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Characterization and Identification of Poliovirus from the Environment in Indonesia 2015

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ABSTRACT

Background: Poliomyelitis is a disease caused by the poliovirus which causes abnormalities in the nervous system, commonly affects children and can be prevented by immunization. Since March 27th, 2014, Indonesia has been declared to be free of polio and anticipate the mutation of poliovirus type 2 (vaccine-derived polioviruses / VDPV). Indonesia will change the use of policy vaccines from triOPV to biOPV by erasing the P2 type before switching from OPV to IPV. Before the policy was implemented, it is needed to have research to determine the level of immunity of children against poliovirus and type of poliovirus which circulate in the area of OPV and IPV in Indonesia.

Methods: The research study was conducted in five cities in five provinces in Indonesia in 2015. The serum was taken on 100 children between 12-59 months and feces were taken towards 150 children in the same age range. The examination of antibody titers in serum performed using the method of neutralization.

Result: Research shows that in regions that apply OPV as routine immunization, about 94.5% of children had antibodies against poliovirus type 1, 96.8% against type 2 and 93.3% of type 3. In areas which implement IPV as routine immunization, children have gained 85% immunity against poliovirus type 1, 92% against type 2 and 93% of type 3. In the environments that implement OPV, poliovirus was found in feces specimens (0.5%) or the mixed waste (58%). All the circulating virus is a virus originated from Sabin vaccine which mostly comes from type 2 (85% of mixed waste and 60% of feces).

Conclusion: From this study, it can be concluded that sabin polio virus is still circulating in the area which applies OPV as routine immunization and poliovirus type 2 is commonly found there. Immunity inflicted on children varies in each region. Children living in a regular immunization using OPV and IPV had immunity for about 85-90%.

Keywords: Polio Antibody, Polio Environment, Circulation Virus

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INTRODUCTION

Poliovirus is a member of the Enterovirus genus, family of *Picornaviridae*, which infects millions of people, especially children, around the world every year.[1,2] Poliovirus itself can cause nervous system disease such as acute flaccid paralysis (AFP, poliomyelitis). Polio Free Certification for the *South East Asia Region* (SEARO) was achieved on March 27, 2014. The last case of wild poliovirus reported in the SEARO region was found in India in February 2011. There has been no detection of wild poliovirus virus, or *Vaccine Derived Polio Virus* (VDPV) in SEARO, including Indonesia, since then. Immunization government programs such as routine immunization, *mopping up*, *catch up* has been done to improve immunization coverage in Indonesia, and it is also held a National Immunization Week (PIN).[3-7]

The vaccine which is given to almost the worldwide is called the oral vaccine or OPV, live attenuated poliovirus. The characteristic of the polio virus which is mostly worried is the return of the wild virus origin from the vaccine virus (revert). This is caused of VDPV. Approximately 1% mutations in poliovirus type 1 and 3 causes poliomyelitis VDPV and consequently caused by the same VDPV with wild poliovirus. While the VDPV of polio vaccine virus type 2, there is only 6 nucleotide changes which have been regarded as VDPV.

Currently, there is no information about the seroprevalence of children in Indonesia. The results of serological studies conducted in children under the age of five after National Immunization Week II in 1998 in Jaya Pura, Irian Jaya, and Kota Waringin Timur, Central Kalimantan showed that 99% of children had antibodies to all three types of poliovirus, with vaccine coverage about 100%. A study conducted by Gendro Wahyuhono et al., about antibody status, in children under five in Bangkalan and Bondowoso in 2009 found that there are only 48% and 57% of children who have antibodies against all three types of poliovirus. It is reported that the immunization coverage from the two districts is less than 80% for routine immunization and above 90% for coverage during National Immunization Week.[8,9]

Since 2004, a study has been conducted to assess poliovirus circulation after implementation of poliovirus inactivated vaccine (IPV) in Yogyakarta. IPV vaccine is an inactivated vaccine given as an injection. IPV use eliminates the possibility of the emergence of VDPV. Serological surveys in 2010 on children who received complete OPV4 immunization in 4 districts and 1 town in Yogyakarta, conducted by National Institute of Health Research and Development, showed that 100% of children have antibodies against all three types of poliovirus with sufficient titers for protection.[8,9] The available seroprevalence data are still sporadic and not exhaustive of the antibody status of children in Indonesia. AFP surveillance carried out as part of the global polio eradication program.[6,10] The aim of the study to determine the level of immunity of children against poliovirus and type of poliovirus which circulate in the area of OPV and IPV in Indonesia.

METHOD

The study was conducted in five cities in five provinces in Indonesia in 2015. The Study was approved by ethical clearance number LB.02.01/5.2/KE320/2015 from National Institute of Health Research and Development, Ministry of Health Jakarta, Indonesia.

