Journal of Infection and Public Health 12 (2019) 625–629

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Post-vaccinated asymptomatic rotavirus infections: A community profile study of children in Surabaya, Indonesia

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ARTICLE INFO

Article history:
Received 8 August 2018
Received in revised form 22 January 2019
Accepted 10 February 2019

Keywords:
Rotavirus
Asymptomatic
Post-vaccination
Genotyping

ABSTRACT

Background: Rotavirus gastroenteritis accounts for significant childhood morbidity and mortality worldwide. Vaccination using RotarixTM (GSK) and RotaTeq® (Merck) was introduced due to the tremendous disease burden. The possibility of asymptomatic infections following vaccinations was poorly understood. This study examined rotavirus cases in post-vaccinated children, their clinical manifestations and the genotypes of isolated strains.

Methods: Stool samples of healthy, vaccinated children under 5 years of age in Surabaya were collected monthly for 1 year between January 2016 and February 2017. Episodes of gastroenteritis were reported, and samples were collected. Rotavirus was identified using multiplex reverse transcription Polymerase Chain Reaction (QIAGEN, Inc., Valencia, CA). Clinical manifestations were measured using the Vesikari score. The genotype was analyzed by Applied Biosystems (Foster, CA).

Results: A total of 109 stool samples were collected from 30 subjects, of which 22 received Rotarix; 8 RotaTeq. Nine out of 109 samples were collected during diarrhea episodes of 8 subjects. Two asymptomatic rotavirus infections were identified by RT-PCR. The genotypes isolated were G1P[8] and G3P[8].

Conclusions: Asymptomatic rotavirus infections can occur in post-vaccinated children. Strains identified were homologous to serotypes eliciting gastroenteritis in unvaccinated children of the same community.

Introduction

Rotavirus infection accounts for significant morbidity and mortality in infants and young children worldwide. The World Health Organization (WHO) estimated 215,000 rotavirus-associated deaths among children under 5 years of age in 2013 [1]. The latest study in Indonesia reported rotavirus as the leading cause of severe acute gastroenteritis in children under 5 years of age, being responsible for 60% of hospitalizations and 41% of clinical visits presenting with diarrhea [2]. So far, 12 G- (G1-G6, G8-G12, G20) and 14 P- (P[1], P[3]-P[11], P[14], P[19], P[25], and P[28]) genotype combinations have been identified in humans, with the global epidemiological emphasis on G1-G4 and G9 [3].

Rotavirus enters humans through the oral route and destroys epithelial cells of mature villi in the small intestine, causing malabsorption and therefore osmotic diarrhea. Animal models also demonstrate the role of the enteric nervous system and NSP4 enterotoxin in inducing osmotic diarrhea. The vomiting center is simultaneously activated although its mechanism remains unclear [4]. Mortality arises when severe dehydration is treated inadequately.

Rotavirus vaccine was introduced in 2006 considering the tremendous global disease burden. Currently, the two licensed vaccines are RotaTeq® (Merck & Co., Inc., West Point, PA, USA) and Rotarix® (GSK Biologicals, Belgium) [5,6].

Many studies have examined the efficacy of rotavirus vaccination in preventing severe gastroenteritis, ranging from 40 to 64% in developing countries [7–10] and >90% in developed countries [11–15]. However, the relationship among rotavirus vaccination, the possible subsequent rotavirus infections in children

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https://doi.org/10.1016/j.jiph.2019.02.015

