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Post-licensure experience with rotavirus vaccination in high and middle income countries; 2006 to 2011☆

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Rotavirus causes one-third to one-half of severe diarrheal disease in children under the age of five years worldwide. In 2006 two rotavirus vaccines became available and, in the intervening years, approximately thirty countries have introduced them into their immunization programs, primarily in high-income and middle-income settings. Major reductions in rotavirus hospitalizations have been observed in a number of these locations, and in select countries, there have been impacts on gastroenteritis mortality associated with rotavirus vaccine introduction. In addition to these direct health benefits, reduced gastroenteritis risk has been documented in unvaccinated groups, including older children and adults, suggesting indirect benefits (i.e. herd immunity). In this paper, we summarize what has been learned from programs studying post-licensure vaccine effectiveness, impact on health-care utilization and death, safety issues (namely, intussception and the detection of adventitious viruses) and the potential selective pressure of vaccination on the diversity of rotavirus genotypes.

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Rotavirus is the leading cause of severe diarrhea in children under the age of 5 years, accounting for 33–49% of diarrhea hospitalizations in countries of all income strata [1]. In high income settings, deaths from rotavirus are uncommon but the disease causes substantial morbidity. For example, in the United States, rotavirus infection was responsible for an estimated 55 000–70 000 hospitalizations per year and half a million emergency room and outpatient clinic visits before vaccine introduction [2]. In lower income countries with suboptimal access to health care, rotavirus is an important cause of childhood mortality and was estimated to cause approximately 450 000 deaths in 2008, or about 5% of all child deaths [3•,4].

Two rotavirus vaccines, a monovalent rotavirus vaccine (RV1, Rotarix®, GlaxoSmithKline Biologicals) and a pentavalent rotavirus vaccine (RV5, RotaTeq®, Merck Vaccines) are approved by the World Health Organization (WHO). The two vaccines differ in their underlying conception, antigenic composition, schedule, and recommended dosages (Table 1). RV1 vaccine contains a single attenuated human strain of the type that is most prevalent globally (G1P[8]) and is administered in a 2-dose series; RV5 contains five bovine-human mono-reassortant rotaviruses with G1, G2, G3, G4, and P[8] human surface antigens and is administered as a 3-dose series. The first dose of either RV1 or RV5 has to be given between 6 and 15 weeks of age and the last dose of the series up to 8 months of age, with at least 4 weeks between doses.

In 2006, the WHO's Strategic Advisory Group of Experts (SAGE) reviewed data from pivotal pre-licensure trials of RV1 and RV5 conducted in Europe and the Americas. Each of these large trials enrolled >60 000 infants to evaluate a risk of intussusception, an adverse event that was associated with a previous rhesus-human reassortant rotavirus vaccine (Rotashield, Wyeth) that was withdrawn from the US market in 1999. The trials did not detect a risk of intussusception in approximately one month following vaccination and that both vaccines were highly efficacious (85–98%) against severe rotavirus disease, conferring protection against a range of rotavirus strains in circulation during the trials. Based on these data, SAGE recommended that rotavirus vaccines be included in the national immunizations programs of countries in these regions [5]. Following successful trials in low income settings of Africa and Asia, SAGE recommended vaccination of infant in all countries worldwide [6], despite the finding that efficacy is lower in low-socioeconomic settings as the burden of disease is considerably greater [7].

Over the past six years, many countries in the Americas, Europe and Australia have adopted rotavirus vaccines into their national immunization schedules. To our knowledge (as of 2011), RV1 and RV5 had been introduced into 27 and 7 programs, respectively, either nationally or

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Table 1

Characteristics of currently licensed rotavirus vaccines

Characteristic	RV5	RV1
Trade name	RotaTeq®	RotaRix®
Manufacturer	Merck Vaccines	GlaxoSmithKline Biologicals
Vaccine composition and parent strain	G1, G2, G3, G4, P1A[8] reassortant strains from bovine strain WC3 (type G6P7[5])	G1P1A[8] from human strain 89–12
Vaccine titre	2.0–2.8 $\times$ 10^6 infectious units per dose, varies depends on serotype	10^6 median cell culture infective dose CCID50 after reconstitution per dose
Dosing schedule (U.S.)	2, 4 and 6 months with first dose given between 6 and 15 weeks of age and the last dose of the series up to 8 months of age, with at least 4 weeks between doses.	2 and 4 months with first dose given between 6 and 15 weeks of age and the last dose of the series up to 8 months of age, with at least 4 weeks between doses.
Volume per dose	2 ml	1 ml

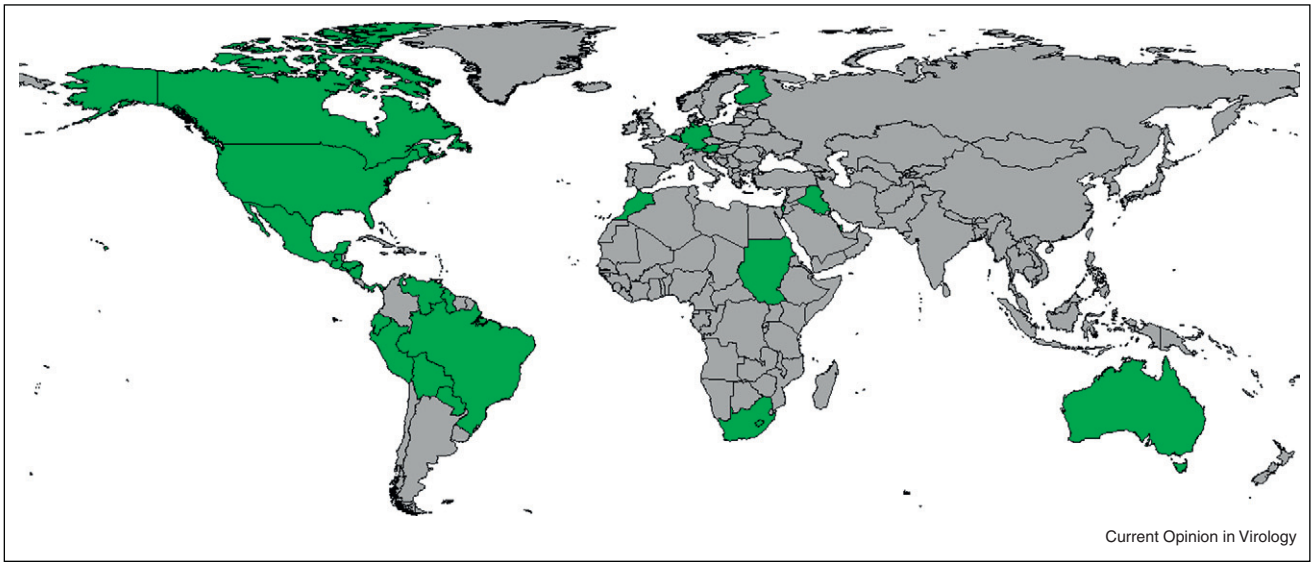
regionally, with both vaccines being available as part of the national program in some countries including the U.S. and Australia (Figure 1) [8]. Some countries have switched from using one vaccine to the other. In this article, we review post-licensure data on effectiveness, duration of protection and safety of rotavirus vaccination from these countries.

