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GENETIC COMPARISON OF H3N2 INFLUENZA VIRUS IN INDONESIA WITH WHO RECOMMENDED STRAINS DURING THE PERIOD 2004 – 2021

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Abstract

Influenza caused by A/H3N2 virus is one commonly found infections in tropical countries. Vaccination policy against A/H3N2 virus is already established for Northern and Southern hemisphere, however, no specified policy is established for tropical countries such as Indonesia. World Health Organization (WHO) has recommended Indonesia to use vaccine formulation designated for Northern hemisphere. Some studies, however, have shown that there were mismatches between recommended vaccine and circulating strains present in Asian countries, which might have caused regional outbreaks. This study was aimed to compare the genetic makeup of WHO recommended vaccine strain with H3N2 virus circulating in Indonesia. A total of 147 HA and 148 NA complete nucleotide sequences from Indonesian population, as well as 14 HA and 14 NA WHO-recommended nucleotide sequences, were downloaded from GISAID. These sequences were subjected to phylogenetic analysis using MEGA 11. Furthermore, antigenicity of these isolates was analyzed using Kolaskar and Tangonkar Prediction method accessible through IEDB website and Vaxijen 2.0. This study revealed that WHO-recommended vaccine strain for southern hemisphere in 2010 were compatible with Indonesian strains circulating in 2009. Furthermore, there were no recommended vaccine strain that matched strain in circulation between 2010 to 2012 and 2019. Kolaskar and Tongaonkar antigenicity prediction analysis had simulated that several mutations rendered some antigenic epitopes of HA and NA protein to be non-antigenic. These findings highlighted the importance of reliable national surveillance network of influenza to prevent regional epidemic and the development of local influenza vaccine to better accommodate Indonesian population.

Keywords: *H3N2, Influenza, WHO, Vaksin, Genetic*

INTRODUCTION

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Influenza is an acute respiratory illness caused by four types of influenza virus (A, B, C, and D) which spreads in all parts of the world (*Influenza (Seasonal)*, n.d.). Annually, it accounts for 3 to 5 million cases of severe illness and approximately 650,000 deaths. The illness can be characterized by symptoms such as fever, dry cough, headache, muscle and joint pain, severe malaise, sore throat and runny nose. Most people are able to recover themselves without medical intervention. High-risk groups such as pregnant women, children under 5 years, elderly, individuals with comorbidity and immunosuppressed condition, as well as health care workers might have more severe outcome than the rest of the population (*Influenza (Seasonal)*, n.d.). Transmission of the virus relies on infectious droplets dispersed into the air when an infected individual cough or sneezes(*Influenza (Seasonal)*, n.d.).

Seasonal epidemics in temperate regions occurs mainly during winter season, while in tropical region, outbreaks might occur irregularly all year round, with potential increase of cases during rainy season (Agustiningsih et al., 2018). Influenza A/H3N2 is one of the influential seasonal viruses which have caused three major influenza outbreaks since 1986. It has resulted in numerous epidemics, significant morbidity, and substantial mortality (Allen & Ross, 2018).

Vaccination is the most recommended method to reduce the morbidity and mortality caused by influenza virus infections, including A/H3N2 virus, as it will trigger immune responses which will provide a certain level of protection (Treanor, 2004). However, it is well known that the antigenicity of A/H3N2 rapidly changing through antigenic drift and antigenic shift of hemagglutinin (HA) and neuraminidase (NA) gene, which ultimately renders most, if not all, immune protection ineffective. Therefore, unlike any other available vaccine, influenza vaccine must be regularly reformulated to keep pace with antigenic changes in HA and NA proteins of the virus in circulation (Treanor, 2004).

World Health Organization (WHO), through Global Influenza Surveillance and Response System (GISRS), recognizes the importance reformulation of vaccine components to reflect currently circulating viral strain (Hay & McCauley, 2018). The system allows WHO to monitor the currently prevalent viral strain and make recommendations on vaccine composition to anticipate potential future antigenic changes. This strategy allows them to avoid a mismatch between vaccine and the strain in circulation, hence reducing the possibility of vaccine being ineffective (Hay & McCauley, 2018).

The recommendations for trivalent influenza vaccine are made in February and September for Northern and Southern hemisphere respectively. However, countries with tropical climate where infection is possible all year long do not have any specific recommendation. The WHO suggested for these countries to use either Southern or Northern hemisphere vaccine formula based on regional epidemiological condition. A study by Agustiningsih *et al*. highlighted the possibilities of new strain to emerge in

tropical region before it spreads into temperate region. This signifies the importance of A/H3N2 monitoring in tropical countries such as Indonesia (Agustiningsih et al., 2018).

