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The Proportion and Properties Change of Seasonal Influenza Virus H3N2 in Indonesia

Abstract

Influenza type B, H3N2, and H1N1pdm09 are the common circulating virus in humans. Recently WHO monitored that there is a change in the subtype proportion that circulating in the northern and southern hemispheres. The H3N2 3C.3a subclade switched to H3N2 3C.2a subclade and they increased in number. This study aimed to identify the current proportion and properties of seasonal influenza virus that circulating in Indonesia.

The study was part of ILI and SARI surveillance program which routinely conducted by the NIHRD. The viruses isolate titer measured by Hemagglutination Assay (HA), and virus strain or subtype was detected by Hemagglutinin Inhibition (HAI) Assay or RT-PCR and confirmed by sequence analysis.

The H3N2 specimens collected in Indonesia in 2014 - 2015 were very difficult isolate (undetected by HA and HAI Assays). The increase in number of H3N2 specimens was not accompanied by an increase in the number of specimens that can be in isolated. However, we detected significant increase of H3N2 by RT-PCR and confirmed as a new subclade (3C.2a) by sequencing. The variation of H3N2 behavior is mostly due to its genetics change. This is in accordance with the trend of global changes that occurred in both the northern and southern hemispheres.

Keywords: H3N2, Seasonal Influenza, Influenza trends.

Background

Seasonal influenza viruses spread in all parts of the world, ranging from the temperate climate up to the tropics. In the temperate climate countries, influenza have more severe symptoms compared to influenza in tropical countries. As in the United States annually 5-20% of the population infected with influenza virus and causes 49,000 deaths [1]. In tropical countries like Indonesia, influenza only causes productivity decrease of the patients. Although in some cases it can lead to hospitalization and a risk factor of death in patients with complications.

Influenza viruses can be divided into three types: A, B and C. Influenza type B (Yamagata and Victoria lineages), H3N2, and H1N1pdm09 are the common viruses circulating in humans, and they are used as the vaccine composition for years. However, influenza virus has a high mutation rate ranging from $1x10^{-3}$ to $8x10^{-3}$ substitutions per site per year, thus the virus strains circulating in the community remain changing [2]. The influenza virus change can occur through two mechanisms: antigenic drift and antigenic shift. Antigenic drift is a small-scale changes in the genes that occur continuously over time. Whereas antigenic shift is a major change in the influenza virus that occurs on the hemagglutinin or neuraminidase protein that resulting in a new type of infectious virus.

Recently WHO monitored that there is a change in the proportion of the subtype that circulating in the northern and southern hemispheres. The H3N2 3C.3a subclade switched to H3N2 3C.2a subclade and they increased in number. The H3N2 3C.2a subclade has some antigenic properties difference from the H3N2 3C.3a subclade. This affects the decrease in the effectiveness of vaccines used based on WHO recommendations.

Based on the results of influenza surveillance in Indonesia, it is known that in the last few years influenza H3N2 specimens are very difficult to isolate (not detected by the Hemagglutinin Inhibition assays). Therefore, it is possible that there is a change of circulating strains in the community. This study aimed to identify the recent proportion and properties of seasonal influenza virus H3N2 that circulating in Indonesia in 2014-2015.

Methods

The study was a part of the national influenza surveillance system that routinely conducted by the National Institute of Health Research and Development (NIHRD) and Center of Diseases Control and Environmental Health (CDC-EH). In the ILI system there are 26 sentinels spread in various provinces as representatives of the 34 provinces in Indonesia. Patients in the public health center with ≥ 38^{o}C fever, cough and symptoms onset no more than 10 days were taken nosal and throat swab samples after they signed the informed consent. The public health centers sent the samples to the regional laboratories to be tested using real time RT-PCR. After that, sampels were sent to the Center for Biomedical and Basic Technology of Health (CBBTH) laboratory as part of the NIHRD for confirmation and virus isolation.

