Rotavirus genotypes in Malaysia and Universal rotavirus vaccination

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Group A rotavirus (RV-A) genotypes isolated in Malaysia was studied to estimate the effectiveness of a universal RV-A vaccination in Malaysia. A simple mathematical model was used, with input from a two-year, two-center, prospective study on hospitalization of RV-A gastroenteritis (RVGE) in young children, published data on RV-A hospitalizations and genotypes, mortality on childhood GE and published genotype-specific efficacy data on two RV-A vaccines. Assuming a 95% vaccine coverage, the overall projected effectiveness was 75.7 to 88.1% for Rotateq® and 78.7 to 90.6% for Rotarix® against RVGE-related hospitalizations. The projected annual reduction in RVGE-related deaths was 27 to 32 deaths (from 34 deaths) for Rotateq® and 28 to 32 deaths annually for Rotarix®. A universal RV-A vaccine is efficacious in reducing RVGE-related hospitalizations and mortality in Malaysia.

Introduction

Group A rotavirus (RV-A) is the leading cause of severe diarrheal disease in infants and young children worldwide.[1,2] Two large clinical trials on two RV-A vaccines, assessed the efficacy and safety of two RV-A vaccines in infants.[3,4] Both vaccines have been found to be highly efficacious in the prevention of severe RV-A gastroenteritis (RVGE) caused by common RV-A genotypes. The pentavalent human-bovine reassortant vaccine (Rotateq®, Merck Co.; RV5) has been effective against genotypes G1, G2, G3, G4 and G9 genotypes,[3] while the monovalent human attenuated RV-A vaccine (Rotarix®, GlaxoSmithKline Biologicals, Rixensart; RV1) was effective against G1P[8], G2[4], G3P[8], G4P[8] and G9P[8].[4,5]

Globally, five G types (G1, G2, G3, G4 and G9) together with P[8] or P[4] represented over 95% of the strains analyzed worldwide.[6,7] However, there is considerably geographical variability, with seasonal and year-to-year fluctuations.[7-9]

In Malaysia, RV-A is the leading cause of childhood acute diarrhea requiring hospitalization.[10-19] It is associated with significant morbidity and financial cost to both government and care-provider.[13,14] Since 2006, two RV-A vaccines have been introduced in Malaysia. It is not included in the national immunization program, but available in the private market. The take up rate was approximately 5% of the total birth cohort in Malaysia.[20]

No formal clinical trial has been conducted to determine the efficacy of RV-A vaccines in Malaysia. The purpose of the present study is to estimate the potential effectiveness of universal RV-A vaccination in Malaysia in reducing hospital admission due to RVGE and RV-A-associated mortality, based on currently available efficacy data.

Results

Part 1 prospective study. During the study period, a total 822 children younger than five years of age were admitted to one of the two participating centers for acute diarrhea. Of these, 279 (34%) were positive for RV-A (**Table 1**). Twenty-eight samples were insufficient for genotype analysis. Of the remaining 251 samples, the commonest RV-A genotype isolated was G1P[8] (82%; **Table 2**). Other genotypes isolated were G2P[4] (7.6%), and G9P[8] (6.3%). A total of 4% of the samples were either mixed or untypeable.

Hospitalizations of childhood acute GE due to RV-A infections. A total of nine studies on hospitalizations due to RV-A infections in Malaysia were identified from the literature.[10-12,14-19] Of the total 8,902 cases of childhood acute GE (including the present study) where stool samples were analyzed for RV-A, the aggregated positive rate for RV-A was 30.3% (**Table 1**).

Common RV-A genotypes in Malaysia. A total of four studies where information on RV-A genotypes determination in Malaysia, in addition to the present study, were identified from the literature (**Table 2**).[11,21-23] The commonest genotypes were G1P[8] 34.7%. A total of 14.4% of the RV-A were either mixed or untypeable.