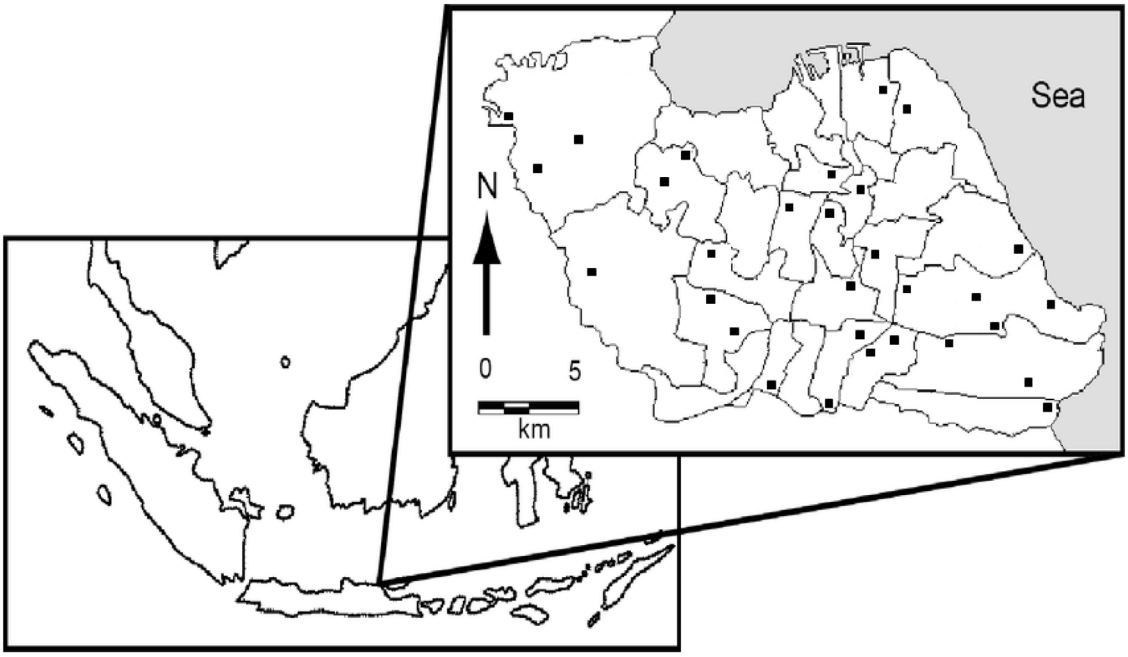

Fig. 1. Map of the participants' residences in Surabaya (Figure adapted from Fig. 1 of [17]).

and changes in local circulating strains are poorly understood. In this study, rotavirus infection status post-vaccination was examined in vaccinated children in Surabaya, Indonesia.

Materials & methods

This study was approved by the Ethics Committee at the Institute of Research and Innovation (*Lembaga Penelitian dan Inovasi*) *Universitas Airlangga* and complied with the National Health and Medical Research Council National Statement for the Ethical Conduct in Research Involving Humans (1999). This was a prospective cohort study of representative vaccinated children under 5 years of age in Surabaya between January 2016 and February 2017. The primary outcome was to identify the presence of rotavirus in the stools of these children and to examine their clinical manifestations.

Community setting and participant recruitment

In Indonesia, rotavirus vaccination is included in the universal immunization schedule under the optional category without government funding [16]. There are no precise data on rotavirus vaccine coverage in Indonesia. Until the end of the study period, these vaccines were relatively new and remained exclusively for those who could afford it.

A total of 30 immunocompetent children under 5 years of age who had completed either RotaTeq® (Merck & Co., Inc., West Point, PA, USA) or Rotarix® (GSK Biologicals, Belgium) vaccinations were recruited from a young mothers' community in Surabaya. Surabaya is the capital city of East Java Province, Indonesia (Fig. 1), and has a warm, tropical climate. The period of recruitment was January to March 2016. Immunocompetence was defined as being HIV negative and not undergoing immunosuppressive or other immunomodulation therapies. The residences of these participants are scattered throughout the city to ensure representative results (Fig. 1).

Stool sample collection

Stool samples from each participant were collected monthly at home in clean containers provided over the one-year study period. An initial stool sample from each study participant was obtained within 2 days after the first meeting with his/her parents. The specific date of each follow-up stool sample was dependent on the day that the parents were willing to provide a sample, ending between December 2016 and February 2017. The majority of parents were unable to provide samples in July and December 2016 due to *Idul Fitri* and the Christmas holidays.

Participants who had diarrhea between monthly collections were also instructed to provide the diarrhea sample. Diarrhea was defined as at least 3 uncommonly loose or watery stools within 24 h [18]. At the time of recruitment, two participants had diarrhea.

Stool specimens were transported promptly in refrigerated boxes to the Viral Diarrhea Laboratory, Institute of Tropical Disease Universitas Airlangga, Surabaya, where they were frozen at −80 °C until tested for rotavirus.

