Detection of Genotype D8 Measles Virus in Indonesia in 2014

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Abstrak

Latar Belakang: *Campak adalah salah satu penyakit menular dan dapat menyebabkan penyakit serius sampai kematian. Campak masih merupakan masalah kesehatan masyarakat karena wabah campak masih terjadi di seluruh Indonesia. Surveilans berbasis laboratorium berikut penyelidikan epidemiologi molekuler memiliki kontribusi besar untuk mencegah wabah campak. Studi sebelumnya telah mendokumentasikan kehadiran genotipe virus campak G2, G3 dan D9 di Indonesia, dan genotipe lainnya seperti B3, D4, D5, D8 dan H1 telah terdeteksi di negara-negara tetangga. Penelitian ini bertujuan untuk mengkarakterisasi virus campak yang menyebabkan wabah di Indonesia pada tahun 2014.*

Metode: *Tujuh puluh empat spesimen urin yang dikumpulkan dari delapan provinsi dan diperiksa secara RT-PCR satu langkah serta metode sekuensing Sanger. Analisis sekuensing dilakukan dengan menggunakan perangkat lunak Bioedit 7.1; DNAstar 7.0 dan MEGA 5.0.*

Hasil: *Hasil PCR menunjukkan 34 dari 74 spesimen klinis positif dari virus campak. Kami menemukan genotipe dari 34 virus campak milik genotipe D8, D9 dan G3.*

Kesimpulan: *Campak pertama genotipe D8 telah terdeteksi dari Indonesia pada tahun 2014 meskipun campak lainnya genotipe masih dapat ditemukan di Indonesia.* ***(Health Science Journal of Indonesia 2016;8(1):1-5)***

Kata kunci: *virus campak, D8 genotipe, wabah, Indonesia*

Abstract

Background: Measles is a highly contagious viral disease. It remains an important cause of death among young children globally, despite the availability of a safe and effective vaccine. Measles is still a public health problem in Indonesia and measles outbreak still reported from many areas throughout Indonesia.. Molecular epidemiology of measles viruses is an important component in outbreak investigations to to monitor the presence of circulating wild–type measles strains.Previous studies in Indonesia have documented the presence of measles virus genotypes G2, G3 and D9 in Indonesia, and the other genotypes such as B3, D4, D5, D8 and H1 have been detected in neighboring countries. This study aims to characterize the measles virus that causing outbreak in Indonesia in 2014.

Methods: Seventy four urine specimens were collected from eight provinces and examined by one step RT-PCR and Sanger sequencing method. Sequencing analysis were conducted using Bioedit 7.1; DNA Star 7.0 and MEGA 5.0 software.

Results: The PCR results showed 34 out of 74 clinical specimens positive of measles virus. We found the genotype of 34 measles viruses belongs to genotype D8, D9 and G3.

Conclusion: The first measles genotype D8 has been detected from Indonesia in 2014 although other measles genotype still can be found in Indonesia. ***(Health Science Journal of Indonesia 2016;8(1):1-5)***

Keywords: Measles virus, D8 genotype, outbreak, Indonesia

Measles is one of the most infectious human diseases and can cause serious illness, lifelong complications and deaths. Measles is still a public health problem in Indonesia since the measles outbreak still occurred throughout the country. Therefore, virology surveillance following molecular epidemiology investigations have a major contribution to prevent and control of measles.[1]There are eight clades (A – H) of measles virus and have been divided into 22 genotypes. Clade A, E and F contain only one genotype, while clade B, C, D, G and H contain multiple genotypes; B1-3, C1-2, D1-10, G1-3, H1-2.[2] Previous studies have documented the presence of measles virus genotypes G2, G3 and D9 in Indonesia, and the other genotypes such as B3, D4, D5, D8 and H1 have been detected in neighboringcountries.[3] Genotype B3 was first detected in 1993 in Gambia but has subsequently been detected in cases from Philippines and Papua New Guinea.[4] Genotypes D4 and H1 appear to have been co-circulating in Australia.[3]Genotype D8 is associated with endemic transmission in India, Bangladesh and Nepal.[5]Genotype D8 also associated with importation in Australia, Japan, Malaysia and Singapore.[6,7]

