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 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 immunogenicity 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 at http://www.who.int/vaccine_research/diseases/influenza/flu_trials_tables/en/index.html

Influenza A(H5N1)

Since their re-emergence in 2003, influenza A(H5N1) viruses have become endemic 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. Despite the emergence of the pandemic A(H1N1) 2009 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 candidate A(H5N1) vaccine viruses.

Influenza A(H5N1) activity from September 2009 to 17 February 2010

A(H5N1) viruses have continued to be detected in birds in Africa, Asia, and the Middle East. Human infections have been reported to WHO from Cambodia, 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 and can be found at http://www.who.int/csr/disease/avian_influenza/guidelines/nomenclature/en/index.html

Viruses characterized from September 2009 to 17 February 2010 belonged to the following clades:

Clade 1 viruses were detected in poultry in Cambodia. Genetic characterization of these viruses showed that they were closely related to clade 1 viruses previously circulating in Cambodia (Figure 1). No antigenic data are available.

Clade 2.2 viruses were detected in poultry in Nepal. Genetically these viruses were most similar to viruses previously detected in the region (Figure 1). No antigenic data are available.

Clade 2.2.1 viruses continue to circulate in poultry in Egypt with sporadic transmission to humans. Viruses isolated during this period were genetically similar to those isolated during 2008 and 2009 (Figure 1). Data are not available on the antigenic properties of the recent poultry viruses, but the human isolates characterized remain antigenically similar to the clade 2.2.1 reference vaccine virus A/Egypt/321/2007 (Table 2) as measured by haemagglutination inhibition (HI).

Clade 2.3.2 viruses were detected in wild birds in China Hong Kong Special Administrative Region (Hong Kong SAR) and the Russian Federation and in poultry in Viet Nam and Nepal. These viruses were genetically similar to clade 2.3.2 viruses isolated in previous years (Figure 1). Antigenically the virus from Hong Kong SAR reacted to antiserum to the clade 2.3.2 reference vaccine virus A/Common Magpie/Hong Kong/5052/2007, albeit with reduced HI titres as compared to the homologous antigen (data not shown).

Clade 2.3.4 viruses were detected in poultry in Viet Nam. These viruses formed two distinct genetic subclades within the 2.3.4 clade (Figure 1). Viruses from one of these subclades reacted well to postinfection ferret antiserum raised against the reference vaccine virus A/Anhui/1/2005 (data not shown); antigenic data are not available for the other.

A(H5N1) candidate vaccine viruses

Based on the available antigenic and epidemiologic data, no new candidate A(H5N1) vaccine viruses are proposed at this time. The available candidate A(H5N1) 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 trial and subsequent stockpiling of vaccines.

Additional A(H5N1) candidate vaccine viruses may be developed 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 at WHO web site http://www.who.int/csr/disease/avian_influenza/guidelinestopics/en/index5.html

Table 1. Influenza A(H5N1) activity reported from September 2009 to 17 February 2010

Country, area or territory	Host	Genetic clade
Bangladesh	Domestic poultry	Unknown
Cambodia	Human (1)*	Unknown
	Domestic poultry	1
China, Hong Kong SAR	Wild birds	2.3.2
Egypt	Humans (14)	2.2.1
	Domestic poultry	2.2.1
India	Domestic poultry	Unknown
Indonesia	Humans (22)#	Unknown
	Domestic poultry	Unknown
Israel	Domestic poultry	Unknown
Myanmar	Domestic poultry	Unknown
Nepal	Domestic poultry	2.2 and 2.3.2
Russian Federation	Wild birds	2.3.2
Viet Nam	Humans (1)	Unknown
	Domestic poultry	2.3.2 and 2.3.4

*number in parentheses denotes number of confirmed cases during this period
represents total cases for 2009 and 2010, no temporal information available

Table 2. : A(H5N1) virus antigenic characterization as measured by haemagglutination inhibition