The SARI surveillance is conducted in 6 hospitals located in 6 big islands. The samples are directly sent to the CBBTH from the hospitals for PCR testing and virus isolation.

Virus isolation performed using the confluent MDCK cells, and incubated for 5 days. The isolates then tested for viral titers with Hemagglutination Assay (HA). If the HA titer ≥ 16, it proceeded with the Hemagglutination Inhibition (HAI) assays to determine the strain in accordance with WHO guidelines [3-5]. We used A/Perth/16/2009-like (H3N2) antisera for HAI assay. Real time RT-PCR was carried out to particularly determine the presence of the H3N2 virus that cannot be detected with the HAI Assays. Virus isolates that were positive in real time RT-PCR proceeded by sequencing to identify the strain of the virus. Indonesian geographic site distribution that covers 1,904,569 km2 areas is drawn in the Figure 6 [6].

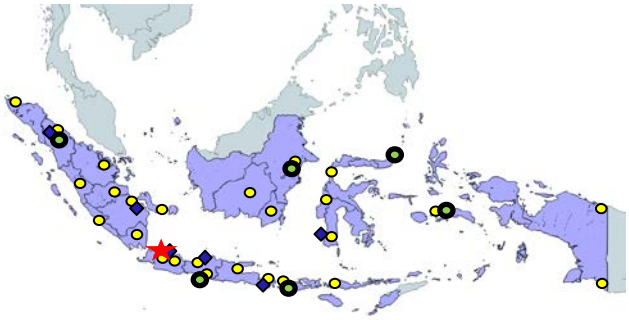

Figure 1. Map of ILI-SARI sites, regional laboratory and CBBTH-NIHRD Indonesia.

ILI sites, : SARI sites, : egional laboratory, CBBTH-NIHRD

Results

During the observation period it is known that the number of PCR positive samples which were obtained from the influenza surveillance activities in 2014 and 2015 (up to week 43) was 984 and 887. The proportion of H3N2 virus in 2014 is 40.1% and 43.1% for 2015. When compared with the data of 2012-2013, the increasing number is clearly shown in Table 1.

Table 1. The proportion of influenza virus circulating in Indonesia from 2013 to 2016.

	2013 (N:878)	2014(N:1285)	2015(N:984)	2016(N:887)*
H3N2	24.3	34.2	40.1	43.1
H1N1pdm09	28.8	20.4	11.3	22.4
Influenza B	46.9	45.4	39.6	34.5

*data up to week 43

The number of H3N2 viruses that can be isolated in Indonesia also decreased by time. It can be observed in Table 2, the H3N2 virus can be isolated in 2013 amounted to 13 isolates and reduced to 4 and 1 and even 0 in 2014, 2015 and 2016, respectively. Although we maintained specimens' cold chain all the way from health centers and hospitals to the regional laboratory and CBBTH laboratory, the length of specimens shipping was mostly more than 8 days (data not shown) which made the isolation success rate low.

Table 2. Number of Influenza Virus Isolated in 2013-2016.

	2013 (N:396)	2014(N:833)	2015(N:528)	2016(N:594)*
H3N2	13	4	1	0
H1N1pdm09	5	9	3	9
Influenza B	42	42	6	30

*data up to week 43

The Indonesian H3N2 specimens collected in 2014-2016 were very difficult to culture and identified using HAI assays. Table 3 shows the number of influenza virus titer that can be isolated (confirmed by HAI assays). Previously H3N2 can be identified with a strong titer, the majority is greater than or equal to 640. But the H3N2 virus circulating in Indonesia is getting harder to detect using HAI assays.

Table 3. HAI titer of isolated H3N2 virus using A/Perth/16/2009-like antisera.