Mortality of RV-A infection in Malaysia. Of the 8,902 cases of childhood acute GE studied, no mortality was reported. A further search yielded two studies on childhood GE mortality from Malaysia.[24,25] Lee WS et al. described ten cases of death due to

Table 1. Hospitalizations due to rotavirus gastroenteritis among children in Malaysia

Year of study	Authors	Refs.	Site(s)	Location of study center	N	% +ve for RV	Mortality due to RV (n)
1981/82	Yap KL, et al.	15	Kuala Lumpur	Urban	587	46%	0
1996	Lee WS, et al.	16	Kuala Lumpur	Urban	297	29%	0
1996–99	Lee WS, et al.	10	Kuala Lumpur	Urban	1724	24%	0
2002	Lee WS, et al.	12	Kuala Lumpur	Both urban	475	23%	0
			Johor Bharu		1367	18%	0
2002–03	Hung LC et al.	11	Kuala Lumpur and Kuching	Urban	1756	35%	0
					912	46%	0
2004	Poo MI and Lee WS	17	Kuala Lumpur	Urban	393	22%	0
2005–06	Tan ADK	19	Penang	Urban	142	54%	0
2005–06	Goh CT et al.	18	Kota Kinabalu	Urban	167	16%	0
2006–07	Chai PF and Lee WS	14	Kuala Lumpur	Urban	260	26%	0
2008–10	Present study		Kuala Lumpur and Kuala Trengganu	Urban and rural	822	34%	0
Overall					**8902**	**30.3%**	**0**

acute diarrhea among 4,689 cases of childhood GE admitted to an urban hospital in Kuala Lumpur over a period of 15 years.[24] None was attributable to RV-A infection.[24] The mortality cases included mostly children residing in the urban area. Hsu VP et al. estimated the disease burden of RV-A in Malaysia, based on nationwide disease admission figures from all government hospitals in 2000–2001.[25] Based on an estimated 2.5 deaths/100,000 children, the authors estimated that each year, there would be 34 children died of RV-A infection.[25] Another nationwide study in Malaysia on the under-five mortality in 2006, involving all government hospitals and rural health centers, showed that a total of 320 deaths were classified under certain infectious and parasitic diseases.[26] Of these, 89 deaths were attributable to acute diarrheal disease.[26] However, no underlying etiological agent was reported.[26] Thus for the purpose of this study, each year, RV-A is estimated to cause approximately 34 childhood deaths in Malaysia.

Projected effectiveness of RV-A vaccines against RVGE-related hospitalizations in Malaysia. The modeled projected effectiveness of both RV5 and RV1 against RVGE-related hospitalizations in Malaysia is shown in **Table 3**. The estimated sensitivity analysis and base case scenario for RV5 are 79.7 and 92.7% respectively,[3] while those for RV1 are 82.8 and 95.4%, respectively.[5]

Projected reduction in RVGE-related deaths in Malaysia. The projected annual reduction in RVGE-related deaths attributable to introduction of both RV5 and RV1, assuming 95% vaccine coverage, was 27 to 32 deaths (from 34 deaths) annually for RV5, and 28 to 32 deaths annually for RV1.

Discussion

Diarrheal diseases remain an important cause of childhood morbidity and mortality in Malaysia. On average, RV-A infections accounted for three of every ten hospitalizations for children GE in Malaysia (**Table 1**). Two nationwide surveys estimated that the annual deaths occurred as a result of acute diarrhea and RVGE were 89 and 34 deaths, respectively.[25,26] The estimated deaths due to RVGE were 2.5 deaths/100,000 children.[25] In contrast, in the United States with a population which is 11 times bigger than in Malaysia, the estimated deaths attributable to RVGE were less than 40 deaths each year.[27] In Taiwan with a population slightly less than of Malaysia, the estimated deaths attributable to RV were seven deaths per year.[28]

Thus improvement can be achieved to reduce RVGE-related deaths in Malaysia further. One of the reasons for the apparently higher RVGE-related deaths in Malaysia as compared with other developed countries is the higher risk of death caused by acute diarrhea in the indigenous population, who are eight times more likely to die of acute diarrhea than other population groups in Malaysia.[26]

