

The genetic match between vaccine strains and circulating seasonal influenza A viruses in Vietnam, 2001–2009

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Background Vietnam is currently developing domestic capability to manufacture influenza vaccines but information on the genetic and antigenic characteristics of locally circulating seasonal influenza viruses is limited. To assess the relevance of WHO recommended vaccine strains to the situation in Vietnam, we analyzed the genetic relatedness of the hemagglutinin (HA) gene of seasonal influenza A viruses circulating in Vietnam from 2001 to 2009 to WHO recommended vaccine strains over the same period.

Methods and Principal findings We sequenced the HA gene of 32 H1N1 and 44 H3N2 seasonal influenza A isolates from laboratory-based sentinel surveillance sites in Hanoi from 2001 to 2005 and from a national influenza surveillance system from 2005 to 2009. H1 and H3 HA phylogenetic trees rooted to vaccine strains A/Beijing/295/1995 (H1N1) and A/Moscow/10/1999 (H3N2), respectively, were constructed with contemporary HA sequences of isolates from neighboring countries. We found some genetic differences between seasonal influenza H3N2 viruses and three WHO influenza vaccine strains recommended for use in the

Northern and Southern Hemispheres for the 2001–2004 and 2007–2008 seasons and close genetic identity of circulating H3N2 strains with the recommended WHO Southern Hemisphere vaccine strains for 2004 and 2009 seasons. The genetic similarity of circulating H1N1 strains with the WHO recommended vaccine strains are described for the study period 2001–2009.

Conclusions The HA gene of seasonal influenza virus strains in Vietnam (especially influenza A/H3N2) showed varying degrees of genetic identity compared with those of the Northern or Southern Hemisphere vaccine strains recommended by WHO. The close relatedness of the HA of Vietnamese strains and contemporary strains from nearby countries indicate a good genetic match of circulating strains during study period. Greater representation of virus isolates from South East Asia in the vaccine strain selection process is desirable of influenza vaccine development in Vietnam.

Keywords Phylogeny, seasonal influenza A virus, vietnam, virologic surveillance, WHO influenza vaccine recommendation strains.

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Introduction

Influenza surveillance with genetic and antigenic characterization of virus isolates has become routine in many countries. Data and viruses submitted to the WHO's Global Influenza Surveillance and Response System (GISRS) from National Influenza Centers (NIC) provides an understanding of the global circulation of influenza viruses and is essential for vaccine strain selection. However, virus strains submitted to GISRS and strains selected for inclusion in Northern and Southern Hemisphere vaccines may not be globally representative. Although GISRS encourages

representative and timely sharing of viruses, specimens from tropical countries are under-represented. There is, therefore, a need to increase the number of viruses submitted for analysis from tropical areas to better assess the relevance of the WHO recommended vaccine strains in tropical countries that are considering the introduction of influenza vaccination.²

The first NIC of Vietnam was established in 2005, at the National Institute of Hygiene and Epidemiology (NIHE), Hanoi. From 2001 to 2005, NIHE conducted limited virologic surveillance in Hanoi to detect influenza infection by virus isolation from nasopharyngeal secretions from ILI

patients.^{1,6–9} The National Influenza Surveillance System (NISS) was established in 2005 and comprises 15 sentinel sites (seven sites located in the Northern Region and eight surveillance sites located in the Southern, Highland, and Central Regions of Vietnam), and four regional laboratories. In global temperate regions, there are two flu season annually, corresponding to the occurrence of winter in the Northern and Southern Hemispheres, and influenza vaccination is delivered annually to protect against the influenza strains emergent during each winter influenza season.^{2,7,8} Unlike those regions, the Hanoi-based surveillance from 2001 to 2006 and the first 5 years of national surveillance in Vietnam from 2006 to 2009 revealed two peaks of seasonal influenza transmission each year, as has been reported in neighboring tropical countries.^{1,3,4,6,7,10,11}

