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

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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 reported periodically by WHO.

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 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 20 September 2011 to 21 February 2012

A(H5N1) viruses have been detected in birds in Africa, Asia, and the Middle East. Human infections have been reported to the WHO from Cambodia, China, Egypt, Indonesia and Viet Nam, 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 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. The updated nomenclature report can be found at http://www.who.int/influenza/gisrs_laboratory/h5n1_nomenclature/en/.

Viruses circulating and characterized from 20 September 2011 to 21 February 2012 belonged to the following clades.

Clade 1.1 viruses were detected in poultry, wild birds and a human in Cambodia, and in poultry and two humans in Viet Nam. Genetic characterization of the HA genes of the human viruses showed that they were closely related to clade 1.1 viruses that circulate in poultry in these countries (Figure 1). The recent human and avian viruses reacted well with post-infection ferret antisera against the clade 1 viruses A/Viet Nam/1203/2004 and/or A/Viet Nam/1194/2004, viruses from which candidate vaccine viruses have been developed (Table 2).

Clade 2.1.3.2 viruses were detected in three human cases in Indonesia. Genetic characterization of the HA genes of these viruses showed that they were similar to previously reported clade 2.1.3.2 viruses isolated from birds and humans (Figure 2). Two of the human viruses were tested in HI assays and reacted well with post-infection ferret antiserum against the clade 2.1.3.2 vaccine candidate virus

produced from A/Indonesia/5/2005 (Table 3). No virologic information is yet available for the other most recent human cases in Indonesia. Clade 2.1.3.1 and 2.1.3.2 viruses were isolated from poultry in Indonesia during 2011.

Clade 2.2.1 viruses continue to circulate in commercial and backyard poultry in Egypt with sporadic transmission to humans. Viruses detected during the period were genetically similar to those isolated previously from poultry and humans (Figure 3). Recent human viruses available for testing reacted with post-infection ferret antiserum against A/Egypt/N03072/2010 (albeit with reduced titres), a virus from which a candidate vaccine virus has been developed.

Clade 2.2.1.1 viruses previously circulated in the commercial poultry sector in Egypt but were not identified in the reporting period.

Clade 2.2.2 viruses were detected in poultry in Bangladesh. Genetically these viruses were similar to viruses detected in this region in previous years. Post-infection ferret antiserum against the clade 2.2 virus A/bar-headed goose/Qinghai Lake/1A/2005, a virus from which a candidate vaccine virus has been developed, reacted well with the recent clade 2.2.2 viruses.

Clade 2.3.2.1 viruses were detected in wild birds in Bangladesh and China Hong Kong Special Administrative Region (China Hong Kong SAR), in poultry in Bangladesh, China, China Hong Kong SAR, the Islamic Republic of Iran, Nepal and Viet Nam, and in a human in China. Those clade 2.3.2.1 viruses belonging to the A/barn swallow/Hong Kong/D10-1161/2010-like genetic group, which included the human virus from China, reacted well with post-infection ferret antiserum to the candidate vaccine virus A/barn swallow/Hong Kong/D10-1161/2010 (Table 4a) and were genetically similar to each other (Figure 4). Some recent clade 2.3.2.1 avian viruses from Bangladesh and Viet Nam belonging to the A/Hubei/1/2010-like genetic group showed reduced reactivity with post-infection ferret antiserum against A/Hubei/1/2010, a virus from which a candidate vaccine virus has been developed. They retained good reactivity with post-infection ferret antiserum against A/common magpie/Hong Kong/5052/2007, a virus from which a candidate vaccine virus has been developed (Table 4b). Increased genetic heterogeneity in HA gene sequence was observed within the A/Hubei/1/2010-like group (Figure 4).

A *Clade 2.3.4.2* virus was isolated from a human in China. Although this virus was genetically distinct from viruses characterised previously (Figure 5), it reacted well with post-infection ferret antiserum against A/Anhui/1/2005, a virus from which a candidate vaccine virus has been developed.

Influenza A(H5N1) candidate vaccine viruses

Based on the current antigenic, genetic and epidemiological data, no new candidate vaccine viruses are proposed. The available and proposed A(H5N1) candidate 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[1].

