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

September 2011

The development of representative 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 periodically reported by WHO. An update of current and completed vaccine clinical trials can be found on the WHO website[1].

Influenza A(H5N1)

Since their re-emergence in 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. This summary provides updates on the characterisation 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 16 February 2011 to 19 September 2011

A(H5N1) viruses have been detected in birds in Africa, Asia, and the Middle East. Human infections have been reported to the WHO from Bangladesh, Cambodia, Egypt and Indonesia, countries in which infections have also been reported in birds (Table 1).

Antigenic and genetic characteristics

The nomenclature for phylogenetic relationships among the haemagglutinin (HA) genes of A(H5N1) viruses has been recently revised 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. The updated nomenclature report will be published in the journal Influenza and Other Respiratory Viruses and on the WHO website.

Viruses circulating and characterised from 16 February 2011 to 19 September 2011 belonged to the following clades.

Clade 1.1 (previously part of clade 1) viruses were detected in poultry and humans in Cambodia and in poultry in Viet Nam. Genetic characterisation of the HA genes of these viruses showed that they were closely related to clade 1.1 viruses that had circulated earlier in these countries. The HA genes from the viruses isolated from humans were located in two genetic groups (Figure 1); the human viruses reacted well with post-infection ferret antisera against the clade 1 viruses A/Viet Nam/1203/2004 and A/Viet Nam/1194/2004 from which candidate vaccine viruses have been developed (Table 5).

[1] http://www.who.int/vaccine_research/diseases/influenza/flu_trials_tables/en/index.html

Clade 2.2.1 viruses continue to circulate in backyard poultry in Egypt with sporadic transmission to humans. All recent human A(H5N1) viruses in Egypt belong to this clade (Figure 2). Clade 2.2.1 viruses were also detected in poultry in Israel. Viruses isolated during this period were genetically similar to those isolated previously. Recent human viruses reacted well with post-infection ferret antiserum against A/Egypt/N03072/2010 from which a candidate vaccine virus has been developed (Table 5).

Clade 2.2.1.1 (previously part of clade 2.2.1) viruses continued to circulate primarily within the commercial poultry sector in Egypt and were isolated from this population during the reporting period. Genetically these viruses were similar to other recent clade 2.2.1.1 viruses (Figure 2).

Clade 2.2.2 (previously part of clade 2.2) viruses were detected in poultry and humans in Bangladesh. Genetically these viruses were similar to viruses detected in this region in previous years (Figure 2). Post-infection ferret antisera against the clade 2.2 viruses A/chicken/India/NIV33487/2006 and A/bar-headed goose/Qinghai Lake/1A/2005, from which candidate vaccine viruses have been developed (Table 5), reacted well with the recent clade 2.2.2 viruses.

Clade 2.3.2.1 (previously part of clade 2.3.2) viruses were detected in wild birds in Bangladesh, Japan and the Republic of Korea, and also in poultry in Bangladesh, China Hong Kong Special Administrative Region (China Hong Kong SAR), India, Japan, Myanmar, Republic of Korea and Viet Nam. Although there is some genetic (Figure 3) and antigenic heterogeneity among viruses of this clade, recently isolated viruses reacted well with post-infection ferret antisera against either A/Hong Kong/6841/2010 (an A/Hubei/1/2010-like virus) or A/barn swallow/Hong Kong/D10-1161/2010 (Tables 2 and 3), from which candidate vaccine viruses have been developed (Table 5).

A *Clade 2.3.4* virus was detected in a poultry carcass in China Hong Kong SAR. Antigenically and genetically this virus was similar to clade 2.3.4 viruses circulating in recent years (Figure 4).

Clade 2.3.4.2 (previously part of 2.3.4) viruses were detected in poultry in Bangladesh and Myanmar. Representative viruses from these countries were genetically similar to each other (Figure 4) but showed reduced reactivity with post-infection ferret antisera against the clade 2.3.4 viruses A/chicken/Hong Kong/AP156/2008, A/duck/Laos/3295/2006, and A/Japanese white eye/Hong Kong/1038/2006 (Table 4), from which candidate vaccine viruses have been developed (Table 5).

Influenza A(H5N1) candidate vaccine viruses

Based on the current antigenic, genetic and epidemiological data, development of a new clade 2.3.4.2 A/chicken/Bangladesh/11rs1984-30/2011-like candidate vaccine virus is proposed. The available candidate A(H5N1) vaccine viruses are listed in Table 5. On the basis of geographic spread, epidemiology and antigenic and genetic properties of the A(H5N1) viruses in particular locations, national authorities may consider the use of one or more of these candidate vaccine viruses for pilot lot vaccine production, for clinical trials and other pandemic preparedness purposes.