Vietnam is developing seasonal influenza vaccine manufacturing capacity, and the implementation of a rational influenza vaccination strategy requires information on the optimal timing and antigenic composition of the vaccine. Because data on the genetic and antigenic characteristics of locally circulating seasonal influenza viruses and the relevance of WHO recommended vaccine strains to the epidemiology of influenza in Vietnam are limited, we have analyzed the hemagglutinin gene of seasonal influenza A/H1N1 and A/H3N2 isolated in Vietnam from 2001 through 2009. The overall objective is to better understand the HA gene match between Vietnamese strains and WHO recommended vaccine strains and to inform the development of influenza vaccination policy in Vietnam.⁶

Materials and methods

Ethical approval

The study was conducted as a part of the Vietnam Ministry of Health approved influenza surveillance system using samples taken from patients presenting to health care facilities with an ILI. The National Institute of Hygiene and Epidemiology and CDC, Atlanta provided ethical committee approval for the study. All participants provided written informed consent.

Sample collection

Patients of any age presenting to one of the sentinel sites (central hospitals, district hospitals, and polyclinics) with an ILI (measured fever $\geq 38^{\circ}\text{C}$ and cough and/or sore throat) within 3 days of onset were eligible for inclusion.¹ Nasopharyngeal swabs (NPS) or throat swabs (TS) were collected by trained nurses using cotton swabs (Hanaco-medical, CO.LTD – Japan). Swabs were stored in in-house viral transport media (VTM).^{1,7} In the sentinel sites in Hanoi, nasopharyngeal swabs (NPS) were collected from outpatients and specimens were transferred to the respiratory virus laboratory of NIHE on the day of collection^{1,2}.

At the 15 sentinel sites of the NISS, throat swabs were collected from the first two ILI patients each weekday (10 swabs per week/per sentinel site).¹ The TS were refrigerated in in-house VTM and transferred on Friday or on Monday of the following week on wet ice to a participating reference laboratory. Participating laboratories included NIHE, the Pasteur Institute, Ho Chi Minh city (IP HoChi Minh); the Pasteur Institute Nha Trang (IPNha Trang); and Tay Nguyen Institute of Hygiene and Epidemiology (TIHE).¹

Viral isolation

Swabs that were positive for influenza A by RT-PCR were selected for viral isolation, with 100 μl of sample inoculated onto MDCK cells (Madin-Darby canine kidney cells – American Type Culture Collection-ATCC) according to NISS protocols.^{1,5,7} Viruses were harvested and stored in -80°C , and all isolates from regional laboratories were transferred to the NIC-NIHE for further analysis.^{1,6,12} All isolates were subtyped using the hemagglutination inhibition assay (HAI) with reference antigens and antisera from the World Health Organization (WHO) reagent kit.^{1,6}

Molecular characterization

Subtyped influenza A isolates with a minimum of 8 HA unit by HA assay were selected for genetic analysis by sequencing at NIC-NIHE, Hanoi.⁶ (Table 2)

RNA extraction and Polymerase Chain Reaction (PCR)

RNA extraction was conducted on 140 μl aliquot of each isolate using the Viral RNA extraction kit (Qiagen-US) according to the manufacturer's instructions. The RNA was transcribed to cDNA using the influenza A virus universal primer (Uni 12) AGC AAA AGC AGG as described.¹³ The hemagglutinin (HA) gene was amplified with segment-specific primers.^{6,7,13}

Nucleotide sequencing and phylogenetic analysis

The PCR products were purified with PCR purification kit (Qiagen-US), and sequencing reactions performed with a Big Dye Terminator v 3.1. Cycle sequencing kit (Applied Biosystem, Foster city, CA, USA) according to manufacturer's instructions. Reaction products were resolved on an ABI 3100 automatic DNA sequencer and sequences assembled using DNA star v 8.0. Sequences were aligned with CLUSTAL X for the major coding regions of HA segments. The sequences of influenza A vaccine viruses were included along with HA sequences from neighboring countries: China, Thailand, Hong Kong SAR, Cambodia, and Japan obtained from the influenza virus resource (National Center for Biotechnology information; <http://ncbi.nlm.nih.gov/genomes/FLU/FLU.html>). Because the HA1 domain of HA, the major antigenic protein of influenza A viruses,

contains all the antigenic sites of HA and is continually under selection pressure driven by the host's immune response, 1130 nucleotides corresponding to the HA1/H1 domain and 1115 nucleotides for the HA1/H3 domain were used to construct phylogenetic trees by maximum likelihood (ML) method with bootstrap supported values. To quantify amino acid sequence diversity of each lineage, we used MEGA 5 software package and blasted against the NCBI database to determine the closest sequence available for representative strains of each lineage. The clade numbering for nucleotide-based and amino acid-based clustering was somewhat different due to synonymous substitutions at the nucleotide level.

