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Original Article

Poliovirus shedding after the first and second doses of trivalent polio vaccines in newborns

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Abstract

Background The trivalent oral polio vaccine (tOPV) produced by *Bio Farma* consists of three live, attenuated poliovirus serotypes (1, 2, and 3). The tOPV stimulates the formation of secretory IgA (sIgA) on the intestinal wall and lumen. The existence of sIgA is considered giving immunity in the intestines, it could prevent the spread of viral replication and thus inhibit the transmission of the polio virus.

Objective To determine the differences in shedding after each of the first two tOPV immunizations in newborns.

Methods This one-way repeated measure study was conducted in newborns from three primary health centers in Bandung, West Java. After administering tOPV to newborns, we assessed the shedding of poliovirus in their stool specimens at 30 days after the first dose and 7 days after the second dose. Data was analyzed using McNemar test with 95% confidence intervals (CI) to differentiate the shedding of poliovirus after the first and second doses.

Results Of 150 children, 128 subjects completed the study. At 30 days after the first tOPV dose, 26 subjects (20.3%) were negative for shedding of poliovirus in stool specimens. Of the 102 subjects who had poliovirus isolated from their stools, the serotypes comprised of polio 1: 10.9%, polio 2: 14.8%, polio 3: 45.3%, polio 1 and 3: 3.1%, polio 2 and 3: 4.7%, and polio 1,2, and 3: 0.8%. At 7 days after the second tOPV dose, there was a significant increase in subjects negative for shedding of poliovirus (78 subjects; 60.9%). Statistical analysis revealed significantly decreased shedding of poliovirus in stool specimens between the first and second doses of tOPV ($P<0.05$).

Conclusion There is a significantly decreased number of subjects with shedding of poliovirus in stool specimens 7 days after the second tOPV dose than at 30 days after the first tOPV dose. **[Paediatr Indones. 2015;55:219-23]**.

Keywords: *tOPV, newborns, shedding of poliovirus, stools*

Since 1980, Indonesia aimed to be free of polio by the year 2005. The Ministry of Health conducted a polio eradication program, the Immunization Development Program (PPI), intensively throughout the regions.[1,2] In 2005, three countries, including Indonesia, which had been declared to be free from polio for two years, suddenly had polio outbreaks. This event indicates that a number of children are prone to poliovirus due to a lack of or incomplete implementation of the immunization schedule.[3-5]

The trivalent oral polio vaccine (tOPV) produced by Bio Farma consists of three live, attenuated poliovirus serotypes (1, 2, and 3), and has been well used in the routine or supplementary immunization program in Indonesia.[1] The tOPV was selected to break the transmission of wild-type poliovirus in the community, as this vaccine stimulates the formation of secretory IgA (sIgA) on the intestinal wall and lumen.[6,7] The existence of sIgA is considered giving immunity in the intestines, it could prevent the spread

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of viral replication and thus inhibit the transmission of the polio virus.[7] The Indonesian Ministry of Health recommends an immunization schedule with the first tOPV dose given to newborns, followed by up to four monthly doses.[2]

The first dose of vaccine in general will lead to the formation of antibodies in small amounts after a longer latent period compared of the secondary vaccination. Growth period perform after latent period, in which the antibody increased rapidly until 4 weeks after first vaccination. Reentry after a certain period of antigen will cause secondary reactions, with the maximum antibodies will attained in just few days. Therefore if poliovirus shedding in stool specimen still remain after 7 days, means that the poliovirus still multiplied, and the gastrointestinal tract failed to form a mucosal immunity.[7] A previous study showed that in newborns, shedding of poliovirus decreased between one week and three weeks after the third dose of tOPV.[8]

The aim of this study was to compare the number of subjects with and without poliovirus shedding in stool at 30 days after the first dose and at 7 days after the second dose of tOPV.

Methods

We conducted a one-way repeated measure study from January to May 2008 at three primary health centers (Garuda, Padasuka, and Puter) based in Bandung, West Java. We used consecutive sampling for collecting subjects. The inclusion criteria were healthy, full term, newborn infants, residing within a relatively short and easily accessible distance (<30 km) from the study clinics and not planning to travel during the study period; born after 37 weeks of pregnancy, of birth weight ≥ 2.5 kg, and had father, mother, or legally acceptable representative properly informed about the study and signed the informed consent form, parents committed to comply with the instructions of the investigator and with the trial schedule, as well as mothers who were at least elementary school graduates. We excluded subjects who concomitantly enrolled or were scheduled to be enrolled in another trial, had a known history of congenital or acquired immunodeficiency (including HIV), had an evolving moderate or severe illness, especially infectious disease or fever (axillary temperature ≥37.5°C), required hospitalization at birth; or were immunized with non-scheduled OPV during the trial.