As the viruses continue to evolve, new A(H5N1) candidate vaccine viruses will be developed and announced as they become available. Institutions, companies and others who wish to receive these candidate vaccine viruses should contact WHO at gisrs-whohq@who.int or the institutions listed in announcements published on the WHO website[2].

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

Table 1. A(H5N1) activity reported from 16 February 2011 to 19 September 2011

Country, area or territory	Host	Genetic clade
Bangladesh	Poultry	2.2.2/2.3.2.1/2.3.4.2
	Wild birds	2.3.2.1
	Human (2*)	2.2.2
Cambodia	Poultry	1.1
	Human (7)	1.1
China Hong Kong SAR	Poultry	2.3.2.1/2.3.4
Egypt	Poultry	2.2.1/2.2.1.1
	Humans (29)	2.2.1
India	Poultry	2.3.2.1
Indonesia	Poultry	unknown
	Humans (7)	unknown
Israel	Poultry	2.2.1
Japan	Wild birds	2.3.2.1
	Poultry	2.3.2.1
Republic of Korea	Wild birds	2.3.2.1
	Poultry	2.3.2.1
Mongolia	Wild bird	unknown
Myanmar	Poultry	2.3.2.1/2.3.4.2
Viet Nam	Poultry	1.1/2.3.2.1

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

Table 2. Antigenic properties of recent A/Hubei/1/2010-like clade 2.3.2.1 A(H5N1) viruses

		Reference ferret antisera			
		cm/HK/07	bhg/Mon/09	HK/6841/10	Anhui/1/05
Reference antigens	Clade				
A/common magpie/Hong Kong/5052/2007	2.3.2.1	**640**	160	320	<10
A/bar-headed goose/Mongolia/x53/2009	2.3.2.1	320	**160**	640	<10
A/Hong Kong/6841/2010*	2.3.2.1	160	80	**320**	<10
A/Anhui/1/2005	2.3.4	20	<10	10	**320**
Test antigens					
A/duck/Viet Nam/NCVD-671/2011	2.3.2.1	20	20	80	<10
A/chicken/Viet Nam/NCVD-703/2011	2.3.2.1	10	40	160	<10
A/chicken/Viet Nam/NCVD-675/2011	2.3.2.1	40	80	160	<10
A/duck/Viet Nam/NCVD-664/2010	2.3.2.1	40	10	80	<10
A/chicken/Viet Nam/NCVD-700/2011	2.3.2.1	10	40	80	<10
A/crow/Bangladesh/1008/2011	2.3.2.1	160	10	320	<10

*A/Hong Kong/6841/2010 is a A/Hubei/1/2010-like virus

Table 3. Antigenic properties of recent A/barn swallow/Hong Kong/1161-2010-like clade 2.3.2.1 A(H5N1) viruses

		Reference ferret antisera		
		dk/VN/1455/06	cm/HK/07	bs/HK/1161/10
Reference antigens	Clade			
A/muscovy duck/Viet Nam/1455/2006	2.3.2	**80**	40	<20
A/common magpie/Hong Kong/5052/2007	2.3.2.1	80	**160**	80
A/barn swallow/Hong Kong/1161/2010	2.3.2.1	<20	40	**160**
Test antigens				
A/goose/Hong Kong/631/2009	2.3.2.1	<20	<20	40
A/oriental magpie robin/Hong Kong/470.1/2011	2.3.2.1	<20	<20	80
A/large-billed crow/Hong Kong/497/2011	2.3.2.1	<20	<20	80
A/black-headed gull/Hong Kong/709/2011	2.3.2.1	<20	<20	80
A/chicken/Hong Kong/884.2/2011	2.3.2.1	<20	<20	80
A/swine/Guangxi/NS592/2011	2.3.2.1	<20	<20	80

Table 4. Antigenic properties of a recent clade 2.3.4.2 A(H5N1) virus

		Reference ferret antisera		
		dk/Laos/3295/06	jwe/HK/1038/06	ck/HK/AP156/08
Reference antigens	Clade			
A/duck/Laos/3295/2006	2.3.4	**640**	20	nt*
A/Japanese white eye/Hong Kong/1038/2006	2.3.4	640	**160**	nt*
A/chicken/Hong Kong/AP156/2008	2.3.4	<10	<10	**640**
Test antigen				
A/chicken/Bangladesh/11rs1984-30/2011	2.3.4.2	20	<10	20

*nt = not tested

Table 5. Status of A(H5N1) candidate vaccine virus development (September 2011)

