Antigenic and genetic characteristics of H5N1 viruses and candidate H5N1 vaccine viruses developed for potential use as pre-pandemic vaccines

The development of representative pre-pandemic H5N1 candidate vaccine viruses by the WHO Global Influenza Programme[1] is being conducted as one step in an overall strategy for pandemic preparedness. This summary presents the current status of the development of new candidate H5N1 vaccine viruses and is intended to provide guidance for national authorities on the production of pre-pandemic vaccine.

The H5N1 viruses chosen for development of pre-pandemic candidate vaccine viruses are representative of antigenically and genetically distinct groups of viruses that have infected humans primarily through contact with ill or dead H5N1-infected birds. These representative candidate H5N1 vaccine viruses have been prepared by reverse genetics and safety tested prior to release for production of pilot vaccine lots that may be used for experimental studies and for stockpiling by governments in advance of a possible H5N1 pandemic, should such a national policy exist. Companies are recommended to consult individual national authorities on the H5N1 strains to be used. Decisions should be based on the epidemiology of the circulating H5N1 viruses that are described below.

Comparison of the previously developed (clade 1 rg A/Vietnam/1194/2004 and rg A/Vietnam 1203/2004)[2] and new candidate H5N1 vaccine viruses and studies of cross-reactivity of these pre-pandemic vaccine viruses and their relationship to newly emerging H5N1 viruses are ongoing, and will be reported periodically by WHO.

Genetic characteristics of recent H5N1 viruses

The haemagglutinin (HA) sequences of the majority of H5N1 viruses circulating in avian species during the past 3 years separated into two distinct phylogenetic clades (genetic groups).[3] Clade 1 viruses circulating in Cambodia, Thailand and Viet Nam were responsible for human infections in those countries during 2004 and 2005. Clade 2 viruses circulated in birds in China and Indonesia during 2003–2004 and subsequently during 2005–2006 spread westwards to the Middle East, Europe and Africa. This latter genetic group of viruses has been principally responsible for human infections during the later part of 2005 and 2006. Six sub-clades of clade 2 have been distinguished, three of which (subclades 1, 2 and 3) also differ in geographical distribution and have been largely responsible for human cases in Indonesia, in countries in the Middle East, Europe and Africa, and in China, respectively (*Fig. 1*).

Antigenic characteristics of recent H5N1 viruses

The antigenic relationships between the HAs of human isolates representative of clade 1 and three subclades of clade 2 were compared by haemagglutination inhibition (HI)

[1] See http://www.who.int/csr/disease/influenza/mission/en/

[2] See http://www.who.int/csr/disease/avian_influenza/guidelines/avian_influenza_prototype_strains/en/index.html

[3] See http://www.cdc.gov/ncidod/EID/vol11no10/05-0644.htm

tests using post-infection ferret antisera. Reciprocal cross-reactions in HI tests demonstrated antigenic similarity of HAs within the same genetic clade and distinguished representatives of different clades (*Table 1*), with the exception of viruses from the Karo cluster[4] represented by A/Indonesia/CDC625/2006. Viruses from this family cluster were antigenically distinguishable from the majority of human isolates represented by A/Indonesia/5/2005 and A/Indonesia/CDC357/2006 (subclade 1), and appeared antigenically more closely related to H5N1 viruses in subclade 2.

New candidate vaccine viruses

Viruses representative of subclade 1 (A/Indonesia/5/2005) and subclade 2 (A/Bar headed goose/Qinghai/1A/2005, A/Whooper swan/Mongolia/244/2005 and A/turkey/Turkey/1/2005) were selected[5] for the preparation of reverse genetics modified reassortant vaccine viruses using the laboratory reference strain A/PR8/34 as donor of the polymerase, nucleoprotein, matrix and non-structural protein genes. HI analysis confirmed that the reassortant candidate vaccine viruses were antigenically similar to the parent viruses and the majority of recent isolates within the same clade. On the basis of more recent data, a subclade 3 vaccine virus is also being prepared from A/Anhui/1/2005.