Subject characteristics and clinical information

Informed consent was obtained from the parents of the participants upon recruitment. Subject characteristics and clinical information were attained directly from the parents of the participants using a questionnaire developed for the study. For diarrhea episodes, clinical severity was assessed using the Vesikari scoring system [19].

Identification of rotavirus

All stool specimens were subsequently thawed and aliquoted. Viral RNA was extracted using a QIAamp Viral RNA Extraction Kit® (QIAGEN, Inc., Valencia, CA) as described in the manufacturer's protocol. Identification of rotavirus was confirmed by a two-step reverse transcription-polymerase chain reaction (RT-PCR) assay against VP7 protein using the corresponding oligonucleotide primer sets (Table 1) according to the WHO manual [20]. Positive controls were also used in this study. The RT-PCR is favored due to its much lower detection threshold, whereby it can identify more asymptomatic infections with significantly lower viral loads [21,22].

Table 1
Primer sets for rotavirus identification [20].

Primer		Sequence (5′–3′)	Position	Amplicon size (bp)
First amplification				
	Beg9	GGCTTTAAAAGAGAGAATTTCCGTCTGG	nt 1-29	
	End9	GGTACACATCATACAATTCTAATCTAAG	nt 1062-1036	1062
Second amplification				
	RVG9	GGTACACATCATACAATTCT	nt 1062-1044	
	aAT8	GTCACACCATTTGTAAATTCG	nt 178-198	885
	aBT1	CAAGTACTCAAATCAATGATGG	nt314-335	749
	aCT2	CAATGATATTAACACATTTTCTGTG	nt 411-435	652
	aDT4	CGTTTCTGGTGAGGAGTTG	nt 480-498	583
	aET3	CGTTTGAAGAAGTTGCAACAG	nt 689-709	374
	aFT9	CTAGATGTAACTACAACTAC	nt 757-776	306

Further genotyping was performed through nucleic acid sequencing and phylogenetic analysis of the VP7 gene. The PCR product was directly sequenced using a BigDye terminator cycle sequencing kit and Applied Biosystems 3500XL Genetic Analyzer (Applied Biosystems, Foster, CA). The sequencing results were then aligned using CLUSTAL X software. The phylogenetic analysis was conducted using Molecular Evolutionary Genetic Analysis (MEGA4) software (http://www.megasoftware.net).

Results

Vaccine types

Eight of 30 (26.7%) study participants received RotaTeq® (Merck & Co., Inc., West Point, PA, USA) while the remaining 22 (73.3%) received Rotarix® (GSK Biologicals, Belgium).

Detection of rotavirus

A total of 109 stool specimens were collected from the 30 study participants. The number of samples obtained from each participant ranged from 1 to 12 over the study course. Rotavirus was detected by RT-PCR in 2 stool specimens collected from 2 different asymptomatic participants. These samples were collected on 21 and 23 February 2016, respectively. On their corresponding collection days, no symptoms of gastroenteritis were reported by these participants. Stool passage frequency and consistency remained normal.

During the course of the study, eight participants experienced diarrhea at least once. One participant reported diarrhea twice, in April and June 2016. Diarrhea episodes ranged from three to ten days. The mean Vesikari score was 7.1. These children were promptly presented to their individual pediatricians and received adequate treatments. This prevented aggravating conditions as reflected by the result of clinical severity scoring. All nine diarrhea specimens were negative for rotavirus.

Characteristics of children with asymptomatic rotavirus infection

Both asymptomatic participants whose stools tested rotavirus-positive were vaccinated with Rotarix® (GSK Biologicals, Belgium). On specimen collection, these participants were 10 and 12 months old. The first asymptomatic participant was male, overweight, received breastmilk until 9 and a half months of age and started formula milk since 2 months of age. He was vaccinated in June (2 months old) and August (4 months old) 2015. The second asymptomatic participant was female with a normal nutritional status, breastfed until the end of te study period (23 months old), and vaccinated in April 2015 (2 months old) and 15 June 2015 (4 months old). Both also had access to safe and clean water, good sanitation and parents with adequate education.

Rotavirus genotypes

On gel electrophoresis, the migration pattern of the first positive sample corresponds to the G1 genotype. However, the second positive sample had shorter RNA migration patterns in both the first and second amplification products, which did not correspond to any G-type. Therefore, further genotyping was conducted.