Available candidate vaccine viruses

Virus	Clade	Institution*	Availability
A/Cambodia/R0405050/2007	1.1	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	2.1.3.2	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	2.2.1.1	CDC	Yes
A/Egypt/2321-NAMRU3/2007	2.2.1	CDC	Yes
A/turkey/Turkey/1/2005	2.2.1	NIBSC	Yes
A/Egypt/N03072/2010	2.2.1	CDC	Yes
A/common magpie/Hong Kong/5052/2007	2.3.2.1	SJ/HKU	Yes
A/chicken/Hong Kong/AP156/2008	2.3.4	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	7.1	CDC	Yes

Candidate vaccine viruses in preparation

Virus	Clade	Institution	Availability
A/chicken/Viet Nam/NCDV-03/2008	7.1	CDC	Pending
A/barn swallow/Hong Kong/D10-1161/2010	2.3.2.1	SJ/HKU	Pending
A/Hubei/1/2010	2.3.2.1	CDC	Pending

Viruses proposed by WHO for candidate vaccine virus preparation

Virus	Clade	Institution
A/chicken/Bangladesh/11rs1984-30/2011-like	2.3.4.2	Pending

* **Institutions:**

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

Influenza A(H9N2)

Influenza A(H9N2) viruses are enzootic in poultry populations in parts of Asia and the Middle East. Although characterisation data on recent A(H9N2) viruses from many regions are limited, the majority of viruses that have been sequenced belong to the G1 clade or the chicken/Beijing (Y280/G9) clade. 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.

Human influenza A(H9N2) infection from 16 February 2011 to 19 September 2011

There has been one human case of A(H9N2) infection detected in Bangladesh in this reporting period. This virus was genetically and antigenically similar to A(H9N2) viruses circulating in poultry in Bangladesh in previous years (Figure 5) but distinct from viruses from which candidate vaccine viruses have been developed (Table 6). Accordingly, the development of an A/Bangladesh/0994/2011-like candidate vaccine virus is proposed (Table 6).

As the viruses continue to evolve new A(H9N2) candidate vaccine viruses will be developed and announced as they become available. Institutions, companies and others who wish to receive these candidate vaccine viruses should contact WHO at gisrs-whohq@who.int or the institutions listed in announcements published on the WHO website[3].

Table 6. Status of A(H9N2) candidate vaccine virus development (September 2011)

Available candidate vaccine viruses				
Virus	**Type**	**Clade**	**Institution***	**Availability**
A/Hong Kong/1073/1999	Wild type	G1	NIBSC	Yes
A/chicken/Hong Kong/G9/1997	Reverse genetics	Y280/G9	NIBSC	Yes
A/chicken/Hong Kong/G9/1997	Conventional reassortant	Y280/G9	CDC	Yes

Candidate vaccine viruses in preparation				
Virus	**Type**	**Clade**	**Institution**	**Availability**
A/Hong Kong/33982/2009 (IBCDC-RG26)	Reverse genetics	G1	CDC	Pending

Viruses proposed by WHO for candidate vaccine virus preparation			
Virus	**Type**	**Clade**	**Institution**
A/Bangladesh/0994/2011-like	Reverse genetics and conventional	G1	CDC/NIBSC

* **Institutions:**
CDC - Centers for Disease Control and Prevention, Unites States of America
NIBSC - National Institute for Biological Standards and Control, Health Protection Agency, United Kingdom of Great Britain and Northern Ireland

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

Swine-Origin Influenza A(H3N2)

Swine influenza A(H3N2) viruses are enzootic in swine herds of North America and other parts of the world. Characterisation of recent A(H3N2) viruses from swine in North America indicates that their HA genes have evolved from the human virus precursors that circulated in the mid-1990s. Isolation of swine-origin influenza viruses (SOIV) A(H3N2) from humans has been reported infrequently. The United States of America reported eight infections due to A(H3N2) SOIV between January 2005 and 15 February 2011.

A(H3N2) SOIV infections from 16 February 2011 to 19 September 2011

There have been four human infections with A(H3N2) SOIV in the states of Indiana (1) and Pennsylvania (3), United States of America, in this period. The HA and neuraminidase genes of these four viruses were similar to those of swine viruses that circulate in the United States of America. Sequencing data indicated that the matrix genes of these viruses were acquired from an A(H1N1)pdm09 virus, unlike SOIV isolates from previous human cases. Antigenic analysis indicated that these viruses were distinct from currently circulating human A(H3N2) viruses but similar to swine A(H3N2) viruses from previous years as well as to A/Minnesota/11/2010 (H3N2) SOIV (Table 7), from which a candidate vaccine virus is under development.