Vaccine efficacy depends on the antigenicity relatedness between viral strain in circulation with the vaccine strains (Moura, 2010). Thus, to elucidate the compatibility of recommended vaccine towards A/H3N2 virus in Indonesia, this study aims to compare the genetic makeup of WHO recommended vaccine strain and A/H3N2 in circulation between 2004 – 2021 using computational analysis based on its antigenicity relatedness.

RESEARCH METHODS

Sequence Acquisition and Alignment

Complete nucleotide sequence of HA and NA gene of influenza virus A/H3N2 was acquired from *Global Initiative on Sharing All Influenza Data* (GISAID) (Elbe & Buckland-Merrett, 2017; Khare et al., 2021; Shu & McCauley, 2017). All isolates were of Indonesia origin with the latest isolation date of August 21st, 2021. Along with Indonesian isolates, all WHO recommended vaccine strains from 2004 up to 2021 were included. All isolates were processed with BioEdit and aligned with vaccine strains using Clustal Omega (Sievers et al., 2011) provided for free by European Molecular Biology Laboratory – European Bioinformatics Institute (EMBL-EBI). Isolates with 100% similarities had their identifiers merged using Biopython (Cock et al., 2009).

Phylogenetic Analysis

Phylogenetic analysis of those isolates was completed by utilizing MEGA 11: Molecular Evolutionary Genetics Analysis version 11 (Tamura et al., 2021). *Neighbor joining* statistical method was chosen with default parameter and bootstrap value set at 1,000. All isolates and vaccine strains were separated into several groups (A – H) based on the recommendation year.

Antigenicity Analysis

Immune Epitope Database and Analysis Resource (IEDB) (Vita et al., 2019), a free accessible online resource tool, was used to predict linear B-cell epitopes on all vaccine strains. The prediction was performed using Kolaskar & Tongaonkar Antigenicity Prediction method of IEDB. Subsequently, predicted epitopes were checked for its antigenicity value via Vaxijen v2.0 server (Doytchinova & Flower, 2007) with the default threshold of 0.4. Isolates with mutation within the reference epitopes were documented and predicted for its B-cell epitopes and antigenicity score using the same methods.

RESULTS AND DISCUSSION

Result

A total of 14 WHO-recommended vaccine strains along with 147 HA and 148 NA gene sequences from Indonesian isolates were retrieved from GISAID. However, it was important to note that the identifier of HA and NA from Indonesian isolates were not necessarily the same. Complete EPI_ISL identifier of both recommended vaccine strains and Indonesian isolates were listed in Table 1.

Table 1. List of EPI_ISL identifier of all vaccine strains and Indonesian isolates used in this study.