Vaccine effectiveness and impact

A number of assessments of vaccine effectiveness against severe rotavirus disease have been undertaken using observational methods (e.g. case-control and cohort studies), and these studies have confirmed good vaccine performance in routine use (for a thorough review, see [8–11]). In high income countries such as the United States [12], Australia [13••], Austria [14] and Israel [15], vaccine effectiveness was on par with estimates from the clinical trials (>85%) and was sustained through 2 years of life. In the upper-middle income Latin American countries of Mexico and Brazil, effectiveness estimates have ranged from 79 to 94%, and in the lower middle income country of El Salvador effectiveness was estimated at 76% [16–19]. Notably, the lowest effectiveness of 46% was observed in Nicaragua, which is the most impoverished setting where vaccine performance in routine use has been evaluated to date [20,21].

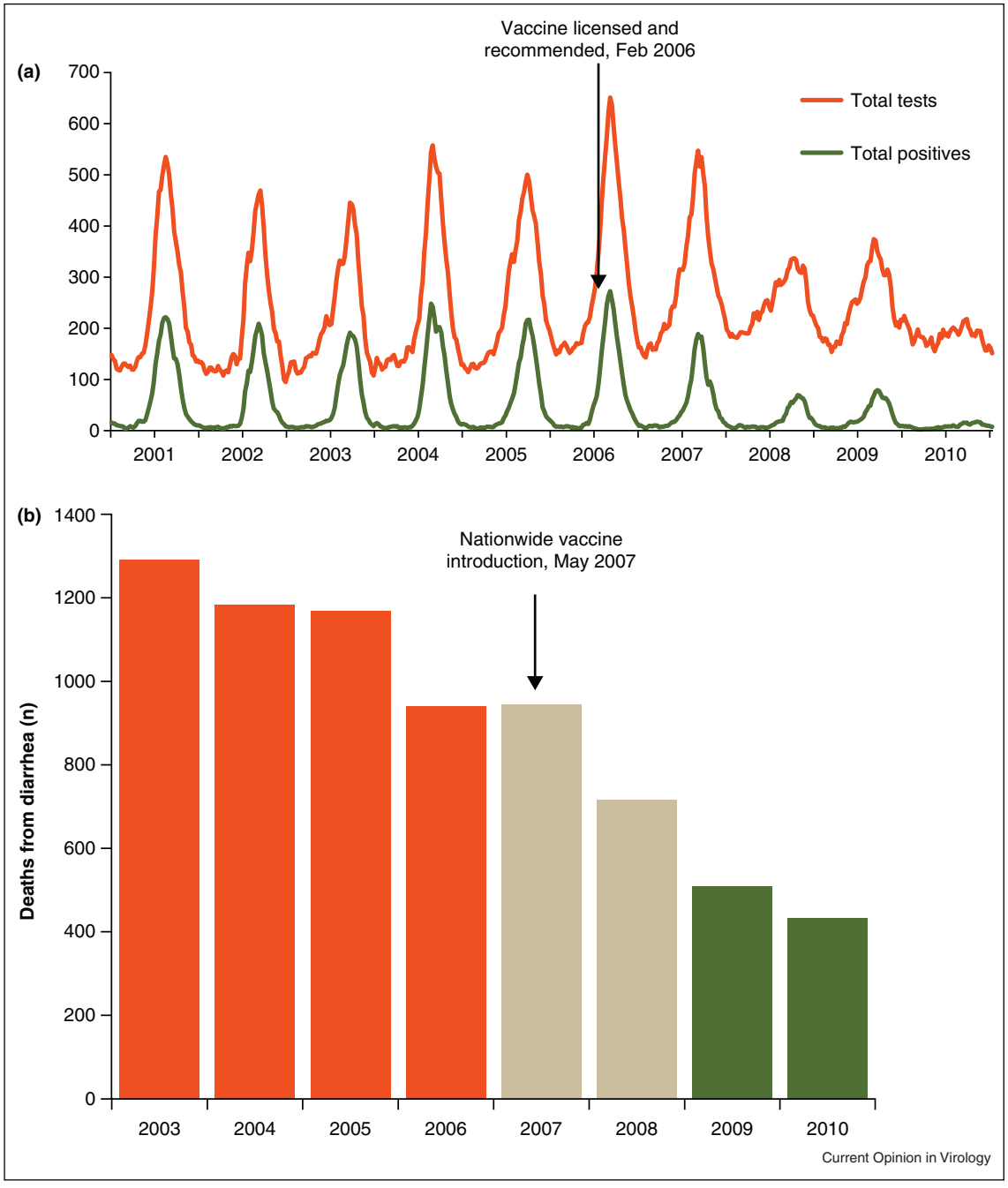
In many countries, substantial declines in rotavirus and/or diarrhea-related hospitalization have been documented following vaccine implementation (Figure 2). In the United States, where vaccine was introduced in 2006, a 46% decline in all-cause diarrhea hospitalizations in children under 5 years of age was observed, equating to an estimated 40 000–60 000 fewer hospitalizations in 2008 [22]. In New South Wales, Australia, where vaccine was introduced in July 2007, rotavirus hospitalizations

Figure 1



Countries with current vaccination programs, 2011. Some countries have both vaccines available as part of the national program (including the U.S. and Australia) and others have switched from the use of RV1–RV5, or vice versa.