[1] http://www.who.int/influenza/vaccines/virus/candidates_reagents/a_h5n1/en/

Table 1. Influenza A(H5N1) activity reported from 20 September 2011 to 22 February 2012

Country, area or territory	Host	Genetic clade
Bangladesh	Poultry	2.2.2/2.3.2.1
	Wild birds	2.3.2.1
Bhutan	Poultry	unknown
Cambodia	Poultry	1.1
	Wild birds	1.1
	Human (1)*	1.1
China	Poultry	2.3.2.1
	Human (2)	2.3.2.1/2.3.4.2
China, Hong Kong SAR	Poultry	2.3.2.1
	Wild birds	2.3.2.1
Egypt	Poultry	2.2.1/2.2.1.1
	Humans (9)	2.2.1 [5]#
India	Poultry	unknown
	Wild bird	unknown
Indonesia	Poultry	unknown
	Humans (7)	2.1.3.2 [2]
Iran (Islamic Republic of)	Poultry	2.3.2.1
Nepal	Poultry	2.3.2.1
	Wild birds	2.3.2.1
Viet Nam	Poultry	1.1/2.3.2.1
	Humans (2)	1.1 [2]

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

#number in brackets denotes the number of viruses for which genetic information is available

Table 2. Antigenic properties of recent influenza clade 1.1 A(H5N1) viruses

		Reference ferret antisera			
		Vn/1203/04	Vn/1194/04	Ck/Vn /NCVD-775/11	Egypt/ 3300/08
REFERENCE VIRUSES	**Clade**				
A/VIETNAM/1203/2004	1	**320**	160	160	5
A/VIETNAM/1194/2004	1	160	**80**	160	5
A/CHICKEN/VIETNAM/NCVD-775/2011	1.1	160	80	**80**	5
A/EGYPT/3300-NAMRU3/2008	2.2.1.1	20	5	5	**5120**
TEST VIRUSES					
A/CHICKEN/VIETNAM/NCVD-359/2009	1.1	320	40	160	5
A/DUCK/VIETNAM/NCVD-363/2009	1.1	320	80	160	5
A/CHICKEN/VIETNAM/NCVD-425/2009	1.1	160	80	160	5
A/DUCK/VIETNAM/NCVD-827/2011	1.1	80	40	80	5
A/CHICKEN/VIETNAM/NCVD-876/2011	1.1	160	80	160	5
A/CAMBODIA/V0401301/2011	1.1	640	1280	640	5
A/VIETNAM/VP12-3/2012	1.1	80	20	160	5

Table 3. Antigenic properties of recent influenza clade 2.1.3.2 A(H5N1) viruses

		Reference ferret antisera			
		Indo/5/05	Indo/5/05 RG2	Anhui/05	Indo/NIHRD 11771/11
REFERENCE VIRUSES	**Clade**				
A/INDONESIA/5/2005	2.1.3.2	**320**	320	40	160
A/ INDONESIA/5/2005 IBCDC-RG2	2.1.3.2	640	**640**	80	160
A/ANHUI/1/2005 IBCDC-RG5	2.3.4.2	320	160	**640**	320
TEST VIRUSES					
A/ INDONESIA/NIHRD11771/2011	2.1.3.2	160	80	10	**1280**
A/ INDONESIA/NIHRD11767/2011	2.1.3.2	160	80	20	640

Table 4a. Antigenic properties of recent influenza clade 2.3.2.1 A(H5N1) viruses

		Reference ferret antisera				
		BS/HK/ 1161/10	Ck/HK/ 729.1/09	Ck/HK/ 5572/10	Ck/HK/ 6841/10	MD/ Vm/1/09
REFERENCE VIRUSES	**Clade**					
A/BARN SWALLOW/HONGKONG/1161/2010	2.3.2.1	**160**	40	80	320	<40
A/CHICKEN HONG KONG/729.1/2009	2.3.2.1	640	**1280**	160	1280	80
A/ CHICKEN HONG KONG /5572/2010	2.3.2.1	40	<40	**640**	320	<40
A/ CHICKEN HONG KONG /6841/2010	2.3.2.1	160	40	160	**640**	<40
A/MUSCOVY DUCK/VIETNAM/1/2009	2.3.4.3	<40	<40	<40	<40	**640**
TEST VIRUSES						
A/GUANGDONG/1/2011	2.3.2.1	40	160	160	160	<40
A/BLACK HEADED GULL/HONG KONG/44/2012	2.3.2.1	40	80	80	80	<40
A/ BLACK HEADED GULL/HONG KONG /6671/2011	2.3.2.1	40	160	80	160	<40
A/LITTLE EGRET/HONG KONG/1237/2012	2.3.2.1	40	80	80	80	<40
A/ CHICKEN HONG KONG /7035.1/2011	2.3.2.1	40	<40	160	160	<40
A/GOOSE/HONG KONG/1771/2012	2.3.2.1	80	<40	80	320	<40