Isolate Type	EPI_ISL Identifier
WHO-recommended vaccine strain (HA and NA genes)	EPI_ISL_111384, EPI_ISL_65217, EPI_ISL_113070, EPI_ISL_154552, EPI_ISL_176458, EPI_ISL_176456, EPI_ISL_134450, EPI_ISL_170149, EPI_ISL_166310, EPI_ISL_176512, EPI_ISL_311688, EPI_ISL_332305, EPI_ISL_413291, EPI_ISL_391201
Indonesian isolates (HA gene)	EPI_ISL_98651, EPI_ISL_135834, EPI_ISL_145127, EPI_ISL_145128, EPI_ISL_145129, EPI_ISL_145130, EPI_ISL_145131, EPI_ISL_145132, EPI_ISL_145133, EPI_ISL_145134, EPI_ISL_145135, EPI_ISL_145136, EPI_ISL_145137, EPI_ISL_145138, EPI_ISL_145139, EPI_ISL_145140, EPI_ISL_145141, EPI_ISL_145142, EPI_ISL_145143, EPI_ISL_145144, EPI_ISL_145145, EPI_ISL_145146, EPI_ISL_145147, EPI_ISL_145148, EPI_ISL_145149, EPI_ISL_145150, EPI_ISL_145151, EPI_ISL_145152, EPI_ISL_145153, EPI_ISL_145154, EPI_ISL_145155, EPI_ISL_145156, EPI_ISL_145157, EPI_ISL_145158, EPI_ISL_145159, EPI_ISL_145160, EPI_ISL_145161, EPI_ISL_145162, EPI_ISL_145163, EPI_ISL_145164, EPI_ISL_145165, EPI_ISL_145166, EPI_ISL_145167, EPI_ISL_145168, EPI_ISL_145169, EPI_ISL_145170, EPI_ISL_145171, EPI_ISL_145172, EPI_ISL_145173, EPI_ISL_145174, EPI_ISL_145175, EPI_ISL_145176, EPI_ISL_145177, EPI_ISL_145178, EPI_ISL_145179, EPI_ISL_145180, EPI_ISL_145181, EPI_ISL_145182, EPI_ISL_145183, EPI_ISL_145184, EPI_ISL_145185, EPI_ISL_145186, EPI_ISL_145187, EPI_ISL_145188, EPI_ISL_145189, EPI_ISL_145190, EPI_ISL_145191, EPI_ISL_145192, EPI_ISL_145193, EPI_ISL_145194, EPI_ISL_145195, EPI_ISL_145196, EPI_ISL_145197, EPI_ISL_145198, EPI_ISL_145199, EPI_ISL_145200, EPI_ISL_145201, EPI_ISL_145202, EPI_ISL_145203, EPI_ISL_145204, EPI_ISL_145205, EPI_ISL_145206, EPI_ISL_145207, EPI_ISL_145208, EPI_ISL_145209, EPI_ISL_145210, EPI_ISL_145211, EPI_ISL_145212, EPI_ISL_145213, EPI_ISL_145214, EPI_ISL_145215, EPI_ISL_145216, EPI_ISL_145217,

	EPI_ISL_145218, EPI_ISL_145219, EPI_ISL_145220, EPI_ISL_145221, EPI_ISL_145222, EPI_ISL_145223, EPI_ISL_145224, EPI_ISL_145225, EPI_ISL_145226, EPI_ISL_166823, EPI_ISL_200738, EPI_ISL_232993, EPI_ISL_232994, EPI_ISL_232995, EPI_ISL_232996, EPI_ISL_234986, EPI_ISL_235206, EPI_ISL_235207, EPI_ISL_235208, EPI_ISL_235209, EPI_ISL_235210, EPI_ISL_289548, EPI_ISL_289549, EPI_ISL_289550, EPI_ISL_289551, EPI_ISL_289552, EPI_ISL_289553, EPI_ISL_289554, EPI_ISL_289555, EPI_ISL_322337, EPI_ISL_322739, EPI_ISL_378549, EPI_ISL_378550, EPI_ISL_378551, EPI_ISL_378552, EPI_ISL_403405, EPI_ISL_403409, EPI_ISL_403419, EPI_ISL_403458, EPI_ISL_493147, EPI_ISL_510016, EPI_ISL_510017, EPI_ISL_510018, EPI_ISL_583902, EPI_ISL_614338, EPI_ISL_614339, EPI_ISL_614340, EPI_ISL_614341, EPI_ISL_614342, EPI_ISL_614343, EPI_ISL_644652, EPI_ISL_718126, EPI_ISL_718127, EPI_ISL_718129
Indonesian isolates (NA gene)	EPI_ISL_20146, EPI_ISL_20149, EPI_ISL_20152, EPI_ISL_20153, EPI_ISL_20161, EPI_ISL_98651, EPI_ISL_135834, EPI_ISL_145127, EPI_ISL_145128, EPI_ISL_145129, EPI_ISL_145130, EPI_ISL_145131, EPI_ISL_145132, EPI_ISL_145133, EPI_ISL_145134, EPI_ISL_145135, EPI_ISL_145136, EPI_ISL_145137, EPI_ISL_145138, EPI_ISL_145139, EPI_ISL_145140, EPI_ISL_145141, EPI_ISL_145142, EPI_ISL_145143, EPI_ISL_145144, EPI_ISL_145145, EPI_ISL_145146, EPI_ISL_145147, EPI_ISL_145148, EPI_ISL_145149, EPI_ISL_145150, EPI_ISL_145151, EPI_ISL_145152, EPI_ISL_145153, EPI_ISL_145154, EPI_ISL_145155, EPI_ISL_145156, EPI_ISL_145157, EPI_ISL_145158, EPI_ISL_145159, EPI_ISL_145160, EPI_ISL_145161, EPI_ISL_145162, EPI_ISL_145163, EPI_ISL_145164, EPI_ISL_145165, EPI_ISL_145166, EPI_ISL_145167, EPI_ISL_145168, EPI_ISL_145169, EPI_ISL_145170, EPI_ISL_145171, EPI_ISL_145172, EPI_ISL_145173, EPI_ISL_145174, EPI_ISL_145175, EPI_ISL_145176, EPI_ISL_145177, EPI_ISL_145178, EPI_ISL_145179, EPI_ISL_145180, EPI_ISL_145181, EPI_ISL_145182, EPI_ISL_145183, EPI_ISL_145184, EPI_ISL_145185, EPI_ISL_145186, EPI_ISL_145187, EPI_ISL_145188, EPI_ISL_145189, EPI_ISL_145190, EPI_ISL_145191,

