Prevalence of poliovirus vaccine strains in randomized stool samples from 2010 to 2018: encompassing transition from the trivalent to bivalent oral poliovirus vaccine

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Abstract Global eradication of poliovirus (PV) has previously relied on the live attenuated oral poliovirus vaccine (OPV). However, in order to eliminate the risk of vaccine-associated paralytic poliomyelitis, the use of OPV will soon be discontinued. Thailand has introduced inactivated polio vaccine since December 2015 and replaced trivalent with bivalent OPV since April 2016. To provide crucial surveillance data during this polio vaccine transition period, poliovirus shedding in stool was performed. A total of 7446 stool samples between 2010 and September 2018 were tested for poliovirus using reverse-transcription polymerase chain reaction. Approximately 0.44% (33/7446) of the samples tested were positive for PV. All positive specimens had more than 99% homology with the Sabin vaccine strain, based on complete VP1 nucleotide sequences. Although trivalent OPV use has been discontinued in Thailand since April 2016, PV type 2 could be detected in stool samples collected in May 2016 but has not been found afterwards. The use of bivalent OPV was able to reduce PV type 2 shedding in stools and could contribute to the reduction of vaccine-associated paralytic poliomyelitis in Thai children.

Keywords Enterovirus · Inactivated polio vaccine · Poliovirus · Stool · Thailand

Introduction

Prior to the development of the poliovirus (PV) vaccine, polio was a devastating childhood disease. Although mild symptoms were characterized by vomiting, headache, myalgia, paralytic poliomyelitis caused debilitating morbidity. PV infects the central nervous system and replicates in the motor neurons of the spinal cord, brainstem, or motor cortex [23]. Humans are the primary natural hosts. Transmission occurs via the fecal–oral route. Infected individuals may shed virus for weeks to months and some immunocompromised people may shed virus for a year or more [12].

The introduction of the Salk inactivated polio vaccine (IPV) in 1955 was followed by the more highly effective Sabin live attenuated trivalent oral polio vaccine (tOPV) in 1961, which included all three attenuated PV strains (PV type 1–3) [3, 8, 28]. The tOPV has proven to be inexpensive to produce, easily administered orally but sensitive to high temperature resulting in the need for a strict cold chain [24]. The World Health Organization (WHO) expansion of tOPV in the vaccine immunization programs has resulted in a drastic decrease in the incidence of poliomyelitis worldwide. As a result of global immunization efforts, only Pakistan, Afghanistan, and Nigeria remained endemic for wild PV (WPV) [27].

Although tOPV has been implemented worldwide, WPV and circulating vaccine-derived PV (cVDPV) are still detected [27]. As a result of viral revertants shedding in some vaccinees, poliovirus was re-introduced into the environment [12]. It can infect immunocompromised individuals and cause poliomyelitis [14]. Such vaccine-associated paralytic poliomyelitis (VAPP) is estimated at approximately 2.9–4.7 annual cases per million births globally, representing one poliomyelitis per 1–2.5 million

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OPV dose [21]. As of June 2018, there were six countries (The Democratic Republic of the Congo, Kenya, Nigeria, Somalia, Syria, and Papua New Guinea) that reported cVDPV detection [13].

Since 1988, the WHO launched the Global Polio Eradication Initiative (GPEI) to interrupt WPV circulation everywhere. The number of countries with endemic poliovirus has reduced from 125 to 3 [27]. Finally, the Global Commission for the Certification of Poliomyelitis Eradication (GCC) has already certified the elimination of WPV type 2 worldwide in September 2015 [1, 10]. WPV type 3 has not been detected since November 2012. Nevertheless, WPV type 1 remains endemic in Afghanistan and Pakistan [13]. Presently, more than 80% of the world's populations live in certified absence WPV transmission, which includes America, Western Pacific, European and South East Asian regions.

To eventually eliminate VDPV contamination, "The Polio Eradication and Endgame Strategic Plan 2013-eliminate VDPV contamination 2018 was developed. It aimed to capitalize on this new opportunity to end all polio disease. It accounts for the pursuit of WPV and cVDPV elimination while planning for the backbone of the polio effort to be used for delivering other health services to the world's most vulnerable children. This is possible with the availability of an improved IPV, which has enhanced potency, increased effectiveness, and no serious systemic adverse reactions compared to the original IPV [9]. IPV is now used exclusively in many higher-income countries in their national immunization programs, which has effectively eliminated WPV and VAPP. This new campaign recommends the administration of at least one IPV dose in all countries [5, 9, 20]. There are major barriers, however, in IPV introduction in low and middle-income countries due to cost, availability, and distribution logistics.

