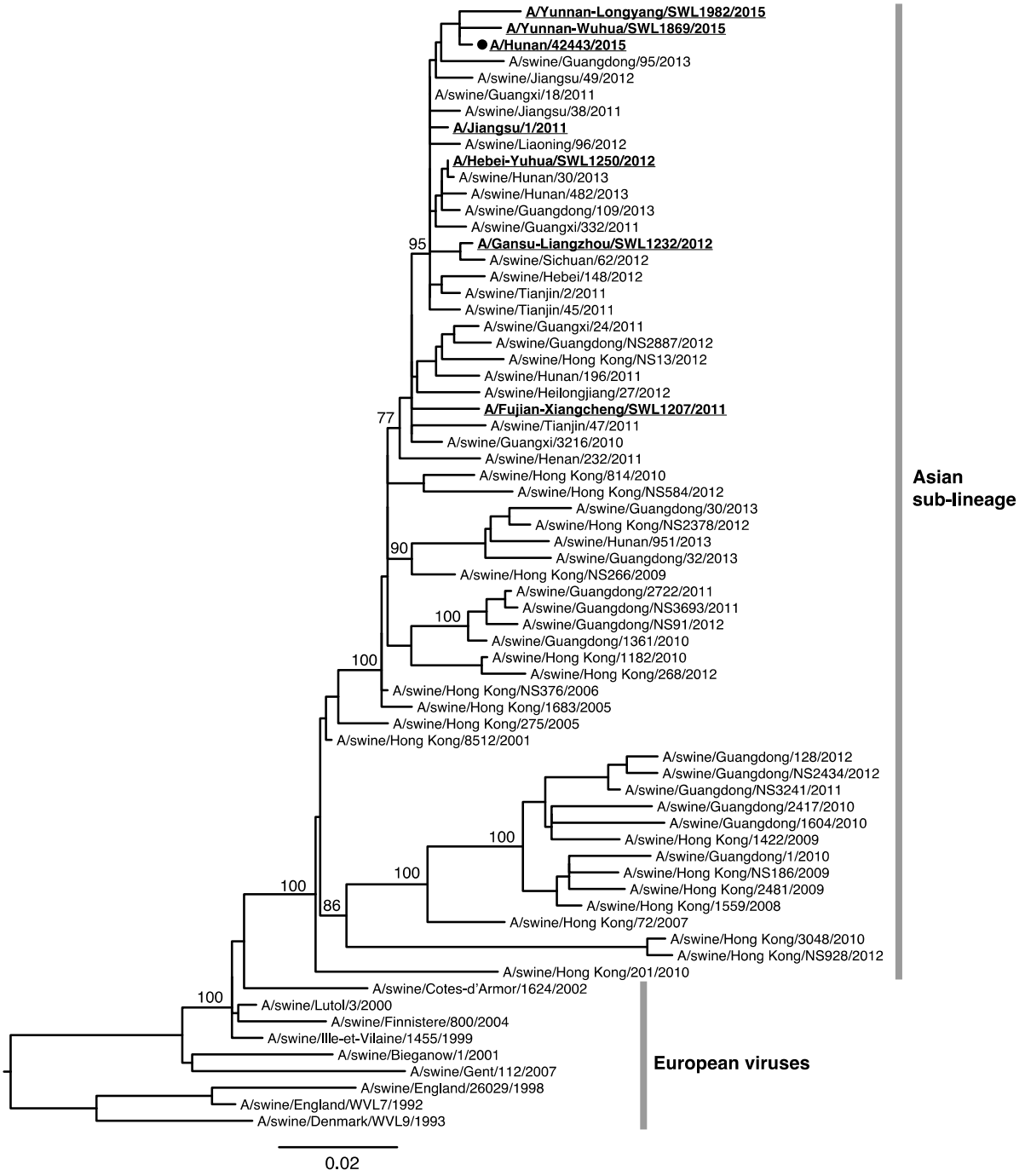

Figure 2. Phylogenetic relationships of HA genes of influenza A(H1) Eurasian avian-like swine lineage. The proposed CVV is indicated by a dot (•). Human viruses are underlined and in bold font. The scale bar represents the number of substitutions per site. Bootstrap supports of topology are shown above selected nodes.

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[7].

Influenza A(H3N2)v activity from 22 September 2015 to 22 February 2016
One case of A(H3N2)v was identified in the United States of America during this reporting period. The individual from New Jersey developed illness in December and recovered following oseltamivir treatment. Virus isolated from the patient, A/New Jersey/53/2015, belonged to cluster IV-A[8] and was related to swine viruses circulating in the United States during 2014-2015.

Despite some genetic diversity between this virus and the closest A(H3N2)v CVV, A/Minnesota/11/2010 (NYMC X-203), it was well-inhibited by ferret antisera raised against A/Minnesota/11/2010. In addition, reactivity of this virus to pooled human sera collected from adults post-vaccination with the 2015-2016 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. 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

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[6] Freidl, GS. et al. Influenza at the animal-human interface: a review of the literature for virological evidence of human infection with swine or avian influenza viruses other than A(H5N1). Euro Surveill. May 8;19(18). 2014
[8] http://onlinelibrary.wiley.com/doi/10.1111/zph.12049/epdf