Nucleotide sequence accession numbers

All sequences newly reported in this study have been deposited in the GenBank database under accession numbers JX506656 to JX506727.

Results

Virus selection

Data from NISS in Vietnam, 2006–2009, indicated year-round circulation of seasonal influenza viruses with frequent cocirculation of influenza A and influenza B (Figure 1). Influenza A activity often peaked during the months of May–November, the hot and rainy season, and

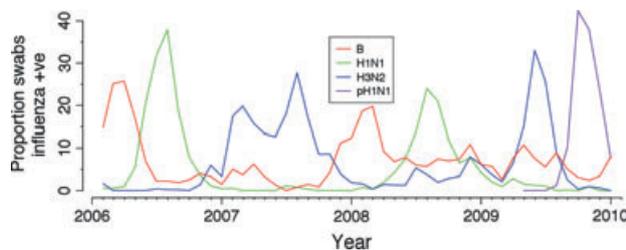


Figure 1. Circulation of influenza viruses in National influenza sentinel surveillance in Vietnam, 2006–2009.

influenza B circulation peaked between December–March^{1,12}. From 2006 to 2009, the NISS identified 1298 A/H1, 1860 A/H3, 1749 B, and 696 A/H1pdm/09 cases by RT-PCR (Table 1). During the study period, only one influenza A subtype, A/H1N1 or A/H3N2, predominated each year with the exception of 2009, when influenza A/H3N2 circulated prior to the appearance and subsequent dominance of A/H1N1/09 pdm (Figure 1). Because of this dominance pattern, we did not isolate influenza A/H1N1 viruses in 2004 and 2009, nor influenza A/H3N2 viruses in 2001 and 2006 (Figure 1, Table 2). Influenza A/H3N2 virus was identified as the predominant circulating influenza A subtype in 2007 by RT-PCR testing; however, viruses recovered from positive H3N2 samples did not reach the required HA titer for confirmation of subtype and sequence analysis. A total of thirty-four (34) A/H1N1 and forty-four (44) of A/H3N2 isolates met the quality criteria for sequencing and were used in this study (Table 2). The samples were collected from Hanoi during 2001–2005 period and various regions of Vietnam from 2006 to 2009 through the NISS (table 2).

HA diversity of seasonal influenza A/H1N1 in Vietnam, 2001–2008

The H1N1 HA phylogenetic tree was developed using 34 HA sequences from Vietnam (2001–2009); the HA sequences from 3 WHO seasonal influenza vaccine recommended strains during the study period (A/New Caledonia/10/1999, A/Solomon Islands/3/2006, and A/Brisbane/59/2007); and 11 H1N1 HA sequences from neighboring countries [Thailand (3), Hong Kong SAR (2), Cambodia (3), China (2), and Japan (1)] from the same period. Using A/Beijing/295/1995 as the tree root, seasonal influenza A/H1N1 viruses collected in Vietnam clustered into four main clades (Figure 2).

Eleven (11) isolates from 2001, 2002, and 2003 clustered in clade I, and nine (9) isolates from 2005, 2006, and 2007 in clade II. Both clade I and II were most closely related to the A/New Caledonia/20/1999 vaccine strain. Clade I and

Table 1. Influenza type/subtype in Vietnam, 2006–2009 (RT-PCR based)

Year	Tested sample (n)	Influenza type/subtype circulation n (%)				
		Total positive sample	A/H1N1 n (%)	A/H3N2 n (%)	A/H1N1pdm/09 n (%)	B n (%)
2006	4625	945	574 (60.7)	56 (5.9)	0 (0)	315 (33.3)
2007	6467	1163	16 (1.4)	875 (75.2)	0 (0)	272 (23.4)
2008	6974	1470	641 (43.6)	211 (14.4)	0 (0)	618 (42.0)
2009	7402	2025	67 (3.3)	718 (35.4)	696 (34.4)	544 (26.9)

