Antigenic and genetic characteristics of influenza A(H5N1) and influenza A(H9N2) viruses for the development of candidate vaccine viruses for pandemic preparedness

February 2011

The development of representative A(H5N1) and A(H9N2) candidate influenza vaccine viruses, coordinated by the World Health Organization (WHO), remains an essential component of the overall global strategy for pandemic preparedness. Comparisons of the candidate vaccine viruses with respect to antigenicity and their relationship to newly emerging viruses are ongoing, and will be updated periodically by WHO. An update of current and completed vaccine clinical trials can be found on WHO website[1].

Influenza A(H5N1)

Since 2003, highly pathogenic avian influenza A(H5N1) viruses have become enzootic in some countries and continue to cause outbreaks in poultry as well as sporadic human infections. The A(H5N1) viruses have diversified both genetically and antigenically leading to the need for multiple candidate vaccine viruses for pandemic preparedness purposes. Despite the emergence of the pandemic 2009 influenza A(H1N1) [A(H1N1)pdm09][2] viruses, the zoonotic and pandemic threats posed by A(H5N1) viruses remain. This summary provides an update on the characterization of A(H5N1) viruses isolated from birds and humans, and the current status of the development of candidate A(H5N1) vaccine viruses.

Influenza A(H5N1) activity from 27 September 2010 to 15 February 2011

A(H5N1) viruses have been detected in birds in Africa and Asia. Human infections have been reported to WHO from Cambodia, China Hong Kong Special Administrative Region (Hong Kong SAR), Egypt and Indonesia, countries, areas or territories that have also reported infections in birds (Table 1).

Antigenic and genetic characteristics

A nomenclature for phylogenetic relationships among the haemagglutinin (HA) genes of A(H5N1) viruses was devised in consultation with representatives of the Food and Agriculture Organization of the United Nations (FAO), the World Organisation for Animal Health (OIE) and WHO. This nomenclature is updated when new genetic clades emerge and can be found on WHO website[3].

Viruses characterized from 27 September 2010 to 15 February 2011 belonged to the following clades.

Clade 1 viruses were detected in poultry and a human case in Cambodia. Genetic

[1] http://www.who.int/vaccine_research/diseases/influenza/flu_trials_tables/en/index.html
[2] Proposed nomenclature. Also see http://www.who.int/csr/disease/influenza/2011_02_recommendation.pdf
[3] http://www.who.int/csr/disease/avian_influenza/guidelines/nomenclature/en/index.html

characterization of these viruses showed that they were closely related to clade 1 viruses previously circulating in Cambodia (Figure 1).

Clade 2.2.1 viruses continue to circulate in poultry in Egypt with sporadic transmission to humans. The HA genes of the Egyptian clade 2.2.1 viruses cluster into two major genetic groups, C and F (Figure 2). All of the recent human cases in Egypt were caused by viruses belonging to group C, which are most frequently found infecting backyard poultry in the country. Viruses isolated during this period were genetically similar to those isolated previously. Recent human viruses reacted well to postinfection ferret antiserum raised against the candidate vaccine virus A/Egypt/N03072/2010 (Table 2).

Clade 2.3.2 viruses were detected in wild birds in Hong Kong SAR, Japan and Republic of Korea, and in poultry in Japan, Nepal, Republic of Korea and Viet Nam. A human case was also detected in Hong Kong SAR. Although the recent viruses are genetically similar to viruses isolated in 2009 and 2010, viruses within clade 2.3.2 form two distinct phylogenetic groups represented by A/Hubei/1/2010 and A/barn swallow/Hong Kong/D10-1161/2010 (Figure 3). The human case from Hong Kong SAR in 2010 belonged to the A/Hubei/1/2010 group. Antigenically, the clade 2.3.2 viruses are heterogeneous and not all viruses react well to postinfection ferret antiserum raised to the candidate vaccine virus A/common magpie/Hong Kong/5052/2007 (Table 3).

Clade 2.3.4 viruses were detected in poultry in Hong Kong SAR, Myanmar and Viet Nam. Although there is genetic heterogeneity amongst clade 2.3.4 viruses, as previously reported, the recent viruses are similar to those detected in prior years (Figure 1). The viruses detected in Hong Kong SAR and Viet Nam reacted well to postinfection ferret antisera raised against the candidate vaccine viruses A/chicken/Hong Kong/AP156/2008 andA/duck/Laos/3295/2006 or A/Anhui/1/2005, respectively (data not shown in this document).