Figure 2



Selected examples of rotavirus vaccine impact. **(A)** U.S. Laboratory Reports. Number of positive and total rotavirus tests from 25 continuously reporting National Respiratory and Enteric Virus Surveillance System (NREVSS) laboratories, by week and year, June 2000–July 2010, 3 week moving average. Adapted from Tate *et al.*, PIDJ 2011 [67]. **(B)** Deaths from diarrhea in Mexico, children ages 0–4 years, 2003–2010, December to May only. Oranges bar represent pre-vaccine years; vaccine was introduced nationwide in May 2007, and 2007 and 2008 are considered transitional years, with low coverage (grey bars). Green bars represent post-vaccine years.
Adapted from Richardson *et al.*, NEJM, 2010 and 2011 [30••,31•].

fell by 75% in 2008–2009 [13••]. In Latin America, where high coverage was rapidly achieved, reductions in all-cause gastroenteritis hospitalizations has been examined by 5 studies in 4 countries, with reductions of 17–51% observed [23•,24•,25–27]. Against the more specific outcome of rotavirus hospitalizations, reductions of 59% were observed in Brazil [28] and 69–81% in El Salvador [29].

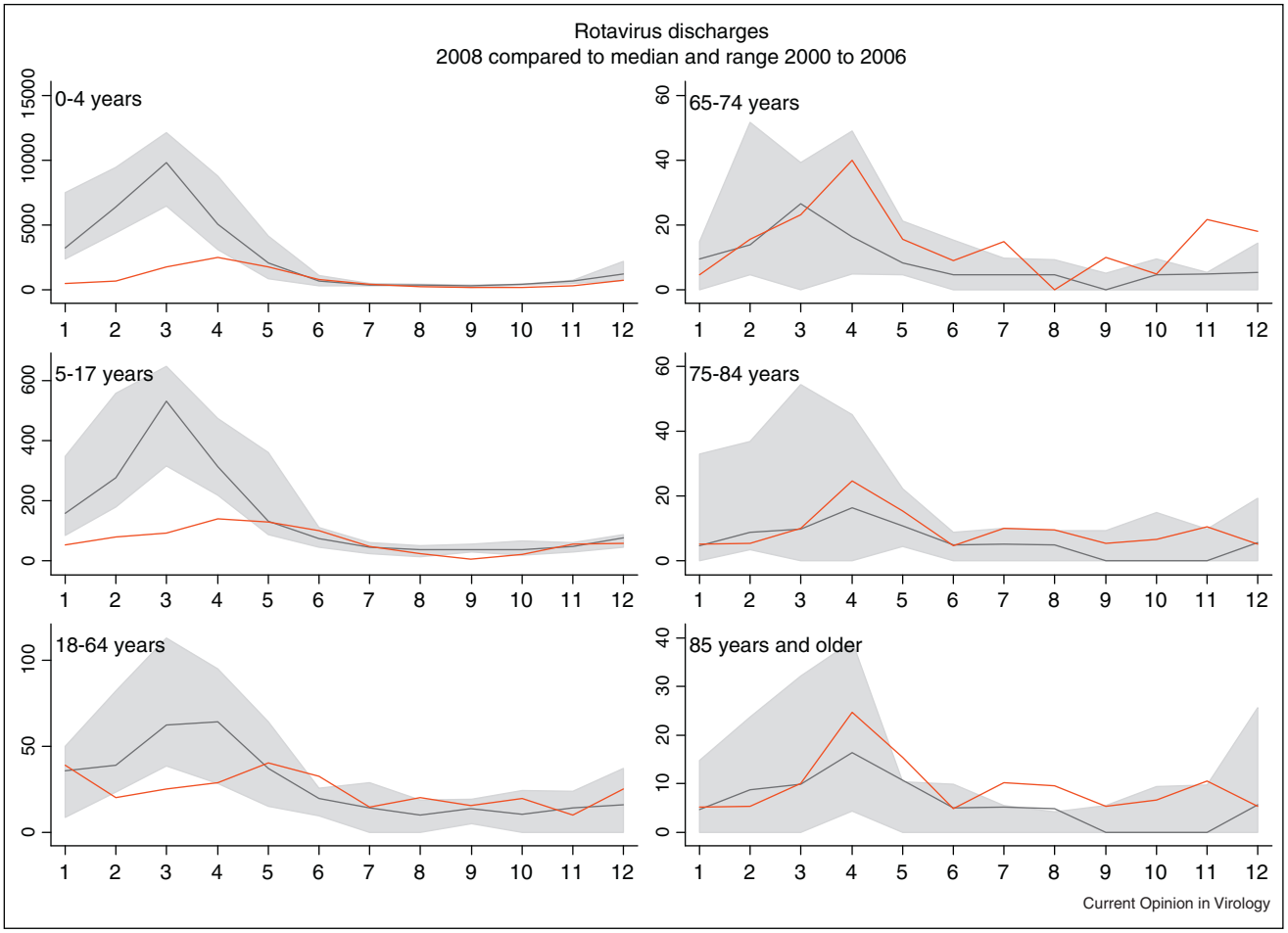
Perhaps most compelling, though, has been the observation of decreased gastroenteritis mortality following introduction of rotavirus vaccination, an outcome that was not evaluated in pre-licensure trials. In Mexico, where nationwide rotavirus vaccination was implemented in 2007, mortality declined by 35% in 2008 compared with pre-vaccine years from 2003 to 2006, with the most pronounced decreases in the vaccinated age groups (<1 year), and during the historic rotavirus season (Figure 2B) [30••]. In subsequent years, these mortality benefits were sustained and broadened to older children aged 1–4 years, who by that time had been eligible for vaccination as infants [31•]. Similar patterns have been observed in Brazil where two separate studies estimated mortality declines of 41% [24•] and 22% [23•]. The latter study in Brazil used statistical methods controlling for the secular declines in mortality that have occurred in Brazil over recent years [32].

Vaccines also appear to have changed the epidemiology of rotavirus by affecting the transmission dynamics of natural infection. First, indirect benefits appear to have occurred in that unvaccinated children have also experienced reduced risk of disease in partially vaccinated populations [13••,22,33•]. These indirect benefits may even extend to older children (5–14 years) and adults (15 years and older), groups not previously well-recognized to have substantial rotavirus burden (Figure 3). [13••,34•] Secondly, in the United States and other temperate climates, RV vaccines have resulted not only in a diminution, but also in a delay in the peak of seasonal activity [14,35–37]. Finally, modelling projections, suggest that biennial peaks (as opposed to annual epidemics) of rotavirus infections may occuring in high-income settings after the introduction of vaccination [35].