Table 2. Number of amino acid difference in the HA segment between WHO recommended vaccine strains and circulating influenza A viruses in Vietnam, 2001–2009

Year	WHO vaccine strains	A/H1N1 isolates (n)	A/H3N2 isolates (n)	Genetic clade	Mean number of amino acid differences
2001	<i>A/New Caledonia/10/1999 (H1N1)</i>	2		I	2.5
	<i>A/Moscow/10/1999(H3N2)</i>		0		
2002	<i>A/New Caledonia/10/1999 (H1N1)</i>	5		I	4.0
	<i>A/Moscow/10/1999(H3N2)</i>		9	la; lb	15.2
2003	<i>A/New Caledonia/10/1999 (H1N1)</i>	9		II	4.0
	<i>A/Moscow/10/1999(H3N2)</i>		8	II; III	19.2
2004	<i>A/New Caledonia/10/1999 (H1N1)</i>	0			
	<i>A/Moscow/10/1999(H3N2)-NH</i>		6	III	21.3
	<i>A/Fujian/411/2002 (H3N2)-SH</i>				2.3
2005	<i>A/New Caledonia/10/1999 (H1N1)</i>	4		II	4.1
	<i>A/Fujian/411/2002 (H3N2)-NH</i>		12	IV; V	3.3
	<i>A/Wellington/01/2004 (H3N2)-SH</i>				4.3
2006	<i>A/New Caledonia/10/1999 (H1N1)</i>	8		II; III	9.8
	<i>A/California/07/04 (H3N2)</i>		0		
2007	<i>A/New Caledonia/10/1999 (H1N1)</i>	2		II; III	10.0
	<i>A/Wisconsin/67/2005(H3N2)</i>		0		
2008	<i>A/Solomon Islands/3/2006 (H1N1)</i>	4		VI	8.4
	<i>A/Wisconsin/67/2005(H3N2)-NH</i>		4	VI	8.6
	<i>A/Brisbane/10/2007(H3N2)-SH</i>				3.2
2009	<i>A/Brisbane/59/2007(H1N1)</i>	0			
	<i>A/Brisbane/10/2007(H3N2)</i>		5	VI	6.8
Total		34	44		

clade II could be differentiated by the amino acid substitutions N171S in clade I and Y269F in clade II relative to A/New Caledonia/10/1999.

The isolates collected in 2006 and 2007 clustered into the new clade III (5 isolates) sharing amino acid changes at T99K, Y111H, R225K, T283N, and K90R. This clade was closely related to the A/Solomon Islands/3/2006 vaccine strain and Cambodia and Thailand viruses collected in the same period. These clade III viruses were displaced by the new clade IV viruses which shared three clade III markers at T99K, Y111H, R225K, T283N, with the addition of R205K. From 2001 through 2007, the A/New Caledonia/10/1999 was recommended by WHO as the vaccine candidate strain, and pair-wise alignment showed the mean number of differences between Vietnamese A/H1N1 isolates ranged from 2.5 amino acids in 2001 to 10.0 amino acids in 2007 (Table 2). The vaccine strain of the 2008 season A/Solomon Islands/3/2006 possessed 8.4 amino acids differences from Vietnamese isolates of the same period (Table 2). However, some of these amino acid differences are due to egg adaptation seen with the A/Solomon Islands/3/2006 vaccine virus that was not seen in the Vietnamese viruses as they were isolated in MDCK cells.

HA diversity of seasonal influenza A/H3N2 in Vietnam, 2002–2009

The H3N2 HA phylogenetic tree was developed using the HA of 44 A/H3N2 isolates collected from 2002 to 2009; the HA of five WHO recommended vaccine candidate strains for Northern and Southern Hemispheres (Moscow/10/1999; Fujian/411/2002; California/07/04; Wisconsin/67/2005 and Brisbane/10/2007) and three WHO seasonal influenza vaccine strains recommended for the Southern Hemisphere only (Wellington/01/2004; Uruguay/716/2007 and Perth/16/2009); and 30 HA sequences from H3N2 isolates from China, Japan, Thailand, and Cambodia. The phylogenetic tree was rooted against the HA sequence of vaccine candidate strain A/Moscow/10/1999.