In anticipation for the change in polio vaccination, infants in Thailand began receiving IPV at month 4 in December 2015 under the existing vaccination regimen of tOPV at month 2, 4, 6, 18, and 48-60. From May 2016 onward, all tOPV stock in the country was withdrawn and its use was replaced by the bivalent OPV (bOPV) devoid of PV type 2 [11, 16]. Overall, polio immunization coverage in Thailand is reportedly 99% [18]. Additionally, the use of bOPV is expected to be discontinued by 2020 whereby all infants would only be immunized with IPV. Given such changes in the vaccine policy, we tested for PV in stool samples in a patient cohort from 2010 to 2018, which encompassed this transition period. This cohort includes patients who provide stool samples for enteric virus testing and therefore served as convenient samples for PV screening. These results are expected to assist in PV surveillance in Thailand, which are crucial for regional PV elimination.

Materials and methods

This study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University (IRB 002/60). The IRB waived the need for written informed consent because samples were de-identified and anonymized. Stool samples conveniently available for enteric virus testing were screened for the presence of PV. A total of 7446 specimens from patients in Bangkok ($n = 4933$) and Khon Kaen province ($n = 2513$) were obtained between January 2010 and September 2018.

Laboratory tests

Stool samples were diluted in phosphate buffer saline to a 10% suspension and centrifuged. Viral nucleic acid was extracted from the supernatant using a viral nucleic extraction kit (GeneAid, Seoul, Korea). Initial screening for enterovirus was performed using pan-enterovirus assay and one-step real-time reverse-transcription polymerase chain reaction (RT-PCR) (SensiFAST, Bioline Reagent, London, UK) to identify EV-A71, CV-A6, and CV-A16 as previously described [22]. Pan-enterovirus positive samples were subjected to complementary DNA (cDNA) synthesis using Improm-II Reverse Transcription system (Promega, Madison, WI), amplified with enterovirus primer sets 5'UTR/VP2 region [6, 17], and sequenced. PV-positive samples were subjected to complete VP1 gene amplification using published primers [15]. Nucleotide sequences obtained from this study were deposited in the GenBank database under the accession numbers MF593155-MF593178.

Phylogenetic analysis

VP1 nucleotide sequences were analyzed using Basic Local Alignment Search Tool (BLAST) on NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), edited using Chromas Lite software version 2.01 (Technelysium, South Brisbane, Australia), and assembled using SeqMan II (DNA STAR, Madison, WI). The phylogenetic tree was constructed using the maximum likelihood and optimal nucleotide substitution models as identified in ModelTest and implemented in the MEGA6.0 software package [25] with a bootstrap value of 1000 replicates. The complete VP1 sequences available in the NCBI database served as references.

The individual site-specific selection pressure on VP1 was measured using the likelihood-based fixed effects likelihood (FEL) and single likelihood ancestor counting (SLAC) methods contained in the HYPHY package of Datamonkey [7]. The significance level for the positively selected site was accepted at < 0.1 (two-tailed binomial distribution).

Table 1 Demographic data of individuals whose samples were PV-positive

	Total specimens	PV type 1	PV type 2	PV type 3
<i>Age (months)</i>				
0 < 2	334	–	1	3
2 < 4	159	5	5	2
4 < 6	211	1	1	2
6 < 18	1626	3	3	2
≥ 18	5116	2	1	2
<i>Province</i>				
Bangkok	4933	7	7	9
Khon Kaen	2513	4	4	2
<i>Sex</i>				
Male	4450	7	9	4
Female	2996	4	2	7
<i>Year</i>				
2010	312	1	–	–
2011	351	1	3	–
2012	1162	2	–	1
2013	264	–	1	–
2014	260	1	–	3
2015	595	1	2	4
2016	1062	3	5	3
2017	1583	–	–	–
2018	1857	2	–	–
Total	7446	11	11	11

Results

Molecular diagnosis

During this study period, 18.8% (1397/7446) of the specimens tested were positive for one-step real-time RT-PCR. Analysis of the 5'UTR/VP2 region revealed that 0.44%

(33/7446) of samples were positive for PV (Table 1). Among the 33 PV-positive specimens, 11 were PV type 1, 11 were PV type 2 and the rest were PV type 3. All positive specimens belonged to patients who did not have symptoms related to poliomyelitis. More than 90 percent were detected in stool samples belonging to children younger than 18 months of age, all of whom were born after the universal polio vaccination. Two PV positive samples (1 each of PV type 2 and 3) were from adults older than 30 years of age whose vaccination histories were not available. During the transition period in 2016, PV was found in all age group; 2–4 months, 4–6 months, 6–18 months, and over 18 months (4, 2, 3 and 2 cases, respectively). Although tOPV has been replaced by bOPV since April 2016, PV type 2 remained detectable in stool samples up to May 2016 but undiscoverable afterwards (Fig. 1). For each of these years, the detection of different PV types did not appear to cluster.