Table 4b. Antigenic properties of recent influenza clade 2.3.2.1 A(H5N1) viruses

		Reference ferret antisera					
		cm/HK/ 5052/07	HK/ 6841/10	Hubei/10	cr/Bang/ 1061/11	ck/Vn/ 700/11	Anhui/05
REFERENCE VIRUSES	**Clade**						
A/COMMON MAGPIE/HONG KONG/5052/2007	2.3.2.1	**320**	320	40	160	40	5
A/HONG KONG/6841/2010	2.3.2.1	320	**160**	80	160	40	5
A/HUBEI/1/2010 PR8 IDCDC RG-30	2.3.2.1	320	320	**320**	160	40	5
A/CROW/BANGLADESH/1061/2011	2.3.2.1	20	80	40	**80**	40	5
A/CHICKEN/VIETNAM/NCVD-700/2011	2.3.2.1	40	160	80	80	**160**	5
A/ANHUI/1/2005	2.3.4	40	20	20	5	80	**320**
TEST VIRUSES							
A/DUCK/VIETNAM/NCVD-760/2011	2.3.2.1	20	80	80	80	160	5
A/DUCK/VIETNAM/NCVD-815/2010	2.3.2.1	320	160	40	80	40	5
A/CHICKEN/BANGLADESH/4070T/2011	2.3.2.1	160	320	80	160	40	5
A/CHICKEN/BANGLADESH/11303/2011	2.3.2.1	320	320	80	160	160	5
A/DUCK/BANGLADESH/4120T/2011	2.3.2.1	320	320	80	320	40	5
A/DUCK/BANGLADESH/4124T/2011	2.3.2.1	160	320	40	80	40	5
A/WILD BIRD/BANGLADESH/315T/2011	2.3.2.1	160	320	40	160	40	5
A/CROW/BANGLADESH/1058/2011	2.3.2.1	160	160	80	160	40	5

Table 5. Status of influenza A(H5N1) candidate vaccine virus development (February 2012)

Candidate vaccine viruses			
Virus	**Clade**	**Institution***	**Available**
A/Cambodia/R0405050/2007	1.1	NIBSC	Yes
A/Viet Nam/1203/2004	1	CDC and SJCRH	Yes
A/Viet Nam/1194/2004	1	NIBSC	Yes
A/duck/Hunan/795/2002	2.1	SJCRH	Yes
A/Indonesia/5/2005	2.1.3.2	CDC	Yes
A/bar-headed goose/Qinghai/1A/2005	2.2	SJCRH	Yes
A/chicken/India/NIV33487/2006	2.2	CDC/NIV	Yes
A/whooper swan/Mongolia/244/2005	2.2	SJCRH	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	SJCRH	Yes
A/Hubei/1/2010	2.3.2.1	CDC	Yes
A/barn swallow/Hong Kong/D10-1161/2010	2.3.2.1	SJCRH	Pending
A/chicken/Hong Kong/AP156/2008	2.3.4	SJCRH	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	SJCRH	Yes
A/goose/Guiyang/337/2006	4	SJCRH	Yes
A/chicken/Viet Nam/NCVD-016/2008	7.1	CDC	Yes
A/chicken/Viet Nam/NCDV-03/2008	7.1	CDC	Pending
Candidate vaccine viruses in preparation			
Virus	**Clade**	**Institution**	**Availability**
A/chicken/Bangladesh/11rs1984-30/2011-like	2.3.4.2	CDC	Pending

* **Institutions distributing the candidate vaccine virus:**

CDC - Centers for Disease Control and Prevention, United States of America
CDC/NIV - Centers for Disease Control and Prevention, United States of America/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
SJCRH - St Jude Children's Research Hospital, United States of America

Influenza A(H9N2)

Influenza A(H9N2) viruses are enzootic in poultry populations in parts of Asia and the Middle East. The majority of viruses that have been sequenced belong either 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.

A(H9N2) activity from 20 September 2011 to 21 February 2012

No human cases of A(H9N2) infections have been reported in the period. A(H9N2) viruses of the G1 clade have been reported in poultry in Egypt for the first time. Viruses from poultry in the Middle East and Bangladesh also belonged to this clade. In China and Viet Nam the majority of the A(H9N2) viruses detected in poultry belonged to the Y280 and Korean clades. Y280-like and G1-like viruses circulate in poultry in China Hong Kong SAR.

As the viruses continue to evolve, new A(H9N2) candidate vaccine viruses will be developed (Table 6) 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].