This study was approved by the Ethics Committee of Dr. Hasan Sadikin Hospital. The protocol was approved by the Internal Committee of Bio Farma and the Institutional Ethics Committee and Indonesian Regulatory Authorities. This trial was conducted in accordance with the latest Edinburg, Scotland revision of the Declaration of Helsinki, ICH Good Clinical Practice guidelines,[9-11] and local regulatory requirements.[12]

The tOPV contains live, attenuated Sabin poliovirus, with one dose corresponding to two drops (0.1 mL). Each dose (batch #202186) contained poliovirus type 1: $\geq 10^{6.0}$ $TCID_{50}$; type 2: $\geq 10^{5.0}$ $TCID_{50}$; and type 3 $\geq 10^{5.8}$ $TCID_{50}$ (TCID= tissue culture infective doses). Two drops were administered directly into the infant's mouth from a multi-dose vial through the dropper. In this study, each subject was given their own vial.

Subjects were given the first dose of tOPV soon after birth, and the second dose on the second visit, 30 days after the first dose. Stool specimens (8g or 1 tablespoon) were collected from all subjects. Prior to arrival at the health center, specimens were stored in a container with an ice pack, to ensure that the temperature was maintained at 0–8 °C. Stool specimens were sent to the health center or collected by the health officer within 24 hours. At the health center, stool specimens were stored at 0–8°C until it was dispatched to the Bio Farma Polio Laboratory. Stool specimens were examined for the presence of poliovirus. Poliovirus was isolated in L20B and RD cell lines. The L20B was the media cell derived from rats. These cells were selective for the poliovirus, producing a characteristic enterovirus cytopathic effect (CPE). Several non-poliovirus were able to produce CPE in L20B (e.g., adenovirus and reovirus), but the CPE was generally different from the CPE induced by poliovirus. The RD was the media cell line derived from human tumor cells (rhabdomyosarcoma). These cells had a high sensitivity to enteroviruses, producing the typical enterovirus CPE. Each positive isolate was then subjected to intratypic differentiation (ITD) by ELISA and probe hybridization, according to the *WHO Polio Laboratory Manual 2004.*[13] Standards, reagents,

and guidelines were the same as the ones used in the *WHO Global Polio Laboratory Network*.[14] The stool was processed according to the standard protocol used by the *National Polio Laboratory*.[15]

McNemar test was used to compare shedding of poliovirus after the first and the second dose of tOPV with 95%CI. The P values of less than 0.05 were considered to be statistically significant.

Results

A total of 150 healthy newborns were enrolled in this study. Only 128 subjects received two doses of the vaccine and completed the study, 72 of whom were male. Of the 22 children who were withdrawn from the study, 9 received another polio vaccines from midwives, 2 moved to another city, and 11 failed to return according to schedule.

Vaccine poliovirus was isolated from subjects' stool specimens 30 days after the first tOPV dose. We found excreted poliovirus in 79.6% of subjects, consisting of 10.9% type 1, 14.8% type 2, 45.3% type 3, 3.1% types 1 and 3, 4.7% types 2 and 3, and 0.8% types 1,2, and 3. At 7 days after the second tOPV dose, we found that shedding of poliovirus decreased to 39.1% of subjects, consisting of 6.3% type 1, 13.3% type 2, 15.6% type 3, 3.1% type 1 and 3, 2.3% type 2 and 3 (**Figure 1**).