Molecular characterization and phylogenetic analysis

In this study, all positive specimens had more than 99% homology with the Sabin vaccine strain based on complete VP1 sequences. The phylogenetic analysis of 24 complete VP1 sequences showed three clusters of PV lineage consistent with 3 PV types (Fig. 2). It also supported the initial PV type assignments by the 5'UTR/VP2 region in which all sequences were closely related to their respective Sabin vaccine strains. No nucleotide deletions or insertions were observed among the PV identified in this study, while most nucleotide substitutions in the coding region were synonymous.

To evaluate the adaptive molecular evolution of PV, we estimated the ratio of non-synonymous to synonymous substitutions (dN/dS) within the VP1 region. The overall

Fig. 1 The distribution of PV identified between 2010 and 2018. Left scale represents the number of PV-positive samples, while the right scale represents the total number of samples screened

Fig. 2 Phylogenetic analysis of each PV type to cluster three lineages based on the complete VP1 region. Nucleotide sequence comparison with the reference strains using the maximum likelihood method implemented in MEGA6. Bootstrap values > 70% are shown at the branch nodes. Scale bar represents a nucleotide substitution rate

dN/dS value was 0.056, while individual dN/dS values were 0.046 (type 1), 0.065 (type 2), and 0.034 (type 3).

Discussion

Official epidemiological surveillance of PV has reported the presence of poliomyelitis in Thailand since 1952 (Fig. 3). The incidence of acute flaccid paralysis (AFP)

possibly linked to PV infection in the country was also monitored beginning in 1992. Although tOPV vaccination in selected Thai provinces began in 1977, implementation nationwide did not occur until 1982 (Fig. 4). Since then, national disease surveillance combined with improved vaccine coverage resulted in a dramatic decrease in the incidence of poliomyelitis in Thailand [3, 26]. The last reported WPV in Thailand occurred in Loei province in April 1997 (identified from a stool sample of a 10-year-old child who had received only one tOPV dose) and the last reported vaccine-derived poliomyelitis case was published in 2003 (PV type 2 identified from an 18-month old boy who completed polio vaccination). As per WHO recommendation, the Thai Ministry of Public Health has implemented the administration of IPV in combination with tOPV or bOPV for childhood vaccination beginning in December 2015.

Although tOPV has contributed to a significant decrease in infection by WPV due to its many advantages, there are several reasons to discontinue tOPV in favor of bOPV. Phenotypic changes in the attenuated PV strains in the tOPV due to their genetic instability could emerge and the resulting variants can cause poliomyelitis [4]. Studies on PV in feces and in sewage have suggested that VDPV replication in populations with low polio vaccine coverage can result in reversions of the attenuating mutations [21]. PV type 2 has been associated with VAPP cases [4]. Given the currently low incidence of polio around the world, the WHO Strategic Advisory Group of Experts on Immunization (SAGE) has determined that the risks of tOPV outweighed the benefits and therefore promoted the use of bOPV as an alternative.

The near elimination of all PV around the world has also made the use of IPV an attractive option. The inactivated IPV also contains all 3 PV types, which provides protection from type 2 lacking in bOPV. Even outbreaks attributed to VDPV type 1 are reported [2, 13]. An increasing number of countries are using IPV for routine vaccinations because it does not cause PV shedding in feces nor contribute to VAPP [28]. For these reasons, IPV has a prominent role in the ongoing efforts towards an eventual global PV eradication.