The analysis showed six different H3N2 clades in Vietnam during 2002–2009 with high genetic homology between isolates circulating in the same year. Of those, clade I was comprised of nine isolates in 2002 and diverged into clade IA and clade IB distinguished by the amino acid substitutions G291D in IA and A147T in IB when compared with A/Moscow/10/1999 strain. An additional four amino acid substitutions (L41I, H91Q, H171T, and Q172H) in the HA gene compared with clade IA resulted

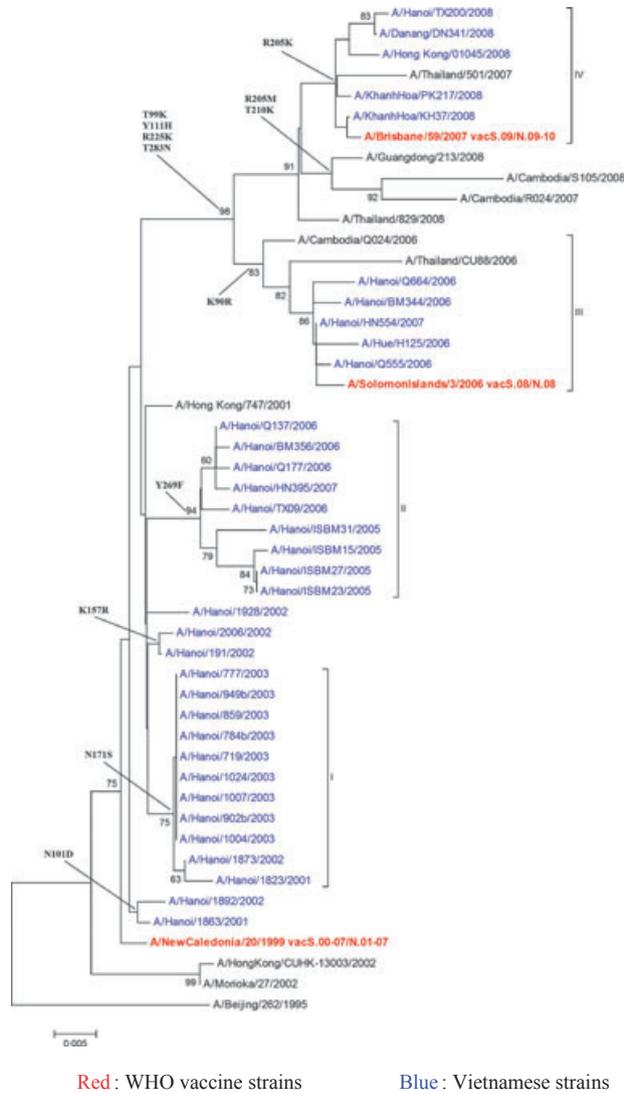


Figure 2. The maximum likelihood phylogenetic tree of the hemagglutinin domain 1(HA1) sequences of influenza A/H1N1 Vietnam strains, 2001–2008.

in six isolates in 2003 being grouped in clade II, which were related to Hong Kong strains from the same year. During the 2001–2004, A/Moscow/10/199 virus was recommended as the H3N2 vaccine strain for Northern and Southern Hemispheres; however, not all Vietnamese isolates in same period (clade I and II) were homologous to this virus (Figure 3). The number of amino acid differences from A/Moscow/10/199 were 15.2 in 2002 and 19.2 in 2003 (Table 2). Clade III comprised five (5) isolates from 2004 and two from 2003 and was closely related to the Wellington/01/2004-like strain used in the Southern Hemisphere vaccine in 2005. In 2004, the mean amino acid difference of circulating Vietnamese H3N2 strains compared with WHO recommended Northern (A/Moscow/10/1999)