Sequencing of the VP7 gene confirmed that the rotavirus genotypes were G1P[8] and G3P[8]. Phylogenetic analysis revealed that isolated strains were closely related to genotypes reported in Japan and Spain, respectively (Fig. 2).

Discussion

Rotavirus remains the global leading cause of childhood hospitalizations and deaths from severe gastroenteritis. Approximately 37% of diarrheal deaths in children under 5 years old is attributed to this infection [1]. As of 2016, 81 countries have included rotavirus vaccines in their national immunization schedules [23]. Indonesia has not been registered in the list due to the optionality of rotavirus vaccine, available only through the private market.

The analysis of stool specimens from post-vaccinated children in the community enabled us to evaluate subsequent rotavirus infections in individuals with rotavirus-specific protection, which has not been performed before. Indonesia is a suitable location to conduct this analysis because given the uneven vaccine coverage setting, the vaccinated population is more likely to be exposed to rotavirus.

In this study, asymptomatic rotavirus infections occurred in two vaccinated children. There are no available comparison data on rotavirus asymptomatic infection in vaccinated children. However, previous studies showed that the prevalence of asymptomatic rotavirus infections in the general population varied from 3% to 30% [24–27] according to community setting and age composition.

Contrastingly, stool samples from diarrhea episodes in this study tested negative for rotavirus. These findings are not consistent with previous studies which demonstrated that severe rotavirus gastroenteritis could occur in 3.7% [7] and 1.9% [10] of post-vaccinated children. A possible explanation for this might be the differences in number of samples used.

Recruitment of study participants was done before knowing their rotavirus infection status, whereas the duration of rotavirus shedding in asymptomatic infection can range from 8 to 25 days [28]. Hence, some children who were potentially infected during the period between specimen collections may not have been detected.

The genotypes of rotavirus identified in this study suggest that these asymptomatic infections were prevalent infections. G1P[8] is the most common dominant genotype worldwide with a prevalence of 52% from 1994 to 2003 [29]. A multi-center study