Strain-specific vaccine effectiveness and potential evolutionary pressure of vaccination

Based on the pre-licensure clinical trial data, there was some controversy as to whether RV1 provided a high level of protection against the completely heterotypic G2P[4]

Figure 3



US rotavirus discharges post-vaccination (2008) compared with pre-vaccination (2000–2006). Rotavirus-coded hospital discharges by month in 2008 (red line) were lower for children (0–17 years) and adults (18–64 years) compared with the month median (black line) and range (grey shaded area) from 2000 to 2008. Data from the Healthcare Cost and Utilization Project's National Inpatient Sample.Figure adapted from Lopman *et al.*, JID 2011 [34•].

genotype. In the Latin American trials, VE against G2P[4] was estimated at 41% (95% CI −79% to 82%) [38]; although the number of cases from this strain was small and the trial was not powered to measure VE against G2P[4]. In Europe, VE was measured to >85% against severe rotavirus gastroenteritis caused all G types through the second year of life [39].

Following routine introduction of RV1, the apparent emergence of G2P[4] strains in Brazil and Australia and a novel G9P[4] strain in Mexico led to concern that the vaccine may be less effective against completely heterotypic strains [40]. In Australia, half of the states used RV1 and half used RV5, and the emergence of G2P[4] was only observed in the RV1 states [40]. However, observational studies in Brazil confirmed that RV1 has similar high effectiveness against RV hospitalizations caused by G2P[4] [17,18]. Likewise, a study in Mexico documented that RV1 was highly effective against RV hospitalizations caused by the fully heterotypic G9P[4] strain, indicating failure to vaccinate led to these cases rather than vaccine pressure [19].

In the US and Australia, G3 emerged as a common strain in several states where RV5 was introduced [40–42], which opened speculation because the clinical trials for RV5 suggested that the neutralization response to G3 was the least robust of all the vaccine strains [43,44]. However, in other US and Australia sites and in Nicaragua, non-G3 strains have been predominant after the use of RV5, and case-control studies have documented high vaccine effectiveness in settings where G3 strains have dominated [40,45].

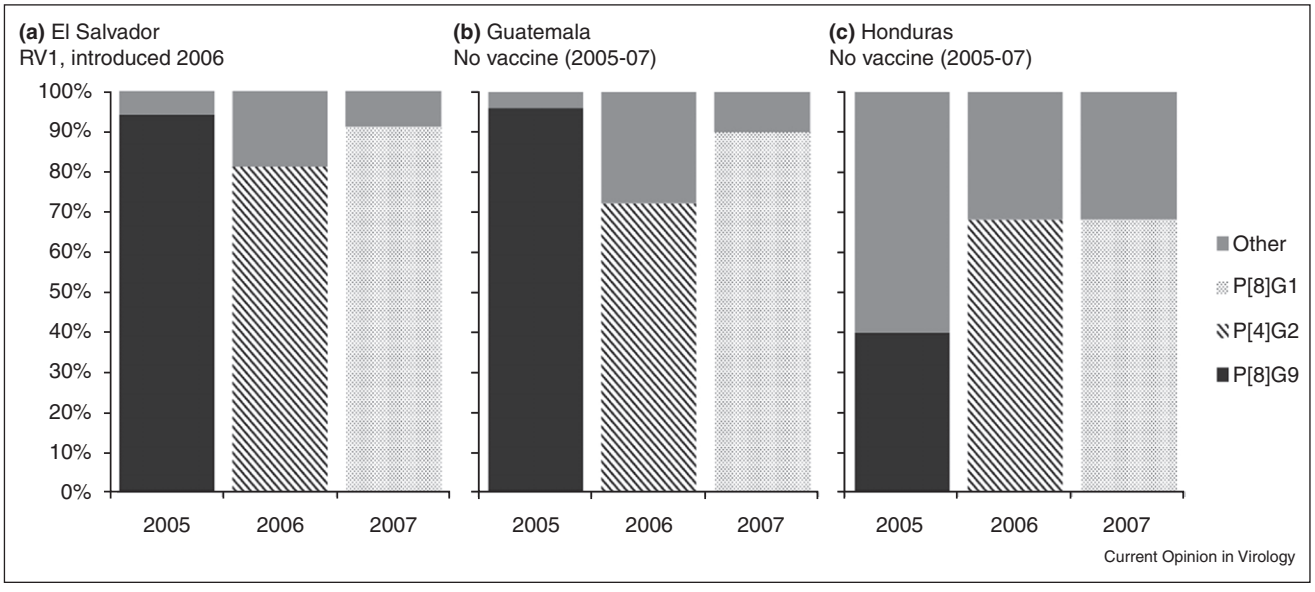
Taken together, these data do not provide any clear indication that rotavirus vaccines are exerting strain selection pressures. Furthermore, observations from Latin America support the suggestion that strain shifts observed following vaccine introduction could be a result of natural variability of strains from year to year as opposed to selection pressure from the vaccine. Similar patterns of G2 (in 2006) and G9 (in 2007) emergence occurred in Honduras and Guatemala (which did not have national vaccination programs) as in El Salvador, where vaccine was used since 2006 (Figure 4) [46]. Nevertheless, mathematical modelling has shown that even modest differences in vaccine effectiveness against specific rotavirus strains could lead to strain selection and that it may take several years for such changes to manifest [47], and thus continued monitoring is key.

Safety issues

Intussusception

While pre-licensure trials of RV5 and RV1 did not show a risk of intussusception (in a 42 or 31 day window after vaccination, respectively), they were only able to evaluate a level of risk similar to that observed with the Rotashield vaccine (1 in 10 000 vaccinees) [43,48]; thus, on-going monitoring is important to assess the possibility of a smaller risk or risk in small time-windows. Indeed, post-licensure evaluations in Mexico (RV1) and in Australia (RV5 and RV1) have both detected a lower intussusception risk on the order to 1 excess intussusception in 50 000–100 000 vaccinees [49,50]. In the U.S., active surveillance has not documented an increased risk of intussusception within 7 and 30 days after any dose of vaccine a population with more than 800 000 doses of RV5

Figure 4



Predominant rotavirus serotypes in El Salvador (**A**, where RV1 was introduced in 2005) and Guatemala **(B)** and Honduras **(C)** where there was no vaccination program from 2005 to 2007.Figure adapted from Patel *et al.*, EID 2008 [46].

administered; however, even this study could only a exclude a risk >1 intussusception case in ~65 000 vaccine recipients and thus the possibility of an intussusception risk similar to that seen in Mexico and Australia remains [51] and should be monitored in the context of overall intussusception risk [52].