Since their first introduction in 2006, many trials have demonstrated the efficacy of RV-A vaccines in reducing severe RVGE and RVGE-related hospitalizations. A trial of RV5 in Vietnam and Bangladesh, two countries with limited health care resources and significant diarrheal mortality rate, the efficacy against severe RVGE was 48%.[29] In developed countries like the United States where RV-A related mortality is low but health care cost associated with physician and emergency room visits and hospitalization was significant, an effectiveness study showed that RV5 prevented 100% of RVGE-related hospitalizations and emergency room visits.[30]

In middle-income countries like Brazil and Mexico, the efficacy of a full RV-A vaccination schedule was projected to be resulting in 76 to 94% reduction in RVGE-related hospitalizations; respectively.[30-33] A time-series analysis in Brazil put the efficacy data on the reduction of hospitalization of acute diarrhea of all causes at 17%, while that of diarrhea-related mortality at 22%.[34]

At present, there is no similar clinical trial or vaccine effectiveness study on RV-A vaccines in Malaysia. The present study

Table 2. Common stereotypes of rotavirus isolated in various sites in Malaysia, 1977 to 2010

Year of study	Authors	Reference	Site(s)	No. tested	Common Rotavirus strains isolated (%)
1977–99	Rasool et al.	20	Kuala Lumpur	360	G4P[8] (71%), G1P[8] (14%), G^{UT}P[8] (11%), G3P[8] (3%)
2000–01	Zuraidah et al.	21	Peninsular Malaysia and Sarawak	157	G4P[8] (65%), G^{UT}P[8] (19%), G1P[8] (6.5%), others (6.5%)
1996, 2007	Zuraidah et al.	22	Kuala Lumpur and Batu Pahat	108	G1P[8] (81%), G^{UT}P[8] (9%), G1P^{UT} (7%), others (3%)
2001–03	Hung LC et al.	11	Kuala Lumpur and Kuching	157	G9P[8] (73.3%), G^{UT}P[8] (10%), G2P[4] (4.5%), G1P[8] (3.8%), Other (P4, P8, G1, G2, G9) (7%)
2008–10	Present study		Kuala Lumpur and Kuala Trengganu	251	G1P[8] (82%), G2P[4] (7.6%), G9P[8] (6.3%), Others (G12P[8], G3P9, G9P9, G3P8) (4%)
1977–2010	**Overall**			**1033**	**G1P[8] (34.7%), G4P[8] (34.6%), G9P[8] (12.7%), G2P[4] (2.5%), G3P[8] (1.1%), others and untypeable 14.4%**

showed that the introduction of either of the two RV-A vaccines is expected to have an effectiveness of between 75.7 to 88.1% for RV5, and 78.7 to 90.6% for RV1, in reducing RVGE-related hospitalizations in Malaysian children. Since the aggregated proportion of all hospitalizations for childhood GE due to RV-A infection was 30.3%, an introduction of a universal RV-A vaccination in Malaysia is expected to result in an approximately 24 to 27% reduction in the overall hospital admission for childhood GE, assuming a 95% vaccine coverage. However, the actual reduction of GE-related hospital admissions would be higher as many studies have shown that introduction of RV-A vaccine also resulted in a significant reduction of non-RVGE-related hospital admission due to acute diarrhea in young children.[3] There would also be a further reduction of RVGE-related deaths in Malaysia.

Thus the projected effectiveness of a universal RV-A vaccination in Malaysia is similar to other middle-income countries where a RV-A vaccination has been implemented, such as that of Brazil and Mexico.[31-35] However, it should be noted that the current projected efficacy is only applicable to the first post RV-A vaccine year and a reduced efficacy is to be expected the following year.[3]