	EPI_ISL_145192, EPI_ISL_145193, EPI_ISL_145194, EPI_ISL_145195, EPI_ISL_145196, EPI_ISL_145197, EPI_ISL_145198, EPI_ISL_145199, EPI_ISL_145200, EPI_ISL_145201, EPI_ISL_145202, EPI_ISL_145203, EPI_ISL_145204, EPI_ISL_145205, EPI_ISL_145206, EPI_ISL_145207, EPI_ISL_145208, EPI_ISL_145209, EPI_ISL_145210, EPI_ISL_145211, EPI_ISL_145212, EPI_ISL_145213, EPI_ISL_145214, EPI_ISL_145215, EPI_ISL_145216, EPI_ISL_145217, EPI_ISL_145218, EPI_ISL_145219, EPI_ISL_145220, EPI_ISL_145221, EPI_ISL_145222, EPI_ISL_145223, EPI_ISL_145224, EPI_ISL_145225, EPI_ISL_145226, EPI_ISL_166823, EPI_ISL_200738, EPI_ISL_232993, EPI_ISL_232994, EPI_ISL_232995, EPI_ISL_232996, EPI_ISL_234986, EPI_ISL_235206, EPI_ISL_235207, EPI_ISL_235208, EPI_ISL_235209, EPI_ISL_235210, EPI_ISL_289548, EPI_ISL_289549, EPI_ISL_289550, EPI_ISL_289551, EPI_ISL_289552, EPI_ISL_289553, EPI_ISL_289554, EPI_ISL_289555, EPI_ISL_322337, EPI_ISL_322739, EPI_ISL_378549, EPI_ISL_378550, EPI_ISL_378551, EPI_ISL_378552, EPI_ISL_493147, EPI_ISL_510016, EPI_ISL_510017, EPI_ISL_510018, EPI_ISL_583902, EPI_ISL_614338, EPI_ISL_614339, EPI_ISL_614340, EPI_ISL_614341, EPI_ISL_614342, EPI_ISL_614343, EPI_ISL_644652, EPI_ISL_718126, EPI_ISL_718127, EPI_ISL_718129

Sequences of vaccine strains and isolates were aligned with Clustal Omega, trimmed with BioEdit, and subsequently, processed with Biopython to merge the all isolates with 100% sequence similarity. A total of 133 HA and 130 NA unique isolates were obtained at the end of this step. All processed sequences were subjected to phylogenetic analysis using MEGA 11, where Neighbor Joining statistical method was selected.

Genetic distance of HA gene among Indonesian isolates and vaccine strains was represented with phylogenetic tree in Figure 1. There were no HA gene isolates between 2004 and 2007 could be found since this study included those with complete nucleotide sequences. Vaccine strain A/Brisbane/10/2007(H3N2)-like virus was used as recommendation in 2008 until 2010 for both hemispheres which matched with viral strain in circulation for 2008 only. Isolates from 2009 had closer relationship with A/Perth/16/2009 (H3N2)-like virus, which was recommended in 2010, a year after. There

were no vaccine strains that matched with isolates from 2010. Recommended vaccine strains for subsequent years were suggested by the WHO the year after.

Genetic distance of NA gene from Indonesian A/H3N2 isolates to the vaccine strain slightly contrasted with the HA gene (Figure 2). Isolates from 2008 were seemed to be split into two separate clusters, however, vaccine strain A/Brisbane/10/2007(H3N2)-like virus was still compatible with the all of those isolates. Several recommendations suggested by WHO were delayed as shown by isolates in 2009, 2012 – 2013, and 2017. Several isolates from 2012, 2010, and 2019 were shown to be incompatible with all of the vaccine strains.