Year / Titer	2013 (n…)	2014 (n…)	2015 (n…)	2016 (n…)
≤ 20	0	0	0	0
40	0	0	0	0
80	2	0	0	0
160	0	1	0	0
320	1	3	0	0
≥ 640	10	0	1	0

*data up to week 43

In 2015 the total number of positive specimens PCR was 594 from 4,494 specimens collected until October 2015. The increasing number of H3N2 specimens was not accompanied by an increasing number of specimens that can be in isolated. There was no H3N2 isolate can be detected by using HA and HAI assays. However, we detect significant increase of H3N2 by RT-PCR. We also found that some of the H3N2 collected in Indonesia was from the subclade of 3C.2a that recently circulates in Northern and Southern hemispheres.

Discussion:

Influenza virus is a virus that is easily transformed from time to time; this led to the composition of the vaccine which should always be taken into account to maintain the effectiveness of the vaccine. In addition, antibodies raised against influenza virus numbers are also decreased over time. In Indonesia, influenza vaccine is usually only used by people who are included in the high risk group such as the prospective pilgrims and particular lab workers.

Based on the results of influenza surveillance in Indonesia, it was known that H3N2 subtype proportion increasing and become dominant when compared with H1N1pdm2009 subtype and influenza type B. It can be an indication of a stronger

match between virus receptor with the host cell surface. HA protein is known to be responsible in the process of attachment to sialic acid on the host cell. NA is liable for removing the terminal sialic acid and destroying receptor for HA. It also accountable for inactivating the inhibitory molecules in the respiratory tract and promote the release of new virus [7]. Thus a genetic change in HA or NA could make the virus more vigorous.

In 2012-2013 the proportion of influenza B dominated influenza virus distribution in Indonesia. Although the number of H3N2 was not high, we managed to identify the virus into the clade of 3C.1. It was in accordance with the WHO for vaccine reference A/Texas/50/2012 [8]. Indonesian H3N2 isolates that grown in 2012-2013 were able to be identified using HAI with the reference sera from WHO. Changes began to occur in Indonesia since 2014, the H3N2 virus becomes difficult to isolate despite increasing proportion of the number of samples in the community. Once we did further tests, we found the virus existence by real-time RTPCR in the isolates, and the sequence was belong to the 3C.2a subclade. It's likely that our samples of Indonesia H3N2 isolates were able to grow, but it cannot be detected by HAI method.

H3N2 virus circulating in northern and southern hemispheres in 2014-2015 has a different antigens profile with the H3N2 virus strain recommended by the WHO for the vaccine. H3N2 has several antigenic parts; the part A or B?? that able to cause a reaction with the antibody. Other part ngga disebut kan? In humans, the majority producing antibodies is against part B antigen. Chambers et al. also showed the experimental results of a mutation that carried on the B antigen can cause inaccuracy of 2014-2015 influenza vaccine [9]. Even a change in an amino acid which is located near the receptor binding site can result in antigenic drift in the HA [10].

Due to the difference between the vaccine strains and circulating viral strains in 2014-2015, seroprotection observed in Finnish health workers who have been vaccinated showed low results 8.9% to 1.3%. When the vaccine strains match then seroprotection ranged from 60.8% to 87.0% [11]. The vaccine mismatched in 2014-2015 in northern hemisphere also occurred in the United Kingdom. Vaccine effectiveness in influenza confirmed patients are only by 3.4% against influenza and 2.3% against H3N2 [12].

In Indonesia vaccine mismatched with circulating virus strains in the community cannot be certainly analyzed, since there are not many Indonesian people who acquired influenza vaccine, only prospective pilgrims and particular lab workers. Therefore it became one of the limitations of our study. Nevertheless our influenza surveillance data can give us an idea about the change in the proportion and properties of H3N2 in Indonesia.

Conclusions

The influenza virus proportion has changed in Indonesia, from dominated by influenza type B into the influenza A(H3N2) in the period of 2012-2015. This is in accordance with the trend of global changes that occurred in both the northern and southern hemispheres. The increase in number of H3N2 specimens was not accompanied by an increase in the number of specimens that can be in isolated. However, we detected the existence of H3N2 by real time RT-PCR that confirmed as H3N2 from 3C.2a subclade.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

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