Table 6. Status of influenza A(H9N2) candidate vaccine virus development (February 2012)

Candidate vaccine viruses				
Virus	**Type**	**Clade**	**Institution***	**Available**
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
A/Hong Kong/33982/2009 (IBCDC-RG26)	Reverse genetics	G1	CDC	Yes

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

Influenza A(H3N2) variant (v)[3]

Eight human infections of A(H3N2)v in four states of the United States of America were detected in the period. Genetically these eight viruses were similar to viruses that circulate in swine in the United States and also to the previously reported 2011 A(H3N2)v viruses[4]. These viruses reacted well with post-infection ferret anti-sera to A/Minnesota/11/2010 and A/Indiana/10/2011, viruses from which candidate vaccine viruses (NYMC X-203 and NYMC X-213, respectively) have been developed. Available candidate vaccine viruses are shown in Table 7.

[2] http://www.who.int/influenza/vaccines/virus/candidates_reagents/a_h9n2/en/
[3] http://www.who.int/influenza/gisrs_laboratory/terminology_ah3n2v/en/
[4] http://www.cdc.gov/flu/swineflu/variant.htm

Influenza A(H1N1)v and A(H1N2)v

Single human infections with A(H1N2)v and A(H1N1)v viruses have been detected in the United States in the reporting period[5]. The A(H1N2)v virus is similar to viruses known to circulate in swine in the United States and has HA, NA and PA genes of human virus origin. The A(H1N1)v virus is a reassortant between endemic North American swine viruses and A(H1N1)pdm09 viruses which are also known to circulate in swine in this region; its HA and NA genes are derived from classical A(H1N1) swine viruses. Candidate vaccine viruses are not proposed at this time based on a risk assessment of the antigenic and genetic characteristics of these A(H1N1)v and A(H1N2)v viruses.

Table 7. Status of influenza A(H3N2)v candidate vaccine virus development (February 2012)

Candidate vaccine viruses			
Virus	**Type**	**Institution***	**Available**
A/Minnesota/11/2010 (NYMC X-203)	Conventional reassortant	CDC	Yes
A/Indiana/10/2011 (NYMC X-213)	Conventional reassortant	CDC	Yes

* **Institutions distributing the candidate vaccine virus:**
CDC - Centers for Disease Control and Prevention, United States of America