These risks are small relative to the benefits conferred by vaccination; for example, in Mexico, Brazil and the U.S. it is estimated that ~300, ~200 and ~80 deaths are averted by vaccination for each death potentially caused by vaccine-associated intussception, respectively [49,53]. Similarly, an estimated ~250, ~1200, and ~1000 rotavirus hospitalizations are averted by vaccination for each intussusception hospitalization potentially caused, respectively. With these overwhelming benefit/risk ratios, the WHO Global Advisory Committee for Vaccine Safety and the U.S. Advisory Committee on Immunization Practices have continued to support recommendations of universal vaccination of healthy infants [53,54].

Adventitious virus contamination

Perhaps in a more unexpected safety arena, in March 2010, a group of researchers using 'deep sequencing' techniques identified nucleic acids from an adventitious porcine circovirus-1 (PCV-1) in RV1 [55••]. PCV is a virus that commonly infects pigs and can be detected in human stool (probably from dietary consumption of pork products), but is not known to cause either infection or illness among humans. Subsequently, the U.S. Food and Drug Administration (FDA) temporarily suspended use of RV1. Further testing of RV5 also revealed PCV-1 and PCV-2 genetic material in that vaccine. The PCV-1 from RV1 was found to include full-length particle-associated genomes, able to grow in cell lines while the PCV-1 and PCV-2 in RV5 were small and non-infectious fragments [56]. It is likely that PCV material was introduced into both rotavirus vaccines through porcine-derived trypsin – a reagent used in the cell-culture growth process of vaccine production – and that the adventitious viruses were present in both vaccines while evaluated during their clinical trials.

On the basis of available evidence of only a theoretical risk of PCV infection among humans and the observed benefits of rotavirus vaccines, the FDA lifted the RV1 restriction in May 2010. The FDA expressed reassurance that the detection of DNA and DNA fragments from PCV in rotavirus vaccines was not likely to cause harm to humans and recommended that information on this topic be provided before vaccination [57]. In the U.S., there was switching to RV5 during the period in which RV1 was suspended [58], but U.S. paediatricians have not ultimately considered the detection of PCV genetic material a barrier to providing either vaccine [59]; reports from some other countries suggest that the finding slowed vaccine uptake [60]. More broadly, this episode raises issues regarding the question of how these highly sensitive new molecular technologies should be used in determining the contamination and potential safety issues of vaccines.

Future directions

Below we outline a number of the key remaining issues for middle and high income settings and highlight where these overlap with challenges in low-income settings. [Developing country-specific challenges will be discussed in detail by Babji and Kang, in this issue [61].

(1) While both vaccines have provided good protection against a broad range of circulating homotypic and heterotypic rotavirus strains, the question of whether vaccination will exert a selective pressure and promote the emergence of new serotypes is not fully answered. Continued monitoring of both the overall incidence and serotype diversity will be needed to address these issues. In low-income settings where there is a greater strain diversity, more rapid emergence of new genotypes (e.g. G8, G9 and, currently, G12), and reduced immunogenicity of vaccines, there remains concern that mass vaccination may facilitate the emergence of new strains. Even if this were to occur, overall reductions in rotavirus disease incidence would still be expected [47], but genotype diversity requires careful monitoring as vaccines are introduced into high burden settings.
(2) On the balance of evidence, it appears that there is a small intussusception risk, at least within one week after the first dose of vaccine, but results are ambiguous from some settings, including the U.S. Monitoring safety, and specifically intussusception risk, will continue to be necessary in high and middle income settings. Of note, in the Latin America study, a risk of intussusception was seen with the first dose of RV1 in Mexico, where inactivated polio vaccine is used, whereas in Brazil, where oral polio vaccine is used, no risk with the first dose of RV1 was seen, while there may have been an even smaller risk associated with the second dose [49]. This has raised the question of whether concomitant administration of oral polio vaccine, which is known to interfere with the take of first dose of RV1, might modify the risk of intussusception [52]. Thus, in developing countries where OPV is widely used, the risk profile could be quite different and requires assessment. Nevertheless, the challenges are formidable to conduct surveillance for such a rare condition in low income settings where health services and informatics are lacking.
(3) It is clear that RV vaccination has resulted in reduced transmission of wild virus, thereby conferring indirect protection (i.e. 'herd-immunity'). By and large, these observations have been made from population surveillance data, rather than in household studies,

for example, so further studies can confirm these findings. The extent of this protection, and the relative role of adults and children in transmission, could have a bearing on the long term equilibrium incidence of disease. How these dynamic effects, including the indirect protection, manifest in low-income/high-burden settings is unknown. Because of differences in population structure and density and social mixing patterns, specific assessment of indirect benefits in low income settings are needed.

Despite the demonstrated success of vaccination programs in the Americas, Australia and a few European nations, only a limited number of countries have introduced RV vaccination programs and cost is probably an important barrier. Additional live and inactivated vaccines from emerging manufactures may be on the market over the next decade, (e.g. [62–66]) providing competition and potentially bringing down cost. Financial barriers to introducing a new vaccine are considerable in both high and low income settings, but of at least equal importance is having a strong public health case that militates for a policy of vaccine introduction. Continued investments to generate data on burden of rotavirus, safety and efficacy of vaccines and impact of programs will be crucial to that end.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Center for Disease Control Prevention (CDC): **Rotavirus surveillance – worldwide, 2001–2008**. *MMWR. Morbidity and Mortality Weekly Report* 2008, **57**:1255-1257.

2. Widdowson MA, Meltzer MI, Zhang X, Bresee JS, Parashar UD, Glass RI: **Cost-effectiveness and potential impact of rotavirus vaccination in the United States**. *Pediatrics* 2007, **119**:684-697.