Some vaccine strains were reused several times; therefore, isolates were reorganized into several groups (A – H) based on the recurring usage of the vaccine strains to simplify the analysis (Table 2). The vaccine strains were analyzed using Kolaskar & Tongaonkar Antigenicity Prediction method from IEDB to find potential epitopes. These epitopes were checked for their antigenicity value with Vaxijen 2.0. with a threshold level of 0.4 afterwards. Vaccine strains sequences were compared with all isolates from the same group to map mutations within epitope region. These point mutations were listed in table 3.

Table 2. WHO-recommended H3N2 vaccine strain for influenza vaccine development.

Year	Hemisphere	Recommended Vaccine Strain	EPI ISL	Group
2004	Southern	A/Fujian/411/2002(H3N2)-like virus	111384	A
2004/2005	Northern	A/Fujian/411/2002(H3N2)-like virus	111384	
2005	Southern	A/Wellington/1/2004(H3N2)-like virus	65217	
2005/2006	Northern	A/California/7/2004(H3N2)-like virus	113070	
2006	Southern	A/California/7/2004(H3N2)-like virus	113070	
2006/2007	Northern	A/Wisconsin/67/2005 (H3N2)-like virus	154552	
2007	Southern	A/Wisconsin/67/2005(H3N2)-like virus	154552	
2007/2008	Northern	A/Wisconsin/67/2005 (H3N2)-like virus	154552	
2008	Southern	A/Brisbane/10/2007(H3N2)-like virus	176458	B
2008/2009	Northern	A/Brisbane/10/2007 (H3N2)-like virus	176458	

2009	Southern	A/Brisbane/10/2007(H3N2)-like virus	176458	
2009/2010	Northern	A/Brisbane/10/2007 (H3N2)-like virus	176458	
2010	Southern	A/Perth/16/2009 (H3N2)-like virus	176456	
2010/2011	Northern	A/Brisbane/10/2007 (H3N2)-like virus	176458	
2011	Southern	A/Perth/16/2009 (H3N2)-like virus	176456	
2011/2012	Northern	A/Perth/16/2009 (H3N2)-like virus	176456	
2012	Southern	A/Perth/16/2009 (H3N2)-like virus	176456	
2012/2013	Northern	A/Victoria/361/2011 (H3N2)-like virus	134450	
2013	Southern	A/Victoria/361/2011 (H3N2)-like virus	134450	
2014-2015	Northern	A(H3N2) virus antigenically like the cell-propagated prototype virus A/Victoria/361/2011	134450	
2014	Southsern	A/Texas/50/2012 (H3N2)-like virus	170149	C
2015/2016	Northern	A/Texas/50/2012 (H3N2)-like virus	170149	
2015	Southern	A/Switzerland/9715293/2013 (H3N2)-like virus	166310	D
2015/2016	Northern	A/Switzerland/9715293/2013 (H3N2)-like virus	166310	
2016	Southern	A/Hong Kong/4801/2014 (H3N2)-like virus	176512	E
2016/2017	Northern	A/Hong Kong/4801/2014 (H3N2)-like virus	176512	
2017	Southern	A/Hong Kong/4801/2014 (H3N2)-like virus	176512	
2017/2018	Northern	A/Hong Kong/4801/2014 (H3N2)-like virus	176512	
2018	Southern	A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus	311688	F

2018/2019	Northern	A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus	311688	
2019	Southern	A/Switzerland/8060/2017 (H3N2)-like virus	332305	G
2019/2020	Northern	A(H3N2) virus to be announced on 21 March 2019	-	
2020	Southern	A/South Australia/34/2019 (H3N2)-like virus	413291	H
2020/2021	Northern	A/Hong Kong/2671/2019 (H3N2)-like virus	391201	
2021	Southern	A/Hong Kong/2671/2019 (H3N2)-like virus	391201	

Table 3. Mutation found on all isolates based on the location of predicted epitopes of recommended vaccine strain