Prevalence of poliovirus detected in other studies also reflected different cohort characteristics and geographical regions. In this study, the majority of PV positive samples were from patients born after the universal polio vaccination. PV was detected in 0.44% (33/7446), which was lower than the rates previously reported [19]. Even though specimens from only two provinces were tested, the sample size was large enough to reflect PV shedding during PV vaccine transition period in Thailand. All three PV types found here were closely related to the Sabin vaccine strains, most of which were identified in children who had

Fig. 3 The annual incidence of poliomyelitis and AFP relative to polio vaccine coverage between 1952 and 2018 in Thailand. Blue bars represent poliomyelitis cases due to natural PV infection, while red

bars represent AFP cases (left scale). Polio vaccine coverage is denoted by the dotted green line (right scale) (color figure online)

Fig. 4 Timeline for PV vaccination in Thailand

received PV vaccine. Moreover, there was no evidence of positive selection, suggesting that nucleotide changes identified in this study were below the thresholds for the possible emergence of VDPV. Interestingly, two PV positive samples were from an adult. Due to the lack of clinical data, we could only assume that these adults had exposure to PV possibly from households with recently vaccinated children or from other environmental exposure.

PV type 2 has not been detected since 2017 possibly due to the fact that presently almost all children received bOPV. The withdrawal of tOPV since in 2016 and the beginning of bOPV and IPV combination has eliminated the circulation of PV type 2. Continued surveillance of PV and AFP is still necessary in order to provide a crucial assessment of VDPV.

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Compliance with ethical standards

Conflict of interest None of the authors have any conflicts of interest to declare.