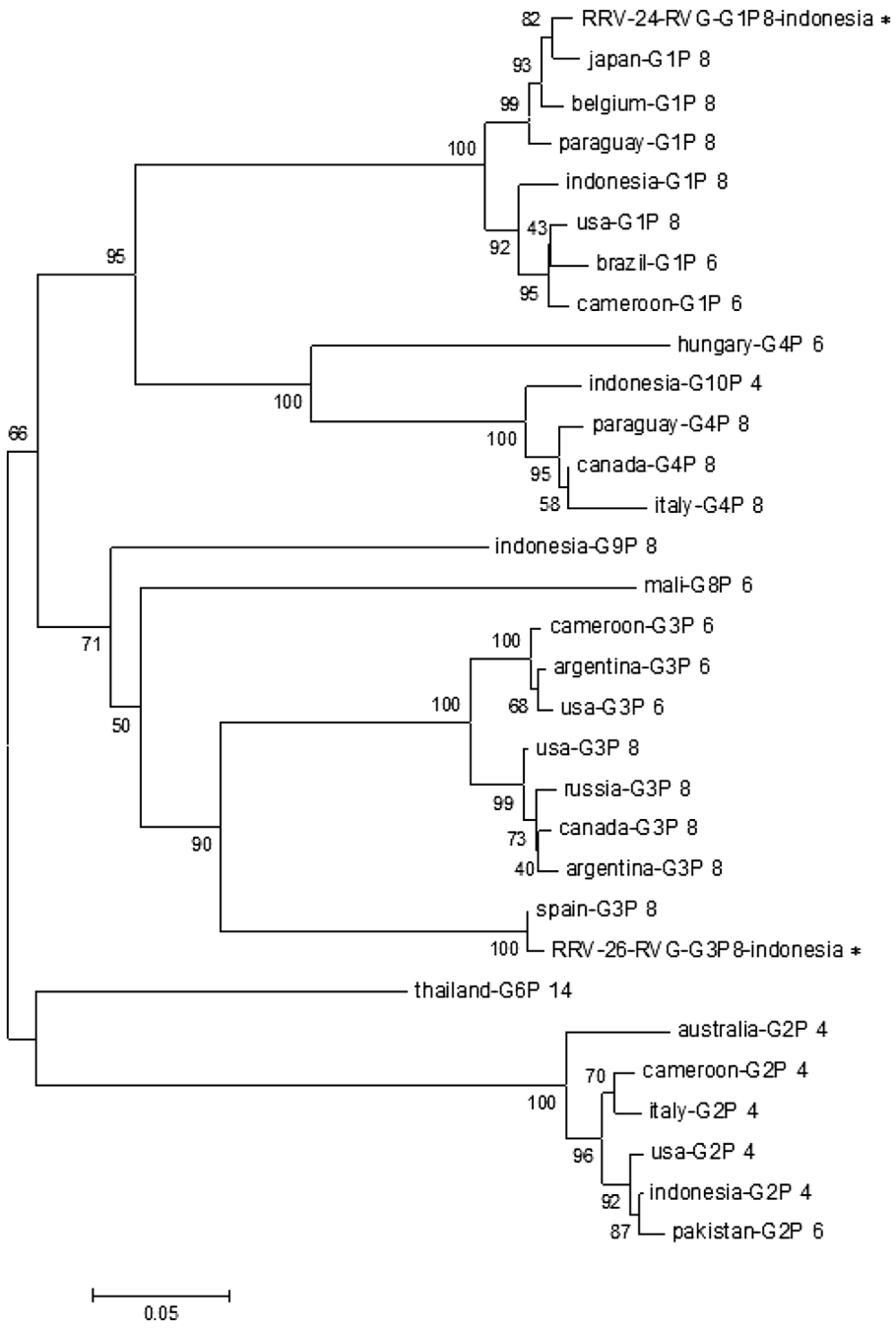

Fig. 2. Neighbor-joining Maximum Composite Likelihood (MCL) Phylogenetic Tree of Human Rotavirus VP7 sequences and other G representative genotypes recognized thus far (partial and/or complete). Numbers on the nodes represent the bootstrap support from 1000 replications. Strains from asymptomatic infection in the present study are denoted by an asterisk.

conducted in Indonesia reported 11% G1P[8] distribution in 2006 [2]. Similarly, G1P[8] accounted for 11.4% of cases in the 2013 one-year-surveillance in Surabaya [30].

In accordance with previous studies, we found a uniquely short RNA migration pattern which, upon further analysis, revealed genotype G3P[8] that is closely related to Spanish equine-like G3P[8] [31]. This finding supports a recent study by *Utsumi et al.* on the predominance of G3 strains with an equine-like VP7 gene and a DS-1-like genetic backbone in Indonesia between 2015–2016 [32]. However, the relationship between these vaccinated children with asymptomatic infection and unvaccinated children with intestinal infectious disease in the aforementioned study is a complex causality dilemma. Nonetheless, it is likely that asymptomatic infections are responsible for the continuing circulation of strains within a community.

These findings suggest that rotavirus infections could still happen in post-vaccinated children. While the number of samples in this study is too small to analyze, it is presumable that both rotavirus vaccines are beneficial in preventing severe gastroenteritis. Former studies showed that the efficacy for both RotaTeq® (Merck & Co., Inc., West Point, PA, USA) and Rotarix® (GSK Biologicals, Belgium) is similar in developing countries [7–10]. Moreover, Rotarix® apparently provides cross-protection for other rotavirus serotypes, despite its monovalent G1P[8] content [33].

In asymptomatic infections, rotavirus could still be excreted in formed stools, although in lower quantities [28], thereby facilitat-

ing its spread within local communities or to other communities when the host travels. With uneven vaccine coverage, unvaccinated children would become more susceptible to severe rotavirus-associated diseases. This study result emphasizes the importance of rotavirus vaccination for children across the globe.

Conclusion

Our study reports asymptomatic rotavirus infections in vaccinated children whose serotypes are homologous to the prevalent serotypes eliciting severe clinical manifestations in unvaccinated children. Complementary to previous studies, our findings emphasize the importance of rotavirus vaccination for all children.

Funding

No funding Sources.

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgements

This research was supported by the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) from Ministry of Education, Culture, Sport, Sciences & Technology in Japan, and Japan Agency for Medical Research and Development (AMED: 18fm0108004h0004). The authors acknowledge Dato' Sri Prof. Tahir for supporting this research through Tahir Professorship, Indonesia.