Recommended use of candidate pre-pandemic H5N1 vaccine viruses

Pre-pandemic vaccines have been produced by manufacturers using clade 1 viruses (rg A/Vietnam/1194/2004 (NIBRG-14) and rg A/Vietman/1203/2004 (CDCRG-1 and SJRG-161052)). Clinical trials have been conducted or are under way in several countries and stockpiling of clade 1 vaccines has begun in some countries. Because it is not known if the next influenza pandemic will be caused by H5N1 viruses or which of the clades or subclades of H5N1 would be responsible, should one occur, clinical trials using clade 1 viruses should continue as an essential element in pandemic preparedness to maximize data available on priming, cross-reactivity and cross-protection by vaccine viruses from different clades and subclades.

On the basis of the geographical spread, the epidemiology, and the antigenic and genetic properties of the H5N1 viruses isolated from humans during the past 12 months, national authorities may recommend the use of one or more of the following H5N1 candidate vaccine viruses for pilot lot vaccine production and subsequent stockpiling of vaccines, should relevant national policies exist:

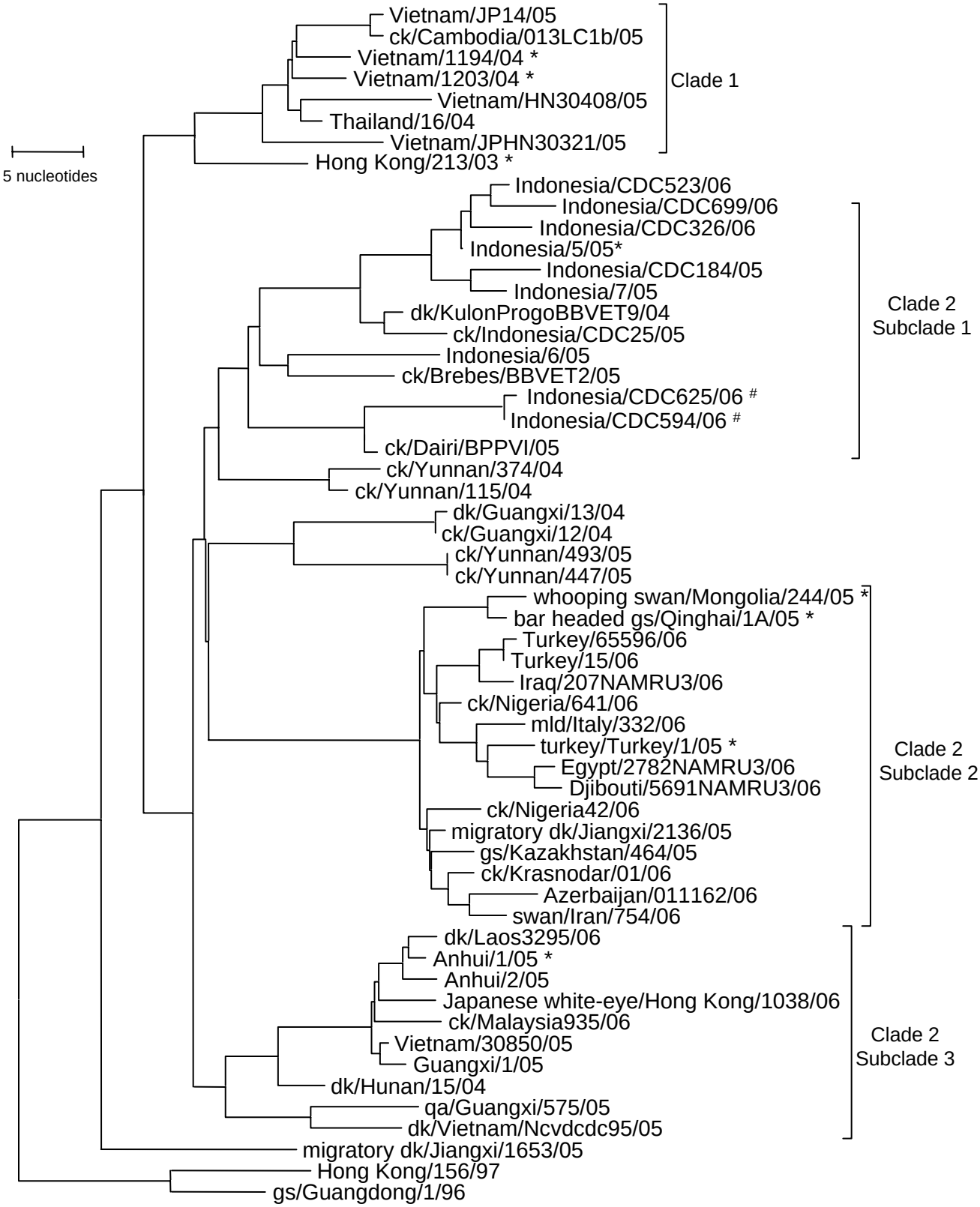
An A/Indonesia/5/2005-like virus
An A/Bar headed goose/Qinghai/1A/2005-like virus[6]
An A/Anhui/1/2005-like virus[7]