[5] http://www.cdc.gov/mmwr/

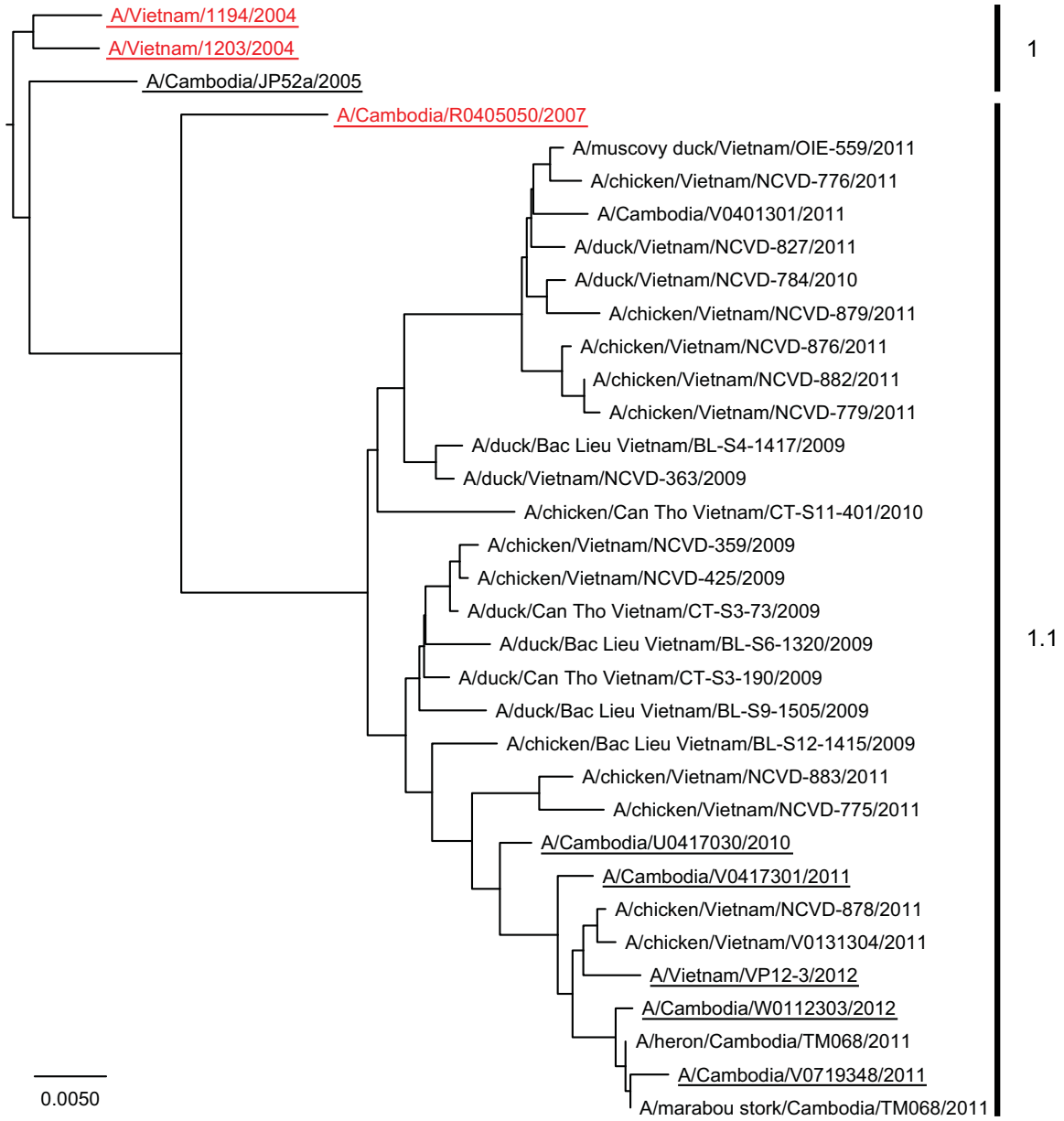

Figure 1. Phylogenetic relationships of A(H5N1) clade 1 and 1.1 virus HA genes showing available (in red) vaccine viruses. Human viruses are underlined. We gratefully acknowledge the contribution of the originating laboratories and countries that have provided samples and/or submitted sequence data to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