In arriving at the projected model of effectiveness, we used the efficacy data of RV5 vaccine, conducted in the United States,[3] rather than that of Vietnam and Bangladesh.[29] The efficacy data for RV1 was based on trials conducted in Singapore, Taiwan and Hong Kong.[5] It should be noted that projected effectiveness model for RV-A vaccine is different in high, middle and low-income settings.[31] The main reason of using the efficacy data from developed countries is both epidemiological and geographical. Geographically, Malaysia was closer to countries like Singapore, Taiwan and Hong Kong.[3,5] In addition, the mortality rate for RVGE in Malaysia was also similar to that of Taiwan.[27]

However, it should be noted that there are differences between the study endpoints of the trials of each RV-A vaccine.[3,5] For the RV5 trial, the efficacy data was expressed in terms of reduction of hospitalization due to RVGE,[3] while for the Asian RV1 trial, the efficacy data was expressed in terms of the prevention of severe RVGE based on a score of 11 using the 20-point Vesikari scale.[5] For the purpose of comparison in the present study, both end-point of trial are treated as equal.

We used a sensitivity analysis to account for variations in efficacy against mixed and untypeable RV-A genotypes, with an assumed efficacy of 90%.[36] This does not have major impact on the projected effectiveness of both RV-A vaccines as the proportion of mixed and untypeable genotypes in the Malaysia was a relative high of 14.4%.

The present study showed that the proportion of acute GE requiring hospital care in Malaysia due to RV-A infection was 30.3%. In addition, mortality related to RVGE was very low. The estimated annual mortality due to RV-A infection in Malaysia was 34 deaths.[25] Thus any universal RV-A vaccination in Malaysia will likely result in significant reduction of hospitalization due to acute GE but not in mortality related to RVGE.

The present study does not address the potential cost-saving of a reduction in direct medical as well as out-of-pocket costs resulting from RV-A vaccination on a national scale. Thus we were unable to estimate the cost-effectiveness of childhood RV-A vaccination in Malaysia. Our previous estimates showed the median cost of providing inpatient care for an episode of RVGE was $212 in 2002,[13] while the out-of-pocket cost incurred by care-provider for an episode of hospitalization for RVGE was $194 in 2006.[14]

In conclusion, the present study showed that both RV-A vaccines are expected to be effective against RVGE-related hospitalizations in Malaysia There will also be a further reduction of RVGE-related mortality. There is a strong case for introducing universal RV-A vaccination in Malaysia. The present study will help policy maker in Malaysia in allocating their health resources in reducing the burden of RV-A disease in Malaysia.

Material and Methods

The present study consists of two parts. Part 1: This is a two-year, two-center, prospective study, conducted on an urban and a rural center to determine the proportion of children hospitalized for

Table 3. Modeled projected effectiveness of two RV vaccines against RVGE-related hospitalizations in Malaysia

Serotypes	Proportion of total serotypes in Malaysia (%)	Serotype-specific of pentavalent RV vaccine (RV5)[A]	Attributable effectiveness: Base case scenario for RV5 (%)[B]	Attributable effectiveness: sensitivity analysis for RV5 (%)[C]	Serotype-specific of monovalent RV vaccine (RV1)[D]	Attributable effectiveness: Base case scenario for RV1 (%)[B]	Attributable effectiveness: sensitivity analysis for RV1 (%)[C]
G1	34.7	0.95	33.0	33.0	1.0	34.7	34.7
G2	2.5	0.88	2.2	2.2	1.0	2.5	2.5
G3	1.1	0.93	1.0	1.0	0.95	1.0	1.0
G4	34.6	0.89	30.8	30.8	0.94[E]	32.5	32.5
G9	12.7	1.0	12.7	12.7	0.92	11.7	11.7
Mixed/ untypeable	14.4	-	13.0	0	-	13.0	0
Overall weighted effectiveness			**92.7**	**79.7**		**95.4**	**82.8**
Overall vaccine effectiveness based on 95% coverage			**88.1**	**75.7**		**90.6**	**78.7**

Note: RV, rotavirus. (A) Pentavalent human-bovine reassortant RV vaccine (Rotateq®, Merck Co.; ref. 3) (B) Base case scenario assumes 90% efficacy against mixed and untypeable serotypes. (C) Sensitivity analysis assumes 0% efficacy against mixed and untypeable serotypes. (D) Monovalent attenuated human RV vaccine (Rotarix®, GlaxoSmithKline Biologicals; ref. 5). The efficacy data for RV1 are for prevention of severe RV gastroenteritis based on a score of 11 using the 20-point Vesikari scale. (E) No efficacy figure for G4 was available, so efficacy for non-G1 was taken

RVGE. Part 2: A systematic search on the RV-A hospitalizations, genotypes and mortality in Malaysia published in the literature.