Statistical analysis with McNemar test revealed that there were significantly more negative subjects at 7 days after the tOPV 2nd dose (60.9%), compared

Table 1. Poliovirus excretion in stool specimens 30 days after the first tOPV dose and 7 days after the second tOPV dose

	Poliovirus excretion		P value
	30 days after the first tOPV	7 days after the second tOPV	
Negative	26	78	0.001
P1	14	8	
P2	19	17	
P3	58	20	
P2+P3	6	3	
P1+P3	4	1	
P1+P2+P3	1	1	
Total	128	128	

P1, P2, P3: poliovirus serotype 1, 2, or 3

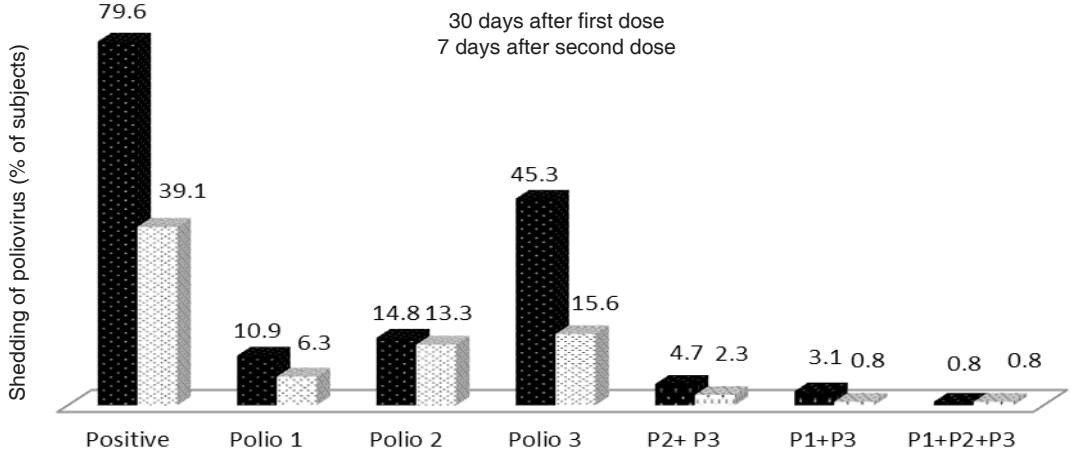

Figure 1. Shedding percentage of poliovirus in stool specimens at 30 days after the first dose and at 7 days after the second dose. [Note:P1, P2, P3 = poliovirus serotype 1, 2, or 3].

to 30 days after the first dose (20.3%), with 95% CI of differences 43 to 75%, P=0.001 (**Table 1**).

Discussion

The Global Polio Eradication Initiative (GPEI) has exclusively relied on the oral poliovirus vaccine (OPV), rather than the inactivated vaccine, primarily because of its superior ability to induce gut mucosal immunity.[16] This vaccine mimics natural infection and induces both circulating and secretory antibodies that protect, not just against paralytic disease, but also against infection.[17]

In this study, we found significantly decreased shedding of poliovirus in stool specimens after the second tOPV dose (95%CI of differences 43% to 75%, P=0.001). However, a study found slightly diminished shedding of poliovirus in 2-month-old infants between 1 week and 3 weeks after the first dose of tOPV (92% vs. 81%, respectively). There was an additional decrease in shedding of poliovirus at 1 week and 3 weeks after the second dose of tOPV (22% *vs*. 5%, respectively).[8] Furthermore, at 7 days after tOPV immunization, another study found poliovirus excretion in infants aged 9 months, to be 96.8%, 98%, and 88% for serotypes 1, 2 and 3, respectively. At 30 days after tOPV immunization, there was a significant decrease in shedding of poliovirus.[18]

Oral polio vaccines have been shown to result in reduced shedding of poliovirus in stool specimens after administration of a subsequent "challenge" dose of the live, attenuated vaccine.[18] Reduction in viral shedding is associated with an increase in poliovirus-specific immunoglobulin A (IgA) secreted in the intestine.[19] Secretory IgA is not found after immunization with inactivated poliovirus vaccine, except among individuals with prior exposure to live poliovirus, which explains the more limited impact of the inactivated vaccine on poliovirus shedding in the intestine after subsequent challenge.[20] As stated in previous studies, the decreased shedding of poliovirus in stool can be used as a proxy for mucosal immunity.[19]

Some limitations of our study were that we did not directly assess mucosal immunity nor did we assess for poliovirus shedding prior to the administration of the first tOPV dose.

In conclusion, there are significantly more subjects whose stool specimens were negative for poliovirus at 7 days after the 2nd tOPV dose than at 30 days after the 1st dose, indicating a decrease in poliovirus shedding at later doses. Therefore, tOPV should be given repeatedly to provide immune respons to polioviruses.

Conflict of interest

None declared.

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