Influenza A(H5N1) candidate vaccine viruses

Based on the current antigenic, genetic and epidemiological data, further clade 2.3.2 candidate vaccine viruses are recommended. Candidate vaccine viruses based on an A/Hubei/1/2010-like virus and an A/barn swallow/Hong Kong/D10-1161/2010-like virus are proposed. The available candidate A(H5N1) vaccine viruses are listed in Table 4. On the basis of the geographical spread, epidemiology and antigenic and genetic properties of the A(H5N1) viruses, national authorities may recommend the use of one or more of these candidate vaccine viruses for pilot lot vaccine production, clinical trials and stockpiling of A(H5N1) vaccines.

For pandemic preparedness purposes, new A(H5N1) candidate vaccine viruses will be developed as the viruses continue to evolve and announced as they become available. Institutions, companies and others who wish to receive these candidate vaccine viruses should contact the WHO Global Influenza Programme at GISN@who.int or the institutions listed in announcements published on the WHO website[4].

[4] http://www.who.int/csr/disease/avian_influenza/guidelinestopics/en/index5.html.

Table 1. A(H5N1) activity reported from 27 September 2010 to 15 February 2011

Country, area or territory	Host	Genetic clade
Bangladesh	Poultry	unknown
Cambodia	Poultry	1
	Human (1)*	1
China, Hong Kong SAR	Wild birds	2.3.2
	Human (1)	2.3.2
	Poultry (chicken carcass)	2.3.4
Egypt	Poultry	2.2.1
	Humans (10)	2.2.1
Indonesia	Poultry	2.1.3
	Humans (2)	2.1.2
Japan	Wild birds	2.3.2
	Poultry	2.3.2
Myanmar	Poultry	2.3.4
Nepal	Poultry	2.3.2
Republic of Korea	Wild birds	2.3.2
	Poultry	2.3.2
Viet Nam	Poultry	2.3.2, 2.3.4

*number in parentheses denotes number of confirmed cases during this period

Table 2. Antigenic properties of clade 2.2.1 A(H5N1) viruses

		tk/Turkey/1/2005	Egypt/2321/2007	Egypt/1394/2007	Egypt/N03072/2010	Egypt/3300/2008
	Clade	**2.2.1-C**	**2.2.1-C**	**2.2.1-C**	**2.2.1-C**	**2.2.1-F**
Reference Antigens						
A/turkey/Turkey/1/2005	2.2.1	**1280**	320	640	1280	10
A/Egypt/2321-NAMRU3/2007	2.2.1-C	320	**320**	320	1280	5
A/Egypt/1394-NAMRU3/2007	2.2.1-C	1280	320	**2560**	5120	20
A/Egypt/N03072/2010	2.2.1-C	640	80	1280	**2560**	10
A/Egypt/3300-NAMRU3/2008	2.2.1-F	10	5	5	10	**5120**
Test Antigens						
A/Egypt/2321-NAMRU3/2007-RG11	2.2.1-C	640	640	320	2560	10
A/Egypt/N01644/2010	2.2.1-C	2560	640	2560	5120	20
A/Egypt/N01982/2010	2.2.1-C	320	160	640	2560	10
A/Egypt/N02038/2010	2.2.1-C	320	40	ND	640	5
A/Egypt/N03071/2010	2.2.1-C	160	80	ND	640	5

Table 3. Antigenic properties of clade 2.3.2 A(H5N1) viruses

	bhg/Qinghai/ 1A/2005	mduck/Viet Nam/ 1455/2006	cmagpie/Hong Kong/ 5052/2007	ck/Hong Kong/ 5572/2010	jwhite eye/Hong Kong/ 1038/2006
Clade	**2.2**	**2.3.2**	**2.3.2**	**2.3.2**	**2.3.4**
Reference Antigens					
A/bar-headed goose/Qinghai/1A/2005	**160**	160	<40	<40	<40
A/muscovy duck/Viet Nam/1455/2006	160	**160**	80	<20	80
A/common magpie/Hong Kong/5052/2007	80	160	**320**	<20	<40
A/chicken/Hong Kong/D10-5572/2010	<40	<40	40	**160**	<40
A/japanese white eye/Hong Kong/1038/2006	1280	640	80	<40	**1280**
Test Antigens (all clade 2.3.2)					
A/chicken/Hong Kong/729/1/2009	80	320	80	40	40
A/chicken/Viet Nam/3/2010	80	160	40	<20	<40
A/Hong Kong/6841/2010	80	160	40	<20	<40
A/barn swallow/Hong Kong/1161/2010	40	80	40	<20	<40
A/black-headed gull/Hong Kong/709/2011	160	80	40	<20	160
A/large-billed crow/Hong Kong/497/2011	<40	<40	40	160	<40
A/oriental magpie robin/Hong Kong/470.1/2011	<40	<40	40	<20	<40