Part 1. *Prospective study.* The prospective component of the study was conducted at the University of Malaya Medical Centre, (UMMC), Kuala Lumpur and at the Sultanah Nur Zahirah Hospital (SNZH), Kuala Trengganu. UMMC is a large, multidisciplinary, teaching hospital situated at Kuala Lumpur, the capital of Malaysia. It caters mainly urban population of Kuala Lumpur. SNZH is located at Kuala Trengganu, the state of Trengganu in the east coast of Peninsular Malaysia. It is a large, multi-disciplinary general hospital, catering mainly semi-urban and rural population. The present study was approved by institutional ethic committees of both centers (Approval number: 55-20-23-1025 and HSNZ.KT.100-23/9).

The prospective study was conducted from 1 April 2008 to 31 March 2010. All children younger than five years of age, admitted to either center with acute diarrhea were enrolled. Acute diarrhea was defined as having loose stools of three or more in a 24-h period, lasting shorter than 10 d. Stool samples were collected and the presence of RV-A antigen was analyzed by enzyme immunoassay (Premier Rotaclone®, Meridian Diagnostics, Inc.).

Rotavirus genotypes identification. Stool samples positive for RV-A were deep frozen at -80°C, and were sent in batches to Murdoch Childrens' Research Institute, for serotype determination. The method for determining RV-A genotypes has been described previously.[9] Briefly, RV-A double-stranded RNA was extracted by using the QIAamp Viral RNA Mini Kit (QIAGEN),

and the genes encoding the VP4 and VP7 proteins were amplified by reverse transcription-PCR (RT-PCR). The VP7 gene segment was amplified by primers VP7-F and VP7-R, and the VP8 subunit of the VP4 gene was amplified by using the primers VP7-F and VP7-R.[37,38] The sequencing was performed by the ABI Prism BigDye Terminator cycle sequencing kit version 3.1 (Applied Biosystems). Sequences were analyzed by the Sequencher program version 4.1 (Gene Codes Corp., Inc.).

Part 2. *Review of rotavirus disease burden in Malaysia.* A literature search on publications on RV-A hospital admissions and serotype determination from Malaysia was conducted. For this, a systematic search on PubMed/Medline, EmBase, Academic Search Premier and ISI Web of Science was conducted for articles published from January 1990 to November 2011. The following keywords were used: rotavirus, Malaysia, Southeast Asia and Asia. The list of publications obtained was narrowed to studies relevant to hospitalizations and serotype circulation.

Rotavirus disease mortality in Malaysia. The list of publications on RV-A hospitalizations in Malaysia obtained in was screened for RV-A-related mortality. A separate literature search on diarrheal mortality in Malaysia was conducted to give a more accurate picture on RV-A-related mortality in Malaysia.

Projecting the effectiveness of RV-A vaccines against RVGE-related hospitalizations in Malaysia. This analysis used a mathematical efficacy projection model which has been previously validated.[36,39] Briefly, to project the impact of RV-A vaccines on the reduction of RVGE-related hospitalizations in Malaysia, we reviewed and summed up the proportion of locally prevalent

genotypes based published surveillance data from 1990 to 2011, supplemented with data derived from the present prospective. Published results on serotype-specific vaccine efficacy from large phase III clinical trials provided the baseline efficacy data for each of the RV-A vaccines marketed in Malaysia.[3,5] To account for unknown efficacy against mixed and non-typeable genotypes, we conducted sensitivity analyses of modeled effectiveness, with a base case scenario of an assumed 90% efficacy against mixed and non-typeable genotypes.[36] This figure was chosen based on the efficacy rate of both RV-A vaccines against the commonly circulated RV-A strains in the literature.[3-5]