As the viruses continue to evolve new SOIV candidate vaccine viruses will be developed and announced as they become available. Institutions, companies and others who wish to receive these candidate vaccine viruses should contact WHO at gisrs-whohq@who.int or the institutions listed in announcements published on the WHO website[4].

Table 7. Antigenic properties of a recent A(H3N2) SOIV

	Reference ferret antisera							
	CA/09	SD/03	Perth/09	Sw/IL/09	KS/09	PA/10	WI/10	MN/10
Reference antigens								
A/California/07/2009 *H1N1pdm09*	**2560**	2560	<10	20	<10	40	<10	<10
A/South Dakota/03/2008 *Human H1N1-SOIV*	2560	**5120**	10	160	160	160	<10	<10
A/Perth/16/2009 *Seasonal H3N2*	<10	<10	**640**	<10	<10	<10	<10	<10
A/swine/Illinois/02907/2009 *Swine H3N2*	<10	<10	<10	**2560**	2560	320	160	80
A/Kansas/13/2009 *Human H3N2-SOIV*	<10	<10	<10	1280	**2560**	320	160	80
A/Pennsylvania/14/2010 *Human H3N2-SOIV*	<10	<10	10	160	320	**1280**	320	640
A/Wisconsin/12/2010 *Human H3N2-SOIV*	<10	<10	<10	160	80	640	**1280**	640
A/Minnesota/11/2010 *Human H3N2-SOIV*	<10	<10	<10	40	40	320	320	**1280**
Test antigens								
A/Indiana/08/2011	20	<10	10	40	80	1280	1280	1280