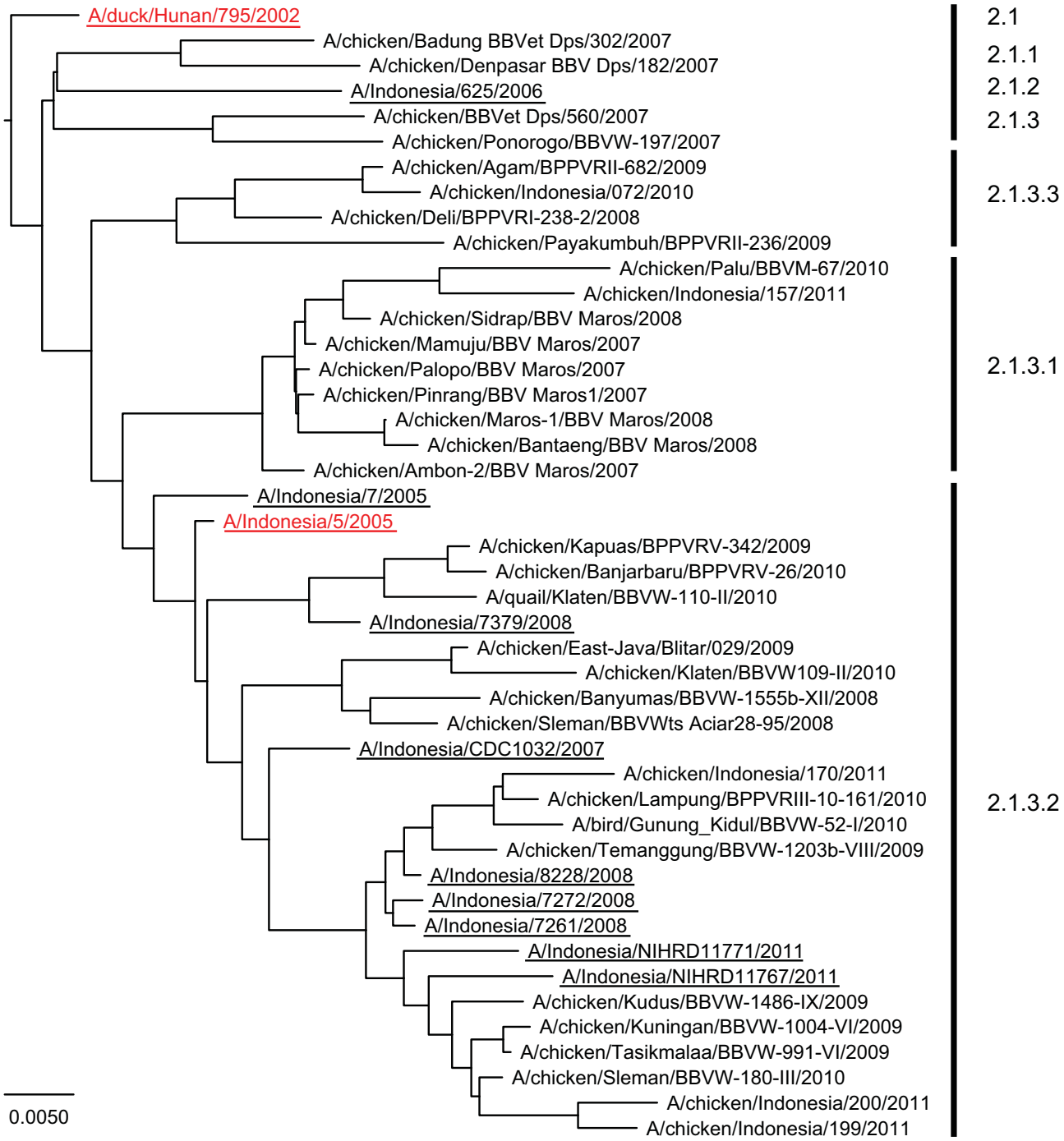

Figure 2. Phylogenetic relationships of A(H5N1) clade 2.1 virus HA genes. Details are as in the legend to figure 1.