Group	Vaccine Strain (EPI ISL)	Mutation on HA Gene	Mutation on NA Gene
A	65217	-	F23L, T69A
B	176458	L7F, S8R, Y9H, Y9N, V14A, F15L, T16A, T16V, K18E, A27T, L31F, S61N, V59I, S61I, P71S, D69N, S70N, I74V, Q73H, Y110H, V128I, L173S, K174N, K174R, P178Q, P178S, V182M, H199L, P210L, Q227R, T228A, I230S, I246V, I258L, I258V, K484T, V521I, V545A, V545F, V545I, A546V, D529E, W530R, A537S, I538T	I20V, T16I, I26T, L52P, C53Y, I65T, T95K, D93G, D127Y, D127N, K128T, F167L, I176M, I176V, I194V, I215V, V240I, G235R, T238A, E258K, V263I, T312I, V317L, S315N, S335G, H336N, D339G, D399N, I418V, I427V
C	170149	L137S	T56I, S315G, D339G
D	166310	D69N, S175Y, K176T, Q213H, Q327H	I233V
E	176512	I4T, Y9C, G65D, Y110N, S112N, D120G, A154S, K158G, F209S, I230T, H327Q, L443I, I538M, V545I, A546V	T19I, T69N, P90Q, G93D, I231V, S311N, D339N

F	311688	Y9H, F15Y, D69N, I83V, Y110H, S112N, A122V, R158G, R158K, N174K, K176T, Y177H, I208T, P210L, Q213H, I230T, A228T, S328R, H327Q, A492V, A546V	G93D, I212V, T238A, V313A
G	332305	Y9C, G158R, T176K, H327Q, A546V	G93D, P126L, M176I, V194I, T238A, T238V, I312V, V313A, S315R
H	413291	I4T, Y9C, T16A, Y110N, D120G, F153S, S154A, T176K, S209F, Y211F, I246V, I258M, G291S, V325I, Q327H, I538M, V545I	I28T, L52P, G93D, L126P, T238A, V313A, R315S, E381G, E381D

Isolates with these mutations were subjected to analysis using Kolaskar & Tongaonkar Antigenicity Prediction method and have their antigenicity evaluated using Vaxijen 2.0. Epitope antigenicity of these isolates were compared with those of vaccine strains. Mutations within HA and NA epitopes that had their antigenicity rendered non-antigenic were summarized in Table 4 and Table 5 respectively. Several epitopes from different isolates completely disappeared due to point mutation outside the antigenic epitopes.

Table 4. Point mutations within antigenic epitopes of HA gene that had been rendered non-antigenic.(*) Epitopes disappeared due to mutation outside the antigenic epitope.

Group by Year	Vaccine Strain (EPI_ISL)	Isolates	Mutation
B	176458	A/Indonesia/NIHRDI-BP116/2010|2010.458 A/Indonesia/NIHRDI-MS285/2010|2010.430	S61N
		A/Indonesia/NIHRDI-MS262/2010|2010.334	V59I
		A/Indonesia/NIHRDI-MS262/2010|2010.334 A/Indonesia/NIHRDI-BM66/2009|2009.532	P210L

		A/Indonesia/NIHRDI-BM78/2009|2009.551 A/Indonesia/NIHRDI-BP103/2010|2010.225 A/Indonesia/NIHRDI-BP116/2010|2010.458 A/Indonesia/NIHRDI-BT226/2009|2009.241 A/Indonesia/NIHRDI-BT77/2009|2009.490 A/Indonesia/NIHRDI-MRK206/2010|2010.315 A/Indonesia/NIHRDI-MRK228/2010|2010.353 A/Indonesia/NIHRDI-MS285/2010|2010.430 A/Indonesia/NIHRDI-NAD182/2010|2010.334 A/Indonesia/NIHRDI-NAD216/2010|2010.548 A/Indonesia/NIHRDI-SMG213/2010|2010.142 A/Indonesia/NIHRDI-TG077/2009|2009.510 A/Jakarta/FLUEJKSV0033/2012|2012.117	
		A/Indonesia/NIHRDI-BT226/2009|2009.241	Q227R
		A/Indonesia/NIHRDI-MRK228/2010|2010.353	N328K
E	176512	A/Indonesia/Nihrd-Dps_302/2016|2016.011	N/A*
		A/Indonesia/Nihrd-Dps_302/2016|2016.011 A/Indonesia/Nihrd-Pal350/2017|2017.170 A/Indonesia/Nihrd-Mks410/2017|2017.551 A/Indonesia/Nihrd-Mks408/2017|2017.052 A/Indonesia/02211874/2017|2017.140	R158G