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

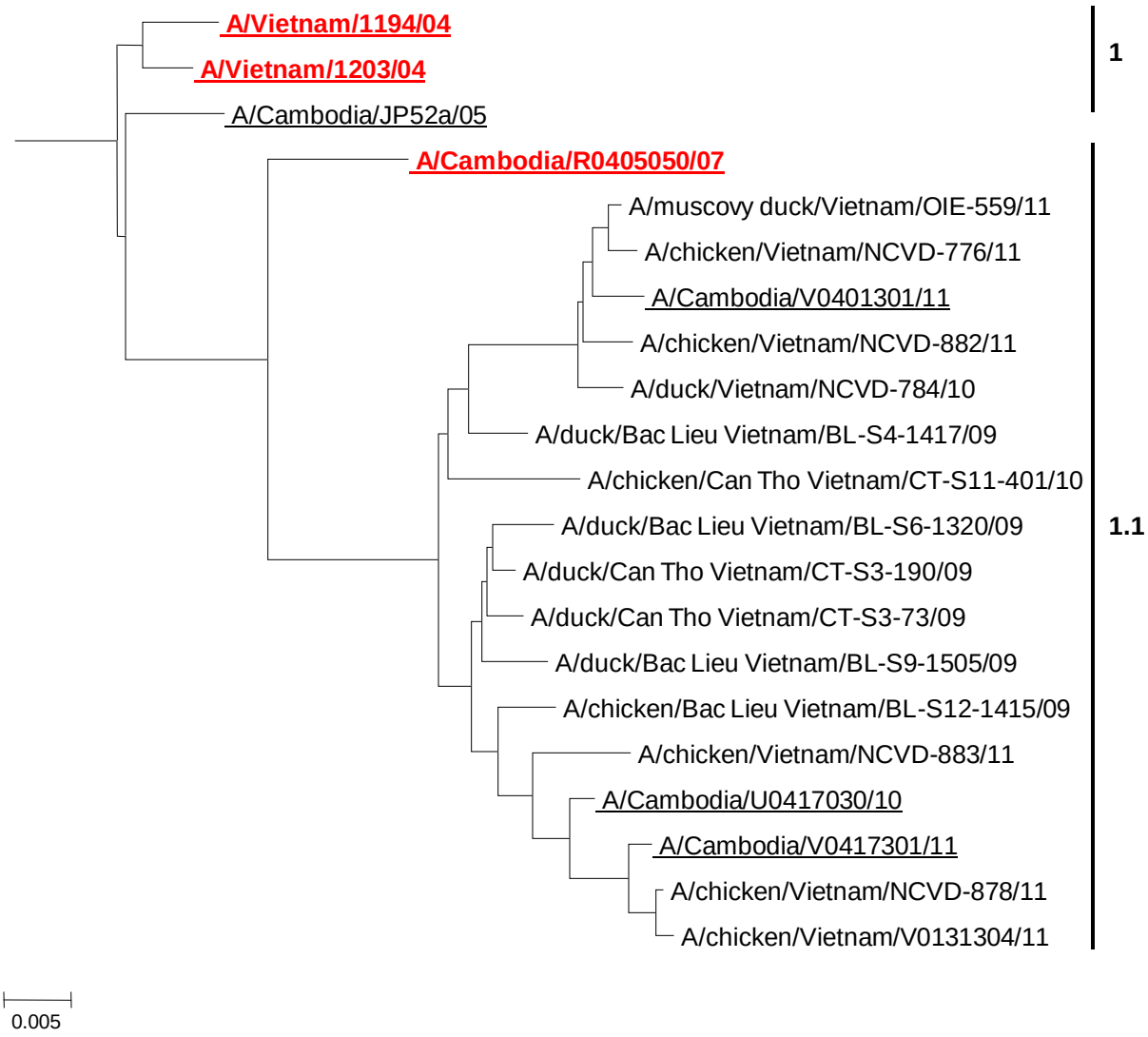

Figure 1. Phylogenetic relationships of A(H5N1) clade 1 and 1.1 virus HA genes showing available (in red) vaccine viruses. Human viruses are underlined. We gratefully acknowledge the contributions of the originating laboratories and countries that have provided samples and/or submitted sequence data to DDBJ, EMBL-Bank, GenBank, GISAID and other public databases. Sequence data have also been provided by the OFFLU network.