	Ferret antisera							
	VN/ 1203	ws/MG	bhg/QI	tk/TK/1	EG/321	EG/394	EG/ 215	EG/ 3300
Clade	**1**	**2.2**	**2.2**	**2.2.1**	**2.2.1**	**2.2.1**	**2.2.1**	**2.2.1**
Reference antigens								
A/Viet Nam/1203/2004	**320**	5	160	80	80	20	10	10
A/w swan/Mongolia/244/2005	5	**1280**	320	640	320	160	80	5
A/bh goose/Qinghai/1A/2005	5	40	**160**	80	10	10	10	5
A/turkey/Turkey/1/2005	5	1280	640	**1280**	640	320	160	5
A/Egypt/321-NAMRU3/2007	10	320	160	320	**320**	80	80	5
A/Egypt/394-NAMRU3/2007	5	640	320	1280	640	**640**	160	5
A/Egypt/0215-NAMRU3/2007	5	640	320	640	320	40	**160**	5
A/Egypt/3300-NAMRU3/2008	5	10	80	40	5	20	20	**40**
Test antigens								
A/Egypt/4526-NAMRU3/2009	5	320	80	320	80	80	10	5
A/Egypt/4979-NAMRU3/2009	5	640	160	640	160	160	20	5
A/Egypt/4822-NAMRU3/2009	5	320	80	10	80	10	10	5
A/Egypt/9539-NAMRU3/2009	5	160	80	320	160	160	40	5
A/Egypt/4395-NAMRU3/2009	5	40	80	160	80	160	20	5
A/Egypt/2039-NAMRU3/2009	5	160	160	640	160	320	80	5
A/Egypt/2752-NAMRU3/2009	5	80	80	320	80	320	20	5
A/Egypt/9538-NAMRU3/2009	5	20	40	160	160	160	5	5
A/Egypt/2563-NAMRU3/2009	5	80	160	320	80	160	20	10
A/Egypt/3228-NAMRU3/2009	5	40	80	160	80	160	20	5
A/Egypt/4396-NAMRU3/2009	5	320	160	640	320	640	80	5
A/Egypt/4394-NAMRU3/2009	5	80	80	320	160	320	20	5
A/Egypt/3450-NAMRU3/2009	5	20	80	320	160	320	20	5
A/Egypt/1310-NAMRU3/2009	5	10	80	320	80	160	10	5

Table 3. Status of A(H5N1) vaccine virus development (February 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/NIAID	Yes
A/Viet Nam/1194/2004	1	NIBSC	Yes
A/duck/Hunan/795/2002	2.1	SJ/HKU/NIAID	Yes
A/Indonesia/5/2005	2.1	CDC	Requires Indonesian Government permission
A/bar-headed goose/Qinghai/1A/2005	2.2	SJ/HKU/NIAID	Yes
A/whooper swan/Mongolia/244/2005	2.2	SJ/NIAID	Yes
A/Egypt/2321/2007	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/NIAID	Yes
A/goose/Guiyang/337/2006	4	SJ/HKU/NIAID	Yes
A/chicken/Viet Nam/NCVD-016/2008	7	CDC	Yes
Reassortants prepared and awaiting regulatory approval			
Virus	**Clade**	**Institution***	**Availability**
A/chicken/India/NIV33487/2006	2.2	CDC/NIV	Pending
A/Egypt/3300-NAMRU3/2008	2.2.1	CDC	Pending
A/common magpie/Hong Kong/5052/2007	2.3.2	SJ/HKU/NIAID	Pending
A/chicken/Hong Kong/AP156/2008-like	2.3.4	SJ/HKU/NIAID	Pending
Viruses proposed by WHO for candidate vaccine preparation			
Virus	**Clade**	**Institution***	
A/chicken/Viet Nam/NCDV-03/2008-like	7	CDC	

