Antigenic and genetic characteristics of influenza A(H5N1) and influenza A(H9N2) viruses and candidate vaccine viruses developed for potential use in human vaccines

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The development of representative influenza A(H5N1) and A(H9N2) candidate 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 their re-emergence in 2003, influenza A(H5N1) viruses have become enzootic in some countries and continue to cause outbreaks in poultry and 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 A(H1N1) virus, 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 A(H5N1) candidate vaccine viruses.

Influenza A(H5N1) activity from 17 February 2010 to 26 September 2010

A(H5N1) viruses have been detected in birds in Africa, Asia, Europe, and the Middle East. Human infections have been reported to WHO from Cambodia, China, Egypt, Indonesia and Viet Nam, countries that have also declared outbreaks 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 novel genetic clades emerge [2].

[1] http://www.who.int/vaccine_research/diseases/influenza/flu_trials_tables/en/index.html
[2] http://www.who.int/csr/disease/avian_influenza/guidelines/nomenclature/en/index.html

Viruses characterized from 17 February 2010 to 26 September 2010 belonged to the following clades.

Clade 1 viruses were detected in poultry and a human in Cambodia. Genetic and antigenic characterization of these viruses showed that they were most closely related to clade 1 viruses previously circulating in Cambodia.

Clade 2.2 viruses were detected in poultry in Bangladesh, Bhutan and Nepal. Genetically, these viruses were most similar to viruses previously detected in the region (Figure 1). Antigenically the viruses from Bhutan and Bangladesh reacted well to postinfection ferret antiserum raised against the vaccine reference virus A/bar-headed goose/Qinghai Lake/1A/2005 (data not shown in this document).

Clade 2.2.1 viruses continue to circulate in poultry in Egypt with sporadic transmission to humans. The HAs of the Egypt 2.2.1 isolates cluster into two major genetic groups (Figure 1) with all of the human cases occurring in 2010 being caused by viruses belonging to one of these groups. Viruses isolated during this period were genetically similar to those isolated during 2009 (Figure 1). Data are not available on the antigenic properties of the recent poultry viruses from Egypt, but the human isolates characterized are antigenically similar to one another. These viruses did not react well to postinfection ferret antiserum raised against the vaccine reference viruses A/Egypt/2321-NAMRU3/2007 and A/Egypt/3300-NAMRU3/2008, but were antigenically more similar to A/Egypt/1394-NAMRU3/2007 (Table 2).

Clade 2.3.2 viruses were detected in wild birds in Bulgaria, China, China Hong Kong Special Administrative Region (Hong Kong SAR), Mongolia and Russian Federation, and in poultry in Nepal, Romania and Viet Nam. These viruses were genetically similar to clade 2.3.2 viruses isolated in 2009 (Figure 1). Antigenically, the viruses from Bulgaria and Romania were similar to the vaccine reference virus A/common magpie/Hong Kong/5052/2007 (Table 3). Viruses from China Hong Kong SAR and Viet Nam were antigenically similar to each other, but antigenically distinguishable from A/common magpie/Hong Kong/5052/2007 (Table 3). A human case was also detected in China. Genetically, this virus was more similar to the viruses isolated from chickens in Viet Nam than to previous human viruses from China. Further antigenic analyses of clade 2.3.2 viruses are required to clarify the need for additional candidate vaccine viruses.

Clade 2.3.4 viruses were detected in poultry in Myanmar, Lao People's Democratic Republic and Viet Nam, and from humans in Viet Nam. The viruses in Viet Nam remain separated into two genetically distinct groups (Figure 1). Viruses from Lao People's Democratic Republic and both genetic groups in Viet Nam reacted well to postinfection ferret antiserum raised against the reference vaccine virus A/duck/Laos/3295/2006 (data not shown in this document).

A(H5N1) candidate vaccine viruses

Based on the current antigenic and epidemiologic data, development of an A/Egypt/1394-NAMRU3/2007-like clade 2.2.1 candidate vaccine virus is proposed. The available A(H5N1) candidate vaccine viruses are listed in Table 3. 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 for pilot lot vaccine production, clinical trials and subsequent stockpiling of vaccines, should such national policies exist.

Additional A(H5N1) candidate vaccine viruses may be developed, for pandemic preparedness purposes, as the viruses continue to evolve and will be announced as they become available. Institutions, companies and others interested in pandemic vaccine development who wish to receive candidate vaccine viruses should contact the WHO Global Influenza Programme at GISN@who.int or the institutions listed in announcements published on the WHO web site[3].