References

[1] Tate JE, Burton AH, Boschi-Pinto C, Parashar UD, Agocs M, Serhan F, et al. Global, regional, and national estimates of rotavirus mortality in children <5 years of age, 2000-2013. Clin Infect Dis 2016;62(Suppl. 2):S96–105.
[2] Soenarto Y, Aman AT, Bakri A, Waluya H, Firmansyah A, Kadim M, et al. Burden of severe rotavirus diarrhea in Indonesia. J Infect Dis 2009;200(s1):S188–94. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1086/605338.
[3] Afrad MH, Matthijnssens J, Moni S, Kabir F, Ashrafi A, Rahman MZ, et al. Genetic characterization of a rare bovine-like human VP4 mono-reassortant G6P[8] rotavirus strain detected from an infant in Bangladesh. Infect Genet Evol 2013;19(July):120–6.
[4] Hagbom M. Rotavirus disease mechanisms diarrhea, vomiting and inflammation — how and why? 2015:1–57, 1463.
[5] Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, et al. Safety and efficacy of a pentavalent human–bovine (WC3) reassortant rotavirus vaccine. N Engl J Med 2006;354(1):23–33. Available from: https://doi.org/10.1056/NEJMoa052664.
[6] Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, Abate H, Breuer T, Clemens SC, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med 2006;354(1):11–22. Available from: https://doi.org/10.1056/NEJMoa052434.
[7] Zaman K, Anh DD, Victor JC, Shin S, Yunus M, Dallas MJ, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. Lancet 2010;376(9741):615–23.
[8] Armah GE, Sow SO, Breiman RF, Dallas MJ, Tapia MD, Feikin DR, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. Lancet 2010;376(9741):606–14. Available from: https://doi.org/10.1016/S0140-6736(10)60889-6.
[9] Tissera MS, Cowley D, Bogdanovic-Sakran N, Hutton ML, Lyras D, Kirkwood CD, et al. Options for improving effectiveness of rotavirus vaccines in developing countries. Hum Vaccin Immunother 2017;13(4):921–7. Available from: https://doi.org/10.1080/21645515.2016.
[10] Madhi SA, Cunliffe NA, Steele D, Witte D, Kirsten M, Louw C, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. N Engl J Med 2010;362(4):289–98.
[11] Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, et al. Safety and efficacy of a pentavalent human–bovine (WC3) reassortant rotavirus vaccine. N Engl J Med 2006;354(1):23–33. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa052664.
[12] Block SL, Vesikari T, Goveia MG, Rivers SB, Adeyi BA, Dallas MJ, et al. Efficacy, immunogenicity, and safety of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine at the end of shelf life. Pediatrics 2007;119(1):11–8. Available from: http://pediatrics.aappublications.org/cgi/doi/10.1542/peds.2006-2058.
[13] Goveia MG, Dinubile MJ, Dallas MJ, Heaton PM, Kuter BJ. Efficacy of pentavalent human-bovine (WC3) reassortant rotavirus vaccine based on breastfeeding frequency. Pediatr Infect Dis J 2008;27(7):656–8.
[14] Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, Abate H, Breuer T, Clemens SC, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med 2006;354(1):11–22.
[15] Vesikari T, Karvonen A, Prymula R, Schuster V, Tejedor JC, Cohen R, et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. Lancet 2007;370(9601):1757–63.
[16] Menteri Kesehatan RI. Peraturan Menteri Kesehatan Republik Indonesia Nomor 12 Tahun 2017 tentang Penyelenggaraan Imunisasi. Kementerian Kesehatan 2017:1–162. Available from: http://hukor.kemkes.go.id/uploads/produk_hukum/PMK_No._12_ttg_Penyelenggaraan_Imunisasi_.pdf.
[17] Nagao Y, Rachmie EM, Ochi S, Padmidewi MM. Geographical association between socioeconomics and age of dengue haemorrhagic fever patients in Surabaya, Indonesia. Dengue Bull 2011;35(January):116–30.