		A/Indonesia/Nihrd-Dps_302/2016|2016.011 A/Indonesia/Nihrd-Mks410/2017|2017.551 A/Indonesia/Nihrd-Mks408/2017|2017.052 A/Indonesia/Nihrd-Plb499/2017|2017.164 A/Indonesia/07301041/2017|2017.575 A/Indonesia/Nihrd-Jbi_248/2016|2016.068 A/Indonesia/Nihrd-Mdn_281/2016|2016.068 A/Indonesia/Nihrd-Btm_185/2016|2016.041 A/Indonesia/Nihrd-Btm190/2016|2016.057	P210L
		A/Indonesia/Nihrd-Pal350/2017|2017.170 A/Indonesia/02211874/2017|2017.140	I208T, P210L, Q213H
		A/Indonesia/Nihrd-Mks408/2017|2017.052	S328R
G	332305	A/Indonesia/BKL0918/2019|2019.000 A/Indonesia/BKL0926/2019|2019.000 A/Indonesia/BKL0928/2019|2019.000 A/Indonesia/COV18.230/2019|2019.000 A/Indonesia/NIHRDSB195083/2019|2019.899	K158G
		A/Indonesia/COV18.230/2019|2019.000	G65D
		A/Indonesia/NIHRDSB195083/2019|2019.899	N/A*
		A/Indonesia/NIHRDSB195083/2019|2019.899	I538M, V545I

		A/Indonesia/NIHRDSB195084/2019|2019.901	A154S, K158G
H	413291	A/Indonesia/NIHRDBJM1205/2020|2020.221 A/Indonesia/NIHRDDPS858/2020|2020.210 A/Indonesia/NIHRDPAL742/2020|2020.085	N/A*
		A/Indonesia/NIHRDBJM1205/2020|2020.221	V325I
		A/Indonesia/NIHRDDPS858/2020|2020.210	I538M, V545I

Table 5. Point Mutations within antigenic epitopes of NA gene that had been rendered non-antigenic. (*) Epitopes disappeared due to mutation outside the antigenic epitope

Group by Year	Vaccine Strain (EPI_ISL)	Isolates	Mutation
B	176458	A/Jakarta/FLUEJKSV0031/2012|2012.085	D339G
		A/Indonesia/Nihrd-Drs490/2012|2012.049	H336N
C	170149	A/Indonesia/Nihrds-_0504/2014|2014.079	D339G
D	166310	A/Indonesia/Nihrd-Mmj337/2015|2015.496	I233V
E	176512	A/Indonesia/Nihrd-Bjm57.159	T69N
		A/Indonesia/Nihrd-Bjm576/2017|2017.159 A/Indonesia/Nihrd-Mks408/2017|2017.052 A/Indonesia/Nihrd-Plb499/2017|2017.164	D339N
F	311688	A/Indonesia/NIHRD-MKS540/2018|2018.123	N/A*
G	332305	A/Indonesia/BKL0918/2019|2019.000 A/Indonesia/BKL0926/2019|2019.000 A/Indonesia/BKL0928/2019|2019.000 A/Indonesia/NIHRDSB195083/2019|2019.899	N/A*

		A/Indonesia/NIHRDSB195084/2019|2019.901	
H	413291	A/Indonesia/NIHRDDPS850/2020|2020.189	R315S
		A/Indonesia/NIHRDDPS850/2020|2020.189	E381G
		A/Indonesia/NIHRDBPP1533/2020|2020.128	E381D
		A/Indonesia/NIHRDSMG722/2020|2020.243	N/A*

Discussion

In the past, a novel A/H3N2 strain first emerged in Hong Kong and quickly led to outbreaks that was associated with more than one million deaths world-wide 3. There was no prior documentation of A/H3N2 infecting humans at that time. Reassortment between HA and PB1 fragment of avian A/H3N2 with NA fragment of A/H2N2 from 1957 were suggested to be the reason of novel A/H3N2 appearance. This novel strain retained the ability of A/H2N2 to interact and transmit between human. These two viral strains continued to co-circulate in human until 1971, where A/H3N2 dominated. These outbreaks had led to enforcement of the immunization policy which include A/H3N2 strain as part of the trivalent influenza vaccine. This was followed by annual release of recommended vaccine strain by WHO on February and September to counter prevalent viral strain circulating in Northern and Southern hemisphere respectively.

The WHO recommended for tropical and subtropical countries to consider which vaccine formulation to be used independently based on the epidemiological condition 15. However, several studies have shown that both recommendations were frequently mismatched in tropical and subtropical region 6,16,17.