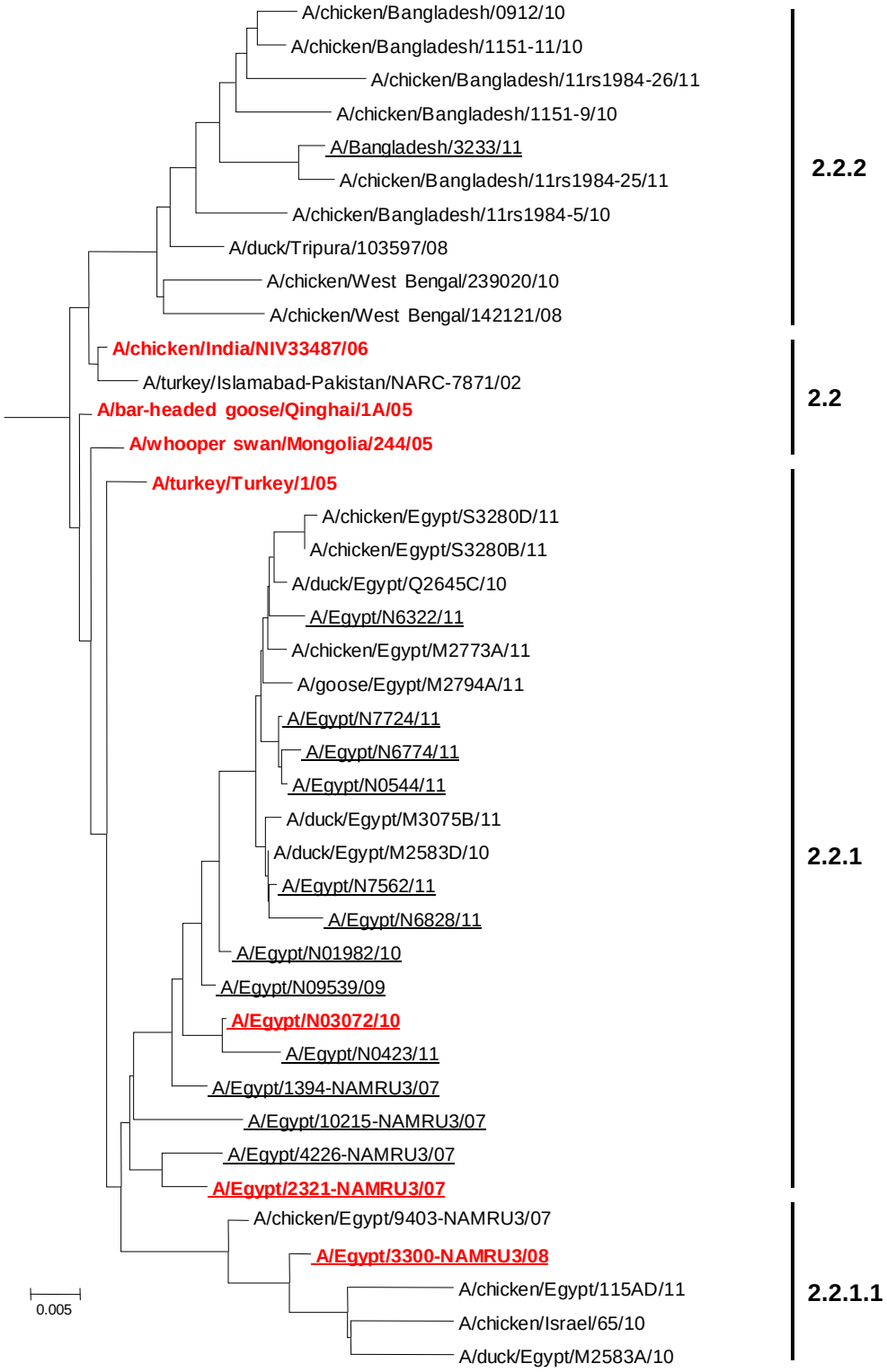

Figure 2. Phylogenetic relationships of A(H5N1) clade 2.2, 2.2.1, 2.2.2, and clade 2.2.1.1 virus HA genes showing available (in red) vaccine viruses. Human viruses are underlined. We gratefully acknowledge the contributions of the originating laboratories and countries that have provided samples and/or submitted sequence data to DDBJ, EMBL-Bank, GenBank, GISAID and other public databases. Sequence data have also been provided by the OFFLU network.