3. • Tate JE, Burton AH, Boschi-Pinto C, Steele AD, Duque J, Parashar UD, the WHOcGRSN: **2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis**. *Lancet Infectious Diseases* 2012, **12**:136-141.
Most recent esdtimates of rotavirus related mortality: 450 000 death annually in children under 5 years. Will be an important benchmark against which to measure the impact of vaccination programs.

4. Center for Disease Control, Prevention (CDC): **Rotavirus surveillance worldwide – 2009**. *Weekly Epidemiological Record* 2011, **86**:174-176.

5. World Health O: **Rotavirus vaccines**. *Weekly Epidemiological Record* 2007, **82**:285-295.

6. Center for Disease Control Prevention (CDC): **Rotavirus vaccines: an update**. *Weekly Epidemiological Record* 2009, **84**:533-540.

7. Nelson EA, Glass RI: **Rotavirus: realising the potential of a promising vaccine**. *Lancet* 2010, **376**:568-570.

8. O'Ryan M, Lucero Y, Linhares AC: **Rotarix(R): vaccine performance 6 years postlicensure**. *Expert Review of Vaccines* 2011, **10**:1645-1659.

9. Tate JE, Patel MM, Cortese MM, Lopman BA, Gentsch JR, Fleming J, Steele AD, Parashar UD: **Remaining issues and challenges for rotavirus vaccine in preventing global childhood diarrheal morbidity and mortality**. *Expert Review of Vaccines* 2012, **11**:211-220.

10. Glass RI, Patel M, Parashar U: **Lessons from the US rotavirus vaccination program**. *JAMA: The Journal of the American Medical Association* 2011, **306**:1701-1702.

11. Glass RI: **Unexpected benefits of rotavirus vaccination in the United States**. *Journal of Infectious Diseases* 2011, **204**:975-977.

12. Staat MA, Payne DC, Donauer S, Weinberg GA, Edwards KM, Szilagyi PG, Griffin MR, Hall CB, Curns AT, Gentsch JR *et al.*: **Effectiveness of pentavalent rotavirus vaccine against severe disease**. *Pediatrics* 2011, **128**:e267-e275.

13. •• Buttery JP, Lambert SB, Grimwood K, Nissen MD, Field EJ, Macartney KK, Akikusa JD, Kelly JJ, Kirkwood CD: **Reduction in rotavirus-associated acute gastroenteritis following introduction of rotavirus vaccine into Australia's National Childhood vaccine schedule**. *Pediatric Infectious Disease Journal* 2011, **30**:S25-S29.

14. Paulke-Korinek M, Rendi-Wagner P, Kundi M, Kronik R, Kollaritsch H: **Universal mass vaccination against rotavirus gastroenteritis: impact on hospitalization rates in austrian children**. *Pediatric Infectious Disease Journal* 2010, **29**:319-323.

15. Muhsen K, Shulman L, Kasem E, Rubinstein U, Shachter J, Kremer A, Goren S, Zilberstein I, Chodick G, Ephros M *et al.*: **Effectiveness of rotavirus vaccines for prevention of rotavirus gastroenteritis-associated hospitalizations in Israel: a case-control study**. *Human Vaccines* 2010, **6**:450-454.

16. Gurgel RQ, Correia JB, Cuevas LE: **Effect of rotavirus vaccination on circulating virus strains**. *Lancet* 2008, **371**: 301-302.

17. Justino MC, Linhares AC, Lanzieri TM, Miranda Y, Mascarenhas JD, Abreu E, Guerra SF, Oliveira AS, da Silva VB, Sanchez N *et al.*: **Effectiveness of the Monovalent G1P[8] Human Rotavirus Vaccine Against Hospitalization for Severe G2P[4] Rotavirus Gastroenteritis in Belem, Brazil**. *Pediatric Infectious Disease Journal* 2011, **30**:396-401.

18. Correia JB, Patel MM, Nakagomi O, Montenegro FM, Germano EM, Correia NB, Cuevas LE, Parashar UD, Cunliffe NA, Nakagomi T: **Effectiveness of monovalent rotavirus vaccine (Rotarix) against severe diarrhea caused by serotypically unrelated G2P[4] strains in Brazil**. *Journal of Infectious Diseases* 2011, **201**:363-369.

19. Yen C, Figueroa JR, Uribe ES, Carmen-Hernandez LD, Tate JE, Parashar UD, Patel MM, Richardson Lopez-Collado V: **Monovalent rotavirus vaccine provides protection against an emerging fully heterotypic G9P[4] rotavirus strain in Mexico**. *Journal of Infectious Diseases* 2011, **204**:783-786.

20. Patel M, Pedreira C, De Oliveira LH, Tate J, Orozco M, Mercado J, Gonzalez A, Malespin O, Amador JJ, Umana J *et al.*: **Association between pentavalent rotavirus vaccine and severe rotavirus diarrhea among children in Nicaragua**. *JAMA: The Journal of the American Medical Association* 2009, **301**:2243-2251.

21. Mast TC, Khawaja S, Espinoza F, Paniagua M, Del Carmen LP, Cardellino A, Sanchez E: **Case-control study of the effectiveness of vaccination with pentavalent rotavirus vaccine in Nicaragua**. *Pediatric Infectious Disease Journal* 2011, **30**:e209-e215.

22. 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**. *Journal of Infectious Diseases* 2010, **201**:1617-1624.

23. • do Carmo GM, Yen C, Cortes J, Siqueira AA, de Oliveira WK, Cortez-Escalante JJ, Lopman B, Flannery B, de Oliveira LH, Carmo EH *et al.*: **Decline in diarrhea mortality and admissions after routine childhood rotavirus immunization in Brazil: a time-series analysis**. *PLoS Medicine* 2011, **8**:e1001024.

24. • Lanzieri TM, Linhares AC, Costa I, Kolhe DA, Cunha MH, Ortega-Barria E, Colindres RE: **Impact of rotavirus vaccination on childhood deaths from diarrhea in Brazil**. *International Journal of Infectious Diseases* 2011, **15**:e206-e210.

25. Molto Y, Cortes JE, De Oliveira LH, Mike A, Solis I, Suman O, Coronado L, Patel MM, Parashar UD, Cortese MM: **Reduction of diarrhea-associated hospitalizations among children aged <5 years in Panama following the introduction of rotavirus vaccine**. *Pediatric Infectious Disease Journal* 2011, **30**:S16-S20.