* CDC- Centers for Disease Control and Prevention, USA
FDA- Food and Drug Administration, USA
NIAID- National Institute of Allergy and Infectious Disease, NIH, 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

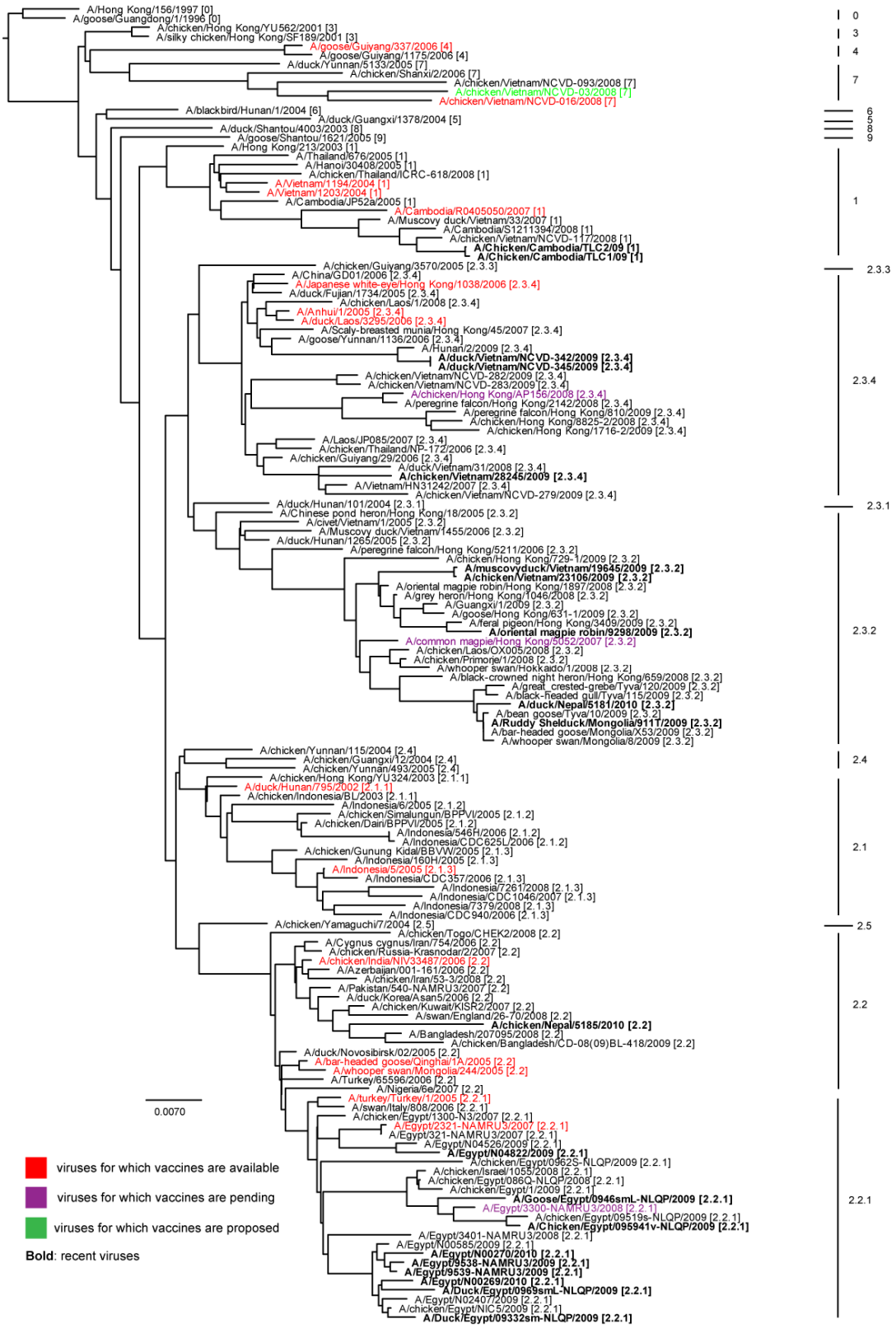

Figure 1. Phylogenetic relationships of A(H5N1) virus HA genes showing availability of vaccine viruses. We gratefully acknowledge the contributions of the originating laboratories and countries that have provided samples and/or submitted sequence data to DDBJ, EMBL-Bank, GenBank, GISAID and other public databases. Sequence has also been provided by the Veterinary Laboratories Agency, Weybridge on behalf of the OFFLU network, the National Laboratory for Veterinary Quality Control on Poultry Production (NLQP), Egypt, and the Pasteur Institute, Cambodia. Recent viruses (where date of isolation known) are shown in bold.

Influenza A(H9N2)

Influenza A(H9N2) viruses are endemic in poultry populations in parts of Asia and the Middle East. These viruses fall within a number of genetically defined HA lineages with the majority of viruses belonging to the G1 and Y280 clades (Figure 2). 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.[1]

This summary provides an update on the characterization of A(H9N2) viruses isolated from humans, and the current status of the development of candidate A(H9N2) vaccine viruses.

Human influenza A(H9N2) infection from September 2009 to 17 February 2010

Two unrelated human infections with A(H9N2) viruses have been reported in 2009 by Hong Kong SAR, one in October in an immunocompromised individual, aged 47, and another in December in a 35-month old child. Both reported recent travel history to mainland China. These individuals presented with mild disease and both recovered.

Antigenic and genetic characteristics.