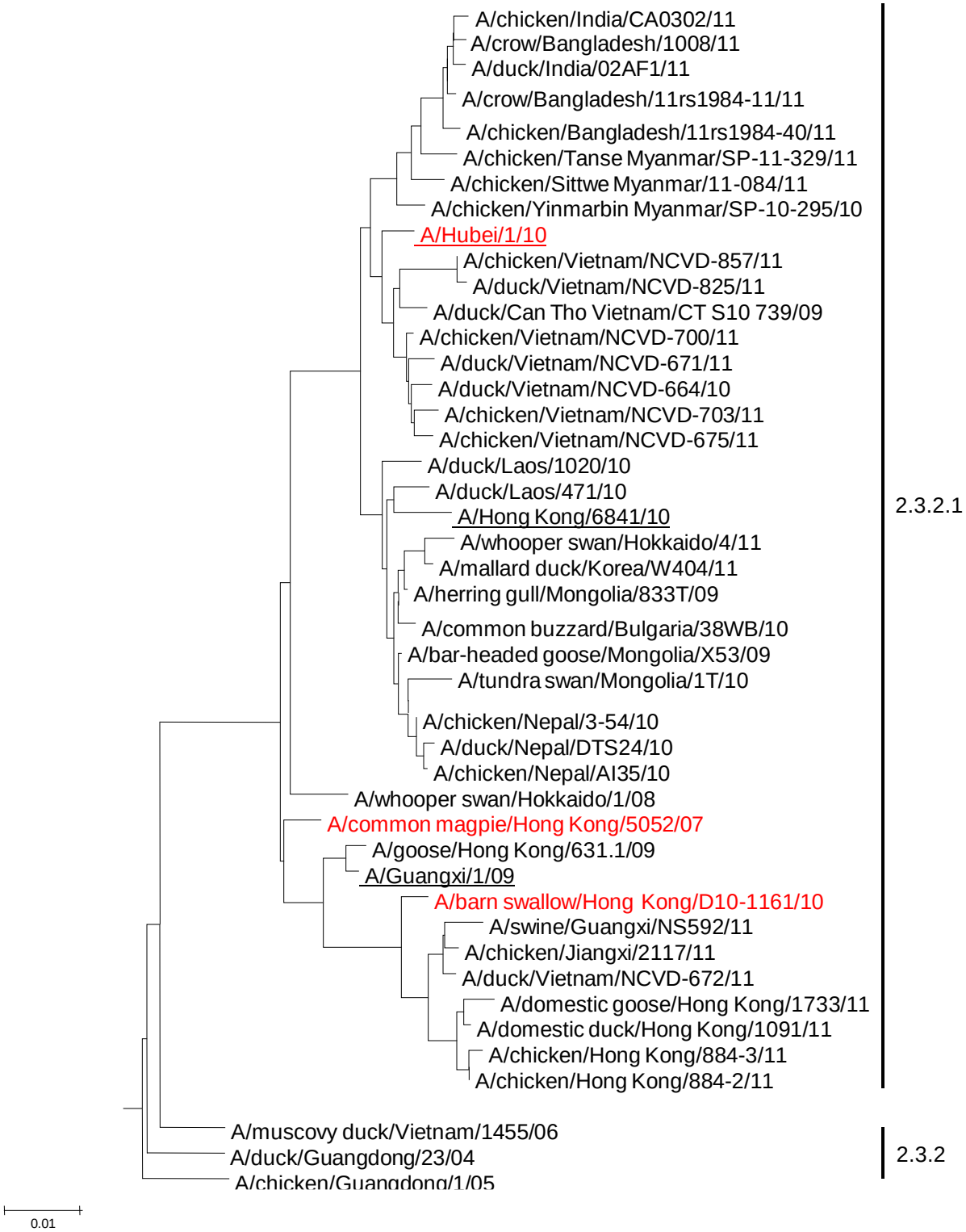

Figure 3. Phylogenetic relationships of A(H5N1) clade 2.3.2, and clade 2.3.2.1 virus HA genes showing available (in red) vaccine viruses. Human viruses are underlined. We gratefully acknowledge the contributions of the originating laboratories and countries that have provided samples and/or submitted sequence data to DDBJ, EMBL-Bank, GenBank, GISAID and other public databases. Sequence data have also been provided by the OFFLU network.

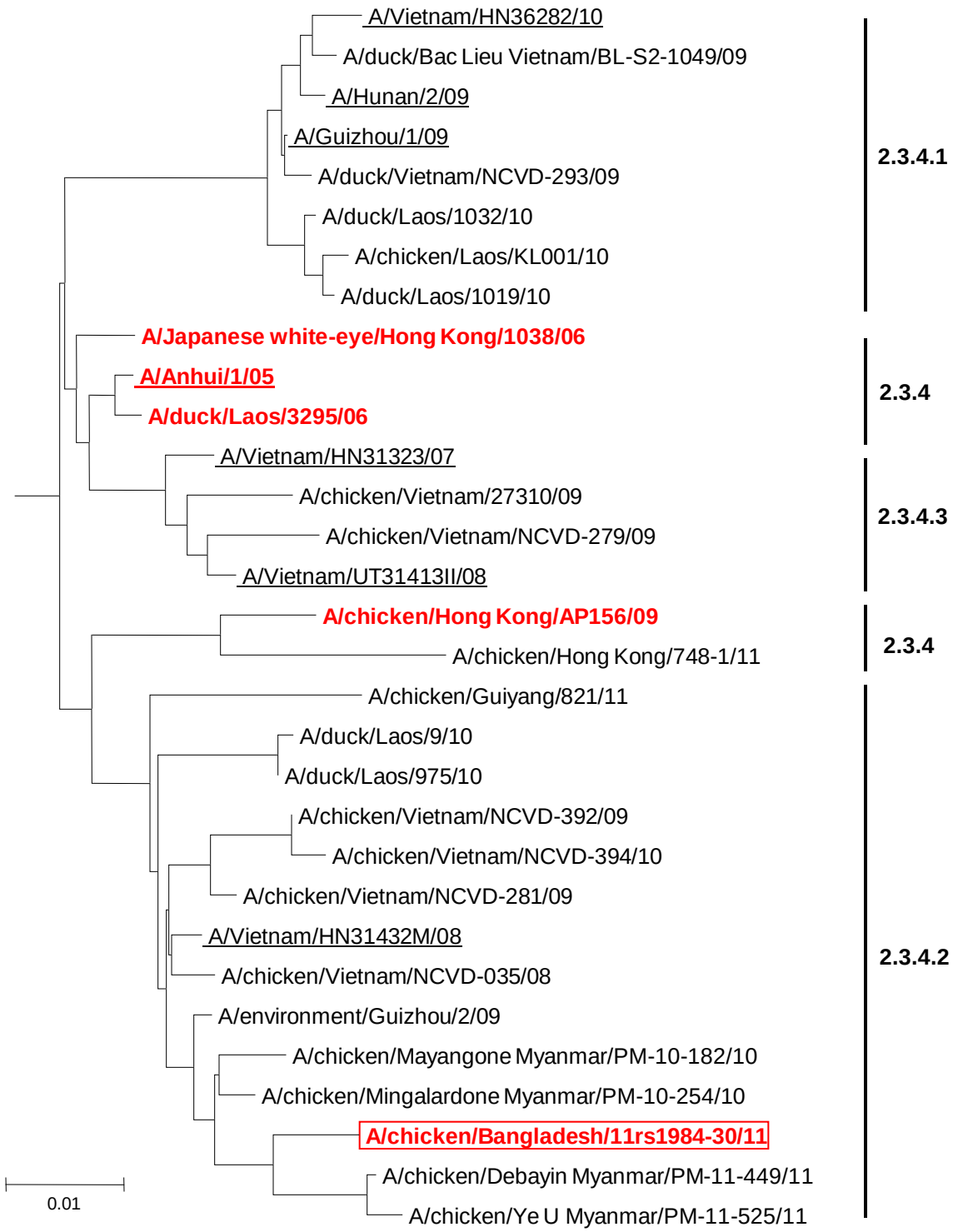

Figure 4. Phylogenetic relationships of A(H5N1) clade 2.3.4, 2.3.4.1, 2.3.4.2, and clade 2.3.4.3 virus HA genes showing available (in red) and proposed (in red boxed) vaccine viruses. Human viruses are underlined. We gratefully acknowledge the contributions of the originating laboratories and countries 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.