26. de Palma O, Cruz L, Ramos H, de Baires A, Villatoro N, Pastor D, de Oliveira LH, Kerin T, Bowen M, Gentsch J *et al.*: **Effectiveness of rotavirus vaccination against childhood diarrhoea in El Salvador: case-control study**. *BMJ: British Medical Journal* 2010, **340**:c2825.

27. Quintanar-Solares M, Yen C, Richardson V, Esparza-Aguilar M, Parashar UD, Patel MM: **Impact of rotavirus vaccination on diarrhea-related hospitalizations among children < 5 years of age in Mexico**. *Pediatric Infectious Disease Journal* 2011, **30**:S11-S15.

28. Safadi MA, Berezin EN, Munford V, Almeida FJ, de Moraes JC, Pinheiro CF, Racz ML: **Hospital-based surveillance to evaluate the impact of rotavirus vaccination in Sao Paulo, Brazil**. *Pediatric Infectious Disease Journal* 2010, **29**:1019-1022.

29. Yen C, Armero Guardado JA, Alberto P, Rodriguez Araujo DS, Mena C, Cuellar E, Nolasco JB, De Oliveira LH, Pastor D, Tate JE *et al.*: **Decline in rotavirus hospitalizations and health care visits for childhood diarrhea following rotavirus vaccination in El Salvador**. *Pediatric Infectious Disease Journal* 2011, **30**: S6-S10.

30. •• Richardson V, Hernandez-Pichardo J, Quintanar-Solares M, Esparza-Aguilar M, Johnson B, Gomez-Altamirano CM, Parashar U, Patel M: **Effect of rotavirus vaccination on death from childhood diarrhea in Mexico**. *New England Journal of Medicine* 2010, **362**:299-305.

31. • Richardson V, Parashar U, Patel M: **Childhood diarrhea deaths after rotavirus vaccination in Mexico**. *New England Journal of Medicine* 2011, **365**:772-773.

References [23•,24•,30••,31•] all demonstrate a decrease in gastroenrteritis mortalirty consistent with what may be expected (in terms of seasonality and age patterns) from rotavirus vaccination.

32. Barreto ML, Teixeira MG, Bastos FI, Ximenes RA, Barata RB, Rodrigues LC: **Successes and failures in the control of infectious diseases in Brazil: social and environmental context, policies, interventions, and research needs**. *Lancet* 2011, **377**:1877-1889.

33. • Payne DC, Staat MA, Edwards KM, Szilagyi PG, Weinberg GA, Hall CB, Chappell J, Curns AT, Wikswo M, Tate JE *et al.*: **Direct and indirect effects of rotavirus vaccination upon childhood hospitalizations in 3 US counties, 2006–2009**. *Clinical Infectious Diseases* 2011, **53**:245-253.

34. • Lopman BA, Curns AT, Yen C, Parashar UD: **Infant rotavirus vaccination may provide indirect protection to older children and adults in the United States**. *Journal of Infectious Diseases* 2011, **204**:980-986.

Taken together, the above two refeerences and reference [13], strongly suggest that rotavirus vaccines are also conferring indirect protection to both unvaccinated children as adults.

35. Pitzer VE, Viboud C, Simonsen L, Steiner C, Panozzo CA, Alonso WJ, Miller MA, Glass RI, Glasser JW, Parashar UD *et al.*: **Demographic variability, vaccination, and the spatiotemporal dynamics of rotavirus epidemics**. *Science* 2009, **325**:290-294.

36. Tate JE, Panozzo CA, Payne DC, Patel MM, Cortese MM, Fowlkes AL, Parashar UD: **Decline and change in seasonality of US rotavirus activity after the introduction of rotavirus vaccine**. *Pediatrics* 2009, **124**:465-471.

37. Hanquet G, Ducoffre G, Vergison A, Neels P, Sabbe M, Van Damme P, Van Herck K: **Impact of rotavirus vaccination on laboratory confirmed cases in Belgium**. *Vaccine* 2011, **29**: 4698-4703.

38. Linhares AC, Velazquez FR, Perez-Schael I, Saez-Llorens X, Abate H, Espinoza F, Lopez P, Macias-Parra M, Ortega-Barria E, Rivera-Medina DM *et al.*: **Efficacy and safety of an oral live attenuated human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in Latin American infants: a randomised, double-blind, placebo-controlled phase III study**. *Lancet* 2008, **371**:1181-1189.

39. Vesikari T, Karvonen A, Prymula R, Schuster V, Tejedor JC, Cohen R, Meurice F, Han HH, Damaso S, Bouckenooghe A: **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**:1757-1763.

40. Kirkwood CD, Boniface K, Barnes GL, Bishop RF: **Distribution of rotavirus genotypes after introduction of rotavirus vaccines. Rotarix(R) and RotaTeq(R), into the National Immunization Program of Australia**. *Pediatric Infectious Disease Journal* 2011, **30**:S48-S53.

41. Boom JA, Tate JE, Sahni LC, Rench MA, Hull JJ, Gentsch JR, Patel MM, Baker CJ, Parashar UD: **Effectiveness of pentavalent rotavirus vaccine in a large urban population in the United States**. *Pediatrics* 2010, **125**:e199-e207.

42. Desai SN, Esposito DB, Shapiro ED, Dennehy PH, Vazquez M: **Effectiveness of rotavirus vaccine in preventing hospitalization due to rotavirus gastroenteritis in young children in Connecticut, USA**. *Vaccine* 2010, **28**:7501-7506.

43. Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, Dallas MJ, Heyse JF, Goveia MG, Black SB *et al.*: **Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine**. *New England Journal of Medicine* 2006, **354**:23-33.

44. Chang CC, Chang MH, Lin TY, Lee HC, Hsieh WS, Lee PI: **Experience of pentavalent human-bovine reassortant rotavirus vaccine among healthy infants in Taiwan**. *Journal of the Formosan Medical Association* 2009, **108**:280-285.

45. Hull JJ, Teel EN, Kerin TK, Freeman MM, Esona MD, Gentsch JR, Cortese MM, Parashar UD, Glass RI, Bowen MD *et al.*: **United States rotavirus strain surveillance from 2005 to 2008: genotype prevalence before and after vaccine introduction**. *Pediatric Infectious Disease Journal* 2011, **30**:S42-S47.