Table 4. Status of A(H5N1) candidate vaccine virus development (February 2011)

Reassortants with regulatory approval

Virus	Clade	Institution*	Availability
A/Cambodia/R0405050/2007	1	NIBSC	Yes
A/Viet Nam/1203/2004	1	CDC and SJ/HKU	Yes
A/Viet Nam/1194/2004	1	NIBSC	Yes
A/duck/Hunan/795/2002	2.1	SJ/HKU	Yes
A/Indonesia/5/2005	2.1	CDC	Requires Indonesian Government permission
A/bar-headed goose/Qinghai/1A/2005	2.2	SJ/HKU	Yes
A/chicken/India/NIV33487/2006	2.2	CDC/NIV	Yes
A/whooper swan/Mongolia/244/2005	2.2	SJ	Yes
A/Egypt/3300-NAMRU3/2008	2.2.1	CDC	Yes
A/Egypt/2321-NAMRU3/2007	2.2.1	CDC	Yes
A/turkey/Turkey/1/2005	2.2.1	NIBSC	Yes
A/common magpie/Hong Kong/5052/2007	2.3.2	SJ/HKU	Yes
A/Anhui/1/2005	2.3.4	CDC	Yes
A/duck/Laos/3295/2006	2.3.4	FDA	Yes
A/japanese white eye/Hong Kong/1038/2006	2.3.4	SJ/HKU	Yes
A/goose/Guiyang/337/2006	4	SJ/HKU	Yes
A/chicken/Viet Nam/NCVD-016/2008	7	CDC	Yes

Reassortants prepared and awaiting regulatory approval

Virus	Clade	Institution*	Availability
A/chicken/Hong Kong/AP156/2008	2.3.4	SJ/HKU	March 2011
A/chicken/Viet Nam/NCDV-03/2008	7	CDC	Pending

Viruses proposed by WHO for candidate vaccine preparation

Virus	Clade	Institution*
A/Egypt/N03072/2010 [#]	2.2.1	CDC
A/Hubei/1/2010-like	2.3.2	CDC
A/barn swallow/Hong Kong/D10-1161/2010-like	2.3.2	SJ/HKU

* **Institutions:**

CDC - Centers for Disease Control and Prevention, USA
CDC/NIV - Centers for Disease Control and Prevention, USA in collaboration with the National Institute of Virology, India
FDA - Food and Drug Administration, USA
NIBSC - National Institute for Biological Standards and Control, Health Protection Agency, UK
SJ - St Jude Children's Research Hospital, USA
SJ/HKU - St Jude Children's Research Hospital, USA in collaboration with the University of Hong Kong, China Hong Kong SAR

[#] A/Egypt/N03072/2010 is an A/Egypt/1394-NAMRU3/2007-like virus, a previously proposed candidate vaccine virus.