Measles is one of the vaccine-preventable diseases, and the world measles vaccination coverage for the first dose was 84%.[8] Indonesia measles vaccination coverage for the first dose of measles vaccine was 97.85% in 2013 and 94.76% in 2014.[9] The available measles vaccine recently should be enough to provide protection since serum from a vaccinated person can neutralized all measles genotypes, although the efficiency varies.[10] Although it was high vaccination coverage, the outbreak still occurred sporadically in several provinces. The number of measles cases was reported from surveillance increasing from 11521 in 2013 to 12943 in 2014.[9] Accordingly, it is necessary to do the molecular testing to determine genotypes of measles virus that cause outbreaks and can help identify the epidemiological association between cases in a region with an outbreak location and discover importation cases. Here we report the complete genome sequence of wild-type measles virus isolated from measles cases during measles outbreaks reported in Indonesia in 2014.

Measles surveillance with laboratory confirmation of suspected cases has been implemented in Indonesia since 2003.[11] The laboratory test has been expand to molecular based for measles genetic characterization. Baseline data on circulating measles virus genotypes are needed for measles elimination, but such data are not available especially in measles outbreak.[9] In this context, we performed a pilot genetic analysis of the measles virus strains that was collected during outbreak measles in 2014. This study aims to characterize the genotypes of measles virus that caused outbreaks in Indonesia during 2014.

METHODS

Measles cases were identified and classified according to the World Health Organization (WHO) and national guidelines.[12] The urine was collected from suspected measles cases reported during January – November 2014 outbreaks. Measles virus were detected by RT-PCR assay[13] at virology laboratory of the National Insitute of Health Research and Development (NIHRD), Jakarta.

The RNA has been extracted directly from the samples using Viral RNA Mini Kit (Qiagen, Hilden, Germany) and performed reverse transcription PCR using a *One Step*RT-PCR kit (Qiagen) with previously described primers.[14] Virus genotyping was based on the 450-nt coding sequence for the carboxyl terminus of nucleoprotein (N) of measles virus, as recommended by the World Health Organization.[15]Amplicons were subjected to bidirectional sequencing using a Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA). We edited and aligned nucleotide sequences using Bio Edit 7.1 and DNA Star 7.0 software.[16,17] Phylogenetic analysis was performed by using the Neighbour Joining method implemented in the MEGA 5 program to compare the determined N gene sequences with the World Health Organization reference sequences.[1]

RESULTS

There were 74 measles suspected cases were reported during the outbreaks from 8 provinces. The data of the cases were presented on the tabel 1.

Urine samples were collected from all the cases. Shipment of the specimens from the outbreak location to NIHRD was done within five days with the cold chain standard procedure. There were 34 out of 74 specimens could be amplified. The specimens' positivity rate by RT-PCR test was 46% (Table.2). All provincesreported positive results, except Kepulauan Riau province.

Table 1. Demography status of measles suspected cases

Demography status	Frequency	Percent (%)
Sex		
Male	43	58
Female	31	42
Age		
Less than 1 year old	13	17.6
1 - 4 years old	27	36.5
5 - 14 years old	26	35.1
15 - 46 years old	8	10.8

Table 2. Urine Specimen 2014 by Provinces

Provinces	n	RT-PCR Positive Results (%)	Genotype
Bangka Belitung	4	1 (25)	D8
DKI Jakarta	2	2 (100)	D8, D9
Jambi	28	12 (43)	D8, D9, G3
Center Kalimantan	5	2 (40)	D9
East Kalimantan	3	2 (67)	D9
Kepulauan Riau	2	0 (0)	-
Riau	9	2 (22)	D9
South Sumatera	21	13 (62)	D8, D9
Total	74	34 (46)	

Phylogenetic analysis revealed measles virus strains circulated in Indonesia are genotype G3, D9 and D8 (Figure 1).