[18] WHO. Essential concepts concerning diarrhoea. In: The treatment of diarrhoea: a manual for physicians and other senior health workers; 2005. p. 1–44. Available from: http://apps.who.int/iris/bitstream/handle/10665/43209/9241593180.pdf?sequence=1.
[19] Ruuska T, Vesikari T. Rotavirus disease in finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. Scand J Infect Dis 1990;22(3):259–67. Available from: https://doi.org/10.3109/00365549009027046.
[20] WHO. Rotavirus characterisation: molecular methods. Manual of rotavirus detection and characterization methods 2009, 143 p.
[21] McLauchlin J, Amar CFL, East CL, Gray J, Iturriza-Gomara M, Maclure EA. Detection by PCR of eight groups of enteric pathogens in 4,627 faecal samples: re-examination of the English case-control Infectious Intestinal Disease Study (1993-1996). Eur J Clin Microbiol Infect Dis 2007;26(5):311–23. Available from: https://doi.org/10.1007/s10096-007-0290-8.
[22] Kang G, Iturriza-Gomara M, Wheeler JG, Crystal P, Monica B, Ramani S, et al. Quantitation of group a rotavirus by real-time reverse-transcription-polymerase chain reaction: correlation with clinical severity in children in South India. J Med Virol 2004;73(1):118–22. Available from: https://doi.org/10.1002/jmv.20053.
[23] PATH. Country national immunization program (NIP) introductions of rotavirus vaccine 2016:1–2. Available from: http://www.vaccineresources.org/files/PATH-Country-Introduction-Table-EN-2016.05.01.pdf.
[24] Barron-Romero BL, Barreda-Gonzalez J, Doval-Ugalde R, Zermeno-Eguia Liz J, Huerta-Pena M. Asymptomatic rotavirus infections in day care centers. J Clin Microbiol 1985;22(1):116–8.
[25] Abiodun PO, Ihongbe JC, Ogbimi A. Asymptomatic rotavirus infection in Nigerian day-care centres. Ann Trop Paediatr 1985;5(3):163–5. Available from: https://doi.org/10.1080/02724936.1985.11748385.
[26] Endara P, Trueba G, Solberg OD, Bates SJ, Ponce K, Cevallos W, et al. Symptomatic and subclinical infection with rotavirus P[8]G9, rural Ecuador. Emerg Infect Dis 2007;13(4):574–80.
[27] Phillips G, Lopman B, Rodrigues LC, Tam CC. Asymptomatic rotavirus infections in England: prevalence, characteristics, and risk factors. Am J Epidemiol 2010;171(9):1023–30.
[28] Mukhopadhya I, Sarkar R, Menon VK, Babji S, Paul A, Rajendran P, et al. Rotavirus shedding in symptomatic and asymptomatic children using reverse transcription-quantitative PCR. J Med Virol 2013;85(9):1661–8.
[29] Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Rev Med Virol 2005;15:29–56. Available from: https://doi.org/10.1002/rmv.448.
[30] Sudarmo SM, Shigemura K, Athiyyah AF, Osawa K, Wardana OP, Darma A, et al. Genotyping and clinical factors in pediatric diarrhea caused by rotaviruses: one-year surveillance in Surabaya, Indonesia. Gut Pathog 2015;7(1):1–10.
[31] Arana A, Miriam A, Montes M, Jere KC, Iturriza-Gomara M, Cilla G. Emergence and spread of G3P[8] rotaviruses possessing an equine-like VP7 and a DS-1-like genetic backbone in the Basque Country (North of Spain), 2015. Infect Genet Evol 2016;44:137–44. Available from: https://doi.org/10.1016/j.meegid.2016.06.048.
[32] Utsumi T, Wahyuni RM, Doan YH, Dinana Z, Soegijanto S, Fujii Y, et al. Equine-like G3 rotavirus strains as predominant strains among children in Indonesia in 2015–2016. Infect Genet Evol 2018;61:224–8. Available from: https://doi.org/10.1016/j.meegid.2018.03.027.
[33] Steele AD, Neuzil KM, Cunliffe NA, Madhi SA, Bos P, Ngwira B, et al. Human rotavirus vaccine Rotarix™ provides protection against diverse circulating rotavirus strains in African infants: a randomized controlled trial. BMC Infect Dis 2012;12(1):213. Available from: BMC Infectious Diseases.