Table 1. A(H5N1) activity report from 17 February 2010 to 26 September 2010

Country, area or territory	Host	Genetic clade
Bangladesh	Domestic poultry	2.2
Bhutan	Domestic poultry	2.2
Bulgaria	Wild birds	2.3.2
Cambodia	Domestic poultry	1
	Human (1)*	1
China	Wild birds	2.3.2
	Human (1)	2.3.2
China, Hong Kong SAR	Wild birds	2.3.2
Egypt	Domestic poultry	2.2.1
	Humans (13)	2.2.1
Indonesia	Domestic poultry	unknown
	Human (5)	unknown
Israel	Zoo bird (Emu)	unknown
Lao People's Democratic Republic	Domestic poultry	2.3.4
Mongolia	Wild birds	2.3.2
Myanmar	Domestic poultry	2.3.4
Nepal	Domestic poultry	2.2
		2.3.2
Romania	Domestic poultry	2.3.2
Russian Federation	Wild birds	2.3.2
Viet Nam	Domestic poultry	2.3.2
		2.3.4
	Human (7)	2.3.4

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

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

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

	eg/Egypt/1162/ 2006	Egypt/2321/ 2007	Egypt/4226/ 2007	Egypt/9539/ 2009	Egypt/1394/ 2007	ck/Egypt/9403 /2007	Egypt/3300/ 2008
Clade	**2.2.1**	**2.2.1**	**2.2.1**	**2.2.1**	**2.2.1**	**2.2.1**	**2.2.1**
Reference Antigens							
A/whooper swan/Mongolia/244/2005 (Clade 2.2)	320	80	80	<10	160	<10	<10
A/bar-headed goose/Qinghai/1A/2005 X PR8 (Clade 2.2)	640	320	80	40	80	20	<10
A/turkey/Turkey/1/2005 (Clade 2.2.1)	320	20	160	<10	320	<10	<10
A/egret/Egypt/1162-NAMRU3/2006	**320**	20	160	<10	320	<10	<10
A/Egypt/2321-NAMRU3/2007	160	**320**	80	<10	80	<10	<10
A/Egypt/4226-NAMRU3/2007	320	40	**80**	20	160	<10	<10
A/Egypt/9539-NAMRU3/2009	80	40	20	**80**	320	<10	<10
A/Egypt/1394-NAMRU3/2007	320	160	80	320	**1280**	<10	<10
A/chicken/Egypt/9403-NAMRU3/2007	20	20	20	<10	<10	**320**	<10
A/Egypt/3300-NAMRU3/2008	<10	<10	<10	<10	<10	80	**1280**
A/Egypt/3300-NAMRU3/2008 RG-13	<10	<10	10	<10	<10	640	2560
Test Antigens							
A/Egypt/N11981/2009	80	<10	10	<10	320	<10	<10
A/Egypt/N01360/2010	80	<10	40	20	320	<10	<10
A/Egypt/N01982/2010	160	160	40	160	320	<10	<10
A/Egypt/N02038/2010	80	<10	<10	20	160	<10	<10
A/Egypt/N02127/2010	80	<10	10	160	320	<10	<10
A/Egypt/N02554/2010	<10	<10	<10	<10	80	<10	<10
A/Egypt/N03071/2010	40	<10	<10	<10	160	<10	<10
A/Egypt/N03072/2010	320	320	160	160	1280	<10	<10

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

	BHG/Qinghai/ 1A/2005	Mduck/Viet Nam/ 1455/2006	CMagpie/HK/ 5052/2007	Grey Heron/HK/ 1046/2008	J white eye/HK/ 1038/2006	chicken/HK/ AP156/2008
Clade	**2.2**	**2.3.2**	**2.3.2**	**2.3.2**	**2.3.4**	**2.3.4**
Reference Antigens						
A/bar-headed goose/Qinghai/1A/2005	**640**	80	40	<40	<40	<40
A/muscovy duck/Viet Nam/1455/2006	80	**80**	80	40	<40	<40
A/common magpie/Hong Kong/5052/2007	40	80	**320**	80	<40	<40
A/grey heron/Hong Kong/1046/2008	80	80	160	**160**	<40	<40
A/Japanese white eye/Hong Kong/1038/2006	640	160	<40	<40	**2560**	<40
A/chicken/Hong Kong/AP156/2008	40	<40	<40	1280	<40	**80**
Test Antigens						
A/common buzzard/Bulgaria/38WB/2010	80	160	160	320	<40	<40
A/chicken/Romania/543-2/2010	40	160	320	320	<40	<40
A/chicken/Viet Nam/3/2010	80	40	40	ND	<40	40
A/duck/Viet Nam/4/2010	<40	<40	<40	ND	<40	<40
A/barn swallow/Hong Kong/1161/2010	40	40	40	ND	<40	<40

Table 4. Status of A(H5N1) candidate vaccine virus development (September 2010)

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/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/common magpie/Hong Kong/5052/2007	2.3.2	SJ/HKU	Expected: October 2010
A/chicken/Hong Kong/AP156/2008	2.3.4	SJ/HKU	Expected: November 2010
A/chicken/Viet Nam/NCDV-03/2008	7	CDC	Pending