Projecting the effectiveness of RV-A vaccines against RV-A-related deaths in Malaysia. The projected reduction in RVGE-related deaths was estimated by applying the projected effectiveness against RVGE hospitalizations to the number of RVGE-related deaths in Malaysia, assuming the vaccine coverage of 95%. The 95% vaccine coverage was used as we anticipated that RV-A vaccine coverage to be the same as the coverage of diphtheria, pertussis and tetanus (DPT), since the RV-A vaccines would likely to be recommended to be administered concurrently with DPT

at 2, 3 and 5 mo of age in Malaysia. DPT vaccine is part of the national immunization program in Malaysia, and is available freely to all eligible population in Malaysia. In 2010, 95% of Malaysian children received 3 doses of DPT.[40] In the base case scenario, it was assumed that 95% of children would be fully immunized and that 5% of children would receive no vaccination.

References

1. Parashar UD, Bresee JS, Glass RI. The global burden of diarrhoeal disease in children. Bull World Health Organ 2003; 81:236; PMID:12764488.

2. Sanchez-Padilla E, Grais RF, Guerin PJ, Steele AD, Burny ME, Luquero FJ. Burden of disease and circulating serotypes of rotavirus infection in sub-Saharan Africa: systematic review and meta-analysis. Lancet Infect Dis 2009; 9:567-76; PMID:19695493; http://dx.doi.org/10.1016/S1473-3099(09)70179-3.

3. Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, et al.; Rotavirus Efficacy and Safety Trial (REST) Study Team. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. N Engl J Med 2006; 354:23-33; PMID:16394299; http://dx.doi.org/10.1056/NEJMoa052664.

4. Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, Abate H, Breuer T, Clemens SC, et al.; Human Rotavirus Vaccine Study Group. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med 2006; 354:11-22; PMID:16394298; http://dx.doi.org/10.1056/NEJMoa052434.

5. Phua KB, Lim FS, Lau YL, Nelson EA, Huang LM, Quak SH, et al. Safety and efficacy of human rotavirus vaccine during the first 2 years of life in Asian infants: randomised, double-blind, controlled study. Vaccine 2009; 27:5936-41; PMID:19679216; http://dx.doi.org/10.1016/j.vaccine.2009.07.098.

6. Hoshino Y, Kapikian AZ. Rotavirus serotypes: classification and importance in epidemiology, immunity, and vaccine development. J Health Popul Nutr 2000; 18:5-14; PMID:11014764.

7. 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; PMID:15484186; http://dx.doi.org/10.1002/rmv.448.

8. O'Ryan M. The ever-changing landscape of rotavirus serotypes. Pediatr Infect Dis J 2009; 28(Suppl):S60-2; PMID:19252426; http://dx.doi.org/10.1097/INF.0b013e3181967c29.

9. Ch'ng LS, Lee WS, Kirkwood CD. Rare rotavirus strains in children with severe diarrhea, Malaysia. Emerg Infect Dis 2011; 17:948-50; PMID:21529427.

10. Lee WS, Veerasingam PD, Goh AYT, Chua KB. Hospitalization of childhood rotavirus gastroenteritis from a Southeast Asian country. J Paediatr Child Health 2003; 39:518-22; PMID:12969206; http://dx.doi.org/10.1046/j.1440-1754.2003.00206.x.

11. Hung LC, Wong SL, Chan LG, Rosli R, Ng AN, Bresee JS. Epidemiology and strain characterization of rotavirus diarrhea in Malaysia. Int J Infect Dis 2006; 10:470-4; PMID:17046306; http://dx.doi.org/10.1016/j.ijid.2006.05.008.