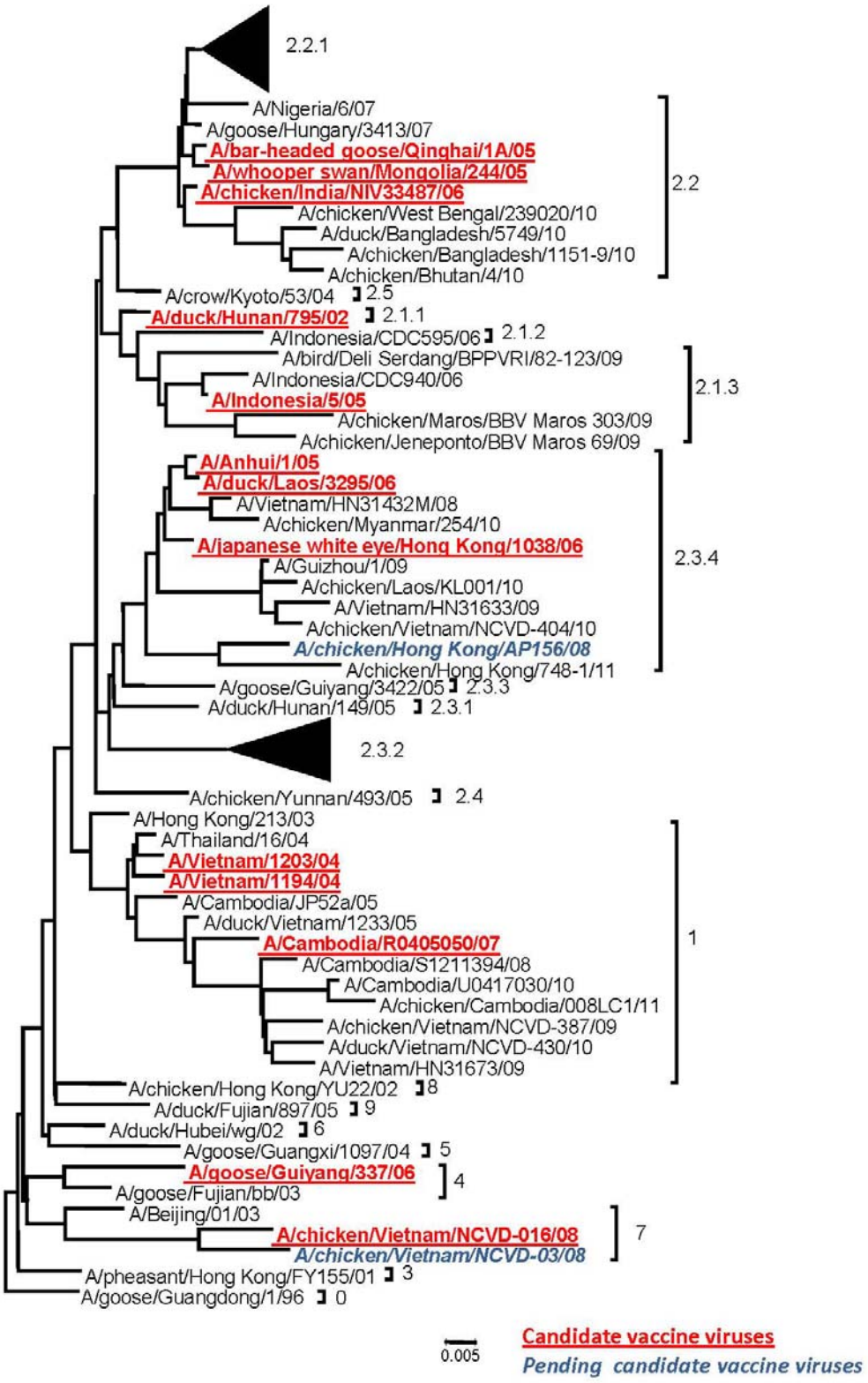

Figure 1. Phylogenetic relationships of A(H5N1) virus HA genes showing available and pending vaccine viruses. We gratefully acknowledge the contributions of the originating laboratories and countries that have provided samples and/or submitted sequence data to DDBJ, EMBL-Bank, GenBank, GISAID and other public databases. Sequence data have also been provided by the OFFLU network and the Pasteur Institute, Cambodia.