Viruses proposed by WHO for candidate vaccine preparation

Virus	Clade	Institution*	
A/Egypt/1394-NAMRU3/2007-like	2.2.1	CDC	Pending

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

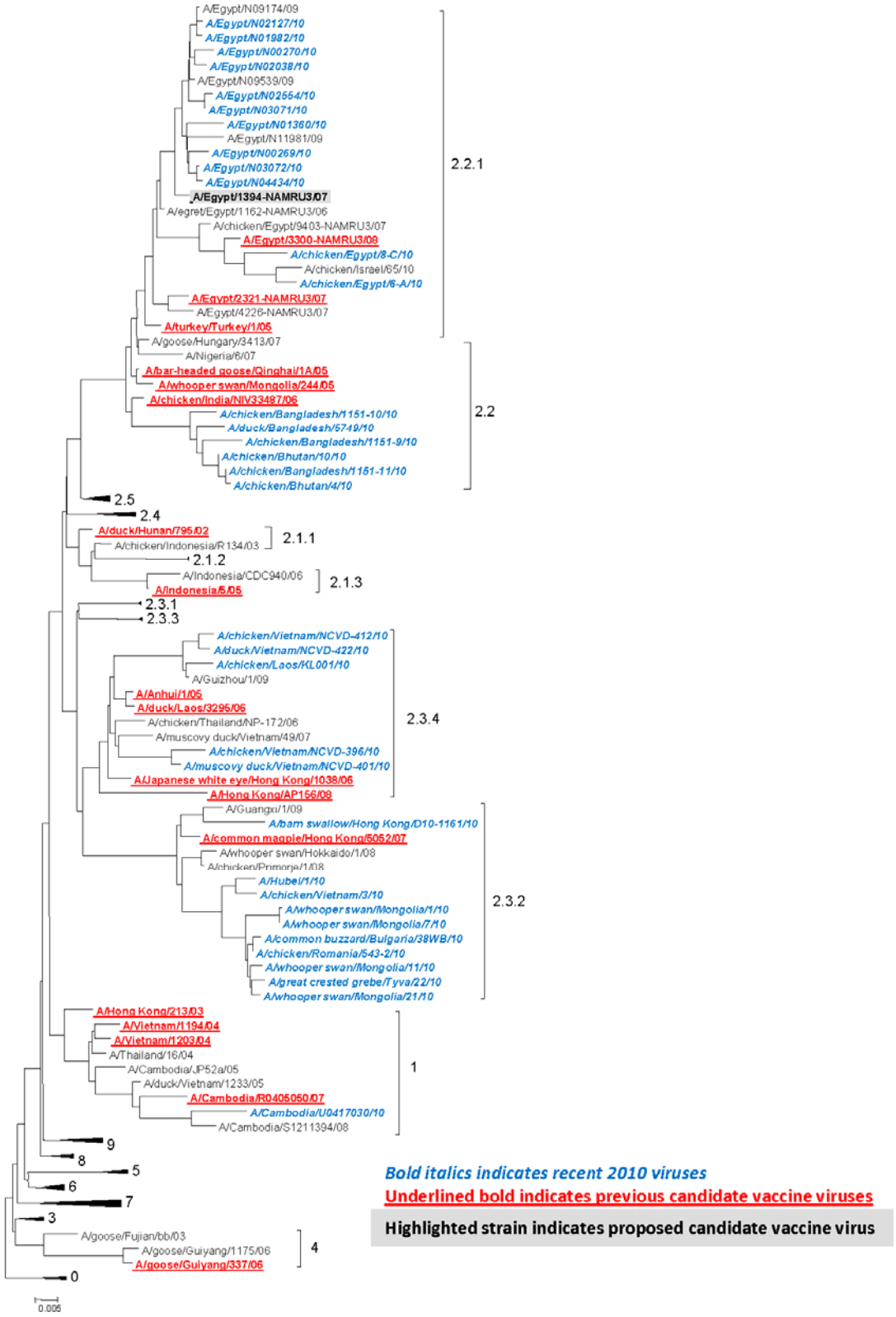

Figure 1. Phylogenetic relationships of A(H5N1) virus HA genes showing available vaccine viruses. We gratefully acknowledge the contributions of the originating laboratories and countries that have provided samples and/or submitted sequence data to the DNA Data Bank of Japan (DDBJ), the European Molecular Biology Laboratory (EMBL)-Bank, GenBank, the Global Initiative on Sharing All Influenza Data (GISAID) and other public databases. Sequence data have also been provided by the World Organisation for Animal Health/United Nations Food and Agriculture Organization Network of Expertise on Animal Influenza (OFFLU) and the Pasteur Institute, Cambodia.

Influenza A(H9N2)

Influenza A(H9N2) viruses are enzootic in poultry populations in parts of Asia and the Middle East. Although characterization data of recent A(H9N2) viruses from many regions are absent, the majority of viruses that have been sequenced belong to the G1 and Y280/G9 clades. 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 17 February 2009 to 26 September 2010

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 viruses proposed during the WHO Consultation on the Composition of Influenza Vaccines for the Northern Hemisphere 2010-2011 (Table 5).

Table 5. Status of A(H9N2) candidate vaccine virus development (September 2010)

Available Vaccine Viruses	Type	Clade	Institution*	Availability
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 reassortant	Y280/G9	CDC	Yes

Proposed Vaccine Virus	Type	Clade	Institution*	
A/Hong Kong/33982/2009	Conventional and reverse genetics reassortants	G1	CDC/NIBSC	Pending

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