12. Lee WS, Ganeswrie R, Karunakaran R, Hassan HH, Puthucheary SD. Rotavirus and other enteropathogens in childhood acute gastroenteritis requiring hospital admission in Malaysia – a study of two centres. J Paediatr Child Health 2006; 42:509-14; PMID:16925536; http://dx.doi.org/10.1111/j.1440-1754.2006.00913.x.

13. Lee WS, Poo MI, Nagaraj S. Estimates of economic burden of providing inpatient care in childhood rotavirus gastroenteritis from Malaysia. J Paediatr Child Health 2007; 43:818-25; PMID:17608648; http://dx.doi.org/10.1111/j.1440-1754.2007.01160.x.

14. Chai PF, Lee WS. Out-of-pocket expenditure associated with rotavirus gastroenteritis requiring hospitalization in Malaysia. Vaccine 2009; 27S:F112-5; http://dx.doi.org/10.1016/j.vaccine.2009.08.069.

15. Yap KL, Sabil D, Muthu PA. Human rotavirus infection in Malaysia. III. A one year survey on the prevalence of rotavirus enteritis in children. Southeast Asian J Trop Med Public Health 1983; 14:467-9; PMID:6673122.

16. Lee WS, Lee SP, Boey CCM. Admission to hospital with gastroenteritis in Malaysia. Singapore Paediatr J 1997; 39:185-90.

17. Poo MI, Lee WS. Admission to hospital with childhood acute gastroenteritis in Kuala Lumpur, Malaysia. Med J Malaysia 2007; 62:109-13; PMID:18246904.

18. Goh CT, Cheah PK, Soo TL, Lee WS. The epidemiology and burden of childhood rotavirus infection in a tertiary hospital in Sabah, Malaysia. Med J Malaysia 2009; 64:146-9; PMID:20058575.

19. Tan ADK. Rotavirus infection in paediatric community-acquired acute gastroenteritis: a retrospective cross-sectional study in a private hospital in Malaysia. Malaysia J Paediatr Child Health 2007; 15:7-15.

20. World Health Organization. Immunization Profile - Malaysia. http://apps.who.int/immunization_monitoring/en/globalsummary/countryprofileresult.cfm?C=mys. Accessed 9 July 2012.

21. Rasool N, Othman RY, Adenan MI, Hamzah M. Temporal variation of Malaysian rotavirus electropherotypes. J Clin Microbiol 1989; 27:785-7; PMID:2470775.

22. Zuridah H, Bahaman AR, Mohd Azmi ML, Mutalib AR. Rotavirus RNA electropherotype in different states in Malaysia for the year 2000 and 2001. Med J Malaysia 2004; 59:153-9; PMID:15559163.

23. Zuridah H, Kirkwood CD, Bishop RF, Bogdanovic-Sakran N, Yap KL. Molecular characterization and epidemiology of rotavirus isolates obtained from children with diarrhoea in Malaysia. Med J Malaysia 2009; 64:193-6; PMID:20527266.

24. Lee WS, Ooi TL. Deaths following acute diarrhoeal diseases among hospitalised infants in Kuala Lumpur. Med J Malaysia 1999; 54:303-9; PMID:11045055.

25. Hsu VP, Abdul Rahman HB, Wong SL, Ibrahim LH, Yusoff AF, Chan LG, et al. Estimates of the burden of rotavirus disease in Malaysia. J Infect Dis 2005; 192(Suppl 1):S80-6; PMID:16088810; http://dx.doi.org/10.1086/431494.

26. Wong SL, Hussain IMI. A study on under five deaths in Malaysia in the year 2006. Clinical Research Centre, Kuala Lumpur, 2008.

27. Glass RI, Kilgore PE, Holman RC, Jin S, Smith JC, Woods PA, et al. The epidemiology of rotavirus diarrhea in the United States: surveillance and estimates of disease burden. J Infect Dis 1996; 174(Suppl 1):S5-11; PMID:8752284; http://dx.doi.org/10.1093/infdis/174.Supplement_1.S5.