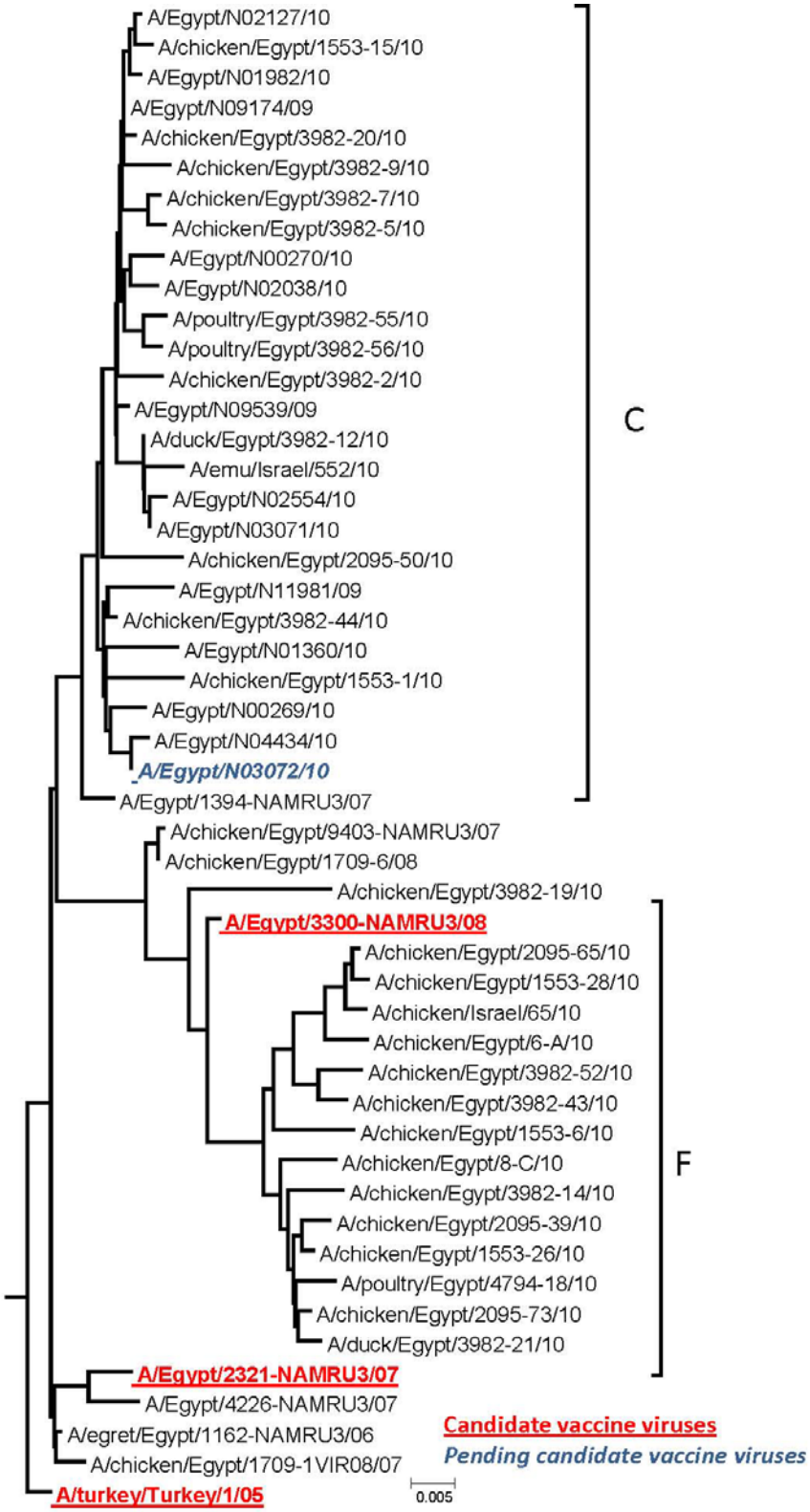

Figure 2. Phylogenetic relationships of A(H5N1) clade 2.2.1 virus HA genes showing available and pending vaccine viruses. We gratefully acknowledge the contributions of the originating laboratories and countries that have provided samples and/or submitted sequence data to DDBJ, EMBL-Bank, GenBank, GISAID and other public databases. Sequence data have also been provided by the OFFLU network.