[4] See http://www.who.int/csr/don/2006_05_31/en/index.html
[5] See http://www.who.int/csr/disease/avian_influenza/guidelinestopics/en/index5.html
[6] Candidate vaccine viruses also include A/turkey/Turkey/1/05 and A/Whooper swan/Mongolia/244/2005.
[7] Candidate vaccine virus in preparation.

Additional pre-pandemic vaccine candidates will be developed as H5N1 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 these prototype strains should contact the WHO Global Influenza Programme at whoinfluenza@who.int or the institutions listed in the publications at the above web site.

Fig. 1 **Evolution of the H5N1 haemagglutinin gene**



* Candidate vaccine reference viruses

Karo family cluster

Table 1. HEMAGGLUTINATION INHIBITION REACTIONS OF INFLUENZA H5N1 VIRUSES

		REFERENCE FERRET ANTISERA												
	STRAIN DESIGNATION	VN/1194	VN/1203	TH/16	IND/5-R	IND/357	IND/625*	TK/15	WS/244-R	BHG/1A-R	TY/TK/1-R	DK/HU/15	ANH/1	GI/1
1	A/VIETNAM/1194/2004 [a]	**640**	160	nd	20	nd	nd	nd	20	20	<20	nd	nd	nd
2	A/VIETNAM/1203/2004 [a]	320	**160**	160	10	<10	80	<10	<10	40	<20	160	10	20
3	A/THAILAND/16/2004 [a]	nd	160	**160**	10	<10	40	<10	<10	40	nd	80	<10	10
5	A/INDONESIA/5/2005 CDC RG-2 [b]	80	<10	<10	**320**	320	160	40	20	80	40	40	40	20
6	A/INDONESIA/CDC357/2006 [b]	nd	40	20	320	**640**	80	40	40	80	nd	20	20	10
7	A/INDONESIA/CDC625/2006 [b] *	nd	40	10	80	40	**1280**	40	40	160	nd	40	40	20
8	A/TURKEY/15/2005 [c]	80	20	<10	40	40	1280	**640**	640	1280	320	20	40	40
9	A/W. SWAN/MG/244/2005 SJRG-163243 [c]	80	20	10	40	80	640	320	**320**	640	320	20	10	10
10	A/B-H GOOSE/QINGHAI/1A/2005 SJRG-163222 [c]	80	10	<10	40	80	320	80	1280	**320**	160	40	20	20
11	A/TURKEY/TURKEY/1/2005 NIBRG-23 [c]	80	<40	nd	80	nd	nd	nd	320	160	**320**	nd	nd	nd
12	A/DUCK/HUNANWG/15/2004 [d]	nd	80	80	20	20	20	<10	<10	20	nd	**160**	160	nd
13	A/ANHUI/1/2005 [d]	nd	40	20	<10	<10	20	10	10	40	nd	160	**640**	160
14	A/GUANGXI/1/2005 [d]	nd	10	20	20	10	10	<10	<10	40	nd	nd	320	**160**

[a] Clade 1

[b] Clade 2, Subclade 1

[c] Clade 2, Subclade 2

[d] Clade 2, Subclade 3

* Karo family cluster - father of 10 y.o. M