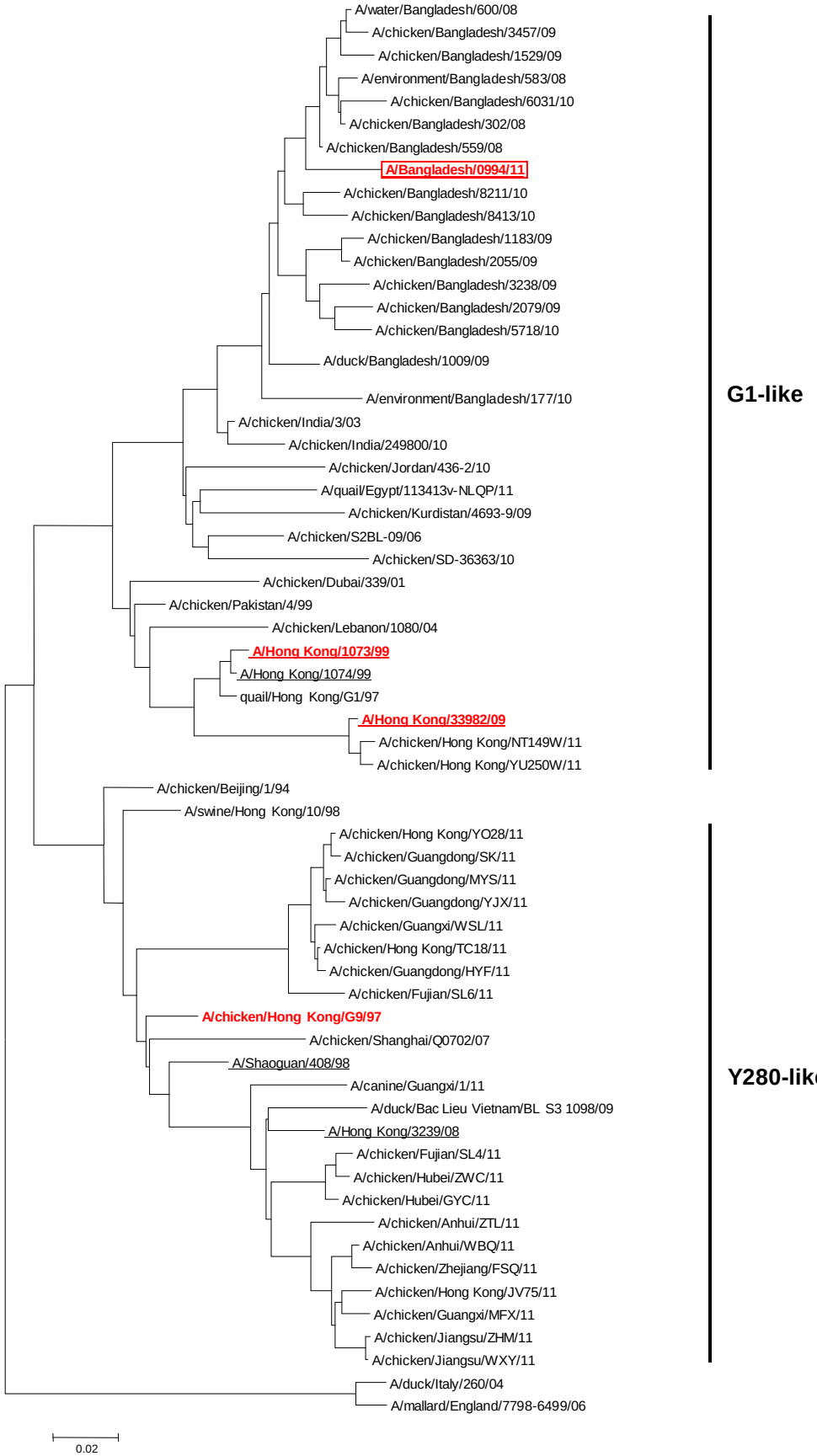

Figure 5. Phylogenetic relationships of A(H9N2) virus HA genes showing available (in red) and proposed (in red boxed) vaccine viruses. Human viruses are underlined. We gratefully acknowledge the contributions of the originating laboratories and countries that have provided samples and/or submitted sequence data to DDBJ, EMBL-Bank, GenBank, GISAID and other public databases. Sequence data have also been provided by the OFFLU network.