Serology

The serum was taken on 100 children between 12-59 months and feces were taken towards 150 children in the same age range. The examination of antibody titers in serum performed using the method of neutralization. Serum diluted ranging from 1/8 to 1/1024 and 25 µL aliquots into 3 well in the 96 well plate and added with a concentration 50TCID50 poliovirus. Virus and serum after incubation for 90 min added Hep2 cells and incubated for up to 5 days.[6]

Environmental surveillance

One liter of liquid waste was collected from integrated waste treatment system in PD Paljaya was taken as much as 1 liter using a grab sampling technique. Samples were delivered to the laboratory in cold conditions and were checked within 24 hours. Waste samples were extracted by using two-phase separation using a mixture of PEG solution, dextran, and NaCl. Sample mixture and reagent were stirred at a temperature of 2-8°C and left for a night in a flask with a vertical condition.[7]

The bottom and middle layer (upper and interphase) were collected and added chloroform and glass beads then centrifuged at a speed of 1000 g for 20 minutes. The supernatant was added penicillin-streptomycin (final concentration 100 IU/ml). 3 ml isolates were inoculated in 5 separated by 25 cm^2 flask containing cells and 1 L20B and 25 cm^2 flask containing RD cells already monolayer. Each vial put 0.5 ml of extract and incubated at 36°C for 5 days. Every day, an observation is conducted to identify the existence of a cytopathic effect (CPE) in the flask. Flask L20B which showed CPE 75% or more is stored in the freezer for examination intatypic Differentiation (ITD) with real-time reverse transcriptase Polymerase Chain Reaction method (RRT-PCR) by using KIT CDC from WHO. RD flask which shows CPE about 75% or more is stored in the freezer to conduct the cultured cells with the new L20B and incubated for 5 days. If the cells show CPE, then lead the ITD test. Enterovirus which only grows in the cells RD is a group of enterovirus with nonpolio virus (non-polio enterovirus / NPEV).[8]

The result of RRT-PCR which show discordant results (positive sabin on ITD examination and negative sabin when confirmed by VDPV screening assay), it is continued by conducting the VP1 sequencing examination.

RESULT AND DISCUSSION

Serology

Examination of antibodies against poliovirus was conducted on children aged 1-5 years. Examination of antibody titers in serum was conducted by using the method of neutralization using Hep2 cells. The antibodies in the serum will neutralize the virus and can be identified by the absence of cytopathic effect (CPE) in cells when it is observed under a microscope Hep2.

Most children have protection against poliovirus with a various level of immunity or against any type of poliovirus one, two, or three. Immunity level of children varies in each province Differences in the type of vaccine used also affects the level of immunity induced in children. A person has protection against poliovirus if he/she has antibody titers ≥ 8. The following table shows the level of immunity among children against poliovirus in the area using OPV or IPV as immunization routine, while still found the children who do not have or low antibodies against third type virus in Yogyakarta and Jakarta.

Environmental surveillance

Poliovirus enters into the human body through the mouth and replicates in the intestine and released into the environment along with the feces. Examination of poliovirus in the environment

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Table 1 The Proportion of Children with Polio Antibody Positive by Province and Type of Virus

Province	Children with antibody Polio (n)				
	PV1	PV2	PV3	PV1+PV2+PV3	No Antibody
Yogyakarta	85/100	92/100	93/100	82/100	4/100
Jakarta	92/100	89/100	86/100	78/100	5/100
West Nusa Tenggara	99/100	100/100	98/100	98/100	0/100
Bali	96/100	99/100	93/100	91/100	0/100
East Java	97/100	99/100	96/100	94/100	0/100

Table 2 The Proportion of Children with Polio Antibody Positive by Age and Province

Ages	Comparison of children who have antibodies to the number of children				
	Yogyakarta	Jakarta	West Nusa Tenggara	Bali	East Java
1 - < 2 years old	15/19	21/27	28/28	16/16	58/61
2 - < 3 years old	32/38	20/23	20/21	30/33	15/17
3 - < 4 years old	14/20	12/19	24/24	26/30	16/16
4-5 years old	20/23	26/31	20/21	20/21	5/6

Table 3 Positive Specimens Poliovirus in the Feces and Sewage WWTP

Provinces	Number of Samples (N)	Polio Virus
Feces		
DKI Jakarta	150	0
DI Yogyakarta	150	0
West Java	150	3
Bali	150	1
West Nusa Tenggara	150	1
WWTP waste		
DKI Jakarta	12	7
DI Yogyakarta	10	0

is conducted through the feces specimens and inlet fluid taken from Waste Water Treatment Plant (WWTP) by using the procedures established by the WHO. Feces specimens were taken from children aged 1-5 years who have received completed polio immunization with specimen collection and immunization within last than 3 months. The sample of a waste inlet from Waste Water Treatment Plant (WWTP) was taken every week in WWTP Sewon Yogyakarta and PD PAL Jaya Jakarta assuming that the polioviruses were circulating there due to its reservoir which is direct household waste.