References

- Adams A, Salisbury DM. Eradicating polio. *Science*. 2015;350(6261):609. <https://doi.org/10.1126/science.aad7294>.
- Akil L, Ahmad HA. The recent outbreaks and reemergence of poliovirus in war and conflict-affected areas. *Int J Infect Dis*. 2016;49:40–6. <https://doi.org/10.1016/j.ijid.2016.05.025>.
- Bahl S, Hasman A, Eltayeb AO, James Noble D, Asia Thapa A. The switch from trivalent to bivalent oral poliovirus vaccine in the south-east region. *J Infect Dis*. 2017;216(suppl_1):S94–100. <https://doi.org/10.1093/infdis/jiw602>.
- Bandyopadhyay AS, Garon J, Seib K, Orenstein WA. Polio vaccination: past, present and future. *Future Microbiol*. 2015;10(5):791–808. <https://doi.org/10.2217/fmb.15.19>.
- Bonnet MC, Dutta A. World wide experience with inactivated poliovirus vaccine. *Vaccine*. 2008;26(39):4978–83. <https://doi.org/10.1016/j.vaccine.2008.07.026>.
- Chansaenroj J, Tuanthap S, Thanusuwanasak T, Duang-In A, Klinfueng S, Thanekongtong N, et al. Human enteroviruses associated with and without diarrhea in Thailand between 2010 and 2016. *PLoS ONE*. 2017;12(7):e0182078. <https://doi.org/10.1371/journal.pone.0182078>.
- Delpont W, Poon AF, Frost SD, Kosakovsky Pond SL. Data-monkey 2010: a suite of phylogenetic analysis tools for evolutionary biology. *Bioinformatics*. 2010;26(19):2455–7. <https://doi.org/10.1093/bioinformatics/btq429>.
- Dowdle WR, De Gourville E, Kew OM, Pallansch MA, Wood DJ. Polio eradication: the OPV paradox. *Rev Med Virol*. 2003;13(5):277–91. <https://doi.org/10.1002/rmv.401>.
- El-Sayed N, El-Gamal Y, Abbassy AA, Seoud I, Salama M, Kandeel A, et al. Monovalent type 1 oral poliovirus vaccine in newborns. *N Engl J Med*. 2008;359(16):1655–65. <https://doi.org/10.1056/nejmoa0800390>.
- Endegue-Zanga MC, Sadeuh-Mba SA, Iber J, Burns CC, Moeletsi NG, Baba M, et al. Importation and outbreak of wild polioviruses from 2000 to 2014 and interruption of transmission in Cameroon. *J Clin Virol*. 2016;79:18–24. <https://doi.org/10.1016/j.jcv.2016.03.025>.
- Garg A, Pattamadilok S, Bahl S. Successes and challenges of expansion of environmental poliovirus surveillance in the WHO South-East Asia Region. *WHO South East Asia J Public Health*. 2018;7(2):122–8. <https://doi.org/10.4103/2224-3151.239424>.
- Guo J, Bolivar-Wagers S, Srinivas N, Holubar M, Maldonado Y. Immunodeficiency-related vaccine-derived poliovirus (iVDPV) cases: a systematic review and implications for polio eradication. *Vaccine*. 2015;33(10):1235–42. <https://doi.org/10.1016/j.vaccine.2015.01.018>.
- Jorba J, Diop OM, Iber J, Henderson E, Zhao K, Sutter RW, et al. Update on vaccine-derived polioviruses—worldwide, January 2017–June 2018. *MMWR Morb Mortal Wkly Rep*. 2018;67(42):1189–94. <https://doi.org/10.15585/mmwr.mm6742a5>.
- Kew O, Morris-Glasgow V, Landaverde M, Burns C, Shaw J, Garib Z, et al. Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. *Science*. 2002;296(5566):356–9. <https://doi.org/10.1126/science.1068284>.
- Kilpatrick DR, Iber JC, Chen Q, Ching K, Yang SJ, De L, et al. Poliovirus serotype-specific VP1 sequencing primers. *J Virol Methods*. 2011;174(1–2):128–30. <https://doi.org/10.1016/j.jviro.2011.03.020>.
- Kosalaraksa P, Chokephaibulkit K, Benjaponpitak S, Pancharoen C, Chuenkitmongkol S, B'Chir S, et al. Persistence of hepatitis B immune memory until 9–10 years of age following hepatitis B vaccination at birth and DTaP-IPV-HB-PRP approximately T vaccination at 2, 4 and 6 months. *Hum Vaccin Immunother*. 2018;14(5):1257–65. <https://doi.org/10.1080/21645515.2018.1426418>.
- Linsuwanon P, Payungporn S, Samransamruajkit R, Posuwan N, Makkoch J, Theanboonlers A, et al. High prevalence of human rhinovirus C infection in Thai children with acute lower respiratory tract disease. *J Infect*. 2009;59(2):115–21. <https://doi.org/10.1016/j.jinf.2009.05.009>.
- O'Connor PM, Allison R, Thapa A, Bahl S, Chunsuttiwat S, Hasan M, et al. Update on polio eradication in the World Health Organization South-East Asia Region, 2013. *J Infect Dis*. 2014;210(Suppl 1):S216–24. <https://doi.org/10.1093/infdis/jit585>.
- O'Reilly KM, Lamoureux C, Molodecky NA, Lyons H, Grassly NC, Tallis G. An assessment of the geographical risks of wild and vaccine-derived poliomyelitis outbreaks in Africa and Asia. *BMC Infect Dis*. 2017;17(1):367. <https://doi.org/10.1186/s12879-017-2443-4>.
- Patel M, Cochi S. Addressing the challenges and opportunities of the polio endgame: lessons for the future. *J Infect Dis*. 2017;216(suppl_1):S1–8. <https://doi.org/10.1093/infdis/jix117>.
- Platt LR, Estivariz CF, Sutter RW. Vaccine-associated paralytic poliomyelitis: a review of the epidemiology and estimation of the global burden. *J Infect Dis*. 2014;210(Suppl 1):S380–9. <https://doi.org/10.1093/infdis/jiu184>.
- Puenpa J, Suwannakarn K, Chansaenroj J, Vongpunsawad S, Poovorawan Y. Development of single-step multiplex real-time RT-PCR assays for rapid diagnosis of enterovirus 71, coxsackievirus A6, and A16 in patients with hand, foot, and mouth disease. *J Virol Methods*. 2017;248:92–9. <https://doi.org/10.1016/j.jviromet.2017.06.013>.
- Racaniello VR. One hundred years of poliovirus pathogenesis. *Virology*. 2006;344(1):9–16. <https://doi.org/10.1016/j.virol.2005.09.015>.
- Shrivastava A, Gupta N, Upadhyay P, Puliyel J. Caution needed in using oral polio vaccine beyond the cold chain: vaccine vial monitors may be unreliable at high temperatures. *Indian J Med Res*. 2012;135(4):520–2.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol*. 2011;28(10):2731–9. <https://doi.org/10.1093/molbev/msr121>.
- Thongcharoen P. Viral diseases in Thailand. *J Infect Dis Antimicrob Agents*. 1988;5(4):196–204.
- Zaffran M, McGovern M, Hossaini R, Martin R, Wenger J. The polio endgame: securing a world free of all polioviruses. *Lancet*. 2018;391(10115):11–3. [https://doi.org/10.1016/S0140-6736\(17\)32442-X](https://doi.org/10.1016/S0140-6736(17)32442-X).
- Zhang Q, Leppold C, Shao Y, Mura Y, Tanimoto T. New poliovirus vaccine schedules. *Lancet*. 2016;388(10059):2477–8. [https://doi.org/10.1016/S0140-6736\(16\)32177-8](https://doi.org/10.1016/S0140-6736(16)32177-8).

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