Genetically the 2009 human isolates belong to the G1 lineage of A(H9N2) viruses and are closely related to each other (Figure 2). A/Hong Kong/33982/2009 was antigenically characterized and this virus reacted with postinfection ferret antiserum to A/quail/Hong Kong/G1/97 although it had reduced reactivity to postinfection ferret antiserum to the available A(H9N2) G1 lineage candidate vaccine virus A/Hong Kong/1073/99 (Table 4).

A(H9N2) candidate vaccine viruses

The currently available candidate A(H9N2) vaccine viruses are listed in Table 5. Based on the available antigenic and epidemiologic data, the development of an A/Hong Kong/33982/2009-like vaccine virus is proposed. On the basis of the geographical spread, epidemiology, and antigenic and genetic properties of the A(H9N2) viruses, national authorities may recommend the use of one or more of these for pilot lot vaccine production, clinical trial and subsequent stockpiling of vaccines.

Additional A(H9N2) candidate vaccine viruses may be developed 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 at WHO web site http://www.who.int/csr/disease/avian_influenza/guidelinestopics/en/index5.html

[1] OIE does not require notification of poultry infections with influenza A(H9N2) viruses.

Table 4. Human A(H9N2) virus antigenic characterization as measured by haemagglutination inhibition

			Ferret antisera	
	quail/HK/G1/97	HK/1073/99	ck/HK/G9/97	sw/HK/9/98
Lineage	G1	G1	Y280	Y280
Reference antigens				
A/quail/Hong Kong/G1/97	**320**	80	<40	<40
A/Hong Kong/1073/99	160	**1280**	<40	<40
A/chicken/Hong Kong/G9/97	<40	40	**2560**	1280
A/swine/Hong Kong/9/98	<40	<40	320	**640**
Test antigens				
A/Hong Kong/3239/2008	<40	40	1280	320
A/Hong Kong/33982/2009	80	80	<40	<40

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

Available Vaccine Viruses	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	NIBSC	Yes
A/chicken/Hong Kong/G9/97 (IBCDC-2)	Conventional reassortant	Y280	CDC	Yes