28. Wu CL, Yang YC, Huang LM, Chen KT. Cost-effectiveness of childhood rotavirus vaccination in Taiwan. Vaccine 2009; 27:1492-9; PMID:19186200; http://dx.doi.org/10.1016/j.vaccine.2009.01.023.

29. Zaman K, Dang DA, 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; 376:615-23; PMID:20692031; http://dx.doi.org/10.1016/S0140-6736(10)60755-6.

30. Curns AT, Steiner CA, Barrett M, Hunter K, Wilson E, Parashar UD. Reduction in acute gastroenteritis hospitalizations among US children after introduction of rotavirus vaccine: analysis of hospital discharge data from 18 US states. J Infect Dis 2010; 201:1617-24; PMID:20402596; http://dx.doi.org/10.1086/652403.

31. Desai R, de Oliveira LH, Parashar UD, Lopman B, Tate JE, Patel MM. Reduction in morbidity and mortality from childhood diarrhoeal disease after species A rotavirus vaccine introduction in Latin America - a review. Mem Inst Oswaldo Cruz 2011; 106:907-11; PMID:22241109.

32. Correia JB, Patel MM, Nakagomi O, Montenegro FM, Germano EM, Correia NB, et al. Effectiveness of monovalent rotavirus vaccine (Rotarix) against severe diarrhea caused by serotypically unrelated G2P[4] strains in Brazil. J Infect Dis 2010; 201:363-9; PMID:20047501; http://dx.doi.org/10.1086/649843.

33. Gurgel RQ, Cuevas LE, Vieira SC, Barros VC, Fontes PB, Salustino EF, et al. Predominance of rotavirus P[4] G2 in a vaccinated population, Brazil. Emerg Infect Dis 2007; 13:1571-3; PMID:18258011; http://dx.doi.org/10.3201/eid1310.070412.

34. Justino MC, Linhares AC, Lanzieri TM, Miranda Y, Mascarenhas JD, Abreu E, et al. Effectiveness of the monovalent G1P[8] human rotavirus vaccine against hospitalization for severe G2P[4] rotavirus gastroenteritis in Belém, Brazil. Pediatr Infect Dis J 2011; 30:396-401; PMID:21150692; http://dx.doi.org/10.1097/INF.0b013e3182055cc2.

35. do Carmo GMI, Yen C, Cortes J, Siqueira AA, de Oliveira WK, Cortez-Escalante JJ, et al. Decline in diarrhea mortality and admissions after routine childhood rotavirus immunization in Brazil: a time-series analysis. PLoS Med 2011; 8:e1001024; PMID:21526228; http://dx.doi.org/10.1371/journal.pmed.1001024.

36. El Khoury AC, Mast TC, Ciarlet M, Markson LE, Goveia MG. Projecting the effectiveness of RotaTeq® against rotavirus-related hospitalizations and deaths in six Asian countries. Hum Vaccin 2011; 7:506-10; PMID:21422820; http://dx.doi.org/10.4161/hv.7.5.14620.

37. Gómara MI, Cubitt D, Desselberger U, Gray J. Amino acid substitution within the VP7 protein of G2 rotavirus strains associated with failure to serotype. J Clin Microbiol 2001; 39:3796-8; PMID:11574622; http://dx.doi.org/10.1128/JCM.39.10.3796-3798.2001.

38. Simmonds MK, Armah G, Asmah R, Banerjee I, Damanka S, Esona M, et al. New oligonucleotide primers for P-typing of rotavirus strains: Strategies for typing previously untypeable strains. J Clin Virol 2008; 42:368-73; PMID:18378188; http://dx.doi.org/10.1016/j.jcv.2008.02.011.

39. Rose J, Singer ME. Projecting vaccine efficacy: accounting for geographic strain variations. Pharmacoeconomics 2008; 26:185-9; PMID:18282014; http://dx.doi.org/10.2165/00019053-200826030-00003.

40. World Health Organization and United Nations Children's Fund. WHO and UNICEF estimates of immunization coverage: 2010 revision. Available at: www.who.int/immunization_monitoring/data/mys.pdf (accessed 3 May 2012).