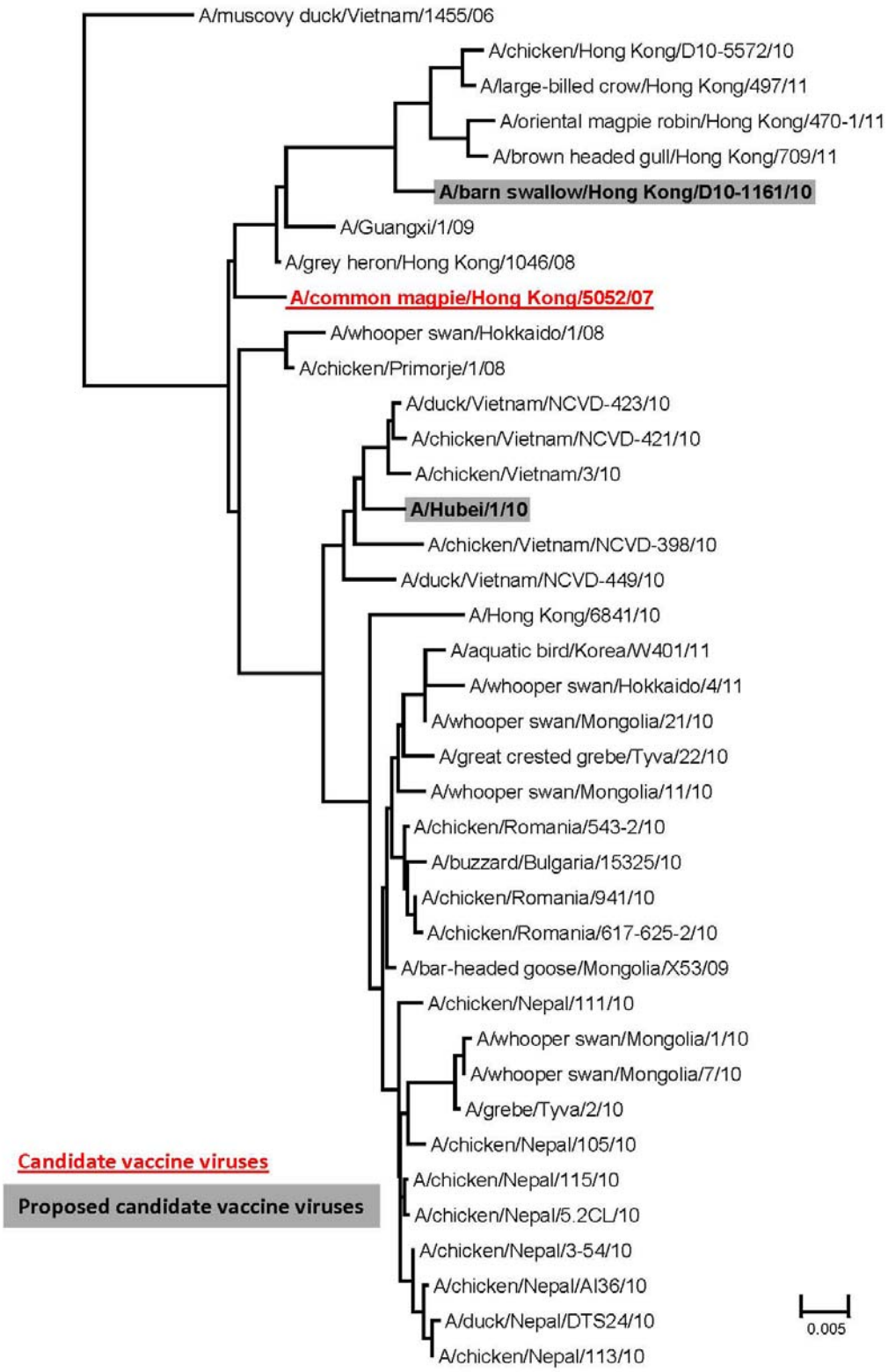

Figure 3. Phylogenetic relationships of A(H5N1) clade 2.3.2 virus HA genes showing available and proposed vaccine viruses. We gratefully acknowledge the contributions of the originating laboratories and countries that have provided samples and/or submitted sequence data to DDBJ, EMBL-Bank, GenBank, GISAID and other public databases. Sequence data have also been provided by the OFFLU network.

Influenza A(H9N2)

Influenza A(H9N2) viruses are enzootic in poultry populations in parts of Asia and the Middle East. Although characterization data on recent A(H9N2) viruses from many regions are limited, the majority of viruses that have been sequenced belong to the G1 or chicken/Beijing (Y280/G9) clades. A(H9N2) viruses analysed from these clades react well to postinfection ferret antisera raised to the candidate vaccine viruses of the corresponding clades (see Table 5). Since 1999, 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.

Human influenza A(H9N2) infection from 27 September 2010 to 15 February 2011

There have been no human cases of A(H9N2) detected in this reporting period. Work continues on the A/Hong Kong/33982/2009 candidate vaccine virus proposed during the WHO Consultation on the Composition of Influenza Vaccines for the Northern Hemisphere 2010-2011[5] (Table 5).

Table 5. Status of A(H9N2) candidate vaccine virus development (February 2011)

Available vaccine viruses				
Virus	**Type**	**Clade**	**Institution***	**Availability**
A/Hong Kong/1073/99	Wild type	G1	NIBSC	Yes
A/chicken/Hong Kong/G9/97 (NIBRG-91)	Reverse genetics	Y280/G9	NIBSC	Yes
A/chicken/Hong Kong/G9/97 (IBCDC-2)	Conventional reassortant	Y280/G9	CDC	Yes
Reassortant prepared and awaiting regulatory approval				
Virus	**Type**	**Clade**	**Institution***	**Availability**
A/Hong Kong/33982/2009 (IBCDC-RG26)	Reverse genetics	G1	CDC	May 2011

* **Institutions:**
CDC - Centers for Disease Control and Prevention, USA
NIBSC - National Institute for Biological Standards and Control, Health Protection Agency, UK

[5] http://www.who.int/csr/disease/avian_influenza/guidelines/201002_H5_H9_VaccineVirusUpdate.pdf