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

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

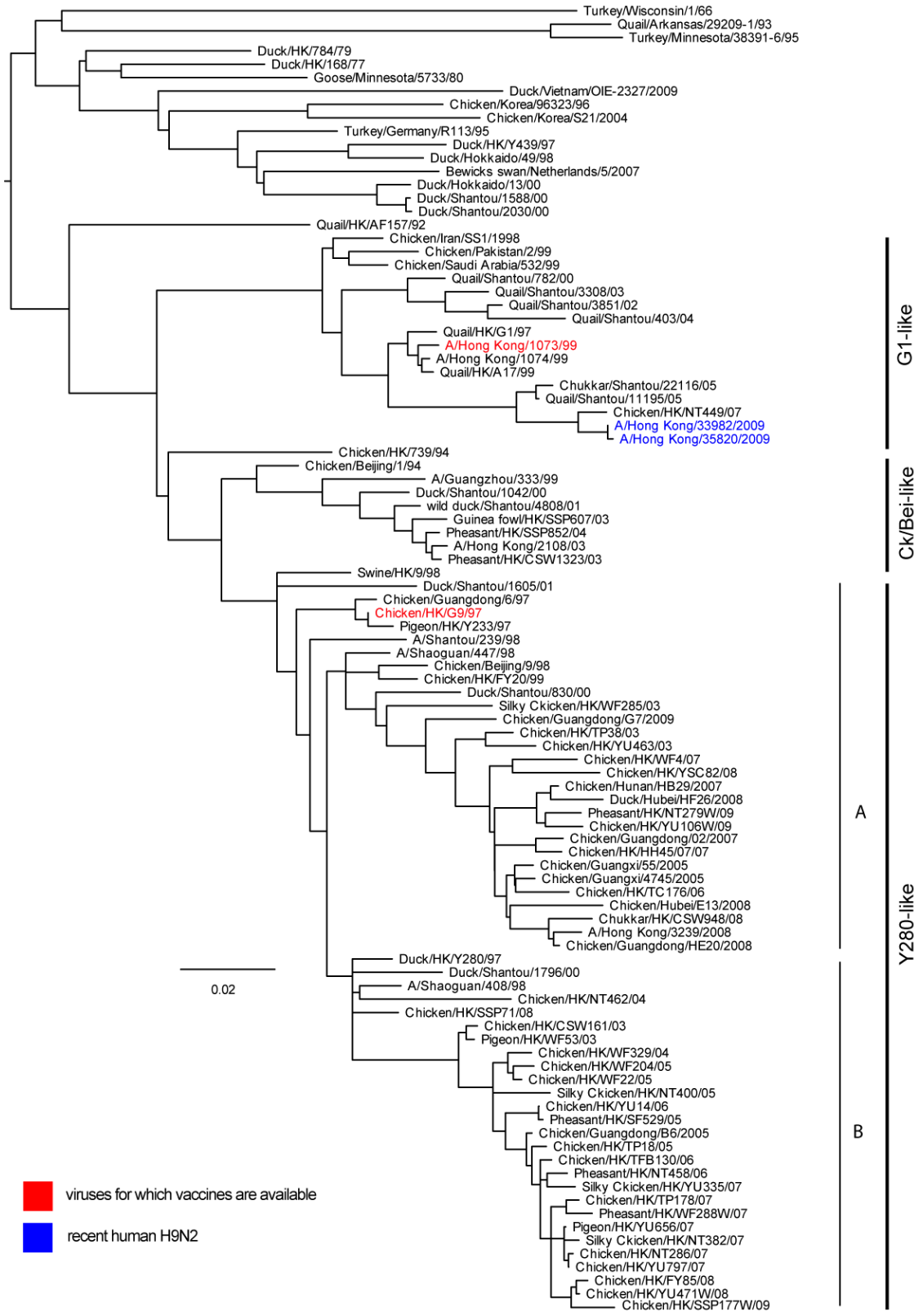

Figure 2. Phylogenetic relationships of A(H9N2) virus HA genes showing availability of vaccine viruses. We gratefully acknowledge the contributions of the originating laboratories and countries that have provided samples and/or submitted sequence data to DDBJ, EMBL-Bank, GenBank, GISAID and other public databases.