Poliovirus is still found in the feces of children who received OPV and living in the area where OPV is applied as routine immunization. Children who get IPV and live in the area of IPV implementation as routine immunization do not excrete poliovirus. The same thing was also found in the liquid waste which is taken at the inlet sewage treatment in Yogyakarta. In Jakarta, however, where OPV is used for routine immunization, poliovirus can be detected (Table 3 and 4).

Characterization of Poliovirus in the Environment

Characterization of poliovirus with Real-time PCR and sequencing method showed that the type of poliovirus found in the environment is PV2 and PV3, which were found in the feces of children as well as the waste liquid inlet in Waste Water Treatment Plant, PD PAL Jaya Jakarta. Most of the circulating virus is poliovirus type 2 where the routine OPV vaccine was switched to bOPV in April 2016.

The result of RRT PCR, type PV2, and PV3 are taken from a children feces specimen which shows identical results with Sabin virus from the vaccine. The result indicates that the poliovirus from the vaccine was going out through the feces and infect children who are not getting an immunization. This reinfection is advantageous to provide passive immunization/secondary. If the virus is circulating in the child's body with abnormal immunity or circulate in areas that have low immunization coverage, it can lead to VDPV mutation which may cause epidemic poliomyelitis.[11]

Different results are shown in the specimen waste inlet PD PAL Jaya where the examination RRT PCR is showing results discordant in poliovirus type 2 because the virus has been found not wild poliovirus but not identical with the virus of polio Sabin/vaccine while poliovirus type PV3 shows the virus is similar to the vaccine virus. Examination sequencing continued with the examination of the samples containing poliovirus type 2. The results of the sequencing of the poliovirus type 2 in VP1 region indicate a change/mutation at nucleotide structure as shown in Table 5. The sample 5, 6, 7 have been done the second test in two difference polio laboratories, and the results remain the same.

Sabin poliovirus from a vaccine is genetically unstable and can become pathogenic virus when it replicates in the human intestine. Virus mutations can happen in the human body with abnormal immunity (immunodeficiency) or when they replicate in the body of the children with low immunization

Table 4 Serotypes of Poliovirus in the Feces and Waste WWTP

Provinces	Polio Positive (n)	Serotype		
		PV1 (n)	PV2 (n)	PV3 (n)
Feces				
West Java	2	0	2	0
Bali	1	0	1	0
West Nusa Tenggara	1	0	1	0
WWTP waste				
DKI Jakarta	7	0	6	1

Table 5 Results of Poliovirus type 2 Sequencing

No of Sample	Sequencing Result of PV2 Sabin Like
Sample 1	2 nucleotides differences (A427G, A505G)
Sample 2	Single nucleotide difference (T428C)
Sample 3	3 nucleotides differences (T428C, T615C, C810T)
Sample 4	4 nucleotides differences (C27T, T66A, G301A, T428C)
Sample 5, 6, 7	5 nucleotides differences (C27T, T66A, G301A, T428C, T900C)
Sample 8	Single nucleotide difference (A427G)

coverage. This mutation is permanent, and it would be accumulated to infect other individuals who have not provided by polio immunization.

The global eradication of poliovirus is still a challenge due to some endemic areas, including Afghanistan and Pakistan.[12,13] In some areas where it has been considered free of the disease, it is still found some outbreaks in China which is bordered by Pakistan. The failure to stop poliomyelitis in endemic countries has resulted in about 200,000 new cases each year worldwide.[14] When poliomyelitis has been eradicated then, it will save the costs at least USD 40-50 million for the next 20 years, especially countries with low income. By conducting the eradication, there will be no longer children who will suffer from incurable paralysis. The improper immunization will result in a sensitivity of more infection, although it is not found any more cases of poliomyelitis in that country. This was the import cases occurred in Indonesia in 2005.[15-17]

Based on WHO recommendations, the change about 1% or more in the nucleotide arrangement can be categorized as *Vaccine Derived Polio Virus* (VDPV), but criteria against viruses PV2 modified in 2011 were the changes more than 0.6% nucleotides in VP1 region compared to the reference can be stated as VDPV. In this study, it is found only 1-5 nucleotides (0.1-0.5%) that have changed, and virus type 2 is still found to be in the Sabin category. VDPV circulation type 2 is found in several countries in the world such as Afghanistan, Nigeria, and Egypt.[18-20]

CONCLUSION

OPV and IPV immunization can induce immunity for about 85-90% of children. Sabin poliovirus is still found in the environment where the OPV is applied as routine immunization. Poliovirus Type 2 is more common, and it is circulating in Jakarta which has mutated 1-5 nucleotides, if the mutation is more than 6 nucleotide, it will be VDPV that can cause paralysis.

CONFLICT OF INTEREST

All authors declare there is no conflict of interest regarding publication of this manuscript.

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