The distribution of measles genotypes can be seen in figure 2. There are two genotypes of which D9 and G3 which are still circulating in Indonesia. The Genotype D9 was detected in 5 provinces and Genotype G3 was only detected in Jambi Province. The Genotype D8 was detected in 4 provinces.

DISCUSSION

Measles virus identification by molecular assay through virology based surveillance prior to 2007 indicated that genotypes G2, G3, and D9 appeared to be the endemic genotypes in Indonesia and East Timor.[18] Genotype G2 has been detected in Indonesia in the early 2000s,[19] but no report for re-circulating.[1] Until recently, G3 and D9 are still circulating in Indonesia. This is understandable because the genetic measles virus is very stable and only slight variations in the sequences that occur in the long term. Mutation rate of measles virus ranged from 4-5 x 10^{-4} substitutions per base per year. The base substitution rate of the measles virus is lower than the estimated rate of substitution of other RNA viruses such as Influenza A, human immunodeficiency virus type 1 (HIV-1) which have ranged between 1.6 x 10^{-3} substitutions per base per year.[20]

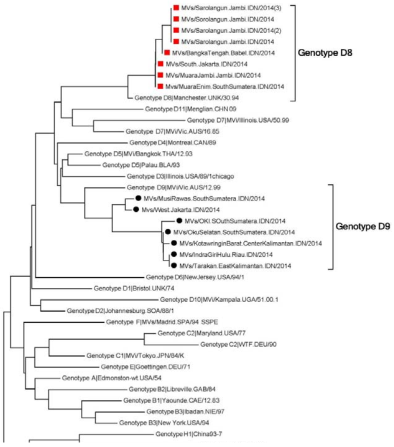

Figure. 1. Phylogenetic Measles virus in Indonesia, 2014 (based on NJ Methods)

Figure 2. Distribution of Measles virus Genotypes in Indonesia, 2014

Interestingly, we detected a novel measles virus strain, D8 genotype, from measles outbreak in several provinces in Indonesia. Mainly, the Genotype D8 was found in Malaysia,[21]Thailand[22] and Singapore.[7] During January – November 2014, Singapore also reported the D8 genotype in local residents. On the otherhand, Singapore also reported one imported cases of D8 genotype from Indonesia.[23] The D8 genotype that was detected in four provinces in Indonesia may be caused by infection of measles virus from outside of Indonesia, likely from neighboring countries. The results of the analysis using the BLAST-ncbi showed measles virus genotype D8 is closely related to the virus found in Kuala Lumpur (Malaysia) and Australia.[24] Nowadays the transportations across country borders are increasing rapidly and that it will increase the infection during the travelling. People from Indonesia can get infections including measles from foreigners and vice versa so measles genotypes that are found can be different from endemic genotype circulated in Indonesia. Since Measles can spread to many people easily by the respiratory route, including airborne transmission.

In 2014 the National Institute of Health Research and Development Ministry of Health Indonesia only received urine samples from 8 provinces in which 4 provinces were found to have genotype D8. The possibility of D8 genotype already existed in Indonesia before 2014 but not detected based on the fact that the focus of surveillance based on IgM detection and the delivery of virological specimens (urine) is still very low and did not cover all provinces. Indonesia has 34 provinces so it is necessary to conduct further studies with samples from a wider `geographic area of Indonesia to determine the spread and genetic diversity of these strains and ascertain their relationship to the global D8 strains. It would also be of interest to determine whether D8 strains co-circulate with G3 and D9 strains, whether they will eventually replace G3 as the predominant genotype in Indonesia.

Although there are many genetic variations of measles virus, but the virus has only one serotype which showed a high similarity of the entire surface antigen of measles virus genotype. Based on this, the antibodies due to vaccination or natural immunity can neutralize the entire genotype measles virus albeit with neutralization capabilities are different.

In conclusion, the first measles genotype D8 has been detected from Indonesia in 2014 alongside with genotype D9 and G3 which are endemic measles genotype in Indonesia. Molecular epidemiology of measles viruses is an important component in outbreak investigations to monitor the presence of circulating wild–type measles strains.

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