46. Patel MM, de Oliveira LH, Bispo AM, Gentsch J, Parashar UD: **Rotavirus P[4]G2 in a vaccinated population, Brazil**. *Emerging Infectious Diseases* 2008, **14**:863-865.

47. Pitzer VE, Patel MM, Lopman BA, Viboud C, Parashar UD, Grenfell BT: **Modeling rotavirus strain dynamics in developed countries to understand the potential impact of vaccination on genotype distributions**. *Proceedings of the National Academy of Sciences of the United States of America* 2011, **108**:19353-19358.

48. Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, Abate H, Breuer T, Clemens SC, Cheuvart B, Espinoza F, Gillard P, Innis BL *et al.*: **Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis**. *New England Journal of Medicine* 2006, **354**:11-22.

49. Patel MM, Lopez-Collada VR, Bulhoes MM, De Oliveira LH, Bautista Marquez A, Flannery B, Esparza-Aguilar M, Montenegro Renoiner EI, Luna-Cruz ME, Sato HK *et al.*: **Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil**. *New England Journal of Medicine* 2011, **364**: 2283-2292.

There appears to be a small risk if intussusception, at least after the first dose of RV5 in Mexico, though the risks of this adverse event is heavily outweghed by the benefits of vaccination.

50. Buttery JP, Danchin MH, Lee KJ, Carlin JB, McIntyre PB, Elliott EJ, Booy R, Bines JE, Group PAS: **Intussusception following rotavirus vaccine administration: post-marketing surveillance in the National Immunization Program in Australia**. *Vaccine* 2011, **29**:3061-3066.

51. Shui IM, Baggs J, Patel M, Parashar UD, Rett M, Belongia EA, Hambidge SJ, Glanz JM, Klein NP, Weintraub E: **Risk of intussusception following administration of a pentavalent rotavirus vaccine in US infants**. *JAMA: The Journal of the American Medical Association* 2012, **307**:598-604.

52. Greenberg HB: **Rotavirus vaccination and intussusception – act two**. *New England Journal of Medicine* 2011, **364**:2354-2355.

53. Centers for Disease Control and Prevention. Advisory Committee on Immunization Practices (ACIP) Summary Report October 27–28, 2010. http://www.cdc.gov/vaccines/recs/acip/downloads/min-archive/min-oct10.pdf.

54. **Rotavirus vaccine and intussusception:report from an expert consultation**. *Weekly Epidemiological Record* 2011, **86**:317–321.

55. •• Victoria JG, Wang C, Jones MS, Jaing C, McLoughlin K, Gardner S, Delwart EL: **Viral nucleic acids in live-attenuated vaccines: detection of minority variants and an adventitious virus**. *Journal of Virology* 2010, **84**:6033-6040.
Detection of an adventitionus agent (porcine circovirus) in RV1, which also led ot the discovery of PCV fragments in RV5, raised an unexpected saftey concern, as well as a general debate about the role of ultra-sensitive molecular techiniquies as a tool for assessing vaccine saftey.

56. McClenahan SD, Krause PR, Uhlenhaut C: **Molecular and infectivity studies of porcine circovirus in vaccines**. *Vaccine* 2011, **29**:4745-4753.

57. US Food and Drug Administration: http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm211101.htm. vol 2/15/2012.

58. Dore DD, Turnbull BR, Seeger JD: **Vaccine discontinuation and switching following regulatory interventions in response to rotavirus vaccine contamination with porcine circovirus DNA fragments**. *Pharmacoepidemiology and Drug Safety* 2012, **21**:415-419.

59. Payne DC, Humiston S, Opel D, Kennedy A, Wikswo M, Downing K, Klein EJ, Kobayashi A, Locke D, Albertin C *et al.*: **A multi-center, qualitative assessment of pediatrician and maternal perspectives on rotavirus vaccines and the detection of Porcine circovirus**. *BMC Pediatrics* 2011, **11**:83.

60. Bouzon Alejandro M, Diez Domingo J, Martinon-Torres F: **Circovirus and impact of temporary withdrawal of rotavirus vaccines in Spain**. *Human Vaccines* 2011, **7**:798-799.

61. Babi S, Kang G: **Rotavirus vaccination in developing countries**. *Current Opinion in Virology*, http://dx.doi.org/10.1016/j.coviro.2012.05.005

62. Vesikari T, Karvonen AV, Majuri J, Zeng SQ, Pang XL, Kohberger R, Forrest BD, Hoshino Y, Chanock RM, Kapikian AZ: **Safety, efficacy, and immunogenicity of 2 doses of bovine-human (UK) and rhesus-rhesus-human rotavirus reassortant tetravalent vaccines in Finnish children**. *Journal of Infectious Diseases* 2006, **194**:370-376.

63. Bhandari N, Sharma P, Taneja S, Kumar T, Rongsen-Chandola T, Appaiahgari MB, Mishra A, Singh S, Vrati S: **A dose-escalation safety and immunogenicity study of live attenuated oral rotavirus vaccine 116E in infants: a randomized, double-blind, placebo-controlled trial**. *Journal of Infectious Diseases* 2009, **200**:421-429.

64. Barnes GL, Lund JS, Mitchell SV, De Bruyn L, Piggford L, Smith AL, Furmedge J, Masendycz PJ, Bugg HC, Bogdanovic-Sakran N *et al.*: **Early phase II trial of human rotavirus vaccine candidate RV3**. *Vaccine* 2002, **20**:2950-2956.

65. Wang Y, Azevedo M, Saif LJ, Gentsch JR, Glass RI, Jiang B: **Inactivated rotavirus vaccine induces protective immunity in gnotobiotic piglets**. *Vaccine* 2010, **28**:5432-5436.

66. Luan le T, Trang NV, Phuong NM, Nguyen HT, Ngo HT, Nguyen HT, Tran HB, Dang HN, Dang AD, Gentsch JR *et al.*: **Development and characterization of candidate rotavirus vaccine strains derived from children with diarrhoea in Vietnam**. *Vaccine* 2009, **27(Suppl. 5)**:F130-F138.

67. Tate JE, Mutuc JD, Panozzo CA, Payne DC, Cortese MM, Cortes JE, Yen C, Esposito DH, Lopman BA, Patel MM *et al.*: **Sustained decline in rotavirus detections in the United States following the introduction of rotavirus vaccine in 2006**. *Pediatric Infectious Disease Journal* 2011, **30**:S30-S34.