This study assessed the compatibility of WHO recommended vaccine strain with circulating strain between 2004 and 2021 using *in silico* method. Due to the lack of actual samples, the robust capability of data analysis and extensive calculation of bioinformatic tools had become indispensable for this study. Using isolates stored in GISAID database, this study provided an important insight on circulating virus in Indonesia compared to vaccine strain.

Phylogenetic analysis of HA and NA genes have revealed that several vaccine strains were incompatible with strains in circulation. Vaccine strain A/Perth/16/2009 (H3N2)-like virus was recommended in 2010 for Southern hemisphere, yet it was fully compatible with HA and NA gene of isolates from 2009. Furthermore, we observed that there was no vaccine strain compatible with isolates from 2010 – 2012 and 2019. In 2020, however, the vaccine strain was found to be compatible for both HA and NA gene.

Further analysis of vaccine strains and Indonesian isolates was done using Kolaskar and Tongaonkar Antigenicity Prediction method and Vaxijen 2.0. Through this analysis, the presence of antigenic epitope for both HA and NA gene from vaccine strain were identified. Mutations within antigenic epitopes of vaccine strains were obtained by comparing these epitopes with isolate sequences. From mutations that have been observed in Indonesian isolates, several mutations have been previously reported by different studies.

Mutation D93G on NA segment of A/H3N2 had been reported by Zhong, *et al*. to be one of many point mutations that drove antigenic drift 18. Another mutation, namely R158G, was reported by Biswas, *et al*. and was likely to influence the antigenicity of A/H3N2 due to its location within the proximity of potential glycosylation site 19. The analysis also revealed that there were mutations which were not found inside the antigenic epitopes of vaccine strain itself, yet caused several antigenic epitopes to completely disappear from Indonesian isolates. The effect of most mutations listed in this study, however, remains elusive due to our lack of actual samples to perform actual laboratory tests.

The actual performance of recommended vaccine formula towards circulating A/H3N2 could not be assessed. However, it could be argued that even if successful influenza vaccination was multifactorial, most of it had been attributed to HA mismatch of vaccine strain and circulating strain 20. Monto, *et al*. highlighted that aside from HA mismatch, neglected NA-specific immunity had been proven to contributes towards protection against influenza independently of HA segment 21. Both HA and NA segment acquired mutation over time during A/H3N2 circulation, which resulted in antigenic drift, reducing vaccine performance.

Several past studies had reported mismatch in A/H3N2 vaccine recommendation and circulating strain. Mott, *et al.* conducted a study about the re-emergence of influenza virus circulation in 2020 within tropical Asia and reported that some viruses from tropical countries belong to different genetic clade than recommended 2020-2021 northern hemisphere influenza vaccine (A/Hong Kong/267/2019) 16. Mott, *et al.* also suggested that that several factors such as surveillance artifact, travel restrictions, and COVID-19 intervention have affected the heterogeneity of influenza activity across countries in tropical Asia 16. Chan *et al.* conducted a study on seasonal influenza virus, especially on influenza A/H3N2 and influenza B between 1996 and 2012 17. He found that vaccine strains recommended by WHO for Northern and Southern hemisphere and viruses circulating in Hong Kong, China, were frequently mismatched.

This study had indicated frequent mismatch between recommended vaccine strain and circulating A/H3N2 strain in Indonesia between 2004 and 2021. Our finding was in line with several previous study that highlighted the mismatch between vaccine strain and circulating strain in tropical countries. Collectively, these studies suggested that there is a need for an improved influenza surveillance and response system for tropical and subtropical countries to better manage A/H3N2 outbreak in the region and formulate vaccine with higher compatibility towards strains in circulation.

There were several limitations to this study, which included the scarcity of influenza data from 2004 – 2006 that prevented this study from assessing compatibility of WHO recommended vaccine for that time period. Furthermore, prediction made in this study was purely based on *in silico* method, which might or might not represent the actual effect of mutation towards the antigenicity and vaccine performance. The effect of undocumented mutations found in Indonesian isolates also remained elusive.

CONCLUSION

This study revealed frequent mismatch between WHO recommended vaccine strains and A/H3N2 in circulation in Indonesia due to antigenic drift. However, due to the use of *in silico* approach, effects of undocumented mutations that caused the reduction or disappearance of antigenic epitopes in Indonesian isolates remained elusive. This highlights the importance of improved national surveillance and response towards A/H3N2 in an attempt to reduce the possibility of influenza outbreak, as well as development of influenza vaccine with higher compatibility for Indonesian population.

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