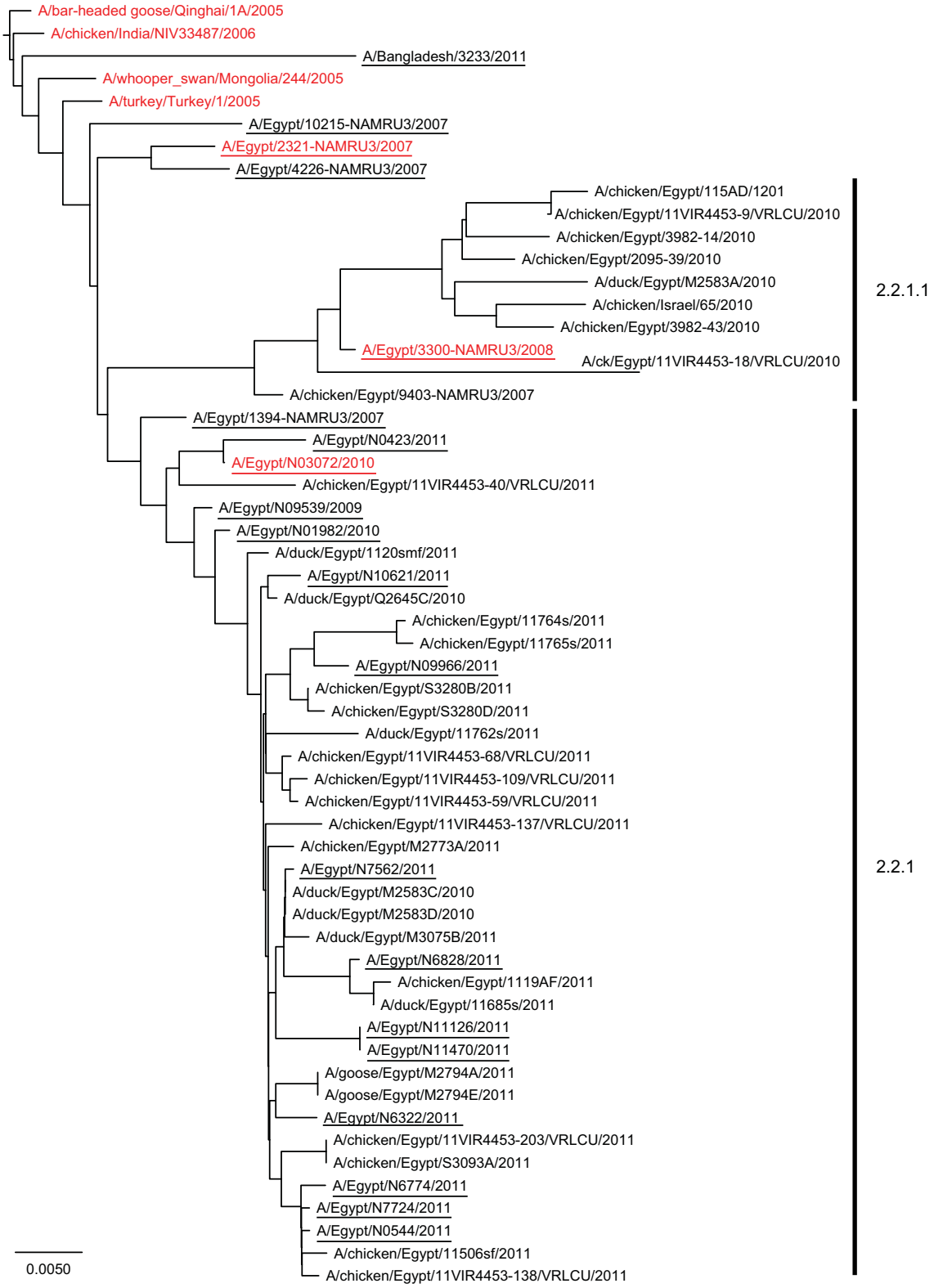

Figure 3. Phylogenetic relationships of A(H5N1) clade 2.2.1 virus HA genes. Details are as in the legend to figure 1.

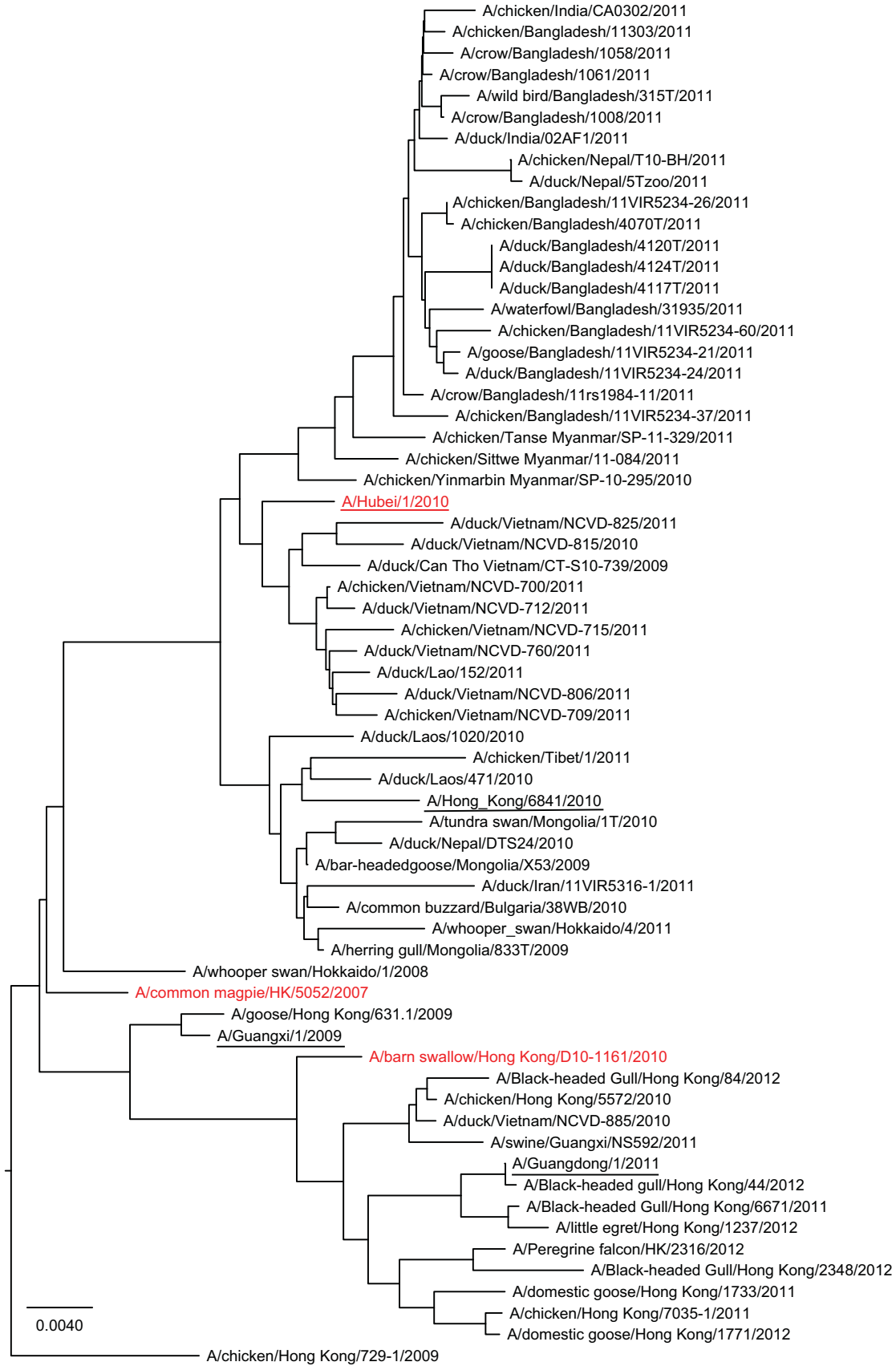

Figure 4. Phylogenetic relationships of A(H5N1) clade 2.3.2.1 virus HA genes. Details are as in the legend to figure 1.