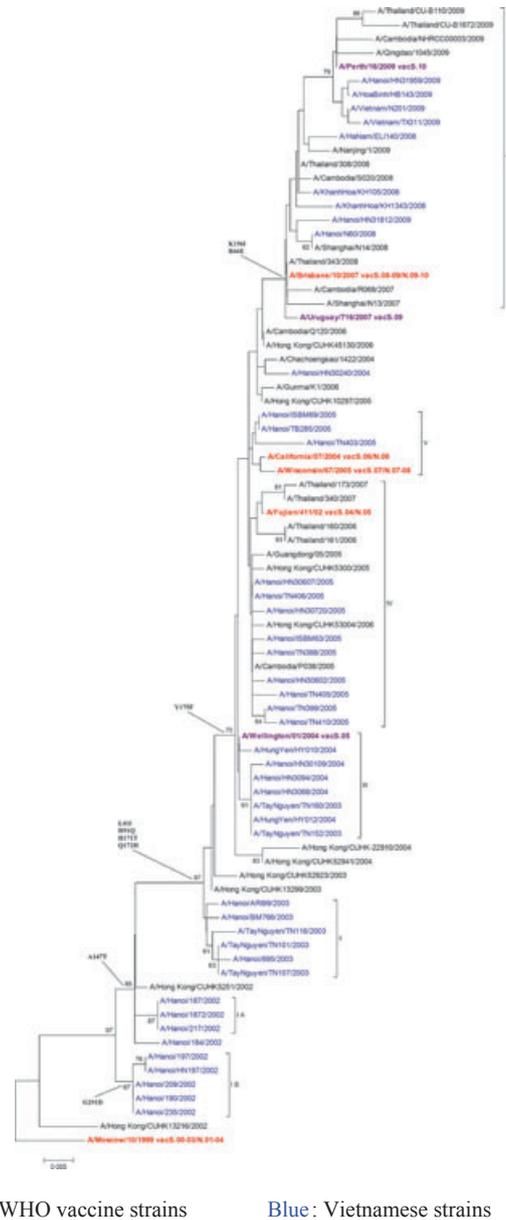


Figure 3. The maximum likelihood phylogenetic tree of the hemagglutinin domain 1 (HA1) sequences of influenza A/H3N2 Vietnam strains, 2002–2009.

and Southern (A/Fujian/411/2002) Hemisphere vaccine strains was 21.3 and 2.3, respectively (Fig 3, table 2). During the 2005 influenza season, we collected twelve H3N2 isolates and most of them (9/12) were grouped in clade IV, with a small number of isolates (3) in clade V which was closely related to the A/Fujian/411/2002 (clade IV), and California/07/04 and Wisconsin/67/2005 (clade V), vaccine strains. The mean amino acid difference of circulating Vietnamese H3N2 strains in 2005 compared with WHO recommended Northern (A/Fujian/411/2002)

and Southern (A/Wellington/01/2004) Hemisphere vaccine strains was 3·3 and 4·3, respectively (Fig 3, Table 2). Although both of the vaccine strains used for clade IV and V comparison were egg grown viruses (which have amino acid substitutions in the egg-derived HA sequences), this is not reflected in large differences in amino acid sequences. Clade VI was comprised of nine isolates in 2008 and 2009, which was distinguished by amino acid substitutions of R66E and K156I and clustered with isolates from Thailand, Cambodia, and China. The Vietnamese isolates were approximately equidistant from the 2008 and 2009 vaccine candidate Brisbane/10/2007. Although grouped in the same clade (VI), H3N2 viruses collected in 2008 and 2009 had a mean number of amino acid differences of 3·2 and 6·8, respectively compared with A/Brisbane/10/2007 (Figure 3, Table 2).

Discussion

We have described influenza A/H1N1 and A/H3N2 HA genetic variation in the period 2001–2009 and found that most isolates clustered in the same clade with other viruses of the same year, with several significant amino acid changes characterizing clade diversity, such as: N171S and Y269F for clade I and clade II of influenza A/H1N1, and R66E and K156I for clade VI of influenza A/H3N2. These clades were similar to isolates of neighboring countries. Since 2001, three WHO influenza A/H1N1 vaccine strains have been recommended, for both northern and southern influenza vaccine formulations. During the period of our study, influenza A/H1N1 viruses circulating in Vietnam segregated into two distinct clades, and there were few amino acid changes in 2001–2005 period (2·5 to 4·1 amino acids) compared with the A/New Caledonia/10/1999 vaccine strain for 2001–2007. This suggests that this vaccine strain may have had a good genetic match with circulating viruses of Vietnam during that time. However, during 2006 and 2007, the genetic divergence of Vietnamese H1N1 isolates compared with the WHO recommended vaccine strain increased, with a mean of 9·8 and 10·0 amino acid differences compared with the WHO recommended vaccine strain. These findings are similar to reports from Thailand, Cambodia, China, and Japan.^{1,3,6–9,14}

Larger genetic variation of HA sequences were observed in A/H3N2 viruses, with six clades being detected during the study period. This is consistent with the known faster evolution of H3N2 viruses compared with H1N1 or influenza B viruses.¹⁵ Our results showed that the WHO vaccine strain recommended for Northern and Southern Hemispheres from 2001 to 2004 (A/Moscow/10/1999) may not have provided optimal protection for the Vietnamese population because all influenza A/H3N2 isolates in that time were genetically diverse (clades I and II) and circulating

viruses had drifted from vaccine strain. In total, eight WHO candidate H3N2 strains were recommended during our study period. The Southern Hemisphere vaccine strains of A/Fujian/411/2002, A/Wellington/01/2004, and A/Brisbane/10/2007 had a good genetic match with strains circulating in Vietnam in years 2004, 2005, and 2008, with very low number of amino acid differences (2·3, 4·3, and 3·2, respectively). The other A/H3N2 vaccine strains recommended for both Northern and Southern Hemispheres in 2006 and 2007 (A/California/07/04 and A/Wisconsin/67/2005) were clustered with Vietnamese strains circulating in 2005. Although, our analysis did not include local H3N2 isolates from 2006 and 2007, during that time strains isolated in Thailand were not closely related to either A/California/07/04 or A/Wisconsin/67/2005 vaccine strains.¹¹ Similarly, the strains circulating in 2009 were more closely related to the 2010 Southern Hemisphere vaccine strain (A/Perth/16/2009), whilst A/Brisbane/10/2007 was recommended as the vaccine strain in 2009. (Figure 3)

Although analysis of the HA gene does provide some indication on the emergence of antigenically divergent strains, inferences about the protection afforded by WHO recommended influenza vaccine strains in Vietnam are limited by the lack of antigenic data, due to the unavailability of essential reagents in the time period prior to participating in the GISRS (2001–2005). Genetic data are, however, useful because some genetic changes can generate significant antigenic changes that drive the evolution of influenza.¹⁶

Full antigenic characterization of viruses from Vietnam and other South East Asian countries is required to ensure that vaccine strains selected by WHO will provide protection in these areas. Such characterization is particularly important given that H3N2 virus may evolve and emerge from an East and Southeast Asia network,¹⁵ the large number of people living in this region, and the more complex epidemiology compared with temperate regions. Therefore, sharing materials, such as epidemiological data and representative isolates, from a variety of countries has important implications for influenza vaccine strain selection and the optimal implementation of influenza vaccination programs.

Vietnam is located in the Northern Hemisphere and is in close proximity to southern China, which has been considered an epicenter of human influenza outbreaks.¹⁷ However, the circulation of seasonal influenza A virus often peaks between May and November in Vietnam,¹ similar to Southern Hemisphere epidemics,¹⁵ and our finding suggest that Vietnamese H3N2 isolates were more similar to WHO Southern Hemisphere vaccine strains in recent years. Thus, strain selection for Southern Hemisphere countries needs to consider strains circulating in Vietnam, and *vice versa*.

Currently, Vietnam lacks a national policy for influenza vaccination as there is insufficient data on the burden of disease and the local epidemiology and virology. Our results provide information on the HA gene that reveals evolution of the virus that might be expected to lead to antigenic change. Complete genome analysis and serological testing (HAI) of influenza A viruses will be necessary to obtain a more comprehensive picture of virus evolution and to guide vaccine strategy in the future in Vietnam.