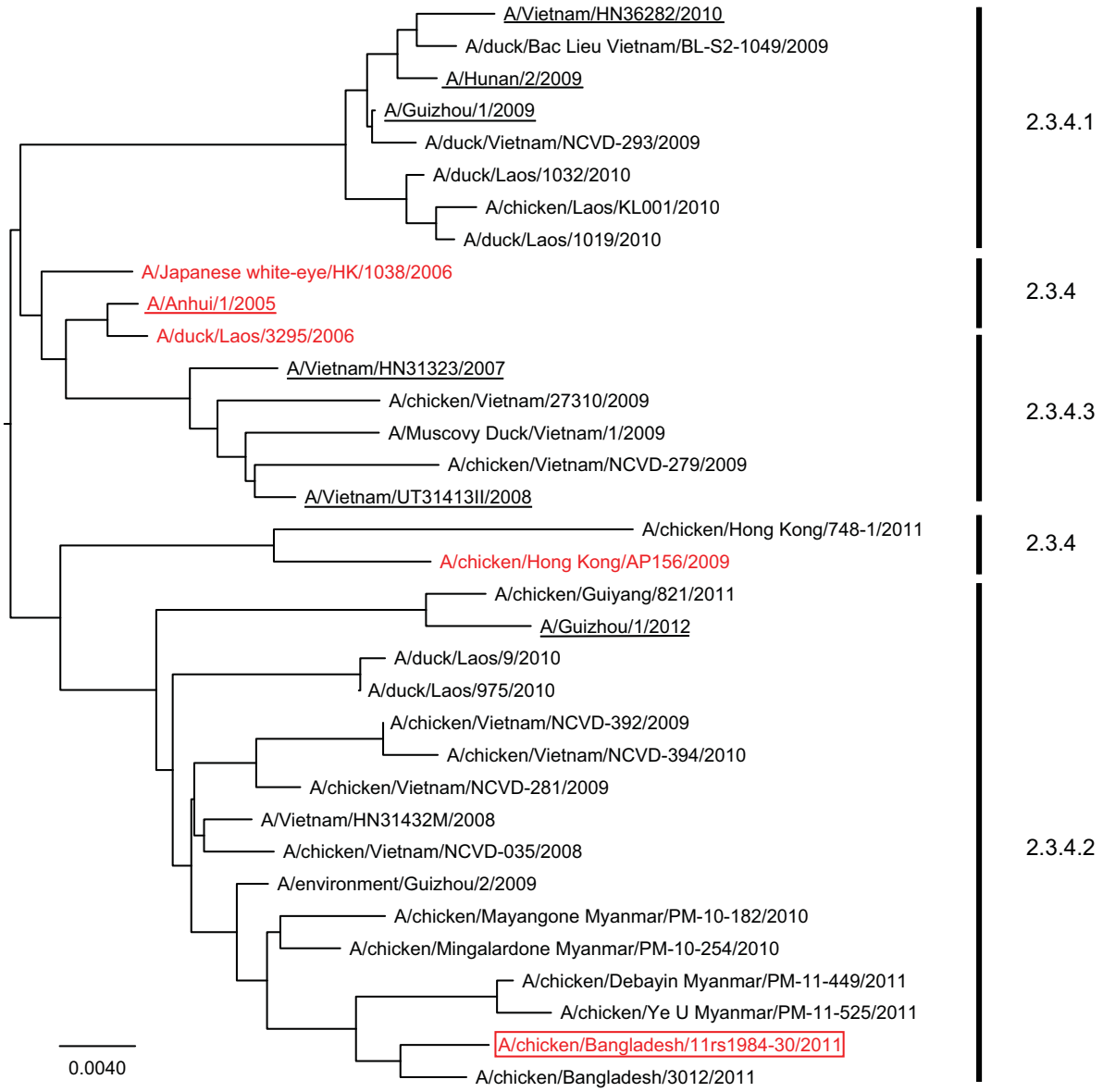

Figure 5. Phylogenetic relationships of A(H5N1) clade 2.3.4 virus HA genes. Details are as in the legend to figure 1. A vaccine virus pending final development is shown boxed in red.