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Author contributions

QML and CDV conceived the study. QML, CDV, HTN, and PMHV designed the study. HLKN, TTL, PMHV, TCN, and CDV undertook the laboratory work. QML, CDV, and PMHV did the phylogenetic analysis. QML wrote the first draft of the study. J.K; D.D; B.K. revised the study. All the authors reviewed and edited drafts of the manuscript and approved the final version.

Competing interests

All authors declare no competing interests.

References

- 1 Nguyen HT, Dharan NJ, Le MTQ *et al.* National influenza surveillance in Vietnam, 2006–2007. *Vaccine*, 2009; 28:398–402.
- 2 World Health Organization. Recommended composition of influenza virus vaccines for use in the 2001–2009. Available at <http://www.who.int/csr/disease/influenza/vaccinerecommendations/en/index>.
- 3 Mardy S, Ly S, Heng S, Vong S, *et al.* Influenza activity in Cambodia during 2006–2008. *BMC Infect Dis*, 2009; 15:9.
- 4 Waichanpen S, Thawatsupha P, Chittagapitch M, Maneewong P, Thanadachakul T *et al.* Influenza viruses circulating in Thailand in 2004 and 2005. *Jpn J Infect Dis*, 2008; 61:321–323.
- 5 Center for Disease Control. Concepts and Procedures for Laboratory Based Influenza Surveillance. Atlanta, GA: U.S. Dept Health Public Health Service, 1982.
- 6 Li D, Saito R, Le MTQ, Nguyen HLK, Suzuki Y *et al.* Genetic analysis of influenza A/H3N2 and A/H1N1 viruses circulating in Vietnam from 2001 to 2006. *J Clin Microbiol*, 2008; 46:399–405.
- 7 Hampson AW. Epidemiological data on influenza in Asian countries. *Vaccine* 1999; 17(Suppl 1):S19–23.
- 8 Saito R, Li D, Shimomura C, Masaki H, Le MQ *et al.* An offseasonal Amantadine-resistant H3N2 influenza outbreak in Japan. *Tohoku J Exp Med* 2006; 210:21–27.
- 9 Muramoto K, Le QM, Phuong SL, Nguyen T, Nguyen TH *et al.* Pathogenicity of H5N1 Influenza A viruses isolated in Vietnam between late 2003 and 2005. *J Vet Med Sci*, 2006; 68:735–737.
- 10 Zhang Y, Zhao X, Huang WJ, Guo JF, Wei HJ *et al.* Study on the antigenicity and HA1 gene characteristics of influenza A viruses during 2004–2008 year in China. *Chin J Prevent Med*, 2009; 43:861–6.
- 11 Suwannakam K, Chieochansin T, Thongmee C, Makkoch J, Prai-nantathavorn K *et al.* Molecular evolution of human H1N1 and H3N2 influenza virus in Thailand, 2006–2009. *PLoS ONE*, 2010; 5:e-9717.
- 12 Nguyen HLK, Saito R, Nghiem HK, Nishikawa M, Shobugawa Y *et al.* Epidemiology of Influenza in Hanoi, Vietnam, from 2001 to 2003. *J Infect*, 2007; 55:58–63.
- 13 Hoffmann E, Stech J, Guan Y, Webster RG, Perez DR. Universal primer set for the full-length amplification of all influenza A viruses. *Arch Virol*, 2001; 146:2275–89.
- 14 Mai LTQ, Wertheim FL, Taylor W, Hoang PVM, Vuong CD *et al.* A new influenza A/H5N1 clade virus with a different antiviral susceptibility profile replaced the old clade in northern Vietnam. *PLoS ONE* 2008; 3:33339.
- 15 Russell CA, Jones TC, Barr IG, Cox NJ, Garten RJ *et al.* The global circulation of seasonal influenza A (H3N2) viruses. [Internet]. *Science* (New York, N.Y.) 2008; 320:340–6.
- 16 Smith DJ, Lapedes AS, de Jong JC, Bestebroer TM, Rimmelzwaan GF *et al.* . Mapping the antigenic and genetic evolution of influenza virus. *Science* 2004; 305:371–376.
- 17 Lin YP, Shu LL, Wright S *et al.* Analysis of the influenza virus gene pool of avian species from Southern China. *Virology*, 1994; 198:557–566.